Optimization of radiotherapy in bladder cancer

2020

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Total word count

30 755 (main body of text including footnotes)

List of abbreviations

5FU	5 Fluorouracil
95% CI	95% Confidence Interval
ADC	Apparent Diffusion Co-efficient
AE	Adverse Events
AJCC	American Joint Committee on Cancer
AP	Anterior-Posterior
ART	Adaptive Radiotherapy
AUA	American Urological Association
BCON	Bladder Carbogen Nicotinamide
BH	Benjaminin-Hochberg
BOLD	Blood oxygenation level dependent
CBCT	Cone Beam Computed Tomography
CO2	Carbon Dioxide
CI	Chief Investigator
CIS	Carcinoma In Situ
CRF	Case Report Form
CSS	Cancer Specific Survival
CTCAE	Common Terminology Criteria for Adverse Events Version
CT	Computed Tomography
CTV	Clinical Target Volume
dATP	Deoxyadenosine triphosphate
DNA	Deoxyribose nucleic acid
DPA	Data Protection Act
DSS	Disease specific survival
DVH	Dose Volume Histogram
DWI	Diffusion Weighted Imaging
EAU	European Association of Urologists

FdUMP	Fluorodeoxyurine Monophosphate
FFPE	Formalin-Fixed Paraffin-Embedded
FGFR3	Fibroblast Growth Factor Receptor 3
FUMP	Fluorouracil Monophosphate
FUTP	Fluorouracil Triphosphate
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
GP	General Practitioner
GTV	Gross Tumour Volume
Gy	Gray
H&E	Haematoxylin and Eosin
HIF	Hypoxia Inducible Factor
HR	Hazard Ratio
HRQoL	Health Related Quality of Life
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICRU	International Commission on Radiation Units
ID	Identification
IGRT	Image guided radiotherapy
IMRT	Intensity modulated radiotherapy
ITV	Internal Target Volume
L/min	Litres per minute
LNMRI	Lymphotropic nanoparticle enhanced MRI
MDT	Multi-Disciplinary Team
mg/m2	Milligram per metre squared
mg/kg	Milligram per kilogram
ml	Millilitre
mm	Millimetre

MIBC	Muscle-invasive bladder cancer
MIUC	Muscle-invasive urothelial cancer
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
MRL	MR Linac
NAC	Neo-Adjuvant Chemotherapy
NHEJ	Non-Homologous End Joining
NICE	National Institute for Health and Care Excellence
NHS	National Health Service
NMIBC	Non Muscle Invasive Bladder Cancer
O2	Oxygen
OAR	Organs at Risk
OS	Overall survival
PARP	Poly (ADP-Ribose) Polymerase
PARPi	PARP Inhibitor
PD-1	Programmed cell death protein 1
PD-L1	Programmed cell death ligand 1
PFS	Progression Free Survival
PIS	Participant / Patient Information Sheet
PLD	Potentially lethal damage
POD	Plan of the Day
POTD+	Plan of the Day Plus
PTV	Planning Target Volume
QoL	Quality of Life
RB	Retinoblastoma protein
R&D	Research & Development
RC	Radical Cystectomy
REC	Research Ethics Committee

RFS	Relapse free survival
RNA	Ribonucleic acid
RRR	Radiotherapy Related Research
RT	Radiotherapy
RTOG	Radiation Therapy Oncology Group
RTP	Radiotherapy Planning
SAE	Serious Adverse Event
SBRT	Stereotactic radiotherapy
SI	Superior-Inferior
SLD	Sub-Lethal damage
SPARE	Selective Bladder Preservation Against Radical Excision
T	Tesla
T2	Transverse Relaxation Time
TMT	Trimodality treatment
TNM	Tumour Nodes Metastasis
TS	Thymidylate Synthase
TURBT	Transurethral Resection of Bladder Tumour
UK	United Kingdom
VI-RADS	Vesical Imaging Reporting And Data System
VMAT	Volumetric modulated arc therapy
XVI	X-Ray Volume Imaging

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Preface

This thesis is the product of various projects assessing different factors involved in the optimization of radiotherapy in bladder cancer. While these projects are related through this central theme, each forms an independent study. The thesis is therefore presented in a journal format. It comprises of a literature review and the four studies undertaken, all of which are presented in the style of a scientific article. This is then followed by concluding remarks and a discussion of future work planned.

The prospective study protocol described in chapter 7 is included in the appendix along with full journal publications and abstracts that resulted from this thesis.

The author was a specialty trainee in Clinical Oncology undertaking a two-year clinical research fellowship. She trained in medicine at Queen's University of Belfast where she graduated in 2009. She obtained Membership of the Royal College of Physicians (MRCP) in 2013 and Fellowship of the Royal College of Radiologists (FRCR) in 2017. She is now a Consultant Clinical Oncologist at The Christie Hospital specialising in urological malignancies.

Acknowledgements

I would like to express my deepest gratitude to my family for their unconditional support over the years.

I am grateful for the patience and guidance of my supervisors, Professor Ananya Choudhury, Professor Peter Hoskin, Dr Andrew McPartlin and Dr Alan McWilliam, without whom completion of the work in this thesis would not have been possible.

I would also like to thank my friends and colleagues at the Radiotherapy Related Research and Clinical Oncology departments at The Christie Hospital, whose support and assistance have been invaluable.

In loving memory of my dearest Papa

Abstract

Introduction: The mainstay of bladder preservation treatment is radiotherapy, and hypoxia modification has been shown to improve outcomes. This thesis aims to (1) investigated the long-term outcomes of hypoxia modification associated with biological features in muscle-invasive bladder cancer; (2) investigate the relationship of parameters on radiotherapy planning (RTP) scan with clinical outcomes of patients following radical radiotherapy for bladder cancer; (3) quantify and compare inter-fraction bladder motion between men and women; (4) prospectively evaluate the association of intra-fraction motion of tumour bed and bladder.

Method: (1) Examine the long-term outcomes of the BCON study and explore the biological features that may aid in patient selection for hypoxia modification including necrosis status, hypoxia gene signature score and molecular subtypes; (2) Conduct a retrospective study on patients who had completed radical chemoradiotherapy, assessing bladder and rectum dimensions on RTP scans and relating that to patients' outcomes; (3) Compare the differences in inter-fraction bladder motion between male and female bladders by measuring motion in six directions. (4) Evaluate the motion of tumour bed and bladder on MRI scans over a time period required to deliver a fraction of MRI guided treatment, and assess potential correlation between the motion of tumour bed and bladder

Results: (1) The long-term benefit of hypoxia modification is sustained and necrosis and hypoxia gene signature score have the potential to select patients who would benefit most. (2) Bladder size is associated with survival outcomes. (3) Mean male and female bladder inter-fraction motion are similar. (4) There is an association between tumour bed and bladder motion in anterior-posterior and superior-inferior directions.

Conclusion: The long-term benefit of hypoxia modification in the presence of necrosis and hypoxia suggest that a biomarker driven study will enable better patient selection for treatment in future. The bladder varies in size, moves in all direction intra- and inter-fraction. The current standard of care of a single treatment plan is not ideal. Adaptive radiotherapy with a library of plans that take into consideration tumour bed and bladder motion will allow for more accurate treatment to be delivered.

1 Introduction

1.1 Epidemiology

Bladder cancer is ninth most common cancer worldwide[1] and the tenth most common cancer in the United Kingdom. It accounts for 3% of all cancer deaths in the UK and is the ninth most common cause of cancer related deaths. Mortality is related to age and rises sharply in both males and females at the age of 60, with rates being higher in males than females[2].

There were 10100 new cases diagnosed in the UK in 2014, and 5400 bladder cancer related deaths in the same year. There is a higher incidence in males than females. More than half of bladder cancer cases each year are diagnosed in patients aged 75 and older, and the incidence increases with age with the highest in people over the age of 90.
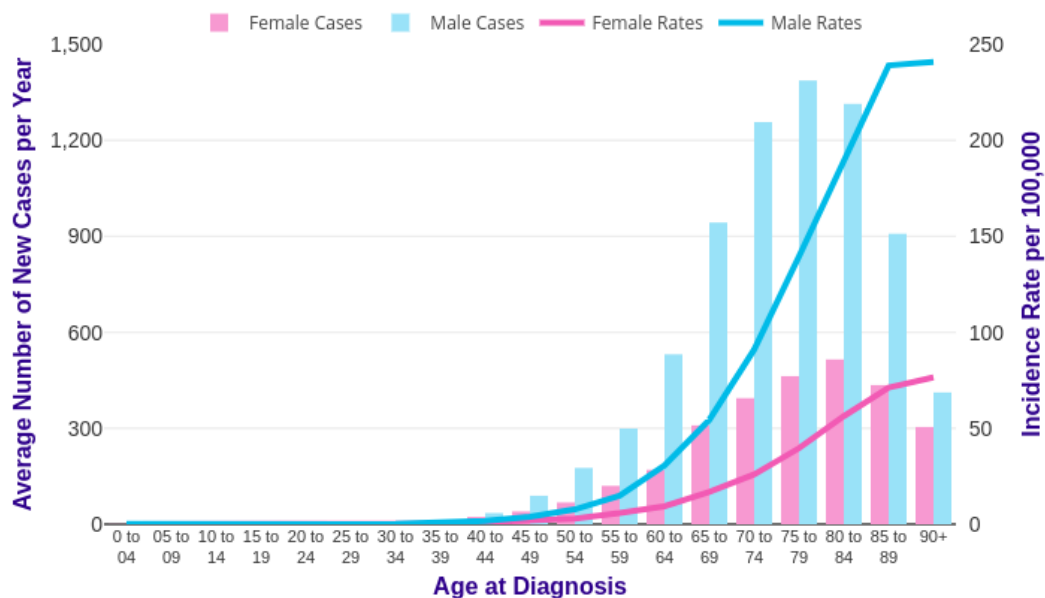
Figure 1 Average number of new cases per year and age-specific incidence rates per 100 000 population, UK (Source: Cancer Research UK)

There are various risk factors associated with the development of bladder cancer, including some lifestyle factors that are potentially avoidable. Smoking is linked to about 37% of bladder cancers in the UK, while 6% is related to occupational exposure and 3% to ionising radiation. There has been a decrease in the incidence of bladder cancer in recent years and this could be linked to a decrease in smoking and exposure to environmental carcinogens[3].

1.2   Anatomy and histopathology

The urinary bladder is a hollow distensible muscular organ. It is a part of the urinary system and serves two main functions – it collects and stores urine temporarily, and assists in the expulsion of urine. Urine from the kidneys enters the bladder via the left and right ureters and exit through the urethra. When the bladder is empty, it sits entirely within the pelvis. As it fills, its shape changes from being flat to being oval and protrudes into the abdomen. The average adult human bladder has the capacity of 300-600ml.
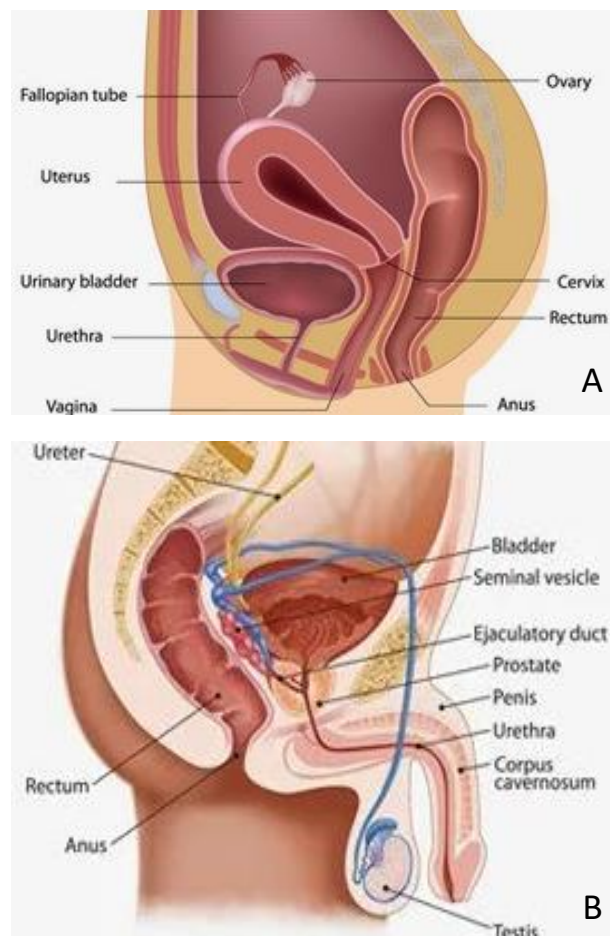
Figure 2 Bladder in relation to other pelvic organs in female (A) and male (B) pelvis (source: http://www.organsofthebody.com/bladder/)

The pubic symphysis lies anterior to the urinary bladder. The fundus (or base) of the bladder lies anterior to the cervix and vagina in females, and anterior to the rectum in males. The prostate is inferior to the bladder in men, and the uterus is superior to the bladder in women[4]. Figure 2 illustrates the position of the bladder in the female and male pelvis respectively.

The bladder wall is made up of four layers – mucosa, submucosa, muscularis propria and adventitia or serosa.

Mucosa	The innermost layer of the bladder is lined with a multilayer epithelium known as urothelium. It is lined with a glycosaminoglycan layer which serves both as an osmotic barrier and an antibacterial coating.
Submucosa	Also known as the lamina propria, this layer consists of connective tissue and a dense capillary plexus that serves as a further barrier
Muscularis propria	The muscularis layer is formed by three layers of smooth muscles
Adventitia or serosa	This connective tissue layer is formed partly by the reflection of the peritoneum and separates the bladder from other organs

Table 1 Layers of the urinary bladder

Up to 95% of bladder cancers diagnosed in the UK originate from the urothelium and are known as urothelial or transitional cell cancers. Urothelial cancers can be non-invasive with flat or papillary lesions or invasive. Invasive cancers are more aggressive.

Squamous differentiation is found in about 20% of bladder cancers and glandular differentiation is found in about 6%. The presence of either squamous or glandular differentiation does not affect the overall prognosis. There has been evidence to support neo-adjuvant chemotherapy in urothelial cancer with squamous differentiation[5]. Other variants like nested and sarcomatoid are known to be more aggressive. Although less common, small cell carcinomas are also known to arise in the bladder. They are characterised by an aggressive clinical course, and tend to carry a poorer prognosis[6].

1.3 Staging

The stage of disease is a vital prognostic factor for progression and OS. It is also an important factor that directs management plans. The most common staging system used for bladder cancer is known as the Tumour, Node, Metastasis (TNM) system which reflects tumour size and degree of bladder wall invasion, nodal involvement and distant metastases. The TNM staging for bladder cancer is described in table 2. A combination of transurethral resection of bladder tumour (TURBT) and imaging modalities such as computed tomography (CT) scans and Magnetic Resonance Imaging (MRI) are used to adequately stage bladder cancer.
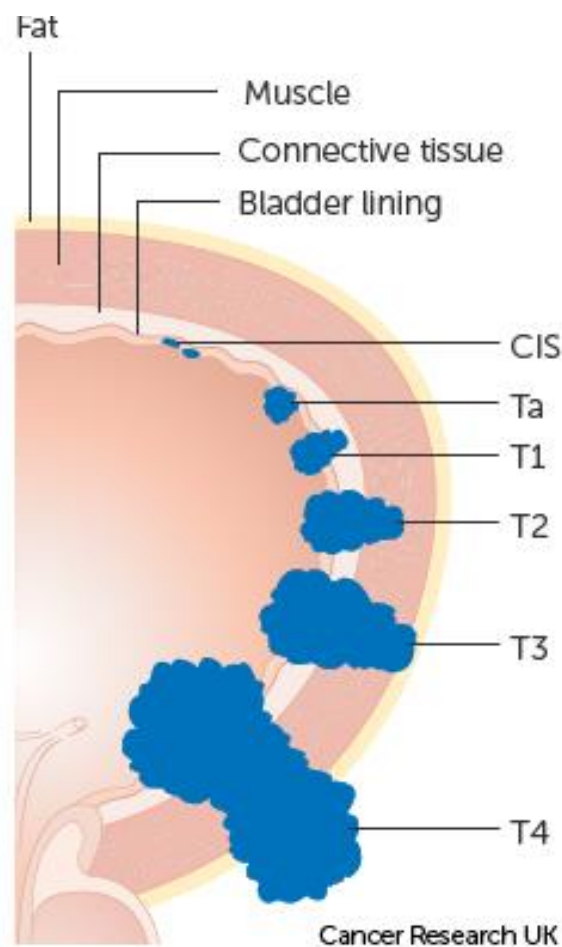
Figure 3 The different T-stages and their relation to the different layers of the bladder. (source: Cancer Research UK)

T – Primary Tumour		
Ta		Exophytic lesions that tend to recur, but not invasive
Tis		Carcinoma in-situ (cancer is in the inner most layer of the bladder and does not invade deeper)
T1		Tumour invading the submucosa or lamina propria
T2		Tumour invades into the muscle layer
	T2a	Superficial invasion
	T2b	Deep invasion
T3		Tumour extends beyond the muscle into perivesical fat
	T3a	Microscopic extension
	T3b	Macroscopic extension
T4		Tumour extending to adjacent organs
	T4a	Tumour invading prostate, vagina, uterus or bowel
	T4b	Tumour fixed to abdominal wall, pelvic wall or other organs
N – Regional Lymph Nodes		
N1		Single lymph node involvement in the true pelvis
N2		Multiple lymph nodes involvement in the true pelvis
M – Distant Metastasis		
M		Distant metastatic disease present

Table 2 The American Joint Committee on Cancer (AJCC) Staging System (8th edition, 2017)

In the absence of nodal or distant metastatic disease, the optimal management of bladder cancer is dependent on the T-staging. Patients with Ta, Tis and T1 disease are known to have non-muscle-invasive bladder cancer (NMIBC) and this is managed with intra-vesical treatments. However, one-third of patients diagnosed with bladder cancer have muscle-invasive disease (MIBC) and these patients require more aggressive treatments. NMIBC and MIBC are regarded as two different diseases both in terms of pathogenetic pathways, treatment and prognosis. This literature review focuses on the muscle-invasive stages of bladder cancer.

1.4 Curative treatment options

The two main treatment options available to patients with localized MIBC are radical cystectomy (RC) and trimodality bladder preservation treatment (TMT). As the choice between TMT and RC is an important one, the differences between the two treatment options and the guideline recommendations should be considered.

1.4.1 Comparing radical cystectomy and trimodality treatment

RC — which involves major abdominopelvic surgery performed under general anaesthesia followed by a prolonged recovery period —is associated with increased operative mortality in elderly patients (age ≥75 years) and can result in long-term changes in QoL to which all patients must adapt. Such QoL changes include the construction of a urostomy or neobladder and negative effects on sexual function. In the long term, 24% of patients with ileal conduits experience stomal problems, and a similar proportion of patients develop renal insufficiency (27%), bowel problems (24%), and urinary tract infections (24%)[7]. With respect to sexual function, a retrospective survey by the Department of Health in England found that, at 1–5 years after diagnosis, 88% of patients reported dissatisfaction with their sex life after RC, whereas only 11% and 17% of patients reported such dissatisfaction following chemoradiotherapy and radiotherapy, respectively[8].

TMT comprises maximal TURBT followed by radiotherapy with concurrent administration of a radiosensitizing agent (often chemotherapy)[9]. Radical radiotherapy to the bladder involves 4–7 weeks of daily treatment and has the potential of causing tiredness, impaired sexual function, and adverse effects related to the bladder and bowel (such as urgency, dysuria and proctitis)[10]. Indeed, a 2018 meta-analysis reported that late Radiation Therapy Oncology Group (RTOG) grade 3 pelvic toxicities, including urinary and gastrointestinal toxicities, occurred in 7% of patients following TMT[11]. Moreover, in the phase III BC2001 trial, which investigated radiotherapy with or without synchronous chemotherapy in patients with MIBC, late RTOG grade 3–4 pelvic toxicities were noted in 4.6% of patients who underwent chemoradiotherapy and in 5.2% of patients who received radiotherapy only at two years after completion of treatment[12]. TMT is also associated with a long-term negative effect on bladder function, specifically decreased bladder capacity in ~3% of patients[13], and cystectomy for intractable bladder symptoms rather than tumour recurrence is required in 1–2% of patients[13], [14]. In a recent report

of the 5-year, patient-reported, health-related quality of life (HRQoL) outcomes from the BC2001 study, there was an early reduction in HRQoL scores at the end of treatment resulting from the impact of acute toxicities, followed by improvement of scores to that of baseline after six months. This remained consistent thereafter. However, only about 70% and 60% of questionnaires were returned at 1 year and 5 years following treatment and there may be a bias of patients who were more well having a greater tendency to complete and return the questionnaires[15].

An important component of TMT is ongoing cystoscopic surveillance with the possibility of salvage cystectomy after disease recurrence, which might be necessary in ~7–15% of patients[12], [14], [16]. A study reported that TMT was associated with a risk of NMIBC recurrence in 25% of patients who had complete response, who might require treatment[17]. However, this retrospective study recruited over a 27 years period from 1986 to 2013. It is therefore likely that a variety of radiotherapy techniques were utilised.

Ideally, RC and TMT should be compared in a prospective randomized controlled trial, but attempts to do so have been unsuccessful owing to poor patient accrual[18]. The early closure of the Selective bladder Preservation Against Radical Excision (SPARE) trial has been attributed to several factors, including the complexity of the patient referral and management pathways (which had multiple specialist teams and centres involved) and the importance of patient preference in a trial that randomizes patients to two distinctly different treatment options.

Large RC series report 5-year OS ranging from 40.2–58%[19]–[21]. Similarly, a meta-analysis showed that patients with MIBC undergoing TMT had good outcomes, despite its use in frail patients, with a complete response rate of 78% and 5-year OS of 56%[22]. In addition, a pooled analysis of six RTOG studies showed that the 10-year disease-specific survival (DSS) was 65% in patients with MIBC following bladder preservation treatment[16].

Various retrospective studies and propensity matched analyses have attempted to compare the outcomes of patients receiving either treatment option. In a population-based retrospective cohort study, the cancer-specific survival (CSS) and OS of patients who underwent RC and TMT were similar following adjustments for covariates[23].

Similarly, a meta-analysis of 29 TMT studies and 30 RC studies found that the 5-year OS was 63% for TMT and 61% for RC patients with T2 disease (P=0.30) and was 45% and 40%, respectively, for patients with >T2 disease (P=0.36)[24]. Conversely, a cohort study from 2018 proposed the contrary — that TMT is associated with poorer OS and DSS than RC[25]. However, patients in the TMT group had received a median of 18 fractions of radiotherapy, suggesting that more than half of patients who allegedly underwent TMT did not, in fact, have curative treatment and, therefore, did not undergo TMT. During the time period studied (December 2011 to December 2013), TMT tended to be recommended for frail patients who were unfit for surgery, a consideration that was also not adequately accounted for in this study.

A number of factors preclude patients from each treatment option. Patients with serious comorbidities (for example, cardiac issues and renal failure) might be unable to tolerate the general anaesthetic and physiological stress associated with major surgery, and some patients might be unable or unwilling to adapt to the lifestyle changes that are required after RC. Similarly, patients with extensive carcinoma in situ (CIS), poor bladder function, or obstruction to their kidneys might not be appropriate candidates for TMT. Moreover, patients who undergo TMT must be prepared for the ongoing cystoscopic surveillance and the ongoing risk, for a minority of patients, that cystectomy will be ultimately required in the event of recurrence or poor residual bladder function.

A retrospective study of patients with MIBC treated with either RC or TMT found that patients who received TMT had markedly better general QoL, bowel function, and sexual function, had fewer concerns about the negative effects of cancer, and had similar urinary symptoms scores[26]. Toxicity rates between the two treatment options have also been reported in the SPARE trial; 70% of patients in the RC arm had Common Terminology Criteria for Adverse Events (CTCAE) grade 3–4 toxicity compared to 36% of patients in the radiotherapy group (P=0.038)[18]. The primary aim of radical treatment for MIBC is to maximise the chance of cure while maintaining a good patient QoL.

1.4.2   Guidelines recommendations

In the UK, specific recommendations in the National Institute for Health and Care Excellence (NICE) guidelines[27], which provide evidence-based guidance for clinical

practice, advocate that patents with MIBC are offered both RC and TMT as curative treatment options. This set of guidelines specifies the need to discuss the evidence for each treatment option in terms of efficacy, potential toxicities, and the influence on QoL. Surgery remains the most common option recommended to patients in Europe and the USA. The European Association of Urology (EAU) guidelines recommend that patients who are fit should be offered RC, reserving TMT for those who are less fit[28]. While the American Association of Urology (AUA) had similar guidelines previously, this has changed. It now recommends that patients who desire to retain their bladder or who are not suitable surgical candidate should be offered TMT[29]. The differences in recommendations are reflected in real world practice. In a survey of 277 radiation oncologists from the USA, 58% treated only 1–3 patients per year with TMT, and 74% primarily treated patients who were deemed unfit for surgery with TMT[30]. Similarly, a survey of 32 Italian centres found that, in the 13 centres that responded, only 12 of 100 patients with bladder cancer in a one-year period received radiotherapy as a primary curative treatment[31]. The main difference between the guidelines is that NICE recommends that patients should be offered both options, whereas the EAU and, until more recently, AUA, guidelines recommend RC over TMT for fit patients.

The choice between TMT and RC is a difficult one for patients and a controversial topic amongst clinicians. As discussed previously, the previous attempt to establish level I evidence with a randomized controlled trial had been unsuccessful.

1.4.3   External beam radiotherapy

External beam radiotherapy is an important aspect of TMT. Ionizing radiation causes both single-stranded and double-stranded deoxyribonucleic acid (DNA) breaks. This in turn results in lethal damage, potentially lethal damage (PLD) and sublethal damage (SLD). As the name suggests, lethal damage is irreversible and would lead to cell death. PLD is damage that can be repaired if the post-irradiation environment changes and SLD is DNA damage that can be repaired spontaneously with time. The repair of SLD is especially important in normal tissue response and should be considered in order to reduce treatment related toxicities.

Radiation dose is expressed in gray (Gy), which is the defined as 1 joule of energy absorbed per 1 kilogram of mass. The total dose of radiation is then delivered in multiple smaller doses over a period of time. This is known as fractionation. Fractionation allows for restoration of normal tissue damage and improvement of radiosensitivity through the concepts of repair, reoxygenation and redistribution.

Cancer cells lack appropriate DNA repair mechanisms to recover from the damage caused by radiation and have less capacity to repair radiation damage but the time between fractions of treatment allow for repair of SLD in normal tissue. As the tumour shrinks in response to treatment, previously hypoxic and radioresistant portions of the tumour may become reoxygenated, rendering them more radiosensitive. Cells in different phases of the cell cycle also display different levels of radiosensitivity. There is greatest radioresistance during the DNA replication phases of the cell cycle and greatest radiosensitivity during mitosis. The time delay between each radiotherapy fraction allows cells to redistribute and progress from one phase to the next, in turn increasing the chances of irradiating cells during the radiosensitive phases and improving the efficacy of treatment. Despite the benefits of having a delay between fractions, the prolongation of treatment time also results in accelerated repopulation. Cytotoxic treatments like radiotherapy can induce an increase in the rate of cell division and reduction in length of cell cycle, thereby increasing proliferation rate. It is therefore vital to achieve a balance of minimising toxicities, improving efficacy and reducing cell proliferation when formulating a curative treatment course.

Although TMT and radiotherapy are often regarded as "second best", reserved for patients too unwell to undergo surgery[30], the use of ionizing radiation in radical treatment of bladder cancer is certainly not a new development. Waters describes a series of 67 cases treated with radiotherapy – implantation (brachytherapy), deep roentgen ray (external beam radiotherapy) or a combination of both in 1923[32]. The idea of radical radiotherapy with salvage cystectomy is also not a new one. Studies since the mid-1970s had been advocating initial radical radiotherapy with close monitoring as opposed to upfront cystectomy, with surgery being held in reserve for patients who do not respond to radiotherapy or whose disease recur[33]–[36].

While brachytherapy may have played a role in bladder cancer treatment in the 1920s, a preference for external beam radiotherapy quickly took hold. Duncan et al described 963 patients with bladder cancer treated with a three-field beam directed technique (one anterior, two posterior oblique fields) while lying prone, at a dose of between 55 to 57.5Gy in 20 fractions over 4 weeks, between 1971 and 1982[34]. While this is not significantly different from the manner in which bladder cancer is treated more than 40 years later, modern radiotherapy techniques have resulted in improved conformality of radiotherapy plans in three-dimensions.
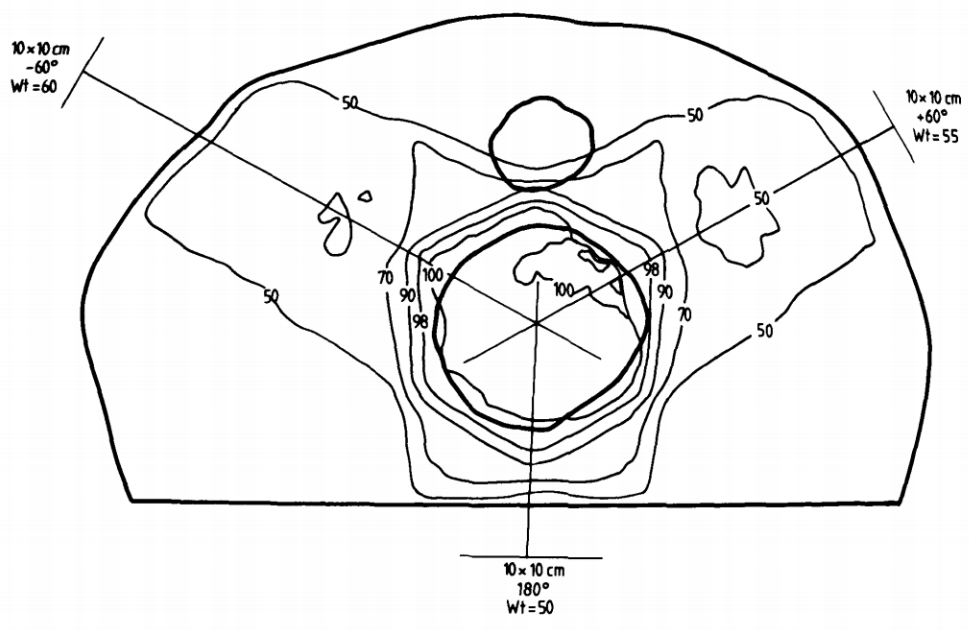
Figure 4 Radiotherapy plan from 1970s with a 3-field technique with patient in a prone position [34].

External beam radiotherapy involves the use of high energy x-ray generated from a linear accelerator to kill cancer cells while minimizing radiation dose to surrounding tissues. Different radiotherapy techniques have been developed over the years to achieve this by delivering treatment with multiple beams of radiation that intersect at the target to achieve a cumulative dose, while spreading out the dose that each individual surrounding organ would achieve.

In the last decade, the use of intensity modulated radiotherapy (IMRT) and volumetric modulated arc therapy (VMAT) has allowed better shaping of dose around the target volume while avoiding organs at risk, potentially reducing both early and late toxicities.

Van Rooijen et al re-planned the radiotherapy of 20 patients who had undergone 3D conformal radiotherapy, examining the effect of IMRT plans and found that while maintaining good target volume coverage, the incidental dose to small intestine was significantly reduced[37]. However, these radiotherapy plans are more complex and time consuming to formulate and deliver.

There is currently no consensus with regards to the dose and fractionation used in curative treatment of bladder cancer. The two major clinical trials in the UK have allowed for either 55Gy in 20 fractions or 64Gy in 32 fractions. The results of a meta-analysis of patients treated in both trials were presented, reporting that the hypofractionated 55Gy in 20 fractions was not inferior 64Gy in 32 fractions. However, these results have not been formally published[38].

1.4.4   Radiosensitisation

Central to bladder preserving treatment is radical radiotherapy with the addition of radiosensitising agents. Radiosensitising agents work in a synergistic or additive manner with radiotherapy to increase cell kill and improve treatment efficacy.

Various phase II studies have investigated the use of different cisplatin-containing regimens (cisplatin alone, cisplatin plus 5-fluorouracil, and cisplatin plus paclitaxel) as radiosensitizers in MIBC, and have reported good complete response rates and long-term DSS that are comparable to that of RC[39]–[42]. However, these were relatively small studies with sample sizes of <100 patients. Patients in these studies were treated in a split course manner, with assessment following an initial induction course of chemoradiotherapy, after which patients were selected for RC or consolidation chemoradiotherapy on the basis of their response to induction treatment. These studies showed encouraging results at the time. Mak et al. conducted a pooled analysis of these phase II trials and a phase III study and demonstrated that these prospective RTOG bladder-preserving protocols result in DSS comparable to that of cystectomy studies [16].

The choice of radiosensitisers has evolved over time. Until the early 2000s, the only randomised phase III clinical trial was a Canadian study that used cisplatin as a radiosensitiser[43]. This small study (n=99) showed an improvement in both the rate of local control and OS with the addition of concurrent cisplatin to perioperative or

definitive radiotherapy, but had limited statistical power (3-year OS 47% versus 33%, p=0.34). As cisplatin causes an increased risk of renal toxicity[44], some clinicians have reservations about the use of cisplatin in patients with MIBC who might already have impaired renal function and other comorbidities. The approach of adding cisplatin to radiotherapy was not widely adopted at the time.

Results from two large randomised trials were published in the last decade. These landmark studies compared outcomes of treatment with radiotherapy alone and with the addition of radiosensitising agents. The phase III BC2001 trial reported an improvement in 2-year locoregional recurrence-free survival (RFS) from 54% to 67% (P=0.03) and in 5-year OS from 35% to 48% (P=0.16) with the addition of concomitant chemotherapy with 5-fluorouracil and mitomycin C to radiotherapy in patients with MIBC[12].

As hypoxic cells are more resistant to radiation, BCON took a different approach to radiosensitisation with the use of hypoxia modifying agents instead of traditional chemotherapy agents. This trial showed that the addition of carbogen and nicotinamide to radiotherapy improved 3 year recurrence-free survival from 43% to 54% (p=0.06) and OS from 46% to 59% (p=0.04)[14].

Another agent that has been tested as a radiosensitiser for bladder cancer is gemcitabine. A phase II study of radiotherapy with concurrent gemcitabine in MIBC reported a complete response rate of 88% at first-check cystoscopy, with an organ preservation rate of 64% and an OS of 72% at three years[45]. In addition, a meta-analysis of eight published studies that evaluated concurrent gemcitabine and radiotherapy, which included a total of 190 patients with MIBC, described a 93% complete response rate at first-check cystoscopy within 12 weeks of completion of treatment, and a 5-year OS of 59%[46]. The NRG/RTOG 0712 study is a phase II study that randomised patients to twice-daily radiation with cisplatin–5-fluorouracil or once-daily radiation with gemcitabine. The primary end point of 3-year distant-metastasis-free survival was reported to be >75% in both arms (78% versus 84%; P=0.73), suggesting that outcomes were comparable[47].

1.4.5   Mechanisms of action of radiosensitisers in bladder radiotherapy

Chemotherapy Agents

Cisplatin is a platinum chemotherapy agent that is used as a radiosensitiser in various cancer subsites. This drug interacts with radiation in various different ways. Radiation results in an increase in cellular cisplatin uptake and there is a synergistic effect due to cell-cycle disruption. It inhibits the repair of DNA damage following irradiation through inhibition of nonhomologous end joining (NHEJ) [48] and blocks cells in the G2 phase where cells are more radiosensitive. Furthermore, cisplatin forms toxic intermediates with radiation-induced free radicals[49].

Mitomycin C has been found to have the greatest effect in tumour control and regrowth delay if administered shortly before radiation. It is also known to be more toxic to radioresistant hypoxic cells and hence would have a synergistic effect with radiation[50].

5FU undergoes anabolism to cytotoxic forms, resulting in both DNA- and RNA-directed effects. 5FU is phosphorylated to form fluorodeoxyurine monophosphate (FdUMP) which inhibits thymidylate synthase (TS). This in turn leads to inhibition of DNA synthesis and repair. 5FU can also be metabolized to fluorouracil monophosphate (FUMP) and then to fluorouracil triphosphate (FUTP), which can be incorporate into RNA. These mechanisms target radioresistant S-phase cells, thereby increasing cell kill when combined with radiotherapy[51].

Like 5FU, gemcitabine is also a pyrimidine antagonist that targets cells in the radioresistant S-phase. It also causes dATP pool depletion. These events lower the threshold for apoptosis with radiation, with an increase in radiation-induced apoptosis with the addition of gemcitabine compared to radiation alone[52].

Hypoxia modification

Carbogen is a mixture of oxygen and carbon, and is used in combination with nicotinamide to improve oxygenation and radiosensitisation. Carbon dioxide has a vasodilatory effect which helps to improve blood flow and hence improved oxygenation.

Nicotinamide is a hypoxia-modifying agent. It reduces intermittent constriction of blood vessels, thereby increasing blood flow to tumours and reduces acute hypoxia. When

combined with breathing high concentration of oxygen, this increases oxygenation and radiosensitivity[53].

Rojas et al. studied the effect of conventional and accelerated radiotherapy fractionation in combination with carbogen with and without nicotinamide [54]. The group found that the dose of radiation required to control 50% of tumour in mice (TCD_{50}) is reduced in mice breathing carbogen compared to air and this is further reduced with the addition of nicotinamide. With regards to conventional fractionation, relative to air-breathing conditions, the enhancement ratio of carbogen breathing was 1.47 and with the addition of nicotinamide this increases to 1.7. A similar effect of carbogen-breathing and nicotinamide relative to air-breathing was seen in the accelerated fractionation cohorts.

Tumour pO_2 measured in 16 patients with Eppendorf pO_2 electrode demonstrated that inhalation of Carbogen with 98% oxygen and 2% carbon dioxide increases tumour oxygenation comparable with the 95% oxygen and 5% carbon dioxide gas mixture. Patients reported less respiratory discomfort with the lower concentration of carbon dioxide[55].

1.5   Margins and motions

Radiotherapy dose prescription is based on the international commission on radiation units and measurement guidelines (ICRU). The gross tumour volume (GTV) is the volume of known disease infiltration. A margin is added on to this to include sub-clinical disease to form the clinical target volume (CTV), and in order to compensate for organ motion and set up errors, a further margin is added on to form the planning target volume (PTV)[56]. The radiotherapy plan would then be aimed at encompassing the PTV, which should receive 95%-107% of the prescribed dose. As a result, the volume receiving any radiation tends to be significantly larger than the original GTV.
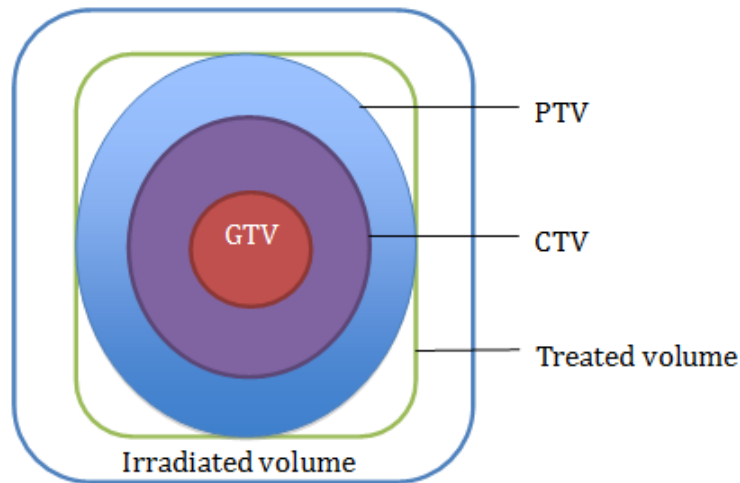
Figure 5 Schematic of volumes described in ICRU 62

In order to reduce radiation dose to surrounding organs at risk (OAR), it is important to minimise the margins added to each volume. However, it is vital to weigh this up against the potential of suboptimal coverage of the target volume.

The urinary bladder is a hollow organ that fills and empties regularly. It changes in shape, size and position due to both internal and external pressure on a regular basis. Unfortunately, the radiotherapy planning (RTP) only offers a snap shot view of the position of the bladder in relation to other pelvic organs, while a course of radical radiotherapy can last up to 7 weeks. In order to ensure adequate coverage of the bladder while avoiding incidental dose to OARs, motion of the bladder and other pelvic organs should be considered in detail.

Various studies have looked into bladder motion during a course of radiotherapy treatment, both in terms of motion between fractions (inter-fraction) and motion during a fraction of treatment (intra-fraction). Table 3 summarises these studies.

Study	Purpose/Methods	Findings
Turner et al (1997)[57]	Inter-fraction bladder motion Comparison of bladder size, shape and motion between RTP and three further CT scans on treatment Series of measurements of organ dimensions and positions made from each of mid-bladder slices for all four scans.	Large variation in bladder size during treatment, but no pattern demonstrated Bladder shape remained stable Change in posterior margin associated with marked rectal diameter change Superior-Inferior changes not measured due to methodology
Nishioka et al (2017)[58] n=29	Inter- and intra-fraction bladder motion Fiducial markers implanted into tumour beds Comparison of caudal vs cranial, anterior vs posterior and left vs right wall tumours and markers movement between fractions and during different time points within a fraction.	Anterior and cranial tumour groups showed larger inter-fractional movement than tumours on the opposite side (not statistically significant). Increase in intra-fraction movement over time.
Dees-Ribbers et al (2014)[59] n=40	Inter- and intra-fraction bladder motion Comparison of the impact of empty and full bladder on bladder wall motion	No significant difference in bladder wall motion in empty and full bladders. Maximum movement in anterior and cranial directions
Fokdal et al (2003) [60] n=15	Inter-fraction bladder motion Compared bladder position on CT scans with different rectal and bladder filling, and post-treatment CT scan to RTP scan.	Bladder and rectum volume impact bladder movements Maximum movement in anterior and cranial directions 2.4cm anterior and 3.5cm cranial margins required to ensure coverage compared to standard isotropic margin of 2cm.

