Clinically Significant Prostate Cancer: Biological and Epidemiological Observations to Improve Cancer-Free and Survival Metrics

by

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A thesis submitted in conformity with the requirements for the
Degree of Doctor of Philosophy (Clinical Epidemiology and Health Care Research),
Institute of Health Policy, Management, & Evaluation,
University of Toronto

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ABSTRACT

Background: Due to very high disease-specific survival following prostate cancer treatment, disease progression (metastasis) and treatment-related complications may significantly affect a patient's life trajectory. Using distinct epidemiologic methodologies, this thesis sought to (1) identify novel microRNA predictors of metastasis following radical prostatectomy; (2) examine the association between local treatment modality (surgery or radiotherapy) and androgen deprivation therapy (ADT) on non-prostate cancer mortality; and (3) study the association between radiotherapy for prostate cancer and secondary malignancies.

Methods: We conducted a matched-case control study of 38 patients who underwent radical prostatectomy using bootstrapping with automated backward selection to identify miRNA sequences which were significantly associated with metastasis. To examine the association between treatment modality and non-prostate cancer mortality, we performed a propensity-score matched, population-based retrospective cohort study of 10,786 men treated for non-metastatic prostate cancer in Ontario between 2002 and 2009. We used the Fine and Gray method with generalized estimating equation survival models with a sandwich variance estimator to calculate the sub-distribution hazard ratio of treatment effect, accounting for ADT exposure in a time-

varying manner. To assess the association between radiotherapy for prostate cancer and the development of secondary cancers, we performed a systematic review and meta-analysis utilizing random-effects models and Mantel-Haenszel weighting.

Results: We identified a panel of five microRNA which were associated with metastasis following surgery (AUC 89.5%, 95% CI 79.5-99.5). Treatment with radiotherapy was independently associated with an increased risk of non-prostate cancer mortality (HR 1.57, 95% CI 1.35-1.83) though ADT exposure was not (p = 0.26 - 0.87 depending on analytic strategy). Radiotherapy was associated with an increased risk of bladder (aHR 1.67, 95% CI 1.55-1.80), colorectal (aHR 1.79, 95% CI 1.34-2.38) and rectal cancers (aHR 1.79, 95% CI 1.34-2.38) but not hematological (aHR 1.64, 95% CI 0.90-2.99) or lung (aHR 1.45, 95% CI 0.70-3.01) cancers.

Conclusions: Varied epidemiologic techniques may be used to characterise outcomes following prostate cancer treatment. These data may inform patients and physicians when making decisions regarding prostate cancer treatment choice.

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CHAPTER 1: INTRODUCTION AND OBJECTIVES

1.1 Study rationale

Prostate cancer is the most common malignancy in men apart from non-melanomatous skin cancer with an estimated 233,000 new cases diagnosed annually in the United States[1] and 23,600 in Canada[2]. Due to the underlying disease biology, early detection and efficacious treatments, five-year relative survival for men newly diagnosed with prostate cancer exceeds 99%[3].

The vast majority of men newly diagnosed with prostate cancer undergo active treatment, whether by surgery (radical prostatectomy), radiotherapy, or androgen deprivation therapy (ADT)[4]. There are many potential outcomes of treatment, both from an oncologic and functional perspective. Considering oncologic outcomes, patients may remain free of disease or may experience recurrence followed by progression to metastasis. Currently, available prognostic factors available include grade, stage and serum prostate-specific antigen. These are insufficient to accurately predict patient outcomes[5,6]. Therefore, there is a significant need for the identification of novel markers of prostate cancer progression and metastasis following treatment. There are advantages and disadvantages to both surgery and radiotherapy in the treatment of localized prostate cancer which patients must consider when deciding on treatment options. These are primarily based on functional outcomes including urinary incontinence, erectile dysfunction[7], and many others[8-10]. ADT may be given as neoadjuvant (prior to primary therapy) or adjuvant (following primary therapy) therapy for patients with locally advanced prostate cancer undergoing radiotherapy[11] and for patients with lymph node metastasis after radical prostatectomy[12] due to improvements in prostate cancer mortality. ADT is associated with an increased risk of cardiovascular[13] and skeletal-related events[14]. It is unclear whether the

choice of surgery or radiotherapy and the provision of ADT affects non-prostate cancer mortality as some have hypothesized[15,16]. Finally, a complication unique to patients undergoing radiotherapy is the development of a treatment-related secondary malignancy. However, the risk of such an event is controversial with some studies reporting an increased risk[17,18] while others report no association[19,20].

Both disease recurrence and functional complications may significantly affect patients' quality of life[21-23]. Given the very high survival following prostate cancer treatment, these issues are of the utmost importance and thus we sought (1) to identify novel predictors of metastasis following surgical therapy; (2) to assess the differential non-prostate cancer mortality among patients undergoing curative local therapy; and (3) to synthesise available data regarding the association between prostate radiotherapy and secondary malignancies.

1.2 Study objectives

Project 1:

To identify novel miRNA sequences which can distinguish patients with metastasis following radical prostatectomy from those who do not develop metastasis.

Project 2:

To determine rates of (a) non-prostate cancer mortality and (b) cardiovascular mortality among men treated with surgery or radiotherapy for non-metastatic prostate cancer and examine the influence of ADT on these rates.

Project 3:

To synthesize current evidence regarding secondary malignancies in patients with prostate cancer and examine the role of radiotherapy in their development.

CHAPTER 2: BACKGROUND

2.1 Prostate cancer epidemiology

Prostate cancer is the most common malignancy in men apart from non-melanomatous skin cancer with an estimated 233,000 new cases diagnosed annually in the United States[1] and 23,600 in Canada[2]. The incidence of prostate cancer peaked in Canada twice, in 1993 and in 2001, coinciding with intensified usage of PSA screening; however, since 2006, the incidence has been declining[2]. Similar phenomena have been observed in the United States with a decline of 2.0% in prostate cancer incidence in recent years[1].

Since 1991, prostate cancer mortality has decreased by more than 40%[24] due to a combination of prostate-specific antigen (PSA) screening and improvements in treatment[24]. Despite this decline, prostate cancer remains the second most common cause of cancer-related death amongst men[1,2].

2.2 Prostate cancer carcinogenesis and risk factors

The underlying disease biology and pathogenesis of prostate cancer are multi-factorial and poorly understood. Demographic, diet and lifestyle, inflammatory, and genetic factors are putative factors thought to be associated with prostate cancer incidence and prognosis.

2.2.1 Demographic factors

Age is a well-established risk factor for prostate cancer. Men under the age of 40 are very unlikely to be diagnosed with the disease while those over the aged of 70 have a greater than 1 in 8 chance of diagnosis[3].

Issues following prostate cancer treatment

A family history of prostate cancer is strongly predictive of a man's risk of prostate cancer. The strongest risk is associated with hereditary prostate cancer, a subset of the disease with an inheritance pattern in keeping with Mendelian transmission of susceptibility genes[25]. To be considered hereditary prostate cancer, a family must have three affected generations, three first-degree relatives affected, or two relatives diagnosed prior to age 55[25]. Family history is an important risk factor even among men for whom a clear hereditary pattern is not demonstrated. Familial prostate cancer refers to clustering of the disease within family groups. Men with one first-degree relative previously diagnosed with prostate cancer have a risk of prostate cancer diagnosis that is two to three times that of individuals without a family history[26]. In addition, the number of affected family members and their age at diagnosis are related to an individual's risk of prostate cancer[27]. While many tumors that exhibit familial, but not hereditary, inheritance likely have an inherited, genetic component, the vast majority of these are not yet recognised. The prognostic importance of a family history following prostate cancer diagnosis and treatment is unclear with most studies finding no significant association[28-30].

Both prostate cancer incidence and mortality have been shown to be significantly related to race. Men of African descent are well recognized as having the highest risk of both prostate cancer diagnosis and mortality[31]. Conversely, men of Asian descent have a decreased risk compared to white men[32]. However, within the United States population, there does not appear to be differences in prostate cancer grade between racial groups[33], though preliminary work suggests that Asian-American men were more likely to present with unfavourable risk disease[34]. The relative components of genetic similarity, socioeconomic factors and shared cultural and environmental characteristics in the racial differences observed are poorly understood. Men moving from low-incidence countries to those with high-incidence experience

a shift in prostate cancer risk towards rates expected in their new country of residence[27]. However, lifestyle differences are likely insufficient to explain differences in prostate cancer risk and underlying genetic factors are likely to be involved[35], as substantial variation in risk allele frequencies was demonstrated between distinct ethnic populations[36].

2.2.2 Diet and lifestyle

The first evidence of the role of diet and lifestyle in prostate carcinogenesis came from ecological studies demonstrating that "Western" nations had higher rates of prostate cancer than developing countries[37,38]. Subsequent studies have demonstrated that as "non-Western" countries adopted lifestyles more in keeping with "Western" mores, rates of prostate cancer increased[39,40]. The association between specific dietary components and prostate cancer risk is unclear and remains a field of active study[41-44]. However, a diet high in fruits and vegetables and low in fat, meats, and dairy has been suggested to be possibly effective in preventing prostate cancer (reviewed by Ma et al.[44]). Specifically, intake of lycopene, cruciferous vegetables, vegetable fats, and coffee may be associated with improved prognosis following a prostate cancer diagnosis[45].

The association between dietary supplementation with specific vitamins and minerals and the risk of prostate cancer has been a topic of considerable interest. The best-known examples are of vitamin E and selenium which were examined the Selenium and Vitamin E Cancer Prevention (SELECT) trial. This study found that neither supplement individually nor in combination reduced the risk of prostate cancer[46]. Despite a hypothesized protective effect, studies of vitamin D have shown no relationship or an increased risk[47,48]. Though not consistent,

increased calcium intake has been associated with an increased risk of aggressive prostate cancer[44,49].

Additional lifestyle factors have been shown to moderate the risk of prostate cancer. Pooled evidence suggests that physical activity may provide a small decrease in the risk of prostate cancer, driven primarily by an effect on advanced disease[50]. Maintenance of a healthy body weight may also improve prostate cancer prognosis[45]. Further, frequent ejaculation has been associated with a decreased risk of prostate cancer, in this case, driven primarily by a decrease in low-risk disease[51].

2.2.3 Medication

There is emerging evidence that commonly used medications including HMG-CoA Reductase inhibitors (statins) and metformin may be associated with a lower risk of prostate cancer mortality following a prostate cancer diagnosis[52]. Other common medications including non-steroidal anti-inflammatory drugs (NSAIDs); anti-hypertensives including beta-blockers, angiotensin-converting enzyme (ACE) inhibitors, and calcium channel blockers; and others including insulin, digoxin, acetylsalicylic acid, and warfarin have also been examined with inconclusive results[52].

2.2.4 Inflammation

Chronic inflammation has been implicated in the development of many cancers[53], including prostate cancer[54,55]. Postulated etiologic factors include infectious agents, dietary carcinogens, hormonal imbalances, and physical and chemical trauma[55]. Intra-prostatic

inflammation, driven by these factors, may result in DNA damage, epithelial cell proliferation and turnover and angiogenesis[55]. Taken together, these factors promote carcinogenesis.

2.2.5 Genetic factors

Over the last 20 years, a significant body of literature has emerged showing that carcinogenesis is in large part the result of genetic and/or epigenetic changes to protein-coding oncogenes and tumor suppressor genes. Prostate cancer is known to have an extraordinarily complex genetic makeup including somatic copy number alterations, point mutations, structural rearrangements and changes in chromosomal number (Table 2.1)[56]. The genetic and epigenetic changes which underlie prostate carcinogenesis may occur at many levels and may occur in the host germline DNA (host factors) or in the tumor genome only (tumour factors).

Epidemiologic evidence suggests that 5-10% of all prostate cancers may be caused by dominantly inherited genetic factors[26]. Of these, there a number of potential hereditary prostate cancer genes including HPC1, HPC2, HPC20, HPCX, PCAP, and CAPB[57,58]. In addition, mutations in BRCA1 and BRCA2 have been shown to increase the risk of clinically-significant prostate cancer[59] and on prostate-cancer specific mortality among men with screen-detected prostate cancer[60]. Studies have identified numerous single nucleotide polymorphisms in prostate cancer susceptibility[61-64]. Even in aggregate, these factors account for only a small proportion of all prostate cancer cases and frequent mutations with prognostic or predictive value have not been identified in prostate cancer, unlike many other solid tumors. Instead, as with many other cancers, research has focused on tumour-level epigenetic changes in expression through mechanisms including messenger RNA, biochemical modification of histones supporting DNA, modification of the DNA itself, and expression of non-coding RNAs, including miRNAs.

Issues following prostate cancer treatment

As miRNA expression levels dynamically reflect tumour biology, miRNA function as complex regulators of genetic expression, and miRNA have a demonstrated role in carcinogenesis, they are considered promising targets for cancer diagnosis and novel therapeutics[65,66]. Further, miRNA are biologically stable due to their resistance to endogenous RNase activity and small size[67]. Therefore, miRNA are stable biomarkers which may reliably be quantitated in formalin-fixed tissue and biological fluids[67]. This both facilitates research endeavours and supports the clinical utility of miRNA-based biomarkers.

Table 2.1. Genetic changes associated with prostate cancer tumorigenesis.

Genetic change	Description	Mechanism	Example
Somatic copy number alterations (SCNAs)	Gain or loss in genetic material	Role in both oncogenic activation and tumor suppressor inactivation	Deletions on chromosome 10q leads to PTEN LOF[11]
Structural rearrangements	Improper repair of DNA breaks leads to intra- and inter-chromosome rearrangement	Rearrangements place otherwise unrelated genes in juxtaposition	Fusion of TMPRSS2:ERG results in oncogenic activation of ERG under the control of the TMPRSS2 androgen-response element[14]
Point mutations	Changes in specific nucleotides or amino acids resulting in altered gene products	Nucleotide changes result in proteins with altered function or stability	HOXB13 G84E variant confers an elevated risk of prostate cancer, specifically early-onset or hereditary through regulation of transcription of AR target genes[46-49]
Single nucleotide polymorphisms (SNPs)	Variation in a single nucleotide differing between individuals or chromosomes	SNPs act as markers in gene-mapping. When occurring within a gene, SNPs may directly affect gene function	SNPs in MSMB have been shown to affect the expression of NCOA4 which is an AR co-activator[61]
miRNA	Small, non-coding RNA molecules which modulate mRNA expression	The majority result in down-regulation though a few cause up-regulation or destruction of the target mRNA	MiR-21 targets PDCD4 and PTEN mRNAs and causes decreased apoptosis[80]

PTEN: phosphatase and tensin homolog; LOF: loss of function; TMPRSS2: transmembrane protease, serine 2; ERG: ETS-related gene; HOXB13: homeobox 13; AR: androgen receptor; MSMB: beta-micro-seminoprotein; PDCD4: programmed cell death 4.

2.2.6 MicroRNA (miRNA)

MicroRNAs (miRNAs) are a class of small non-coding RNA which modulate messenger RNA (mRNA) expression through direct binding. The 5' end of the miRNA binds via a targeting "seed" region to a complementary sequence in the 3' mRNA transcript. The strength of this bond depends on the sequence and number of seeds. For the most part, miRNA-mRNA interactions result in down-regulation though a small number cause either up-regulation or complete destruction of the mRNA target.

MiRNA are initially transcribed as a longer primary transcript (pri-miRNA) by RNA polymerase III[68]. Subsequently, they undergo modification and cleavage in order to produce the next precursor, pre-miRNA. This precursor is then exported from the nucleus to the cytoplasm for further processing. The enzyme Dicer cleaves the pre-miRNA product into a mature 19-24 nucleotide duplex[69]. One strand of this duplex (mature miRNA) is incorporated in the RNA-induced silencing complex (RISC)[69]. Where there is perfect or near perfect complementarity between the miRNA and the 3' UTR of the target mRNA, the RISC cleaves the target mRNA. In the case of imperfect matching, there may be either translational silencing of the target or reduction in the amount of target mRNA[69].

Significantly less research focus has been dedicated to understanding factors which regulate miRNA expression, compared with that expended to understand the regulatory effects of miRNA. Recently, Gulyaeva and Kushlinskiy reviewed mechanisms of miRNA expression regulation[70]. While there are complex, multilevel effects which depend on cell type physiologic context, regulation of miRNA expression can be categorized as transcriptional or post-transcriptional[70]. First, miRNA expression may be regulated through miRNA processing. As discussed in the prior paragraph, pri-miRNA are modified prior to export from the nucleus.

Defects in these modifications (cleavage and adenylation) may affect miRNA expression and function. Additionally, miRNA editing, such as adenosine-to-inosine RNA editing has been shown to affect the stability and function of miRNA[71]. Further, defects in the enzymes (Drosha, Dicer, and others) involved in miRNA processing may also contribute to aberrations in miRNA function. This has been shown to contribute to the oncogenesis of nonepithelial ovarian cancers[72]. There are a number of proteins with chaperone-like functions for miRNA. Among these, Argonaute proteins haven been demonstrated to increase mature miRNA expression, decrease miRNA degradation and increase miRNA half-life[73,74]. Ribonucleases may also contribute to miRNA stability[75]. MiRNA expression may be modulated in concert with the expression of host genes where the miRNA is encoded through the action of transcription factors and DNA methylation[70]. Finally, miRNA expression have be regulated by various physiological and pathologic stimuli, including endogenous hormones, cytokines, pharmacologic interventions, and hypoxia[70].

The role of miRNA in cancer was first demonstrated in leukemia[76]. Since then, it has been discovered that altered expression of miRNA contributes to most, if not all, human cancers. Furthermore, it has been found that miRNA may either initiate carcinogenesis or drive disease progression[76].

Unlike somatic DNA mutations, miRNA expression is dynamic and both miRNA expression and target may vary within the same cell depending on time or circumstance. This allows for significant signal amplification as a single protein may act via a small number of miRNAs to influence many genes[77].

Alterations in miRNA expression may themselves be driven by either genetic or epigenetic changes. Many miRNAs are located in genetically unstable sites where they are

prone to deletion or rearrangement in cancer[78]. In addition, miRNA function may be affected by mRNA mutation in the target site. Epigenetically, many miRNA genes are located next to CpG islands where they may be prone to epigenetic silencing. This phenomenon has been documented to be relevant in urologic malignancy[79-82].

MiRNA genes may be located either within coding mRNAs or in the intergenic region. Approximately one-third are clustered while the remainder are solitary. In clusters, single events may affect several miRNAs and subsequently thousands of protein targets.

Porkka et al. published the first report describing miRNA expression in prostate cancer in 2007[83]. They compared benign and malignant cells are found that many miRNAs were either up or down regulated. Hundreds of reports have subsequently looked at the role of miRNA in prostate cancer and over 30 unique miRNAs have been implicated.

Change in miRNA expression have been implicated in many of the key events in carcinogenesis. These are briefly reviewed below.

2.2.6.1 Apoptosis avoidance

One of the most important events in carcinogenesis is the avoidance of apoptosis. Thus far, at least 10 different miRNAs have been found to be involved in this process. In many cases, this follows a cascade pattern.

In prostate cancer specifically, miR-21 has been found to target both PDCD4 (programmed cell death 4) and PTEN (phosphatase and tensin homologue) mRNAs in order to decrease apoptosis. Furthermore, miR-21 contributes to apoptosis through the p53 network in a mechanism that seems to be preserved throughout many malignancies[84].

A recurrent theme in miRNA mediated genetic expression is multiple targeting and feedback loops. In apoptosis avoidance, this is seen in the miR-34 family whose expression is partly controlled by p53[85]. Loss of p53 activity results in decreased miR-34a expression which subsequently decreases targeting of the SIRT1 (silent information regulator 1) locus. As a result, up-regulated SIRT1 results in further down-regulation of p53 and decreased apoptosis. Due to this, miR-34a/b/c are down-regulated and induce their own effects.

2.2.6.2 Cellular pathways

Apart from apoptosis avoidance, cell cycle regulation, intracellular signalling, DNA repair and adhesion/migration are all affected by miRNA. *In vitro* experiments have shown that there is up-regulation of miR-221/222 in the PC3 cell line[86]. By targeting p27(kip1), these miRNAs induce cell proliferation through inhibition of this cell cycle checkpoint. Furthermore, miR-15a and miR-16-1 are down regulated in a majority of prostate tumors[87]. This results in an up-regulation of cyclin D1 which facilitates the G1/S transition and cellular proliferation. In addition, these miRNAs target WNT3a so their loss results in WNT activation which is carcinogenic. There is significant evidence that there is an interaction between miRNAs and key carcinogenic events – for example, miR-21 up-regulation can reduce apoptosis, induce proliferation and assist cell migration[88].

2.2.6.3 Androgen signalling

MiRNAs are intricately involved in a complex feedback loop involving androgen signalling. Androgen responsive miRNAs modulate the androgen pathway. For example, mi-125b contains an androgen-responsive element (ARE) within its promoter[89]. *In vitro* studies

have shown that miR-125b up-regulation leads to androgen-independent growth in LNCaP cells and decreases apoptosis through targeting of BAK1, BBC3, and p53[90]. MiR-21 also contains an ARE in its promoter and, through multiple channels, may be involved in androgen insensitivity. MiR-141 was recently found to be the most strongly regulated by androgen signalling in cell culture and xenografts and is also over-expressed in prostate cancer[91]. Interestingly, miR-141 is up-regulated in human prostate cancer. In addition, miR-146a acts upon ROCK1, a kinase involved in the development of castrate resistant prostate cancer. Sun et al. found that there was up-regulation of miR-221/222 in androgen-resistant versus androgen-sensitive cells[92]. Manipulation of the levels of these miRNAs altered the cellular response to dihydrotestosterone (DHT), as measured by PSA and promoted the development of androgen-independence.

There is also crosstalk between miRNAs and other signally pathways through shared transcription factors. ERBB-2 (Her2-neu) is a tyrosine kinase receptor that is over-expressed in some prostate cancers. Loss of miR-331-3p appears to up-regulate ERBB-2 expression. *In vitro* expression of miR-331-3p suppressed ERBB-2 expression and prevented androgen signalling[93]. This occurred in an androgen receptor (AR)-independent manner and was enhanced by the administration of bicalutamide. Looking at networks of related genes, Wang et al. found that miR-331-3p was among the central 20 RNAs altered between low- and high-risk prostate cancers[94].

2.2.6.4 Epithelial-mesenchymal transition

We have previously demonstrated the miR-301a directly targets p63, a tumor suppressor, in order to promote epithelial-mesenchymal transition[95]. As with other miRNA discussed above, miR-301a acts through a cascade: inhibition of p63 leads to miR-205 down-regulation which

releases ZEB 1 and ZEB 2 from inhibition and results in suppression of E-cadherin. This mechanism was found to be valid in both cell lines and patient-derived tumors.

In summary, aberrations of miRNA expression have been found to affect numerous pathways which are important in the pathogenesis of prostate cancer. Further research offers the opportunity to identify miRNA expression patterns which may be useful in prostate cancer diagnosis, prognosis, and treatment.

2.3 Prognostic factors

2.3.1 General considerations regarding prognostic factors in oncology

Prognostic factors are clinical or biological characteristics which are objectively measurable and provide information regarding the likely outcome of a disease process[96]. In oncology, prognostic factors may fall into a series of categories and serve many purposes. Prognostic factors may be tumor-related, host-related or environment-related[97]. Tumor-related prognostic factors are the best studied and described. Traditionally, these include histologic type and grade, local tumor extent, and metastatic disease. Over the past decade, extensive molecular research has expanded this category to include serum tumor markers, hormone receptors, proliferation markers, and genetic mutations. Host-related prognostic factors include age, gender, ethnicity, comorbidity, and performance status. Though these may or may not be related to the presence of the cancer, they have a profound impact on disease treatment and overall prognosis. Age is a well described prognostic factor in oncology which has also been found in prostate cancer[98,99] as has black ethnicity[100]. Finally, environmental factors such as access to

health care, health care policy, quality of care delivered and choice of treatment are external to the patient but may have a significant impact in their outcome.

Prognostic factors may be used to group patients into homogenous populations, understand the natural history of cancer, to compare or predict the results of treatments, identify patients with either favourable or adverse features, and/or plan follow-up. In order to offer the potential for clinical benefit, a prognostic factor must be identifiable either at the time of diagnosis or early in the disease process in order for management and patient counselling to be modified. Patients who are at high risk for cancer progression or recurrence based on prognostic factors have the greatest potential to benefit from both initial and subsequent adjuvant therapy. In contrast, those who are determined to be at low risk could be spared the toxicity of unnecessary treatment.