Meijer et al (2003)[61] n=10	Interfraction bladder motion Compared bladder position on weeks 1, 3 and 5 of treatment Empty bladder protocol	Maximum movement in posterior and cranial directions.
Foroudi et al (2012) [62] n=50	Intrafraction bladder motion Bladder motion compared on daily pre-treatment and weekly post-treatment CBCT Empty bladder protocol	Maximum movement in anterior and cranial directions. 1.2cm anterior and 1.25cm superior margins required to account for intrafraction motion
McBain et al (2009)[63] n=15	Intrafraction bladder motion Cine-MRI scans on 2 occasions with bladder contoured at 3 different time points. Empty bladder protocol	Dominant source of motion was due to bladder filling. Maximum movement in anterior and cranial directions

Table 3 Summary of studies on bladder motion

Meijer et al studied inter-fraction bladder motion in 10 patients at RTP scan, and then at 3 different time points. It is interesting to note that this is the only study that concluded that movement was maximal in the posterior and cranial (superior) direction when other studies are consistent in their findings that the bladder moved most in the anterior and superior directions. The differences that may have resulted in this finding between this study and the others were two-fold. Firstly, this study has comparatively smaller sample size, and secondly, while the bladder on the RTP scans were contoured by 7 different radiation oncologists, the bladder on the follow up scans were contoured by a single observer. The combination of small sample size and inter-observer variability may have contributed to the difference in anterior/posterior motion results.

This suggests that we should move away from the use of isotropic margins so as to improve coverage in the anterior and superior directions, while reducing unnecessary dose to the rectum posteriorly. As we move towards online adaptive radiotherapy, the question regarding the predictability of bladder motion and feasibility of deformable

registration is one to consider. With increasing interest in differential dose to tumour bed, it is important to examine the potential difference between tumour bed and whole bladder movement. Furthermore, taking into account the differences in male and female pelvic anatomy described previously, it is also vital to consider the potential differences in male and female bladder motion.

1.6   Image-guided and adaptive radiotherapy

Effective radiotherapy is dependent on accurate delivery of a curative dose to the target volume, encompassing both macroscopic and microscopic disease, while avoiding unnecessary radiation to the surrounding tissues as much as possible. As discussed previously, the bladder's function is to fill, store and empty on a regular basis. Hence, there is a constant change in its shape and position, posing a challenge to precise delivery of radiation treatment.

1.6.1   Image-guided radiotherapy

Cone beam computed tomography (CBCT) scans are low dose CT images taken on a linear accelerator during treatment. These images have revolutionised radiotherapy as they allow tracking of the patient's anatomy and strategies to be employed to modify treatment in response to anatomical changes within the patient. This allows the radiotherapy team to visualise what was previously an invisible target.

Patients who undergo radical radiotherapy to the bladder traditionally have a maximum transurethral resection of bladder tumour (TURBT) and a GTV is often not defined as the tumour has been removed and the tumour bed is difficult to visualise on the RTP scan. The entire bladder is defined as the CTV and treated to the same dose of radiation. A margin of 15 to 20mm is added to the CTV to account for bladder expansion and motion. The RTP scan and CBCT are matched based on bony anatomy during treatment to ensure that the patient's position is reproduced as much as possible. As the position of surrounding normal tissues is relative to the bladder, this method results in treating larger volumes of normal tissue when the bladder is small with a risk of inadequate coverage of the bladder when the bladder is large. It also assumes uniform expansion of the bladder, which is not the usual case *in vivo*. In a study of 141 patients undergoing bladder radiotherapy with regular CBCT, following a match to bony anatomy, 44% of patients

required further intervention in order to improve target coverage including 10% who required repeat planning to accommodate for a small or large bladder[64]. This study explores the effect of inter-fraction motion and demonstrates that the manner in which the bladder and its surrounding organs move is often not adequately accommodated by the current CTV to PTV margin. The intra-fraction motion is not yet accounted for and the real magnitude of the problem may be bigger.

1.6.2   Fiducial markers

Studies have looked into the use of markers such as lipiodol or gold fiducial markers in image guided radiotherapy (IGRT)[65]. Gold seeds have been used in other cancer subsites, such as oesophageal, prostate and breast, to guide radiotherapy[66]–[68]. Garcia et al reported on the use of gold fiducial markers in 16 patients who underwent chemoradiotherapy, outlining that 98% of markers stayed in place until the end of treatment and that the use of these markers resulted in a reduction in bladder area treated with high-dose radiation[69].

Lipiodol markers are radio-opaque liquid markers. Chai et al demonstrated that the use of lipiodol markers is a feasible and useful way of tracking bladder tumour during radiotherapy[70]. Apart from feasibility, lipiodol markers have also shown to improve accuracy in IGRT, Søndergaard et al also showed that 5mm and 3mm inter- and intra-fraction shifts respectively is required to match on to lipiodol spots, suggesting that the use of these markers can help to improve accuracy[71]. Furthermore, Pos et al. showed that the matching on lipiodol is more precise than grey value or manual registration[72]. In a relatively small study of 5 patients, Freilich et al explored the impact of lipiodol markers on inter-observer variability regarding the size and location of the tumour bed, and found that lipiodol reduces inter-observer variability[73].

While the use of fiducial markers allows for better matching and hence accuracy, it is not without problems. The insertion of these markers requires patients to undergo a further invasive procedure, which may in turn delay definitive treatment. There is also the risk of markers moving following insertion.

1.6.3   Adaptive radiotherapy

Different adaptive strategies have been developed in order to improve target coverage while reducing unnecessary dose to surrounding tissues. The "plan of the day" (POD) adaptive radiotherapy strategy involves the formulation of multiple treatment plans and the best plan being selected on the day of treatment based on the CBCT findings. A treatment plan is designed based on the patient's initial radiotherapy planning scan. This plan is then modified, generating 3 concentrically grown clinical target volumes – small, medium and large. Prior to each day's treatment a CBCT scan is undertaken and the most suitable plan that provides the best target coverage and the least dose to OARs is selected.

This has been investigated in several studies and has shown promising results for both feasibility and clinical outcomes [74]–[76]. Hafeez et al reported on the findings of treating 55 patients who were not suitable for daily radiotherapy or surgery with weekly hypofractionated treatment using the POD approach[74]. In this group of less fit patients, 82% of patients completed treatment and local disease control was achieved in 60% of patients, with a 4.3% rate of grade 3 late toxicity at 12 months. The low rate of late toxicity appears encouraging but may not truly reflect the long-term impact of treatment as only 42% of patients were alive at the 12 months timepoint. While this study has promising results, the aim of treatment in this group of patients is for local disease control rather than long term cure.

Improved normal tissue sparing with this method has also been demonstrated, with a 30% reduction of planned target volume (PTV) in patients treated with adaptive radiotherapy compared to the non-adaptive approach [75]. This demonstrates that adaptive radiotherapy has the potential to reduce radiation dose to uninvolved surrounding tissues, but the clinical impact of this is not discussed and remains the important factor in any proposed treatment.

Another adaptive radiotherapy approach is known as the composite method. In this approach, only one treatment plan is developed from the RTP scan initially. The patient is treated with this plan for the first few fractions of treatment, during which time CBCT images are taken. These CBCTs are then averaged to generate a composite plan, which is

used for subsequent fractions of treatment. While this method corrects changes in bladder volume and position during the relatively longer time period between the planning scan and start of treatment, it does not account for the random errors that occur with changes between fractions[76] or further changes in the bladder after the composite plan has been designed.

While it is an improvement from using a single plan, current adaptive radiotherapy approaches assume uniform bladder movement and expansion, and do not consider intra-fraction changes. The generation of multiple radiotherapy plans is labour-intensive and the daily choice of plans is dependent on subjective assessment by the treating team. Furthermore, while we are concerned about reducing radiation dose to surrounding tissue, it is not known if the incidental dose that patients currently receive actually plays a positive role towards their long-term disease status.

1.6.4   Tumour bed boost

Considering that the basis of radiotherapy lies with delivering the maximum radiation dose to a high-risk region while minimising that to surrounding tissues, the practice of treating the whole bladder to the same dose is incongruous. A study of 149 patients randomised to whole bladder or partial bladder radiotherapy found that a reduction in treatment volume allowed for higher radiation dose to be delivered, but survival outcomes were similar[77]. Furthermore, BC2001 included a comparison of reduced high dose tumour focused volume to standard high dose whole bladder radiotherapy[78]. This sub-study reported no significant differences in late toxicity or rates of recurrence, but it should be noted that the bladder sparing effect in BC2001 was modest as the protocol called for a 1.5cm margin around the tumour within an empty bladder. CBCT had not yet been wholly developed and as such on-treatment imaging was of poor quality.

The RAIDER trial (NCT02447549) is a phase II, three arm randomised trial of adaptive radiotherapy for bladder cancer. This trial investigates both the feasibility and impact of adaptive radiotherapy and dose escalated tumour boost in radical radiotherapy to the bladder. It has recently closed to recruitment and results are awaited. This will be of interest as we move forward in optimising the delivery of radical radiotherapy and improve patient outcome.

1.7   MRI in bladder cancer

Magnetic resonance imaging (MRI) exploits the high hydrogen content of the human body by using a strong magnetic field and radiofrequency pulses to align hydrogen proton axes. The resulting processing magnetic dipole then generates a detectable radiofrequency, which is used to create images. Different tissues have different hydrogen density and relaxation time, thereby emitting radio waves of varying intensity[79].

Compared to computed tomography (CT), MRI produce scans with better soft tissue contrast, which in turn allows better identification and localization of disease and surrounding organs at risk. MRI also allows both anatomical and functional images, which provides more information about the disease. Different pulse sequences allow for image contrast to be manipulated when imaging different structures.

However, it takes a significantly longer amount of time to obtain MRI images, and involves the patient being in a confined space during the scan time. This gives rise to increased risk of image distortion from patient motion. Furthermore, patients with certain implanted medical devices or metallic fragments are not able to undergo MRI scans.

1.7.1   Anatomical imaging

The superior soft tissue contrast on MRI scans is important in its use in the staging of bladder cancer. On T2 weighted images, bladder tumour and bladder muscularis layer can be differentiated. It can be determined if the muscularis layer is intact and therefore allowing the radiologist to accurately determine if the cancer is superficial or muscle invasive[80].

MRI allow for images to be acquired in different planes and accurate evaluation for the bladder tumour is not limited by its location within the bladder[81]. For example, tumours on the lateral wall can be better evaluated with coronal images while those on the anterior or posterior wall can be better evaluated with sagittal images.

MRI with gadolinium enhancement has an overall accuracy of 85% in distinguishing between non-muscle invasive bladder cancer (NMIBC) and muscle invasive bladder cancer (MIBC), 82% accuracy in classifying organ-confined disease and 96% accuracy in

diagnosing lymph node involvement[82]. In comparison, CT scans only have an accuracy rate of 49% in predicting the depth of bladder wall invasion[83].

1.7.2   Functional imaging

Apart from anatomical information, MRI allows for functional or biological information to be obtained by imaging perfusion through the use of diffusion-weighted imaging (DWI), oxygenation through blood oxygen level dependent (BOLD) scans and macrophage functions with lymphotropic nanoparticle enhanced MRI (LNMRI).

DWI examines the diffusion of water molecules (Brownian motion) and reflects the cell density in the region examined. As tumours have a greater cell density, there is greater restriction in diffusion and hence a lower apparent diffusion co-efficient (ADC). In addition to structural information, DWI-MRI provide quantitative information to further aid tumour assessment[84]. The ADC value may be useful in determining the aggressiveness of tumours, with a lower ADC value found in MIBC and high-grade tumours[85]. In addition to its use in diagnosis, DWI-MRI has also been shown to predict response to chemoradiotherapy with a multivariable analysis identifying ADC value as the only significant and independent predictor of sensitivity to chemoradiotherapy[86]. However, it is important to consider the limitations of DWI-MRI in bladder cancers as water diffusion is also impeded in non-cancerous tissues such as neurological tissues, lymphatic tissues and areas of fibrosis and can lead to misdiagnosis.

BOLD MRI scans utilises the difference in magnetism of deoxyhaemoglobin and oxyhaemoglobin. As deoxyhaemoglobin is paramagnetic and oxyhaemoglobin is diamagnetic, oxygenated blood appears brighter on T2 weighted images. MRI scan sequences can be manipulated to be sensitive to the level of deoxyhaemoglobin. Therefore, by increasing oxygenation through the breathing of carbogen (95% oxygen and 5% carbon dioxide) during a BOLD MRI scan can help to identify patients who would benefit from hypoxia modification during treatment[87].

LNMRI utilises macrophage function within normal lymph nodes to differentiate between benign and metastatic lymph nodes. Lymph node specific nanoparticles are administered prior to the scan, and are phagocytosed by functioning macrophages, causing them to accumulate within benign lymph nodes. As a metastatic lymph node would have poorer

macrophage function, the nanoparticles do not accumulate, and there would be a difference in signal intensity[80].

These new developments in functional imaging hold much potential, but are not currently used in clinical practice due to the lack of access and availability and more importantly, the lack of validation in large cohorts of patients.

1.7.3   MRI-guided radiotherapy

The superior soft tissue contrast of MRI scans makes it ideal for radiotherapy planning and image guidance. The recent development of MRI-guided radiotherapy machine, which would allow for online adaptive radiotherapy has resulted in much excitement.

There is limited literature on the role of MRI guided radiotherapy in bladder cancer. Vestergaard et al has assessed different MRI-guided adaptive radiotherapy techniques with regards to target coverage, and concluded that online re-optimization allows normal tissue sparing and should be considered for use in bladder cancer[88]. While this is optimistic, it must be recognised that this study was in a relatively small patient group of 9 patients, with only one female patient.

The role of MRI guided radiotherapy has been explored in other urological tumours. For example, Hegde et al. reports that MRI guidance with a cobalt-60 radiotherapy system is an alternative to fiducial marker in prostate stereotactic radiotherapy (SBRT) in a single patient case report[89].

There have also been reports of MRI guided radiotherapy use in other cancer subsites. For example, Chen et al. reports a single institution experience of treating 18 patients with head and neck cancer with IMRT and on-board MRI, and found that the clinical outcome is comparable to contemporary IMRT series[90]. Padgett et al. found that online adaptive MRI guided lung stereotactic radiotherapy (SBRT), with deformably propagated contours that were edited by clinicians, provides better target conformality and reduces dose to normal tissue [91]. However, this small study included only three patients and 13 fractions of treatment in total.

MRI guided radiotherapy is still in its infancy. Online adaptive radiotherapy requires time and manpower unless the system is designed to generate an adaptive plan with

deformable registration and has the ability to carry out effective and efficient software based quality assurance[92]. The additional time required to deliver treatment may also have an impact on intra-fraction motion, which would need to be taken into consideration as well. MRI based studies would need to be carried out to examine bladder movement, and adaptive radiotherapy strategies would need to be studied in a large and varied patient population.

1.8   Radiomics and imaging biomarkers

Radiological imaging is commonly used in the diagnosis of cancer and in monitoring of response to treatment. They aid clinical decisions regarding disease treatment. Radiologists use the different tissue contrast obtained in various imaging modality to decide on diagnosis. However, a large amount of information is gathered during imaging studies that are not visible to naked eye[93]. Recent advancement in computer technology means interpretation of images is no longer limited to what the radiologist can see. Radiomics refers to the high throughput extraction of advanced quantitative data from radiological images creating a high dimensional data set, followed by data mining and analysis of these data supports clinical decision[93], [94]. In turn, this contributes to a further step towards the practice of precision medicine.

1.8.1   Imaging versus tissue biopsy

Biopsies involves small samples of a tumour and do not account for the spatially heterogenous nature of most tumours[95]. This could easily result in misrepresentation. On the other hand, radiological imaging allows the examination of an entire tumour, and the extraction and analysis of quantitative features of both the tumour and its surroundings. Relating the radiomics features to pathological features or clinical outcomes will in turn help to overcome the issues of under-sampling[94].

1.8.2   Radiomics workflow

The radiomics workflow involves a four-step process. High quality images are obtained and the area of interest is identified through segmentation. This could refer to an area of disease or normal tissue. Quantitative features are then extracted, and subsequently analysed[95].

Different features can be extracted from a single set of images. This includes three different categories. Tumour intensity histogram-based features involves the conversion of the 3-dimensional data of a segment into a single histogram (e.g. Hounsfield unit for a CT scan), describing the distribution of voxel intensities within an image. The morphological features describe the geometric shape of the segment, total volume and total surface area. Lastly, the texture features describe the distribution of grey levels within an image[93], [96].

1.8.3   Radiomics in bladder cancer

There have been limited number of studies into role of radiomics in bladder cancer, but findings have nonetheless been interesting.

CT radiomics

Two different studies have been published with regards to the use of CT radiomics in bladder cancer.

Cha et al. explored the role of response assessment using radiomics with deep learning in the setting of neo-adjuvant chemotherapy[97]. CT scans prior to and after neo-adjuvant chemotherapy were evaluated with three different radiomics' predictive models and the findings compared to expert radiologist assessment. This study found that there was no significant difference between the radiologists' and the predictive models' overall assessment of residual tumour following neo-adjuvant chemotherapy. While an obvious criticism of this study would be that disease response is often not seen following neo-adjuvant chemotherapy as patients had maximum trans-urethral resection of tumour (TURBT) prior to commencement of chemotherapy, it is important to note that the main finding is that the deep learning radiomics predictive models' evaluation of disease response is similar to that of trained radiologists.

Wu et al. demonstrated the value of CT radiomics texture features in the prediction of lymph node metastases[98]. A comparison of radiomics features obtained from pre-operative CT scans was made with post-operative pathological lymph node status, thereby developing a radiomics signature. This was shown to accurately predict lymph node involvement even in situations where the contrast enhanced CT scans were reported as lymph node negative.

MRI radiomics

Studies have shown that textural features from MRI scans are significantly different between cancer tissue and uninvolved bladder wall, and also between the uninvolved bladder wall of patients and those of healthy volunteers[99]. Moreover, the combination of textural features and apparent diffusion coefficient (ADC) can also help to distinguish between high and low grade non-muscle invasive bladder cancer (NMIBC)[100].

Clinical applications

Current findings have demonstrated that radiomics may be useful in accurate diagnosis and grading of bladder cancer, and that with further validation, could be useful in clinics.

However, little has been done to look at the potential for monitoring or predicting response to treatment. Modern radiotherapy techniques have an increased flexibility in dose delivery with intensity-modulated radiotherapy (IMRT) and volumetric-modulated arc therapy (VMAT). While these techniques are used to improve more anatomically conformal contours, radiomics could translate to biological conformality in future.

Limited work has been done in the field of radiomics and imaging biomarkers in bladder cancer and we are still a long way away from realising the goal of biological conformality. There is a need to first isolate useful imaging biomarkers in bladder cancer and to further validate them in different independent cohorts of patients in order to demonstrate their reliability, thereby narrowing down and identifying robust imaging biomarkers to be used in clinical practice.

1.9   Molecular biomarkers in bladder cancer

Over the past decade, unprecedented developments have been made in cancer genetics and genomics, enabling the exploration of molecular biomarkers in different cancer types, including MIBC. Importantly, non-invasive and invasive bladder cancers have been found to be different diseases with distinct pathogenetic pathways[101], [102]. Invasive tumours are believed to originate from CIS and are associated with dysregulation of the p53 and retinoblastoma (RB) pathways whereas non-invasive tumours are associated with *FGFR3* and *HRAS* mutations[102]–[104]. Importantly, such molecular biomarkers might have prognostic and predictive value in bladder cancer, and could inform treatment strategies
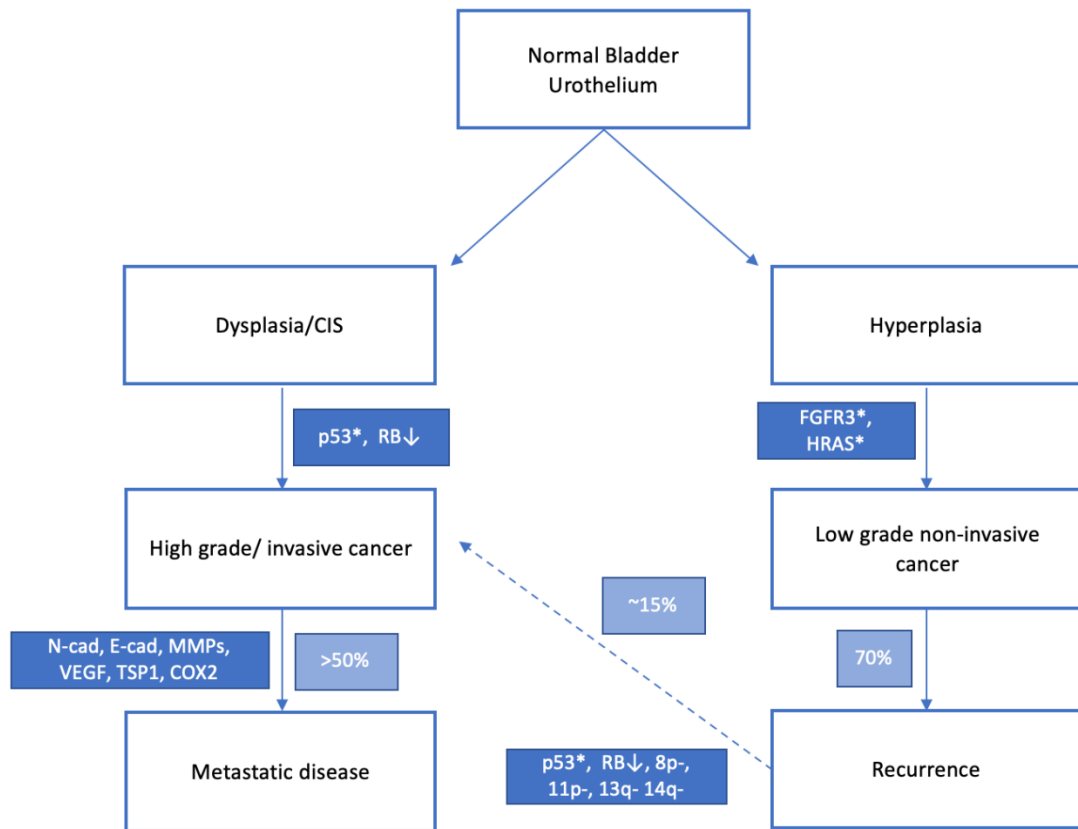
Figure 6 The divergent pathogenetic pathway of urothelial cancers. Adapted from Wu et al[102]

1.9.1 Prognostic biomarkers

Prognostic biomarkers are biological features of a tumour that provide information about the general outcome of the disease and might help to identify patients who require treatment intensification. Importantly, despite predicting outcomes, prognostic biomarkers do not predict response to a specific treatment or intervention. Several potential prognostic biomarkers have been studied in urothelial bladder cancer, but they are not in routine clinical use as they have not been adequately validated and their clinical relevance has not yet been determined.

The tumour suppressor p53 is an important gatekeeper in G1–S cell cycle progression and has a key role in regulating the cell growth and division[105]. Mutational inactivation of the *TP53* gene results in an altered p53 phenotype, and *TP53* alterations were found to be associated with increased risk of disease recurrence and a poor prognosis in patients with bladder cancer[106]–[108]. In bladder cancer, individual alterations in the levels of p53, cyclin-dependent kinase inhibitor 1 (also known as p21) and phosphorylated RB are associated with early recurrence and poor prognosis, whereas combinations of these

alterations further enhance this prediction[109]. Indeed, the 5-year recurrence and survival rates were 93% and 8%, respectively, in patients with all three alterations compared with 23% and 70%, respectively, for those with a single alteration.

Gene expression profiling has been used to identify genes that might aid in bladder cancer diagnosis and in predicting recurrence and progression[101], [110]–[112]. Smith et al.[113] developed a 20-gene model that identified patients at high and low risk of lymph node metastasis, independent of age, gender, pathological tumour stage, and lymphovascular invasion. As lymph node involvement is an important prognostic factor in bladder cancer recurrence and survival[114], the ability to accurately predict lymph node metastases using this model, after adequate validation, could prove invaluable and could help to select patients for neoadjuvant chemotherapy prior to definitive treatment.

Various groups have identified basal and luminal subtypes in MIBC through whole genome mRNA expression profiling, echoing the molecular subtyping in breast cancers[115]. The cancer genome atlas (TCGA) identified four different molecular subtypes – clusters I to IV. The group found that the cluster III subtype was enriched with breast basal markers while clusters I and II showed similarity to breast luminal A subtypes[116].

Sjödahl et al. used gene expression data from 308 samples (NMIBC and MBC) to defined five major subtypes: urobasal A, genomically unstable, urobasal B, squamous cell like and highly infiltrative, and demonstrated that urobasal B and squamous cell-like subtypes have the worse prognosis[117]. Their findings were validated in two independent cohorts.

Using a meta-dataset of 262 high grade MIBC tumours and a further smaller independent dataset as validation, a gene signature was developed to classify MIBC tumours into luminal and basal subtypes[118]. The authors found that within the validation cohort, basal tumours had worse OS and disease specific survival outcomes. However, it should be noted that the validation cohort was relatively small comprising of only 49 patients.

Interest has been growing in the role of the immune system in disease outcome and treatment response. Lymphocytic infiltration has been reported to be related to clinical outcome in various cancer types[119]. In a cohort of patients with MIBC undergoing

radical chemoradiotherapy to the bladder, pre-treatment lymphopaenia was associated with poor outcome, a finding that was also observed in a separate cohort of patients with advanced bladder cancer undergoing palliative chemotherapy for advanced urothelial cancer[120].

Programmed cell death protein 1 (PD-1), a cell surface receptor expressed on T cells, has gained importance in various cancer types. In line with its role in immune regulation, interaction of PD-1 with its ligands, such as programmed cell death 1 ligand 1 (PD-L1), results in downregulation of the immune response[121]. By expressing PD-1 ligands, tumours can exploit this immune checkpoint in order to evade immune detection. PD-L1 expression in bladder cancer has been shown to be associated with the risk of disease progression and decreased survival[122]–[124]. The use of immune checkpoint inhibitors (ICIs) targeting either PD-L1 or PD-1 in the metastatic setting has improved survival outcomes, particularly in patients with high PD-L1 expression[125]–[127].

Receptor tyrosine kinases (RTKs) regulate a number of cellular processes, including cell proliferation and differentiation, and have an active role in cancer development and progression[128]. Overexpression of human epidermal growth factor receptor 2 (HER2; also known as ERBB2) is associated with aggressiveness and poor prognosis in urothelial cancer, specifically lymphovascular invasion, disease recurrence, and decreased DSS and OS[129], [130].

Prognostic biomarkers could possibility be used for the identification of patients with a poor prognosis who might require treatment intensification. However, although some biomarkers have both prognostic and predictive value (for example, PD-L1 expression), the prognostic value of most of the aforementioned biomarkers does not necessarily translate into predictive values and, therefore, they might not be useful in guiding optimal treatment for an individual patient.

1.9.2 Predictive biomarkers

Predictive markers are biological features that predict response to an intervention or treatment. Despite still being at the developmental stage, predictive biomarkers have been identified that might aid patient selection for treatment options in bladder cancer, specifically for identifying patients who might respond well to radiotherapy or who might

be better suited to surgery, and also in identifying patients in who neoadjuvant chemotherapy would be useful. However, further studies are now required to validate their predictive power and clinical value.

Apart from its prognostic value, molecular subtyping has been shown to predict response to neoadjuvant chemotherapy. Choi et al. demonstrated that p53-like subtype of tumours has little response to neoadjuvant chemotherapy. However, this was a small study of 100 patients, of which 9 were of p53-like subtype. These findings were not validated[131].

A further multi-institution study of tumour samples prior to neoadjuvant chemotherapy stratified tumours into four subtypes – claudin-low, basal, luminal-infiltrated and luminal, and validated the findings in an independent cohort[132]. The survival outcomes were compared to that of a non-neoadjuvant chemotherapy dataset. Luminal tumours were found to have the best while claudin-low tumours were associated with worse outcomes regardless of neoadjuvant chemotherapy. Neoadjuvant chemotherapy made the most difference improvement in OS in basal tumours. While there was a large sample size and the molecular subtyping results were validated, the study compared survival outcomes across different datasets.

Whole transcriptome analysis of TURBT samples from 136 TMT patients and 223 NAC and RC patients found that molecular subtype did not have an impact on DSS or OS in the TMT cohort, but claudin-low subtype was associated with worse outcomes in the NAC and RC cohort[133]. The study also studied two gene signatures of immune infiltration, T-cell inflamed gene expression and IFN-gamma gene expression, and showed that higher expression of immune signature is associated with improved DSS in the TMT cohort but not in the NAC and RC cohort. Stromal infiltration was found to be associated with worse outcomes in the NAC and RC cohort. While the results provide an interesting potential of predicting outcomes with treatment, they should be treated cautiously. The two cohorts do not have balanced patient characteristics. The RC cohort had a lower median age, with a greater proportion of female patients and higher T stage. The gene signature expression scores were dichotomised, but it is unclear how the cut off scores were determined. Most importantly, the results require further validation in prospective study before any change in practice should be considered.

Double-strand break repair protein MRE11 is involved in activation of the DNA damage response (DDR) by forming part of the MRE11–RAD50–NBS1 (MRN) complex, which has an important role in detecting double-stranded DNA damage and repair[134]. On the basis of their role in DDR, increased expression of MRN complex proteins would be expected to predict poor radiosensitivity, but different studies have concluded the opposite. The expression of MRE11 has been shown to be predictive for DSS following radical radiotherapy in both a test and validation cohort, with high MRE11 expression being associated with improved 3-year DSS in patients with MIBC after radiotherapy. This predictive value could be due to the relationship between low MRE11 expression and impaired checkpoint arrest and/or reduced apoptosis, resulting in increased radioresistance [135]. In the same study, MRE11 expression was found not to be associated with survival outcomes in patients who underwent RC, a finding that has been further validated in a separate patient cohort[136]. Thus, MRE11 expression might be a useful predictive biomarker for stratifying patients to receive either surgery or bladder-preserving treatment. However, further validation of these findings in other cohorts has been disappointing owing to problems associated with assay reproducibility[137] and, therefore, MRE11 is not in current routine clinical use as a biomarker for patient selection.

The RTOG has reported that patients with HER2-positive MIBC have a poor response to chemoradiotherapy; analysis of tumour samples from 55 patients enrolled in 4 RTOG bladder cancer studies revealed that HER2 expression status was associated with response rate following chemoradiotherapy[138]. In a biomarker-selective, nonrandomized study, patients with HER2-positive MIBC were treated with HER2 antibody trastuzumab in addition to chemoradiotherapy with paclitaxel, whereas HER2-negative patients were treated with chemoradiotherapy only. Complete response rates were similar between groups (72% in HER2-positive group versus 68% in HER2-negative group) despite expectations that response rates would be lower in HER2-positive patients[139]. This was a small study (*n*=76) and the radiosensitising regimen used was non-standard, but the findings suggest the possibility of using HER2 status as a predictive biomarker for the addition of trastuzumab in order to improve outcomes in this patient group.

In addition to concurrent chemoradiotherapy, another option for radiosensitisation in TMT is the use of hypoxia modification instead of chemotherapy. As a step towards biological stratification for the choice of radiosensitizer, retrospective analysis of tumour samples from the phase III BCON study (which evaluated the addition of concurrent carbogen and nicotinamide to radiotherapy) has enabled exploration of associations between tissue biomarkers and clinical outcomes and led to the identification of a number of potentially important predictive biomarkers for response to concurrent hypoxia modification. Specifically, necrosis, carbonic anhydrase-IX (CA-IX), hypoxia-inducible factor-1α (HIF-1α), and a 24-gene signature have been shown to predict improved outcomes in patients treated with carbogen and nicotinamide. Eustace et al.[140] examined a variety of histopathological features in tumour samples from 231 patients enrolled in the BCON trial, and found that necrosis and expression of the hypoxia marker CA-XI independently predicted OS benefit from hypoxia modification. Another study that evaluated HIF-1α expression by immunohistochemistry in tumour samples showed that patients with high HIF-1α expression who were treated with hypoxia modification in combination with radiotherapy had a marked improvement in local relapse-free survival compared with those treated with radiotherapy alone, whereas no improvement was observed with hypoxia modification in patients with low HIF-1α expression[141]. Similarly, a 24-gene signature that identified hypoxic MIBC tumours predicted benefit from the addition of hypoxia modification to radiotherapy[112].

In summary, a number of different biomarkers that predict response to bladder-preserving treatments in MIBC have been identified, mostly using retrospective data from randomised controlled trials, but few have been validated. With appropriate validation, these predictive biomarkers could aid clinicians and patients in the decision between treatment options and in formulating appropriate management plans. Validated biomarkers that predict response to RC will be invaluable and could enable the development of algorithms to aid clinical decision making regarding definitive treatment options.

1.10   Optimising radiotherapy in bladder cancer

Patient outcomes in bladder cancer have improved over the years[16] and this can be attributed to improvement in TURBT techniques, radiotherapy delivery and supportive care. In order to optimise patients' chances of bladder preservation, we need to improve on the accuracy of radiotherapy delivery. The bladder moves within the pelvis due to external pressure from surrounding organs, and it changes in shape and size from internal filling and emptying. By exploiting modern imaging techniques and advanced radiotherapy methods, we could better visualise the bladder, learn to predict its movement in both male and female pelvis, and adapt treatment more appropriately. This would translate to the maximum dose of radiation to target areas to optimise the chance of local disease control, while minimizing dose to normal tissue to reduce toxicity. As we evaluate the long-term outcomes of hypoxia modification in bladder cancer radiotherapy, we can improve patient selection for this radiosensitisation regimen.

2   Hypothesis

Organ preservation treatment in bladder cancer can be personalised through improved accuracy with image guided radiotherapy and biologically informed use of hypoxia modification.

3   Aims

The aims of this thesis are as follows:

1   To investigate the associations between long-term outcomes of hypoxia modification and biological features in muscle invasive bladder cancer.
2   To investigate the relationship of parameters on radiotherapy planning scan on the clinical outcomes of patients who underwent radical radiotherapy for bladder cancer.
3   To quantify and compare inter-fraction bladder motion between men and women, looking for gender differences using CBCT.
4   To prospectively evaluate the association of intra-fraction role of tumour bed and bladder.

4 Long-Term outcomes of hypoxia modification in bladder cancer in relation to hypoxia biomarkers and molecular subtypes

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Conception or design of the work: YS, AC, PH

Acquisition of data: YS, PH, LY

Analysis of data: YS, HM, LY, BL

Interpretation of data: YS, HM, LY

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4.1 Abstract

4.1.1 Introduction

Organ preservation in muscle-invasive bladder cancer (MIBC) is achieved through radiotherapy with the addition of a radiosensitiser. Oxygen makes radiation induced DNA damage permanent and increases cell kill. Hypoxic tumours are therefore more radioresistant. Biological features that reflect the presence of hypoxia such as necrosis and gene expression scores could potentially select patients that would best benefit from hypoxia modification. Furthermore, there has been increasing interest in molecular subtyping in bladder cancer. Like in breast cancer, basal and luminal bladder tumours display a different course of disease and may respond differently to treatments. The Bladder CarbOgen Nicotinamide (BCON) study showed that the use of hypoxia modification resulted in an improvement of 3-year overall survival. We examine the long-term outcomes of the BCON study and explore the biological features that may aid in patient selection for hypoxia modification. We also present the first study into the effect of hypoxia modification in radiotherapy for basal and luminal bladder cancers.

4.1.2 Methods

Patients with histologically proven non-metastatic muscle-invasive, or high grade non-invasive, transitional cell carcinoma of the bladder were recruited to the BCON study and randomised to receive radical radiotherapy (RT) alone or radical radiotherapy with the addition of nicotinamide and carbogen (RT+CON). Centres were contacted for the long-term survival outcomes of patients. Tumour samples were examined for presence of

necrosis. Whole transcriptomic analysis was carried out and a hypoxia gene signature score was determined for each sample based on a previously developed 24-gene signature. Tumours were also stratified into basal and luminal molecular subtypes. Kaplan-Meier analyses were used to determine long term survival for these biological features. Multivariable analysis was carried out to adjust for known prognostic factors.

4.1.3   Results

There is sustained benefit of hypoxia modification in bladder radiotherapy in the long term, with p-values overall in this moderate sample of patients falling just short of 0.05. Tumours with necrosis present and/or high hypoxia gene signature scores benefited from hypoxia modification. In the presence of necrosis, 5-year OS was 53% (95% CI 42-67%) in RT+CON and 33% (95% CI 22-50%) in RT alone. Similarly, with high hypoxia score, 5-year OS was 51% (95%CI 41-62%) in RT+CON and 34% (95%CI 23-49%) in RT alone. There was an improvement in OS with the addition of CON in the basal group (HR 0.58 (0.32-1.06) p=0.08) but not in the luminal group (HR=0.96 (0.58-1.61) p=0.88) (figure 5).

4.1.4   Conclusion

Our study shows that necrosis, hypoxia gene signature score and molecular subtypes are predictive biomarkers in bladder radiotherapy with hypoxia modification. This would allow the development of biologically stratified management plans as opposed to being dependent on patient and clinician bias. Further qualification of our findings in the form of a biomarker driven study is required prior to a move into routine clinical use.

4.2 Introduction

The optimum strategy for organ preservation in muscle-invasive bladder cancer (MIBC) involves maximum transurethral resection of bladder tumour (TURBT), radiotherapy and radiosensitisation. The Bladder CarbOgen Nicotinamide (BCON) study opened in 2000 and randomised patients to receive radiotherapy alone or radiotherapy with carbogen and nicotinamide (CON). This study demonstrated a 13% improvement in 3-year overall survival (OS)[14].

4.2.1 Hypoxia

Tumour hypoxia occurs as a result of structural abnormalities of tumour blood vessels, size of tumour resulting in poor oxygen diffusion or disturbed microcirculation[142]. This leads to the activation of hypoxia inducible factors (HIFs), which in turn play important roles in the tumour's invasive and metastatic properties by affecting glycolysis, mitosis, apoptosis and angiogenesis[143].

The interplay between presence of oxygen and effect of radiotherapy is an issue that has been known about for over a century. This was first described by Schwarz in 1909 when he showed that the effect of radiation to the skin is reduced with compression but this was attributed to the lack of perfusion at the time. Mottram demonstrated in the 1930s that cells closer to blood supply had greater damage compared to cells further way away when the same dose of radiation is applied[144], [145].

Ionizing radiation causes cell death through direct DNA damage and indirect formation of free radicals from water molecules. The majority of damage caused by photon radiotherapy is indirect. Radiation causes the formation of highly reactive free radicals which in turn react with DNA to cause structural damage[146], [147]. This results in various types of DNA damages single and double strand DNA breaks and base damages[148], [149]. DNA damage causes arrest in cell cycle and the activation of various DNA repair pathways. For example, double strand breaks require homologous repair (HR) or non-homologous end joining (NHEJ), while single strand breaks are repaired through single strand repair pathway and base damages through base excision repair pathway. Radiosensitivity is dependent on the cell's ability to repair.

Oxygen causes "fixation" of damage induced by radicals by forming organic peroxide, thereby making damage permanent and result in cell kill. This is known as the oxygen effect. The oxygen enhancement ratio (OER) is the ratio of doses under hypoxic conditions to doses under aerated conditions to produce the same effect. Up to three times the dose of radiation is required to result in the same cell kill in hypoxic cells as in normoxic cells[150]. Therefore, improving oxygenation of cancer cells increase their radiosensitivity.

4.2.2   Hypoxia modification

BCON studied the impact of hypoxia modification in the form of carbogen and nicotinamide. Nicotinamide is the soluble, active form of vitamin B3. Carbogen is a high oxygen content gas mixture of 95% oxygen and 5% carbon dioxide. As explained in chapter section 1.4.5, tumour oxygenation is similar when a 98% oxygen and 2% carbon dioxide gas mixture was used with the additional benefit of patients experiencing less respiratory discomfort. Therefore, the higher oxygen concentration mixture was used in BCON.

Oxygenation of tissues is dependent on perfusion of blood, oxygen concentration in blood and the diffusion of oxygen across capillary walls. High concentration oxygen in carbogen results in increased arterial pO_2 and therefore a greater diffusion gradient, and subsequently improved diffusion of oxygen into tissue. Carbon dioxide causes vasodilation and may thereby improve tissue perfusion[151].

The radiosensitising effect of carbogen is further enhanced with the addition of nicotinamide, the amide form of vitamin B3, niacin. In a murine study, it was shown that the nicotinamide reduced mean arterial blood pressure by about 50%[152]. Nicotinamide reduces acute hypoxia by reducing transient vasoconstriction thereby reducing acute hypoxia[53]. Nicotinamide is also the pre-cursor to nicotinamide adenine dinucleoside (NAD+), which is a PARP substrate. PARP is an important enzyme in DNA repair induced by radiation. The inhibition of PARP by nicotinamide increases clonogenic cell kill[153].

4.2.3   Molecular subtypes

There has been increasing interest in molecular subtyping of solid tumours. For example, molecular subtyping in breast cancer has allowed a better understanding of the disease, treatment and prognosis[154], [155]. Recent years have seen various research groups classify MIBC into molecular subtypes through whole genome mRNA expression profiling. Different subtyping classifications have been developed, but at the highest level, MIBC can be categorised into basal and luminal subtypes. Seiler et al showed that basal tumours benefitted from neoadjuvant chemotherapy while luminal tumours did not[132]. Molecular subtypes do not affect the rate of complete response after TMT[133].

4.2.4   Patient selection

Radiosensitisation regimens in bladder radiotherapy can be through the use of chemotherapy or hypoxia modification. There is currently no optimum method of selecting patients for either option. Previous studies into the role of MRE11 as a predictive biomarker in bladder radiotherapy has not been conclusive[135], [137]. Our group had previously shown that necrosis and hypoxia gene signature scores were independently predictive of survival outcomes with hypoxia modification [140], [156].

We aim to examine the potential of selected biomarkers to predict sustained benefit of hypoxia modification in the long-term.

4.3   Methods

The BCON study recruited patients with non-metastatic muscle-invasive, or high grade non-invasive, histologically proven transitional cell carcinoma of the bladder. Patients were randomised to receive radical radiotherapy (RT) alone or radical radiotherapy with the addition of nicotinamide and carbogen (RT+CON). The study included patients with localised disease and who were able to tolerate a closed carbogen breathing system. Patients with distant or nodal metastases, or who have contraindications to carbogen or nicotinamide use were excluded from the study. The detailed inclusion and exclusion criteria were described in the original publication[14] and are listed in table 4. Informed consent to the study, including consent for tumour sample collection and analysis, were obtained prior to randomisation.

Inclusion criteria:
Age over 18 years
Histologically proven transitional cell carcinoma of the bladder
Muscle invasive carcinoma (stage T2 or T3) of any grade, high grade (G3) superficial bladder carcinoma (stage T1), or prostatic invasion (T4)
Ability to give informed consent
Capable of complying with the use of a closed breathing system delivering carbogen through either a mask or a mouthpiece with nasal clip
Exclusion criteria:
Squamous or adenocarcinoma of the bladder
Locally advanced T4b carcinoma
The presence of distant metastasis or enlarged pelvic lymph nodes on CT staging scan of the pelvis
Co-existing respiratory disease with reduced respiratory drive which would make delivery of 95% oxygen contra-indicated
Impaired renal or hepatic function resulting in serum creatinine or bilirubin more than twice the normal range
Ischaemic heart disease or peripheral vascular disease requiring treatment with angiotensin-converting enzyme (ACE) inhibitors

Table 4 Inclusion and exclusion criteria for BCON study

4.3.1 Treatment

Radiotherapy was delivered with either a conventional fractionation of 64 Gy in 32 fractions over 6 and a half weeks or a hypofractionated regimen of 55 Gy in 20 fractions over 4 weeks. Patients underwent radiotherapy planning (RTP) scan with the bladder empty. The treatment volume was defined on this scan and included the bladder and extra-vesical tumour extension, with a 1 to 2cm margin. A three or four field treatment plan using at least 6MV x-rays was used. Conformal planning with multi-leaf collimators (MLC) or fixed block techniques were allowed. Treatment was carried out daily, 5 times a week, treating all fields each day.

Patients randomised to receive hypoxia modification received oral nicotinamide 60mg/kg 1.5 to 2 hours before radiotherapy each day. Nicotinamide dose was reduced to 40mg/kg in the event of significant nausea. This was returned to the higher dose if treatment was well tolerated or discontinued if it was not. Patients also inhaled 15L/min of carbogen (2% carbon dioxide, 98% oxygen) throughout each fraction. Carbogen was delivered via a face mask with an air tight seal or mouthpiece with nasal clip. Carbogen breathing continued even if nicotinamide was stopped.

4.3.2   Long-term outcomes

Centres that enrolled patients in the BCON study were contacted for updates on disease and survival status. A pro forma with information required and detailed instructions about the definition of each section were sent to all centres. This included information about local recurrence (superficial and/or invasive recurrence), cystectomy, metastatic disease, date last seen, whether patient is alive, date and cause of death (if applicable).

4.3.3   Biological features

Pre-treatment tumour samples were obtained from the study and analysed for the presence of necrosis and hypoxia gene signature score. Tissue samples were obtained through transurethral resection of bladder tumour (TURBT). Samples were formalin-fixed and paraffin-embedded.

One 4µm haematoxylin and eosin (H&E) stained section from each FFPE block was analysed for necrosis. This was characterised by increased tissue eosinophilia, nuclear breakdown and loss of tissue architecture. Necrosis was scored as present (of any amount) or absent, as is used routinely in histopathology reporting for other cancers. Each sample was assessed by one of two experienced histopathologists, with no cross checking between scorers[140].

RNA was extracted from BCON FFPE samples using the RecoverAll Total Nucleic Acid Isolation Kit (Life Technologies, Warrington, UK) and profiled using human exon 1.0 ST array. Transcriptomic analysis was performed.

A hypoxia gene signature was derived with a seed-based co-expression network and tested in publicly available bladder cancer cohorts. This is known as the West-24

Signature. Details are available in previously published literature[112]. In summary, a training cohort was obtained from the cancer genome atlas project (TCGA)[157]. Genes shown to be hypoxia regulated and validated in clinical cohorts were identified from a recent review[158]. Spearman correlation was calculated for each pair of candidate genes and a bladder cancer specific hypoxia co-expression network was constructed by pooling together interactions above a threshold of 0.5. As tumour hypoxia was associated with aggressive tumour biology and poor prognosis, genes that were upregulated and associated with poor prognosis in the TCGA training cohort were selected. The 24 gene signature was identified and validated in six other independent bladder cancer cohorts. West-24 signature scores were then derived for each BCON tumour sample. In this analysis, we explored the impact of the hypoxia gene signature scores on two levels – Patients were deemed to have high or low hypoxia based on the median cohort hypoxia score. Analysis was also carried out based on the hypoxia score as a continuous variable.

A 47-gene mRNA subtype signature (BASE47) which had been shown to consistently stratify tumours into basal and luminal subtypes was used to stratify samples into the two subtypes[118]. Gene differential expression analysis was performed with the LIMMA package (v3.32.2). We evaluated the impact of molecular subtypes on survival outcomes.

4.3.4   Statistical analysis

Statistical analysis was carried out with R v3.6.3. OS was calculated from time of randomisation to time of death or time last known to be alive. Relapse free survival (RFS) was defined as time from randomisation to time of local or distant disease recurrence or death. Patients who withdrew consent or did not receive allocated treatment were excluded from the analysis. Kaplan-Meier method was used to analyse survival.

Multivariable analysis was carried out to adjust for known prognostic factors.

Chi-squared test is used to determine correlation between the presence of necrosis and the two molecular subtypes studied. Wilcoxon signed rank test is used to determine the correlation between West-24 signature score and the two other categorical biomarkers – presence of necrosis and molecular subtypes.

4.4 Results

333 patients were recruited to the BCON study between November 2000 and April 2006. 165 were randomised to radiotherapy (RT) alone and 168 to radiotherapy with hypoxia modification (RT+CON). 9 patients have been excluded from this analysis due to not receiving the allocated treatment or withdrawal of consent. A total of 324 patients were included in this analysis. This is illustrated in the CONSORT diagram (figure 7). We obtained updated survival data for 301 (92.9%) patients. Patient characteristics and demographic details are shown in table 5. The patient characteristics were well-matched between both arms.
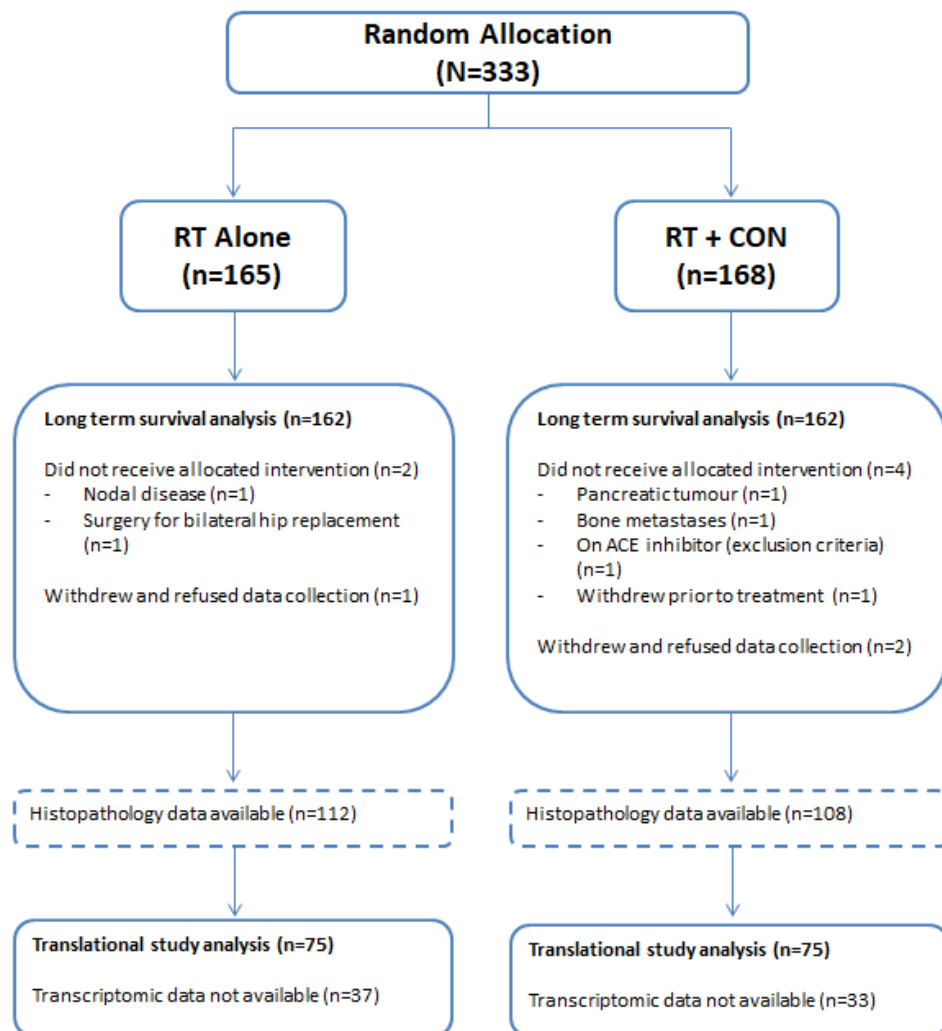
Figure 7 CONSORT diagram of BCON study

	Main trial			Translational study		
	RT (n=162)	RT+CON (n=162)	p-value	RT (n=75)	RT+CON (n=75)	p-value
Age (years)						
median (range)	74 (51-90)	74 (44-89)	0.615	75 (51-86)	75 (51-89)	0.781
Gender – N (%)						
male	128 (79)	131 (81)	0.781	55 (73)	59 (79)	0.566
female	34 (21)	31 (19)		20 (27)	16 (21)	
Grade – N (%)						
2	21 (13)	25 (15)	0.493	11 (15)	14 (19)	0.475
3	140 (86)	136 (84)		64 (85)	60 (80)	
Unknown	1(<1)	1 (<1)		0 (0)	1 (1)	
T-Stage – N (%)						
1	14 (9)	15 (9)	0.623	0 (0)	10 (13)	**0.007**
2	103 (64)	112 (69)		54 (72)	52 (69)	
3	38 (23)	29 (18)		19 (25)	11 (15)	
4	6 (4)	6 (4)		2 (3)	2 (3)	

Missing	1 (<1)	0 (0)		0 (0)	0 (0)	
Tumour Bulk – N (%)						
Complete De-bulking	67 (41)	62 (38)	0.390	32 (43)	33 (44)	0.712
Partial De-bulking	43 (27)	56 (35)		25 (33)	23 (31)	
Biopsy	45 (28)	40 (25)		15 (20)	18 (24)	
Missing	7 (4)	4 (2)		3 (4)	1 (1)	
Haemoglobin (g/dL)						
median (range)	13.7 (9.3 – 17.0)	14.0 (9.5-17.2)	0.318	13.7 (9.8-16.9)	14.1 (9.5-17.2)	0.340
missing– N (%)	3	2		1 (1)	1 (1)	
Hypertension – N (%)						
No	112 (69)	113 (70)	0.605	48 (65)	55 (73)	0.291
Yes	49 (30)	49 (30)		27 (36)	20 (27)	
Missing	1 (<1)	0 (0)		0 (0)	0 (0)	
Diabetes – N (%)						
No	151 (93)	147 (91)	0.358	72 (96)	68 (91)	0.326
Yes	10 (6)	15 (9)		3 (4)	7 (9)	

Missing	1 (<1)	0 (0)		0 (0)	0 (0)	
IHD – N (%)						
No	149 (92)	143 (88)	0.259	70 (93)	70 (93)	1.000
Yes	12 (7)	19 (12)		5 (7)	5 (7)	
Missing	1 (<1)	0 (0)		0 (0)	0 (0)	
Hypoxia Scores						
median (range)				4.68 (4.44-5.21)	4.65 (4.33-5.18)	**0.022***
Necrosis – N (%)						
No				39 (52)	32 (43)	0.327
Yes				36 (48)	43 (57)	
Molecular Subtyping – N (%)						0.623
Basal				37 (49)	33 (44)	
Luminal				38 (51)	42 (56)	

Table 5 Patient characteristics of BCON study. *There is a significant difference in hypoxia score between the two arms.

Median follow up was 10.3 years. OS and RFS are shown in figures 8. One patient in the RT+CON arm progressed during treatment and treatment was stopped, therefore does not have RFS time. The 5 years OS is 49% (95%CI 42-57%) in RT+CON patients and 40% (95%CI 33-48%) in the RT alone patients (HR 0.8 (95% CI 0.61-1.04), p=0.08). With regards to RFS, this was 60% (95%CI 52-69%) with RT+CON and 51% (95%CI 43-60%) with RT alone (HR 0.74 (95%CI 0.52-1.04) p=0.06). (Figure 8)
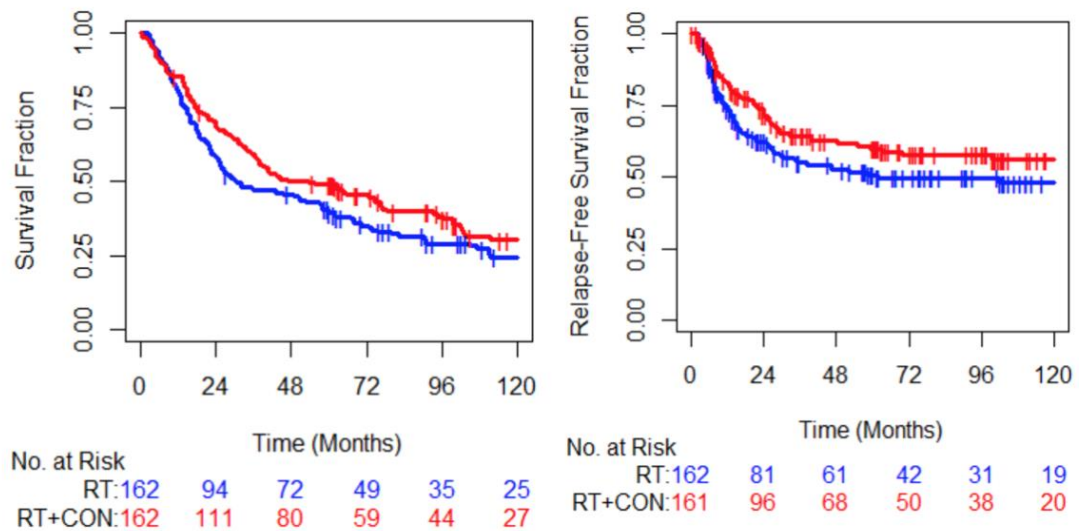
Figure 8 Overall survival and relapse free survival curves of RT vs RT+CON

Biological features (necrosis, hypoxia score and molecular subtypes) were available in 150 patients, 75 from each arm of study (table 5). There was a greater number of patients with T1 disease in the RT+CON arm compared to the RT alone in this group.

4.4.1   Necrosis

In the absence of necrosis, OS was similar in both treatment arms (HR 1.20 (0.70-2.04), p=0.5). 5-year OS was 43% (95% CI 31-61%) in RT+CON and 49% (95% CI 37-65%) in RT alone. The median OS was 37 months (95% CI 24-141 months) and 57 months (95%CI 32-112 months) respectively. This is illustrated in figure 9A. There was no significant difference in RFS (0.52 (0.25-1.12), p = 0.094).

In the presence of necrosis, there was a statistically significant difference in OS (HR 0.59 (0.36-0.99), p=0.04). 5-year OS was 53% (95% CI 42-67%) in RT+CON and 33% (95% CI 22-50%) in RT alone. The median survival was 65 months (95%CI 32-112 months) and 22

months (95% CI 15-68 months) respectively. This is illustrated in figure 9B. There is no significant difference in RFS (1.49 (0.72-3.10), p = 0.283).

The interaction between survival outcomes and necrosis, remains following adjustments for tumour bulk, haemoglobin, T stage, tumour grade and molecular subtype (table 6).

	Univariable			Multivariable (N = 144/E = 110)	
	N/E	HR (95% CI)	p-value	HR (95% CI)	p-value
Tumour Bulk	146/114				
Partial v Complete		0.98 (0.63-1.50)	0.911	0.99 (0.63-1.54)	0.962
Biopsy v Complete		0.84 (0.51-1.37)	0.472	0.97 (0.59-1.62)	0.916
Haemoglobin	148/114	0.97 (0.86-1.09)	0.608	0.97 (0.85-1.10)	0.622
T-Stage					
3/4 v 1/2	150/115	0.88 (0.56-1.36)	0.552	0.77 (0.47-1.24)	0.283
Grade					
2 v 3/4	150/115	1.24 (0.77-2.02)	0.381	1.11 (0.65-1.89)	0.703
Interaction	150/115				
Necrosis x Treatment Arm*		1.56 (0.93-2.61)	0.089	1.69 (0.97-2.94)	0.065
Treatment Arm*		1.00 (0.63-1.56)	0.986	0.98 (0.62-1.56)	0.930
Mol. Subtype	150/115				
Luminal vs Basal		1.16 (0.80-1.69)	0.422	1.18 (0.79-1.76)	0.417

**Yes v No; * RT v RT/CON

Table 6 Univariable and multivariable analysis of survival outcomes and presence of necrosis
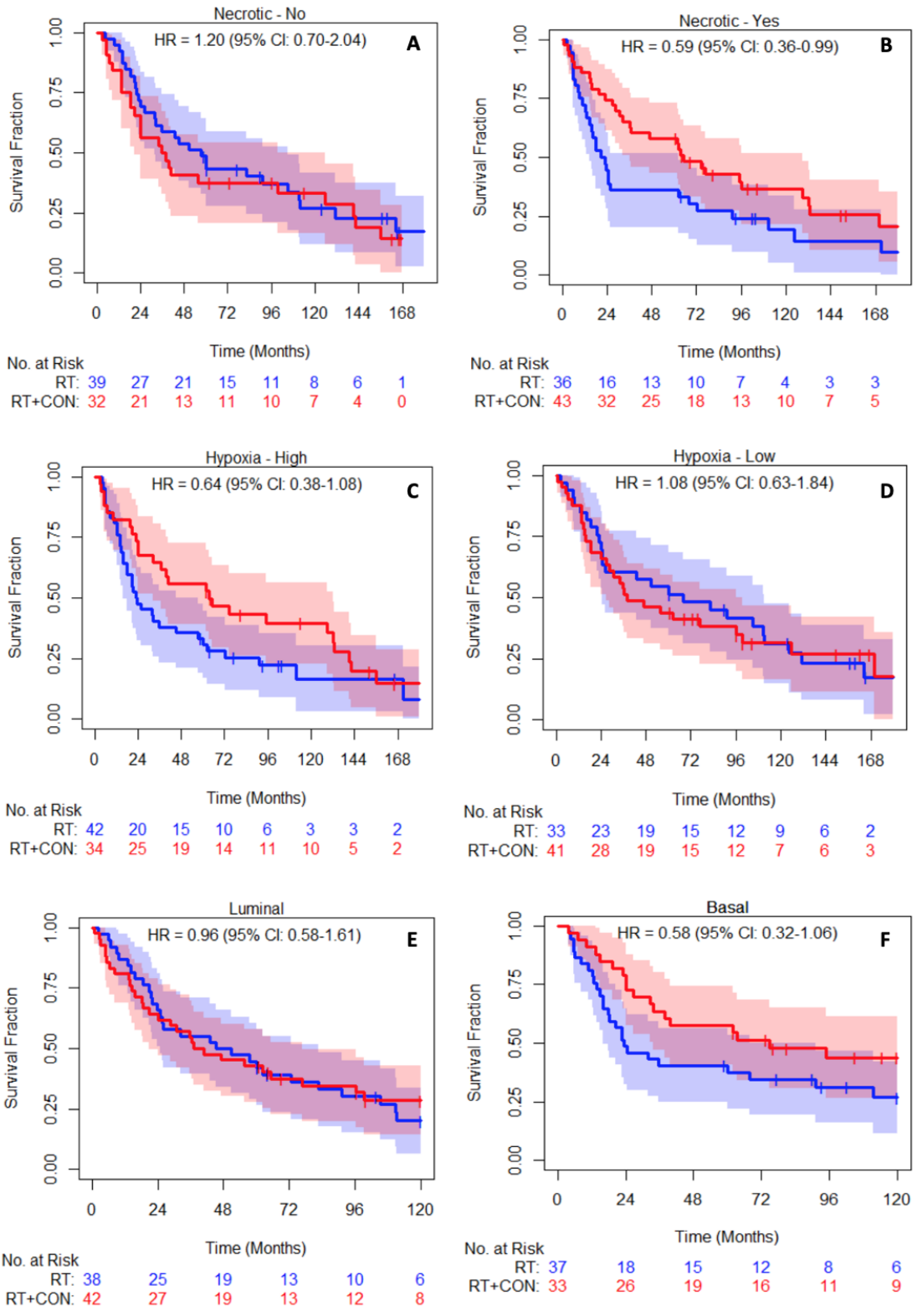
Figure 9 Kaplan Meier curves showing survival probability in both treatment arms based on necrosis (A and B), hypoxia (C and D) and molecular subtype (E and F) groups.

4.4.2 West-24 Signature Score (Hypoxia Score)

West-24 signature score analysis was carried out with categorisation of patients into high and low hypoxia groups based on the median score value, and also with West-24 signature score as a continuous variable.

OS outcomes were similar in both treatment arms in the low hypoxia group (HR 1.08 (0.63-1.84), p=0.8). 5-year OS was 51% (95% CI 41-62%) in RT+CON and 51% (95% CI 39-68%) in RT alone (figure 9C). The median OS in this group was 38 months (95% CI 29-126 months) and 68 months (25-124 months) respectively. This is illustrated in figure 9C. There is no difference in RFS (HR 1.00 (0.46-2.21), p = 0.987)

In the high hypoxia group, 5-year OS was 51% (95%CI 41-62%) in RT+CON and 34% (95%CI 23-49%) in RT alone (figure 9D). The median OS was 64 months (37-133 months) and 23 months (18-60 months) respectively (HR 0.64 (0.38-1.08), p=0.09). This is illustrated in figure 9D. There is no difference in RFS (0.84 (0.41-1.68), p=0.615).

When assessing the relationship of survival and hypoxia score as a continuous variable, the 5-year survival probability of patients receiving RT alone decreased with increasing hypoxia score. The 5-year survival probability remained constant despite hypoxia score in those who underwent RT+CON. A visual representation of this interaction is demonstrated in figure 10.

The interaction between survival outcomes and hypoxia score remain following adjustments for tumour bulk, haemoglobin, T stage, tumour grade and molecular subtype (table 7).
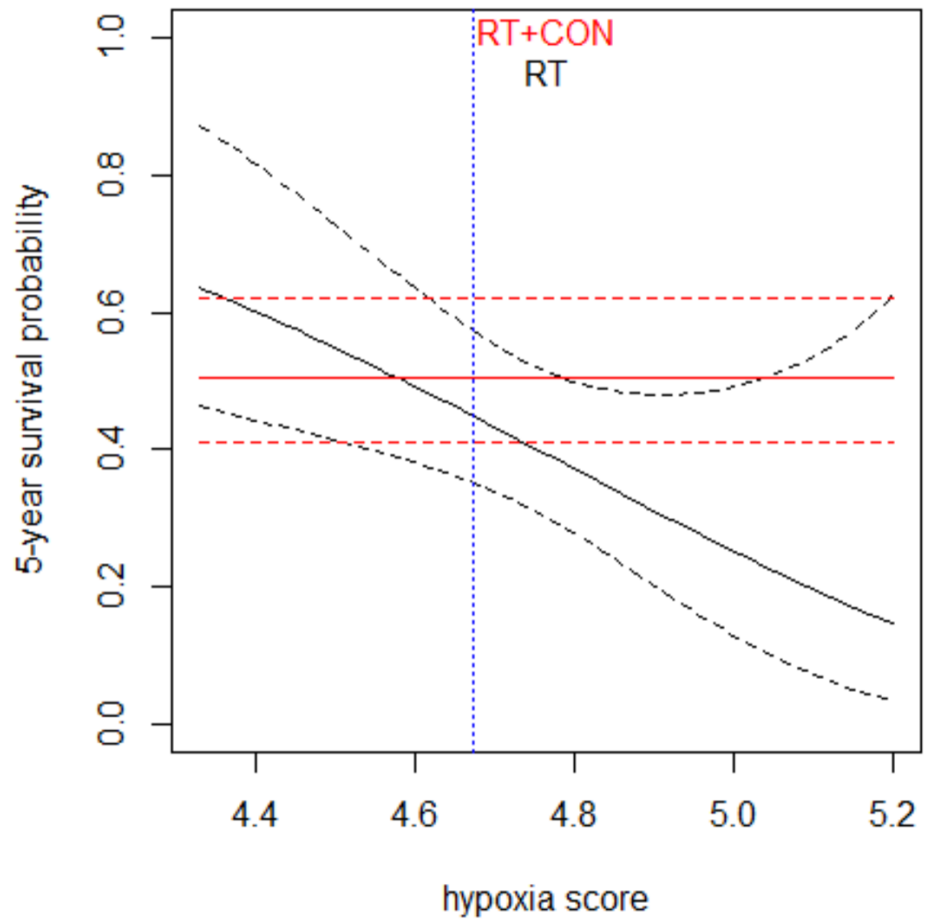
Figure 10 Plot showing the 5-year survival probability as a function of hypoxia score based on whether a patient had RT+CON (red) or RT alone (black). The median hypoxia score is illustrated as a dotted blue vertical line.

	Univariable			Multivariable (N = 144/E = 110)	
	N/E	HR (95% CI)	p-value	HR (95% CI)	p-value
Tumour Bulk	146/114				
Partial v Complete		0.98 (0.63-1.50)	0.911	1.06 (0.68-1.65)	0.795
Biopsy v Complete		0.84 (0.51-1.37)	0.472	1.09 (0.64-1.84)	0.752
Haemoglobin	148/114	0.97 (0.86-1.09)	0.608	0.98 (0.86-1.12)	0.768
T-Stage					
3/4 v 1/2	150/115	0.88 (0.56-1.36)	0.552	0.75 (0.46-1.22)	0.250
Grade					
2 v 3/4	150/115	1.24 (0.77-2.02)	0.381	1.12 (0.66-1.91)	0.665
Interaction	150/115				
Hypoxia Cat. x **Treatment Arm***		1.63 (0.97-2.72)	0.065	1.88 (1.04-3.41)	0.038
Treatment Arm*		0.95 (0.59-1.52)	0.819	0.90 (0.55-1.48)	0.673
Mol. Subtype Luminal vs Basal	150/115	1.16 (0.80-1.69)	0.422	1.26 (0.84-1.91)	0.265

** High v Low; * RT v RT/CON

Table 7 Univariable and multivariable analysis of survival outcomes and hypoxia scores

4.4.3   Molecular Subtype

In luminal subtypes, OS was similar in both treatment arms (HR=0.96 (0.58-1.61) p=0.88) (figure 5). 5-year OS was 43% (95% CI 30-61%) in RT+CON and 45% (95% CI 31-64%) in RT alone. The median OS was 39 months (95% CI 24-99 months) and 48 months (95% CI 25-91 months) respectively. This is illustrated in figure 9E. There was no significant difference in RFS (HR 0.63 (0.32-1.25), p = 0.185).

In basal subtype, there was a difference in OS (HR 0.58 (0.32-1.06) p=0.08). 5-year OS was 58% (95%CI 43-77%) in RT+CON and 38% (95%CI 25-57%)in RT alone. The median OS in the basal group was 75 months (95% CI 34-NR months) with RT+CON and 23 (95% CI 18-112 months) respectively. This is illustrated in figure 9F. There is no significant difference in RFS (HR 1.49 (0.65-3.45), p = 0.350).

The interaction between survival outcomes and molecular subtype was not significant following adjustments for tumour bulk, haemoglobin, T stage, tumour grade and hypoxia score (table 8).

There is an association between molecular subtype and West-24 signature score (ROC AUC = 0.82 (0.75-0.89) p<0.001) (figure 11) and but no association between molecular subtype and necrosis (p=0.234) or between West-24 signature score and necrosis (ROC AUC 0.63 (0.54-0.72) p<0.001).
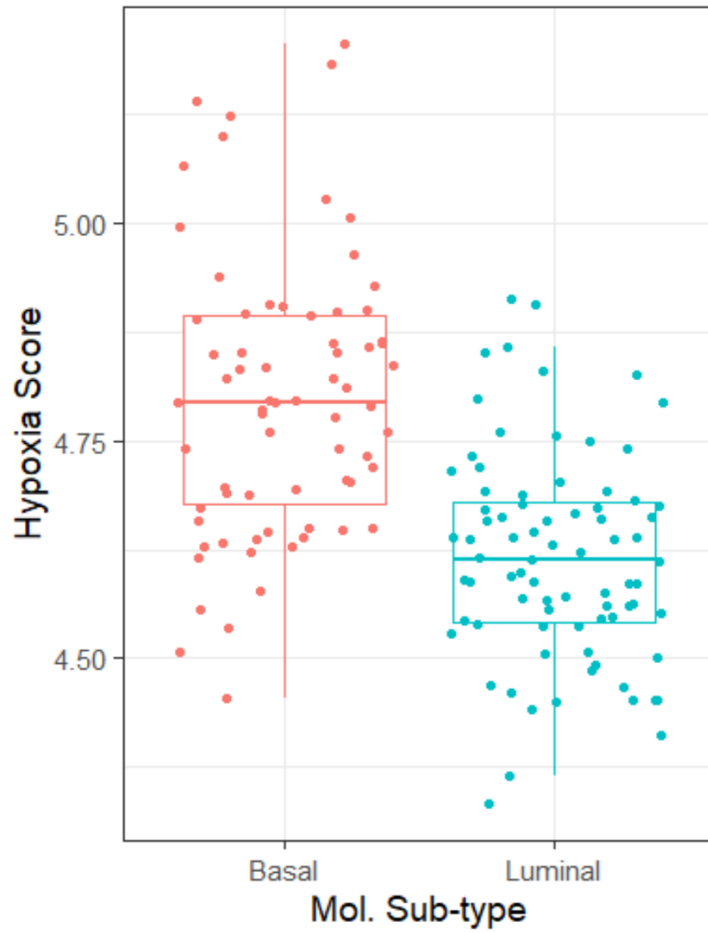
Figure 11 Distribution of hypoxia score for basal and luminal molecular sub-types. Box and whisker plot is also shown with 25th, 50th and 75th percentiles as solid horizontal lines.

	Univariable			Multivariable (N = 144/E = 110)	
	N/E	HR (95% CI)	p-value	HR (95% CI)	p-value
Tumour Bulk	146/114				
Partial v Complete		0.98 (0.63-1.50)	0.911	1.13 (0.72-1.79)	0.590
Biopsy v Complete		0.84 (0.51-1.37)	0.472	1.00 (0.58-1.73)	0.995
Haemoglobin	148/114	0.97 (0.86-1.09)	0.608	0.94 (0.82-1.08)	0.388
T-Stage					
3/4 v 1/2	150/115	0.88 (0.56-1.36)	0.552	0.75 (0.45-1.25)	0.267
Grade					
2 v 3/4	150/115	1.24 (0.77-2.02)	0.381	1.18 (0.66-2.12)	0.569
Interaction	150/115				
Mol. Subtype ** **x Treatment Arm***		0.62 (0.36-1.05)	0.077	0.62 (0.35-1.10)	0.101
Treatment Arm*		0.99 (0.63-1.56)	0.975	1.09 (0.67-1.77)	0.739
Hypoxia Score*	150/115	1.99 (0.61-6.53)	0.258	3.06 (0.85-11.07)	0.088

** Luminal vs Basal; * RT v RT/CON;***mean centered (mean = 4.7)

Table 8 Univariable and multivariable analysis of survival outcomes and molecular subtype

4.5   Discussion

Patients with localised muscle-invasive bladder cancer have various options for curative treatment. There has to be a scientifically sound approach to personalise treatment and guide clinicians and patients in their decisions regarding treatment plans. Our study explores the use of biomarkers to predict benefit from hypoxia modification in bladder radiotherapy.

The BCON study closed to recruitment in April 2006 and with a 5 -year median follow-up, the study reported improvements in OS and relapse free survival outcomes at 3 years with the addition of hypoxia modification to bladder radiotherapy. The median age at randomisation was 74 (44-90), the advanced age at randomisation would explain the limited median OS in each subgroup. With a median follow up of 10.3 years, we demonstrated that there is a trend of sustained benefit with hypoxia modification.

Hypoxia is an important biological feature that drives cancer cell behaviour [159]–[162] and a poor prognostic factor in various solid cancers. Tumour hypoxia induces various pathways including the hypoxia inducible factor (HIF), PI3K/AKT/mTOR, MAPK and NFκB pathways. The absence of oxygen causes stabilisation and accumulation of HIF-1α. This results in enhancement of cell survival via increase in levels of growth factor and inhibition of pro-apoptotic pathways, angiogenesis and tumour neovascularisation via VEGF pathway, and also the induction of cell migration and invasion. The upregulation of hypoxia-inducible factor 1α (HIF-1α) and tumour-associated carbonic anhydrase in bladder cancer suggests that hypoxia plays an important role in bladder cancer[163], [164]. HIF-1α has also been shown to predict tumour recurrence and progression in bladder cancer[165][166].In addition to its impact on tumour cell survival, mobility and metastasis, hypoxia also has an impact on radioresistance as oxygen causes permanent fixation of damage caused by radiation through the formation of organic peroxide.

Our study results are in line with that of published literature regarding the poor outlook of patients with hypoxic features. In the RT alone cohort, without an attempt to alter oxygenation, patients with necrosis or high hypoxia scores have a poorer OS than those without these features.

Due to anatomical restrictions, the measurement of hypoxia in bladder tumours in vivo is difficult. Measuring hypoxia directly would require the insertion of Eppendorf electrode probe into tumour with the aid of cystoscopy and general anaesthetic. This is not practical in the clinical setting and is associated with risks of an invasive procedure. Hypoxia can be measured indirectly with blood oxygen dependent (BOLD) MRI scans. This utilises the difference in magnetism of deoxyhaemoglobin and oxyhaemoglobin. As deoxyhaemoglobin is paramagnetic and oxyhaemoglobin is diamagnetic, oxygenated blood appears brighter on T2 weighted images. MRI scan sequences can be manipulated to be sensitive to the level of deoxyhaemoglobin[87]. Oxygen-enhanced MRI (OE-MRI) measures the change in relaxation time (R_1) which is related to tumour hypoxia. This allows identification of hypoxic regions within a tumour. While both functional imaging techniques can translate to useful imaging biomarkers, further validation is required. In addition, there could be significant logistical challenges involved. MIBC patients undergo maximum TURBT as part of their diagnostic work up, a BOLD MRI or an OE-MRI following maximum resection of the tumour will therefore not be a true reflection of tumour hypoxia status.

Necrosis is considered the endpoint of hypoxia as the lack of oxygen results in ischaemic injury which in turn causes cell death. Tumour necrosis has been shown to be associated with poorer prognosis following radical cystectomy in bladder cancers[167]. Coagulative necrosis results from chronic hypoxia and is characterised by increased tissue eosinophilia, nuclear breakdown and ultimately, the loss of tissue architecture[168]. The presence of necrosis can therefore be determined through careful examination of a haemotoxylin and eosin (H&E) stained sample of a tumour sample by an experienced histopathologist. In this study, we found that in patients with no necrosis, the addition of hypoxia modification to radiotherapy did not make a difference their survival probability at 5 years. In those with necrosis, the addition of hypoxia modification to radiotherapy improved survival outcomes, indicating that the presence of necrosis could help select patients for hypoxia modification.

Exploring genes regulated by hypoxia in cancer cell lines or previous hypoxia signature genes validated in clinical cohorts, we were able to develop the West-24 signature and a score was calculated for each tumour as the median of expression levels over all[156].

This hypoxia gene signature score provides both an objective and quantitative measure of hypoxia. With a hypoxia score lower than the median, survival probability at 5 years is similar with and without hypoxia modification. In patients with high hypoxia scores, survival is poor with radiotherapy alone. However, this improves with the addition of hypoxia modification, suggesting that when the radioresistance resulting from hypoxia is mitigated, patents response to treatment improves. The use of a median score as cut-off to impact on choice of radiosensitiser requires careful consideration. Those with lower than median score may also benefit from hypoxia modification, albeit to a smaller degree.

When assessing the hypoxia score as a continuous variable, we demonstrate that prognosis decreases with increasing hypoxia score if they receive RT alone, but hypoxia score does not affect prognosis if hypoxia modification is administered. This graph suggests that for low hypoxia values, there is little difference between RT and RT+CON. There is a clearer separation of the two curves and as the hypoxia score increases. This nomogram is an illustration of the potential benefit of hypoxia modification for patients, depending on their hypoxia score. There is potential for this to be a useful nomogram for clinical use, enabling patients and clinicians to consider the use of hypoxia modification.

In recent years, various groups have identified basal and luminal subtypes in MIBC through whole genome mRNA expression profiling, echoing the molecular subtyping in breast cancers[115], [116], [118], [169]. However, these published classifications were derived from different datasets and methods used. Despite earlier attempts to reach a consensus in this field, the studies considered continued to vary in numbers and names of subtypes[170], [171]. This resulted in a barrier for collaboration in research and a transfer to clinical practice. At the highest level, MIBC can be classified into basal and luminal subtypes. We have therefore utilised this classification in our analysis.

A study had found that basal tumours had worse OS and disease specific survival outcomes[118]. Seiler et al showed that basal tumours benefitted from neo-adjuvant chemotherapy while luminal tumours did not[132]. In a study of 136 patients who underwent TMT and 223 who underwent neoadjuvant chemotherapy (NAC) followed by radical cystectomy (RC), samples were classified into luminal, luminal-infiltrated, basal and claudin-low subtypes[133]. Classification using TCGA subtypes found that the

majority of luminal and luminal-infiltrated tumours were classified as TCGA luminal subtypes while basal and claudin-low subtypes were classified as TCGA basal subtype. There was no difference in outcomes across all subtypes in the TMT group, but outcomes were worse in the claudin-low subtype in the NAC/RC group. The study also found that tumours with higher level of immune infiltration in the TMT group had improved outcomes, while higher level of immune infiltration is associated with NAC/RC.

To our knowledge, our study is the first to examine the difference in outcomes between molecular subtypes following hypoxia modification. We demonstrated that basal tumours benefited from hypoxia modification while luminal subtypes did not. Taking into consideration various other studies also showed that basal tumours have worse outcomes, it is likely that luminal tumours follow a more indolent course and intensifying treatment through radiosensitisation would not have an impact on long term outcome. In comparison, basal tumours are more aggressive and any attempt to improve outcomes though systemic treatment or radiosensitisation can result in marked improvement.

Our study is not without its limitations. The sample size included in the biological features analysis is less than half of the total patients recruited to the study. Inclusion of other tumour samples within the study would improve precision for the difference observed.

Tumour necrosis appears to be straightforward biomarker that could potentially stratify patients, there are three issues with the use of necrosis in patient selection. The assessment of necrosis is a subjective one and therefore dependent on the expertise of individual histopathologist. In our study, each sample is scored by one histopathologist and not cross-checked. Secondly, only a small part of the tumour is examined and tumour heterogeneity is not accounted for. Lastly, necrosis is a qualitative factor and unlike the West-24 signature score which provides a quantitative measure, it does not illustrate the differing amount of benefit patients would gain from hypoxia modification depending on the amount or severity of hypoxia.

The West-24 signature score allows a quantitative measurement of hypoxia as illustrated by the nomogram we present. However, the nomogram requires further validation as it was not included in our previous study where the prognostic value of the median score was validated.

The use of a median score to stratify patients into high and low hypoxia groups allow a simple way to separate those who would benefit from hypoxia modification and those who would not. However, a median score is dependent on the patient cohort. Less than half the patient cohort of the main study was included in the development of the 24 gene hypoxia signature score. The median score may change if more samples were included. In clinical practice, the median score may differ depending on the target patient cohort. Therefore, it is important to determine if the median score is dependent on sample numbers and patient cohort through increasing sample size and further validation in order to work towards a more robust method of determining the cut off score.

Whole transcriptome analysis was utilised to develop both the 24 gene hypoxia signature score and to determine the molecular subtype of each sample. Transcriptome analysis is dependent on RNA extraction from tumour samples. This is in turn is affected by sample and technological factors. The age of tissue sample, storage conditions and sample handling can affect the quality of RNA extracted. In a study of RNA extraction from frozen samples of oral tumours and fresh oral mucosa, it was found that the mean RNA concentration was significantly lower in samples stored for a long time[172]. Similarly, the initial handling of tissue prior to storage has also been shown to affect RNA yield[173]. Patients were recruited to the study over a decade ago and the FFPE blocks were obtained from their diagnostic procedures. The large time lapse may have had an impact of the amount and integrity of RNA extracted. Furthermore, RNA extraction is operator dependent and the technique utilised may also affect results. We utilised the RecoverAll Total Nucleic Acid Isolation Kit and human exon 1.0 ST array and results will differ with different kits and array [174]. The careful storage, specialist skillset and equipment required translates to a costly method of determining biomarker status consideration is made to move this to wider clinical use.