While molecular prognostic factors have been gaining increasing prominence in oncology, the clinical scenario which is perhaps the most analogous and informative to prostate cancer has been that of breast cancer. Historically, tumor grade and stage were the only prognostic factors available[101]. However, over the past few decades, a number of gene arrays have been introduced which allow both prognostication as well as treatment stratification in breast cancer[102]. Estrogen receptor (ER), progesterone receptor (PgR) and human epidermal growth facts 2 (HER2), and the Ki-67 proliferation index are the most prominent of these and can be used to define specific subtypes of breast cancer[102]. These have been used to characterize the natural history of subtypes of breast cancer and to make treatment decisions such as the provision of chemotherapy and endocrine therapies[103]. It has also allowed the development of biologically targeted therapies (eg. anti-HER2 antibodies, Herceptin) directed at these targets[103].

While it is clear from other tumor sites that prognostic factors can significantly alter cancer management, efforts to transition predictive factors for primary and adjuvant treatment outcomes in prostate cancer from research to clinical applications have thus far proven unsuccessful.

2.3.2 Prognostic factors in prostate cancer

Patients with clinically localized prostate cancer may experience a phenotypically wide spectrum of natural history ranging from indolent tumors which will never require treatment to highly aggressive, metastatic and ultimately fatal cancer. Distinguishing between these remains one of the most important open questions in the management of these patients. Histologic tumor grade, tumor stage, and prostate specific antigen (PSA) level at the time of diagnosis have been reliably shown to be important in prognostication and useful in patient management[104].

2.3.2.1 Traditional, clinical factors

Tumor stage is assessed using the American Joint Committee on Cancer TNM staging system based on the local extent of the tumor (T), the extent of spread to lymph nodes (N), and the presence of metastasis[104]. Since it was first introduced in 1977, this system has undergone multiple iterations and is now in its 7th edition[105]. Local tumor extent is evaluated on a scale from 1 to 4 based on its size and anatomic involvement; regional nodal status is assessed as involved or uninvolved; and metastasis is assessed as present or absent (Table 2.2). Tumor stage is a strongly prognostic for biochemical recurrence, metastasis, prostate cancer-specific mortality, and overall survival[106].

Table 2.2. TNM classification of prostate cancer.

T: Primary tumor	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Clinically in-apparent tumor, not palpable or visible by imaging
T1a	Tumor incidental finding in ≤ 5% of resected tissue
T1b	Tumor incidental finding in > 5% of resected tissue
T1c	Tumor identified by needle biopsy
T2	Tumor confined to the prostate
T2a	Tumor involving ≤ half of one lobe
T2b	Tumor involving > half of one lobe, but not both lobes
T2c	Tumor involving both lobes
T3	Tumor extends through prostate capsule
T3a	Extracapsular extension (ECE), including microscopic bladder neck involvement
T3b	Tumor involving seminal vesicle(s)
T4	Tumor involving adjacent structures (external sphincter, rectum, levator, pelvic wall)
N: Regional lymph nodes	
NX	Regional lymph nodes cannot be assessed
N0	No evidence of regional lymph node metastasis
N1	Evidence of regional lymph node metastasis
M: Distant metastasis	
MX	Distant metastasis cannot be assessed
M0	No evidence of distant metastasis
M1	Evidence of distant metastasis
M1a	Non-regional lymph nodes
M1b	Bone
M1c	Other, visceral sites

Tumor grade, quantified using the Gleason scoring system[107], is the strongest clinical prognostic factor in patients with clinically-localized prostate cancer[108]. The Gleason score is a low-power microscopic assessment of tumour histology including tumour differentiation, architecture and morphology. The more abnormal the tumor architecture, the higher the Gleason score. The evaluating pathologist determines the characteristics of the two most common growth patterns within the tumor. A score from one to five is assigned to each and the Gleason score is derived from the sum of these. If the third most common pattern is of a higher grade than the second most common, the first and third most common growth patterns are summed to calculate

the Gleason score. While patterns 1 and 2 were initially described, these are no longer commonly used[109]. Gleason 6 is the lowest grade currently used in clinical practice.

Prostate specific antigen (PSA) is a glycoprotein synthesized in both prostate epithelial and prostate cancer cells[110]. PSA is a member of the tissue kallikrein family and functions as an androgen-regulated serine protease[111]. Following secretion into seminal fluid, proPSA is activated to PSA and functions to cleave semenogelins to allow for seminal liquefaction[111]. Among the fraction of PSA which enters vascular circulation, the majority is bound by protease inhibitors ("bound PSA") while a small proportion undergoes enzymatic inactivation and circulates in an unbound form ("free PSA")[111]. PSA was introduced into clinical use as a serum marker for prostate cancer in 1987[112]. Apart from the significant controversies regarding its use in prostate cancer screening (discussed in detail below), pre-operative PSA level is associated with pathological tumor stage[113], biochemical recurrence, and prostate cancer specific mortality[114].

Despite their value, the currently available prognostic factors of tumor stage, histologic grade and PSA are insufficient to adequately risk stratify patients[5,6]. These factors have been combined to create risk groups and nomograms in order to more accurate estimate the prognosis for patients with prostate cancer[108]. These combinations have been consistently shown to out-perform the predictive ability of any single factor. The goal of these classification schema is to categorized patients who have similar clinical outcomes[115].

The best known risk categorization is the D'Amico Risk Classification[116]. This classification divides men into low, intermediate, and high-risk categories for recurrence following prostate cancer treatment on the basis of pre-operative serum PSA level, clinical stage, and biopsy Gleason score (Table 2.3). However, it does not account for multiple risk

factors. Another widely used risk classification is the University of California – San Francisco Cancer of the Prostate Risk Assessment (UCSF – CAPRA) score[117]. CAPRA assessment yields a score between 0 and 10 which has been shown to predict biochemical recurrence[118], metastasis, prostate-cancer specific death, and overall survival[119]. This score is derived from the age at diagnosis, PSA level at diagnosis, Gleason score, clinical stage, and tumor volume (Table 2.4). Patients with CAPRA scores of 0 to 2 are classified as low-risk, 3 to 5 as intermediate risk, and 6 to 10 as high risk[117].

Table 2.3. D'Amico Risk Classification.

	Pre-operative PSA		Clinical Stage		Gleason score
Low-risk	≤ 10 ng/mL	*and*	≤ T2a	*and*	≤ 6
Intermediate-risk	10 – 20 ng/mL	*or*	T2b	*or*	7
High-risk	> 20 ng/mL	*or*	≥ T2c	*or*	≥ 8

Table 2.4. The University of California – San Francisco Cancer of the Prostate Risk Assessment (UCSF – CAPRA) score criteria.

Variable	Variable strata	Points assigned
Age at diagnosis	< 50 years	0
	≥ 50 years	1
PSA at diagnosis	≤ 6 ng/mL	0
	6.1 – 10 ng/mL	1
	10.1 – 20 ng/mL	2
	20.1 – 30 ng/mL	3
	> 30 ng/mL	4
Gleason score	No pattern 4 or 5	0
	Secondary pattern 4 or 5	1
	Primary pattern 4 or 5	3
Clinical Stage	T1 or T2	0
	T3a	1
Tumor volume	< 34%	0
(% of biopsy cores involved with cancer)	≥ 34%	1

In addition to these risk classifications, a number of groups have developed nomograms, diagrammatic representations of complex, multivariable models, to predict prostate cancer-related outcomes have been developed. While many of these have been developed to estimate the risk of prostate cancer, and high-grade prostate cancer, diagnosis among patients prior to transrectal-ultrasound guided prostate biopsy[120-122], others are prognostic for patients following diagnosis. The best known and most widely used of these are the Kattan and Stephenson nomograms which may be used pre-operatively or post-operatively to predict the risk of biochemical recurrence following radical prostatectomy based on routine clinical data[123-126]. Further nomograms from the same group have demonstrated the ability to pre-operatively predict the probability of a "trifecta" following radical prostatectomy (cancer-free, continent, and potent)[127].

In addition to risk classification schemes and nomograms, a number of other types of prediction tools have been proposed for prognostication in patients diagnosed with prostate cancer, including artificial neural networks, probability tables, and classification and regression tree analyses[128]. These are not widely used.

2.3.2.2 Novel molecular-based markers

Many molecular and genetic factors have been examined in an attempt to provide more meaningful prognostication for patients with prostate cancer. As with nomograms, there are many molecular biomarkers which have been developed to aid in prostate cancer diagnosis, including the 4Kscore[129], an aggregate score of four kallikreins (total PSA, free PSA, intact PSA, and human kallikrein 2), PCA3 (prostate cancer antigen 3)[130], a non-coding RNA gene

product, and PHI (prostate health index, a mathematical combination of total, free and [-2]pro-PSA)[131].

There are a greater number of prognostic molecular biomarkers including Prolaris, Oncotype Dx Prostate, Decipher and ProMark. Each of these utilizes different biology and provides differing clinical information. Analogous to the intent of the Partin tables, ProMark seeks to assist in pre-treatment decision making by estimating the probability of favorable pathology, defined as surgical Gleason score ≤ 3 + 4 and localised disease (≤ pT2)[132]. To do so, the ProMark test relies upon an 8-biomarker proteomic assay of biopsy tissue. Similarly, the Oncotype DX Prostate utilizes prostate biopsy tissue in order predict adverse pathology[133]. In addition, it has proven useful in predicting biochemical recurrence and the development of metastases[134]. Following an assessment of 732 potential genes, the Oncotype DX Prostate test comprises a Genomic Prostate Score based on a 17-gene assay. The Prolaris test, also referred to as the cell-cycle progression (CCP) score, is a mathematical product derived from the average, normalized expression of 31 genes involved in the regulation of cell cycle progression based on RNA expression[135]. It is among the most widely examined and published prognostic factors in prostate cancer. It has been shown to predict biochemical recurrence following radical prostatectomy[135,136] and prostate-cancer specific death for patients undergoing watchful waiting after transurethral resection of the prostate[135]. In a survey of treating physicians, the Prolaris test was found to significantly modify patient management[137]. Finally, the Decipher Prostate Cancer Classifier Test (also known as the Genomic Classifier) is a prognostic marker developed to predict clinical metastases following radical prostatectomy based on the expression of 22 RNA[138]. In validation studies, the Decipher Classifier has been shown to correlate with biochemical recurrence, metastasis (including rapid metastasis within five years of surgery[139])

and prostate-cancer specific mortality[140,141]. Further, the Decipher Classifier has additive value when combined with clinical risk factors[139,141]. In addition, the Decipher Classifier provides prognostic information regarding the development of metastases among men with biochemical recurrence following surgery[142] and among men undergoing post-operative radiotherapy[143,144]. As a result, the Decipher Classifier may be used in order to identify patients who may benefit from adjuvant radiotherapy[143]. Finally, the Decipher Classifier may aid in the identification of patients who are unlikely to respond to salvage radiotherapy[145]. Similar to the Prolaris test, there is evidence that the additional information provided by the Decipher Classifier may significantly affect patient management[146].

Despite their value, there are many limitations to the molecular factors which have been examined thus far. Frequently, there are systematic errors in the design and execution of the discovery studies[147]. First, many biomarkers are developed without a clear clinical or research question which they seek to address. This is reflected in the wide variety of outcomes reported in the studies assessing the available tests. Further, the majority have been developed using pathological findings at the time of prostatectomy or biochemical recurrence as the endpoint[108]. The limitations of biochemical recurrence as an outcome will be discussed later (*Section 2.5.1 Oncologic outcomes*). More clinically relevant research questions include (1) distinguishing patients with clinically-significant prostate cancer and a low or indeterminate PSA level from those with clinically-insignificant disease or benign prostatic hyperplasia; (2) distinguishing between disease destined to progress from that which will have an indolent course; and (3) identifying patients with metastatic disease, prior to radiographic evidence[147]. In addition, the majority of biomarkers have been tested among patients who have undergone radical local treatment, despite the need for biomarkers for prognostication in men undergoing active

surveillance[108]. Finally, there is a significant publication bias in biomarker development studies with selective non-reporting[147].

2.3.2.3 MiRNA as prognostic factors

While no microRNA-based prognostic factors are yet commercially available in prostate cancer, they offer greater promise. As there is a low rate of mutations with prognostic value in prostate cancer, models based on dynamic or epigenetic changes may be more useful. Expression patterns of microRNA have recently been shown to be important prognostic factors in epithelial ovarian cancer progression[148] and penile cancer progression[149].

Reverse transcriptase polymerase chain reaction (RT-PCR) using stem-loop primers has been shown to measure miRNA expression with high specificity and sensitivity[150]. Further, the biologic stability of miRNA make them ideal for research and clinical use as they may be quantitated from both formalin-fixed tissue and biological fluids[67]. Messenger RNA (mRNA) are prone to degradation due to mRNA cleavage, which affects the accuracy of mRNA quantification[151]. In contrast, Jung et al. demonstrated that miRNA stability is robust across a variety of experimental and clinical conditions[151]. Thus, accurate quantification of miRNA expression is feasible, even among samples with degraded RNA fractions in which mRNA quantification is unreliable. Of particular relevance to urologic disease, miRNA have been shown to be stable in urine, across a number of storage conditions[152].

We have previously identified a panel of five miRNAs which was predictive of biochemical recurrence and metastasis following radical prostatectomy[153]. Other authors have examined the prognostic role of other miRNA in prostate cancer[154-159]. However, none of these have transitioned to commercialization or clinical practice. We believe that the selection of

research question including both a relevant patient cohort and important outcome with both patient- and population-level implications will allow for more rationale and clinically-important biomarker discovery.

2.4 An overview of prostate cancer identification and treatment

2.4.1 Prostate cancer screening

Historically, prostate cancer was typically diagnosed at an advanced stage[160]; in the 1970s, the majority of patients had clinical metastases at the time of diagnosis[161]. Since the introduction of PSA-based prostate cancer screening, there has been a significant stage migration with a much greater proportion of patients newly diagnosed with prostate cancer harbouring clinically localized disease[160,162].

The adoption of PSA-based prostate cancer screening started, particularly in the United States, prior to rigorous trials demonstrating a benefit to PSA screening. Since this time, there have been two large studies to assess the effect of PSA screening on prostate cancer mortality: the European Randomized Study of Screening for Prostate Cancer (ERSPC)[163] and the U.S.-based Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial[164]. Significant differences exist between the trials, in large part due to the fact that PSA had been widely adopted in the US during the study interval while it did not have such uptake in Europe. As a result, there is significant contamination of the control group in PLCO compared with ERSPC[165].

The most mature data from the ERSPC has 13 years of follow up[166]. Based on these data, the absolute risk reduction in prostate cancer mortality from PSA screening was 0.11 per 1000

person years or 1.28 per 1000 men randomized. This risk reduction has increased with increasing duration of follow up. Additional analysis has shown an absolute risk reduction of metastatic disease was 3.1 per 1000 men randomized[167]. In a subgroup of the ERSPC with longer follow-up, the absolute risk reduction in prostate cancer mortality was 4.0 per 1000 men randomized[168]. This corresponds to a number needed to screen of 293 and number needed to diagnose of 12 in order to prevent one prostate cancer death.

In contrast, the results of the PLCO trial no absolute or relative benefit to PSA screening. While there are concerns that the trial did not compare screening to no screening and that the trial would be unlikely to find a benefit even if a significant one existed[169], it remains often cited.

Based in large part on the results of the PLCO trial, both the United States Preventative Services Task Force[170] and the Canadian Task Force on Preventative Health Care[171] have recommended against PSA screening. This is based on an assessment that prostate cancer screening resulted in the avoidance of 0 to 1 prostate cancer deaths per 1000 men screened[170], a minimal benefit. Further, they cited evidence of significant harms based on PSA-based prostate cancer screening due to false-positive PSA results which confer a risk of psychological harm in addition to medical evaluation including biopsy[170]. In addition, they considered there to be, at minimum, a small harm associated with prostate biopsy due to pain, bleeding and infectious risk. The panels also concluded that there was significant evidence of at least moderate over-diagnosis and resultant overtreatment among patients undergoing PSA-based screening. The CTFPHC further cited harms of treatment, whether by surgery, radiotherapy, or androgen-deprivation therapy[171]. These will be discussed in greater detail below.

Thus, due to a perceived lack of benefit and presence of significant harms, the both the USPSTF and CTFPHC concluded, with moderate certainty, that the benefit of PSA-based screening did not outweigh the harms and thus recommended against PSA-based screening for prostate cancer[170].

2.4.2 Prostate cancer diagnosis

For men with suspicion of prostate cancer based on elevated PSA levels or suspicious findings on digital rectal examination, prostate needle biopsy may be undertaken in order to make the diagnosis[115]. The vast majority of biopsies are performed with a transrectal approach, though perineal biopsy is an alternative. Ultrasound guided biopsy is considered the standard of care[115] though recent evidence suggests that MRI/ultrasound fusion guided biopsy may increase the detection of clinically significant prostate cancer while decreasing the diagnosis of low-risk disease[172].

2.4.3 Prostate cancer treatment

The treatment of clinically-localized, screen-detected prostate cancer is controversial. Many men, particularly older men with low-risk prostate cancer, will not benefit from active intervention[173]. Thus, treatment options including conservative management strategies (active surveillance and watchful waiting) and active intervention (surgical radical prostatectomy (RP), external beam radiotherapy (EBRT), brachytherapy, and androgen-deprivation therapy (ADT))[115].

Among the conservative management strategies, the intent and intensity of observation during the period of conservative management differs. Watchful waiting is a form of non-

curative therapy in which conservative management is offered until the development of disease progression with present or imminent disease-related symptomatology[115]. Treatment is then administered in order to preserve quality-of-life, without intention of cure. In contrast, active surveillance refers to a period of active disease observation (involving repeated physical examination, PSA determination, repeated biopsy, and imaging) which continues until there is disease progression. Treatment is then administered with curative intent[173]. Thus, the goal of active surveillance is to minimize or delay treatment-related toxicity without compromising the chance of cure.

Radical prostatectomy (RP) is the complete surgical removal of the prostate gland, seminal vesicles and adjacent tissue to such a degree as is necessary to obtain negative margins. A bilateral pelvic lymph node dissection is often performed in conjunction with RP, based on the risk of lymph node involvement as determined using the Partin tables[174]. Typically, lymph node dissection is undertaken for men with a probability of lymph node involvement of 3% or greater[175]. These patients typically have Gleason sum ≥ 7 or PSA ≥ 10 ng/mL[175]. Radical prostatectomy is the only treatment which has been shown in a randomized controlled trial (SPCG-4) to confer a benefit in overall and cancer-specific survival for patients with clinically-localized prostate cancer, compared to watchful waiting[176]. In addition to survival benefits, surgery reduced the risk of metastatic disease[176]. However, these benefits were not confirmed in a similar study (PIVOT)[177]. Wilt et al. randomized 731 men recruited from US Veterans Affairs Hospitals with screen-detected localized PCa to surgery or observation. With a median follow-up of 10 years, there was no significant difference in overall or PCa mortality. They did find a benefit to surgery among men with PSA levels above 10 ng/mL and those with higher risk disease. The conflicting conclusions between the SPCG-4 and PIVOT trials are likely explained

by differences in patient populations (inclusion of proportionally more men with low-risk disease and more men with significant comorbidities in the PIVOT trial), duration of follow-up (significantly longer in the SPCG-4 trial), and potential differences in outcomes associated with watchful waiting (SCPG-4) and active surveillance (PIVOT). The results from PIVOT may also be less generalizable as non-PCa related mortality in the PIVOT study greatly exceed that of patients at American centres of excellence[178], the Surveillance, Epidemiology, and End Results (SEER) registry over the same time period[179], and European cohorts[180]. In the SPCG-4 trial, the benefit of surgery has continued to increase as ongoing follow-up has accrued.

Radiotherapy is a commonly employed alternative to surgery for patients undergoing active treatment for clinically localized prostate cancer. Currently there are no randomized controlled trials comparing radiotherapy to a conservative management strategy. Radiotherapy may be administered via external beam radiotherapy (EBRT) or brachytherapy. The technique of EBRT has evolved over the last decades and intensity-modulated radiotherapy (IMRT) is currently considered the gold standard for the provision of EBRT[115]. Brachytherapy may be administered by low-dose rate (LDR) or high-dose rate (HDR) technique. LDR brachytherapy utilizes permanent radioactive seeds which are implanted in the prostate[181]. In contrast, HDR brachytherapy is performed with the temporary insertion of a radioactive source into the prostate. This may be administered in a single or in multiple settings. HDR brachytherapy is often co-administered with EBRT[182,183].

Prostate cancer proliferation is dependent on androgenic stimulation[184]. In 1941, Huggins demonstrated that suppression of testosterone results in the regression of both primary and metastatic prostate cancer[184]. Since that time, androgen deprivation therapy (ADT) has been an important component of prostate cancer treatment. Androgen deprivation may be obtained

either through the suppression of the release of testicular androgens or the inhibition of their actions. Suppression of the release of testicular androgens may be achieved through surgical castration (bilateral orchiectomy) or medical therapy. Luteinising-hormone releasing hormone (LHRH) agonists are the most widely used form of medical therapy. More recently an LHRH antagonist has entered clinical practice. Historically, estrogens were used but these are no longer considered standard of care due to severe side effects[185]. Anti-androgens, inhibitors of androgenic action, may be used in combination with LHRH-agonists (collectively known as complete androgen blockade, CAB) or on their own. Whether steroidal or non-steroidal, these compounds compete with androgens at the receptor, thus impeding androgenic activity. While it was initially indicated for patients with metastatic disease[186], ADT has been used for patients with clinically-localized disease. However, primary ADT has been shown not to improve survival[187,188] and is thus not recommended for patients with localized disease. However, ADT is used as adjuvant or neoadjuvant therapy for patients undergoing surgery or radiotherapy.

The combination of radiotherapy with androgen deprivation therapy (utilizing luteinising-hormone releasing hormone, LHRH) has been shown to improve overall survival as compared with radiotherapy alone, based on a number of randomized controlled trials[189-191]. The addition of ADT has become the standard of care for patients with locally-advanced prostate cancer undergoing radiotherapy, based on the results of EORTC 22863[189], and for patients with intermediate-risk localized disease, based on the results of RTOG 94-08[192]. Long-term ADT (consisting of 2 or 3 years of therapy) is recommended for patients with locally advanced disease rather than short-term therapy (6 months)[193]. However, among patients with localized disease, short-term ADT appears sufficient[194]. Finally, ADT has been shown to improve survival in patients with lymph node metastasis after radical prostatectomy[12].

There are a number of other modalities which are less commonly accepted for the treatment of clinically-localized prostate cancer. These include focal and whole gland treatments employing cryotherapy, high-intensity focused ultrasound (HIFU), photodynamic therapy, radiofrequency ablation and electroporation[115]. These are typically considered experimental therapies and are not routinely recommended in guidelines[115].

2.4.3.1 Prostate cancer treatment recommendations

Treatment recommendations for patients newly diagnosed with prostate cancer depend on the prostate cancer risk classification[195]. For patients with low-risk disease, watchful waiting should be offered to patients who are not eligible for curative therapy and those with a short life expectancy[115]. For patients with life expectancy in excess of 10 years, active surveillance, radical prostatectomy, IMRT, or LDR brachytherapy are reasonable treatment options according to European Association of Urology, National Comprehensive Cancer Network and Cancer Care Ontario guidelines[115,196,197]. However, as of 2015, both Cancer Care Ontario and the American Society of Clinical Oncology (ASCO) recommend active surveillance for these patients[198,199].

A similarly wide variety of treatment options are supported by guidelines for patients with intermediate-risk localized prostate cancer. For patients undergoing radical prostatectomy, most should undergo pelvic lymph node dissection based on their risk of lymph node involvement[174]. For patients desiring radiotherapy treatment, IMRT, LDR and HDR brachytherapy may all be used for treatment of intermediate-risk disease[115]. Patients undergoing IMRT are recommended to have combination therapy with short-term ADT. For those unable to tolerate ADT due to medical comorbidity, dose-escalated IMRT or combined brachytherapy and IMRT may be reasonable. For patients with favorable intermediate-risk prostate cancer, LDR

brachytherapy may be a reasonable option[181]. For others, combined LDR brachytherapy with EBRT and ADT may be offered. For patients with intermediate and high-risk disease, the recently reported ASCENDE-RT showed those who received LDR brachytherapy in addition to 1 year of ADT and pelvic radiotherapy had lower rates of biochemical recurrence compared with patients receiving ADT, pelvic radiotherapy and EBRT prostate boost[200]. Finally, HDR brachytherapy, most often in combination with EBRT, may be offered to these patients[201].