Apart from the subjectivity surrounding identification of necrosis, the technical issues and cost surrounding the transcriptome analysis, it is also vital to consider that these biological features have not been adequately validated. Although the prognostic value of the gene signature had been validated in independent cohorts[112], the predictive value had not. Similarly, while recent years have seen much interest in molecular subtyping of

bladder cancer, the effect of hypoxia modification in different subtypes has not been qualified.

We have presented the potential impact of three different biomarkers on the long-term outcomes of hypoxia modification. However, due to the relatively small cohort in our study, it had not been possible to determine the value of a composite biomarker comprising two or three of the phenotypes presented.

The Cancer Research UK's prognostic and predictive biomarker roadmap recommends that biomarker driven randomised trials should be carried out prior to a biomarker's introduction into routine clinical practice[175].

Radiosensitisation in bladder cancer is most common with chemotherapy regimens. We have demonstrated the possibility of identifying potentially useful predictive biomarkers in hypoxia modification cohorts. This is an initial step in personalising treatment in MIBC. Similar studies in chemoradiotherapy may also find biomarkers that predict benefit with chemotherapy radiosensitisation. In combination, a treatment decision algorithm could be developed such that treatment plans best suited to a patient and most effective for the cancer biology can be formulated.

5 Radical Radiotherapy to Bladder –Do Bladder and Rectal Size on Radiotherapy Planning Scan Matter?

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Conception or design of the work: YS, AMcW

Acquisition of data: YS, AMcW

Analysis of data: YS, HM

Interpretation of data: YS, HM

Drafting and editing text: YS, AMcP, PH, AC, AMcW

5.1 Abstract

5.1.1   Introduction

Radiotherapy is the mainstay of curative treatment for bladder cancer patients opting for organ preservation. Historically, the entire empty bladder is included in as single clinical target volume. An empty bladder reduces dose to organs at risk and improves reproducibility. Advances in imaging and radiotherapy techniques allow the possibility of delivering higher radiation dose to tumour beds. Such techniques require the filling of bladders to separate bladder walls. The effect of bladder size on clinical outcomes is unknown. Studies in prostate cancer have shown the impact of rectal dimension on radiotherapy planning (RTP) scan on biochemical failure. The impact of rectal dimensions in bladder radiotherapy is not known. We evaluate the association of rectal and bladder dimensions on bladder RTP scan with survival outcomes.

5.1.2   Methods

This retrospective study included patients treated with radical concurrent chemoradiotherapy for urothelial carcinoma of the bladder in a tertiary cancer centre from 2010 to 2014. The whole bladder and extra-vesical extension of tumour was treated to a uniform dose of 52.5Gy in 20 fractions with weekly concurrent gemcitabine. An empty bladder imaging and treatment protocol was used. Superior-inferior length and anterior-posterior diameter of the rectum corresponding to superior, inferior and mid-point of the planned target volume (PTV) were measured. Bladder volume was measured on RTP scan. Overall survival was defined as time from start of treatment to death and

patients still alive were censored at time last known alive. Progression free survival was defined as time to local or metastatic progression. Cox proportional hazard ratio was used to investigate the association of bladder and rectal dimensions with outcomes

5.1.3   Results

132 patients were included in this study. With a median follow-up of 74.1 months, the median OS of all patients was 73.2months (95% CI 58.7-NR) and median progression free survival (PFS) was not reached. A larger log (bladder volume) on RTP scan was associated with poorer overall survival (OS) (HR 1.78 (95% CI 1.07-2.94), p=0.03) and PFS (HR 1.62 (95% CI 0.99-2.63), p=0.05). Larger superior-inferior (SI) distance and rectal volumes was associated with poorer OS (HR 1.42 (95% CI 1.14-1.78), p=0.002 and HR 1.59 (95% CI 1.06-2.37), p=0.02 respectively) and PFS (HR 1.39 (95%CI 1.12-1.73), p=0.003 and HR 1.46 (95%CI 0.98-2.16), p=0.06 respectively). This was not statistically significant following multivariable analysis. Rectal anterior-posterior (AP) diameters at three positions corresponding to PTV and mean cross sectional area (CSA) are not associated with survival outcomes.

5.1.4   Conclusion

Rectal AP diameters and mean CSA are not associated with survival outcomes. Rectal SI distance and volumes reflect bladder volume. Advances in radiotherapy technique allow more precise treatment plans. As clinical trials adopt bladder-filling protocols, it is vital that the impact of bladder volume on clinical outcomes is considered. Our relatively small study shows that bladder volume is not related to survival in a multivariable analysis. Factors like hydronephrosis and CIS impact survival, and should be considered in formulation of management plans. Bladder volume in this study reflects poor bladder emptying which may differ from planned bladder filling. Further evaluation in a prospective patient cohort with planned bladder filling will improve our understanding of the impact of bladder size on outcomes in the modern era.

5.2   Introduction

Around one-third of bladder cancer patients present with locally advanced muscle-invasive bladder cancers and require aggressive treatments in the form of either radical cystectomy or trimodality bladder preserving treatment[27]. Radiotherapy is the mainstay of curative treatment for those who opt for bladder preservation treatment[12], [14], [176]. Therefore, optimal delivery of radiotherapy in this group of patients is especially important.

When considering radiotherapy to any part of the body, factors affecting motion and position of the target volumes are vital. The urinary bladder is a muscular organ that sits amongst pelvic organs. It changes in shape, size and position as a result of internal filling and external pressure[177]. Hence, radiotherapy to the bladder can be challenging.

5.2.1   Bladder volume

Historically, the entire bladder and any extra-vesical extension of tumour are included in a single clinical target volume (CTV), and a 1.5 to 2cm margin is added to form planning target volume (PTV). The bladder is a highly elastic structure and as it fills, volume can increase to over 500 millilitres. A larger bladder on radiotherapy planning (RTP) scan compared to on-treatment can result in increased treatment volume and unnecessary dose to normal tissue while the contrary would in geographical miss. Either scenario could impact treatment outcomes. Hence, patients are advised to empty their bladders prior to radiotherapy planning (RTP) and treatment. This improves reproducibility, and reduces the overall treatment volume and dose to organs at risk.

Advances in imaging and radiotherapy techniques in recent years allow visualisation of the bladder wall and delivery of high dose to the tumour bed. Such techniques require planned bladder filling to separate bladder walls for tumour bed boost. While clinical trials investigate the benefits of tumour bed boost, the effect of this increase in bladder volume on patients' clinical outcomes is unknown.

5.2.2   Rectal dimensions

The bladder is a pelvic organ that is situated immediately anterior to the rectum. A large rectum on RTP scan can potentially result in the bladder being more anterior at planning than on treatment if the rectum empties prior to treatment and reduces in size. A filled

rectum would be difficult to reproduce accurately on treatment resulting in poor target coverage. The impact of rectal filling and motion on prostate radiotherapy has been extensively evaluated. Studies in prostate cancer have demonstrated that a large rectum on radiotherapy planning scans is associated with a greater risk of geographical miss and therefore worse clinical outcomes. Regardless of the use of implanted markers, prostate cancer patients with a greater rectal cross sectional area[178] or rectal diameter[179] on RTP scans had a greater risk of biochemical failure in 5 years. Furthermore, the combination of distended rectum on RTP scan and diarrhoea on treatment (and hence a change in rectal dimensions on treatment) was also shown to have a detrimental impact on outcome in patients with high risk prostate cancer[180]. Since diarrhoea is also a common acute toxicity in bladder radiotherapy, this may also be the case in bladder cancers.

In this study, we evaluate the association of bladder and rectal size with patients' clinical outcomes. We hypothesize that rectal diameter and bladder volume would not be associated with survival outcomes.

5.3   Methods

This retrospective study included patients who had undergone radical radiotherapy for urothelial carcinoma of the bladder with concurrent chemotherapy in a tertiary cancer centre between May 2010 and December 2014. Patients underwent maximum transurethral resection of bladder tumour (TURBT) prior to radiotherapy planning.

5.3.1   Radiotherapy planning

All patients underwent radiotherapy planning scan and treatment with an empty bladder protocol – patients were instructed to empty their bladder immediately prior to RTP scan and each fraction of treatment. Patients underwent RTP scan in the supine position with three localisation tattoos (one anterior and two laterals) and intravenous contrast. 3mm CT slices were obtained from L2 to pelvic floor. The clinical target volume (CTV) was contoured by the patient's treating consultant clinical oncologist. The CTV included the whole bladder and any extra-vesical extension of bladder tumour. An isotropic expansion of 1.5cm was added to CTV to form PTV. Organs at risk (OAR) including the rectum was contoured by experienced radiographers.

5.3.2   Treatment

Patients were treated with 52.5Gy external beam radiotherapy in 20 daily fractions on consecutive week days over a four week period with three-dimensional conformal radiotherapy technique. All patients had intravenous gemcitabine chemotherapy at a dose of 100mg/m^2 body surface area (BSA) on days 1, 8, 15 and 22 of treatment. This was administered at least two hours before radiotherapy treatment on the same day.

5.3.3   Rectal and bladder dimension

We calculated the volume of CTV contoured as a proxy of bladder volume at RTP. We also calculated the rectal volume, and measured the anterior-posterior (AP) diameter of the rectum at three points on the RTP scan corresponding to the most superior, inferior and mid-point of PTV – RectumSup, RectumInf and RectumMid respectively (figure 12). The superior-inferior (SI) distance between RectumSup and RectumInf points were also measured. Mean cross sectional area (CSA) was calculated by dividing rectal volume by SI distance.
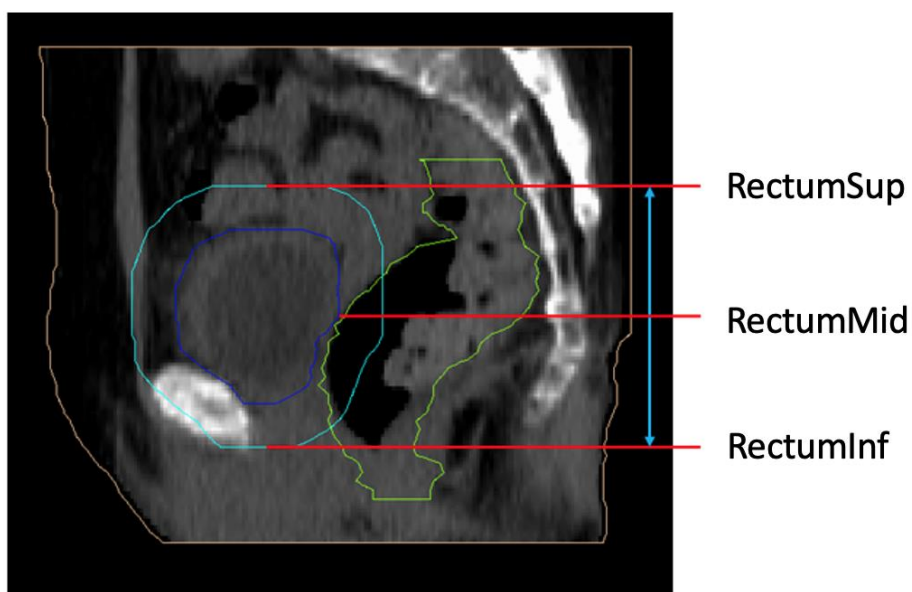
Figure 12 Image illustrating the definition of points (RectumSup, RectumMid and RectumInf) at which rectal AP diameter was measured. Green contour – rectum; dark blue contour – CTV; light blue contour – PTV.

5.3.4   Statistical analysis

Data analysis was carried out with R version 3.5.1[181]. Overall survival (OS) and progression free survival (PFS) survival probability was calculated with Kaplan Meier method and Cox's proportional hazard ratio was used to investigate the association of bladder and rectal parameters with survival probability. Univariable and multivariable analyses were carried out. PFS was defined as time from start of chemoradiotherapy to local muscle-invasive or metastatic recurrence. OS was defined as time from start of chemoradiotherapy to death, patients still alive were censored at time last known to be alive.

5.4   Results

138 patients with histologically proven urothelial carcinoma of the bladder underwent concurrent chemoradiotherapy at the tertiary cancer centre during the study period. RTP scans of 6 patients were not available and they were excluded from the study.

5.4.1   Patient characteristics

Of the 132 patients included in this study, one had high grade T1 disease and all others had muscle-invasive disease (T2-T4a). One patient had an indeterminate para-aortic node at diagnosis, but this did not change following neo-adjuvant chemotherapy and was therefore deemed not to be a metastatic lesion. No other patients had distant metastatic disease at diagnosis. 5 patients did not complete radiotherapy but patients had at least 16 of the planned 20 fractions of treatment. 74 patients had neoadjuvant chemotherapy – 72 patients had gemcitabine with either cisplatin or carboplatin, 2 patients had methotrexate, vinblastine, doxorubicin and cisplatin. Four patients did not complete neo-adjuvant chemotherapy due to treatment related toxicities – one stopped after one cycle, three stopped after two cycles. Patient characteristics are summarised in table 9.

5.4.2   Survival outcomes

With a median follow-up of 74.1 months, the median OS of all patients was 73.2months (95% CI 58.7-NR) and median PFS was not reached. 5 years OS was 56.7% (95% CI 48.9-65.9%) while 5 years PFS was 69% (95% CI 60.9-78.1%). Kaplan-Meier charts illustrating OS and PFS are shown in figure 13.

5.4.3 RTP parameters

The mean bladder volume was 129.95cm^3 (range 39.23-433.25cm^3). The mean rectal SI distance was 4.11cm (range 1.20-7.80cm). Mean AP distance at the RectumSup, RectumMid and RectumInf points were 2.78cm (range 0.33-6.67cm), 3.39cm (range 1.31-5.86cm) and 2.94cm (range 0.49-5.86cm) respectively. Rectal volume ranged from 8.14 to 137.6cm3 with a mean of 41.46cm3. The mean PTV volume was 130.24cm3(39.23-433.25).

Total patients (n)	132
Median age	71 years (43-88)
WHO performance status (PS) 0 1 2 3	 72 50 9 1
T stage T1 T2 T3 T4 Not documented	 1 94 31 5 1
CIS Yes No	 15 117
Hydronephrosis Yes No	 19 113
ACE-27 0 1 2 3 Not documented	 37 45 20 24 18
Neo-adjuvant chemotherapy Yes (MVAC Gemcitabine/Platinum) No	 74 (2 72) 58
Completed concurrent chemotherapy Yes No	 98 34
Completed radiotherapy Yes No	 127 5

Table 9 Patient characteristics
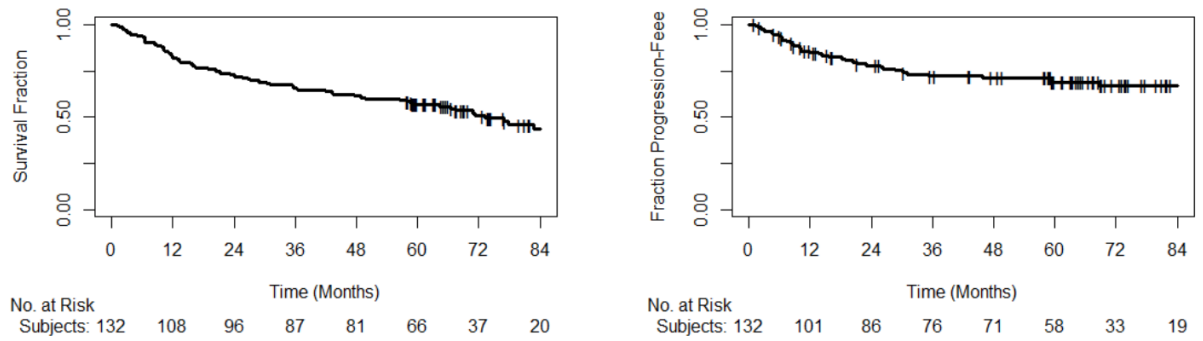
Figure 13 Overall survival and progression free survival probability

5.4.4 Relationship of RTP parameters to survival outcomes

Due to the large range of bladder volume, a log scale of the volume was used in the statistical analysis. A larger log (bladder volume) on RTP scan was associated with poorer OS (HR 1.78 (95% CI 1.07-2.94), p=0.03) and PFS (HR 1.62 (95% CI 0.99-2.63), p=0.05). This was not statistically significant following multivariable analysis, adjusting for known prognostic factors – age, presence of carcinoma in situ (CIS), performance status (PS), prior neo-adjuvant chemotherapy, pre-treatment white cell count (WCC) and hydronephrosis (table 10). CIS, PS and hydronephrosis were the only factors that continued to show an association with OS and PFS following in multivariable analysis.

Rectal AP diameters at three positions corresponding to PTV and mean CSA do not have an association with patients' survival outcomes. Larger SI distance and rectal volumes correlate with poorer OS (HR 1.42 (95% CI 1.14-1.78), p=0.002 and HR 1.59 (95% CI 1.06-2.37), p=0.02 respectively) and PFS (HR 1.39 (95%CI 1.12-1.73), p=0.003 and HR 1.46 (95%CI 0.98-2.16), p=0.06 respectively). The association of SI distance and PFS remained borderline statistically significant following multivariable analysis (HR 1.29 (95%CI 0.998-1.67), p=0.05) but the confidence intervals cross 1.

	OS			PFS		
Univariable analysis	HR	95% CI	p-value	HR	95% CI	p-value
Log (Bladder Vol)	1.78	1.07-2.94	0.03	1.62	0.99-2.63	0.05
Age	1.04	1.01-1.07	0.02	1.02	0.99-1.05	0.09
WHO PS	1.70	1.21-2.38	0.002	1.60	1.16-2.20	0.004
CIS	2.41	1.26-4.61	0.01	2.30	1.21-4.38	0.01
Neo-adjuvant chemotherapy	0.54	0.33-0.87	0.01	0.62	0.39-0.99	0.04
Pre-treatment WCC	1.11	1.01-1.23	0.03	1.12	1.02-1.23	0.02
Hydronephrosis	2.33	1.32-4.11	0.003	2.44	1.41-4.22	0.002
Multivariable analysis						
Log (Bladder Vol)	1.52	0.88-2.62	0.13	1.47	0.86-2.50	0.15
Age	1.02	0.98-1.05	0.35	1.01	0.97-1.04	0.74
CIS	2.60	1.33-5.06	0.005	2.39	1.23-4.63	0.01
WHO PS	1.47	1.03-2.10	0.04	1.43	1.01-2.01	0.04
Neo-adjuvant chemotherapy	0.76	0.43-1.37	0.36	0.83	0.47-1.46	0.51
Pre-treatment WCC	1.08	0.96-1.21	0.20	1.09	0.97-1.21	0.14
Hydronephrosis	2.17	1.21-3.89	0.009	2.18	1.24-3.83	0.007

Table 10 Univariable and multivariable analyses of correlation between bladder volume and survival outcomes

5.5   Discussion

There is potential for a patient's anatomy to vary from one moment to the next due to respiratory motion, organ filling or muscle relaxation. However, radiotherapy plans are typically generated from a snapshot view known as the RTP scan. It is therefore crucial that the RTP scan is an accurate reflection of the patient's anatomy, with easy reproducibility during treatment. Immobilization techniques can help to ensure a patient's position is replicated externally, but internal organ motion is more difficult to predict and repeat. Bladder motion is affected by its filling and emptying and by the motion of surrounding pelvic organs.

Studies in other pelvic cancers like the prostate have demonstrated the impact of rectal dimensions on clinical outcomes[178]–[180]. Biochemical failure was significantly higher in patients with increased mean rectal CSA, rectal volumes or rectal diameters. In these studies, the rectum was contoured from the anus to the rectosigmoid flexure, and the mean CSA was calculated by dividing the rectal volume by the superior-inferior rectal length. As a common acute toxicity of pelvic radiotherapy is diarrhoea, the emptying of a previously filled rectum could cause posterior displacement of organs anterior to it, resulting in a geographical miss. In our study, we defined rectal dimensions based on its relation to the PTV, thereby examining the section of the rectum whereby movement will most likely impact on displacement of the target volume. Unlike published studies in prostate cancer, we found the rectal diameter have no association with clinical outcomes. This could be attributed to the difference in CTV to PTV margins used in prostate and bladder radiotherapy. The radiation dose in prostate radiotherapy is higher than that in bladder radiotherapy (78Gy vs 64Gy in 2Gy per fraction). In order to minimise rectal toxicities, a smaller posterior CTV to PTV margin of 0.5 to 1cm is used. As the bladder expands and changes in volume, and the radiation dose used is smaller, the margin used in bladder cancer is an isotropic 1.5cm margin. Hence, a posterior displacement of the target volume during treatment in bladder radiotherapy is less likely to result in geographical miss.

Our study found that a large rectal SI distance and rectal volume are related to poorer survival outcomes. While the association is no longer statistically significant following multivariable analysis, the p-value in the association of SI distance to outcome remains

low in this relatively small patient cohort. Due to our definition of rectal SI distance, it is a reflection of PTV length as opposed to true rectal length. Similarly, the rectal volume is also a reflection of PTV length. As described previously, the PTV is generated from a uniform expansion margin added to CTV. Therefore, a large PTV length and volume is a reflection of a large bladder and/or large extra-vesical extent of tumour. The negative impact of a tumour with large extra-vesical extension is obvious, however, a large SI rectal distance would only result from a large extra-vesical tumour extension if the tumour was on the superior or inferior aspect of the bladder. Hence, it is more likely that the result signifies a possible association between large bladders and poor outcomes.

As advanced radiotherapy techniques allow more precise treatment plans and delivery of tumour bed boost, planned bladder filling protocols are used to separate bladder walls. The impact of bladder volume on survival outcomes is an important factor to examine. Our study found that while there appeared to be an association on univariable analysis, bladder volume is not related to survival once baseline characteristics are adjusted for in a multivariable analysis. Bladder volume in this study reflects poor bladder emptying which may be differ from planned bladder filling. The statistically significant association of CIS and hydronephrosis on survival outcomes further suggests that it is renal and bladder function that should be taken into account when formulating management plans.

This is a retrospective study on a single patient cohort, treated in a single centre. There has been no inter-observer validation of bladder or rectal contours. Furthermore, the impact of change in bladder size, rectal size and rectal gas on dosimetry has also not been taken into account. Therefore, further studies taking these uncertainties into account, involving a patient cohort with planned bladder filling, will need to be undertaken before any firm conclusions can be drawn.

6 Comparison of Inter-fraction bladder motion in male and female pelvises

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Conception or design of the work: YS, PH, AC

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6.1   Abstract

6.1.1   Introduction
The bladder is a mobile organ that moves with external pressure from surrounding pelvic organs and with internal pressure through filling and emptying. Differences in male and female anatomy and physiology have a potential impact on bladder motion. This study compares the differences in bladder motion between the two sexes.

6.1.2   Methods
This is a retrospective study of patients who underwent radical radiotherapy to the bladder for muscle-invasive bladder cancer. Three patients' scans were contoured by two independent observers to check for interobserver variability measured by Dice coefficient. The bladder was contoured on radiotherapy planning (RTP) scan and cone beam computed tomography (CBCT) at start of and during the final week of treatment. The most extreme points in each of six directions were identified on RTP and CBCT delineations. The distances between these points were used as proxy of bladder wall movement. Mean motion between RTP to first fraction (RTPtoWk1), RTP to week 4 (RTPtoWk4) and week 1 to week 4 (Wk1toWk4) and changes in motion over the course of treatment between male and female patients were analysed using R version 3.5.1.

6.1.3   Results
There is minimal difference in observers' contours with a mean Dice coefficient of 98.1% (94.2-99.5%). Bladder wall motion is observed in all directions. There is a statistical

difference in mean motion between RTPtoWk1 and RTPtoWk4 in the superior (p=0.003), right lateral (p=0.02), left lateral (0.003) and anterior directions (p=0.05) for all patients. The difference in mean bladder motion in superior (p=0.01), right lateral (p=0.001), left lateral (p=0.008) and posterior (p=0.04) directions at RTPtoWk1 and RTPtoWk4 are statistically significant in female patients but not in males. There is statistically significant difference in mean right lateral movement at RTPtoWk1 between female and male bladders (p=0.04). There is a moderate correlation in inferior and anterior bladder motion between RTPtoWk1 and RTPtoWk4 in female patients but strong correlation in male patients.

6.1.4   Conclusion

Bladder motion is seen in all directions. There is little difference in mean motion between female and male patients. However, there are differences in the change in bladder motion during a course of treatment between the two sexes. Further studies into female and male differences in intra-fraction motion will be required to validate these results.

6.2   Introduction

Radiotherapy is a localized treatment, tailored to each patient's anatomy. The basis of modern radiotherapy planning lies in accurate definition of the target volumes. The gross tumour volume (GTV) encompasses the visible tumour and the clinical target volume (CTV) includes both GTV and probable microscopic disease. Lastly, the planning target volume (PTV) adds a further margin to CTV to take into consideration the net effect of any possible geometrical variation and inaccuracies such as patient and organ movement or disparity in patient setup. A large CTV to PTV margin would result in increased unnecessary dose to surrounding organs while a small margin could translate to a risk of inadequate treatment.

The convention of bladder radiotherapy in the UK is to formulate the CTV by extending any visible GTV to include the entire bladder[12], [14]. Bladder motion is an important factor to consider in the margin added forming PTV. The bladder is a mobile organ that moves with external pressure from surrounding pelvic organs and also with internal pressure as it fills and empties. Due to its mobility, a large 1.5-2cm margin is often added to CTV to account for the geometrical variations.

As described in section 1.2, the urinary bladder stores urine collected from the kidneys temporarily then assists in micturition with contraction of the musculature and relaxation of the sphincters. This causes variation in its shape and size. When empty, the bladder is almost flat. It can fill to an oval shape, extending above the symphysis pubis. The anatomical relationship of the bladder differs between males and females.

The bladder is immediately anterior to the rectum in the male pelvis. It is hence pushed forward and upwards when the rectum is distended[60]. The prostate lies inferior to the bladder, surrounding the prostatic urethra as it exits the bladder. Hypertrophy and movement of the prostate affect bladder emptying and position respectively. In the female pelvis, the bladder lies anterior to the upper part of the vagina, cervix and uterus. When the bladder is empty, the uterus rests over its superior aspect. The close proximity of the female internal genitalia to the bladder results in bladder position being affected by movement of the cervix and uterus and vice versa. The male urethra is longer than the

female urethra (18-20cm vs 3-4cm), and in order to facilitate the greater voiding pressure required, the male detrusor muscle is thicker than that of the female.

Apart from external pressures from surrounding organs, bladder motion is also affected by bladder size. This is a result of internal pressure from bladder filling from urine collected by the kidneys. Urine production is influenced by renal physiology and this differs between males and females. The glomerular filtration rate (GFR) is a measure of renal function. Males have higher GFR then females[182]. Vasopressin regulates the excretion of water, and atrial natriuretic peptide and renin-angiotensin-aldosterone system regulate salts clearance[183]. Sex hormones have an influence on these hormones and therefore, the excretion of salt and water differ between males and females as well.

The differences in male and female pelvic anatomy and renal physiology have potential impact on bladder motion. Adaptive radiotherapy techniques like plan of the day (POD) and composite plans have been developed to improve target coverage and reduce unnecessary dose to surrounding tissues. However, neither traditional nor adaptive radiotherapy planning takes into account the difference between male and female pelvises. The same CTV to PTV margins are applied to male and female patients undergoing bladder radiotherapy.

Various studies have looked into bladder motion but little is known about the variability in bladder motion between male and female pelvises. Our study compares male and female bladder movement during radiotherapy. In the era of adaptive radiotherapy, we also examine the relationship of bladder motion from RTP to start of treatment and RTP to final week of treatment, and the potential difference of this relationship in male and female pelvises. We hypothesise that there is no difference between male and female bladder movement.

6.3   Methods

This retrospective study included 42 patients who had undergone radical radiotherapy for muscle-invasive bladder cancer at a tertiary cancer centre. Approval was granted to collect and analyse patient data by the UK Computer Aided Theragnostic (ukCAT) Research Database Management Committee (REC reference: 17/NW/0060).

6.3.1   Contouring Validation

Three patients' scans (two males and one female) were contoured by two independent observers (AA and YS) to check for inter-observer variability. Both observers were senior clinical oncology fellows with experience in bladder radiotherapy and pelvic organs contouring.

6.3.2   Radiotherapy technique

All patients underwent radiotherapy planning scan and treatment with an empty bladder protocol – patients were instructed to empty their bladder immediately prior to radiotherapy planning scan and each fraction of treatment. Patients were treated with 52.5Gy external beam radiotherapy in 20 daily fractions on consecutive weekdays over a four-week period with three-dimensional conformal radiotherapy technique (3DCRT). The CTV included the whole bladder and any extra-vesical tumour extension. 1.5cm isotropic margins were added to form PTV. Cone beam computed tomography (CBCT) scans were taken during the first 3 fractions of treatment and weekly thereafter.

6.3.3   Bladder contouring and quantifying motion

The urinary bladder was contoured on RTP scan and CBCTs from the first fraction and final week of treatment. Rigid registration was performed using bony landmarks. Motion was measured in six directions – superior, inferior, right lateral, left lateral, anterior and posterior. Motion was measured between RTP scan and fraction 1 CBCT (RTPtoWk1), RTP scan to week 4 CBCT (RTPtoWk4) and fraction 1 to week 4 CBCT (Wk1toWk4) in these six directions. The distance between the most extreme points in each direction were measured and used as proxy of bladder wall motion (figure 14). The direction of motion was taken into account. Using the CT scan earlier in the time points of assessment as reference, motion in the direction of assessment was deemed to be positive and motion opposite to the direction of assessment was negative. The average motion in each direction was calculated for each patient (AveMotion).

6.3.4   Statistical Analysis

Dice coefficient was measured for the scans contoured by the two independent observers to check for interobserver variability.

Data analysis was carried out with R version 3.5.1[181]. Histograms of motion in each direction between the time points described were compared to that of a normal probability curve to establish normal distribution of data. Comparison of mean male and female bladder motion in each direction was analysed using a two-sample t-test. Pearson coefficient was calculated to examine the relationship between RTPtoWk1 and RTPtoWk4 motion.
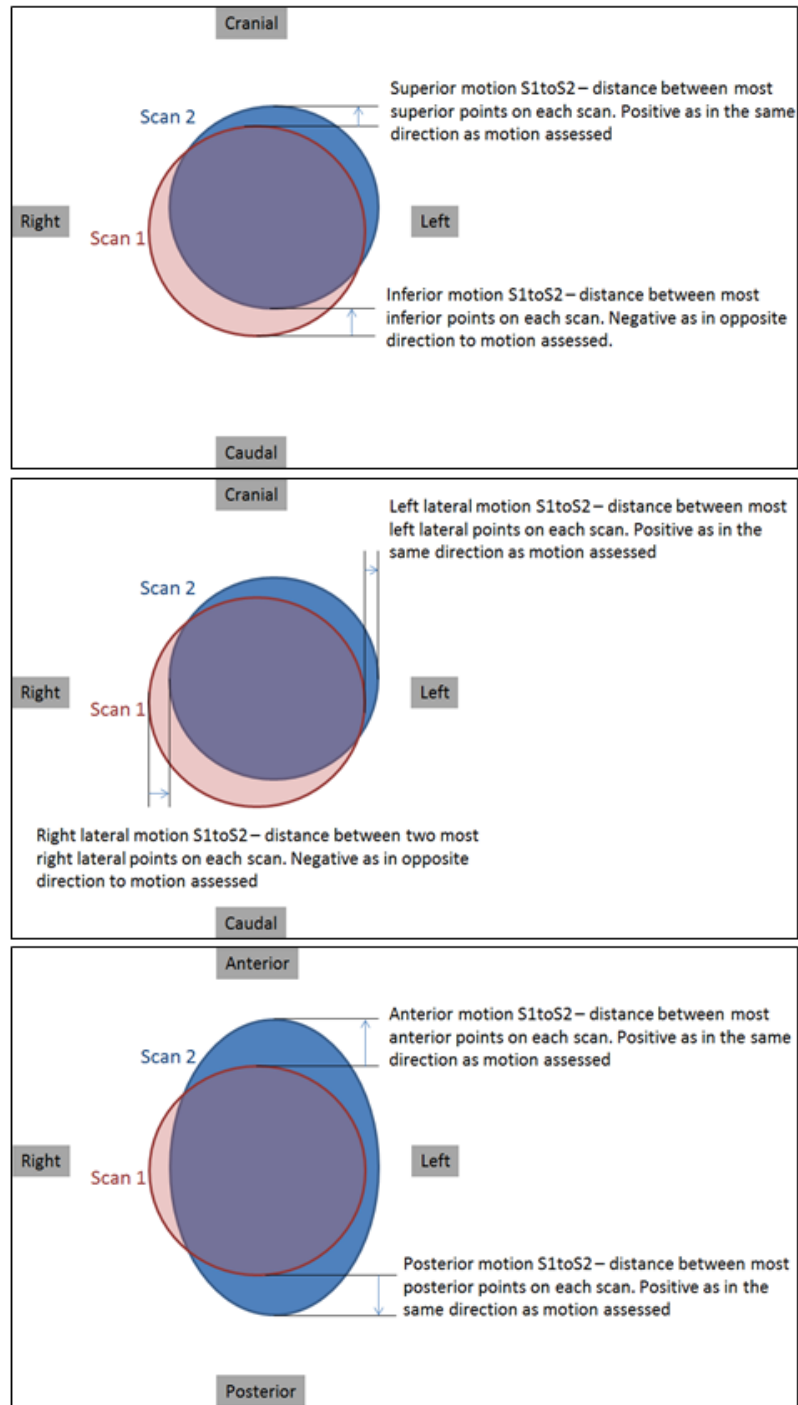
Figure 14 Schematic of definition of motion in six directions measured from Scan 1 to Scan 2 (S1toS2)

6.4   Results

Forty-two patients were included in this retrospective study – 19 female and 23 male patients. No female patients had prior hysterectomy and no male patients had prior prostatectomy.

6.4.1   Interobserver variability

RTP and CBCT scans from three patients were contoured by two independent observers. There was minimal difference between observers' contours in the nine scans assessed. The mean Dice coefficient was 98.1% (94.2-99.5%). A single observer contoured subsequent patients' scans.

6.4.2   Mean bladder motion in study population

The bladder was contoured on each patient's RTP scan, fraction 1 CBCT and week 4 CBCT. A total of 126 contours were assessed. Motion is seen in all six directions, with the biggest magnitude in the superior, anterior and posterior directions. The mean motion of all patients was measured as described above. This is summarised in table 11.

Direction	RTPtoWk1 Mean (SD) (cm)	RTPtoWk4 Mean (SD) (cm)	Wk1toWk4 Mean (SD) (cm)	AveMotion Mean (SD) (cm)
Superior	0.23 (0.84)	-0.29 (0.66)	-0.51 (1.10)	-0.19 (0.44)
Inferior	-0.02 (0.65)	0.03 (0.74)	0.05 (0.63)	0.02 (0.49)
Right lateral	0.19 (0.51)	-0.07 (0.49)	-0.26 (0.63)	-0.05 (0.33)
Left lateral	0.12 (0.50)	-0.17 (0.42)	-0.29 (0.66)	-0.11 (0.28)
Anterior	0.72 (0.96)	0.36 (0.72)	-0.37 (0.79)	0.24 (0.48)
Posterior	0.26 (0.90)	-0.10 (0.95)	-0.36 (1.20)	-0.06 (0.63)

Table 11 Mean and standard deviation of motion in all patients in six directions

Apart from motion in the inferior direction, mean bladder motion from RTP scan to start of treatment in all patients was in the direction measured, indicating an outward motion of bladder walls. However, bladder motion from RTP scan to week 4 of treatment and from week 1 to week 4 were in the opposite direction measured, indicating an inward motion of bladder walls. There is statistical difference in mean motion in the superior (0.23 vs -0.29cm, p=0.003), right lateral (0.19 vs -0.07cm, p=0.02), left lateral (0.12 vs -0.17cm, p=0.003) and anterior directions (0.72 vs 0.36cm, p=0.05) between RTP to start of treatment and RTP to week 4. There is a difference in posterior motion but this is not statistically significant (0.26 vs -0.10cm, p=0.08) (table 12). Accounting for multiple hypothesis testing using the Benjamini-Hochberg (BH) step-up procedure to limit false discovery rate to 10%, all four significant results remain positive discoveries.

Direction	RTPtoWk1 Mean (SD) (cm)	RTPtoWk4 Mean (SD) (cm)	p-value
Superior	0.23 (0.84)	-0.29 (0.66)	0.003*
Inferior	-0.02 (0.65)	0.03 (0.74)	0.74
Right lateral	0.19 (0.51)	-0.07 (0.49)	0.02*
Left lateral	0.12 (0.50)	-0.17 (0.42)	0.005*
Anterior	0.72 (0.96)	0.36 (0.72)	0.05*
Posterior	0.26 (0.90)	-0.10 (0.95)	0.08

Table 12 Comparison of mean motion of all patients from RTP to start of treatment and RTP to final week of treatment.

6.4.3 Comparison between female and male bladder motion

The difference in mean bladder motion in superior (0.14 vs -0.49cm, p=0.01), right lateral (0.33 vs -0.15, p=0.001), left lateral (0.19 vs -0.18, p=0.008) and posterior (0.52 vs -0.11cm, p=0.04) directions from RTP scan to start of treatment and RTP scan to week 4 is statistically significant in female pelvises. Accounting for multiple hypothesis testing using the BH step-up procedure to limit false discovery rate to 10%, results were no longer statistically significant. Results were not statistically significant in male pelvises (table 13).

Female	RTPto Wk1 (SD) (cm)	RTPto Wk4 (SD) (cm)	p-value	Male	RTPtoWk1 (SD) (cm)	RTPtoWk4 (SD) (cm)	p-value
Superior	0.14 (0.78)	-0.49 (0.73)	0.01*	**Superior**	0.30 (0.90)	-0.12 (0.56)	0.06
Inferior	0.13 (0.72)	0.00 (0.82)	0.62	**Inferior**	-0.14 (0.58)	0.05 (0.68)	0.30
Right lateral	0.33 (0.47)	-0.15 (0.37)	0.001*	**Right lateral**	0.07 (0.52)	-0.01 (0.57)	0.63
Left lateral	0.19 (0.30)	-0.18 (0.48)	0.008*	**Left lateral**	0.07 (0.62)	-0.16 (0.37)	0.15
Anterior	0.64 (0.78)	0.30 (0.83)	0.20	**Anterior**	0.79 (1.10)	0.41 (0.62)	0.16
Posterior	0.52 (0.86)	-0.11 (0.97)	0.04*	**Posterior**	0.05 (0.89)	-0.08 (0.95)	0.63

Table 13 Comparison of motion in six directions from RTP to start of treatment and RTP to last week of treatment in female and male bladders

	Female (SD) (cm) (n=19)	Male (SD) (cm) (n=21)	p-value		Female (SD) (cm) (n=19)	Male (SD) (cm) (n=21)	p-value
RTPtoWk1				**RTPtoWk4**			
Superior	0.14 (0.78)	0.30 (0.90)	0.55	**Superior**	-0.49 (0.73)	-0.12 (0.56)	0.08
Inferior	0.13 (0.72)	-0.14 (0.58)	0.20	**Inferior**	0.00 (0.82)	0.05 (0.68)	0.83
Right lateral	0.33 (0.47)	0.07 (0.52)	0.09	**Right lateral**	-0.15 (0.37)	-0.01 (0.57)	0.36
Left lateral	0.19 (0.30)	0.07 (0.62)	0.40	**Left lateral**	-0.18 (0.48)	-0.16 (0.37)	0.84
Anterior	0.64 (0.78)	0.79 (1.10)	0.61	**Anterior**	0.30 (0.83)	0.41 (0.62)	0.63
Posterior	0.52 (0.86)	0.05 (0.89)	0.09	**Posterior**	-0.11 (0.97)	-0.08 (0.95)	0.93
Wk1toWk4				**AveMotion**			
Superior	-0.63 (1.09)	-0.42 (1.05)	0.53	**Superior**	-0.33 (0.49)	-0.08 (0.37)	0.08
Inferior	-0.13 (0.80)	0.20 (0.42)	0.12	**Inferior**	0.00 (0.55)	0.03 (0.45)	0.83
Right lateral	-0.48 (0.52)	-0.08 (0.67)	0.04*	**Right lateral**	-0.10 (0.24)	-0.01 (0.38)	0.36
Left lateral	-0.37 (0.47)	-0.22 (0.79)	0.45	**Left lateral**	-0.12 (0.32)	-0.10 (0.24)	0.82
Anterior	-0.34 (0.81)	-0.38 (0.78)	0.88	**Anterior**	0.20 (0.55)	0.28 (0.42)	0.63
Posterior	-0.63 (1.21)	-0.13 (1.17)	0.18	**Posterior**	-0.07 (0.64)	-0.06 (0.63)	0.92

Table 14 Comparison of mean motion of female and male bladders between timepoints

There is statistically significant difference between female and male bladder motion in the right lateral direction from start of treatment to the final week (-0.48 vs -0.08, p=0.04) as shown in table 14. However, there is no difference from RTP scan to either start or final week, and also no statistically significant difference in average motion between the two sexes. On further examination of this result, this difference is accounted for by two outliers, one in each group, of movement in opposite directions.

With regards to superior motion, there is a difference between female and male bladder motion from RTP to final week and also in average motion, but this is not statistically significant (-0.49 vs -0.12cm, p=0.08). There is no difference from RTP to start of treatment or between start and final week of treatment.

Similarly, a difference in posterior motion is seen from RTP to start of treatment but this is not statistically significant (0.52 vs 0.05 cm, p=0.09). There is no difference in posterior motion between other timepoints and in average motion.