There is no consensus on the optimal treatment of patients with high-risk, localized prostate cancer. Most acknowledge that these patients will often require multi-modal therapy. Guidelines indicate that radical prostatectomy with extended pelvic lymph node dissection is a "reasonable first step"[115]. For patients opting for primary radiotherapy, dose-escalated IMRT with long-term ADT is recommended[115]. HDR brachytherapy with EBRT may also be offered to these patients.

Finally, for those with locally-advanced disease, historically, surgery was discouraged[195] but more recent guidelines have suggested that surgery may be reasonable[115]. This often necessitates a multi-modal approach to treatment. For patients undergoing radiotherapy, long-term ADT is necessary[189]. Primary ADT, without radiotherapy, has been shown to be inferior to radiotherapy plus ADT[202].

2.5 Outcomes following prostate cancer treatment

2.5.1 Oncologic outcomes

Following treatment with curative intent with surgery or radiotherapy, patients may experience disease recurrence and progression to metastasis. As patients are followed after

treatment with serial serum PSA measurements, the first sign of disease recurrence is a rise in PSA, known as biochemical recurrence. There exist innumerable definitions of biochemical recurrence (BCR): a systematic review of the literature in 2007 identified 53 different definitions for BCR following radical prostatectomy and 99 different definitions for BCR following radiotherapy[203]. The most common definition of BCR following surgery was a PSA level > 0.2 ng/mL while the most common definition following radiotherapy was three consecutive rises in PSA (American Society of Therapeutic Radiology and Oncology (ASTRO) definition). Since that time, American Urological Association definition (PSA ≥ 0.2 ng/mL confirmed on two separate occasions) and the Phoenix criterion (a rise in PSA ≥ 2 ng/mL above the nadir PSA level) have become the most commonly accepted definitions of BCR following surgery and radiotherapy, respectively[203,204].

Clinically, while BCR is important as it often begins a cascade of therapy which results in significant costs and quality of life detriments[205-207], only a small percentage of men with biochemical recurrence will have systemic progression or die of their disease[208]. For patients initially treated with radical prostatectomy, salvage radiotherapy is typically offered at the time of biochemical recurrence based on data demonstrating durable cancer control[209] and improved prostate-cancer specific survival[210]. For patients initially treated with radiotherapy, the vast majority (up to 90%) will receive palliative therapy with ADT[211]. A select few may be eligible for salvage prostatectomy[212].

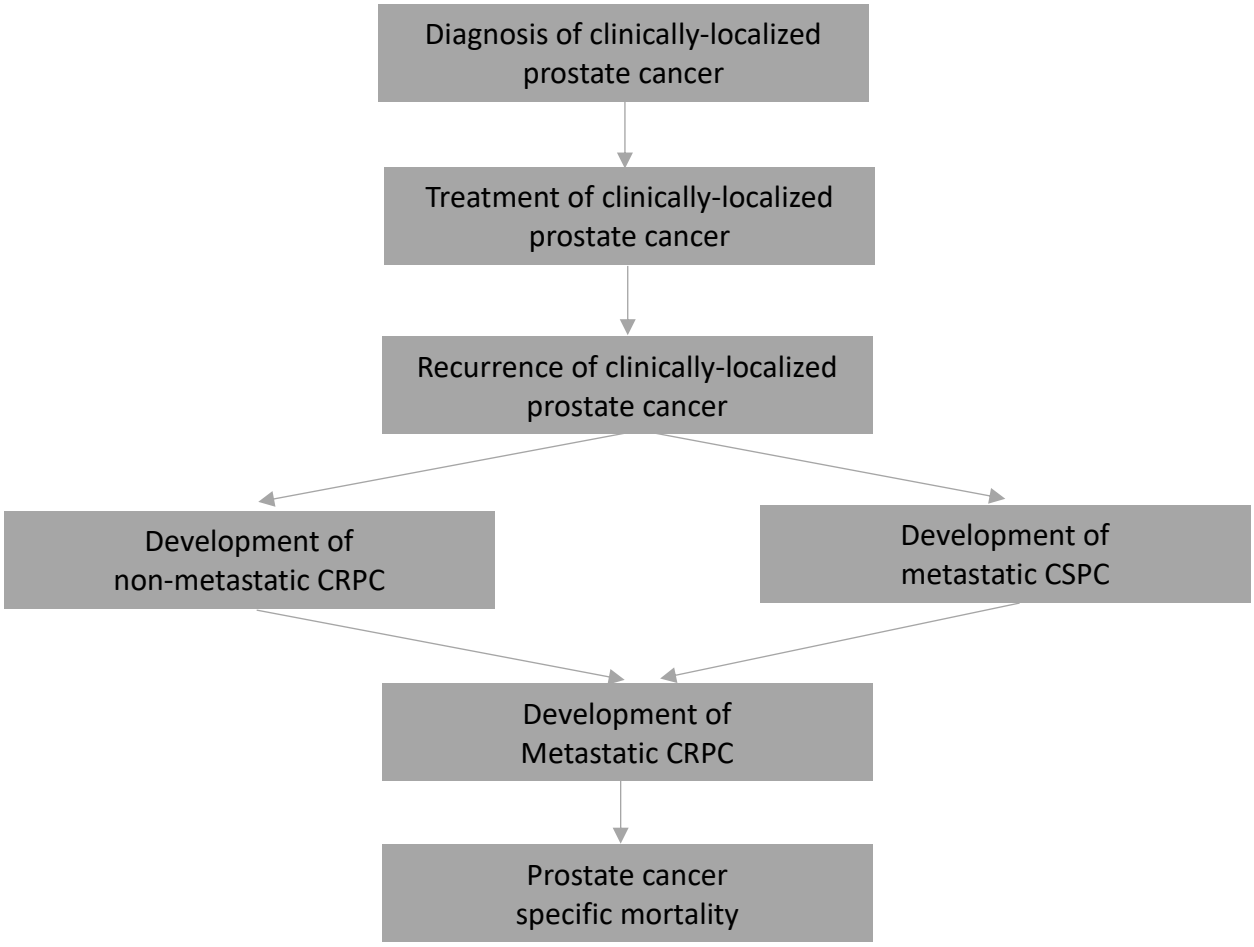
While BCR is an important clinical event, most notably as it triggers further therapy, it has many limitations as a research outcome. The first limitation, as stated above, is that only a small percentage of men with biochemical recurrence will have systemic progression or die of their disease[208]. Second, the vast number of BCR definitions[203] makes it difficult to compare

outcomes between studies. Finally, given the intrinsically different definitions of BCR for patients treated initially with surgery and radiotherapy, the use of BCR to compare outcomes following treatment with the two modalities is problematic. Nielsen et al. showed that the use of the Phoenix criterion (nadir + 2 ng/mL) as a definition of BCR for patients following radical prostatectomy systematically overestimated biochemical-recurrence free survival[213]. As a result, metastasis-free survival or, even better, prostate-cancer specific mortality, are more robust research outcomes for comparative effectiveness studies between prostate cancer treatment modalities. Despite this, many investigators have designed studies to assess BCR as the primary endpoint, likely due to its relatively high frequency and early appearance[214].

Following biochemical recurrence, patients may progress to metastatic disease or may develop resistance to ADT (castrate resistance) prior to the development of metastasis[215], a disease state known as non-metastatic castrate resistant prostate cancer (NM-CRPC) (Figure 2.1). Our understanding of the natural history of NM-CRPC is relatively limited. Following the development of castration resistance, approximately one third of patients will develop metastases within two years[216] and nearly 60% will develop metastases within five years[217]. Additionally, 20% of patients will have died within two years of the development of NM-CRPC[216]. There are no treatments that have been shown to improve survival for patients with NM-CRPC[217]. For patients with metastatic disease, there are many therapies that have been shown to improve overall survival[218-224]. Metastasis-free survival is a better research outcome than BCR given its more proximal association with prostate-cancer specific and overall mortality[225]. ADT has been shown to significantly delay the development of metastases among patients at high risk of metastatic progression[189]. As a much higher proportion of patients with clinically-localized prostate cancer treated with radiotherapy receive ADT as compared with

those treated with surgery[226], metastasis-free survival remains a problematic outcome in studies comparing the effectiveness of surgery and radiotherapy, particularly when follow-up periods are less than five years.

Figure 2.1. Flow chart of prostate cancer related health states following diagnosis of clinically-localized disease. At any prostate-cancer related health state, patients are at risk for non-prostate cancer related mortality.



Note: CRPC = castrate-resistant prostate cancer; CSPC = castrate-sensitive prostate cancer.

Death from prostate cancer, or prostate-cancer specific mortality, remains a significant cause of death for patients with advanced prostate cancer[225]. As prostate cancer can be reliably

ascertained as a cause of death from administrative records[227], prostate-cancer specific mortality and overall mortality are the most objective and clinically-relevant outcomes.

2.5.1.1 Comparative oncologic outcomes following prostate cancer treatment

Despite the clinical, patient-level, and health care systems importance, there is a relative lack of high quality clinical data regarding the comparative effectiveness of surgery and radiotherapy in the treatment of prostate cancer. The biochemical recurrence rates following radical prostatectomy and brachytherapy have been compared in a single center, randomized controlled trial[228]. Among 174 men with outcome data for assessment, biochemical recurrence free survival rates were similar (radical prostatectomy 91.0% and brachytherapy 91.7%). Numerous observational studies have compared biochemical recurrence free survival between various surgical and radiotherapy modalities[229-231]. The utility of these data must be considered in the context of the known problems of BCR as an endpoint for comparative studies, as discussed above.

There have been three randomized controlled trials which have been published assessing survival outcomes in the treatment of prostate cancer. The first dates from the pre-PSA era and was conducted by the Uro-Oncology research group[232]. Fifty-six patients received radiation while 41 underwent surgery. The primary outcome was first treatment failure as defined by elevation of acid phosphatase levels or bony or parenchymal disease. They found a significant advantage to radical prostatectomy ($p=0.037$). More recently, the Japanese Study Group for Locally Advanced Prostate Cancer randomized 95 patients with T2b-3N0M0 patients to external beam radiotherapy or radical prostatectomy with common androgen deprivation therapy[233]. While they found improved biochemical recurrence free survival, clinical progression free

survival, cause specific survival, and overall survival for surgery compared to radiotherapy, none of these comparisons were statistically significant. However, due to methodologic limitations and the evolution of medical practice, neither of these studies are currently used to inform decision making regarding prostate cancer treatment. Other trials have closed prematurely due to poor accrual[234] and there was concern that patients were unwilling to leave their treatment to chance[235]. Recently, the Prostate cancer screening and Treatment (ProtecT) study reported survival outcomes for 1643 patients randomized to monitoring (n=545), surgery (n=553), and radiotherapy (n=545)[236]. The investigators found no significant difference in their primary outcome of prostate cancer specific mortality (p=0.48) with 8 attributable deaths in the monitoring group, 5 in the surgery group and 4 in the radiotherapy group[236]. Rates of clinical progression (p<0.0001) and metastasis (p=0.004) were significantly higher among patients undergoing monitoring as compared to active treatment[236]. Meaningful comparisons between surgery and radiotherapy are limited in this study cohort as the trial is underpowered and patients with low-risk disease (77%) are over-represented among the study cohort[237]. In light of this, we have performed a meta-analysis of observational studies assessing the association between primary treatment modality (surgery vs radiotherapy) and overall and prostate-cancer specific mortality[15]. Utilizing pooled results of 95,791 patients for the outcome of overall mortality and 118,830 patients for prostate cancer specific mortality, we found an association between radiotherapy treatment and increased risk of death (overall mortality: HR 1.63, 95% CI 1.54 – 1.73; prostate-cancer specific mortality: HR 2.08, 95% CI 1.76 – 2.47). These findings were robust to subgroup and sensitivity analyses including prostate cancer risk categorization, study accrual period, radiotherapy modality (EBRT or brachytherapy), duration of follow-up, and geographic region of study accrual[15]. While observational data cannot account for

unmeasured confounding in the manner of a randomized controlled trial, the included studies were of low to moderate risk of bias and many provided analyses of patients matched on all identifiably relevant patient and tumor characteristics including propensity score matching techniques. This meta-analysis represents Level 2a evidence, although the limitations to account for unmeasured confounding continue to be a problem for these studies[238].

2.5.2 Functional outcomes

2.5.2.1 Local treatment-related complications

Radical prostatectomy is associated with a risk of peri-operative mortality ranging from 0.005% and 0.5%, based on population-based studies[239-242]. Further intra- and peri-operative complications of radical prostatectomy depend on surgical modality (open, laparoscopic or robotic) and include bladder neck contracture (1.0 – 4.9%), anastomotic leak (1.0 – 4.4%), infection (0.8 – 4.8%), organ injury (0.4 – 2.9%), ileus (0.3 – 1.1%), and deep vein thrombosis (0.2 – 1.4%)[243].

The best characterized and most frequently discussed complications of prostate cancer treatment are urinary incontinence and erectile dysfunction. For patients undergoing radical prostatectomy, recent systematic reviews have estimated 12-month urinary incontinence and erectile dysfunction rates ranging from 8 – 11%[244] and 10 – 46%[245], respectively. Again, surgical modality may significantly affect rates of these outcomes[244,245]. These complications may also occur following radiotherapy[246]. The best data on the comparative continence and potency outcomes following prostate cancer treatment comes from the Prostate Cancer Outcomes Study[7]. Utilizing a sample of 3533 patients enrolled from six Surveillance,

Epidemiology, and End Results (SEER) sites, the investigators completed surveys at 6 months and/or 12 months following diagnosis. In the primary report, 1655 patients with clinically-localized disease who received surgical or radiotherapy treatment within 1 year of diagnosis and completed follow-up surveys beyond 2 years were examined. While men who underwent surgery were significantly more likely to report urinary incontinence at 2 years (odds ratio (OR) 6.22, 95% CI 1.92 – 20.29) and 5 years (OR 5.10, 95% CI 2.29 – 11.36), this difference became non-significant at 15 years (OR 2.34, 95% CI 0.88 – 6.23)[7]. Similarly, patients undergoing surgery reported significantly higher rates of erectile dysfunction at 2 years (OR 3.46, 95% CI 1.93 – 6.17) and 5 years (OR 1.96, 95% CI 1.05 – 3.63), but this became non-significant at 15 years (OR 0.38, 95% CI 0.12 – 1.22) as the vast majority of all men had developed erectile dysfunction by that time[7].

Due to the field effects of radiotherapy on the bladder and bowels, both external beam radiotherapy and brachytherapy significantly affect the bowel and rectal, as well as urinary domains of health-related quality of life[247]. While the majority of these are transient, they may persist for many years after treatment[247]. Typically, these symptoms are worse for patients undergoing EBRT than those receiving brachytherapy[248]. In the PCOS cohort, patients treated with radiotherapy report significantly higher rates of bother due to bowel symptoms than those treated with surgery, from 2 years to 15 years following treatment[7]. While bowel urgency was significantly more common among men undergoing radiotherapy at 2 years (OR 2.56, 95% CI 1.47 – 4.55) and 5 years (OR 2.13, 95% CI 1.19 – 3.85), there was no significant difference at 15 years (OR 1.02, 95% CI 0.47 – 2.22).

More recently, we have described rates of other local, treatment-related complications including minimally-invasive urologic procedures, rectal-anal procedures, major surgeries and

secondary malignancies. Primary treatment with radiotherapy as compared to surgery was associated with an increased risk of rectal-anal procedures (HR 2.72) and major surgeries (HR varying by time: 1.15 at 1 year to 3.68 at 5 years following treatment) but lower risk of minimally-invasive urologic procedures[8]. After propensity-score adjustment to account for baseline difference between the groups, patients receiving radiotherapy had increased long-term risk of minimally-invasive urologic procedures (hazard ratio (HR) varying by time: 0.50 at 1 year to 6.93 at 5 years following treatment), rectal-anal procedures (HR 2.64), and major surgeries (HR varying by time: 1.02 at 1 year to 3.56 at 5 years following treatment)[9]. While the use of post-operative radiotherapy contributed to increased complication rates, patients undergoing both surgery and radiotherapy had lower rates of rectal anal procedures and open surgeries than patients undergoing radiotherapy but higher rates of minimally-invasive urologic procedures[207]. When taken on an intention-to-treat basis, the initial decision to begin therapy with surgery was associated with lower risk of all of these outcomes in the long-term[207]. As many of these complications may recur, we also examined rates of these complications using a counting process with negative binomial regression, in an independent cohort of patients[10]. In this analysis, we found that radiotherapy treatment was associated with increased rates of urologic procedures (relative rate (RR) 1.25, 95% CI 1.2 – 1.3) and rectal-anal procedures (RR 1.4, 95% CI 1.4 – 1.5) but lower rates of major surgeries (RR 0.9, 95% CI 0.8 – 0.9).

Complications following prostate cancer treatments may require hospitalization for management, in the absence of a procedural intervention. These include genitourinary or gastrointestinal bleeding, infection, and urinary obstruction[8]. Treatment with radiotherapy was associated with increased hospitalizations in time-to-first event analysis (HR varying by time: 0.86 at 1 year to 10.8 at 5 years following treatment)[8], after propensity-score matching (HR

varying by time: 0.85 at 1 year to 37.6 at 5 years following treatment)[9], and when assessed using a counting process (RR 1.8, 95% CI 1.8 – 1.9)[249]. Patients treated with both surgery and post-operative radiotherapy had lower rates of hospitalization than those treated with radiotherapy alone[207] and, when taken on an intention-to-treat basis, the initial decision to begin therapy with surgery was associated with lower risk of hospitalization from two years following treatment onwards[207], as would be expected given the 100% rate of hospitalization in the first year for patients undergoing surgery.

Finally, radiotherapy may be associated with increased rates of secondary cancers[17,18,250]. However, other studies have not demonstrated this association[19,20] and reviews on the subject are conflicting[251-254]. While an increased risk of bladder and rectal cancer is often demonstrated[115], we found increased risks of lung cancer, hematological cancers, and cancers at other sites[8], which persisted following propensity-score matching[9].

2.5.2.2 Systemic treatment-related complications

In additional to local treatment-related toxicity, patients undergoing prostate cancer treatment are also at risk for systemic toxicity. The best recognised of these are the effects of ADT but emerging evidence suggests that radiotherapy may independently contribute to complications outside the pelvis.

The adverse effects of ADT relate mechanistically to the suppression of testosterone. ADT has been associated with detriments in bone health, cardiovascular disease, sexual function, mental health, and others. ADT causes decreased bone-mineral density due to disruptions in sex hormone synthesis[255] and this has been associated with an increased risk of skeletal-related events both in historical[14] and more modern cohorts[226].

Issues following prostate cancer treatment

The metabolic harms of ADT have been established from both prospective and population-based sources[256]. ADT has been shown to drive metabolic derangement through nearly every aspect of the metabolic syndrome. Even short durations of ADT have been demonstrated to result in decreased insulin sensitivity and increased circulating insulin levels[257]. Further, after a year of therapy, ADT is associated with weight gain, increased body fat percentage, and decreased lean body mass in addition to rises in serum triglycerides[258]. While not directly linked to hypertension, ADT has been associated with increased atrial stiffness[259]. The first study to highlight the metabolic effects was published in 2006 by Keating et al. and showed an increased risk of diabetes, myocardial infarction, and sudden cardiac death among men receiving ADT[260]. Subsequent studies have confirmed these findings[226,261] and further data has shown that men receiving ADT have a higher risk of fatal cardiovascular events[262], although this last finding is controversial[263,264]. Further research associated ADT with other cardiovascular outcomes including stroke, peripheral arterial disease, and venous thromboembolism[265,266].

Through suppression of circulating testosterone, ADT may cause sexual dysfunction through both a loss of sexual interest (libido) and decreased erectile function[267]. This has been estimated to effect more than 90% of men undergoing ADT. ADT has also been associated with decreases in penile length[268] and testicular size[269] which may be psychologically distressing and associated with treatment regret.

Androgen deprivation may have significant effects on the brain. First, hypogonadism has been associated with significant cognitive declines in longitudinal[270], observational[271], and interventional studies[272]. Further, ADT has been associated with increased rates of depression, emotional lability, and anxiety[273,274]. This is in keeping with other research among men without

cancer which demonstrates that hypogonadism is associated with depression[275,276]. A retrospective review suggested that up to 28% of men treated with ADT may be newly diagnosed with psychiatric illness, most commonly depression[277]. After a year following treatment, ADT was associated with significant impairments in health-related quality of life and with greater psychological distress than a conservative management strategy while no differences were found between either surgical or radiotherapy intervention and conservative management[278].

Finally, ADT may be associated with significant fatigue, gynecomastia, hot flashes and anemia[256]. The use of adjuvant ADT, along with EBRT or brachytherapy, may potentiate adverse effects on bowel and sexual function, as well as vitality[247,279].

There is growing evidence that radiotherapy may also exert systemic effects, affecting most of the domains discussed above. That radiotherapy has effects beyond the treatment field is relatively well-established[280] and likely contributes to the beneficial oncologic effects of radiotherapy. A combination of local effects to the femur and pelvis along with systemic effects may explain an observed association between radiotherapy and fracture risk which has been demonstrated among women with pelvic malignancies[281,282]. There is recent evidence demonstrating an association between radiotherapy and fracture risk in men treated for prostate cancer[226], though others have not demonstrated this relationship[283]. Etiologically, radiotherapy results in damage and occlusion of the periostic microcirculation[284] and a reduction in the number of osteoblasts[285], both of which result in bone atrophy. Further, radiotherapy induces a pro-inflammatory state through the induction of pro-inflammatory cytokines[286]. Inflammation has been implicated in the development of osteoporosis in a number of studies[287-291].

Additionally, radiotherapy may independently promote the development of cardiovascular disease. While the risk of radiotherapy induced cardiac disease appears to be greatest for patients with Hodgkin's disease and breast cancer undergoing thoracic radiation[292], abdominal radiotherapy has been associated with cardiovascular disease in patients with testicular cancer, even after excluding patients receiving mediastinal radiation[293]. Mechanistically, radiotherapy may induce cardiovascular disease through fibrosis,[294] intimal thickening, proteoglycan deposition, inflammatory infiltration[295] and radiation-nephropathy induced hypertension[296] in contrast with intimal plaque formation which drives spontaneous and age-related atherosclerosis[297]. Further, as described above, radiotherapy may induce a systemic inflammatory response, mediated by cytokines. Both epidemiologic and clinical evidence has demonstrated a strong and reproducible relationship between markers of inflammation and the development of cardiovascular events[298-302]. Recently, we showed a possible independent association between radiotherapy for clinically-localized prostate cancer and the development of coronary artery disease, myocardial infarction, and sudden cardiac death[226].

Finally, external beam radiotherapy has been associated with an increased risk of major depressive disorder[303] though this has not been extensively studied and the validity of this finding remains to be demonstrated.

2.6 Thesis overview

Prostate cancer is the most common malignancy in men apart from non-melanomatous skin cancer. Due to a combination of the underlying disease biology, early detection and treatment, five-year relative survival for men newly diagnosed with prostate cancer exceeds 99%[3] and a large proportion of men diagnosed with prostate cancer will die of other causes, chiefly cardiovascular disease.

The majority of men newly diagnosed with clinically-localized prostate cancer undergo active treatment, whether by surgery or radiotherapy. Given the long natural history of the disease, both oncologic and functional events following prostate cancer treatment may significantly affect a patient's life trajectory. In this dissertation, we use a variety of epidemiologic techniques to explore important outcomes following prostate cancer treatment including disease progression to metastasis, treatment-induced mortality, and secondary malignancies.

CHAPTER 3: IDENTIFICATION OF A NOVEL MICRORNA PANEL ASSOCIATED WITH METASTASIS FOLLOWING RADICAL PROSTATECTOMY FOR PROSTATE CANCER

3.1 Abstract

Objective: To identify one or more novel microRNA sequences which are associated with metastasis following radical prostatectomy for clinically localized prostate cancer.

Design: Case control study.

Data sources and study population: Patients who developed clinical evidence of metastatic disease following surgery (cases) and patients who showed no evidence of metastasis or biochemical recurrence at least 5 years following surgery (controls) as identified from a single center, institutional database. Cases and controls were matched for tumor grade and duration of follow-up.

Exposure: Differential expression of microRNA sequences, as determined from whole miRNome analysis.

Main outcome measure: Metastasis.

Results: Among 585 patients in our institutional database, we identified 32 patients who developed metastasis following radical prostatectomy. Of these, 28 were matched to a suitable control. 19 pairs of patients had sufficient sample for analysis. We identified a total of 2792 unique miRNA. Of these, 497 sequences had sufficient expression for analysis. Bootstrapping with backward selection identified a panel of 5 miRNAs which were associated with metastasis. A risk score derived from weighted expression levels of these 5 miRNAs was strongly associated with metastasis (AUC 89.5%, 95% CI 79.5-99.5%).