6.4.4   Relationship of motion between time points

There is moderate correlation between RTPtoWk1 and RTPtoWk4 motion in the inferior and anterior directions (r=0.59, p<0.01 in both directions). Correlation is very weak in other directions. (Figure 15)
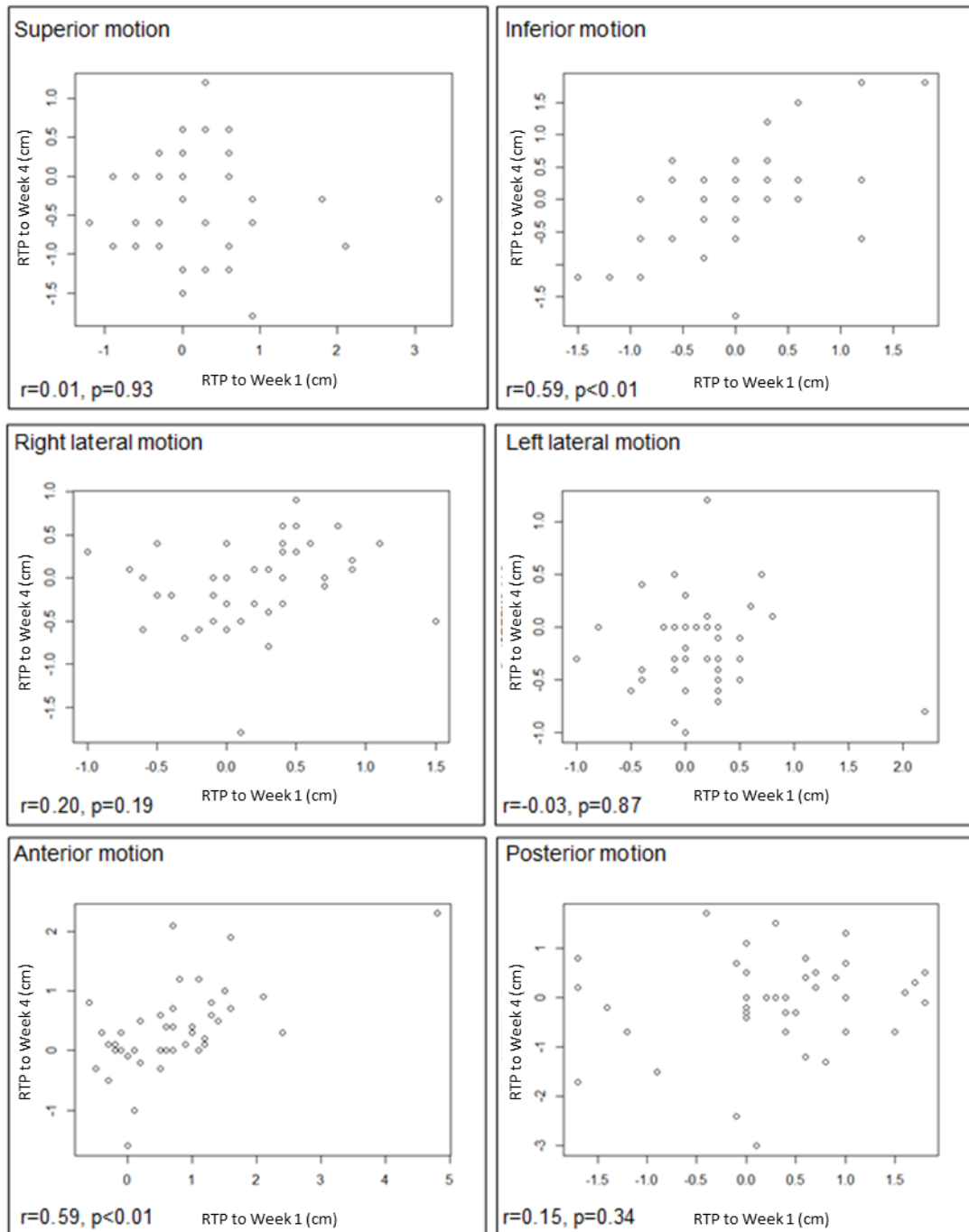
Figure 15 Scatter plots illustrating the correlation of motion of all patients in six direction between RTPtoWk1 (x-axis) and RTPtoWk4 (y-axis) with corresponding Pearson coefficients (r) and p-values.

In terms of differences between female and male pelvises, there is moderate correlation between RTPtoWk1 and RTPtoWk4 motion in inferior direction in females (r=0.47, p=0.04) and strong correlation in males (r=0.79, p<0.01). This is similar in the anterior direction; there is moderate correlation females (r=0.49, p=0.03) and strong correlation in males (r=0.72, p<0.01). Correlation is very weak in other directions. (Figure 16)

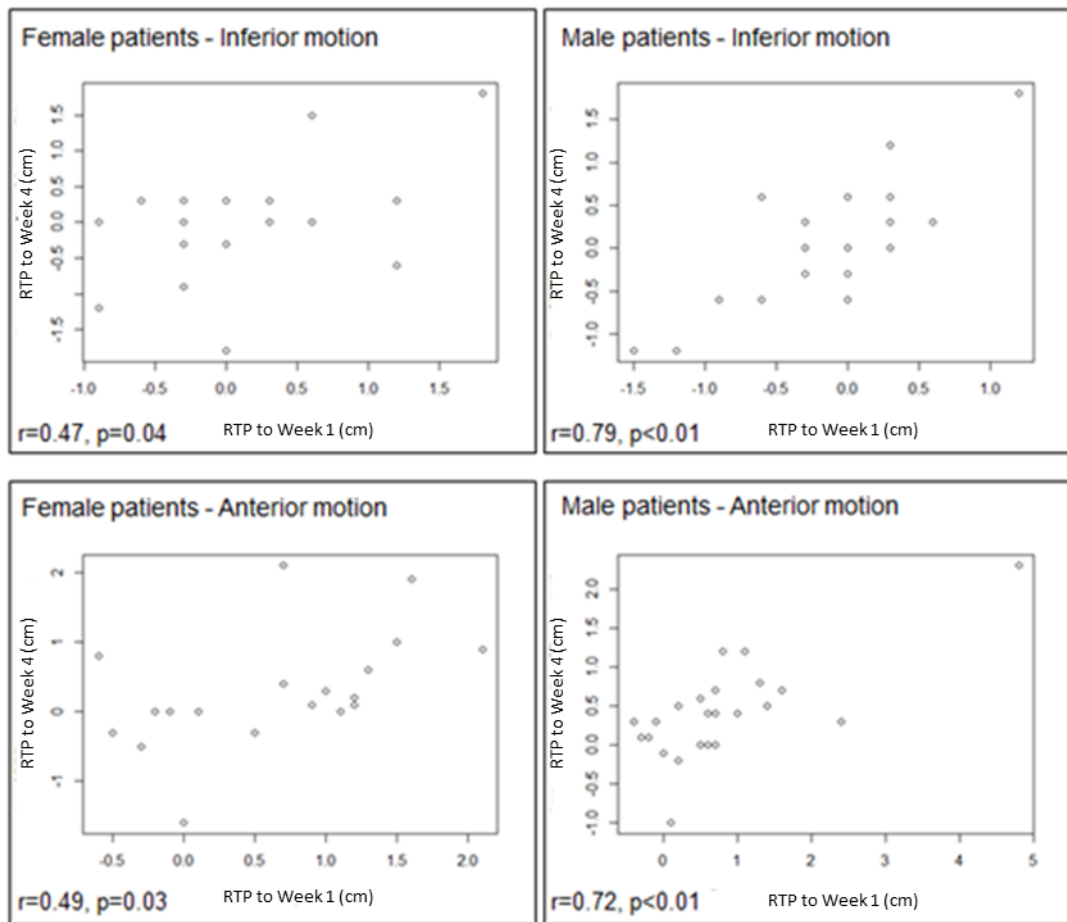Figure 16 Correlation of motion in female (left) and male (right) bladders in inferior and anterior directions between RTPtoWk1 (x-axis) and RTPtoWk4 (y-axis) with corresponding Pearson coefficient (r) and p-values

6.5   Discussion

Organ motion has an impact on accurate delivery of radiotherapy. The bladder is a highly deformable organ with potential to change in size and shape during a course of radiotherapy[184]. Relatively large margins have been applied to ensure adequate target coverage. Our study has shown that the bladder moves in all directions. There is little difference in mean bladder motion between male and female pelvises. Accounting for multiple hypothesis testing, there are no significant differences in the change in motion during a course of radiotherapy between the two sexes.

Various authors have studied inter-fraction bladder motion in radiotherapy. The maximum motion had been found to be in the superior and anterior directions[59], [60], [185], [186] and also to a lesser extent, in the posterior direction[61], [187]. When assessing motion between the three time intervals (RTPtoWk1, RTPtoWk4 and Wk1toWk4), our study found that the greatest magnitudes of motion are in the directions as described in published literature.

Despite the differences in pelvic anatomy and renal physiology, there had been no previous studies examining difference in bladder inter-fraction motion with males and females. We have shown that motion in male bladders remained consistent during radiotherapy, with no difference at the start and end of treatment. The pattern of movement in female bladders is different. Movement in all directions Wk1toWk4 are negative, indicating an inward motion of bladder wall. This could be due to better compliance to bladder emptying instructions or the effect of treatment related radiation cystitis causing a reduction in bladder capacity. This change is also seen in male bladders although the difference is not significant. While the magnitude of mean change in bladder motion is small, it could warrant a reduction of superior and posterior CTV to PTV margins in female patients, thereby reducing dose to small bowel and rectum with a potential impact on treatment related bowel toxicities.

There is a correlation between bladder movement early on in treatment and during the final week of treatment in the anterior and inferior directions, but no correlation in other directions. This is important in the modern era of adaptive radiotherapy. The composite method utilises information from CBCTs during the first week of treatment to formulate a

composite CTV and radiotherapy plan to be used for the rest of treatment. This assumes that the bladder behaves in the same way throughout the course of treatment. As described above, this may not be the case. The positive correlation of anterior and inferior motion at the beginning and end of treatment may allow a better formulation of treatment margins in these directions.

While we have shown the difference in change in motion between female and male bladders, there are limitations within our study and further research needs to take place to better understand the difference in female and male bladder motion such that more accurate margins can be applied and treatment improved.

This study only included CBCTs at the start and a week 4 as it was a retrospective study and there was limited imaging data available. A larger study including CBCTs during the second and third week of treatment may give us a better understanding of how the bladder moves during an entire course of radiotherapy. As patients treated at this centre only had CBCTs prior to treatment, we have only been able to examined differences in inter-fraction bladder motion. Intra-fraction bladder motion is influenced by bladder filling[188]. The difference in renal physiology between the two sexes may translate to differences in intra-fraction motion and should be studied. This would require CBCTs at start and end of treatment fractions or MRI scans spanning the time taken for treatment delivery.

The convention for bladder radiotherapy in the UK utilises an empty bladder protocol. However, this is changing. There is increasing interest in drinking protocols in order to fill bladders, separate bladder walls, thereby allowing delivery of high dose radiation boost to tumour beds. The use of drinking protocols may have an impact on bladder motion and affect male and female bladders differently. Therefore, similar studies should also be carried out in patient cohorts with planned bladder filling.

The rationale for our study stems from differences in female and male pelvic anatomy, the main distinctions being the presence and position of internal genitalia. All patients included in this study had intact internal genitalia. It is not certain how previous hysterectomy or prostatectomy would impact bladder motion.

Despite its limitations, to our knowledge, this is the first study to examine differences between female and male bladder motion. It is has shown that there are differences in bladder motion during a course of treatment. This is an initial step towards improved personalisation of treatment for patients. Further research taking into account different treatment time points, intra-fraction motion, planned bladder filling and previous surgery would be invaluable.

7 Assessment of intra-fraction bladder and tumour bed movement in a pilot MRI study

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7.1   Abstract

7.1.1   Introduction

Radiotherapy to the bladder most commonly involves treating the whole bladder and any extra-vesical extension of tumour with a uniform dose. There is increasing interest in adaptive radiotherapy and dose escalation to tumour beds. MRI guided linear accelerator allows improved visualisation of tumour bed and online adaptive treatment. However, this increases the intra-fraction time and it is vital to consider the intra-fraction movement of bladder and tumour bed. We hypothesize that as the bladder fills and expands during this period of time, there is a correlation between bladder wall and tumour bed expansion in the same direction.

7.1.2   Method

Patients with histologically confirmed diagnoses of muscle-invasive bladder cancer planned for curative chemoradiotherapy were included in this study. Patients underwent MRI scans weekly during treatment. Each MRI scan included three T2 sequences. Whole bladder and tumour bed were contoured each week by a single observer. Bladder and tumour bed volumes and inner surface area were calculated from the contours at each timepoint. The largest superior-inferior (SI), anterior-posterior (AP) and left-right (LR) extent of each contour were also calculated and their differences compared. Lastly the change in radial distance of tumour bed, which would reflect the tumour bed thickness, was compared with change in bladder volume. The mean difference in volume and

diameters across the timepoints were compared with one-way analysis of covariance (ANCOVA) using R v3.6.2.

7.1.3   Results

13 (12 males, 1 female) patients were recruited to the study from July 2018 to June 2019. The median age was 63 (34-79) and patients had T2-T3b disease at diagnosis. Three patients withdrew from the study due to medical reasons. The remaining patients underwent four MRI scans each. Change in bladder volume is correlated to the change in tumour bed volume (p=0.02) and tumour bed thickness (p<0.01). Change in bladder SI and AP distances and internal surface area correlated with change in tumour bed SI and AP distances and internal surface area (p<0.01). There is no correlation in change in bladder and tumour bed LR distances (p=0.66)

7.1.4   Conclusion

The bladder is a highly mobile organ. Understanding the correlation between tumour bed and bladder motion allows for formulation of an appropriate library of plans to account for intrafraction motion.

7.2   Introduction

Radiotherapy allows patients with muscle-invasive bladder cancer (MIBC) the chance of cure while keeping an intact bladder. The unpredictable manner in which the bladder volume varies is a challenge in bladder radiotherapy, and this is overcome by applying a large margin to prevent a geographical miss. The most commonly used technique in the UK is to define the whole bladder and any extra-vesical extension of tumour as the CTV, then apply a uniform expansion margin of 1.5 to 2cm around this to account for bladder motion and expansion. This technique was developed at a time when on treatment imaging modalities were poor and visualisation of the bladder was poor and even more difficult to accurately identify the tumour bed following maximum transurethral resection of bladder tumour (TURBT).

The basis of radiotherapy lies with delivering maximum radiation dose to a high-risk region and minimise dose to surrounding tissues. The current practice in bladder radiotherapy is therefore suboptimal. The relatively large margin translates to uninvolved organs being included in the high dose region when the bladder is small, and irradiating the bladder to a uniform dose does not take into account the position of the original tumour.

The urinary bladder is a muscular balloon that fills and empties, thereby changing shape and position constantly. While some anatomical structures can be immobilised during radiotherapy, the bladder cannot. Hence, the study of its motion during each fraction of treatment, as well as between each fraction of treatment is important. Studies have suggested that bladder filling during treatment result in three dimensional changes in bladder wall positions, with maximum movement in the anterior and superior walls[63], [184], [186].

After decades of stagnation, bladder radiotherapy is finally evolving. With the development of image guided radiotherapy (IGRT), it is now possible to formulate multiple radiotherapy plans and apply the best plan for the patient's anatomy on the day, thereby adapting the radiotherapy plan for each patient. While current ART methods may account for inter-fraction motion, it does not correct for intra-fraction motion. With plan selection in POD, there is increased time spent on the treatment couch and therefore

intra-fraction time. Foroudi et al reported 18% of patients with at least one post-treatment CBCT indicating that the bladder had filled and extended outside the PTV[189]. Murthy et al. showed that there was geographical miss on post-treatment CBCT of the superior bladder wall in 13.8% and anterior bladder wall in 10.3% of all patients treated[190]. It is therefore important that as we move into utilising ART and reduced margins, intra-fraction motion is studied and taken into account.

RAIDER (ISRCTN 26779187) is a phase II randomised trial investigating the effect of dose escalated tumour boost radiotherapy in bladder cancer. However, the visualisation of tumour bed on CT imaging remains poor. Shi et al demonstrated an ability to distinguish between tumour and normal bladder on T2 weighted MR images[99]. This allows accurate delineation of tumour beds and high-risk regions for disease recurrence, leading to the potential for dose escalation in these areas and dose reduction in the rest of the organ. Compared to normal bladder wall, the tumour bed may have had previous resection leading to healing and scarring. This may impact its elasticity and motion. In the era of differential dose to tumour bed, it is also important to consider the motion of this particular area of the bladder.

Lipiodol and fiducial markers allow the tracking of the tumour bed and studies have demonstrated that the tumour bed moves as the bladder fills over time[65], [71]. However, little is known about the correlation between tumour bed and bladder movement, and hence, whether a different expansion margin needs to be applied to the tumour bed and bladder when planning for a higher dose boost to the tumour bed. Furthermore, the use of lipiodol and fiducial markers in radiotherapy translates to an additional invasive procedure for patients.

With regards to online imaging modalities, the quality of CBCT images does not allow for adequate visualisation of tumour bed. However, with the advent of MRI guided linear accelerators, there is now an ability to better visualise tumour bed movement during treatment in a non-invasive manner. Each fraction of treatment on MRI guided linear accelerator (MRL) takes around 30minutes and the intra fraction motion of tumour bed and bladder is important.

An improvement in the understanding of bladder wall and tumour bed movement is key to delivering accurate radiotherapy, and is especially important in terms of adaptive radiotherapy and tumour bed boost in the future. Through the use of MRI scans at different timepoints during a course of radical radiotherapy, we evaluate bladder and tumour bed motion over a 30-minute period. We hypothesize that as the bladder fills and expands during this period of time, there is a correlation between bladder wall and tumour bed expansion in the same direction.

The study aims to recruit 20 patients and this report represents an interim analysis.

7.3   Methods

Patients with histologically confirmed diagnoses of muscle-invasive bladder cancer planned for curative chemoradiotherapy were included in this study. Exclusion criteria included any contraindications to magnetic resonance imaging (MRI), contraindications to hyoscine butylbromide and previous cystectomy.

Patients either had standard treatment with external beam radiotherapy at a dose of 52.5-55 Gy in 20 daily fractions to the whole bladder over four weeks or participated in the RAIDER study. RAIDER randomised patients to standard radiotherapy, adaptive tumour focused radiotherapy and adaptive tumour boost radiotherapy. Only patients randomised to one of the two adaptive arms were recruited to this imaging study. All patients had radiosensitisation in the form of gemcitabine weekly at 100mg/m^2 administered intravenously 2-4 hours prior to that day's radiotherapy treatment.

Patients underwent MRI scans weekly during treatment. Week 1 scan (MRI 1) was carried out on day 1 of treatment and subsequent scans (MRI 2, MRI 3 and MRI 4) on any day on the subsequent weeks. No contrast agents were used. Patients took oral hyoscine butylbromide 10mg prior to each MRI scan to minimise the impact of peristalsis on image quality. They were also instructed to fully empty their bladders and drink 350mls of water 30min before MRI scan.

Approval was obtained from the local ethics committee REC 18/NW/0352.

7.3.1   Scan sequence

MRI scans were carried out on 1.5 Tesla AERA Siemens scanner in the same position as for the radiotherapy planning (RTP) scans – supine position on flat table top, with knee support if applicable. Spine coil and anterior flexible coil on coil bridge were positioned close to but not touching the patient. Three 3D T2 sequences were carried out during each scan. Coronal and transverse DIXON sequences were performed in between each T2 sequence. There was a 30 minutes interval between first and third T2 sequence. 30 minutes was also the estimated time required to deliver a single fraction of adaptive radiotherapy on the MRI guided linear accelerator.

7.3.2   Contouring

Whole bladder and tumour bed were contoured on Raystation 7R on the first and third T2 sequence each week by a single observer who was experienced in bladder contouring. Tumour bed was contoured with the aid of information from cystoscopy reports and diagnostic CT urogram images and reports. Visible tumour in close proximity to the tumour bed was included within the tumour bed contours. In one patient, a separate tumour focus was seen and this was contoured separately.

7.3.3   Data Analysis

Bladder and tumour bed volumes and inner surface area were calculated from the contours at each timepoint. The largest superior-inferior, anterior-posterior and left-right extent of each contour were also calculated and their differences compared. Lastly the change in radial distance of tumour bed, which would reflect the tumour bed thickness, was compared with change in bladder volume. A visual inspection was carried out to ensure that the change in dimension calculated was similar to the change observed. The mean difference in volume and diameters across the timepoints were compared with one-way analysis of covariance (ANCOVA) using R v3.6.2.

7.4   Results

7.4.1   Patient Characteristics

13 (12 males, 1 female) patients were recruited to the study from July 2018 to June 2019. The median age was 63 (34-79) and patients had T2-T3b disease at diagnosis. One patient was found to have squamous cell cancer and underwent radical cystectomy, and

therefore withdrew from the study. Another withdrew from the study prior to any MRI scan due to deteriorating health, while another had chronic confusion and was not able to adhere to study instructions and withdrew following MRI 1. All patients included in the final analysis were male.

The remaining 10 patients underwent four MRI scans. One patient was not able to complete MRI 2 due to backpain and therefore did not have a third T2 sequence that week. Instead of the difference between timepoint 1 and 3, the difference between timepoint 1 and 2 was calculated for this patient on week 2.

Patient demographics and tumour positions (based on diagnostic imaging and cystoscopy reports) are listed in table 1.

Patient ID	Age	Sex	T Stage	Tumour Position
001	63	M	3a	Right postero-lateral
003	79	M	3b	Left postero-lateral
004	54	M	2	Right postero-lateral
005	60	M	2	Right lateral
007	72	M	3a	Left lateral
008	72	M	3b	Left antero-lateral
009	76	M	2	Posterior
010	61	M	3b	Left postero-lateral
011	34	M	2	Left postero-lateral
013	58	M	2	Superior (dome)

Table 15 Patient demographics and tumour positions

7.4.2   Change in volumes

Bladder volumes ranged from 70.1 to 399.2 cc and tumour bed volume 6.8 to 15.0 cc.

Figure 17 illustrates the difference variation in contoured bladder and tumour bed

volumes for each patient across time points each week.
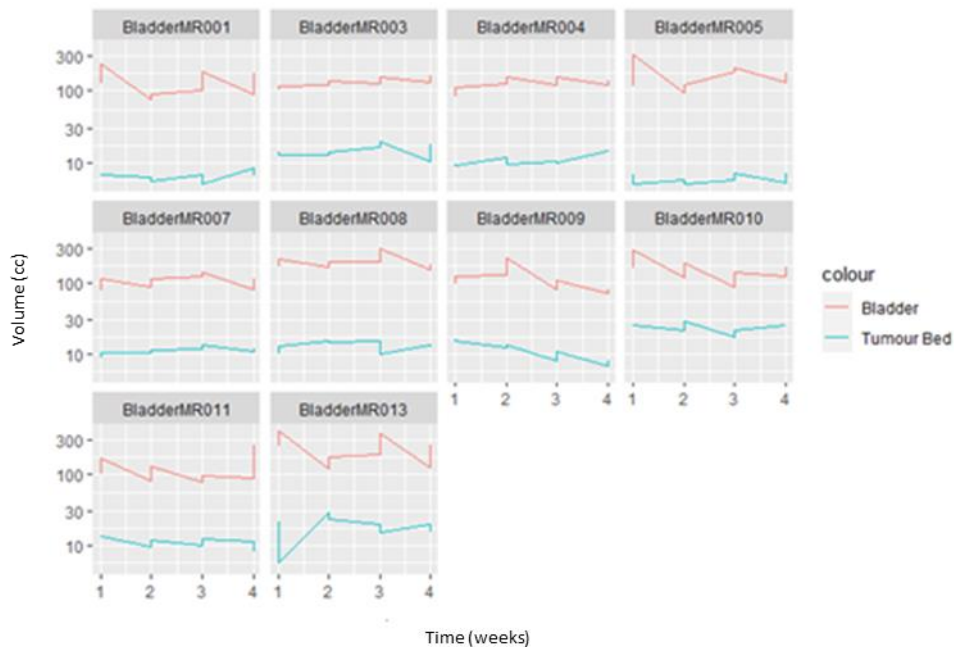
Figure 17 Change in contoured bladder (red) and tumour bed (blue) volumes (y-axis) each week
(x-axis) for each patient.

The bladder volume increases from timepoint 1 to timepoint 3 in all patients each week

but there is variability with tumour bed volume. Mean differences in bladder and tumour

volumes are shown in table 16. The change in bladder and tumour volume from timepoint

1 to timepoint 3 (30 minute interval) remains similar throughout a course of treatment. In

patients 7, 9, 10 and 11, the changes in bladder volume corresponded to similar changes

in tumour bed volumes. The opposite is observed in patients 1, 4, 8 and 13, while there is

a mixture from week to week in patients 3 and 5. With repeated measures ANCOVA, the

change in bladder volume is correlated to the change in tumour bed volume (p=0.02)

	Week 1	Week 2	Week 3	Week 4
Bladder Volume Change Mean (Range)	82 (16, 202)	47 (-8, 109)	57 (16, 178)	58 (10, 163)
Tumour Bed Volume Change Mean (Range)	-1 (-15, 3)	3 (-9, 16)	0 (-5, 5)	0 (-5, 7)

Table 16 Change in mean bladder and tumour bed volumes between both timepoints each week

7.4.3   Change in diameters

Figure 18 Change in maximum superior-inferior diameter (y-axis) of bladder (red) and tumour bed (blue) over time (x-axis) for each patient

There is a similar trend in change of superior-inferior (SI) diameters of bladder and tumour bed in most patients apart from patients 4 and 13. With repeated measures ANCOVA, the change in SI diameter of the bladder is correlated to that of tumour bed (p<0.01) (figure 18).

As patient 13 had a superior tumour, the SI distance measures a change in tumour thickness. Repeat analysis with exclusion of patient 13 continues to show a correlation of bladder and tumour bed SI distance (p<0.01)
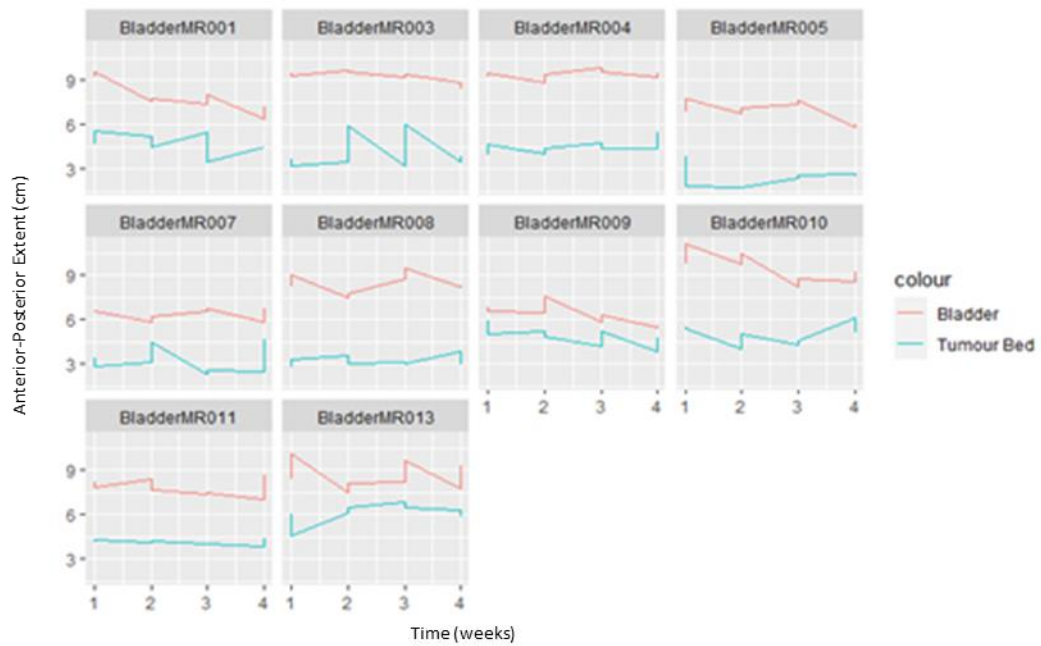
Figure 19 Change in maximum anterior-posterior diameter (y-axis) of bladder (red) and tumour bed (blue) over time (x-axis) for each patient

The changes in maximum anterior-posterior (AP) diameter of bladder and tumour bed shows a more varied pattern, with some similarities in patients 4, 9, 10, and 13. With repeated measures ANCOVA, the change in superior-inferior diameter of the bladder is correlated to that of tumour bed (p<0.01) (figure 19).

As patient 9 had a posterior tumour, the AP distance measures a change in tumour thickness. Repeat analysis with exclusion of patient 9 continues to show a correlation of bladder and tumour bed SI distance (p<0.01).
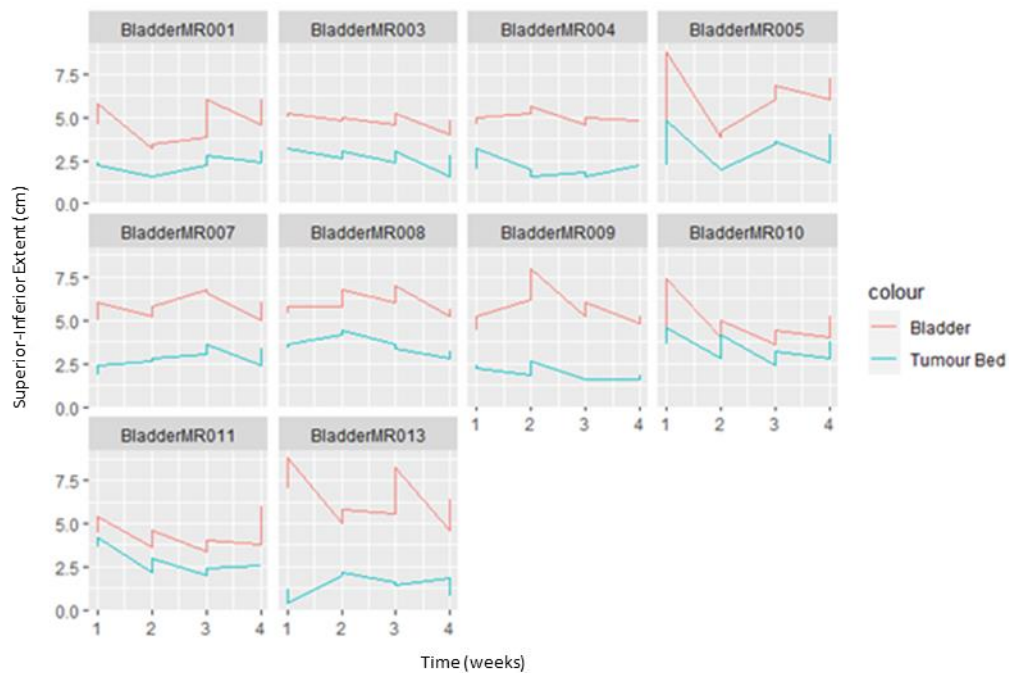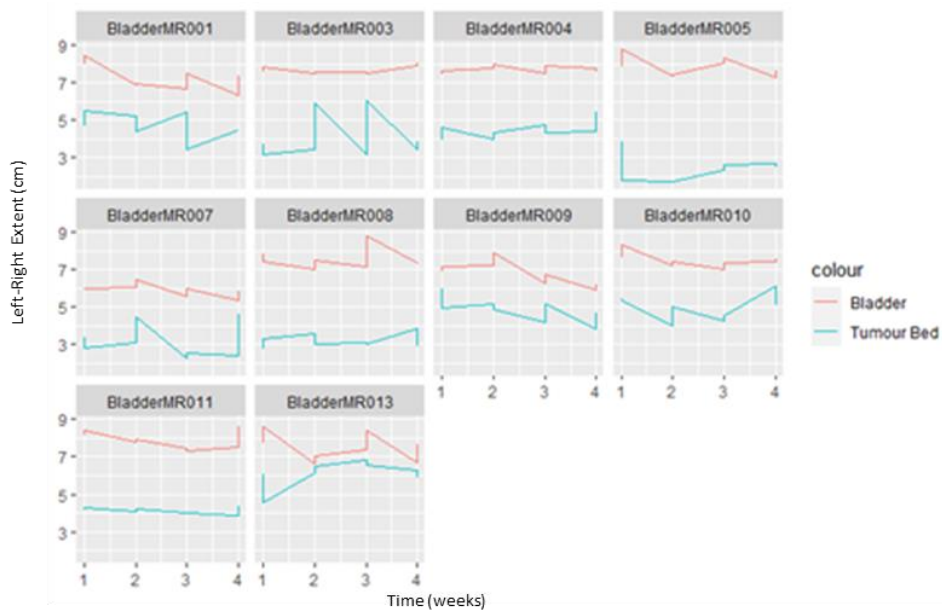
Figure 20 Change in maximum left-right diameter (y-axis) of bladder (red) and tumour bed (blue) over time (x-axis) for each patient

There is little similarity in the change in left-right diameter of bladder and tumour bed at each timepoint on observation. This lack of correlation is confirmed with a repeated measures ANCOVA (p=0.66) (figure 20).
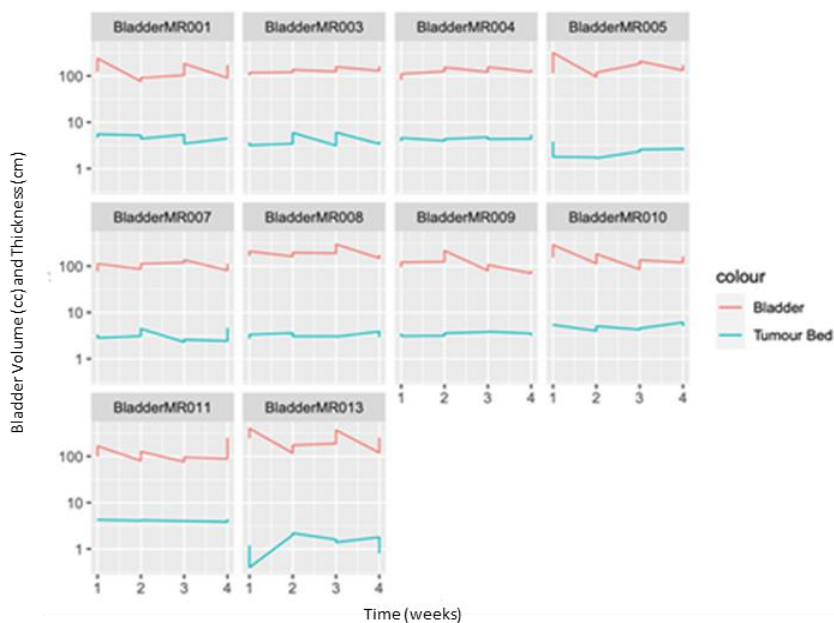
Figure 21 Change in bladder volume (red) and tumour bed thickness (blue) over time for each patient

The changes in bladder volume and tumour bed thickness shows a more varied pattern, with some similarities in patients 3, 4 and 10. With repeated measures ANCOVA, the change in bladder volume correlated to that of tumour bed thickness (p<0.01) (figure 20).

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7.4.4   Change in surface area
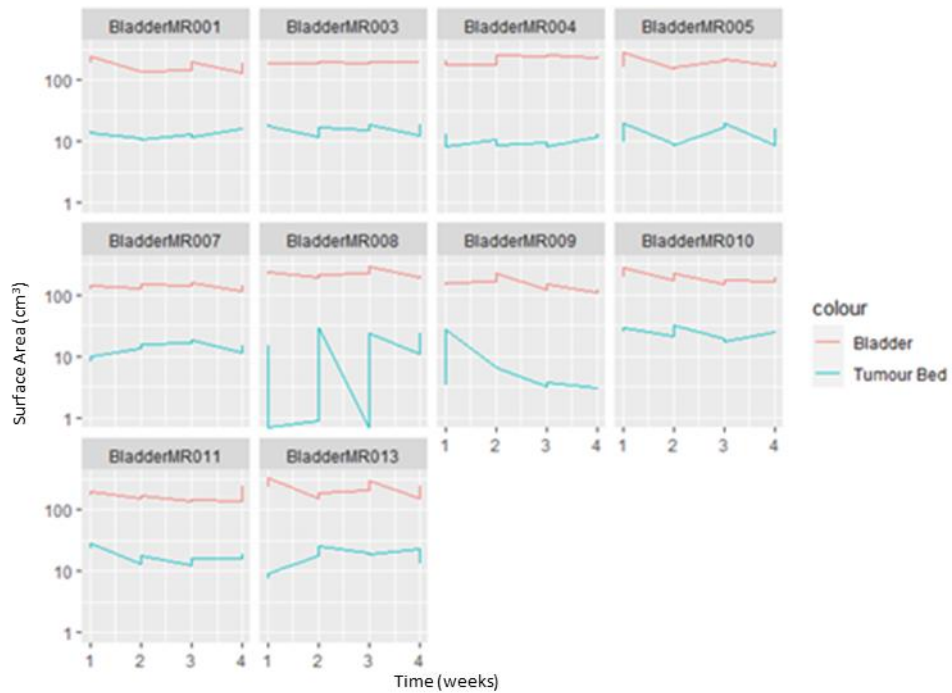
Figure 22 Change in internal surface area (y-axis) of bladder (red) and tumour bed (blue) over time (x-axis) for each patient.

There is a similar trend in change of internal surface area of bladder and tumour bed in most patients apart from patients 8, 9 and 13. With repeated measures ANCOVA, the change in internal surface area of the bladder is correlated to that of tumour bed (p<0.01).

7.5   Discussion

A challenge in delivering differential dose to tumour bed in bladder cancer is visualisation of the tumour bed. This was difficult on CBCT but MRL has allowed for improved on treatment image quality, better visualisation, and also online adaptation to changes in shape and size of targets. However, this requires online contouring of the target. For a highly mobile organ like the bladder, the target would have changed further while this takes place. This highlights the importance of intrafraction motion in bladder radiotherapy. An understanding of motion during an appropriate intra-fraction time period would contribute to the development of suitable expansion margins for both bladder and tumour bed.

This study uses T2 MRI sequences to compare bladder and tumour bed motion over a time period required to deliver a single fraction of treatment on the MRL. Our results show that changes in tumour bed volume and surface area are related to changes in bladder volume and surface area, and that changes in tumour bed AP and SI diameters are related to the same diameters in the bladder. Similarly, changes in tumour bed thickness are related to changes in bladder volume.

The lack of correlation between changes in left-right diameter is likely due to the position of tumour beds. Apart from patients 9 and 11, all other tumour beds were on the lateral aspects of the bladders. Changes in left-right diameter of the tumour beds reflect changes in tumour bed thickness. In comparison, changes in tumour bed AP and SI diameters reflect changes in surface area.

Bladder radiotherapy is conventionally carried out with an empty bladder due to its easier replicability and to minimise radiation dose to surrounding tissues. In order to allow differential dose to tumour bed and whole bladder, the bladder needs to be filled to move bladder walls apart. A drinking protocol was utilised in this study. Patients were instructed to fully empty their bladder and to drink 350ml of water 30 minutes before the start of each MRI scan. Despite adhering to the protocol, it should be noted that each patient's bladder volume differed from week to week. This highlights the challenge of predicting bladder filling and maintaining a constant bladder size during radiotherapy.

Our study is limited by the small sample size. While 40 MRI scans and 80 sequences were analysed, these images are from 10 individual patients. There is a limit with the variability of bladder filling, tumour location and tumour stage that may not be accounted for in this small group of patients. In particular, there were no patients with T4 cancers, where tumour bed motion may differ due to invasion of surrounding organs. All 10 patients included in the analysis were male. As bladder cancer is more common in men than women, recruiting female patients is a challenge. While it was described in chapter 6 that the mean inter-fraction motions of male and female bladders are similar, it is not known if the intra-fraction motions are also alike.

Due to logistical constraints, the MRI scans in this study were not performed on the MRL but on diagnostic 1.5 Tesla MRI scanners. We tried to simulate conditions on the MRL with flat table tops and knee supports, scanning in treatment position and also in the time period estimated to deliver a fraction of treatment. However, there may be differences in scanner specifications leading to variation in image quality compared to those obtained on an MRL. There may also be operator dependent differences as scans on diagnostic scanners are carried out by diagnostic radiographers who may be less used to ensuring patients are in treatment positions compared to their therapeutic counterparts.

Contouring is observer dependent. The bladders and tumour beds were contoured by a single observer with information from diagnostic CT urograms and cystoscopies. While the observer had experience in contouring bladder for radiotherapy planning, this would have been on CT images instead of MRI images. The difficulty in tumour bed delineation, errors in interpreting MRI images may have led to biases which were not compensated for. The use of multiple independent observers may result in different outcomes. In particular, it is noted in the surface area calculation for patient 8 that the tumour bed contours were not in contact with that of bladder contours at 3 timepoints and it was therefore not possible to calculate tumour bed surface areas. However, with regards to the other aspects of the analysis, the study correlates the extent of bladder and tumour bed, we therefore do not expect these errors to have a large effect on our results.

While we were able to demonstrate the correlation in magnitude of tumour bed and bladder motion, the direction of motion would enable formulation of appropriate margins

(isotropic or otherwise) in treatment planning to ensure adequate coverage of target volume without unnecessary irradiation of normal tissues. This could be achieved by measuring distance in six directions from a reference point at the different timepoints, and comparing the difference. This would reflect the distance moved in each direction.

Through this study, we have a better understanding of tumour bed and bladder motion. The correlation between the two suggest that a planning margin added to tumour bed CTV to account for intra-fraction motion should be a proportion of that used for bladder. The preferred on-treatment imaging modality to visualise tumour bed remains an MRI but in the absence of an MRL, our results suggest that bladder motion may predict tumour bed motion. However, this study requires expansion to include a larger sample size, inter-observer variability and also to take into account direction of motion.

8 Concluding remarks

The UK has been at the forefront of organ preservation treatment in bladder cancer, leading in two major practice changing trials. However, there has been little change in radical bladder cancer treatment in the past decade despite advances in systemic treatments and radiotherapy technique. This is an area of unmet clinical need.