Conclusions: Based on a genome wide analysis of microRNA expression, we identified a novel panel of 5 miRNAs which are strongly associated with prostate cancer metastasis following radical prostatectomy. Further validation is required prior to clinical applicability.

3.2 Introduction

Patients with clinically localized prostate cancer may experience a wide spectrum of phenotypes, ranging from indolent tumors which will never require treatment to highly aggressive and lethal disease. Distinguishing between these remains one of the most important questions in the management among these patients. Current prognostic factors including tumor stage, histologic grade (Gleason score), and pre-operative serum prostate specific antigen (PSA) level are insufficient to accurately predict patient outcomes[5,6].

Molecular prognostic factors have significantly altered the management of patients with many solid tumors. MicroRNAs (miRNAs), small non-coding RNA which modulate messenger RNA expression, have shown promise as prognostic biomarkers in many cancers[149,304-306]. Numerous groups have examined the prognostic role of miRNA in prostate cancer[154-159]. However, no miRNAs or miRNA-based panels have yet transitioned to clinical use. We sought to identify a panel of miRNA which were predictive of the development of metastasis following radical prostatectomy for patients with clinically localized prostate cancer.

3.3 Methods

3.3.1 Study design

We conducted a matched case-control study in order to identify a novel panel of microRNA sequences which could predict metastasis following radical prostatectomy. Cases were patients who developed metastasis following radical prostatectomy and controls were patients who were cancer-free following surgery.

3.3.2 Data sources and study population

We utilized a well-established, institutional research ethics board approved database of 585 patients who were treated with radical prostatectomy for clinically-localized prostate cancer between 1990 and 2000 at Sunnybrook Health Sciences Centre, a single, tertiary care hospital. Written, informed consent was obtained from all patients for the use of biologic materials and clinical information. Trained data abstractors systematically reviewed each patient's medical record. Standardized data entry forms were used to compile an institutional, prostate cancer-specific database. Within this database, metastasis was defined as evidence of bony lesions identified on radionuclide bone scan or evidence of extra-pelvic lymphadenopathy or visceral lesions on computed tomography imaging of the abdomen, pelvis and chest.

Within this dataset, we identified patients diagnosed with metastases following surgery. Patients with metastases were matched to patients with no evidence of disease recurrence (ie. no metastasis and no biochemical recurrence) based on a hard match comprising Gleason score from radical prostatectomy and duration of follow-up. Inclusion of other variables in the matching process, including age, pathologic stage, nodal status, pre-operative prostate specific antigen (PSA) and margin status, resulted in insufficient matches for analysis. Gleason score was selected for matching as it is the strongest independent predictor of recurrence and metastasis[153]. Patients who developed metastasis following radical prostatectomy who were unable to be matched to a suitable control were excluded. Similarly, where there was insufficient biologic sample for analysis, the pair was excluded.

3.3.3 Whole miRNome analysis

For each patient selected, genito-urinary pathologists re-reviewed the formaldehyde-fixed paraffin-embedded (FFPE) radical prostatectomy specimen. A representative slide was selected and the corresponding tumor block was identified. The largest tumor focus was identified and a sample was collected by micro-dissection from the area of the block with the highest tumor-to-stroma ratio.

3.3.4 RNA Extraction and Small RNA Enrichment

Two FFPE cores with a diameter of 1 mm and a maximum length of 3 mm (after trimming of excess paraffin) were used for total RNA extraction. FFPE cores were manually ground in a 1.5ml conical tube using plastic pellet pestles. Total RNA was then extracted using the Recoverall Total Nucleic Acid Isolation Kit for FFPE (ThermoFisher Scientific) in accordance with the manufacturer's manuals. The concentration and integrity of extracted total RNA were ascertained using Qubit 2.0 fluorometer with Qubit RNA HS Assay kit (ThermoFisher Scientific) and Agilent 2100 Bioanalyzer with RNA6000 Nano chip (Agilent Technologies), respectively. The small RNA fraction was enriched from 1 μg of extracted RNA using the Magnetic Beads Cleanup Module (ThermoFisher Scientific). All procedures were carried out in accordance with the manufacturer's manuals. The quality and quantity of samples that are enriched for small RNA were assessed by Agilent 2100 Bioanalyzer with the Small RNA chip (Agilent Technologies).

3.3.5 cDNA Library Construction

Complementary DNA (cDNA) libraries were constructed from the enriched small RNA using the Ion Total RNA-Seq Kit v2 (ThermoFisher Scientific), in accordance with the manufacturer's manuals. Ion Adaptor Mix v2 were hybridized and then ligated to small RNA followed by reverse transcription to generate a cDNA library. The cDNA products were purified and size-selected with the Magnetic Beads Cleanup Module and then PCR amplified with barcoded primers for 14 cycles. The barcoded cDNA libraries were purified and size-selected with the Magnetic Beads Cleanup Module and the yield and size distribution of cDNA libraries were assessed by Agilent 2100 Bioanalyzer with the High Sensitive DNA chip (Agilent Technologies).

3.3.6 Ion S5XL Sequencing and Data Analysis

The barcoded cDNA libraries were diluted to 30pM and 8 libraries were pooled for each run. Template-positive ion sphere particles containing clonally amplified DNA were generated and enriched using the Ion 540 Chef kit with the Ion Chef instrument (ThermoFisher Scientific). Ion Torrent sequencing was performed for 160 flows on an Ion S5XL Sequencer with Ion 540 Chips.

Ion Torrent platform-specific pipeline software (Torrent Suite version 5.0.4; ThermoFisher Scientific) was used to remove polyclonal, low quality and adapter dimer reads and then separate barcoded reads for each sample. Ion Torrent platform-specific Small RNA Analysis plug-in (v5.0.5) was used to analyze micro RNA reads. Reads were aligned to mature micro RNAs (mirBase build 21) using bowtie2 aligners. Unmapped reads were further aligned to the hg19 Human genome reference to rescue miRbase unaligned reads and count other RNA

molecules. The miRNA expression levels were quantified as the number of reads mapped to individual miRNAs normalized by the total number of mapped reads in miRBase per sample.

3.3.7 Statistical analysis

Baseline characteristics of patients who did and did not develop metastasis were described using medians and interquartile ranges for continuous variables and proportions for categorical variables. Patients who developed metastasis and controls were compared using the Wilcoxon rank sum test for continuous variables and Fisher's exact test for categorical variables.

We then undertook variable selection in order to identify miRNA sequences which were significantly differentially expressed between patients who developed metastasis and controls using bootstrapping with automated backward selection[307]. To do this, first, we excluded sequences where the mean reads per million (RPM) was less than 10 for both cases and controls as sequences with these low expression levels are unlikely to provide reproducible results during qPCR validation[153]. Second, we performed screening: we identified sequences which were associated with metastasis with a $p<0.05$ on univariate logistic regression models. Among the sequences identified on screening, we conducted multivariable logistic regression with backwards selection to identify significant independent predictors of metastasis. Variables were retained in the model if the significance of association was $p<0.05$. We performed bootstrapping (sampling with replacement for a total sample of N) for a total of 1000 repetitions[307]. We identified the frequency with which each sequence was included in the predicted models. Sequences which were maintained in greater than 100 bootstrapped samples were considered important predictors.

Among the selected sequences identified using bootstrapping with automated backward selection, we examined whether a parsimonious combination could be identified. We compared the predictive ability of each combination of sequences using the Score criterion (higher Score considered better).

Using the combination of variables identified previously, we developed a risk score using the linear combination of the expression level of each selected miRNA sequence, weighted by the regression coefficient derived from a univariate logistic regression model[304,305].

All statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

3.4 Results

We identified 32 patients who developed metastasis following radical prostatectomy. Of these, 28 were matched to a control based on Gleason score and duration of follow-up and 4 were excluded due to an inability to identify a suitable match. Of the 28 matched pairs, there was an insufficient sample for analysis in at least one member of 9 pairs. Thus, the analytic cohort comprised 19 pairs of patients matched on Gleason score and duration of follow-up (Figure 3.1). A greater proportion of patients who developed metastasis following radical prostatectomy had pathological stage T3 disease and positive lymph nodes (Table 3.1). Among patients who developed metastasis, the median time to metastasis following radical prostatectomy was 4.84 years (interquartile range: 2.34 – 7.75 years).

Figure 3.1. Cohort derivation.

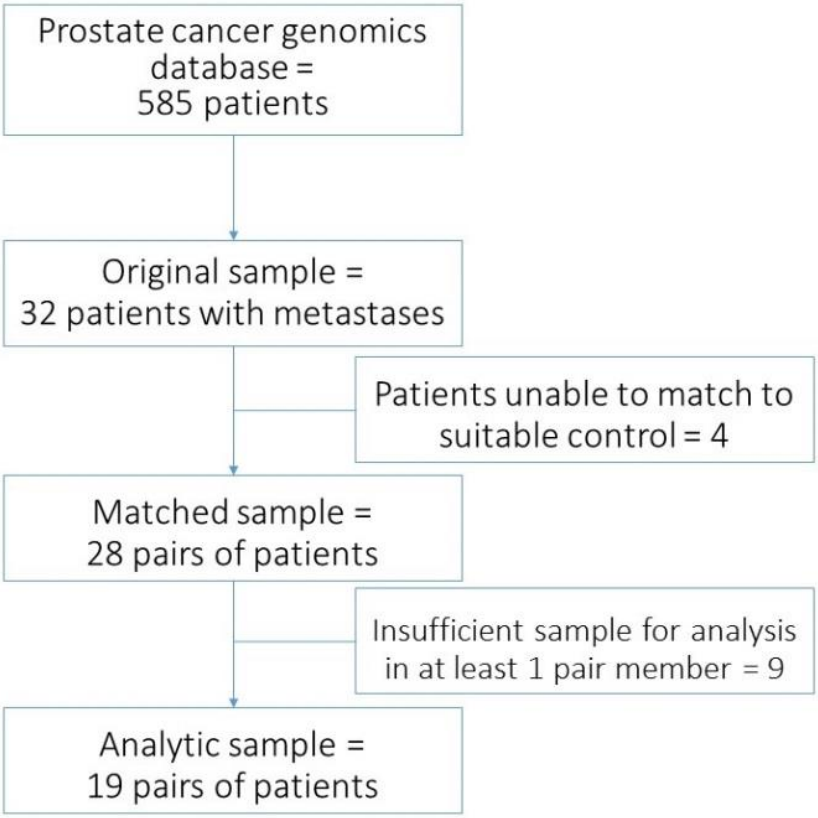


Table 3.1. Baseline demographic characteristics of patients in the discovery cohort.

	Controls (no metastasis)	Cases (metastasis)	p-value
Sample (n)	19	19	
Age (median, IQR)	63.9 (61.7-67.0)	62.7 (59.1-67.6)	0.54
Gleason score (n,%)			1.00
6	1 (5.3)	1 (5.3)	
7	8 (42.1)	8 (42.1)	
8-10	10 (52.6)	10 (52.6)	
Pathological Stage (n,%)			0.0006
T2	11 (57.9)	1 (5.3)	
T3	8 (42.1)	18 (94.7)	
Nodal status (n,%)			0.004
Negative	6 (31.6)	10 (52.6)	
Positive	0	5 (26.3)	
Missing	13 (68.4)	4 (21.1)	
Positive margins (n,%)	8 (42.1)	10 (52.6)	0.75
PSA (median, IQR)	7.3 (4.0-10.0)	10.0 (4.3-15.1)	0.30

We identified 2792 unique miRNA which were expressed in the prostate tumors of patients in the analytic cohort. Of these, 1920 had very low levels of expression in both cases and controls (less than 1 RPM) and a further 375 had low levels of expression in both groups (RPM between 1 and 10). Therefore, after exclusion of 2295 sequences with low expression (82.2%), there were 497 miRNA sequences (17.8%) with adequate expression which were used for further analysis.

We then undertook bootstrapping with backward selection to identify predictors of metastasis. On univariate screening, 99 sequences were associated with metastasis with a p-value < 0.05. Based on bootstrapping with 1000 repetitions, 28 miRNA sequences were retained in at least one bootstrapped sample, 21 sequences were retained in at least 50 bootstrapped samples and 5 miRNAs were retained in at least bootstrapped 100 samples. The five miRNA sequences retained in at least 100 bootstrapped samples were miR-17-3p, miR-27a-3p, miR-200a-3p, miR-375, and miR-376b-3p. Each of these sequences was up-regulated in patients who developed metastasis as compared to those who remained cancer-free (Table 3.2).

Table 3.2. Expression level (RPM) of five miRNAs identified to predict metastasis.

MiRNA sequence	Controls (no metastasis)	Cases (metastasis)	Differential expression
miR200a_3p	2288.8	5340.88	2.33
miR375	6849.91	12278.4	1.79
miR376b_3p	12.2533	20.6362	1.68
miR17_3p	195.04	323.011	1.66
miR27a_3p	4004.76	5350.32	1.34

We examined all possible permutations of the five miRNAs in order to ascertain if a parsimonious panel could be identified. The combination of five miRNAs had the highest Score criterion, indicating that this combination resulted in the greatest predictive ability (Table 3.3).

Table 3.3. All possible regression models using five miRNA sequences identified by bootstrapping with backward selection.

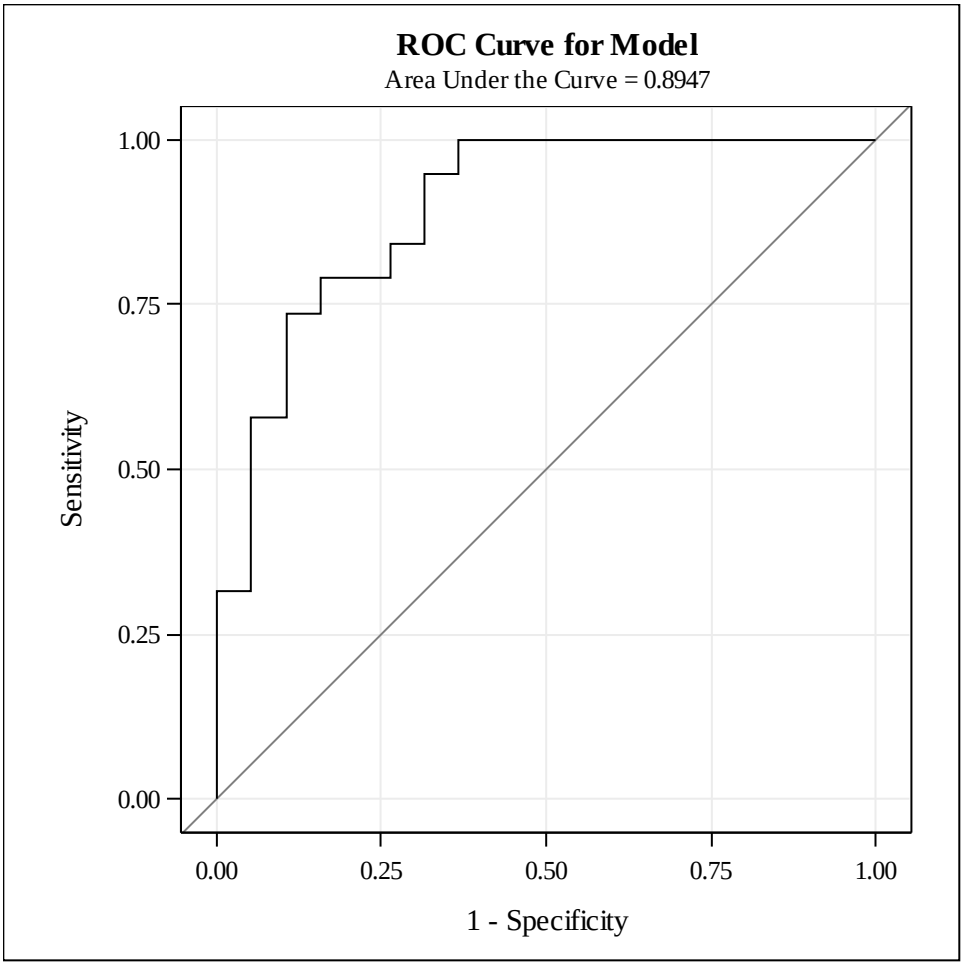
Number of variables	Score criterion	Variables Included in Model
5	**15.9406**	miR-200a-3p miR-375 miR-376b-3p miR-17-3p miR-27a-3p
4	**15.5868**	miR-200a-3p miR-375 miR-376b-3p miR-27a-3p
4	**15.1226**	miR-200a-3p miR-375 miR-376b-3p miR-17-3p
4	**14.5503**	miR-200a-3p miR-375 miR-17-3p miR-27a-3p
3	**14.4445**	miR-200a-3p miR-375 miR-376b-3p
3	**14.3435**	miR-200a-3p miR-375 miR-27a-3p
4	**14.3236**	miR-375 miR-376b-3p miR-17-3p miR-27a-3p
4	**13.4773**	miR-200a-3p miR-376b-3p miR-17-3p miR-27a-3p
3	**13.4388**	miR-375 miR-376b-3p miR-17-3p
3	**12.854**	miR-200a-3p miR-376b-3p miR-17-3p
3	**12.5932**	miR-375 miR-17-3p miR-27a-3p
3	**12.4601**	miR-200a-3p miR-376b-3p miR-27a-3p
3	**12.4207**	miR-375 miR-376b-3p miR-27a-3p
3	**11.7461**	miR-376b-3p miR-17-3p miR-27a-3p
3	**11.6345**	miR-200a-3p miR-17-3p miR-27a-3p
3	**11.5436**	miR-200a-3p miR-375 miR-17-3p
2	**11.3916**	miR-200a-3p miR-376b-3p
2	**11.0667**	miR-376b-3p miR-17-3p
2	**10.9695**	miR-375 miR-27a-3p
2	**10.8897**	miR-200a-3p miR-375
2	**10.8624**	miR-200a-3p miR-27a-3p
2	**10.5273**	miR-375 miR-376b-3p
2	**9.482**	miR-17-3p miR-27a-3p
2	**9.0914**	miR-375 miR-17-3p
2	**8.7627**	miR-200a-3p miR-17-3p
2	**7.9928**	miR-376b-3p miR-27a-3p
1	**7.242**	miR-200a-3p
1	**6.1081**	miR-17-3p
1	**6.0519**	miR-376b-3p
1	**6.022**	miR-27a-3p
1	**5.647**	miR-375

We then derived a risk score based on the linear combination of the expression level of each selected miRNA sequence, weighted by the regression coefficient derived from a univariate logistic regression model[304,305]. The risk score was:

(0.00635 x miR-17-3p) + (0.000548 x miR-27a-3p) + (0.000466 x miR-200a-3p) + (0.000144 x miR-375) + (0.1121 x miR-376b-3p).

This miRNA panel yielded an area under the curve (AUC) of 89.5% (95% CI 79.5-99.5%) to predict prostate cancer metastasis (Figure 3.2).

Figure 3.2. Receiver operating curve for five miRNA panel to predict metastasis.



3.5 Discussion

In this study, we identified a novel panel of miRNA comprising miR-17, miR-27a, miR-200a, miR-375, and miR-376b which is strongly associated with the development of metastasis following surgery for localized prostate cancer (AUC 89.5%). Each of the miRNAs identified for inclusion in the panel has previously been associated with carcinogenesis, though not all have been associated with prostate carcinogenesis. MiR-375 is well-described in the carcinogenesis and progression pathways of prostate cancer[308,309] and has been proposed as both a diagnostic[310] and a prognostic[311] biomarker for prostate cancer. Increased expression of miR-375 has been found in prostate cancer cells, as compared to normal cells[308,309], indicating a pro-oncogenic role despite anti-invasive and anti-epithelial-mesenchymal transition properties[312]. Further, upregulation of miR-375 has been shown to be associated with disease recurrence in breast cancer[313]. In contrast, decreased expression of miR-375 has been associated with many other tumor sites including hepatocellular carcinoma, gastric cancer, esophageal cancer, head and neck cancers, lung cancer, and cervical cancer indicating a tumor suppressor role at these sites[314]. MiR-17-3p and its corresponding mature strand have been shown to enhance prostate tumor growth and invasion through the suppression of TIMP metallopeptidase inhibitor 3 (TIMP3)[315]. Further work has shown that miR-17 may act in the carcinogenesis pathway of hepatocellular carcinoma[316], gallbladder cancer[317], glioblastoma[318], breast cancer[319], and colorectal cancer[320]. However, others have shown that miR-17-3p may act as a tumor suppressor in prostate cancer[321]. We found that miR-17-3p was over-expressed in the tumors of patients who developed metastasis (differential expression 1.66). Thus, further work is necessary to understand its role in prostate carcinogenesis. Similar complexity exists for miR-27a-3p. While, to our knowledge, there are no studies linking changes in miR-27a-3p expression to prostate

carcinogenesis, there is evidence that it functions in promoting the development and progression of other urologic malignancies including renal cell carcinoma[322] and bladder cancer[323,324] in addition to other tumors including gastric cancer[325], esophageal cancer[326], pancreatic cancer[327], colorectal cancer[328], and glioma[329]. However, in hepatocellular carcinoma, miR-27a-3p appears to have a tumor suppressive role[330]. Again, we demonstrated increased expression in patients who developed metastatic disease (differential expression 1.34). Similar to miR-27a-3p, there are, to our no knowledge, no studies assessing the association between miR-200a-3p expression and prostate carcinogenesis. Mir-200a-3p appears to be involved in the development and progression of gastric cancer[331], hepatocellular carcinoma[332], lung cancer[333], colorectal cancer[334], HPV-induced tonsillar cancer[335], while it suppresses development of renal cell carcinoma[336] and negatively correlated with the aggressiveness of gliomas[337]. Further, miR-200a-3p may play a role in the development of chemotherapy resistance[338]. MiR-200a-3p has also been implicated in the development of polycystic ovarian syndrome[339]. Finally, we are unaware of any direct associations between miR-376b-3p expression and prostate cancer or general carcinogenesis. Among three studies returned on a PubMed search for "miR-376b-3p" are manuscripts demonstrating its performance as a biomarker for periodontitis[340], its down-regulation in mouse models of chronic obstructive pulmonary disease[341], and its role in the central nervous system response to hypoxia[342]. While these citations are seemingly oblique to carcinogenesis, miR-376b-3p is associated with TGF-β1 which is a well-recognized initiator and regulator of epithelial-mesenchymal transition[343]. TGF-β1 has been directly implicated in prostate cancer treatment response and prognostication[344,345]. Of the identified miRNAs, miR-17 and miR-27a have previously been included in predictive panels for colon cancer diagnosis and

prognosis[320,328] and miR-375 has been included in a panel for prostate cancer diagnosis, but not prognostication[310].

Previous studies have sought to identify miRNA which may be important in prostate cancer prognosis and many have been identified[154-159]. However, these have not been used in routine clinical practice. This likely reflects at least two issues: first, validation of the findings and secondly, the use of biochemical recurrence, a surrogate outcome. Kristensen et al. have recently published their work identifying miRNAs for prostate cancer diagnosis and prognosis. Following derivation in a discovery cohort, they validated their findings in an independent group of patients within their institution (validation cohort 1) and using a publicly available dataset (validation cohort 2)[346]. They identified a panel of three miRNAs (miR-185-5p, miR-221-3p, and miR-326) for prognosis. As with other authors, they used biochemical recurrence as an outcome. While this is appealing due to its relatively high frequency and early appearance[214], only a small percentage of men with biochemical recurrence will have systemic progression or die of their disease[208]. Thus, metastasis, as used in our study, is a more clinically-relevant endpoint. In our previous study examining miRNA for prostate cancer prognostication, we considered a composite endpoint of biochemical recurrence and metastasis in our discovery cohort[153], while in this study we specifically identified patients who developed radiographic evidence of metastatic disease. While the prior panel was independently prognostic for the prediction of metastases[153], further study showed that many of the miRNA included in the panel were much more strongly associated with biochemical recurrence than metastasis[95].

The miRNA panel identified in this analysis is currently undergoing further validation work using independent patient cohorts from the University of British Columbia and l'Université Laval and publicly available datasets including The Cancer Genome Atlas[347].

Such a panel, once validated, may allow for the provision of appropriate subsequent therapies after surgery. In a similar vein, Zehentmayr et al. have proposed using miR-375 expression levels to guide post-operative therapy following breast conserving surgery[313]. Future research will be undertaken to assess whether this panel can be applied to transrectal-ultrasound guided prostate biopsy tissue or serum samples in order to risk stratify patients prior to local treatment.