Bladder preservation is currently delivered in a generic manner with little personalization for the individual patient. With improved patient selection and radiotherapy adaptation, organ preservation treatment in bladder cancer can be personalised through greater accuracy with image guided radiotherapy and biologically informed use of hypoxia modification.

8.1 Individualisation of radiosensitiser with biological factors

The BCON study was conducted in the early 2000s, and reported improvement in overall survival outcomes with the addition of carbogen and nicotinamide (CON) to radiotherapy. Within the long-term outcomes update, we evaluated the benefit of hypoxia modification with CON in the long term, and the potential role of molecular biomarkers in patient selection.

The improvement in overall survival benefit of hypoxia modification in bladder radiotherapy continues with 10-years median follow up. With the use of molecular biomarkers, it is demonstrated that the benefit of hypoxia modification is seen in the presence of necrosis and high hypoxia score. There is also a trend in benefit with basal molecular subtypes. This study shows that the use of biomarkers enables selection of the patients who would benefit most, thereby allowing biologically driven treatment decisions.

Moving forward, a prospective biomarker driven study will further qualify the use of hypoxia biomarkers in the clinical setting. Selecting the appropriate biomarker for use in the future study is important and therefore there is a need to consider the differences in biomarkers investigated. Necrosis is categorical and therefore perhaps the most straightforward biomarker to be used in the clinical setting, both in terms of clinician interpretation and communication to patients. However, the determination of necrosis

status is observer and sample dependent. The absence of necrosis and vice versa determined by a single observer in a small tumour sample may not accurately reflect the overall necrosis status. The study demonstrated a clear association between molecular subtype and hypoxia score. Hypoxia score is presented on a continuous scale and produced from transcriptome analysis. This score is determined in an objective manner, quantifies hypoxia and allows for the degree of benefit from hypoxia modification to be demonstrated. Therefore, when planning for a large phase III clinical trial, the more robust hypoxia score should be utilized.

The main treatment choice in curative treatment for bladder cancer is between RC and TMT. MRE11 expression was previously felt to have the potential to stratify patients to receive either surgery or bladder preservation but validation of the initial findings had been disappointing. It is therefore important that further work is undertaken to develop a scientific manner to guide patients.

CON is the only hypoxia modification regimen investigated in bladder radiotherapy in a phase III randomized trial setting. From a practical perspective, the administration of nicotinamide tablets coupled with inhalation of high concentration of oxygen is easily achieved in the radiotherapy treatment setting. Other techniques like hyperthermia involve heating tumour cells to a specified temperature during radiotherapy. Heat not only has an impact on the repair of radiation-induced DNA damage, but also induces more cell kill in radioresistant hypoxic cells and increases tumour blood flow and reoxygenation. However, hyperthermia treatment is invasive and would be a logistical challenge to deliver.

In the past two decades, other drugs have been developed. Cells exposed to acute hypoxia have impaired homologous recombination (HR) repair of DNA damage. PARP is an important enzyme in the repair process. Hence, HR events are synthetically lethal in the presence of PARP inhibitor (PARPi) resulting in increased clonogenic cell kill. Pre-clinical study in muscle-invasive bladder cancer cell lines showed that the addition of a PARPi to radiotherapy radiosensitised all evaluated cell lines to a dose enhancement ratio of 1.22-2.27[191]. In a clinical setting, there are ongoing studies regarding the radiosensitising role of PARPi different cancer subtypes.

8.2   Individualisation of radiotherapy with physical factors

Three studies were carried out to assess the physical factors involved in bladder radiotherapy – RTP parameters, inter-fraction motion and intra-fraction motion.

An important aspect of accurate radiotherapy is the RTP scan. As clinical trials adopt bladder filling protocols to enable differential dose to tumour beds, the impact of bladder size on clinical outcomes is an important consideration. By investigating the relationship of bladder and rectal dimensions on RTP scan and clinical outcomes, this study demonstrated that a large bladder on RTP scan was associated with worse OS. With multivariable analysis, the effect of large bladder is no longer statistically significant but known prognostic factors like presence of CIS and hydronephrosis remains predictive of outcome.

The results from this study should be considered with caution as it was conducted with an empty bladder protocol. A large bladder on RTP scan would be a reflection of inadequate bladder emptying, which could, in turn, be due to poorer bladder function or issues with treatment adherence. This differs from planned bladder filling. Hence, bladder size should not currently be taken into consideration in terms of patient selection for bladder preservation treatment. Within the original retrospective study, toxicity data was not captured. As RTP parameters impact on radiotherapy plans and hence the potential for increased dose to normal organs, toxicity is an important consideration. The current results suggest that a similar study with planned bladder filling should be carried out and that the impact of RTP parameters on toxicity should also be included.

The bladder changes in shape, size and position with internal and external pressure. Its motion is therefore an important consideration as we attempt to improve accuracy in radiotherapy and consider personalization of treatment based on physical factors.

Differences in male and female pelvic anatomy may impact bladder motion but this has not previously been investigated. This retrospective study found that the mean inter-fraction motion is similar in both sexes during a course of radiotherapy, but there is a difference in the change in motion between both sexes from the start to end of treatment. While male bladder motion remains consistent throughout treatment, there is an inward motion of the female bladder in all directions towards the end of treatment.

This study also demonstrated correlation between bladder motion in the beginning and end of treatment in the anterior and inferior directions and the lack of correlation in other directions.

Adaptive radiotherapy is important in the treatment of highly mobile targets like the bladder. Current adaptive radiotherapy techniques use information from RTP scans to generate a library of plans, or include information from the beginning of treatment to generate a composite plan. The study has allowed for better understanding of similarity and differences in bladder motion within male and female pelvises during a course of radiotherapy. The results from this study require further validation in a separate patient group, with inclusion of a greater variety of patients such as those with previous pelvic surgery. With validation of results, there could be further consideration when planning treatment such as considering additional "small" plans for female bladders towards the end of treatment, or the utilization of information on anterior and inferior motion from the beginning of treatment to generate planning margins for plans to be used later.

As bladder radiotherapy trials investigate the impact of differential dose to tumour bed by increasing radiation dose to the high-risk area and reducing dose to the unaffected bladder, it is vital that the tumour bed motion is considered in relation to bladder motion.

In a small study of ten patients, the intra-motion of tumour beds and bladder observed on 40 MRI scans during the time required to deliver a single fraction of treatment on an MRI-guided linear accelerator is analysed. Results from this study showed that there is an association between tumour bed and bladder motion in the change in AP and SI dimensions, the change in surface area, and also an association between tumour bed thickness and bladder volume.

The study is limited by the small sample size and the lack of consideration for interobserver variability. As the study is expanded to include a greater number of patients from both sexes, the results will help guide the formulation of CTV to PTV margins for a library of plans and true online adaptation of treatment. The information from these MRI scans can also be analysed for changes in imaging features during a course of treatment.

Tumour beds are difficult to visualize on CT imaging. The study demonstrated that the potential for tumour bed to be adequately visualized on T2 MRI sequences and including further observers will help to validate this. Differential dose to tumour bed is currently being investigated as part of the randomized control trial, RAIDER. If the results are promising and this moves into standard of care, MRI-based planning for bladder cancer should be considered in order for accurate delineation. With standard linac-based treatment, the tumour bed cannot be accurately seen on CBCT but with MRI guided linear accelerators, online images can visualize the tumour bed and be used to guide treatment.

The ability to identify tumour bed on MRI scans coupled with the availability of MRI guided linear accelerators hold much potential. For example, imaging features in contoured tumour beds can now be analysed in relation to biological features from the tumours removed from the same area, allowing a correlation between imaging and molecular biomarkers to be investigated. The change in imaging features during a course of treatment can also be studied to allow for the possibility of functional adaptation in addition to physical adaptation of treatment.

While the three studies investigated different physical aspects of bladder radiotherapy, the main observation is that the bladder varies in size, moves during a fraction of treatment and between fractions as well. This shows that the current standard of care where a single treatment plan is generated with a large CTV to PTV margin is not ideal. Adaptive radiotherapy with a library of plans that takes into consideration changes in bladder and tumour bed motion during each fraction and throughout each treatment course will allow for more accurate treatment to be delivered, with a lower incidental dose to surrounding organs.

8.3   Future directions

As most cancer subsites move from curative surgery with significant impact on quality of life to organ preservation treatments that maintain quality of life without compromising on the chances of cure, the urological oncology community has a duty of care to bladder patients to move further in this regard as well. This can be achieved through improvement in patient selection and personalization of management plans.

While the previous attempt at a randomized trial between RC and TMT failed due to poor accrual, there remain opportunities to evaluate biomarkers that could predict benefit from either treatments and from selection of radiosensitisers. Propensity matched analysis carried out had suggested equivalence in outcomes. There is current work investigating the relationship of hypoxia score and clinical outcomes in a bladder preservation cohort with chemotherapy radiosensitisation. Carrying out a similar study in surgical cohorts would be invaluable. A propensity matched analysis of radical cystectomy, hypoxia modification and chemotherapy radiosensitisation cohorts with the inclusion of hypoxia score as a factor should be carried out next. This would help inform clinicians in patient selection for these vastly different treatment modalities.

Advances in computer technology now allows for identification of imaging biomarkers. There is now an opportunity to relate imaging and molecular features, which could translate to less invasive methods of gaining more information about each cancer and planning treatment. A prospective study with biomarker stratification between chemotherapy and hypoxia modification radiosensitisation, with online adaptation on an MRI guided linear accelerator will allow for the qualification of available biomarkers.

Functional imaging information can be obtained and analyzed in relation to molecular biomarkers allowing the development of imaging biomarkers during the study and any change in imaging features during a course of treatment can also be identified. The variability in scanners across centres resulted in a challenge in reproducing and validating imaging features but a large proportion of the MRI guided linear community form a consortium, using the same scanner and linear accelerator. This allows for the potential to reduce variability in images obtained and greater opportunity for collaboration.

The development of imaging biomarkers will in turn form the basis of a further imaging and biomarker driven study, with the potential for early changes in treatment plans or treatment intensification based on on-treatment functional imaging.

The future of radiotherapy in bladder cancer is one where there is both anatomical and biological adaptation of treatment. The use of molecular biomarkers to select patients and plan treatment, coupled with the adaptation of treatment based on changes during a

course of radiotherapy brings along the exciting potential of improving outcomes in bladder cancer through true personalization of management plans.

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Study Protocol

A pilot study using Magnetic Resonance Imaging (MRI) to assess bladder motion during radiotherapy treatment

Version 2.0
4th July 2018

Version 2.0
4th July 2018
IRAS ID: 240130

SIGNATURES/PROTOCOL APPROVAL

A pilot study using MRI to assess bladder motion during radiotherapy treatment

This document describes a pilot study using MRI to assess bladder motion during radiotherapy treatment and provides information about procedures for entering patients into it. This protocol should not be used as a guide for the treatment of patients outside the study. Every care was taken in drafting this protocol; however corrections and/or amendments may be necessary. Care must be taken to use the most up to date and approved version. This study will adhere to the principles outlined in the ICH Good Clinical Practice guidelines. The study will be conducted in compliance with the protocol, the Data Protection Act (DPA Z6364106), The Declaration of Helsinki, Human Tissue Act (2004), the Research Governance Framework (2005) and other regulatory requirements as appropriate.

Chief Investigator
Dr Yee Pei Song, Research Fellow, University of Manchester

Signed: ………………………………… Date: …………………………………

I ………………………………… as Chief Investigator for 'A pilot study using MRI to assess bladder motion during radiotherapy treatment' to be conducted at The Christie NHS Foundation Trust confirm that I will be responsible to ensure that all members of the local study team are appropriately trained on the study protocol and have the relevant qualifications and experience to carry out their role in accordance with the study protocol.

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List of abbreviations

ADC	Apparent Diffusion Co-efficient
AE	Adverse Events
CBCT	Cone Beam Computed Tomography
CO_2	Carbon Dioxide
CI	Chief Investigator
CRF	Case Report Form
CTCAE v3.0	Common Terminology Criteria for Averse Events Version 3.0
CT	Computed Tomography
CTV	Clinical Target Volume
DPA	Data Protection Act
DVH	Dose Volume Histogram
DWI	Diffusion Weighted Imaging
FFPE	Formalin-fixed paraffin-embedded
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
GP	General Practitioner
H&E	haematoxylin and eosin
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ID	Identification
ITV	Internal Target Volume
L/min	Litres per minute
MDT	Multi-Disciplinary Team
mg/m^2	Milligram per metre squared
mg/kg	Milligram per kilogram
ml	Millilitre
mm	Millimetre
MIBC	Muscle-invasive bladder cancer
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
MRL	MR Linac
NHS	National Health Service
O_2	Oxygen
OARs	Organs at Risk
PIS	Participant / Patient Information Sheet
POTD	Plan of the Day
POTD+	Plan of the Day Plus
PTV	Planning Target Volume
R&D	Research & Development
REC	Research Ethics Committee
RRR	Radiotherapy Related Research
RT	Radiotherapy
RTP	Radiotherapy Planning
SAE	Serious Adverse Event
T	Tesla
T2	Transverse Relaxation Time
TMT	Trimodality treatment

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TNM	Tumour Nodes Metastasis
TURBT	Transurethral Resection of Bladder Tumour
UK	United Kingdom
XVI	X-Ray Volume Imaging

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Study Summary

Title	A pilot study using MRI to assess bladder motion during radiotherapy treatment
Brief introduction	Patiens with muscle invasive bladder cancer (MIBC) have the option of surgical or non-surgical treatments with similar chances of long-term cure. The standard non-surgical treatment for muscle invasive bladder cancer (MIBC) is concurrent chemo-radiotherapy. This treatment is associated with long term side effects in around a third of patients with up to 12% suffering from grade 3-4 toxicity. Effective radiotherapy depends on delivering a curative dose to the target whilst minimising dose to surrounding tissues to reduce toxicities. As an organ that constantly varies in shape and position, achieving this in bladder irradiation is challenging. Cone beam Computed Tomography (CBCT) has allowed visualisation of soft tissue on treatment and hence image-guided treatment and improved accuracy, but the image quality of CBCT is suboptimal for distinguishing soft tissue boundaries. On the other hand, MRI scans produce superior soft tissue definition and visualisation of tumour bed. This would in turn allow for various ways of optimising treatment and potentially improving outcome. There have been a number of studies evaluating pelvic organ motion in bladder cancer as well as assessing different adaptive radiotherapy strategies. These have included individualized margins, plan of the day and adaptive techniques. Most of these studies have been carried out using CBCT imaging which is often poor quality with limited soft tissue contrast. MRI offers better visualization of the tumour bed and organs at risk (OARs). As a result, the utilisation of MRI in radiotherapy could allow for increased radiation dose to the tumour bed while maintaining minimal dose to surrounding soft tissue. As stated previously, there are two main options for curative treatment of MIBC. With no scientific method of biological stratification, the choice of treatment is dependent on clinician and patient preference. There is a clinical unmet need to find ways of personalising treatment for patients with bladder cancer. Despite improvements in trimodality treatment (TMT) outcomes with the addition of radio-sensitising agents, the outcomes of bladder cancer patients remain poor. Advancements in radiotherapy techniques have allowed more accurate delivery of treatment and the possibility of dose intensification of high-risk regions. Appropriate identification of patients for dose intensification is vital. Biological sampling allows for identification of biomarkers that predict treatment outcomes. Radiomics is the identification of quantitative features from medical images and allows identification of imaging biomarkers. This study will explore the role of MRI imaging in adaptive radiotherapy for bladder cancer with development of a number of theoretical treatment strategies. In addition, the study also proposes to relate biological and radiological features in order to move towards personalising treatment.

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Design	Single site, non-randomised basic science study
Objectives	- To develop and test a MRI protocol suitable for soft tissue delineation, volumetric imaging over the duration of plan adaptation/delivery and diffusion weighted imaging (DWI). - To develop patient specific models of bladder filling; investigate intra-patient consistency and the feasibility of predicting the bladder and tumour bed position at the time of treatment. - To investigate different adaptive strategies; feasibility, scope for tumour bed boost, margin reduction and normal tissue toxicities. - To measure early changes in DWI imaging during radiotherapy. - Perform a feasibility study to compare features found on MRI scans with data generated from genomic analysis.
Eligibility	Inclusion Criteria - Histologically confirmed diagnosis of muscle invasive bladder cancer - Treatment with primary curative intent - Undergoing external beam radiotherapy (+/-chemotherapy) - Age over 18 Exclusion Criteria - Any contraindications to MRI identified after MRI safety screening including completion of an MRI Safety Screening Form - Any contraindications to Hyoscine Butylbromide (Buscopan) - Previous cystectomy - Unable to tolerate MRI scans - Metastatic disease - Pregnancy
Study Methods	Patients will undergo standard treatment during the study with four weeks of external beam radiotherapy (20 fractions). They will be treated with weekly Gemcitabine (100mg/m^2) if clinically appropriate. As part of standard treatment patients will have a staging CT scan at diagnosis and a radiotherapy planning CT. Cone beam imaging will also be performed. Response will be assessed as standard with cystoscopy at 3 months and CT scan at 12 months. As part of the study patients will undergo 4 additional MRI scans. These will take place in the 1st, 2nd, 3rd and 4th weeks of treatment. These MRI scans will include anatomical images – with full and empty bladder as well as cine data (with a scan every minute for 10 minutes) to assess intra fraction motion. They will also include a DWI sequence, which will be used to assess if early prediction of response is possible. The frequency of cone beam imaging will be increased from 6 scans (minimum) as standard of care to 20 scans to allow for daily imaging. Further cone beam CT (CBCT) scans may be required if there are issues with target and/or normal organs coverage detected on CBCTs taken during treatment to ensure accurate delivery of treatment. The 1st MRI scan will be contoured to outline the tumour bed, clinical target volumes (CTVs) and OARs. A variety of planning strategies will be developed including standard planning target volume (PTV) margins, a plan of the day (POTD) approach, a POTD+ as well as an online adaptation model. These models will be

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	used to assess coverage of CTV and PTV, and the feasibility of increasing radiation dose to the tumour bed, as well as dose to OARs using the scans obtained during the radiotherapy treatment. The practicality of each approach will also be assessed. Inter and intra fraction organ and tumour bed motion will also be analysed in order to develop patient specific models. In an optional aspect of this study, patients will be consented for collection of archived tumour samples obtained at time of diagnosis. A section will be taken for haematoxylin and eosin (H&E) staining to confirm the presence of tumour and determine the percentage of tumour material. The biology of the tumour sample will be analysed by whole transcriptome sequencing using next generation RNA sequencing technology. From this gene expression signatures known to correlate with aspects of tumour biology (in particular; hypoxia, proliferation and DNA-damage response) will be evaluated. Radiomics features will be identified from the weekly MRI scans patient have as part of the main study. Patients' response to treatment will be correlated to the genomic and radiomic features identified.
Study Duration	18 months
Sample Size	20 patients each undergoing 4 MRI scans & 20 CBCTs 10 of the 20 patients for will also be consented for optional aspect of the study
Funding	Funding for additional scans and imaging will be from departmental funds. Funding for tumour block collection and RNA sequencing from Action for Bladder Cancer grant.

Introduction

Background

Over 10,000 patients are diagnosed with bladder cancer in the UK every year. For patients who present with localised muscle invasive disease (T2-T4aN0M0) the standard treatment is either radical cystectomy (RC) or tri-modality bladder preservation treatment with maximal trans-urethral resection of bladder tumour (TURBT) followed by concurrent chemotherapy and radiotherapy to the whole bladder. Appropriate patients will also undergo neo-adjuvant chemotherapy prior to concurrent chemotherapy and radiotherapy. A meta-analysis has shown that the complete response rate of tri-modality treatment (TMT) is 78%, with 5 year overall survival of 56%[1]. However, this treatment is associated with long term side effects in around a third of patients with up to 12% suffering from grade 3-4 toxicity.

Effective radiotherapy is dependent on delivering a curative dose to the target, encompassing the macroscopic and potential microscopic tumour whilst minimising dose to surrounding tissues to reduce toxicities. The bladder fills and empties on a regular basis, resulting in constant variation in shape and position. This makes accurate delivery of treatment challenging. Cone beam computed tomography (CBCT) has allowed visualisation of soft tissue on treatment and hence image guided treatment and improved accuracy, but the image quality of CBCT is suboptimal for distinguishing soft tissue boundaries. On the other hand, magnetic resonance imaging (MRI) produces superior soft tissue definition which would in turn allow for various ways of optimising treatment and potentially improve outcome.

Furthermore, the basis of radiotherapy lies with delivering the maximum radiation dose to a high risk region while minimising that to surrounding tissues, but the practice of treating the whole bladder to the same dose is incongruous. The ability to better visualize soft tissues and anticipate movement would allow for higher dose boosts to be delivered to tumour beds and reduction in expansion margins. Shi et al demonstrated an ability to distinguish between tumour and normal bladder on T2 weighted MR images[2]. This will enable accurate delineation of tumour beds and high risk regions for disease recurrence, leading to the potential for dose escalation in these areas and dose reduction in the rest of the organ.

Conventionally, the radiotherapy plan and CBCT are matched based on bony anatomy and a margin of >10mm is added to the target to account for bladder expansion and movement. This method results in treating larger volumes of normal tissue when the bladder is small and there is a risk of suboptimal coverage of the bladder when the bladder is large. It also assumes uniform expansion of the bladder which is not the usual case in vivo. A "plan-of-the-day" adaptive radiotherapy strategy has been investigated in various studies. A series of 3 target volumes (small, medium, large) are designed based on the patient's initial radiotherapy planning scan. The treatment team selects the most appropriate plan for the patient each day based on the day's CBCT image. This method does not account for intra-fraction changes and movement of pelvic organs which can be better seen on MRI scans.

Apart from structural information, MRI also gathers physiological or functional information. For example, diffusion-weighted MRI images reflect cell density and tumour load while dynamic contrast-enhanced images reflect the oxygen permeability and radiosensitivity. Functional imaging in other solid organs has allowed exploration of dose modulated techniques, adjusting dose to match

variation in radiosensitivity. This could be developed in the muscle-invasive bladder cancer setting to allow for further improvement in radiotherapy planning.

The Christie NHS Foundation Trust is one of seven sites worldwide within the Atlantic consortium that is developing the MR-linac (MRL) prior to clinical release in mid-2018. The MRL allows MR images of patients to be acquired before, during and following radiotherapy (RT). MRL could result in a further improvement in adaptive radiotherapy techniques with the development of an accurate and reliable algorithm for organ deformation, allowing for true online adaptation. These approaches would result in a greater dose of radiation to high risk regions while limiting dose to adjacent organs, improving clinical outcomes through better disease control and reduction in long term toxicities.

As stated previously, the two options of curative treatment for MIBC are TMT or RC, with similar long-term outcomes. NICE recommends that both options be offered to patients, acknowledging that TMT offers better quality of life. With no scientific method of biological stratification, the choice of treatment is dependent on clinician and patient preference. There is a clinical unmet need to find ways of personalising treatment for patients with bladder cancer.

Despite improvements in TMT outcomes with the addition of radio-sensitising agents, the outcomes of bladder cancer patients remain poor. Advancements in radiotherapy techniques have allowed more accurate delivery of treatment and the possibility of dose intensification of high-risk regions. Increasing radiation dose can result in greater treatment-related toxicities. Appropriate identification of patients for dose intensification is vital.

Biological sampling allows for identification of biomarkers that predict treatment outcomes. A number of biomarkers have been studied, but none have reached the clinic[3].

Radiomics is an area of increasing interest. This is the identification of quantitative features from medical images. Diffusion-weighted magnetic imaging reflects cell density and can be used to direct radiotherapy dose to different parts of the tumour[4] and monitor response to treatment during[5].

This study will explore the role of MRI in adaptive radiotherapy for bladder cancer with development of a number of theoretical treatment strategies. In addition, the study also proposes to relate biological and radiological features in order to move towards personalising treatment.

Rationale for the proposed study

There is some evidence from dosimetric studies that adaptive radiotherapy techniques can be used to reduce margins and reduce doses to organs at risk. Most studies have been carried out using CT (some using cone beam imaging.) The development of a MR-linac offers the opportunity to utilise MRI in a radiotherapy setting, prior to and during treatment, with benefits including identification of tumour bed, better soft tissue delineation and no additional radiation dose for imaging. Furthermore, with the ability to identify quantitative features from MRI scans, it is an opportunity to relate biological and radiological features in order to enable patients to benefit from more personalised treatment in future.

Preliminary Work

Previous work has been carried out to create a bladder MRI protocol with suitable image quality in healthy volunteers. This protocol includes anatomical images to allow radiotherapy planning and to assess inter fraction motion as well as a cine sequence to assess intra fraction motion.

Study Hypotheses

- MRI guided adaptive strategies for external beam radiotherapy on the MR-linac will facilitate tumour bed boost, margin reduction and reduced patient toxicity.

- Patient specific bladder filling can be modelled, predicting the position of the tumour bed at the point of treatment. This will allow reduction of margins, and for these to be personalised for individual patient and for each fraction of treatment.

- Diffusion weighted MRI can be used to identify response early in treatment to allow for individualised plan adaption.

Biological and radiological features can be used to move towards personalising treatment.Study objectives

Primary Objective

To develop a potential MRI guided adaptive radiotherapy strategy which is practical and leads to adequate coverage of CTV, tumour bed boost and reduced dose to OARs.

Secondary Objectives

To develop a patient specific model of bladder filling, tumour bed movement and pelvic organ motion to allow individualised margins and planning strategies.

To determine if DWI MRI can be used to predict early response to treatment.

Tertiary Objective

Perform a feasibility study to compare features found on MRI scans with data generated from genomic analysis.

Study Design

This is a Basic Science Study which does not include an investigational medicinal product or other treatment. The aim of the study is to develop a practical MRI adaptive radiotherapy strategy. Patients will undergo additional MRI scans (in radiotherapy planning position with a flat top scanner and bridging coil, on a diagnostic MRI scanner). These scans will not be used for medical decision making.

The first study MRI scan will be used as a planning scan (with full and empty bladder) and will be contoured to develop tumour bed volume, CTV and OARs. These will be used to develop a number of planning strategies including a standard clinical plan with fixed PTV, individualised Internal Target Volume (ITV) based plans and also a variety of plan of the day strategies (including a plan of the day

plus where information from further MRIs will be used to develop further plans if required). These plans will be assessed using the MRI scans obtained during treatment. Coverage of the tumour bed and CTV as well as the doses to OARs will be calculated for each adaptive strategy. The use of daily cone beam CT scans will allow for assessment of tumour bed and organ motion each day and ensure that the images from the MRI scans at 4 time points are representative of actual organ motion.

A patient specific model of tumour bed movement, bladder filling and bladder position will be created using volumetric imaging over a number of fractions and used to develop a personalised margin recipe. An attempt will be made to stratify patients into groups with a large range of bladder and tumour bed movement and those where the bladder and tumour bed shows little positional changes.

The final part of the study will use DWI gradients to assess if it is possible to predict early response to treatment.

The study begins with the consent of the first participant and will be complete when the final participant has had their post-treatment cystoscopy (3 months following completion of treatment) and all data processing has been completed.

There is also an optional translational aspect of the study that patient can consent to. This is a feasibility study to compare features found on MRI scan with data generated from genomic analysis of tumour samples in order to move towards personalising treatment for MIBC.

Selection of Study Participants

Inclusion Criteria
- Histologically confirmed diagnosis of bladder cancer
- Treatment with primary curative intent
- Undergoing external beam radiotherapy (+/-chemotherapy)
- Age over 18 years

Exclusion Criteria
- Any contraindications to MRI identified after MRI safety screening including completion of an MRI Safety Screening Form
- Any contraindications to both Hyoscine Butylbromide (Buscopan)
- Previous cystectomy
- Unable to tolerate MRI scans
- Metastatic disease
- Pregnancy

Concomitant Medications

It is common clinical practice for patients to have Hyoscine Butylbromide (Buscopan) prior to MRI scans of the pelvis in order to reduce intestinal peristalsis and hence optimise image quality. Patients will be asked to take Hyoscine Butylbromide 30 minutes prior to each MRI scan. These medications are well tolerated and are routinely used with diagnostic MRI scans. There is a <1/100 chance of skin reactions, tachycardia, dry mouth, dyshidrosis with this medication and a <1/1000 chance of urinary retention with Hyoscine Butylbromide. Patients who have contraindications to Hyoscine Butylbromide will not be recruited to the study.

Expected Toxicity

No additional toxicity is expected as part of this study as treatment will be standard of care.
MRI (including DWI) is carried out in routine clinical practice without any known adverse events. No contrast agents will be used.

The expected toxicity from radiotherapy treatment doses of radiation are listed below. These adverse events **are not to be reported** for this study, since they are incurred by the standard of care.

Radiotherapy/chemotherapy for Bladder Cancer:

Acute chemotherapy/radiotherapy toxicity: CTCAE v3.0 grades 0-5
Fatigue / Lethargy
Radiation dermatitis
Cystitis
Proctitis
Diarrhoea
Nausea/vomiting
Fistula formation
Neutropaenia (+/-fever)
Anaemia
Thrombocytopaenia
Hearing impairment
Renal dysfunction

Late chemotherapy/radiotherapy morbidity: CTCAE v3.0 grades 0-5
Bowel urgency/ diarrhoea
Urinary urgency/cystitis
Fistula formation
Vaginal stenosis
Small bowel dysfunction (malabsorption/obstruction)

Safety Reporting

The study does not involve any new treatments. Anatomical and diffusion weighted imaging (DWI) is routinely used in clinical MRI imaging protocols. We therefore do not anticipate any adverse or serious adverse events directly relating to the scanning protocol. In the unlikely event a serious adverse event should occur which is considered to be directly related to the scanning protocol, the CI will be contacted to assess the causality and expectedness of the event.

Definitions
The following definitions apply in this protocol:

An Adverse Event (AE) for this study includes any untoward medical occurrences in a participant which **have a causal relationship** with the study related procedures. (see table 1 for causal relationships)

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A Serious Adverse Event (SAE) for this study is any untoward medical occurrence in a participant that:

- requires inpatient hospitalisation or prolongation of existing hospitalisation **(due to additional MRI scans)**.
- results in persistent or significant disability/incapacity **(due to additional MRI scans)**
- results in a congenital anomaly/birth defect **(due to additional MRI scans)**
- is life threatening **(due to additional MRI scans)**
- results in death **(within 90 days of last MRI scan and is due to additional MRI scans)**

Serious Adverse Events that are both **serious and unexpected** are subject to expedited reporting to the Research Ethics Committee (REC). The following list details all adverse events that should be recorded throughout the study.

- The study team are not expecting there to be any serious adverse events which specifically relate to the study procedures.
- Serious adverse events relating to radiotherapy treatment are not required to be reported for this study.

Reporting

Relevant AE's and SAE's for all patients should be captured from the moment a patient is registered onto the study for the duration of the study only. Should any AEs occur they will be recorded in the Case Report Form (CRF). Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning AE reporting should be directed to the Chief Investigator (CI) in the first instance.

Serious adverse events

All events meeting the criteria for seriousness as defined above must be reported immediately (within 24 hours of knowledge) by the study team to the CI using the Study SAE form. Any new information collected after sending the initial report should also be forwarded to the CI when available. The CI should assign the causality and expectedness of the event. Additional information should be sent to the CI if the reaction has not resolved at the time of reporting.

Study specific exceptions to expedited SAE notification and reporting

Radiotherapy treatment related toxicities, disease progression or events related to disease progression are **not** considered to be SAEs and should **not** be reported as SAEs. Adverse events relating to other anti-cancer treatments, that the patient may be receiving are **not** to be reported. Any events related to chemotherapy are not considered to be SAEs and should **not** be reported on the CRF. Due to the seriousness of the disease in this study, the following situations that fulfil the standard definition of an SAE **are excluded** from expedited notification on an SAE form.

- Elective hospitalisation to simplify treatment or procedures
- Elective hospitalisation for pre-existing conditions that, in the investigator's opinion, have not been exacerbated by study treatment
- Hospital admission related to disease progression
- Hospital admission related to radiotherapy and/or chemotherapy (delivery of treatment or toxicity)

Causality

Most adverse events and reactions that occur in this study, whether they are serious or not, will be expected treatment related toxicities due to the RT treatment, previous chemotherapy or due to

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expected disease progression NOT due to the study related procedures. The assessment of causality should be made using the definitions in table 1 below.

Relationship	Description	Response
Unrelated	There is no evidence of any causal relationship	Yes or No
Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the study treatment). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatment).	Yes or No
Possible	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the study treatment). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant treatments).	Yes or No
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.	Yes or No
Definitely	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.	Yes or No
Not assessable	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.	Yes or No

Table 1. List of factors used to determine causality of AE from this study.

Reporting SAEs
All serious adverse events occurring during the study should be faxed to the Chief Investigator on 0161 446 8111, within 24 hours of the study team becoming aware of them.

Expedited reporting by the CI
The CI will notify the Sponsor and the REC of all events that are unexpected and related to the study treatments occurring in the study procedures within 15 days of notification.

Recruitment of Study Participants

Participant recruitment will only commence when the study has:
- Documented REC, and other regulatory approvals
- Confirmation of capability and capacity to run the study from the local R&D department
- A signed site agreement and all other essential documents are in place
- Final approval from the sponsor

Identifying Participants
Potential participants will be identified via the multi-disciplinary team (MDT)/referral letters by members of the clinical team. They will be approached and given written information during their first oncology appointment. When they attend for their radiotherapy planning scan, they will be consented and screened for the study.

Consenting Participants

Patients who are suitable for the study will be invited to take part and will be provided with a Patient Information Sheet (PIS) and Informed Consent Form (ICF), and will have the opportunity to discuss the study in detail with a clinician before deciding whether to participate.

The person taking consent will be Good Clinical Practice (GCP) trained, suitably qualified and experienced and will have been delegated this duty by the CI on the delegation log. The person taking consent will either be a consultant clinical oncologist, clinical fellow, registrar or nurse clinician who has had suitable training to do so.

After being given verbal and written information about the study, patients will be given sufficient time to consider participation (there is no minimum waiting period for this study) and the opportunity to raise any questions they may have. Three copies of the consent forms will be completed (one copy will be given to the patient), one copy will be retained in the hospital notes and a further copy will be stored in the Investigator Site File.

If new safety information results in significant changes in the risk/benefit assessment, the consent form will be reviewed and updated if necessary. All subjects, including those already being treated, would be informed of the new information, given a copy of the revised form and asked to give their consent to continue in the study.

The consent process includes some optional consent points which are in addition to the standard informed consent. The participant may choose not to consent to any, or all, of these optional parts without being excluded from the trial. These optional consents are for:

- Donation of archived tissue samples collected before starting in the trial.
- Permission for transfer of anonymised scan images to Elekta for work to improve radiotherapy treatment
- For name and contact details to be retained in order to be sent a summary of main findings of the study.

Screening for Eligibility

- Medical history and physical examination
- Assessment of performance status
- Satisfactory completion of MRI safety screening form
- Pregnancy test where appropriate

Randomisation

Randomisation is not applicable for this study.

Registration

Once a patient is deemed eligible for the study and has consented to participate, they will be registered and given sequential ID numbers. These ID numbers will be used to identify all collected anonymised imaged data.

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Discontinuation/Withdrawal of Participants

Patients will be taken off the study for the following reasons:

• Patients withdraw their consent to the study.

• Patients cannot tolerate the MRI scan because they are claustrophobic or for any other reason.

• Image artefacts which are the result of *technical issues*, rendering scans useless.

Patients can withdraw consent at any time without giving any reason, as participation in the research is voluntary, without their care or legal rights being affected. After withdrawal, no further data will be collected on or in relation to the participant.

The CI will be contacted as soon as a decision has been made to withdraw a patient from the study.

If a participant withdraws from the study prior to completion of the 4th study MRI scan then a new participant will be recruited onto the study.

Methodology

Data Acquisition

Patients will undergo CT planning scans as per standard of care. Cone beam imaging (CBCT) will be performed on a daily basis (increasing the number of scans from a minimum of 6 scans per patient to 20), with the correct high dose protocol to allow for adequate imaging quality, as per department protocol. Further CBCT scans may be required if there are issues with target and/or organs at risk (OAR) coverage detected on CBCTs taken during treatment to ensure accurate delivery of treatment.The first study MRI (MR-1) scan will take place on the first or second day of radiotherapy treatment; the second, third and fourth study MRI (MR-2, MR-3 and MR-4) scan will take place weekly during the second, third and fourth week of treatment respectively. These scans will be scheduled around radiotherapy treatments which will minimise patient travel and time spent in hospital.

Image contouring

The initial MRI scan (MR-1) will be used as a simulated planning scan to develop theoretical radiotherapy planning strategies. Tumour bed, CTV and OARs will be contoured on axial T2 sequences by the clinical fellow for both full and empty bladder scans. The daily cone beam CTs will also be contoured to ensure that the MRI scans represent an accurate representation of daily organ motion.

MR-2, MR-3 and MR-4 will be used to evaluate potential planning strategies. Tumour bed, CTV and OARs will be contoured and used to assess coverage of CTV and used to estimate doses that would be given to OARs depending on the simulated treatment protocols. The 'cine' sequences will also be contoured and will be used to develop patient specific models of pelvic organ motion.

Tumour bed boost

Due to image quality, the tumour bed cannot be visualised on CT scans and are not routinely treated to a higher dose. A tumour bed PTV will be generated from MR-1 and a theoretical plan formulated to investigate the feasibility of increasing radiation dose to the tumour bed while maintaining OAR dose limits.

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Adaptive radiotherapy strategies

Radiotherapy strategies assessed will include a standard PTV with a 10-15mm expansion (current clinical standard of care). A further approach will be to create a tumour bed ITV and whole bladder ITV with individualised margins using information gathered from MR-1.

The first adaptive strategy developed will be a library plan of the day (POTD) which involves creating a series of plans, typically three, that aim to encompass the likely tumour bed and bladder positions that will be encountered. These will be developed using MR-1 to create plans dependant on bladder filling - the most appropriate plan will be assessed for coverage of tumour bed and bladder volume for each MR-2, MR-3 and MR-4. Radiation induced changes are known to affect the bladder motion over treatment, these additional MR I time-points will allow the plan coverage to be assessed, particularly of the tumour bed. Additionally, bladder coverage will be compared using the daily high quality CBCT images obtained during treatment.

Full on-line re-optimisation will also be assessed.

Additional analyses may be performed using the anonymized images at discretion of the investigators.

Optional translational aspect

This is an optional aspect of the study. Patients have diagnostic transurethral resection of bladder tumour (TURBT) as part of their routine clinical work up. Formalin-fixed paraffin-embedded (FFPE) tumour blocks will be obtained from the samples collected during this initial TURBT.

A section will be taken for haematoxylin and eosin (H&E) staining to confirm presence of tumour and determine percentage of tumour material. The biology of the tumour sample will be analysed by whole transcriptome sequencing using next generation RNA sequencing technology. From this gene expression signatures known to correlate with aspects of tumour biology (in particular; hypoxia, proliferation and DNA-damage response) will be evaluated.

Radiomics features will be identified from the weekly MRI scans patient have as part of the main study. Patients' response to treatment will be correlated to the genomic and radiomic features identified.

Other methodology

Sequences from MR-1 (empty/full bladder and 'cine') will be used to create a patient specific model of bladder filling, and therefore to create a personalised margin recipe. Changes in this motion across treatment can be assessed on the subsequent weekly MRI scans. These models will be used to assess if it is possible to stratify patients into groups with a large range of tumour bed and bladder movement and those where there is a small range of tumour bed and bladder movement.

To assess DWI imaging an Apparent Diffusion Coefficient (ADC) map will be created using 4 gradient values on each of the available MRI scans. A region of interest will be determined with maximum tumour volume (standardised for each patient over each scan). A variety of ADC parameters will be assessed.

Participants will be asked for their consent for the collection of tissue and samples for future research. Analysis will be conducted as part of a separate funding application. It is the sites responsibility to ensure that all samples are anonymized and labelled in accordance with the General Data Protection Regulation (GDPR).