In addition to their use as biomarkers, identification of miRNA associated with metastasis following radical prostatectomy may allow for further biologic insights into prostate carcinogenesis and progression. We have previously used epidemiologic observations such as those included in this manuscript to guide such research into biologic mechanisms, including understand the biology of miR-301a[95] and miR-182[348]. Using miRwalk 2.0, a publicly available online databased of miRNA-target interactions[349,350], thousands of potential gene targets were identified for the five miRNA included in this panel. These include genes which have previously been implicated in carcinogenesis, including ABL2, a proto-oncogene[351]. Further bioinformatics work will be required to understand the complex miRNA-gene interactions through which these miRNA exert their biologic effects.

There are limitations to the use of miRNA profiling from prostate cancer tissue. Foremost is the intra-tumoral heterogeneity among tumor foci. Most patients with prostate cancer have a tumor foci with varying Gleason scores. We[153], and others[352,353], have shown that miRNA expression varies by Gleason grade. Further study remains necessary to determine the best method to identify representative samples for miRNA profiling. In this study, we used a section of the largest tumor focus with the highest tumor-to-stroma ratio. Kristensen et al. employed a similar strategy in which they selection representative areas with >90% tumor

involvement[346]. Methodologically, most studies use the magnitude of differential expression with or without clinical judgement for selection of predictors for inclusion in panels. As we sought to develop a strongly predictive model, we employed a selection strategy, bootstrapping with automated backwards selection, which relied on the strength of association for inclusion.

3.6 Conclusions

Using a genome wide analysis of microRNA expression, we have identified a novel panel of five microRNAs which are associated with prostate cancer metastasis following radical prostatectomy. However, the strengths of these findings are limited due to sample size and require external validation prior to clinical applicability.

CHAPTER 4: NON-PROSTATE CANCER AND CARDIOVASCULAR MORTALITY FOLLOWING PROSTATE CANCER TREATMENT: THE ROLE OF PRIMARY TREATMENT MODALITY AND ANDROGEN DEPRIVATION THERAPY

4.1 Abstract

Background: There are many observational studies comparing survival rates between patients undergoing radiation and surgery for non-metastatic prostate. The addition of androgen-deprivation therapy improves prostate-cancer specific mortality, but it is unknown how it effects non-prostate cancer specific mortality.

Objective: To assess rates of non-prostate mortality, including cardiovascular mortality, among patients with non-metastatic prostate cancer treated with surgery or radiotherapy, while considering the role of androgen deprivation therapy (ADT) as a potential confounder or indirect causal pathway.

Design: Population-based, propensity-score matched retrospective cohort study.

Data sources and study population: Men treated for non-metastatic prostate cancer in Ontario, Canada between 2002 and 2009. Patients treated with surgery were matched 1:1 with those treated with radiotherapy based on age, general and specific comorbidities, previous cardiovascular events, and demographic factors.

Main outcome measures and analysis: Non-prostate cancer mortality. Secondary outcomes were cardiovascular mortality and ischemic cardiac events. We used the Fine & Gray sub-distribution method with generalized estimating equations to compare outcomes while accounting for competing risks. We accounted for ADT exposure as a time-varying binary and cumulative exposure covariate.

Results: Of 20,651 eligible men, we matched 10,786 (5393 pairs). Patients treated with radiotherapy were more likely to receive ADT at some point during their treatment than those receiving surgery (34% vs 17%, respectively; p<0.0001). The 5- and 10-year cumulative incidence of non-prostate cancer mortality was higher among patients who underwent primary radiotherapy (4% and 12%, respectively) than those who underwent surgery (2% and 8%, respectively; aHR 1.57, 95% CI 1.35-1.83, p<0.0001). Patients treated with radiotherapy were also at increased risk of cardiovascular death (aHR 1.74, 95% CI 1.27-2.37) and ischemic cardiac events (aHR 1.13, 95% CI 1.03-1.24). ADT, whether operationalized as a binary or cumulative dose exposure, was not significantly associated with outcomes (p = 0.26 – 0.81). In a *post-hoc* analysis, we restricted our cohort to patients treated with radiotherapy to reduce selection biases. We found no association between ADT and any of our outcomes in this subgroup.

Conclusions: Among patients carefully matched on the basis of baseline cardiovascular risk, those treated with radiotherapy had an increased risk of non-prostate cancer mortality, cardiovascular mortality, and ischemic cardiac events. This did not appear to be mediated by ADT exposure. Due to the observational nature of the data, the potential for confounding remains.

4.2 Introduction

Five-year relative survival for men newly diagnosed with prostate cancer exceeds 99%[3]. As a result, unlike for patients newly diagnosed with more fatal tumours, competing risks of death are significant. Heart disease remains the most common cause of death among men in general[3] and among men diagnosed with prostate cancer[354]. Further, men with prostate cancer are at increased risk of both fatal and non-fatal cardiovascular disease, as compared to age-matched counterparts in the general population, regardless of treatment modality[355].

The association between androgen deprivation therapy (ADT) and cardiovascular disease is well-established[13,356]. More recently, both treatment with radiotherapy (compared to surgery) and receipt of ADT were shown to increase the risk of coronary artery disease, myocardial infarction, and sudden cardiac death among older men treated for clinically-localized prostate cancer[226].

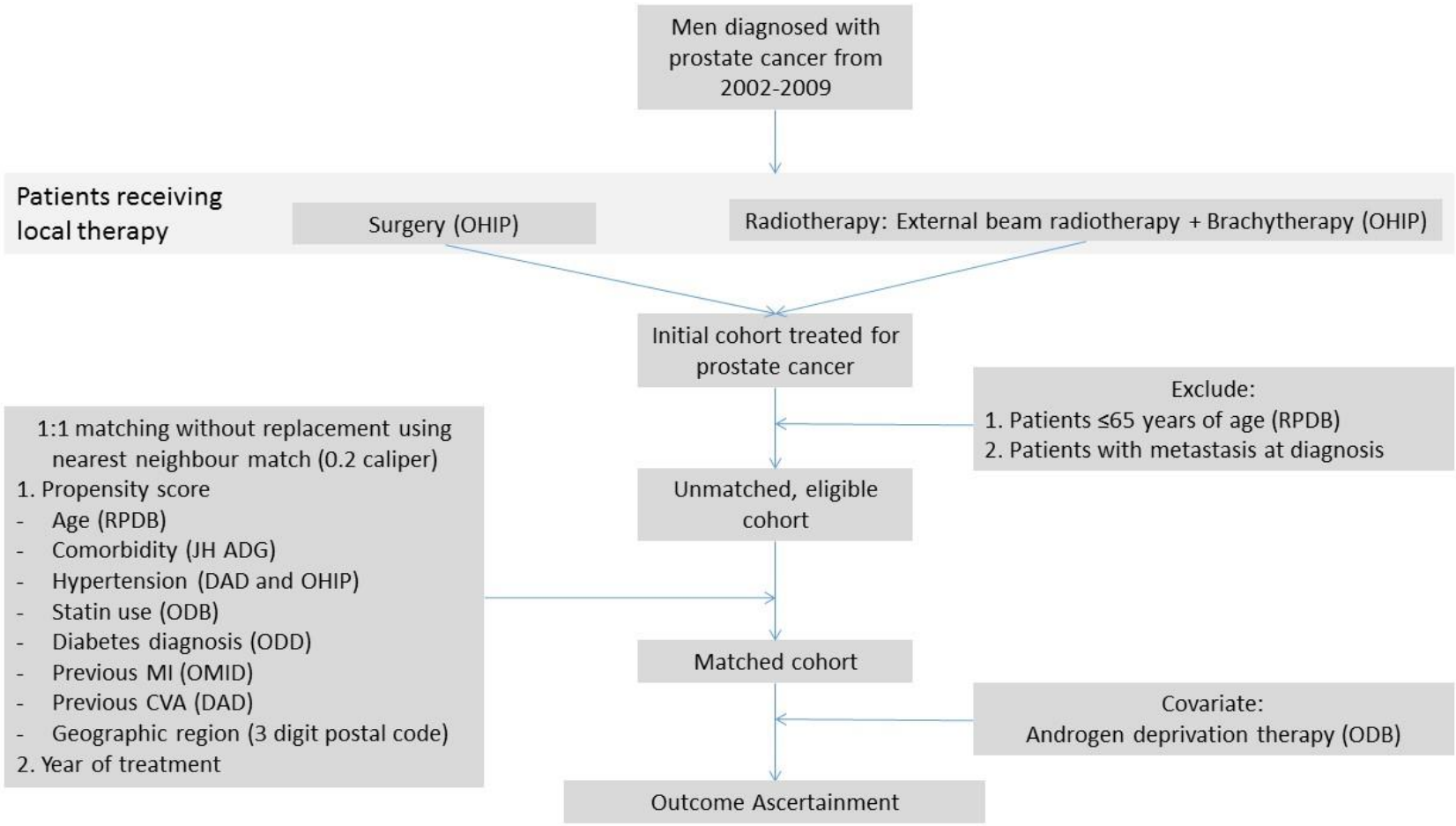
ADT has been advocated for many patients undergoing radiotherapy. Recently, there has been increasing frequency and duration of ADT use among patients undergoing external beam radiotherapy[357]. Ehdaie and Eastham have postulated that patients treated with radiotherapy, compared to surgery, may be at higher risk of mortality either due direct radiation effects, or indirectly through ADT[16]. We hypothesized that cardiovascular disease may explain this observed relationship. Therefore, we examined non-prostate cancer mortality, cardiovascular mortality, and ischemic cardiac events in men over the age of 66 treated with radiotherapy as compared to those treated with surgery while considering ADT as an indirect causal pathway.

4.3 Methods

4.3.1 Study design and setting

We conducted a population-based, retrospective cohort study of men aged 66 and older diagnosed with prostate cancer from April 1, 2002 to December 31, 2009 in Ontario, Canada using administrative hospital data, physician billing codes, and cancer registry data from the Institute of Clinical Evaluative Sciences (ICES). The study design is outlined in Figure 4.1. In Ontario, all citizens receive health care, financed by the single government payer Ontario Health Insurance Program (OHIP). Outpatient pharmaceuticals are provided to citizens aged 65 years and older through the Ontario Drug Benefit. Men were followed from the date of primary treatment until death or March 31, 2013. This study was approved by the Sunnybrook Health Sciences Centre Research Ethics Board (#203-2015).

Figure 4.1. Flow diagram of cohort derivation.



4.3.2 Data Sources

Using ICES as a data repository, we linked a number of administrative datasets including the Ontario Cancer Registry (OCR) which has been estimated to capture more than 95% of cancers in Ontario[358]; Ontario Drug Benefit (ODB) which provides information on all outpatient pharmaceuticals for patients aged 65 years and older[359]; the Canadian Institute for Health Information (CIHI) Discharge Abstract Database (DAD) which contains records for each hospitalization[360]; the CIHI National Ambulatory Care Reporting System (NACRS) which contains records for ambulatory and emergency room visits; the Ontario Health Insurance Plan (OHIP) which tracks claims paid for physician billings, laboratories, and out-of-province providers (OHIP fee codes are provided for specific procedures with specific indications)[361]; the Ontario Myocardial Infarction Database (OMID) which contains validated data on all Ontario patients hospitalized with a most responsible diagnosis of acute myocardial infarction[362-364]; the Ontario Diabetes Database (ODD) which provides validated data on all Ontarians diagnosed with diabetes[365]; the Ontario Registrar General – Death database for vital status and cause of death information; and the Registered Persons (RPDB) for demographic information. While no administrative dataset is free of information bias, each of our data sources has been validated and found to have good sensitivity and specificity.

4.3.3 Study participants

We identified men aged 66 years and older diagnosed with prostate cancer in the Ontario Cancer Registry (dxcode 185, ICD-10 C61) over the study interval. As prescription medication information is available beginning at age 65, age 66 was selected to ensure that patients were not exposed to ADT prior to study entry and to ensure we could accurately ascertain the

duration of ADT exposure. As we want to examine the influence of primary treatment modality, we included patients treated with radical prostatectomy (OHIP billing code S651 or Canadian Classification of Health Interventions (CCI) 1.QT.91), external beam radiotherapy (OHIP billing codes X310, X311, X312, X313, A343, A340, A341, K013 or CCI 1.SQ.27.JA, 1.QT.27.JA, 1.QT.27.JA-DA, 1.QT.27.JA-DB, 1.QT.27.JA-DC, 1.QT.27.JA-DE, 1.QT.27.JA-DG, 1.QT.27.JX) or brachytherapy (OHIP billing codes S640, X323, X324, X325, X313 and J138 same day or CCI 1.QT.26.BA-EB, 1.QT.26.BA-EC, 1.QT.26.HA, 1.QT.26.HA-EB, 1.QT.26.HA-EC, 1.QT.26.LA, 1.QT.26.LA-EB, 1.QT.26.LA-EC, 1.QT.53.HA-EM, 1.QT.53.LA-EM) within 1 year of their initial diagnosis. As radiotherapy can be given with either curative or palliative intent, we excluded patients diagnosed with metastatic disease (OCR diagnostic code 198).

4.3.4 Exposure

We examined patients treated with any form of radiotherapy (external beam or brachytherapy) as our primary exposure. *A priori* sub-group analyses were conducted to assess each of these treatment modalities separately. The control group comprised men treated with radical prostatectomy.

In addition to local therapy, we examined the use of ADT as a key exposure. We used the ODB to identify LHRH agonists and antagonists administered from the date of diagnosis until death or the study end date (Appendix 4.1). We modeled ADT as both a time-dependent binary exposure and a time-dependent cumulative exposure. In the time-dependent binary models, patients could move from a non-exposed to an exposed status over time. Once exposed, patients were considered exposed for the duration of follow-up. In the time-dependent

cumulative exposure models, we partitioned both exposure and follow-up time for each exposed man into categories. First, each exposed man contributed time and outcome status to the first category until he reached 6 months of cumulative exposure, the second from 6 to 12 months, the third from 12 to 18 months, the fourth from 18-24 months, the fifth from 24-36 months, and the sixth thereafter. We then repeated this procedure with a less granular approach and categorized exposure into durations of 0-12 months, 12-24 months and greater than 24 months.

The specific procedural codes employed in this study have not be validated; however, previous work has demonstrated that the data sources employed may reliably capture procedures[361,366]. The OBD is a reliable source of prescription information with an error rate of 0.7%[359].

4.3.5 Outcomes

The primary outcome measure was non-prostate cancer mortality as ascertained from the Ontario Registrar General – Death (ORGD). Our secondary outcome measures included cardiovascular mortality and a composite of ischemic heart disease-related cardiovascular events including myocardial infarction, coronary syndrome, angina pectoris, angiography, angioplasty, and coronary artery bypass grafting. The composite ischemic heart disease outcome was ascertained using the CIHI DAD and CIHI NACRS databases using ICD-10 diagnostic codes, the OHIP database using physician billing codes, and the Ontario Myocardial Infarction Database (Appendix 4.2).

While prostate cancer has not been validated as a cause of death in the Ontario Cancer Registry (OCR), cause of death has been validated for both head and neck cancers (kappa = 0.79)[367] and breast cancer (kappa = 0.88)[368]. Other authors have used Ontario Registrar

General's database in the past for cause of death[369] and cause-specific death[370] ascertainment. Each of the data sources used for our ischemic heart disease-related cardiovascular event outcome have been validated: OMID has demonstrated accuracy in excess of 94% for acute myocardial infarction; the CIHI DAD has good sensitivity and specificity for myocardial infarction[371] though this is somewhat lower for angina[371]; OHIP data for angiography, angioplasty, and coronary artery bypass grafting showed over 99% agreement on cross-validation[372].

4.3.6 Covariates

Demographic covariates of interest include age, comorbidity, previous diabetes diagnosis, previous hypertension diagnosis, previous dyslipidemia treatment, previous cardiovascular events, and geographic region. The Johns Hopkins ADG score was determined via the linked administrative data holdings from the year prior to prostate cancer diagnosis and was used to measure comorbidity. Further, we identified patients diagnosed with diabetes using the ODD (binary measure). We used a validated administrative algorithm to identify hypertension (binary measure)[373]. Further, we assessed dyslipidemia by identifying patients with more than 6 months of continuous prescription of statin medications in the two years prior to index date, using the ODB (binary measure; drug identification numbers in Appendix 4.3). We used OMID and CIHI DAD and NACRS databases to ascertain if patients had a myocardial infarction or cerebrovascular accident in the five years prior to the index date (binary measure for each).

4.3.7 Matching

To adjust for biases from non-random allocation to surgery and radiation, we employed propensity-score matching. The propensity score comprised age, comorbidity score, diabetes diagnosis, hypertension, dyslipidemia, previous myocardial infarction, previous cerebrovascular accident, year of treatment and geographic region of residence (measured by 3-digit postal code). Propensity score matched pairs were created without replacement in a 1:1 match using the greedy algorithm and a caliper length of 0.2. We examined the similarity of the radiotherapy and surgery groups following matching using standardized differences. Standardized differences of less than 10% were considered adequate balance between the groups[374]. Due to limitations of the available databases, we were unable to include tumor factors such as prostate specific antigen, tumor stage, and tumor grade (Gleason score) in the matching process.

As ADT exposure is affected by the choice of treatment modality, it would be inappropriate to include in the matching process[375]. Instead, it was included in the regression models.

4.3.8 Statistical analysis

We used descriptive statistics to summarize the demographic characteristics of the cohort, stratified by local treatment modality.

We examined the total treatment effect of radiotherapy, as compared to surgery, on non-prostate cancer mortality. We assessed the cumulative incidence function with potential outcomes including non-prostate cancer mortality (outcome of interest), prostate cancer mortality, and alive at study end and compared outcomes for patients treated with surgery and radiotherapy using Gray's test[376]. We used the Fine and Gray sub-distribution method[377] with

generalized estimating equation survival models with a sandwich variance estimator (marginal models) in order to calculate the sub-distribution hazard ratio (sdHR) of primary treatment effect.

We then examined the effect of primary treatment modality (radiotherapy vs. surgery) and ADT in time-dependent multivariable generalized estimating equation survival models with a sandwich variance estimator (marginal models) using the Fine and Gray sub-distribution method in order to calculate the sub-distribution hazard ratios (sdHRs) for both primary treatment modality and ADT (both binary and cumulative exposure). In order to examine cumulative exposure, we categorized the duration of ADT exposure into clinically-meaningful categories as described in *Section 4.3.4 Exposure*.

We repeated our analysis for the secondary outcomes of cardiovascular mortality and ischemic heart disease-related cardiovascular events. In the competing risks models assessing cardiovascular mortality, potential outcomes included the outcome of interest, prostate cancer mortality, non-prostate cancer / non-cardiovascular mortality, and alive at study end. For ischemic heart disease-related cardiovascular events, we assessed only the first event as both risk factors and ongoing risks of future events are likely to change following an event. Outcomes of interest for this analysis included ischemic cardiac event prior to death, death prior to ischemic cardiac event, and alive without ischemic cardiac event.

For each analysis, the matching identifier was used as a clustering variable. The global null hypothesis was assessed using the Wald test. We verified the assumptions underlying the model: multicollinearity (where relevant) using the variance inflation factor with a cut-off of 4; the proportionality assumption using Schoenfeld residuals; influential observations using the deviance residual (removed only where biologically implausible); and over-specification by

looking for separation of data points. No violations of these assumptions were identified. Statistical significance was set at $p<0.05$ based on a two-tailed comparison. Statistical analyses were performed using Enterprise Guide 6.1 (SAS Institute Inc., Cary, NC, USA).

4.3.9 Subgroup and sensitivity analyses

In order to quantify the effect of prostate cancer treatments on our outcomes of interest, we matched each patient in the study cohort (whether treated by surgery or radiotherapy) to 5 members of the general public using a hard age match and a propensity-score comprising comorbidity score, diabetes diagnosis, hypertension, dyslipidemia, previous myocardial infarction, previous cerebrovascular accident, and geographic region of residence (measured by 3-digit postal code). Each matched patient was assigned an index date which corresponded to the date of treatment of the corresponding prostate cancer patient. We repeated the analyses using the control patients as the referent.

As residual selection bias following propensity-score matching may have affected our conclusions, we examined the effect of ADT on the primary and secondary outcomes among all patients treated with radiotherapy in the pre-matched cohort. We operationalized ADT exposure as a time-varying binary and 3-level categorical exposure as detailed in *Section 4.3.4 Exposure*. Within this group, we explored effect modification due to a prior history of myocardial infarction, comorbidity, and age on the effect of ADT.

Finally, as a sensitivity analysis, we assumed no difference in non-prostate cancer mortality between patients treated with surgery and radiotherapy. We then quantified the prevalence and strength of association necessary for a potential unmeasured binary residual

confounder to fully explain the observed differences between patients treated with radiotherapy and surgery using the technique of Lin, Psaty, and Kronmal[378].

4.4 Results

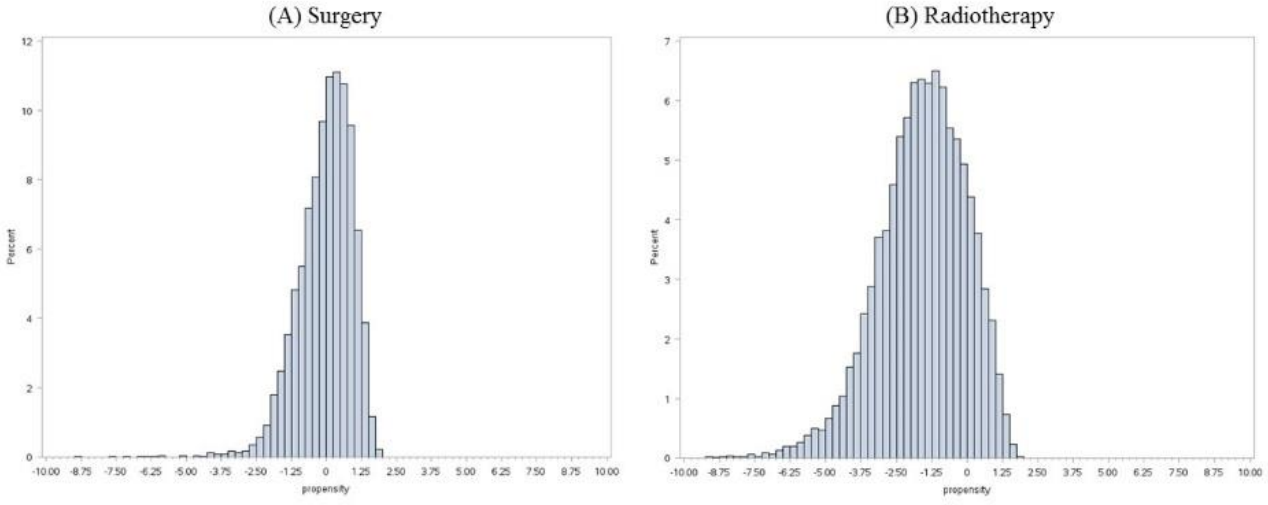
Between April 2002 and 2009, we identified 20,651 men aged 66 years and older who underwent surgery or radiotherapy for non-metastatic prostate cancer. Of these, 6851 (33.2%) received radical prostatectomy and 13,800 (68.2%) received radiotherapy as their primary treatment modality. Prior to propensity-score matching, patients treated with radiotherapy were older and had greater levels of comorbidity, both in aggregate and for the specific comorbidities considered (hypertension, dyslipidemia, and diabetes; Table 4.1). The distribution of propensity-scores for patients treated by surgery and radiotherapy, prior to matching, is shown in Figure 4.2.

Table 4.1. Baseline characteristics of patients treated for non-metastatic prostate cancer with surgery or radiotherapy, before and after propensity score matching.