Study Flow Chart

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┌─────────────────────────────────────────────────────────┐
│ Patient with stage II-III muscle invasive bladder cancer │
│        suitable for treatment with radical               │
│               chemoradiotherapy                          │
└─────────────────────────────────────────────────────────┘
                          ↓
┌─────────────────────────────────────────────────────────┐
│ MRI safety questionnaire and patient information sheet    │
│                      given                                │
└─────────────────────────────────────────────────────────┘
                          ↓
┌─────────────────────────────────────────────────────────┐
│            Informed consent to participate                │
└─────────────────────────────────────────────────────────┘
                          ↓
┌─────────────────────────────────────────────────────────┐
│      MR-1 on 1st or 2nd day of radiotherapy               │
└─────────────────────────────────────────────────────────┘
                          ↓
┌─────────────────────────────────────────────────────────┐
│      MR-2 during 2nd week of radiotherapy                 │
└─────────────────────────────────────────────────────────┘
                          ↓
┌─────────────────────────────────────────────────────────┐
│      MR-3 during 3rd week of radiotherapy                 │
└─────────────────────────────────────────────────────────┘
                          ↓
┌─────────────────────────────────────────────────────────┐
│      MR-4 during 4th week of radiotherapy                 │
└─────────────────────────────────────────────────────────┘
                          ↓
┌─────────────────────────────────────────────────────────┐
│               Completion of treatment                     │
└─────────────────────────────────────────────────────────┘
                          ↓
┌─────────────────────────────────────────────────────────┐
│   Analysis of data and dissemination of trial results     │
└─────────────────────────────────────────────────────────┘
```
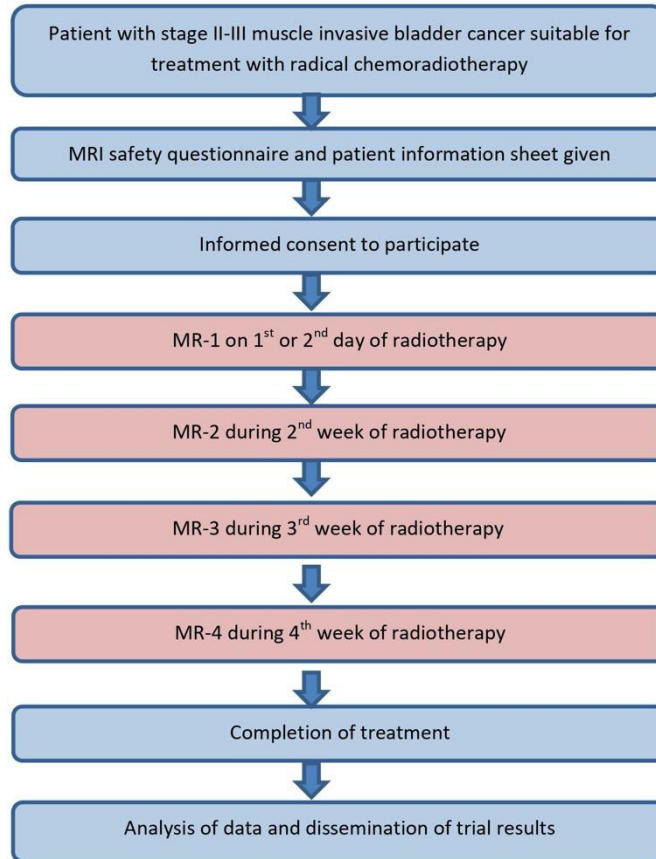
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Summary of Examinations

Each patient will have four additional MRI scan sessions. Patients will be scanned on a Siemens 1.5 T Aera wide bore scanner on a flat table top with a coil bridge. Patients will be scanned in the radiotherapy planning position. Patients will be asked to empty their bladder, drink 350mls of water and take 10mg oral Hyoscine Butylbromide 30minutes prior to the scan.

The scan protocol will include an initial high resolution anatomical sequence to provide visualisation of OARs and tumour (with empty bladder). T1, T2 and DWI MRI sequences will be acquired over a period of 35 minutes. These will be followed by a further high resolution anatomical sequence to represent pelvic organ position with a full bladder.

Each MRI scan is expected to last approximately 45 minutes per patient. Each scan slot will last for 1 hour which includes time to position the patient correctly and a patient safety screening check to determine whether the patient is suitable for MRI scanning.

Cone beam images will be performed as per the standard departmental protocol but will take place on a daily basis during external beam radiotherapy.

	Baseline	Week -1	Week 1	Week 2	Week 3	Week 4	Week 16
Standard of care	Clinical Review	RTP scan & consent	External beam RT (+/- chemotherapy)				Cystoscopy
Written Consent		X					
XVI			Daily cone beam CT imaging				
MRI Scan			MR-1	MR-2	MR-3	MR-4	

Outcome measures

Primary Outcome Measure

Assessment of different adaptive radiotherapy strategies with regard to coverage of CTV and dose to OARs. To identify the best approach, with regards to coverage and practicality, as a starting point for further studies.

Secondary Outcome Measure

Correlation between DWI imaging on MRI at the beginning of and during treatment and clinical response to treatment on post treatment cystoscopy.

Assessment of individual bladder filling model – number of MRI sequences required and ability to stratify patients into large or small tumour bed and bladder motion.

Perform a feasibility study to compare features found on MRI scans with data generated from genomic analysis.

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Confidentiality and Data Protection

Confidentiality

All identifiable records will be kept in a secure storage area with limited access. Clinical information will not be released without the written permission of the participant, except as necessary for monitoring and auditing by the Sponsor, its designee, Regulatory Authorities, or the REC.

The Investigator and trial site staff involved with this trial will not disclose or use for any purpose other than performance of the trial, any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the trial. Prior written agreement from the Sponsor or its designee will be obtained for the disclosure of any confidential information to other parties.

Data Protection

All investigators and site staff involved with the study will comply with the requirements of the Data Protection Act 1998 with regard to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles.

Computers used to collate the data will have limited access measures via user names and passwords.

Published results will not contain any personal data that could allow identification of individual participants.

All images will be anonymised, i.e. all identifiable information will be removed. Sharing of anonymised images will be at the discretion of the Chief Investigator. Anonymised images may also be shared with Elekta and members of the MR-linac consortium to allow for software and workflow development.

Publication Policy

The results of this study will be submitted to peer review journals for publication and will also be presented at national / international conferences.

Participants will be provided with a contact address and email from which to obtain results from the study and copies of publications.

Statement of Indemnity

The University of Manchester will arrange insurance for research involving human subjects that provides compensation for non-negligent harm to research subjects occasioned in circumstances that are under the control of The University of Manchester, subject to policy terms and conditions. In addition the study will be covered by the NHS insurance and indemnity scheme.

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Trial Conduct

Protocol Amendments

Any changes in research activity (except those necessary to remove an apparent, immediate hazard to the participant) will be reviewed and approved by the CI and submitted in writing to the University of Manchester, and to the appropriate REC, Regulatory Authority and local R&D for approval prior to enrolment into an amended protocol.

Protocol Violations/ Deviations/ Serious Breaches

Investigators will not implement any deviation from the protocol without agreement from the CI, the University of Manchester and appropriate REC, Regulatory Authority and R&D department except where necessary to eliminate an immediate hazard to trial participants.

Trial Record Retention

All trial documentation will be retained and archived in accordance with the existing regulations and the University of Manchester/The Christie's standard operating procedures. All trial documentation will be held by the investigator in a way that will facilitate the management of the trial, audit and inspection. They should be retained for a sufficient period (at least 5 years) for possible audit or inspection. Documents should be securely stored and access restricted to authorised personnel.

End of Trial

The Chief Investigator and the trial team have the right at any time to terminate the trial for clinical or administrative reasons. The end of the trial will be reported to the University of Manchester and the REC within the required timeframe if the trial is terminated prematurely. Investigators will inform participants of any premature termination of the trial and ensure that the appropriate follow up is arranged for all involved. A summary report of the trial will be provided to the University of Manchester and the REC within the required timeframe.

Ethical and Regulatory Requirements

The study will be conducted in full conformance with principles of the Declaration of Helsinki, Good Clinical Practice (GCP) and within the laws and regulations of the UK.

The sponsor will ensure that the trial protocol, participant information sheet, consent form, GP letter and submitted supporting documents have been approved by a research ethics committee, the Health Research Authority and the R & D department, prior to any participant recruitment. The protocol and all agreed substantial protocol amendments, will be documented and submitted for ethics committee favourable opinion prior to implementation.

The CI and sponsor will ensure that the main REC is notified that the trial has finished (either as expected or prematurely) within required timeframes with summary reports to be provided as required.

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Statistical considerations

Statistical Analysis

Dose volume histogram (DVH) parameters will be calculated for tumour bed and CTV coverage as well as dose to organs at risk (including rectum) for each strategy. The V95 (volume receiving at least 95% of total dose) will be used for tumour bed volume, CTV and OARs. The volume of PTV and OARs will also be calculated. The averages per patient of the DVH parameters and PTV/OAR volumes obtained by each strategy will be compared using the student paired T test.

For assessment of DWI, average ADC maps will be created for a variety of parameters. Correlation between imaging metrics and baseline clinical features will be evaluated using the Mann Whitney test and confirmed using multivariate analysis if correlation is found.

Sample Size

20 patients will have 4 MRI scans each. Each scan will include imaging with a full and empty bladder. Therefore there will be 80 MRI data sets in total. As this is a pilot study a formal sample size calculation has not been performed. This study is hypothesis generating but the sample size of 20 patients each with 4 scans should allow us to collect sufficient data to assess change in CTV (clinical target volume) to PTV (planning target volume) with mean and standard deviation.

If a participant withdraws from the study before completion of all 4 MRI scans then a new participant will be recruited onto the study. Additionally, if the patient cannot tolerate the entire scan, e.g. because they are uncomfortable, then the data for those scans obtained will still be used for analysis.

Translational aspect (optional) - 10 of the 20 patient will also be recruited into this study and appropriately consented. They will undergo standard treatment.

Funding

Funding for additional imaging (to take place at The Christie NHS Foundation Trust) will come from within the academic supervisor's University of Manchester departmental budget. Funding for the translational aspect of this study will come from Action for Bladder Cancer.

References

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[3]   A. Choudhury *et al.*, "MRE11 expression is predictive of cause-specific survival following radical radiotherapy for muscle-invasive bladder cancer.," *Cancer Res.*, vol. 70, no. 18, pp. 7017–26, Sep. 2010.

[4]   U. A. van der Heide, A. C. Houweling, G. Groenendaal, R. G. H. Beets-Tan, and P. Lambin, "Functional MRI for radiotherapy dose painting.," *Magn. Reson. Imaging*, vol. 30, no. 9, pp. 1216–23, Nov. 2012.

[5]   S. Yoshida *et al.*, "Diffusion-weighted magnetic resonance imaging in management of bladder cancer, particularly with multimodal bladder-sparing strategy.," *World J. Radiol.*, vol. 6, no. 6, pp. 344–54, Jun. 2014.

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Participant Information Sheet

A pilot study using Magnetic Resonance Imaging (MRI) to assess bladder motion during radiotherapy treatment

We invite you to take part in a research study for patients with bladder cancer, which will involve four extra MR scans

- Before you decide whether to take part, it is important to understand why the research is being done and what is involved.

- Please take time to read this information carefully and discuss it with your family and friends if you wish.

- You are free to decide whether you would like to take part or not. If you decide not to, this will not affect the care you receive.

- Please ask us if anything is unclear.

What is the study about?

- Radiotherapy is targeted on the bladder.

- The bladder can move and expand within the pelvis for a number of reasons including how full your bladder is.

- We do scans during radiotherapy to make sure we are covering the right area. These are usually a type of short CT scan known as Cone Beam CT (CBCT) scans. Patients normally have a minimum of 6 of these CBCT scans, although they have more if movement is detected. On average, patients have 10 CBCT scans during their treatment.

- During the study, we will do one CBCT scan every day just before your radiotherapy

- We also want to see if a different type of scan – an MRI scan will be helpful. The bladder and its surrounding structures can be seen better on MRI scan.

- These MRI scans will not be used to change your treatment – it will be planned and carried out normally

- We will use the MRI and CBCT scans to try out some different ways of planning radiotherapy to account for the movement and expansion of the bladder. This is called adaptive radiotherapy. It is hoped that this could be used in the future to target radiotherapy

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treatment more accurately.

- We also want to see if we can use special images from the MRI scans to see early if a cancer is responding to the treatment – this part of the study will take place after the end of your treatment. We will not be able to use the images from the trial to tell you if your cancer is responding to treatment.

- Twenty patients with bladder cancer will be included in this study.

Optional aspect

- There are two options for treating muscle invasive bladder cancer – surgery or a combination of chemotherapy and radiotherapy

- Currently, there is no scientific way of helping doctors and patients choose between these two options.

- Some biological features of patients' tumour samples (like the presence of a particular protein) have the potential to predict how they respond to certain treatments.

- We hope to identify features from your tumour sample taken when you were initially diagnosed with bladder cancer and compare them with changes found on your CT or MRI scans

- For example, the presence of a particular protein or chemical in your tumour sample with a change on your MRI scan.

- We hope that this will help to predict response to treatment in future patients.

- This is extra aspect of the study entirely optional and will not affect your participation in the rest of the study or your treatment.

What would taking part involve?

- If you agree to take part in the study you will be asked to sign a consent form.

- You will be asked to drink 350ml of water (just over half a pint) before your radiotherapy planning scan, MRI scans and each daily treatment.

- You will have a short CBCT scan on the radiotherapy machine just before your radiotherapy every day. This will take about 3 minutes.

- You would normally have a CBCT scan between one and three times a week if you were not in this study. As part of the study, you will have one CBCT scan a day.

- If the CBCT scan shows that the treatment is not covering the right

area accurately, your position may be changed slightly and a further CBCT will be taken to ensure that we are covering the right area.

- Normally you do not have MRI scans as part of your treatment but as part of the study you will have four extra MRI scans.

- The scans will take place at The Christie hospital on the same day as you attend for radiotherapy treatments.

- The first MRI scan will take place on the first or second day of treatment. You will then have an MRI scan once a week during the course of radiotherapy treatment.

- Some people find the scanner a little claustrophobic, and it can be noisy. Please ask us if you are concerned about this.

- Optional: If you agree to the optional aspect of the study, we will contact the hospital where you had the tumour sample taken (before diagnosis). The sample will be anonymised (all personal identifiers will be removed) before it is processed.

What are the possible benefits of taking part?

There will be no direct benefit to you from taking part in this study. **Your treatment will not change.** Your help will enable us to improve radiotherapy treatment for bladder cancer patients in the future.

What are the possible disadvantages and risks of taking part?

- As you will be having a number of extra short CBCT scans before your radiotherapy, you will have a small increase in the radiation dose you receive.

- This extra dose is less than the dose of radiation that you will receive as part of the radiotherapy to treat your cancer.

- In terms of the associated risk, if 99 people between the ages of 30-39 receive this extra dose of radiation, it is estimated that it would cause a second cancer to occur in one of these people over their lifetime. This risk decreases with people who are older. In people between the ages of 70-79, this risk of second cancer reduces to 1 case in 524 people receiving the extra dose of radiation.

- MRI scans are painless, no injections are required and there is no radiation.

- The extra MRI scans will take place on days you are in the hospital

for radiotherapy. The scan will take about an hour and there may be a wait between the radiotherapy and MRI scan.

- Some patients feel claustrophobic in the scanner but our staff will be there to reassure you. Only patients who are suitable for an MRI scan will take part.

Further information

What is an MRI scan?

An MRI scan is a way to get detailed pictures of the body (using a strong magnet). This sort of scan does not use radiation.
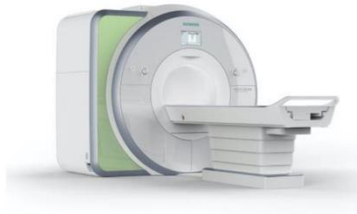
You may or may not have had an MRI scan whilst your cancer was being diagnosed. You lie on a flatbed that slides into the scanner which is shaped like a doughnut. It is important to try and lie still during the scan.

You can talk to the radiographer who is controlling the scanner at all times via an intercom. If you are struggling the scan can be stopped.

We will ask you to empty your bladder and drink some water just before your scan. We will also ask you to take a tablet called Hyoscine Butylbromide (Buscopan) just before your scan. This is to help your bowels relax but you will not feel any different. This medication is well tolerated, but if you develop any side effects such as skin reaction, fast heart rate or dry mouth, please inform us and this medication can be stopped. If you have problems with glaucoma or previous allergic reactions with Hydroscine Butylbromide, please inform us as well. You will not need to drink any contrast dye or have any injections during the scan.

The scan takes up to one hour.

What if something goes wrong?

MRI scanners have been used many times worldwide with no reports of any harm.

In the event that something does go wrong and you are harmed during the research you may have grounds for a legal action for compensation against the University of Manchester or The Christie NHS Foundation Trust but you may have to pay your legal costs.

The normal National Health Service complaints mechanisms will still be available to you. If you wish to complain formally, you can do this by contacting the Patient Advice and Liaison Service on 0161 446 8217.

By taking part in the study you do not waive any of your legal rights.

What will happen if I don't want to carry on with the study?

You are free to withdraw from the study at any time. This will not affect your care. Any anonymised data and scans already taken will still be used but we will not collect any additional information.

In the unlikely event that, during the study, you are unable to give your consent to continue (for example, due to an accident, another major illness or if you require surgery) the research team will keep the anonymised data and scans already collected and continue to use them confidentially in connection with the study. This may include further ethically approved research after the current project has ended.

Complaints

Minor Complaints - If you have a minor complaint then please contact the research team (details below) in the first instance who will do their best to answer your questions.

Dr Yee Pei Song
0161 446 8273
yeepei.song@christie.nhs.uk

Formal complaints – If you wish to make a formal complaint or if you are not satisfied with the response you have gained from the researchers in the first instance the please contact the Research Governance and Integrity Manager, Research Office, Christie Building, University of Manchester, Oxford Road, Manchester, M13 9PL, by emailing:

research.complaints@manchester.ac.uk or by telephoning 0161 275 2674 or 275 2046.

Will my information be kept confidential?

Yes. Your name will not appear in any publications. Your medical records (which contain personal information) will be reviewed by the clinical care team to assess if you are able to enter the study.

Once you have joined the study you will be assigned a unique reference number that will be used to label the images we collect, to preserve your anonymity. The research team will be able to link the collected information back to you with this reference number. The research team will also look at the routine scans that you have had during your treatment and any follow-up scans and procedures you have after treatment for a period of 12 months. All study data will be kept secure with restricted access.

What will happen to the information collected about me?

All data collected about you will be anonymous (all information that could identify you will be removed).

After the study has finished, all the data collected as part of the study will be kept and stored according to research regulations and the University of Manchester and The Christie's standard procedures. All study data will be kept at The Christie for a period of at least 15 years, in case it is needed for audit or inspection. All the data will be kept securely and access will be restricted to authorised personnel. The images collected as part of this study may be used in future research with appropriate approvals. At the end of the 15 year period, all research data will be destroyed.

The scan pictures (with all your personal details removed e.g. your name) may also be shared with Elekta, a company that make radiotherapy equipment, to help develop new computer software which should speed up the process of planning treatment for future patients. Elekta have offices based both within and outside the EU where data protection criteria may be different to the UK, however, no identifiable data will be sent to Elekta.

What about my personal information?

The Christie NHS Foundation Trust will collect information from you and your medical records for this research study in accordance with our instructions.

The Christie will keep your name, NHS number, hospital number and contact details confidential and will not pass this information to University of Manchester. The Christie will use this information as needed, to contact you about the research study, and make sure that relevant information about the study is recorded for your care, and to

oversee the quality of the study. Certain individuals from University of Manchester and regulatory organisations may look at your medical and research records to check the accuracy of the research study. The University of Manchester will only receive information without any identifying information. The people who analyse the information will not be able to identify you and will not be able to find out your name, NHS number, hospital number or contact details.

The Christie will keep identifiable information about you from this study for 15 years after the study has finished.

Will my GP be informed?

Yes, your GP will be informed that you are taking part in the study.

What will happen to the results of this study?

The results of this study will be used by the Chief Investigator (Dr Yee Pei Song) as part of her Research Doctorate or MD thesis.

We will also communicate the results of this study to the participants through publications. We expect the results to be available 12 months after the last patient has finished participating in the study. Your name will not appear in any of these reports. The research team will ask if you would like us to send you a copy of the publications or a summary of the research when the study has finished. Your name and hospital number will be kept on record securely (on a password protected hospital database) to enable us to check your records for up-to-date contact details in order to contact you. Alternatively, you may contact Dr Yee Pei Song (details below) for a copy of the results.

Who is organising and funding this study?

This study is being organised by researchers at the University of Manchester, who is the Research Governance Sponsor, and The Christie NHS Foundation Trust. The extra scans are paid for from internal department funds.

Who has reviewed the study?

All research in the NHS involving patients is reviewed and approved by an independent group of people called a Research Ethics Committee to protect your safety, rights, wellbeing, and dignity. The local research and development department at The Christie has also given approval. This study has also been independently peer-reviewed.

What happens now?

If you are interested in participating in this study, or require further information, please contact your clinical team or the researchers (details as shown below).

Thank you for considering to help with our research

Contact us

For further information about this study please contact:

Study Investigators:

Dr Yee Pei Song
0161 446 8273
yeepei.song@christie.nhs.uk

Dr Ananya Choudhury
0161 446 3000

Consent Form

A pilot study using Magnetic Resonance Imaging (MRI) to assess bladder motion during radiotherapy treatment

Hospital Number:_____

Patient Identification Number for this trial:_____

		Initials
1	I confirm that I have read and understand the Bladder MRI Study Patient Information Sheet dated 4th July 2018, Version 3 for the above study and that I have been provided with a copy to keep. I was able to consider the information, ask questions and had them answered satisfactorily.	
2	I understand that my participation is entirely voluntary, that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.	
3	I understand that if I withdraw from the study, or lose the capacity to give consent to continue on the study, the research team will keep the anonymised data and scans already collected and continue to use them confidentially in connection with the study.	
4	I give permission for a nominated person from the research team to look at my medical records to get information on my medical history, diagnosis, treatment and progress following treatment. I understand the information will be kept confidential.	
5	I understand that relevant sections of my medical notes and study data may be looked at by individuals from the University of Manchester, The Christie NHS Foundation Trust and regulatory agencies for auditing and monitoring purposes. I give permission for these people to access to my records.	
6	I give permission for any anonymised (with all personal information removed) scan images and trial data to be used by the study researchers for this study and possibly in future research subject to appropriate approvals.	
7	I agree to my GP being informed of my participation in this study.	
8	I agree to take part in the above study.	

OPTIONAL:

1	I give permission for the research team to obtain, store and further analyse tumour sample(s) collected during my diagnosis. I give permission for these individuals to have access to my sample(s), (but not any personal identifying information about me. I offer my tumour sample(s) as a gift).	
2	I agree to the possible transfer of scan images to Elekta; a radiotherapy company who are carrying out work to improve radiotherapy treatment, and that this may involve transfer of anonymised data outside the EU where data protection criteria may be different.	
3	I would like a summary of the main findings from the study. I understand that my name and hospital number/contact details will be kept to send the summary.	

_____   _____   _____
Printed name of Patient   Date   Signature
(BLOCK CAPITALS)

_____   _____   _____
Printed name of person   Date   Signature
Taking consent
(BLOCK CAPITALS)

When completed, original form to be kept in Investigator Site file, one copy to be kept in the medical notes and one copy to be given to the patient

Thank you very much for agreeing to participate in this research

11 Appendix 2: Publications/Presentations

The following all relate to the work presented in this MD thesis. Abstracts or full articles of peer-reviewed journal publications are appended where available.

11.1   Publications

"Does the use of chemoradiation enable preservation of the bladder in bladder cancer?"

Y Song, A Vinayan, P Hoskin (Accepted for publication in BJUI Knowledge)

"Organ preservation in bladder cancer: an opportunity for truly personalised treatment"

Y Song, A McWilliam, P Hoskin, A Choudhury

Nat Rev Urol 16, 511-522 (2019)

11.2   Presentations

"Hypo-fractionation in muscle-invasive bladder cancer: an individual patient data meta-analysis of the BC2001 and BCON trials"

N Porta, Y Song, E Hall, A Choudhury, R Owen, R Lewis, S Hussain, N D James, R A Huddart, P Hoskin

Oral presentation at ASTRO 2019, Chicago, September 2019

"Hypoxia modification in bladder preservation: relating long term outcomes to necrosis and hypoxia"

Y Song, H Mistry, L Yang, S Chin, C West, A Choudhury, P Hoskin

Oral presentation at ESTRO 38, Milan, April 2019

"Long term outcomes of hypoxia modification in bladder preservation: update from BCON trial"

Y Song, H Mistry, A Choudhury, P Hoskin

Oral presentation at ASCO GU 2019, San Francisco, February 2019

11.3   Posters

"Impact of bladder size at radiotherapy planning scan on survival"

<u>Y Song</u>, A Choudhury, A McPartlin, P Hoskin, A McWilliam

Poster accepted to ESTRO 2020, Vienna, August 2020

"Gender inequality in bladder motion"

<u>Y Song</u>, E Vasquez Osorio, A McWilliam, A McPartlin, A Choudhury

Poster at ESTRO Meets Asia 2018, Singapore, December 2018

Organ preservation in bladder cancer: an opportunity for truly personalized treatment

Yee Pei Song [1,2]*, Alan McWilliam[1,2], Peter J. Hoskin[1,3] and Ananya Choudhury[1,2]

Abstract | Radical treatment of many solid tumours has moved from surgery to multimodal organ preservation strategies combining systemic and local treatments. Trimodality bladder-preserving treatment (TMT) comprises maximal transurethral resection of the bladder tumour followed by radiotherapy and concurrent radiosensitizing treatment, thereby sparing the urinary bladder. From the patient's perspective, the choice of maintaining quality of life without a negative effect on the chances of cure and long-term survival is attractive. In muscle-invasive bladder cancer (MIBC), the evidence shows comparable clinical outcomes between patients undergoing radical cystectomy and TMT. Despite this evidence, many patients continue to be offered radical surgery as the standard-of-care treatment. Improvements in radiotherapy techniques with adaptive radiotherapy and advances in imaging translate to increases in the accuracy of treatment delivery and reductions in long-term toxicities. With the advent of novel biomarkers promising improved prediction of treatment response, stratification of patients for different treatments on the basis of tumour biology could soon be a reality. The future of oncological treatment lies in personalized medicine with the combination of technological and biological advances leading to truly bespoke management for patients with MIBC.

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https://doi.org/10.1038/ s41585-019-0199-x

Urothelial bladder cancer is the most common cancer of the urinary system[1]. In the UK, it is the ninth most common cancer and is responsible for 3% of cancer-related deaths[2]. The incidence of urothelial bladder cancer is higher in males than in females and increases with age, with more than half of new cases diagnosed in patients older than 75 years of age. Approximately one-third of patients present with muscle-invasive bladder cancer (MIBC) at diagnosis and require a more aggressive treatment than those with non-muscle-invasive bladder cancer (NMIBC). Traditionally, radical cystectomy, which includes surgical removal of the bladder and other pelvic organs followed by reconstruction of the urinary tract, has been the standard-of-care treatment for MIBC[3]. However, radical cystectomy is associated with potential complications (severe blood loss, infections, paralytic ileus and issues with wound healing)[4] and long-term consequences that could have a negative effect on a patient's quality of life (QOL)[5].

Over the past decade, curative oncological strategies have moved from extensive surgery to organ-preserving treatments in different cancer types, ranging from head and neck malignancies to anal cancer. Trimodality bladder-preserving treatment (TMT) comprises maximal transurethral resection of the bladder tumour (TURBT) followed by radiotherapy and concurrent radiosensitizing treatment, thereby allowing patients to keep their own bladders. Data from meta-analyses and propensity-matched analyses have now led to an increasing acceptance that patients undergoing TMT and radical cystectomy have comparable long-term clinical outcomes[6,7]. Importantly, patients should be informed about treatment options in a manner that will enable them to choose the optimal strategy that is most suitable for them. Advances in different aspects of non-surgical oncology, including adaptive and image-guided radiotherapy techniques and prognostic and predictive biomarkers, will enable the delivery of personalized management plans.

In this Review, we examine the main curative treatment options in MIBC by comparing TMT and radical cystectomy and discussing the role of neoadjuvant and adjuvant systemic treatments, the importance of radiosensitization, recent advancements in radiotherapy techniques in MIBC and the discovery of imaging and tissue biomarkers and their potential prognostic and predictive roles.

Curative treatment options

The two main treatment options available to patients with localized MIBC are radical cystectomy and TMT. As the choice between TMT and radical cystectomy is an important one, the differences between the two treatment options and the guideline recommendations should be considered.

Radical cystectomy versus TMT. Radical cystectomy, which involves major abdominopelvic surgery performed under general anaesthesia followed by a prolonged recovery period, is associated with increased operative mortality in elderly patients (age ≥75 years) and can result in long-term changes in QOL to which all patients must adapt. Such QOL changes include the construction of a urostomy or neobladder and negative effects on sexual function. In the long term, 24% of patients with ileal conduits experience stomal problems, and a similar proportion of patients develop renal insufficiency (27%), bowel problems (24%) and urinary tract infections (24%)[8]. With respect to sexual function, a retrospective survey by the Department of Health in England found that, at 1–5 years after diagnosis, 88% of patients reported dissatisfaction with their sex life after radical cystectomy, whereas only 11% and 17% of patients reported such dissatisfaction following chemoradiotherapy and radiotherapy, respectively[5].

TMT comprises maximal TURBT followed by radiotherapy with concurrent administration of a radiosensitizing agent (often chemotherapy)[9]. Radical radiotherapy to the bladder involves 4–7 weeks of daily treatment and has the potential of causing tiredness, impaired sexual function and adverse effects related to the bladder and bowel (such as urgency, dysuria and proctitis)[10]. Indeed, a 2018 meta-analysis reported that late Radiation Therapy Oncology Group (RTOG) grade 3 pelvic toxicities, including urinary and gastrointestinal toxicities, occurred in 7% of patients following TMT[11]. Moreover, in the phase III BC2001 trial, which investigated radiotherapy with or without synchronous chemotherapy in patients with MIBC, late RTOG grade 3–4 pelvic toxicities were noted in 4.6% of patients who underwent chemoradiotherapy and in 5.2% of patients who received radiotherapy only at 2 years after completion of treatment[12]. TMT is also associated with a long-term negative effect on bladder function, specifically decreased bladder capacity in ~3% of patients[13], and cystectomy for intractable bladder symptoms rather than tumour recurrence is required in 1–2% of patients[13,14]. An important component of TMT is ongoing cystoscopic surveillance with the possibility of salvage cystectomy after disease recurrence, which might be necessary in ~7–15% of patients[3,12,14]. In addition, TMT was associated with a risk of NMIBC recurrence in 25% of patients who had complete response, who might require treatment[15].

Ideally, radical cystectomy and TMT should be compared in a prospective randomized controlled trial, but attempts to do so have been unsuccessful owing to poor patient accrual[16]. The early closure of the Selective bladder Preservation Against Radical Excision (SPARE) trial has been attributed to several factors, including the complexity of the patient referral and management pathways (which had multiple specialist teams and centres involved) and the importance of patient preference in a trial that randomizes patients to two distinctly different treatment options.

Large radical cystectomy series report 5-year overall survival (OS) metrics ranging from 40.2% to 58%[17–19]. Similarly, a meta-analysis showed that patients with MIBC undergoing TMT had good outcomes, despite its use in frail patients, with a complete response rate of 78% and 5-year OS of 56%[20]. In addition, a pooled analysis of six RTOG studies showed that the 10-year disease-specific survival (DSS) was 65% in patients with MIBC following bladder preservation treatment[3].

Various retrospective studies and propensity-matched analyses have attempted to compare the outcomes of patients receiving either treatment option. In a population-based retrospective cohort study, the cancer-specific survival and OS of patients who underwent radical cystectomy and TMT were similar following adjustments for covariates[7]. Similarly, a meta-analysis of 29 TMT studies and 30 radical cystectomy studies found that the 5-year OS was 63% for TMT and 61% for radical cystectomy for patients with T2 disease ($P = 0.30$) and was 45% and 40%, respectively, for patients with >T2 disease ($P = 0.36$)[21]. Conversely, a cohort study from 2018 proposed the contrary — that TMT is associated with poorer OS and DSS than radical cystectomy[22]. However, patients in the TMT group had received a median of 18 fractions of radiotherapy, suggesting that more than half of patients who allegedly underwent TMT did not, in fact, have curative treatment and, therefore, did not undergo TMT. During the time period studied (December 2011 to December 2013), TMT tended to be recommended for frail patients who were unfit for surgery, a consideration that was also not adequately accounted for in this study.

A number of factors preclude patients from each treatment option. Patients with serious comorbidities (for example, cardiac issues and renal failure) might be unable to tolerate the general anaesthetic and physiological stress associated with major surgery, and some patients might be unable or unwilling to adapt to the lifestyle changes that are required after radical cystectomy. Similarly, patients with extensive carcinoma in situ (CIS), poor bladder function or obstruction to their kidneys might not be appropriate candidates for TMT. Moreover, patients who undergo TMT must be prepared for the ongoing cystoscopic surveillance and the ongoing

risk, for a minority of patients, that cystectomy will be ultimately required in the event of recurrence or poor residual bladder function.

A retrospective study of patients with MIBC treated with either radical cystectomy or TMT found that patients who received TMT had markedly better general QOL, bowel function and sexual function; had fewer concerns about the negative effects of cancer; and had similar urinary symptoms scores[23]. Toxicity rates between the two treatment options have also been reported in the SPARE trial; 70% of patients in the radical cystectomy arm had Common Terminology Criteria for Adverse Events (CTCAE) grade 3–4 toxicity compared with 36% of patients in the radiotherapy group ($P = 0.038$)[16]. The primary aim of radical treatment for MIBC is to maximize the chance of cure while maintaining a good patient QOL. As discussed, both radical cystectomy and TMT as curative treatment options might result in serious adverse effects and, therefore, the long-term effect on QOL is an important consideration.

Guideline recommendations. In the UK, specific recommendations in the National Institute for Health and Care Excellence (NICE) guidelines[24], which provide evidence-based guidance for clinical practice, advocate that patents with MIBC are offered both radical cystectomy and TMT as curative treatment options. This set of guidelines specifies the need to discuss the evidence for each treatment option in terms of efficacy, potential toxicities and the influence on QOL. Surgery remains the most common option recommended to patients in Europe and the USA[25,26]. The European Association of Urology (EAU) and American Association of Urology (AUA) guidelines recommend that patients who are fit should be offered radical cystectomy, reserving TMT for those who are less fit; this recommendation is reflected in real-world practice. In a survey of 277 radiation oncologists from the USA, 58% treated only 1–3 patients per year with TMT and 74% primarily treated patients who were deemed unfit for surgery with TMT[27]. Similarly, a survey of 32 Italian centres found that, in the 13 centres that responded, only 12 of 100 patients with bladder cancer treated with radiotherapy in a 1-year period received radiotherapy as a primary curative treatment[28]. The main difference between the guidelines is that NICE recommends that patients should be offered both options, whereas the EAU and AUA guidelines recommend radical cystectomy over TMT for fit patients.

The choice between TMT and radical cystectomy is a difficult one for patients and a controversial topic among clinicians. Ideally, level I evidence from phase III randomized controlled trials would help to inform future guidelines. However, previous attempts at such a study have been unsuccessful.

Systemic therapy

As distant metastases are the most common cause of treatment failure following local therapy[29], systemic treatment (chemotherapy and immunotherapy) before or following definitive local treatment with radical cystectomy or TMT has a role in improving the long-term outcomes of patients with MIBC.

Neoadjuvant chemotherapy. A systematic review and meta-analysis from 2005 reported a 5% improvement in 5-year OS and a 9% improvement in 5-year DSS with the use of platinum-based neoadjuvant chemotherapy before radical cystectomy or radiotherapy[30]. Another early randomized controlled trial conducted by the Southwest Oncology Group (SWOG) compared the use of three cycles of neoadjuvant methotrexate, vinblastine, doxorubicin and cisplatin (MVAC) with no neoadjuvant treatment before radical cystectomy in patients with MIBC. Despite a nonsignificant improvement of 6% in 5-year OS in the MVAC group, the trial reported a statistically significant improvement in pathological complete response of 23% ($P < 0.001$)[31]. In 2011, a phase III International Collaboration of Trialists study randomized 976 patients with MIBC to receive either neoadjuvant chemotherapy with cisplatin, methotrexate and vinblastine (CMV) or no neoadjuvant chemotherapy before definitive treatment with radical cystectomy, perioperative radiotherapy or radiotherapy alone. This study demonstrated that neoadjuvant CMV led to a 6% improvement in 10-year OS ($P = 0.037$)[32]. As these studies were undertaken before the adoption of radiosensitizing agent treatment with radiotherapy, the role of neoadjuvant chemotherapy in this setting has yet to be adequately tested.

The RTOG 8903 group carried out a phase III clinical trial to evaluate the benefit of neoadjuvant chemotherapy with CMV before chemoradiotherapy, the findings of which suggested that neoadjuvant chemotherapy did not improve survival outcomes[33]. However, the study was closed to recruitment early owing to high rates of severe neutropenia and sepsis, and only 67% of patients recruited to the experimental arm completed treatment with no or minor protocol deviation. In the BC2001 trial, 31.3% of patients who received chemoradiotherapy had undergone prior neoadjuvant platinum-based chemotherapy, but this treatment did not seem to affect locoregional disease-free survival outcomes[12].

The choice of neoadjuvant chemotherapy regimen has also evolved. The aforementioned prospective randomized trials evaluated MVAC or CMV, but trials from the past two decades have increasingly investigated the use of gemcitabine and cisplatin (GC) owing to its favourable toxicity profile and the similarity in survival outcomes between GC and MVAC in a phase III study of patients with advanced or metastatic MIBC[34]. A retrospective study comparing GC and MVAC in the neoadjuvant setting demonstrated similar pathological and survival outcomes before radical cystectomy and superextended pelvic lymph node dissection[35]. In the past 10 years, dose-dense MVAC (ddMVAC) has replaced classic MVAC. Classic MVAC is administered in 4-week cycles whereas ddMVAC is delivered every 2 weeks with granulocyte colony-stimulating factor (G-CSF) in order to ameliorate the resulting myelosuppression, which enables the same dose of doxorubicin and cisplatin to be delivered in half the time. A further retrospective study reported similar outcomes with ddMVAC, classic MVAC and GC[36], whereas another study suggested that ddMVAC yields better rates of complete pathological response and survival than GC[37].

Considering the retrospective nature of these studies and the lack of randomization, a prospective trial would be helpful to determine the optimal neoadjuvant chemotherapy regimen.

Neoadjuvant chemotherapy has been shown to improve pathological response and survival outcomes before radical cystectomy and radiotherapy, but limited evidence exists for its role before chemoradiotherapy. However, given the role of systemic agents in reducing metastatic recurrence and given the published data showing that it is well tolerated before chemoradiotherapy[38], neoadjuvant chemotherapy is standard treatment for bladder preservation in the UK[24].

Neoadjuvant immunotherapy. Immunotherapy with immune checkpoint inhibitors (ICIs) is an emerging treatment modality in MIBC. In the metastatic setting, the anti-programmed cell death 1 (PD-1) antibody pembrolizumab was shown to improve median OS by 2.9 months and was associated with fewer treatment-related adverse events than chemotherapy in the second-line setting[39]. Accordingly, interest has been growing regarding the role of immunotherapy in the neoadjuvant setting[40]. Indeed, the phase II PURE-01 study investigated the role of neoadjuvant pembrolizumab before radical cystectomy and reported that patients with high PD-L1 expression had higher rates of complete pathological response with pembrolizumab than patients with low PD-L1 expression[41].

Despite the emerging trend towards exploring the potential of ICIs in various cancer types and although their role in the neoadjuvant setting in MIBC is certainly interesting, additional investigation is required before we can confidently establish its efficacy.