	Before propensity-score matching			After propensity-score matching		
	Surgery	**Radiotherapy**	**Std Diff**	**Surgery**	**Radiotherapy**	**Std Diff**
Sample size	N=6,851	N=13,800		N=5,393	N=5,393	
Age at diagnosis						
Mean ± SD	68.92 ± 2.63	72.99 ± 4.46	1.11	69.45 ± 2.68	69.46 ± 2.78	0
Median (IQR)	68 (67-70)	73 (70-76)	1.15	69 (67-71)	69 (67-71)	0.01
Hypertension (n,%)	5,149 (75.2%)	11,183 (81.0%)	0.14	4,166 (77.2%)	4,187 (77.6%)	0.01
Active statin use (n,%)	654 (9.5%)	3,256 (23.6%)	0.38	622 (11.5%)	636 (11.8%)	0.01
Diabetes (n,%)	1,176 (17.2%)	3,115 (22.6%)	0.14	1,003 (18.6%)	1,067 (19.8%)	0.03
History of MI (n,%)	68 (1.0%)	301 (2.2%)	0.1	65 (1.2%)	76 (1.4%)	0.02
History of stroke (n,%)	18 (0.3%)	135 (1.0%)	0.09	18 (0.3%)	15 (0.3%)	0.01
Year of treatment (n,%)						
2002	710 (10.4%)	1,542 (11.2%)	0.03	580 (10.8%)	571 (10.6%)	0.01
2003	717 (10.5%)	1,617 (11.7%)	0.04	587 (10.9%)	623 (11.6%)	0.02
2004	770 (11.2%)	1,648 (11.9%)	0.02	614 (11.4%)	610 (11.3%)	0
2005	839 (12.2%)	1,623 (11.8%)	0.01	648 (12.0%)	632 (11.7%)	0.01
2006	936 (13.7%)	1,754 (12.7%)	0.03	732 (13.6%)	707 (13.1%)	0.01
2007	988 (14.4%)	1,784 (12.9%)	0.04	770 (14.3%)	763 (14.1%)	0
2008	834 (12.2%)	1,682 (12.2%)	0	636 (11.8%)	644 (11.9%)	0
2009	795 (11.6%)	1,581 (11.5%)	0	614 (11.4%)	633 (11.7%)	0.01
2010	262 (3.8%)	569 (4.1%)	0.02	212 (3.9%)	210 (3.9%)	0
Comorbidity score (ADG sum)						
Mean ± SD	8.39 ± 2.98	9.12 ± 3.11	0.24	8.56 ± 3.00	8.62 ± 2.99	0.02
Median (IQR)	8 (6-10)	9 (7-11)	0.23	8 (6-11)	8 (6-11)	0.02

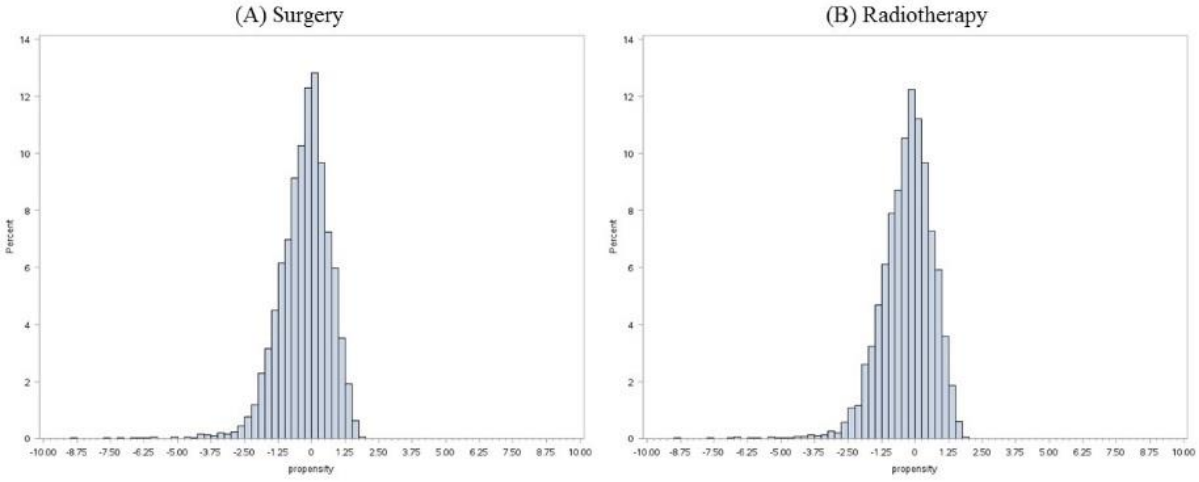
Note: Std diff = standardized difference; SD = standard deviation; IQR = interquartile range; ADG = Aggregated Diagnosis Groups.

Figure 4.2. Distribution of propensity scores among patients undergoing (a) surgery and (b) radiotherapy, prior to matching.



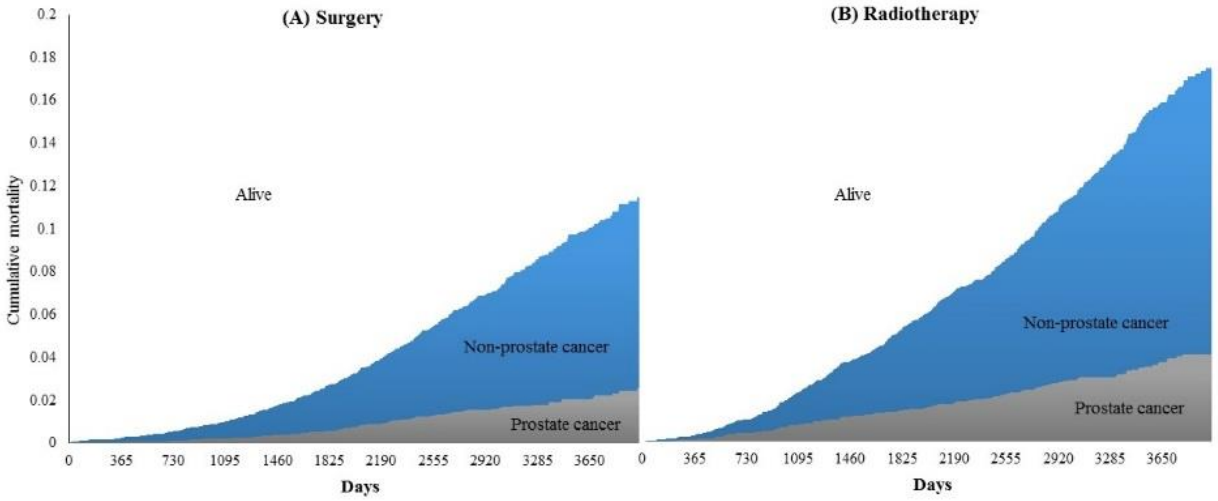
Propensity-score matching yielded 5393 matched pairs. The distribution of propensity-scores for patients treated by surgery and radiotherapy, following matching, is shown in Figure 4.3. The two groups were well balanced with respect to demographic characteristics (Table 4.1). Among the matched cohort, the median age was 69 years and the median ADG comorbidity sum was 8, indicative of moderate levels of comorbidity (Table 4.1). The median duration of follow-up was similar between patients initially treated with surgery (7.42 years; interquartile range (IQR) 5.60-9.54 years) and radiotherapy (7.43 years; IQR 5.48-9.50; p=0.23). Patients treated primarily with radiotherapy were more likely to also receive ADT as some point during their treatment course than those receiving surgery (34% vs 17%, respectively; p<0.0001). Further, for those receiving ADT, the median duration for patients treated with radiotherapy (6.5 months; interquartile range 3.4-15.3 months) was longer than that for patients treated with surgery (6.3 months; interquartile range 3.3-12.2 months; p<0.0001).

Figure 4.3. Distribution of propensity scores among patients undergoing (a) surgery and (b) radiotherapy, following matching.



After accounting for baseline differences through matching, the 5- and 10-year cumulative incidence of non-prostate cancer mortality were higher among patients who underwent local treatment with radiotherapy (4% and 12%, respectively) as compared those who underwent surgery (2% and 8%, respectively; $p<0.0001$; Figure 4.4). Thus, the absolute difference in mortality was 2% at 5 years and 4% at 10 years, both in favour of patients treated with surgery.

Figure 4.4. Survival and cumulative incidence of prostate cancer and non-prostate cancer mortality among patients treated with (a) surgery and (b) radiotherapy for non-metastatic prostate cancer.



In a competing risks analysis accounting for prostate-cancer specific mortality and the effect of clustering due to matching, radiotherapy was associated with a 57% increased risk of non-prostate cancer mortality (Table 4.2). After accounting for ADT exposure using a time-varying binary exposure, patients treated with radiotherapy remained at significant risk of non-prostate cancer mortality compared to those treated with surgery (Table 4.2). Similarly, when considering ADT as a time-varying cumulative exposure, radiotherapy remained a significant risk factor for non-prostate cancer mortality whether ADT was categorized in three or six strata (Table 4.2). In each analysis accounting for ADT treatment, ADT exposure was not significantly associated with non-prostate cancer mortality (p-value = 0.26 – 0.87).

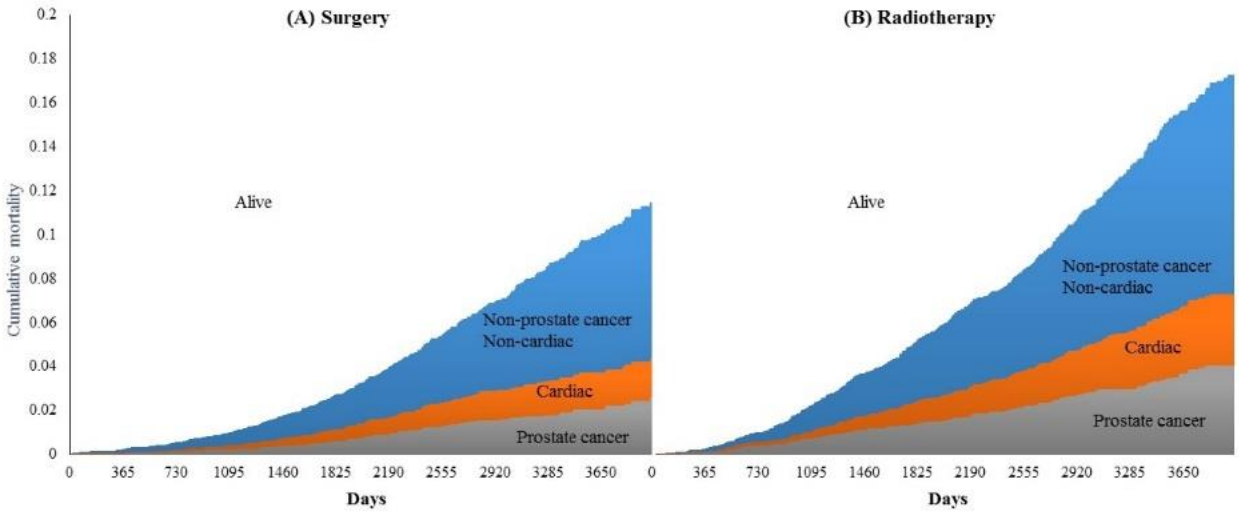
Similarly, while accounting for both prostate cancer mortality and non-prostate / non-cardiovascular mortality, patients treated with radiotherapy had higher rates of cardiovascular mortality ($p<0.0001$; Figure 4.5). In competing risk models, patients treated with radiotherapy had a significantly increased risk of cardiovascular mortality compared to those treated with surgery (Table 4.2). This effect persisted whether accounting for ADT as a time-varying binary

exposure, as a three-level time-varying cumulative exposure, or a six-level time-varying cumulative exposure (Table 4.2). In each analysis accounting for ADT, the ADT exposure was not significantly associated with cardiovascular mortality.

Table 4.2. Competing risks analysis examining non-prostate cancer mortality, cardiovascular mortality, and ischemic cardiac events for patients treated with surgery or radiotherapy.

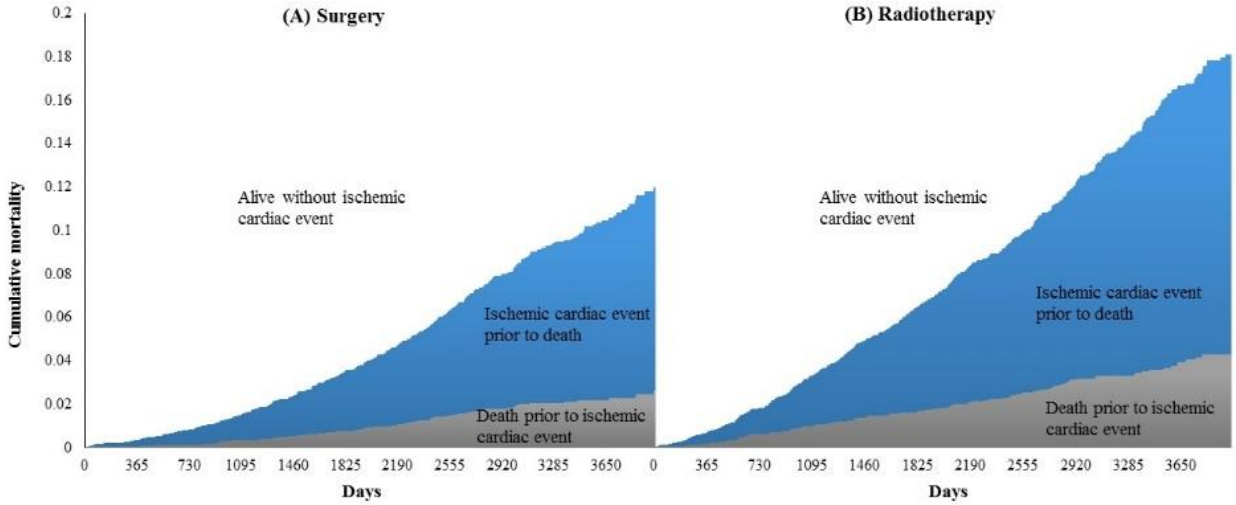
	Primary outcome : Non-prostate cancer death (sdHR, 95% CI)	***Secondary outcome:*** Cardiovascular death (sdHR, 95% CI)	***Secondary outcome:*** Ischemic cardiac event (sdHR, 95% CI)
Univariate competing risk model			
Surgery	Referent	Referent	Referent
Radiotherapy	1.57 (1.35-1.83)	1.74 (1.27-2.37)	1.13 (1.03-1.24)
Competing risk model accounting for time-varying binary ADT exposure			
Surgery	Referent	Referent	Referent
Radiotherapy	1.57 (1.35-1.83)	1.74 (1.27-2.37)	1.13 (1.03-1.24)
Competing risk model accounting for time-varying cumulative ADT exposure (3 categories)			
Surgery	Referent	Referent	Referent
Radiotherapy	1.56 (1.34-1.82)	1.78 (1.30-2.42)	1.13 (1.03-1.24)
Competing risk model accounting for time-varying cumulative ADT exposure (6 categories)			
Surgery	Referent	Referent	Referent
Radiotherapy	1.57 (1.35-1.83)	1.75 (1.28-2.38)	1.13 (1.03-1.24)

Figure 4.5. Survival and cumulative incidence of prostate cancer, cardiovascular, and non-prostate cancer / non-cardiovascular mortality among patients treated with (a) surgery and (b) radiotherapy for non-metastatic prostate cancer.



Finally, we examined ischemic cardiac events while accounting for any-cause mortality. The cumulative incidence of ischemic cardiac events prior to death was significantly higher among patients treated with radiotherapy than surgery (p=0.0003; Figure 4.6). Patients treated with radiotherapy were at approximately 13% increased risk compared to those treated with surgery, whether the model did not include ADT, included ADT as a time-varying binary exposure, or included cumulative ADT exposure as a time-varying cumulative dose exposure (Table 4.2). As with previous analyses, ADT exposure was not significantly associated with ischemic cardiac events.

Figure 4.6. Cumulative incidence of ischemic cardiac events while accounting for the competing risk of all-cause mortality among patients treated with (a) surgery and (b) radiotherapy for non-metastatic prostate cancer.



We performed subgroup analysis examining external beam radiotherapy (EBRT; n = 4748) and brachytherapy (n = 742) separately. Median follow-up was shorter for patients treated with brachytherapy (6.50, IQR 5.17-8.74 years) as compared to those treated with EBRT (7.51, IQR 5.52-9.54 years) or surgery (7.42 years, IQR 5.60-9.54 years; p<0.0001). ADT was administered more commonly to patients receiving EBRT (n = 1726, 36.4%) than those treated with brachytherapy (n = 143, 19.3%) or surgery (n = 930, 17.3%; p<0.0001). Among those receiving ADT, patients treated with EBRT had a longer median use (6.6 months, IQR 4.2-15.6 months) than those treated with brachytherapy (3.3 months, IQR 3.3-7.1 months) or surgery (6.3 months, IQR 3.3-12.2 months; p<0.0001). Patients treated with external beam radiotherapy had a significantly increased risk of non-prostate cancer mortality, cardiovascular mortality, and ischemic cardiac events compared to those treated with surgery, while those treated with brachytherapy did not have a significantly increased risk (Table 4.3). In each of these analyses, ADT exposure, whether binary or cumulative dose, was not associated with non-prostate cancer mortality (p-values 0.25 – 0.95).

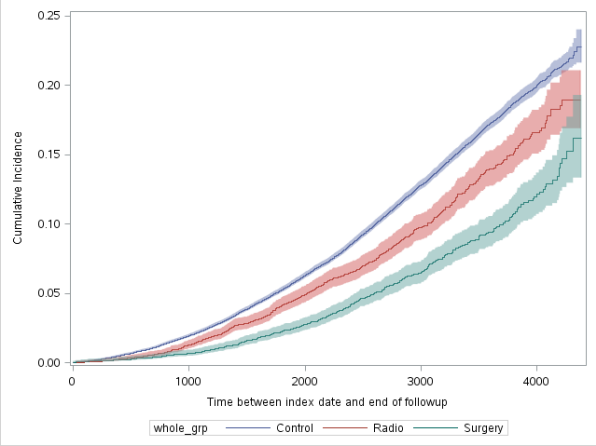
Table 4.3. Sub-group analysis examining non-prostate cancer mortality, cardiovascular mortality, and ischemic cardiac events for patients treated with surgery, brachytherapy and external beam radiotherapy.

	Primary outcome : Non-prostate cancer death (sdHR, 95% CI)	***Secondary outcome:*** Cardiovascular death (sdHR, 95% CI)	***Secondary outcome:*** Ischemic cardiac events (sdHR, 95% CI)
Univariate competing risk model			
Surgery	Referent	Referent	Referent
Brachytherapy	0.75 (0.48 – 1.17)	0.26 (0.06 – 1.06)	1.14 (0.92-1.40)
External beam radiotherapy	1.67 (1.44 – 1.95)	1.94 (1.42 – 2.65)	1.13 (1.03-1.24)
Competing risk model accounting for time-varying binary ADT exposure			
Surgery	Referent	Referent	Referent
Brachytherapy	0.75 (0.47 – 1.17)	0.26 (0.06 – 1.06)	1.14 (0.92-1.40)
External beam radiotherapy	1.68 (1.44 – 1.95)	1.94 (1.42 – 2.64)	1.13 (1.03-1.24)
Competing risk model accounting for time-varying cumulative ADT exposure (3 categories)			
Surgery	Referent	Referent	Referent
Brachytherapy	0.75 (0.48 – 1.18)	0.26 (0.06 – 1.07)	1.14 (0.92-1.40)
External beam radiotherapy	1.66 (1.43 – 1.94)	1.95 (1.43 – 2.67)	1.13 (1.03-1.24)
Competing risk model accounting for time-varying cumulative ADT exposure (6 categories)			
Surgery	Referent	Referent	Referent
Brachytherapy	0.75 (0.47 – 1.17)	0.26 (0.06 – 1.06)	1.14 (0.92-1.40)
External beam radiotherapy	1.67 (1.44 – 1.95)	2.00 (1.46 – 2.73)	1.13 (1.03-1.24)

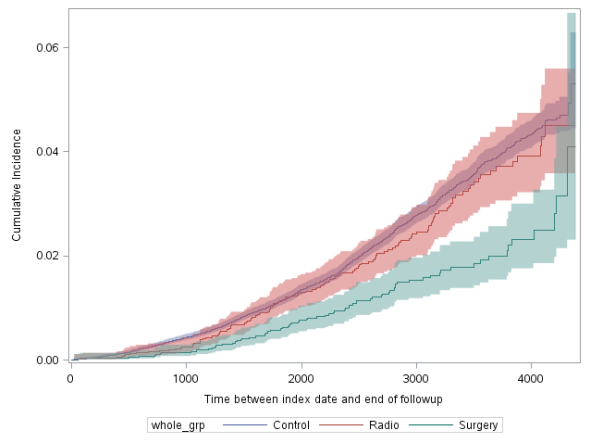
We then matched patients treated for prostate cancer 1:5 with men in the general population. We identified a total of 53,722 men from the general population who were matched to 5372 patients treated with surgery and 5373 patients who were treated with radiotherapy. The cumulative incidence of non-prostate cancer mortality was higher in men from the general population than men with prostate cancer treated either with surgery or radiotherapy ($p<0.0001$; Figure 4.7). Compared with men in the general population, those treated for prostate cancer with surgery had a 47% decreased risk of non-prostate cancer mortality (sdHR 0.53, 95% CI 0.47-0.59) and those treated with radiotherapy had a 21% decreased risk (sdHR 0.79, 95% CI 0.72-0.86). The cumulative incidence of cardiovascular mortality was significantly lower among patients treated with surgery than those treated with radiotherapy or from the general population ($p<0.0001$). Compared to men in the general population, men treated with surgery had a 45% decreased risk of cardiovascular mortality (sdHR 0.55, 95% 0.44-0.69) while those treated with radiotherapy had similar risk (sdHR 0.93, 95% CI 0.78-1.10). As with non-prostate cancer mortality, rates of ischemic cardiac events significantly differed ($p<0.0001$) with the highest rates among men in the general population, followed by those treated by radiotherapy, and then those treated surgically (Figure 4.7). Compared to men in the general population, patients treated with surgery had a 24% decreased risk (sdHR 0.76, 95% CI 0.70-0.81) and those treated with radiotherapy had a 12% decreased risk (sdHR 0.88, 95% CI 0.82-0.94).

Figure 4.7. Cumulative incidence of (a) non-prostate cancer mortality (b) cardiovascular mortality and (c) ischemic cardiac events among men treated with surgery or radiotherapy for prostate cancer or matched men from the general population.

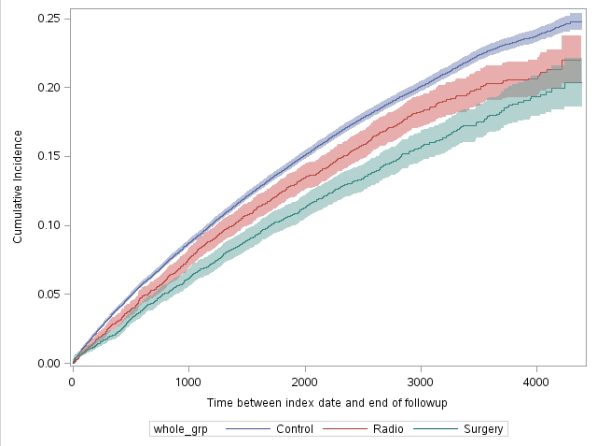
(A)



(B)



(C)



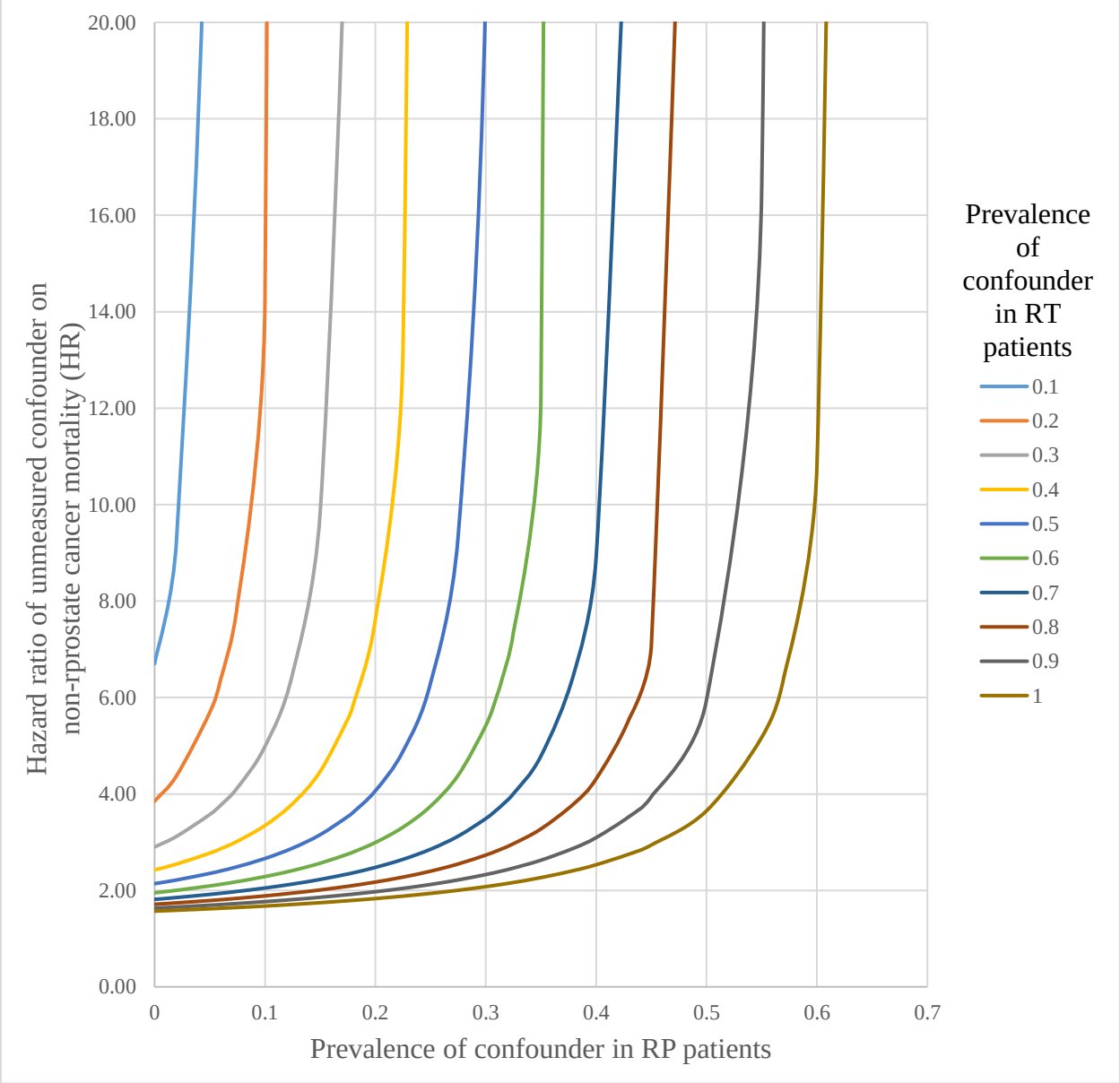
In order to further explore the effect of ADT, we conducted a subgroup analysis among patients treated with radiotherapy in the unmatched cohort. We found no significant effect of ADT on non-prostate cancer mortality (sdHR 1.15, 95% CI 0.79 – 1.68), cardiovascular mortality (sdHR 1.14, 95% CI 0.59 – 2.20) or ischemic cardiac events (sdHR 1.15, 95% CI 0.98 – 1.35) among this cohort. We did not observe clinically important effect modification due to a history of previous myocardial infarction, comorbidity, or age (Table 4.4). Results from operationalizing ADT exposure as a 3-level categorical variable did not differ from those derived from operationalizing ADT exposure as a binary variable.

Table 4.4. Subgroup analysis exploring effect modification of the effect of ADT on non-prostate cancer death, cardiovascular death and ischemic cardiac events due to a history of previous myocardial infarction, comorbidity, and age. Results presented are the sdHR (95% CI) of time-varying binary ADT exposure compared to non-exposure.

	Non-prostate cancer death (sdHR, 95% CI)	Cardiovascular death (sdHR, 95% CI)	Ischemic cardiac events (sdHR, 95% CI)
Stratification according to history of prior myocardial infarction			
No previous MI	HR 1.16 (0.79-1.70)	HR 1.18 (0.61-2.30)	1.14 (0.97-1.34)
Previous MI	HR 0.81 (0.15-4.40)	No events in ADT	1.30 (0.54-3.09)
Stratification according to comorbidity			
ADG 0-5	2.10 (0.46-8.88)	No events in ADT	2.04 (11.18-3.53)
ADG 6-8	1.11 (0.55-2.24)	1.02 (0.28-3.73)	0.95 (0.70-1.28)
ADG 9-11	1.36 (0.77-2.42)	1.42 (0.55-3.65)	1.36 (1.06-1.75)
ADG 12+	0.82 (0.36-1.89)	0.87 (0.21-3.62)	0.99 (0.72-1.37)
Stratification according to age			
65-69 years	1.96 (0.88-4.39)	2.34 (0.62-8.81)	1.17 (0.84-1.62)
70-74 years	0.77 (0.36-1.64)	0.69 (0.16-2.97)	1.08 (0.83-1.40)
75+ years	1.16 (0.68-1.95)	1.09 (0.43-2.75)	1.20 (0.93-1.54)

Finally, to address potential residual selection biases, we conducted a sensitivity analysis to quantify the magnitude of effect necessary for a confounder to obviate the observed effect of radiotherapy as compared to surgery on non-prostate cancer mortality across a wide spectrum of confounder prevalence. At one extreme, a confounder with a hazard ratio of 3.65 would have to have to be present in all radiotherapy patients (100%) and only half of surgery patients (50%; Figure 4.8). If the prevalence in the surgical population rose to 60%, the effect required to obviate the findings rises to a hazard ratio in excess of 10.5. At the other extreme, an incredibly infrequent confounder which occurs in 10% of patients receiving radiotherapy and no patients receiving surgery (0%) would have to have a hazard ratio of 6.7. Thus, any hypothetical residual confounder would have to be both strongly associated with non-prostate cancer mortality (HRs in excess of 2.5) and have highly differential prevalence in order to nullify the observed effect (Figure 4.8).

Figure 4.8. Graphical representation of sensitivity analysis assessing the effect of a hypothetical unmeasured confounder.



4.5 Discussion

As prostate cancer-specific mortality is very low for patients undergoing treatment for localized disease, competing causes of mortality are significant. In this matched, population-based cohort study, we found that (1) patients who were treated with radiotherapy for non-metastatic prostate cancer were at increased risk of non-prostate cancer mortality and cardiovascular mortality compared to patients treated with surgery and (2) androgen deprivation therapy (ADT), whether operationalized as a binary or cumulative dose exposure, was not associated with these outcomes. Unmeasured confounding still remains an important reason to potentially bias these findings.

From observational studies, there is consistent evidence that patients who receive radiotherapy in the treatment of prostate cancer have significantly shorter overall survival than those who undergo surgery[15]; however, no differences were recently demonstrated in the ProtecT study cohort[236]. We hypothesized that treatment-related cardiovascular mortality, whether directly due to radiotherapy or indirectly through to co-administration of ADT, may account for this.

There is an extensive literature examining the association between androgen deprivation therapy and cardiovascular events[260]. While much of this literature has not distinguished between patients with localized and metastatic disease for whom baseline risks may differ, we recently examined the risk of cardiovascular events among men treated for clinically localized prostate cancer in the Surveillance, Epidemiology, and End Results (SEER) database[226]. We found that treatment with ADT was independently associated with an increased risk of coronary heart disease and sudden cardiac death, but not myocardial infarction[226]. There is also strong

biologic mechanistic evidence to support the relationship between ADT and adverse cardiovascular effects[379].

Despite this well-established relationship between ADT and cardiovascular events, there is little evidence to support a relationship between ADT and cardiovascular-related mortality. In this analysis, we found that there was no significant association between ADT and non-prostate mortality or cardiovascular mortality, whether ADT was operationalized as a time-varying binary exposure or a time-varying cumulative dose exposure. Previously, *post hoc* analyses of both RTOG 94-08 and EORTC 22863 have found no increase in cardiovascular mortality among men receiving ADT in addition to radiotherapy[189,380]. Pooling of 4141 patients from 8 randomized controlled trials found no association between ADT exposure and cardiovascular mortality[381]. However, there may be subgroups of men for whom ADT is associated with an increased risk of cardiovascular mortality. Among those with moderate or severe comorbidity at the time of treatment, D'Amico et al. demonstrated that the addition of ADT to radiotherapy resulted in significantly increased risk of cardiovascular and overall mortality[382]. Further, Kovtun et al. found a significant interaction between African American ethnicity and ADT use in the risk of non-prostate cancer mortality[383].

The relationship between radiotherapy for prostate cancer and cardiovascular disease is more controversial. Recently, using the SEER dataset, we found that treatment with radiotherapy was possibly associated with an increase risk of coronary artery disease, myocardial infarction and sudden cardiac death among patients with clinically localized prostate cancer treated between 2000 and 2008[226]. This effect was restricted to patients treated with external beam radiotherapy. These results corroborate previous work by Gandaglia et al. which demonstrated that patients treated for non-metastatic prostate cancer between 1995 and 2009

with external beam radiotherapy were at increased risk of cardiovascular events[384]. Mechanistically, there is reason to consider radiotherapy a risk factor for cardiovascular disease. While this risk is greatest among patients receiving thoracic and mediastinal radiotherapy[292], likely due to direct toxic effects, abdominal radiotherapy in the treatment of testis cancer has also been associated with an increased risk of cardiovascular disease[293]. This is likely mediated through two distinct pathways. First, radiotherapy is well-recognised to exert systemic effects through so-called radiation-induced abscopal effects in which radiotherapy-attributable effects may occur far from the irradiated site independent of direct exposure[280]. While these effects may have beneficial oncologic outcomes, they may also contribute to treatment-related toxicity. Further, localized radiotherapy has been demonstrated to inevitably result in exposure of the entire body to a sub-therapeutic radiotherapy dose[385]. This generalized radiotherapy dose as well as the localized treatment dose has been shown to induce proinflammatory mediators[286,386]. There is strong epidemiologic and clinical evidence of a relationship between systemic inflammation and cardiovascular events[298-302]. Second, radiotherapy may induce cardiovascular disease in exposed vessels through fibrosis,[294] intimal thickening, proteoglycan deposition, inflammatory infiltration[295] and radiation-nephropathy induced hypertension[296]. Peripheral vascular disease has been shown to be associated with cardiovascular mortality, with risk ratios ranging from 2-6, as well as overall mortality[387-390].

Conversely, the observed increase in cardiovascular mortality among men treated with radiotherapy may simply reflect unaccounted selection bias. We examined the risk of non-prostate cancer mortality and cardiovascular mortality among a matched sample of the general population. We found that patients treated with either surgery or radiotherapy for prostate cancer had decreased risks of non-prostate cancer mortality as compared to the general population. This

reflects in part residual differences between the populations which are not accounted for by matching, in keeping with a healthy-user bias where patients screened and subsequently treated for localized prostate cancer are healthier than the general population. While these studies have typically been performed among patients treated with radical prostatectomy[391,392], this observation has been shown to also apply to those treated with radiotherapy[393,394]. Further, we conducted sensitivity analysis to quantify the prevalence and strength of a hypothetical residual confounder in order to negate the observed differences between patients treated with surgery and radiotherapy. This analysis found that the differential prevalence and strength of association is sufficiently large that it is unlikely that such a confounder, or combination of confounders, exists. Thus, the present results may be explained by a true effect of radiotherapy, magnified by residual confounding.

In addition to its population-based, generalizable nature, a strength of this study the extensive risk-adjustment performed, when compared to other administrative data sources. While previous studies relying on SEER-Medicare data (including our own) have accounted for comorbidity as a composite, measured by Charlson score, we accounted for numerous cardiovascular risk factors (hypertension, dyslipidemia using statin medication use as a proxy, diabetes, history of myocardial infarction, and history of stroke) individually using extensively validated algorithms and data sources in addition to a generalized comorbidity score, the Johns Hopkins Aggregate Disease Groups score[395], with better discrimination properties than the Charlson score[396]. Further, we examined ADT cumulative dose exposure as previous work has shown that increasing duration of ADT is associated with increased risk of cardiovascular events[397]. We utilized time-dependent models to account for ADT exposure in order to address the potential for immortal time bias[398].

Limitations include the aforementioned risk of selection bias associated with observational data. Due to limitations in the available data, tumor characteristics including grade and stage were not included. However, while these are clearly associated with prostate cancer mortality, it is not clear that these would confound the association with our outcomes. Finally, this analysis was limited to men aged 66 years and older at the time of local prostate cancer therapy. Therefore, these results may not be generalizable to younger populations.

Methodologically, we relied upon propensity score matching to account for baseline differences between patients treated with surgery and radiotherapy. Propensity-score matching has been used widely used to assess oncologic[399-401] and functional outcomes of localized prostate cancer treatments[7,9,10,402]. Theoretically, propensity score matching is appealing due to its ability to reduce the impact of treatment-selection bias in observational data[403]. However, it relies upon observed covariates. Thus, while we achieved very good balance among measured confounders, the potential for unmeasured confounding remains[404]. Further, for inclusion in a propensity-score matched analysis, a suitable match (propensity score within 0.2) is required. Subjects without a suitable match are excluded. In this study, 1458 patients treated with surgery (21.2%) and 8407 patients treated with radiotherapy (60.9%) were excluded from analysis. As a result, the conclusions only apply to patients for whom both radiotherapy and surgery are reasonable options. Thus, incomplete matching decreases the generalizability of the findings and diminishes study power[405]. In contrast, inexact matching increases the risk of residual confounding[405]. The use of caliper decreases the risk of so-called “bad matching” and increases matching quality[406] compared to a nearest-neighbour approach. Widening of the caliper increases the risk of residual confounding as well due to decreased similarity within matched

pairs. Given the large sample size available in this cohort, we opted to allow incomplete matching in order to reduce the magnitude of residual confounding.

Stukel and colleagues have suggested that instrumental variable analysis may produce less biased estimates of treatment effect than propensity-score matching[407]. This technique uses the relationship between the exposure and an instrumental variable to capture the causal effect of the exposure on the outcome[408]. However, the use of instrumental variable analysis is limited by the ability to identify an appropriate instrumental variable, most notably that the instrumental variable cannot influence the outcome either directly or indirectly[408]. While instrumental variable analysis has been employed in the study of prostate cancer treatment outcomes, its widespread use has been limited by the lack of quality instruments. Vickers, a noted epidemiologist, biostatistician and research methodologist, has eloquently argued that the instrumental variable analysis confers little additional information when compared with more traditional statistical methods[409]. Further, there are at least two limitations to the use of instrumental variable analysis in medical research, according to Korn and Freidlin[410]. First, the variability in estimates of treatment effect are much larger in clinical research than they are in economics and econometrics, where the use of instrumental variable analysis is more widely accepted. Second, instrumental variable analysis relies on a series of unverifiable assumptions which, while different from those underpinning propensity-score based analyses, are no less important. Further, the interpretation of the results of an instrumental variable based analysis are not intuitive to most physicians. The estimate represents the average effect in a "marginal population" and not to the population in general. For these reasons and others, Kuo, Montie and Shahinian found that the differences between such an instrumental variable based analysis and other approaches, such as propensity score matching, is not likely applicable to clinical

decisions, but rather suited for policy making[411]. Stukel et al. similarly concede that instrumental variable based analyses are more relevant to policy matters than specific clinical questions[407].

4.6 Conclusions

In conclusion, among a large, population-based matched cohort of patients undergoing curative treatment for localized prostate cancer, we demonstrated no increased risk of cardiovascular mortality associated with ADT treatment. Further, we found that primary local therapy with radiotherapy was associated with an increased risk of cardiovascular disease. While residual confounding and selection biases may affect these results, the magnitude of effect necessary to negate these findings is such that such biases are unlikely to account for the observed differences.

CHAPTER 5: SECONDARY MALIGNANCIES FOLLOWING RADIOTHERAPY FOR PROSTATE CANCER: A SYSTEMATIC REVIEW AND META-ANALYSIS

5.1 Abstract

Objective: To determine the association between radiotherapy exposure in the treatment of prostate cancer and subsequent secondary malignancies.

Design: Systematic review and meta-analysis of observational studies.

Data sources: Medline and EMBASE through April 6, 2015 without restriction to year or language.

Study Selection and Exposure: Comparative studies assessing the risk of secondary malignancies in patients exposed or unexposed to radiotherapy in the course of prostate cancer treatment were selected by two reviewers independently with any disagreement resolved by consensus.

Data extraction and synthesis: Two reviewers independently extracted study characteristics and outcomes. Risk of bias was assessed using the Newcastle-Ottawa Scale. Outcomes were synthesized using random-effects models and Mantel-Haenszel weighting. Unadjusted odds ratios (uOR) and multivariable adjusted hazard ratios (aHR), where available, were pooled.

Main outcome measures: Secondary cancers of the bladder, colorectal tract, rectum, lung and hematologic system.

Results: Of 3,056 references retrieved, 21 studies were selected for analysis. The majority of included studies were large, multi-institutional reports but had moderate risk of bias. EBRT was the most commonly assessed radiotherapy modality; 13 studies used surgically treated patients

as controls and 8 used non-radiated controls. The length of follow-up among studies varied. There was increased risk of bladder (4 studies, aHR 1.67, 95% CI 1.55 to 1.80), colorectal (3 studies, aHR 1.79, 95% CI 1.34 to 2.38), and rectal cancers (3 studies, aHR 1.79, 95% CI 1.34 to 2.38), but not hematological (1 study, aHR 1.64, 95% CI 0.90 to 2.99) or lung cancers (2 studies, aHR 1.45, 95% CI 0.70 to 3.01) following radiotherapy compared to those unexposed to radiotherapy. The odds of secondary cancer varied depending on radiotherapy modality – treatment with external beam radiotherapy was consistently associated with increased odds while brachytherapy was not. Among the patients who underwent radiotherapy, the highest absolute rates reported for bladder, colorectal and rectal cancers were 3.8%, 4.2%, and 1.2%, respectively, while the lowest reported rates were 0.1%, 0.3% and 0.3% from individual studies. **Conclusion:** Among the studies examined, radiotherapy for prostate cancer was associated with higher risk of developing secondary malignancies of the bladder, colon, and rectum compared to patients unexposed to radiotherapy, but the reported absolute rates were low. Further studies with longer follow-up are required to confirm these findings.

5.2 Introduction

Active treatment options for patients diagnosed with clinically-localized prostate cancer include surgery or radiotherapy[412]. Each option is associated with side effects including urinary incontinence and erectile dysfunction[7,402]. Recently, other treatment-related complications resulting in hospital admissions, genitourinary and rectal-anal procedures, and major surgeries were described[9,207,413]. A unique complication for patients undergoing radiotherapy is the possibility of development of a secondary malignancy.

Studies assessing the risk of secondary cancers following radiotherapy for prostate cancer have reported either an increased risk of secondary malignancies[17,18] or no association between radiotherapy and secondary malignancies[19,20]. One previous review concluded a negligible risk of secondary malignancies after radiotherapy[251] whereas other reviews concluded that this is an important risk for both patients and physicians to consider[252-254]. A previous meta-analysis lacked data from a number of important recent publications[414].

While direct radiation carcinogenesis has long been accepted[415], there is evidence that prostate irradiation may contribute to carcinogenesis outside of the irradiated field through radiation scatter and radiation-induced genetic alterations without direct exposure due to increased reactive oxygen species[416-418] and changes in gene expression in what has been termed the "bystander effect"[419]. Thus, our primary objective was to systematically review and meta-analyze available data on the association between radiotherapy and the development of secondary malignancies of the bladder, colorectal tract, lung and hematological system in patients with prostate cancer compared with other treatments.

5.3 Methods

5.3.1 Research question

Is there an association between radiotherapy and development of secondary malignancies in patients treated for prostate cancer? Does this association vary by type of radiotherapy?

5.3.2 Types of participants and exposure

We reviewed studies reporting on patients with confirmed adenocarcinoma of the prostate treated with commonly-utilized forms of radiotherapy including conformal external beam (EBRT), intensity-modulated (IMRT), brachytherapy, or a combination of modalities. We included studies irrespective of dose and duration of radiotherapy. Controls were non-irradiated patients including those who were treated with surgery, other prostate cancer treatments, or received no therapy. We conducted a subgroup analysis using only controls treated with surgery. When the comparator group was unclear, the study was excluded.

5.3.3 Outcome

Our primary outcome was the development of one or more histologically-unique secondary cancers of the bladder, colorectal tract, rectum, lung and hematologic system, excluding metastatic tumors. Studies reporting on rectal cancer were included in the colorectal cancer analysis as well as in the rectal cancer analysis.

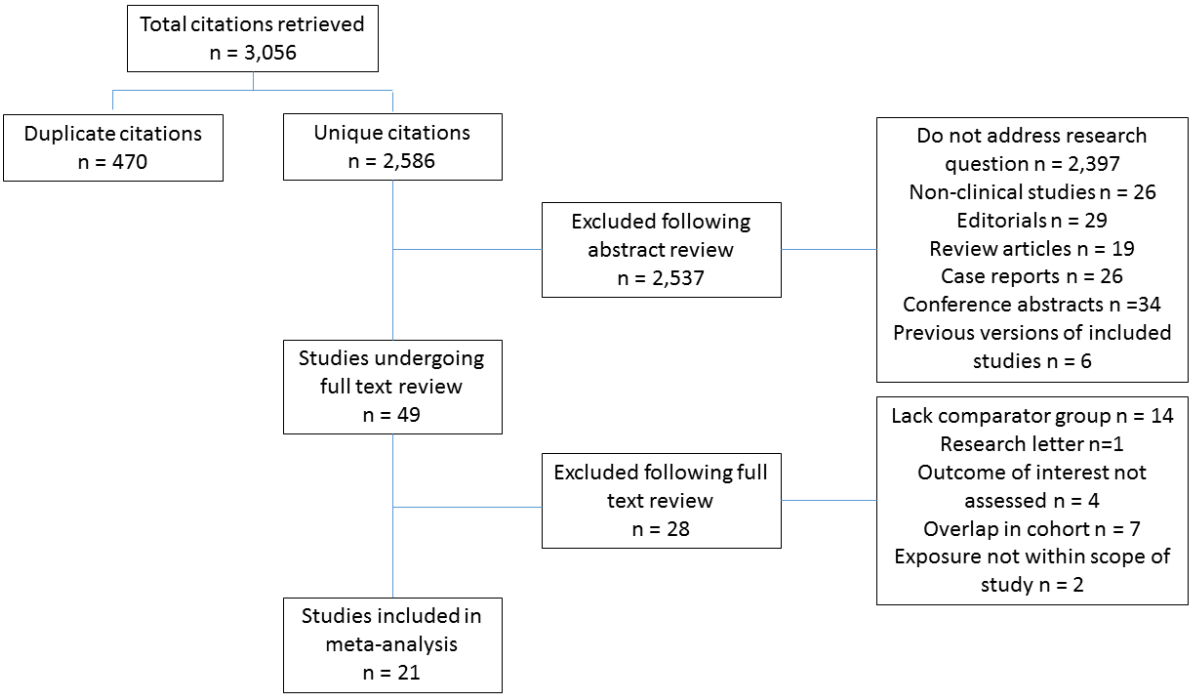
It is argued that time (lag period) must elapse between the date of radiation exposure and the development of a secondary cancer in order for that tumor to be considered radiation-

induced[253]. Historically, this has been defined as five years[253,420-422]. There were differences in the use and application of the length of the lag period from the time of treatment to secondary cancer diagnosis among included studies. To address differences in how studies handled the lag period, we conducted separate analyses stratifying by inclusion of studies without respect to lag period, to only those using a five-year lag period, and to only those using a ten-year lag period.

5.3.4 Types of studies

We included cohort and case-control studies. We excluded case series which lacked non-radiated comparators. Other publications on the topic including basic science papers, review articles, editorials, articles not dealing with radiation-induced malignancy, conference abstracts, early versions of data later published, and non-standard treatment (such as cryotherapy) were excluded (Figure 5.1). Where there was more than one publication resulting from the same patient cohort, to prevent the duplication of patients from one cohort, for each of our analyses we selected one study based on a hierarchical assessment of comparability of study groups, definition of radiation exposure, time period of study (preference for more recent), and number of patients (Appendix 5.1).

Figure 5.1. Flow diagram outlining search strategy and final included and excluded studies.



5.3.5 Methods of review

We used Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) and Meta-analysis of Observational studies in Epidemiology (MOOSE) guidelines for reporting of this systematic review and meta-analysis[423,424].

5.3.6 Search strategy

Medline and EMBASE databases were searched using the OvidSP search platform for studies indexed as of April 6, 2015, with the help of a professional librarian. A detailed search strategy for each database is available in Appendix 5.2. References from review articles, editorials and included studies were reviewed and cross-referenced to ensure completeness. Studies in any language were included. Conference abstracts were excluded.

5.3.7 Selection and data extraction

Two authors performed study selection (Figure 5.1), the primary author of this dissertation and a colleague (Alyson Mahar, Doctoral Student in Clinical Epidemiology, Queen's University). Titles and abstracts were used to screen for initial study inclusion. Full-text review was used where the abstract was insufficient to determine if the study met the inclusion or exclusion criteria. Final agreement on study inclusion was made by discussion and consensus with other authors. Two reviewers performed all data extraction including evaluation of study characteristics, risk of bias and outcome measures. Key variables were selected based on clinical and methodological relevance. The data abstraction form was pilot tests by two authors to ensure completeness. Discrepancies were resolved through consensus. Authors were contacted when suitable data were not available.