Adjuvant chemotherapy. Various attempts have been made to evaluate the benefit of adjuvant chemotherapy following radical cystectomy in patients with advanced bladder cancer. Unfortunately, however, early trial closure and poor accruals to these studies meant that firm conclusions could not be drawn[42–44]. A meta-analysis of 491 patients from six eligible trials found a 9% improvement in 3-year OS[45]. The authors concluded that, owing to various factors, the evidence was insufficient to change clinical practice. These factors include small patient numbers (491 patients; 238 deaths), the early closure of four trials, poor compliance with trial protocol (a large proportion of patients in two trials did not receive chemotherapy despite randomization to the chemotherapy group or received regimens other than those described in the protocols) and the lack of information about salvage chemotherapy for patients whose disease progressed. An updated meta-analysis has since been published but was based on summary statistics of each study as opposed to individual patient information. This meta-analysis found borderline statistical significance ($P = 0.049$) for improvement in OS with a 23% relative decrease in the risk of death when treated with adjuvant chemotherapy, but a large variation in the hazard ratio across the included studies was noted[46]. In addition, an exploratory analysis of a European Organisation for Research and Treatment of Cancer (EORTC) study that closed after recruitment of 284 of a planned 660 patients demonstrated a benefit in 5-year OS for adjuvant chemotherapy after radical cystectomy (79.5% versus 59.0%; $P = 0.012$) in node-negative patients, which constituted only 30% of recruited patients[44]. Owing to the lack of robust evidence in the adjuvant setting, neoadjuvant chemotherapy should continue to be the preferred option after radical cystectomy.

Developments in radiosensitization

Radical radiotherapy, with the addition of radiosensitizing agents to improve clinical outcomes, is central to bladder-preserving treatment. These radiosensitizing agents function in a synergistic manner with radiotherapy, increasing cell death and, therefore, improving the efficacy of treatment[47]. The different mechanisms of action of commonly used radiosensitizers are illustrated in BOX 1. Radiosensitizers can be used either as single agents or in combination with other agents.

Box 1 | **Mechanisms of action of radiosensitizers**

Radiosensitizers are agents that function in a synergistic manner with radiotherapy to increase cell kill. Commonly used radiosensitizers include chemotherapy (single-drug or multidrug regimens) or other regimens such as hypoxia modification.

5-Flurouracil
- Targets radioresistant cells in S phase

Gemcitabine
- Targets radioresistant cells in S phase
- Lowers threshold for radiation-induced apoptosis[116]

Cisplatin
- Inhibits DNA damage repair through formation of intrastrand and interstrand crosslinks
- Radiation results in increased cellular uptake of cisplatin
- Blocks cells in the radiosensitive G2 phase of the cell cycle[117]
- Forms toxic intermediates with radiation-induced free radicals[118]

Mitomycin C
- Toxic to radioresistant cells, thereby increasing cell kill

Carbogen (95% oxygen and 5% carbon dioxide)
- Increases the oxygen concentration in the blood and the tumour
- Synergizes with radiation owing to the oxygen effect, whereby radiotherapy causes DNA damage with free radicals and oxygen makes these damages permanent by 'fixing' them
- Carbon dioxide has a vasodilatory effect
- Carbogen in combination with nicotinamide further reduces hypoxia

Nicotinamide
- Hypoxia-modifying agent
- Reduces intermittent constriction of blood vessels, thereby improving oxygenation
- Reduces acute hypoxia

Various phase II studies have investigated the use of different cisplatin-containing regimens (cisplatin alone, cisplatin plus 5-fluorouracil and cisplatin plus paclitaxel) as radiosensitizers in MIBC and have reported good complete response rates and long-term DSS that are comparable to that of radical cystectomy[48–51]. However, these were relatively small studies with sample sizes of <100 patients. Patients in these studies were treated in a split-course manner, with assessment following an initial induction course of chemoradiotherapy, after which patients were selected for radical cystectomy or consolidation chemoradiotherapy on the basis of their response to induction treatment. These studies showed encouraging results at the time. Mak et al.[3] conducted a pooled analysis of these phase II trials and a phase III study and demonstrated that these prospective RTOG bladder-preserving protocols result in DSS comparable to that of cystectomy studies.

Until the early 2000s, the only randomized clinical trial was a Canadian study that evaluated cisplatin monotherapy as a radiosensitizer[52]. This small study ($n = 99$) showed an improvement in both local control rate and OS with the addition of concurrent cisplatin to preoperative or definitive radiotherapy but had limited statistical power (3-year OS 47% versus 33%; $P = 0.34$). As cisplatin causes an increased risk of renal toxicity[53], some clinicians have reservations about the use of cisplatin in patients with MIBC who might already have impaired renal function and other comorbidities.

Over the past 10 years, two large randomized trials from the UK comparing radiotherapy alone with the addition of radiosensitizing agents have been published. The phase III BC2001 trial reported an improvement in 2-year locoregional recurrence-free survival (RFS) from 54% to 67% ($P = 0.03$) and in 5-year OS from 35% to 48% ($P = 0.16$) with the addition of concomitant chemotherapy with 5-fluorouracil and mitomycin C to radiotherapy in patients with MIBC[12]. As cellular hypoxia increases resistance to radiation, the bladder carbogen nicotinamide (BCON) trial took a different approach to radiosensitization with the use of hypoxia-modifying agents instead of traditional chemotherapy agents. This trial showed that the addition of concurrent carbogen and nicotinamide to radiotherapy improved 3-year RFS from 43% to 54% ($P = 0.06$) and OS from 46% to 59% ($P = 0.04$) in patients with locally advanced disease[14].

Another agent that has been tested as a radiosensitizer for bladder cancer is gemcitabine. A phase II study of radiotherapy with concurrent gemcitabine in MIBC reported a complete response rate of 88% at first-check cystoscopy, with an organ preservation rate of 64% and an OS of 72% at 3 years[54]. In addition, a meta-analysis of eight published studies that evaluated concurrent gemcitabine and radiotherapy, which included a total of 190 patients with MIBC, described a 93% complete response rate at first-check cystoscopy within 12 weeks of completion of treatment and a 5-year OS of 59%[55]. The phase II NRG/RTOG 0712 study randomized patients to twice-daily radiation with cisplatin–5-fluorouracil or once-daily radiation with gemcitabine. The primary end point of 3-year distant-metastasis-free survival was reported to be >75% in both arms (78% versus 84%; $P = 0.73$), suggesting that outcomes were comparable[56].

In summary, the addition of radiosensitizers to radical radiotherapy of the bladder improves patient survival outcomes and should be considered in bladder-preserving treatments.

Radiotherapy to the bladder

Central to TMT is the use of radical radiotherapy. As an organ that changes in shape, size and position, it is important to consider bladder motion, different techniques used to accommodate this variability and the benefit of advances in technology.

Challenges in bladder radiotherapy. Effective radiotherapy is dependent on delivering a curative dose of radiation to the macroscopic tumour and any potential microscopic extension while minimizing the dose to normal tissues. A course of radical radiotherapy for MIBC spans 4–7 weeks, depending on the fractionation protocol used. Before treatment, patients undergo CT imaging, known as the radiotherapy planning (RTP) scan, which enables identification of the clinical target volume (CTV) and organs at risk (OARs) and formulation of the optimal radiotherapy plan. Various radiotherapy protocols are in use internationally. The current convention in the UK is for the entire bladder to be treated with the same dose; therefore, the CTV includes the whole bladder and any extravesical extension of the tumour[12,14]. Other treatment protocols might include an interim assessment of treatment response and, subsequently, reduction in the initial CTV to high-risk areas such as the tumour bed in patients with good response or proceeding to radical cystectomy in the event of inadequate response to initial treatment[3,57].

Regardless of the radiotherapy plan and protocol used, the urinary bladder does not remain static during the entire course of radiotherapy, both between treatments (interfraction) and during each radiotherapy treatment (intrafraction)[58]. As a hollow organ that fills and empties on a regular basis, the shape, size and position of the bladder changes as a result of both internal and external pressures. To overcome this anatomical variation and ensure consistent coverage during treatment, a safety margin is added to the CTV, forming the planning target volume (PTV). Consequently, a potentially large amount of normal tissue is irradiated when the bladder is relatively empty, whereas some of the CTV could be missed if the bladder is very full; different radiotherapy departments have different drinking protocols to ameliorate this risk. When treatment starts, daily low-dose CT images, known as cone-beam CTs (CBCTs), are taken using the linear accelerator, and the bony anatomy on the RTP and CBCT scans is matched to ensure that the patient is treated in the same position. Various studies have investigated bladder motion during a course of radiotherapy. Apart from a small study of ten patients[59], all other studies have concluded that maximum movement is in the anterior and cranial directions[58,60–63] (TABLE 1). These studies have given us a better understanding of bladder movement, thereby providing an important basis for adaptive radiotherapy.

Adaptive radiotherapy. Different adaptive radiotherapy strategies that account for internal anatomical changes have been developed to improve target coverage while reducing unnecessary doses to surrounding tissues.

The 'plan of the day' (POD) strategy involves the formulation of multiple treatment plans, with the best plan selected on the day of treatment on the basis of the CBCT scan findings[64]. The treatment plans are designed on the basis of the patient's initial RTP scan, which is then modified to generate three isocentrically grown CTV plans — a small, medium and large plan (FIG. 1). Before each day's treatment, a CBCT scan is performed and the most suitable plan that provides the best target coverage and the lowest dose to OARs is selected.

Several studies investigating the POD strategy have reported promising results regarding both feasibility and clinical outcomes[65–67]. Hafeez et al.[65] reported the outcomes of 55 patients who were unsuitable for daily radiotherapy or surgery and, therefore, treated with weekly hypofractionated radiotherapy using the POD approach. Among this group of patients who were unfit for radical treatment, 82% completed treatment and local disease control was achieved in 60% of patients, with a 4.3% rate of grade 3 late toxicity at 12 months. Improved normal tissue sparing with the POD method has also been demonstrated, with a 30% reduction in PTV in patients treated with adaptive radiotherapy compared with those treated using a nonadaptive approach[66]. Thus, adaptive radiotherapy has the potential to reduce the radiation dose to uninvolved surrounding tissues.

Another adaptive radiotherapy approach is known as the composite method[68]. In this approach, only one treatment plan is initially developed from the RTP scan. The patient is treated with this plan for the first few fractions of treatment, during which CBCT imaging is performed. These CBCTs are then averaged to generate a composite plan, which is used for subsequent fractions of treatment. This method corrects changes in bladder volume and position during the relatively longer time period between the RTP scan and the start of treatment than the POD approach, but it does not account for the random errors that occur with internal anatomical changes in the bladder between fractions or further changes in the bladder after the composite plan has been designed[67].

Despite an improvement over the use of a single plan, current adaptive radiotherapy approaches assume uniform bladder movement and expansion and do not consider intrafraction anatomical changes in the bladder. Furthermore, the generation of multiple radiotherapy

Table 1 | Studies on bladder motion

Study	Patients (n)	Methods	Findings	Refs
Interfraction and intrafraction bladder motion				
Nishioka et al. (2017)	29	• Fiducial markers implanted into tumour beds • Compared marker movement between caudal versus cranial, anterior versus posterior and left versus right wall tumours between fractions and at different time points within a fraction	• Anterior and cranial tumour groups showed larger interfractional movement than tumours on the opposite side (not statistically significant) • Increase in intrafraction movement over time in the cranial–caudal direction in cranial and anterior tumour groups • Increase in intrafraction movement in the left–right direction in right-sided tumour groups	58
Dees-Ribbers et al. (2014)	40	• Compared the effect of an empty and full bladder on bladder wall motion	• No statistically significant difference in bladder wall motion between patients with empty and full bladders • Maximum bladder movement was noted in the anterior and cranial directions	60
Intrafraction bladder motion				
Foroudi et al. (2013)	50	• Bladder motion compared on daily pretreatment and weekly post-treatment CBCT scans • Empty bladder protocol	• Maximum bladder movement was noted in the anterior and cranial directions • 1.2 cm anterior and 1.25 cm superior margins were required to account for intrafraction motion	61
McBain et al. (2009)	15	• Cine MRI scans on two occasions with bladder contoured at three different time points • Empty bladder protocol	• Main cause of motion was bladder filling • Maximum bladder movement was noted in the anterior and cranial directions	62
Interfraction bladder motion				
Fokdal et al. (2004)	15	• Compared bladder position on CT scans with different rectal and bladder filling protocols • Compared bladder position on post-treatment CT scan with that of the RTP scan	• Bladder and rectum volume influenced bladder movements • Maximum movement was noted in the anterior and cranial directions • 2.4 cm anterior and 3.5 cm cranial margins were required to ensure coverage compared with a standard isotropic margin of 2 cm	63
Meijer et al. (2003)	10	• Compared bladder position on weeks 1, 3 and 5 of treatment • Empty bladder protocol	• Maximum movement was noted in the posterior and cranial directions	59

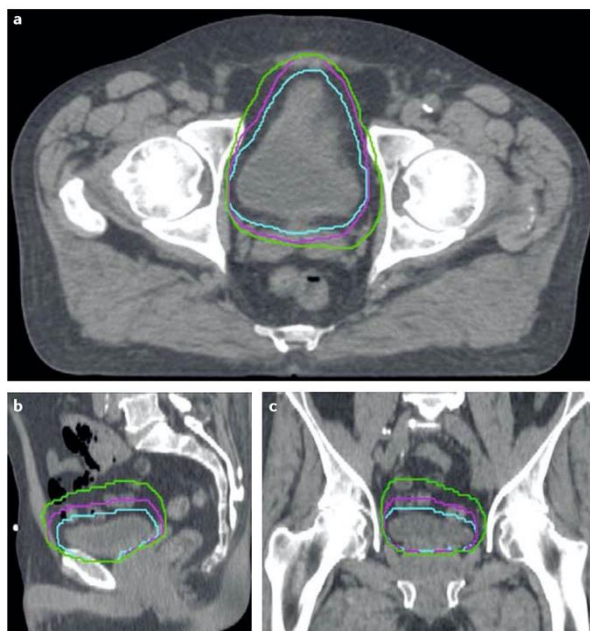CBCT, cone-beam CT; RTP, radiotherapy planning.

Fig. 1 | **Adaptive radiotherapy with the POD strategy.** The axial (part **a**), sagittal (part **b**) and coronal (part **c**) CT images show the clinical target volume (CTV) plans for the 'plan of the day' (POD) adaptive bladder radiotherapy strategy. The initial treatment planning CT scan is modified to generate three isocentrically grown CTV plans — a small (blue), medium (purple) and large (green) plan. Before each day's radiotherapy treatment, a cone-beam CT scan is performed and the most suitable CTV plan that provides the best target coverage and the lowest dose to organs at risk is selected.

plans is labour-intensive and the daily choice of treatment plan is dependent on subjective assessments made by the treatment team. The RAIDER trial[69] is an ongoing phase II randomized study of adaptive radiotherapy in the radical treatment of MIBC that will investigate both the feasibility and influence of adaptive radiotherapy and dose-escalated adaptive tumour boost radiotherapy. Patients will be randomized to receive treatment on the standard single radiotherapy plan, adaptive radiotherapy with the POD approach or adaptive radiotherapy with the POD approach and dose-escalated tumour bed boost. RAIDER is an important study as we continue to optimize the delivery of radical radiotherapy to improve patient outcomes. Patient recruitment is anticipated to end in June 2019.

MRI-guided radiotherapy. CBCT imaging has enabled image-guided and adaptive radiotherapy and, in turn, has improved accuracy while a patient is on treatment. However, the image quality of this form of low-dose CT imaging is poor. Identification of the urinary bladder is relatively easy on CBCT imaging compared with other techniques such as MRI, but distinguishing soft tissue boundaries and accurately defining surrounding organs

such as gastrointestinal structures and genitalia are more difficult with CBCT. By contrast, MRI produces superior soft tissue contrast, which offers consequent advantages for defining the target volume (CTV) and for daily evaluation of the anatomical structures for image guidance to ensure treatment reproducibility[70] (FIG. 2). The next generation of linear accelerators combined with MRI are in clinical use. In addition to increased accuracy of anatomical coverage, improved visualization of soft tissues enhances identification of the tumour bed and, therefore, the potential delivery of high-dose boosts to this high-risk region[71].

T2-weighted MRI is one of the basic pulse sequences of an MRI scan and enables tumour tissues to be distinguished from normal bladder tissues[72]. Thus, this technique could enable the accurate identification and delineation of the tumour bed and high-risk regions for disease recurrence, leading to the potential for dose escalation in this area. Improved imaging on treatment would also improve the accuracy of radiotherapy delivery, enabling a reduction in expansion margins. The combination of these two advantages of T2-weighted MRI will enable the safe delivery of a high dose of radiation to the tumour bed.

A study of different MRI-guided adaptive radiotherapy techniques with regard to target coverage concluded that online re-optimization enables normal tissue sparing and should be considered for use in bladder cancer[73]. Patients in this study were treated with a hypofractionation regimen with one treatment each week. The study assessed target coverage and normal tissue sparing with respect to intrafraction motion by performing MRI scans at 2-minute intervals over 10-minute periods on a weekly basis, before each treatment, and applying different margins. The original treatment plan was then modified and adapted to the first MRI scan of that week. However, as this was a relatively small study with only nine patients (eight male patients and one female patient), further confirmatory studies are required.

The role of MRI-guided radiotherapy has been explored in other urological tumours. In a systematic review, McPartlin et al.[74] summarized the potential role of MRI in prostate radiotherapy, particularly in terms of the detection of intrafraction motion. MRI-guided treatment has also shown encouraging results in other cancer types. For example, adaptive MRI-guided lung stereotactic body radiotherapy (SBRT) during each fraction of treatment has been found to provide better target conformality, thereby reducing the radiation dose to normal tissues[75], whereas MRI-guided radiotherapy for re-irradiation of the head and neck region enabled the use of smaller CTV to PTV margins and improved accuracy[76].

Apart from anatomical information, MRI can also yield functional or biological information by imaging perfusion with the use of diffusion-weighted MRI (DWI) and oxygenation through blood-oxygen-level-dependent (BOLD) MRI. In turn, this technique has the potential of identifying areas that require high doses of radiation or patients who would benefit from hypoxia modification.
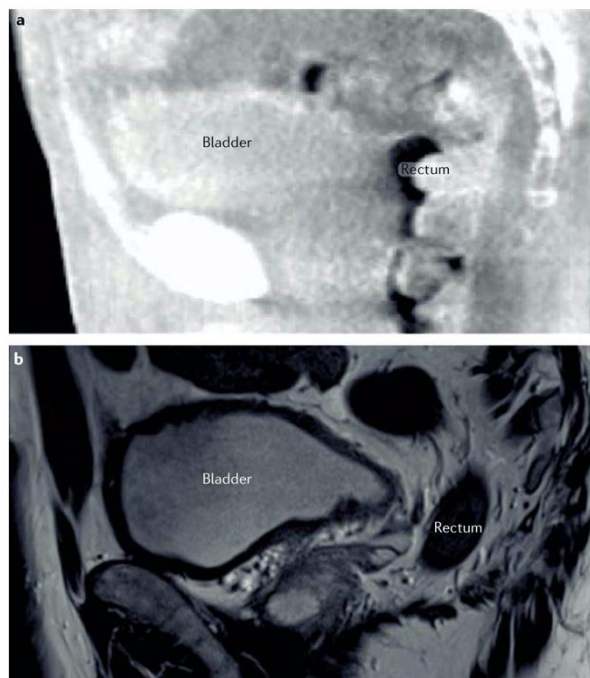
Fig. 2 | **Comparison of CBCT and MRI images of the pelvis.** Cone-beam CT (CBCT) (part **a**) and MRI images (part **b**) of the pelvis are shown. The bladder and rectum are as labelled to illustrate the difference in soft tissue visualization between the two imaging modalities.

DWI provides quantitative information to aid tumour assessment[77]. DWI examines the diffusion of water molecules (Brownian motion) and reflects the cell density in the region examined. DWI differs from T2-weighted MRI, which provides information only on water content. As tumours have a greater cell density than normal tissues, they have a greater restriction in diffusion and a lower apparent diffusion coefficient (ADC). The ADC value might be useful in determining the aggressiveness of tumours, with a low ADC value found in MIBC and high-grade tumours[78]. DWI has also been shown to predict response to chemoradiotherapy, with a multivariate analysis having identified ADC value as the only statistically significant and independent predictor of sensitivity to chemoradiotherapy[79]. However, the limitations of DWI in bladder cancers represent an important consideration given that water diffusion is also impeded in noncancerous tissues such as neurological tissues, lymphatic tissues and areas of fibrosis, which can lead to misdiagnosis[80].

BOLD MRI exploits the difference in magnetism of deoxyhaemoglobin and oxyhaemoglobin. As deoxyhaemoglobin is paramagnetic and oxyhaemoglobin is diamagnetic, oxygenated blood appears brighter on T2-weighted MRI and, therefore, MRI sequences can be manipulated to be sensitive to the level of deoxyhaemoglobin. Thus, increasing blood oxygenation through the inhalation of carbogen (95% oxygen and 5% carbon dioxide) during a BOLD MRI scan can help to identify patients who would benefit from a hypoxia-modifying agent during treatment[81].

MRI-guided radiotherapy is still in its infancy. Online adaptive radiotherapy requires time and is labour-intensive — unless the system is designed to generate an adaptive plan with deformable registration by warping the image obtained on treatment to fit the reference RTP image — and has the ability to perform effective and efficient software-based quality assurance[74]. In order to achieve software-based quality assurance in bladder radiotherapy, additional MRI-based studies examining bladder movement are needed, and adaptive radiotherapy strategies would need to be studied in large and varied patient populations.

Biomarkers

Over the past decade, unprecedented developments have been made in cancer genetics and genomics, enabling the exploration of molecular biomarkers in different cancer types, including MIBC. Importantly, noninvasive and invasive low-grade bladder cancers have been found to be different diseases with distinct pathogenetic pathways[82,83]. Invasive tumours are believed to originate from CIS and are associated with dysregulation of the p53 and retinoblastoma (RB) pathways whereas noninvasive tumours are associated with *FGFR3* and *HRAS* mutations[83–85]. Importantly, such molecular biomarkers might have prognostic and predictive value in bladder cancer and could inform treatment strategies.

Prognostic biomarkers. Prognostic biomarkers are biological features of a tumour that provide information about the general outcome of the disease and might help to identify patients who require treatment intensification. Importantly, despite predicting outcomes, prognostic biomarkers do not predict response to a specific treatment or intervention. Several potential prognostic biomarkers have been studied in urothelial bladder cancer, but they are not in routine clinical use as they have not been adequately validated and their clinical relevance has not yet been determined.

The tumour suppressor p53 is an important gatekeeper in G1–S cell cycle progression and has a key role in regulating cell growth and division[86]. Mutational inactivation of the *TP53* gene results in an altered p53 phenotype, and *TP53* alterations were found to be associated with increased risk of disease recurrence and a poor prognosis in patients with bladder cancer[87–89]. In bladder cancer, individual alterations in the levels of p53, cyclin-dependent kinase inhibitor 1 (also known as p21) and phosphorylated RB are associated with early recurrence and poor prognosis, whereas combinations of these alterations further enhance this prediction[90]. Indeed, the 5-year recurrence and survival were 93% and 8%, respectively, in patients with all three alterations compared with 23% and 70%, respectively, for those with a single alteration.

Gene expression profiling has been used to identify genes that might aid in bladder cancer diagnosis

and in predicting recurrence and progression[82,91–93]. Smith et al.[94] developed a 20-gene model that identified patients at high and low risk of lymph node metastasis, independent of age, gender, pathological tumour stage and lymphovascular invasion. As lymph node involvement is an important prognostic factor in bladder cancer recurrence and survival[95], the ability to accurately predict lymph node metastases using this model, after adequate validation, could prove invaluable and could help to select patients for neoadjuvant chemotherapy before definitive treatment.

Interest has been growing in the role of the immune system in disease outcome and treatment response. Lymphocytic infiltration has been reported to be related to clinical outcome in various cancer types[96]. In a cohort of patients with MIBC undergoing radical chemoradiotherapy to the bladder, pretreatment lymphopenia was associated with poor outcome, a finding that was also observed in a separate cohort of patients with advanced bladder cancer undergoing palliative chemotherapy for advanced urothelial cancer[97].

PD-1, a cell surface receptor expressed on T cells, has gained importance in various cancer types. In line with its role in immune regulation, interaction of PD-1 with its ligands, such as programmed cell death 1 ligand 1 (PD-L1), results in downregulation of the immune response[98]. By expressing PD-1 ligands, tumours can exploit this immune checkpoint in order to evade immune detection. PD-L1 expression in bladder cancer has been shown to be associated with the risk of disease progression and decreased survival[99–101]. The use of ICIs targeting either PD-L1 or PD-1 in the metastatic setting has improved survival outcomes, particularly in patients with high PD-L1 expression[39,102,103].

Receptor tyrosine kinases (RTKs) regulate a number of cellular processes, including cell proliferation and differentiation, and have an active role in cancer development and progression[104]. Overexpression of human epidermal growth factor receptor 2 (HER2; also known as ERBB2) is associated with aggressiveness and poor prognosis in urothelial cancer, specifically lymphovascular invasion, disease recurrence and decreased DSS and OS[105,106].

Prognostic biomarkers could possibly be used for the identification of patients with a poor prognosis who might require treatment intensification. However, although some biomarkers have both prognostic and predictive value (for example, PD-L1 expression), the prognostic value of most of the aforementioned biomarkers does not necessarily translate into predictive values; therefore, they might not be useful in guiding optimal treatment for an individual patient.

Predictive biomarkers. Predictive markers are biological features that predict response to an intervention or treatment. Despite still being at the developmental stage, predictive biomarkers have been identified that might aid patient selection for treatment options in bladder cancer, specifically for identifying patients who might respond well to radiotherapy or who might be better suited to surgery. However, further studies are now required to validate their predictive power and clinical value.

Double-strand break repair protein MRE11 is involved in activation of the DNA damage response (DDR) by forming part of the MRE11–RAD50–NBS1 (MRN) complex, which has an important role in detecting double-stranded DNA damage and repair[107]. On the basis of their role in DDR, increased expression of MRN complex proteins would be expected to predict poor radiosensitivity, but different studies have concluded the opposite. The expression of MRE11 has been shown to be predictive for DSS following radical radiotherapy in both a test and validation cohort, with high MRE11 expression being associated with improved 3-year DSS in patients with MIBC after radiotherapy. This predictive value could be due to the relationship between low MRE11 expression and impaired checkpoint arrest and/or reduced apoptosis, resulting in increased radioresistance[108]. In the same study, MRE11 expression was found not to be associated with survival outcomes in patients who underwent radical cystectomy, a finding that has been further validated in a separate patient cohort[109]. Thus, MRE11 expression might be a useful predictive biomarker for stratifying patients to receive either surgery or bladder-preserving treatment. However, further validation of these findings in other cohorts has been disappointing owing to problems associated with assay reproducibility[110]; therefore, MRE11 is not in current routine clinical use as a biomarker for patient selection.

The RTOG has reported that patients with HER2+ MIBC have a poor response to chemoradiotherapy; analysis of tumour samples from 55 patients enrolled in four RTOG bladder cancer studies revealed that HER2 expression status was associated with response rate following chemoradiotherapy[111]. In a biomarker-selective, nonrandomized study, patients with HER2+ MIBC were treated with anti-HER2 antibody trastuzumab in addition to chemoradiotherapy with paclitaxel, whereas HER2− patients were treated with chemoradiotherapy only. Complete response rates were similar between groups (72% in HER2+ group versus 68% in HER2− group) despite expectations that response rates would be lower in HER2+ patients[112]. This study was small (n = 76), and the radiosensitizing regimen used was non-standard, but the findings suggest the possibility of using HER2 status as a predictive biomarker for the addition of trastuzumab in order to improve outcomes in this patient group.

In addition to concurrent chemoradiotherapy, another option for radiosensitization in TMT is the use of hypoxia modification instead of chemotherapy. As a step towards biological stratification for the choice of radiosensitizer, retrospective analysis of tumour samples from the phase III BCON study (which evaluated the addition of concurrent carbogen and nicotinamide to radiotherapy) has enabled exploration of associations between tissue biomarkers and clinical outcomes and led to the identification of a number of potentially important predictive biomarkers for response to concurrent hypoxia modification. Specifically, necrosis, carbonic anhydrase IX (CAIX), hypoxia-inducible factor 1α (HIF1α) and a 24-gene signature have been shown to predict improved outcomes in patients treated with carbogen and nicotinamide. Eustace et al.[113] examined a
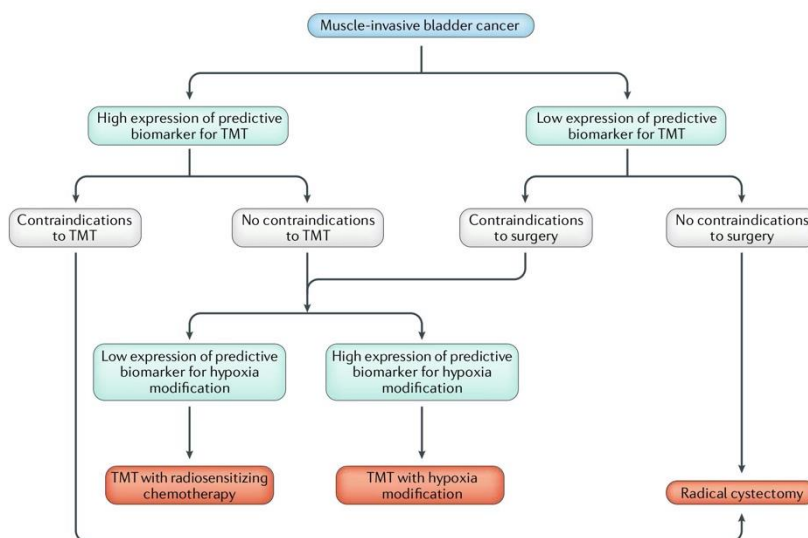
Fig. 3 | **Putative algorithm for personalized treatment with validated biomarkers.** The algorithm depicts a putative example illustrating the potential of a personalized treatment strategy using validated biomarkers. In the future, the expression of a predictive biomarker for trimodality bladder-preserving treatment (TMT) might help patients and clinicians decide between TMT and radical cystectomy. Furthermore, as discussed in the main text, predictive biomarkers to hypoxia modification such as double-strand break repair protein MRE11, carbonic anhydrase IX (CAIX), necrosis and a 24-gene hypoxia signature might also aid further decision with regard to choice of radiosensitizers. Importantly, this model is highly speculative and the listed biomarkers have not been adequately tested and should not be used in clinical practice until additional biomarker validation studies have been carried out.

variety of histopathological features in tumour samples from 231 patients enrolled in the BCON trial and found that necrosis and expression of the hypoxia marker CAIX independently predicted OS benefit from hypoxia modification. Another study that evaluated HIF1α expression by immunohistochemistry in tumour samples showed that patients with high HIF1α expression who were treated with hypoxia modification in combination with radiotherapy had a marked improvement in local relapse-free survival compared with those treated with radiotherapy alone, whereas no improvement was observed with hypoxia modification in patients with low HIF1α expression[114]. Similarly, a 24-gene signature that identified hypoxic MIBC tumours predicted benefit from the addition of hypoxia modification to radiotherapy[93].

In summary, a number of different biomarkers that predict response to bladder-preserving treatments in MIBC have been identified, mostly using retrospective data from randomized controlled trials, but few have been validated. With appropriate validation, these predictive biomarkers could aid clinicians and patients in the decision between treatment options and in formulating appropriate management plans. Biomarkers that predict response to radical cystectomy will be invaluable and could enable the development of algorithms to aid clinical decision-making regarding definitive treatment options (FIG. 3).

Conclusions

Advances in TURBT techniques, radiotherapy delivery and supportive care over the past decade have improved the outcomes of patients with bladder cancer receiving organ preservation treatments[3,57]. As discussed in this Review, meta-analyses and retrospective studies have suggested that response rates and survival outcomes of patients undergoing radical cystectomy and TMT are comparable. Thus, patients should be offered both options. Unfortunately, despite the potential influence on QOL, treatment choice is currently largely dependent on the centre at which the patient is treated[115] and the clinician's usual practice. We must look to exploit the ability to stratify patients using predictive biomarkers and improve the accuracy of radiotherapy delivery with new technologies, which will hopefully enable informed shared decision-making by clinicians and patients, thereby improving access of bladder preservation treatments to appropriate patients. When counselling patients and discussing potential treatment plans, a scientific approach will allow the clinician to offer patients truly bespoke management plans that are based on robust evidence. Importantly, this method would empower patients to make informed decisions on treatment choice that would have an effect on their long-term outcomes and QOL.

Published online: 13 June 2019

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Hypo-fractionation in muscle-invasive bladder cancer (MIBC): an individual patient data meta-analysis of the BC2001 and BCON trials

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Oral presentation at ASTRO 2019, Chicago, September 2019

Purpose/Objectives

There are two radiotherapy (RT) fractionation schedules used in the UK for treatment of MIBC: 64Gy in 32 fractions (f) given over 6.5-weeks and a hypo-fractionated schedule of 55Gy in 20 f over 4-weeks. Using an α/β ratio of 10, the EQD2 of 55Gy/20f is only 58.4Gy but the biological effect may be greater than this if the α/β is lower and the shorter overall treatment time reduces the effects of repopulation. The long term outcomes of several studies suggest that response, survival and toxicity of the two schedules are comparable, but there is no published direct comparison. This work was aimed to assess whether 55Gy/20f is non-inferior to 64Gy/32f in terms of invasive loco-regional control (ILRC) (pre-specified non-inferiority (NI) margin hazard ratio (HR)=1.25), and late bladder and bowel toxicity in MIBC patients (pre-specified NI margin for absolute difference 10%). Secondary endpoints included overall survival.

Materials/Methods

Individual patient data (IPD) were obtained from two prospective multicenter randomized controlled trials: BC2001 (NCT00024349), which assessed addition of chemotherapy to RT, and BCON (NCT00033436), which investigated adding hypoxia-modifying therapy to RT. In both trials fractionation schedule (64Gy or 55Gy) was according to local standard practice. One-stage IPD meta-analysis models both for time to event (ILRC/OS) and binary outcomes (toxicity) were used. Trial differences and clustering due to within-center correlation were accounted for in the models, as well as adjustment for randomized treatment received, baseline imbalances and potential confounding from relevant prognostic factors.

Results

782 patients (456 BC2001 326 BCON; 376 64Gy 406 55Gy) were included (mean age 72 years, 80% stage T1/2); median follow-up was 120 months. Both trials showed consistent benefit in ILRC by the addition of a radiosensitiser (combined HR 0.65, 95% confidence interval (CI) [0.49, 0.87]).

Patients who received 55Gy had a 29% lower risk of invasive ILRR than those who received the 64Gy schedule (adjusted HR=0.71, [CI 0.52, 0.96]); this benefit was seen when analysis was restricted to patients receiving RT alone (adjusted HR 0.72 [CI 0.49-1.05]). No differences in OS were found (adjusted HR=0.87, CI [0.72, 1.06]). The 2 fractionation schedules had a similar toxicity profile, with a difference in absolute risk of

experiencing a grade 3 or 4 late bladder or rectum symptom of - 3.82%, CI [-11.88%, 4.24%]).

Conclusions: In this IPD meta-analysis, the hypo-fractionated 55Gy/20f schedule was non-inferior to conventionally fractionated 64Gy/32f in terms of ILRC, OS and late toxicity. Superiority of 55Gy over 64Gy was shown for ILRC but not OS. Results indicate that hypo-fractionated RT is a safe and effective alternative to conventional RT in the primary treatment of MIBC.

Hypoxia Modification In Bladder Preservation: Relating Long Term Outcomes To Necrosis And Hypoxia

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Oral presentation at ESTRO 38, Milan, April 2019

Introduction: The BCON trial showed that the addition of carbogen and nicotinamide (CON) significantly improved recurrence free survival (RFS) and overall survival (OS) rates (Hoskin et al., 2010). Twelve years after the trial closed to recruitment, the long-term clinical outcomes and their relationship to hypoxia markers are reported.

Methods: An updated analysis of patients with bladder cancer treated in the BCON trial was undertaken. Cox regression was carried out to relate clinical outcomes to previously published biomarkers: a 24 gene signature hypoxia score (Yang et al., 2017) and necrosis status (Eustace et al, 2013).

Results: 333 patients were included in the original study. 12 patients were excluded from the analysis. Necrosis and hypoxia score were available for 148 of the remaining patients (73 RT+CON, 75 RT alone). There was a significant improvement in RFS at 5 years (41% vs 33%, p=0.040) which was maintained at 10 years (27% vs 20%). The 5 year OS was 49% vs 40% (p=0.068) with a continued effect seen at 10 years (32% vs 24%). The hypoxia score was prognostic in the RT alone group (p=0.041), but not in the RT+CON group (p=0.634). Necrosis status was a less strong prognostic indicator in the RT alone group (p=0.079) and had no effect in the RT+CON group. The prognostic value of both hypoxia score and necrosis remained following adjustment for other known prognostic factors. Hypoxia scores (p=0.013) (Figure 1) and presence of necrosis (p=0.05) were both independent predictors of benefit from hypoxia modification in the RT+CON group.

Conclusion: With long-term follow up, there continues to be an advantage in RFS and OS with the addition of CON to radiotherapy, with a statistically significant improvement in 5 year RFS. These findings confirm the significant impact of hypoxia modification on long-term survival for bladder cancer patients undergoing organ preservation treatment. Similarly, the presence of necrosis and hypoxia predicts long-term benefit from hypoxia modification. A prospective biomarker driven clinical trial based on this data is now required to validate the use of hypoxia modification in patients most likely to benefit.

Long Term Outcomes of Hypoxia Modification In Bladder Preservation Treatment: Update from BCON Trial

Y Song, H Mistry, A Choudhury, P Hoskin
Oral presentation at ASCO GU 2019, San Francisco, February 2019

Introduction: The mainstay of radical organ preserving treatment in muscle-invasive bladder cancer is radiotherapy (RT). Radiosensitising agents have been shown to improve treatment response and survival. Chemoradiotherapy is the most commonly used regime, however, hypoxia modification has also been shown to be effective. BCON is a phase III clinical trial which showed that the addition of carbogen and nicotinamide (CON) significantly improved recurrence-free survival (RFS) and overall survival (OS) (Hoskin et al, 2010). The long term outcomes twelve years after the trial closed to recruitment are reported.

Methods: Outcomes from the original BCON trial were updated and analysed. Patients excluded from the original analysis were excluded once again. Cox regression analysis adjusted for known prognostic factors was performed.

Results: 333 patients were randomized to receive RT alone or RT+CON. 9 patients from the RT+CON arm and 3 from RT alone arm were excluded from the analysis. The median age of patients in both arms was 74 (51-90) and the median follow-up was 10.3 years. There was a statistically significant improvement in 5 year RFS (41% vs 33%, p=0.04) with the difference maintained at 10 years (27% vs 20%). The 5 year OS was 49% vs 40% (p=0.068) with a continued effect seen at 10 years when OS was 32% vs 24%. These differences remained following adjustment for known prognostic factors.

Conclusions: The survival advantage for CON in the original analysis which demonstrated a significantly improved 3 years OS (59% vs 47%, p=0.020) is maintained in long term follow up. At 5 years there is a statistically significant improvement in RFS and at 5 and 10 years the addition of hypoxia modification to radiotherapy continues to show an effect on overall survival, These results confirm a sustained benefit of hypoxia modification in bladder preservation treatment for muscle-invasive bladder cancer.

Impact of bladder size at radiotherapy planning scan on survival

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Poster accepted to ESTRO 2020, Vienna, August 2020

Purpose or Objective: Radiotherapy is the mainstay of curative treatment for bladder cancer patients opting for organ preservation. Historically, the entire empty bladder is included in a single clinical target volume (CTV). An empty bladder aims to reduce dose to organs at risk and improve reproducibility. Advances in imaging and radiotherapy techniques allow visualisation of the bladder wall and delivery of higher radiation dose to the tumour bed. Such techniques require bladder-filling to separate bladder walls for tumour bed boost. We evaluate the impact of bladder size in radiotherapy planning (RTP) scans on outcomes.

Material and Methods: This retrospective study included all patients treated with radical chemoradiotherapy for urothelial carcinoma of the bladder in a tertiary cancer centre from 2010 to 2014. An empty bladder imaging and treatment protocol was used. The whole bladder and extravesicle extension of tumour was treated to a uniform dose of 52.5Gy in 20 fractions with weekly chemotherapy. Bladder volume was measured on RTP scan. Overall survival (OS) was defined as time from start of treatment to death and patients still alive were censored at time last known alive. Progression free survival (PFS) was defined as time to local or metastatic recurrence. Cox proportional hazard ratio was used to investigate the association of bladder volume with outcomes.

Results: 132 patients were included in this study. One patient had high grade T1 disease and all others had muscle-invasive cancers. None had distant metastases. 5 patients did not complete radiotherapy but all had at least 16 of planned 20 fractions. 79 patients had neoadjuvant chemotherapy. With a median follow up of 74.1 months, the median OS of patients was 73.2 months (58.7-108.4). Median PFS was 64.3 months (35.5-108.4). Mean bladder volume was 109.50cm^3 (39.2- 433.3). Due to the large range of bladder volumes, a log scale was used. Larger log(bladder volume) on RTP scan was associated with poorer OS (HR 1.78 p=0.03) and PFS (HR 1.71 p=0.03). This is not clinically significant after multivariate analysis. (Table 1).

	OS			PFS		
Univariate analysis	HR	95% CI	p-value	HR	95% CI	p-value
Log (Bladder Vol)	1.78	1.07-2.94	0.03	1.71	1.05-2.79	0.03
Age	1.04	1.01-1.07	0.02	1.03	1.00-1.06	0.06
CIS	2.41	1.26-4.61	0.01	2.25	1.18-4.29	0.01
Neo-adjuvant chemotherapy	0.54	0.33-0.87	0.01	0.60	0.38-0.96	0.03
Pre-treatment WCC	1.11	1.01-1.23	0.03	1.12	1.02-1.23	0.02
Hydronephrosis	2.33	1.32-4.11	0.003	2.39	1.38-4.13	0.002
Multivariate analysis						
Log (Bladder Vol)	1.44	0.85-2.45	0.18	1.45	0.87-2.43	0.15
Age	1.02	0.99-1.06	0.25	1.01	0.98-1.05	0.40
CIS	2.80	1.45-5.41	0.002	2.58	1.34-4.97	0.004
Neo-adjuvant chemotherapy	0.71	0.40-1.27	0.24	0.75	0.43-1.31	0.31
Pre-treatment WCC	1.10	0.98-1.23	0.11	1.10	0.99-1.23	0.07
Hydronephrosis	2.21	1.23-3.97	0.008	2.29	1.29-4.04	0.004

Table 1. Univariate and multivariate analysis of correlation between survival outcomes and bladder volume

Conclusion: Advances in radiotherapy technique allow more precise treatment plans. As clinical trials adopt bladder-filling protocols, it is vital that the impact of bladder volume on clinical outcomes is considered. Our relatively small study shows that bladder volume is not related to survival in a multivariate analysis. Factors like hydronephrosis and CIS impact survival, and should be considered in formulation of management plans. Bladder volume in this study reflects poor bladder emptying which may differ from planned bladder filling. Further evaluation in a prospective patient cohort with planned bladder filling will improve our understanding of the impact of bladder size on outcomes in the modern era.

Gender Inequality In Bladder Motion

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Poster at ESTRO Meets Asia 2018, Singapore, December 2018

Introduction: The central challenge in delivering bladder radiotherapy is in accurately predicting bladder motion. The shape, size and position of the urinary bladder is affected by internal pressure from bladder filling and external pressure from other pelvic organs. In order to account for bladder motion, a relatively large margin is added to the clinical target volume. This results in potentially increased dose of radiation to normal tissue when the bladder is small and inadequate coverage of the bladder when it is big. Various studies have looked into bladder motion during a course of treatment. However, little is known about the variability in bladder motion in male and female pelvises. We hypothesise that there is no difference in bladder movement in male and female pelvis.

Methods: 16 (13 male, 3 female) patients treated radically with concurrent chemo-radiotherapy were included in this retrospective study. The bladder was contoured on the original radiotherapy planning (RTP) scan, cone beam computed tomography (CBCT) at fraction 1 and a further CBCT towards the end of treatment. The distance in which the bladder walls had moved from RTP scan was measured in the anterior, posterior, superior, inferior, left and right directions. The average movement in each direction is then compared using SPSS v25

Results: There is bladder wall movement in all directions. The difference in movement between male and female patients is greatest in the posterior direction. In female patients, the average movement posteriorly ranged from 0.37cm at first fraction to 1.23cm at later fractions while in male patients, this remained relatively stable. There was no marked difference in movement of male and female bladders in other directions.

Conclusion: It is important to consider organ motion so as to deliver radiotherapy accurately. As the bladder sits amongst other pelvic organs, it is vital to consider the difference between male and female pelvis. The presence of a mobile uterus can easily affect bladder position between each fraction of treatment, and similarly the comparatively less mobile prostate posteriorly may account for the marked reduction in posterior motion of a male bladder. This study provides preliminary results when exploring the different way bladders may behave in male and female pelvis. Data collection continues in order to increase the sample size and determine whether a significant association exist.