5.3.8 Risk of bias assessment

We used the Newcastle-Ottawa Scale for risk of bias assessment. This scale assesses risk of bias in three domains[425]: 1) selection of the study groups; 2) comparability of groups; and 3) ascertainment of exposure and outcome[426]. Studies with score >7 were considered having low risk of bias, score of 5-7 having moderate risk of bias and score of <5 having high risk of bias.

5.3.9 Statistical analysis

Meta-analysis was performed using Review Manager 5.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014) software. We assessed the adjusted hazard ratio (aHR) and unadjusted odds ratio of developing a secondary malignancy between

participants treated with radiotherapy and controls. We first analysed studies including any form of radiotherapy, stratified by studies that used controls groups comprising non-radiated patients and surgically-treated patients.

Due to the clinical heterogeneity inherent in our data, random-effects models were used for all meta-analyses. Given the relatively rare nature of our events, Mantel-Haenszel weighting was used[427]. For adjusted hazard ratios, the inverse variance technique was used. Statistical heterogeneity was calculated using I^2 values[428].

As a *post-hoc* analysis, we assessed the absolute risk difference between patients treated with radiotherapy and controls. We expressed this as the difference per 100 patients.

5.3.10 Subgroup Analysis and Exploration of Heterogeneity

We performed *a priori* subgroup analyses by examining studies restricting to external beam radiotherapy (EBRT) and restricting to brachytherapy. For each of these analyses, stratification by control group (no radiation and surgery) was undertaken. In order to further explore heterogeneity, we conducted meta-regression using the Newcastle-Ottawa risk of bias score as a continuous variable in random effects models for all comparisons comprising five or more studies.

5.3.11 Ethical approval

Ethics approval was obtained from the University of Toronto Health Research Ethics Board (#31250). As aggregate data was used, patient consent was not deemed necessary.

5.4 Results

A total of 3,056 references were retrieved from our literature search (Figure 5.1). After full text review of 49 manuscripts, 21 reports were selected for inclusion (Table 5.1). Twenty-eight studies were excluded and the reasons are highlighted in Figure 5.1. Of note, 24 reports derived from the United States Surveillance, Epidemiology, and End Results (SEER) cohort were identified in our literature search. These studies overlapped in their inclusion criteria, study intervals, patient selection and outcome measures. To prevent the duplication of patients from the SEER cohort, we selected a single study to represent the SEER cohort for each comparison as outlined in Appendix 5.1. The studies utilized in each of the analyses are outlined in Appendix 5.3.

We obtained unpublished data from the principal author of one study which was included[429]. In a second case, we were unable to obtain necessary information for inclusion in a sub-group analysis but the published data were adequate for our primary analysis[430].

Table 5.1. Characteristics of included studies.

Author	Data source (study interval)	Follow-up	Lag time	Study size	Type of radiation	Control group	Crude incidence rate of secondary cancers, by treatment modality (%)				
							Bladder	Colorectal	Rectal	Lung	Hematologic
Abdel-Wahab (2008)[431]	SEER (1972-2002)	median 4.3-4.7 years	1 year	108,452	EBRT, brachytherapy, brach+	No radiation and no surgery	RT: 1.2 EBRT: 1.5 Brach: 0.5 Brach+: 0.8 Ctrl: 0.9	RT: 1.7 EBRT: 2.0 Brach: 0.8 Brach+: 1.0 Ctrl: 1.6	RT: 0.3 EBRT: 0.4 Brach: 0.2 Brach+: 0.2 Ctrl: 0.3	RT: 1.9 EBRT: 2.3 Brach: 0.9 Brach+: 1.1 Ctrl: 1.7	RT: 1.0 EBRT: 1.2 Brach: 0.6 Brach+: 0.6 Ctrl: 0.9
Abern (2013)[432]	SEER (1988-2007)	median 65 months	1 year	275,200	EBRT, brachytherapy, brach+, XRT NOS	Surgery	RT: 1.4 EBRT: 1.6 Brach: 0.9 Brach+: 1.3 Ctrl: 0.7	NR	NR	NR	NR
Baxter (2005)[433]	SEER (1973-1999)	mean > 9 years	5 years	85,815	EBRT	Surgery	NR	RT: 1.7 Ctrl: 1.0	RT: 0.4 Ctrl: 0.3	NR	NR
Berrington de Gonzales (2011)[17]	SEER (1973-2002)	mean 12 years	5 years	200,163	EBRT, brachytherapy, brach+	No radiation	RT: 1.3 Ctrl: 0.9	RT: 1.3 Ctrl:1.2	RT: 0.5 Ctrl: 0.4	RT: 1.9 Ctrl: 1.7	NR
Bhojani (2010)[434]	Quebec, CA (1983-2003)	NR	Multiple	17,845	EBRT	Surgery	RT: 2.3 Ctrl: 2.1	NR	RT: 1.1 Ctrl: 0.7	RT: 3.5 Ctrl: 2.9	NR
Boorjian (2007)[435]	CaPSURE (1989-2003)	median 39 months	30 days	9681	EBRT, brachytherapy	Surgery, other	RT: 1.3 Ctrl: 0.9	NR	RT: 0.4 Ctrl: 0.3	NR	NR
Brenner (2000)[18]	SEER (1972-1993)	median 4 years	2 month	122,123	"Radiation"	Surgery	RT: 0.9 Ctrl: 0.9	RT: 1.4 Ctrl: 1.6	RT: 0.4 Ctrl: 0.4	RT: 1.6 Ctrl: 1.5	RT: 0.2 Ctrl: 0.2*
Davis (2014)[436]	SEER (1992-2010)	NR	10 years	106,879	EBRT	No radiation	RT: 1.3 Ctrl: 0.9	RT: 1.2 Ctrl: 0.8	RT: 0.4 Ctrl: 0.2	RT: 1.3 Ctrl: 1.1	RT: 1.0 Ctrl: 0.9
Hinnen (2011)[437]	Utrecht, Netherlands (1989-2005)	median 7.5 years	0	1888	Brachytherapy	Surgery	RT: 1.4 Ctrl: 1.4	RT: 2.1 Ctrl: 2.3	RT: 0.8 Ctrl: 1.2	RT: 1.4 Ctrl: 1.3	RT: 0.7 Ctrl: 0.7

Huang (2011)[438]	William Beaumont, MI and SEER (1984-2005)	mean 7.4 years	NR	17,264	EBRT (2D/ 3DCRT, IMRT), brachytherapy, brach+	Surgery	NR	NR	NR	NR	NR
Huo (2009)[439]	SEER (1973-2005)	NR	NR	635,910	EBRT, brachytherapy	No radiation	NR	RT: 1.2 Ctrl: 1.2	RT: 0.4 Ctrl: 0.4	NR	NR
Margel (2011)[430]	Israel Cancer Registry (1982-2005)	median 11.2 years	6 months	29,593	"Radiation"	Surgery, other/ none	NR	NR	RT: 1.2 Ctrl: 0.6	NR	NR
Moon (2006)[440]	SEER (1973-1999)	median 10.0 years	5 years	140,767	EBRT, brachytherapy, brach+, XRT NOS	No radiation	RT: 1.4 EBRT: 1.4 Brach: 1.2 Brach+: 1.1 Ctrl: 0.9	RT: 1.7 EBRT: 1.7 Brach: 0.6 Brach+: 1.6 Ctrl: 1.4	RT: 0.4 EBRT: 0.4 Brach: 0.1 Brach+: 0.5 Ctrl: 0.3	RT: 2.0 EBRT: 2.0 Brach: 1.2 Brach+: 1.7 Ctrl: 1.6	RT: 1.0 EBRT: 1.0 Brach: 0.3 Brach+: 0.9 Ctrl: 0.9
Nam (2014)[413]	Ontario, CA (2002-2009)	NR	5 years	32,465	EBRT	Surgery	RT: 0.1 Ctrl: 0.1	RT: 0.3 Ctrl: 0.1	NR	RT: 0.2 Ctrl: 0.04	RT: 0.1 Ctrl: 0.1
Nieder (2008)[441]	SEER (1973-1990)	median 49 months	6 month	243,082	EBRT, brachytherapy, brach+	Surgery	RT: 1.1 EBRT: 1.3 Brach: 0.5 Brach+: 0.8 Ctrl: 0.7	NR	RT: 0.4 EBRT: 0.4 Brach: 0.2 Brach+: 0.3 Ctrl: 0.3	NR	NR
Pickles (2002)[429]	BC Tumor Registry (1984-2000)	median 3.1-5.3 years	2 months	39,261	EBRT	No radiation	RT: 0.6 Ctrl:0.5	RT: 2.4 Ctrl: 1.1	NR	RT: 2.1 Ctrl: 1.2	RT: 0.9 Ctrl: 0.6
Rapiti (2008)[442]	Geneva Cancer Registry (1980-1998)	median 7.4 years	5 years	1134	EBRT	No radiation	RT: 1.1 Ctrl: NR	RT: 4.2 Ctrl: 0.9	RT: 0.8 Ctrl: 0.5	RT: 1.1 Ctrl: NR	RT: 1.5 Ctrl: NR
Singh (2008)[443]	Syracuse VA Center (1996-2003)	NR	6 months	626	EBRT, brachytherapy, brach+	No radiation	RT: 3.8 Ctrl: 1.7	NR	NR	NR	NR
Singh	SEER	median	Multiple	555,337	EBRT	Surgery,	RT: 1.5	NR	NR	NR	NR

(2010)[444]	(1973-2005)	48.4-93.6 months				none	Ctrl: 1.2				
Van Hemelrijck (2014)[445]	Zurich Cancer Registry (1980-2010)	NR	0	20,559	Radiation	Surgery, ADT, other	RT: 1.5 Ctrl: 1.2	RT: 1.5 Ctrl: 1.8	RT: 0.4 Ctrl: 0.5	RT: 1.1 Ctrl: 1.6	RT: 1.4 Ctrl: 1.0
Zelefsky (2012)[20]	MSKCC (1998-2001)	median 90-113 months	NR	2658	EBRT (IMRT), brachytherapy	Surgery	RT: 1.2 EBRT: 1.3 Brach: 1.0 Ctrl: 1.2	RT: 1.5 EBRT: 1.2 Brach: 1.9 Ctrl: 0.7	RT: 0.5 EBRT: 0.6 Brach: 0.5 Ctrl: 0.7	RT: 1.2 EBRT: 1.3 Brach: 0.7 Ctrl: 1.6	RT: 1.2 EBRT: 1.2 Brach: 1.2 Ctrl:1.1ǂ

Abbreviations: SEER = Surveillance, Epidemiology and End Results; CA = Canada; CaPSURE = Cancer of the Prostate Strategic Urologic Research Endeavor; MI = Michigan; VA = Veterans Administration; MSKCC = Memorial Sloan Kettering Cancer Center; EBRT = external beam radiotherapy; Brachy = brachytherapy; NR = not reported; XRT NOS = radiotherapy not otherwise specified; 2DCRT = 2-dimensional conformal radiotherapy; 3DCRT = 3-dimensional conformal radiotherapy; IMRT = intensity modulated radiotherapy; brach+ = brachytherapy and EBRT; brach = brachytherapy; Ctrl = control. Notes: * - hematologic cancers restricted to leukemias for Brenner et al.; ǂ - hematologic cancers restricted to lymphoma for Zelefsky et al.

5.4.1 Study description

Characteristics of included studies are described in Table 5.1. Eighteen studies were large, multi-institutional reports and three were single centre studies[20,438,443]. Conformal EBRT was the most commonly assessed radiotherapy modality. There were insufficient data to distinguish between 2-dimensional, 3-dimensional and intensity modulated radiotherapy, although the majority of included studies assessed 3-dimensional conformal EBRT. There were considerable differences in the definition and use of a lag period before outcome ascertainment (Table 5.1). Length of follow-up varied significantly between included studies (Table 5.1). Thirteen studies (62%) included surgically treated patients as the comparator; and 8 studies (38%) used "no radiation" or "no radiation and no surgery" control groups (Table 5.1). Crude incidence of individual secondary cancers ranged from 0.2 to 2.3% for patients treated with external beam radiotherapy, 0.1 to 2.1% for patients treated with brachytherapy, 0.2 to 1.7% for patients treated with brachytherapy and external beam boost, and 0.3 to 2.3% for patients not exposed to radiotherapy; however, these rates varied significantly between studies (Table 5.1). The majority of studies did not specify whether the reported bladder cancers were superficial or invasive. For studies reporting adjusted hazard ratios, covariates included in the model varied significantly between studies though all included age at diagnosis as a covariate (Table 5.2).

Table 5.2. Covariates included in multivariate Cox proportional hazard models for studies reporting adjusted hazard ratios.

Study	Factors included as covariates in model		
	Demographic	**Clinical**	**Comorbidity**
Abern[432]	Age at diagnosis Race Year of diagnosis Geographic region	Prostate Ca grade Follow-up duration	
Baxter[433]	Age at diagnosis Race Year of diagnosis Geographic region		
Bhojani[434]	Age at diagnosis Year of treatment		Charlson Comorbidity Index
Boorjian[435]	Age at diagnosis Race Education Income Relationship status	Pre-operative PSA level Biopsy Gleason score Clinical stage D'Amico risk group	Hypertension Heart disease Diabetes Stroke history Lung disease Smoking status Cardiovascular comorbidity Body mass index
Hinnen[437]	Age at diagnosis		
Huang[438]	Age at diagnosis	Follow-up duration	
Singh (2010)[444]	Age at diagnosis Race	Tumor grade	

Notes: PSA = prostate-specific antigen

5.4.2 Risk of bias assessment

The majority of studies were deemed to be of moderate risk of bias (Table 5.3). Commonly identified concerns include a lack of explicit demonstration that the outcome was not present at the start of the study, the length of follow-up and attrition bias.

Table 5.3. Newcastle-Ottawa Scale for risk of bias assessment of studies included in the meta-analysis.

Study	Selection				Comparability	Outcome			Overall
	Representativeness of exposed cohort	Selection of non-exposed	Ascertainment of exposure	Outcome not present at start		Assessment of outcome	Adequate follow-up length	Adequacy of follow-up	
Abdel-Wahab[431]	1	1	1	1	2	1	0	0	7
Abern[432]	1	1	1	0	1	1	1	0	6
Baxter[433]	1	1	1	1	2	1	1	0	8
Berrington de Gonzales[17]	1	1	1	1	2	1	1	0	8
Bhojani[434]	1	1	1	0	2	1	0	0	6
Boorjian[435]	1	1	1	0	2	1	0	0	6
Brenner[18]	1	1	1	1	2	1	1	0	8
Davis[436]	1	1	1	0	1	1	1	0	6
Hinnen[437]	1	1	1	0	1	1	1	0	6
Huang[438]	1	0	1	0	1	1	1	0	5
Huo[439]	1	1	1	1	2	1	1	0	8
Margel[430]	1	1	1	1	1	1	1	0	7
Moon[440]	1	1	1	1	2	1	1	0	8
Nam[413]	1	1	1	0	2	1	1	0	7
Nieder[441]	1	1	1	1	2	1	0	0	7
Pickles[429]	1	1	1	1	1	1	0	0	6
Rapiti[442]	1	1	1	1	2	1	1	1	9
Singh (2008)[443]	1	1	1	0	2	1	1	0	7
Singh (2010)[444]	1	1	1	1	2	1	1	0	8
Van Hemelrijck[445]	1	1	1	1	2	1	0	1	8
Zelefsky[20]	1	1	1	0	1	1	1	1	7

5.4.3 Bladder Cancer

On unadjusted analysis with no restriction to lag period, we found increased odds of bladder cancer (9 studies, 555,873 participants, unadjusted OR 1.39, 95% CI 1.12 to 1.71; I^2=56%; Figure 5.2). The results were similar when we restricted to only studies using a five-year lag period (3 studies, 397,416 participants, unadjusted OR 1.30, 95% CI 1.19 to 1.42; I^2=0%; Table 5.4) or a ten-year lag period (2 studies, 99,362 patients, unadjusted OR 1.89, 95% CI 1.65 to 2.16, I^2=0%). After multivariable adjustment, we found an elevated risk for bladder cancer in those treated with radiotherapy (4 studies, adjusted HR 1.67, 95% CI 1.55 to 1.80; I^2=0%).

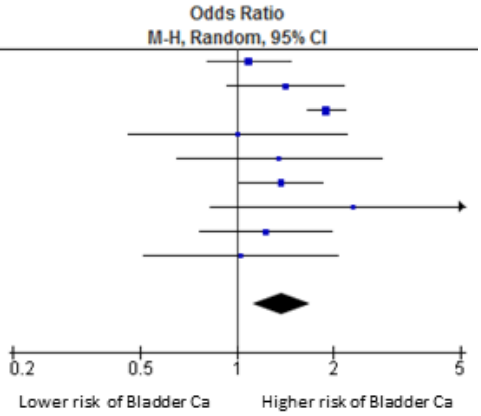
Absolute differences in bladder cancer risk between radiotherapy exposed and unexposed patients ranged from 0 to 0.6 cancers per 100 patients, depending on radiotherapy modality, comparator group and lag period (Table 5.5).

Figure 5.2. Forest plots of studies assessing the risk of bladder cancer following any radiotherapy compared with no radiation by (a) no restriction to lag period; (b) restriction to studies using a 5-year lag period; (c) restriction to studies using a 10-year lag period; and (d) studies reporting adjusted hazard ratios.

A

Study or Subgroup	Radiation Events	Radiation Total	No Radiation Events	No Radiation Total	Weight	Odds Ratio M-H, Random, 95% CI
Bhojani 2010	69	3008	120	5693	16.4%	1.09 [0.81, 1.47]
Boorjian 2007	33	2471	68	7210	12.5%	1.42 [0.94, 2.16]
Davis 2014	343	25569	506	71242	22.3%	1.90 [1.66, 2.18]
Hinnen 2011	17	1187	10	701	5.5%	1.00 [0.46, 2.21]
Nam 2014	17	16595	12	15870	6.1%	1.36 [0.65, 2.84]
Pickles 2002	62	9890	134	29371	16.3%	1.38 [1.02, 1.86]
Singh 2008	8	210	7	416	3.6%	2.31 [0.83, 6.47]
Van Hemelrijck 2014	23	1577	64	5381	10.8%	1.23 [0.76, 1.99]
Zelefsky 2012	16	1310	16	1348	6.6%	1.03 [0.51, 2.07]
Total (95% CI)		**61817**		**137232**	**100.0%**	**1.39 [1.12, 1.71]**
Total events	588		937			

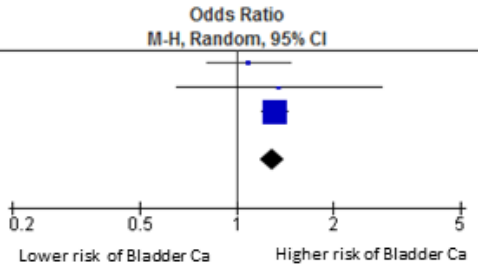
Heterogeneity: Tau² = 0.05; Chi² = 18.32, df = 8 (P = 0.02); I² = 56%
Test for overall effect: Z = 3.04 (P = 0.002)



B

Study or Subgroup	Radiation Events	Radiation Total	No Radiation Events	No Radiation Total	Weight	Odds Ratio M-H, Random, 95% CI
Bhojani 2010	69	3008	120	5693	8.8%	1.09 [0.81, 1.47]
Nam 2014	17	16595	12	15870	1.4%	1.36 [0.65, 2.84]
Singh 2010	748	123053	1076	233197	89.8%	1.32 [1.20, 1.45]
Total (95% CI)		**142656**		**254760**	**100.0%**	**1.30 [1.19, 1.42]**
Total events	834		1208			

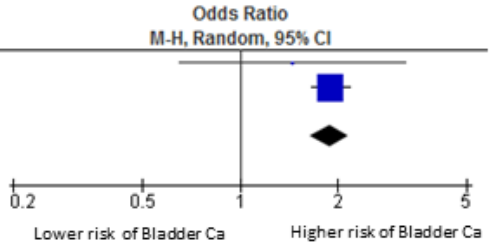
Heterogeneity: Tau² = 0.00; Chi² = 1.43, df = 2 (P = 0.49); I² = 0%
Test for overall effect: Z = 5.77 (P < 0.00001)



C

Study or Subgroup	Radiation Events	Radiation Total	No Radiation Events	No Radiation Total	Weight	Odds Ratio M-H, Random, 95% CI
Bhojani 2010	9	630	19	1921	2.9%	1.45 [0.65, 3.22]
Davis 2014	343	25569	506	71242	97.1%	1.90 [1.66, 2.18]
Total (95% CI)		**26199**		**73163**	**100.0%**	**1.89 [1.65, 2.16]**
Total events	352		525			

Heterogeneity: Tau² = 0.00; Chi² = 0.43, df = 1 (P = 0.51); I² = 0%
Test for overall effect: Z = 9.16 (P < 0.00001)



D

Study or Subgroup	log[Hazard Ratio]	SE	Weight	Hazard Ratio IV, Random, 95% CI
Abern 2013	0.5306	0.0406	89.6%	1.70 [1.57, 1.84]
Bhojani 2010	0.3365	0.1446	7.1%	1.40 [1.05, 1.86]
Boorjian 2007	0.4637	0.2521	2.3%	1.59 [0.97, 2.61]
Hinnen 2011	0.1222	0.3836	1.0%	1.13 [0.53, 2.40]
Total (95% CI)			**100.0%**	**1.67 [1.55, 1.80]**

Heterogeneity: Tau² = 0.00; Chi² = 2.75, df = 3 (P = 0.43); I² = 0%
Test for overall effect: Z = 13.30 (P < 0.00001)

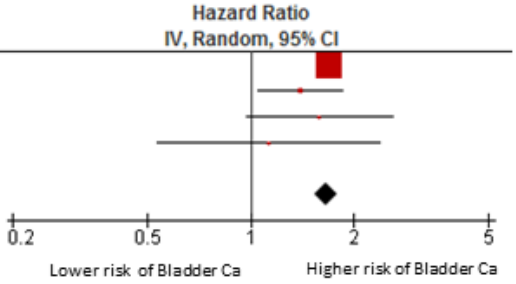


Table 5.4. Pooled odds/hazard estimates for secondary tumor sites stratified by radiotherapy modality and comparator group.

	No lag restriction			5-year lag			Adjusted Hazard ratio	
	Studies	N	OR (95% CI)	Studies	N	OR (95% CI)	Studies	HR (95% CI)
BLADDER CANCER								
Any XRT vs no XRT	9	555873	1.39 (1.12 to 1.71)#	3	397416	1.30 (1.19 to 1.42)#	4	1.67 (1.55 to 1.80)#
EBRT vs no XRT	6	186854	1.37 (1.05 to 1.77)#	3	397416	1.30 (1.19 to 1.42)#	2	1.62 (1.20 to 2.20)#
Brach vs no XRT	3	161889	1.25 (1.10 to 1.42)#	1	95826	1.45 (0.88 to 2.39)	2	1.04 (0.52 to 2.09)
Any XRT vs surgery	6	692487	1.37 (1.02 to 1.84)*#	2	41166	1.12 (0.85 to 1.48)	2	1.62 (1.38 to 1.91)#
EBRT vs surgery	5	259521	1.39 (0.93 to 2.07)*	2	41166	1.12 (0.85 to 1.48)	2	1.63 (0.90 to 2.96)
Brach vs surgery	2	160001	1.26 (1.11 to 1.43)#	N/A			1	0.66 (0.11 to 3.96)
COLORECTAL CANCER								
Any XRT vs no XRT	10	228965	1.68 (1.33 to 2.12)#	4	242878	1.94 (1.07 to 3.50)*#	3	1.79 (1.34 to 2.38)#
EBRT vs no XRT	8	217396	1.78 (1.38 to 2.29)#	4	177061	1.93 (1.04 to 3.57)*#	2	1.41 (0.78 to 2.56)*
Brach vs no XRT	3	135716	0.99 (0.39 to 2.53)*	1	95826	0.15 (0.02 to 1.07)	1	0.96 (0.43 to 2.13)
Any XRT vs surgery	7	332953	1.45 (1.07 to 1.96)#	3	127396	1.57 (0.91 to 2.70)*	2	1.41 (0.78 to 2.56)*
EBRT vs surgery	6	282014	1.52 (1.14 to 2.03)#	3	127396	1.57 (0.91 to 2.70)*	2	1.41 (0.78 to 2.56)*
Brach vs surgery	2	133828	1.12 (0.19 to 6.66)*	N/A			N/A	
RECTAL CANCER								
Any XRT vs no XRT	8	157239	1.62 (1.26 to 2.08)#	3	204064	1.68 (0.90 to 3.15)*	3	1.79 (1.34 to 2.38)#
EBRT vs no XRT	6	145670	1.64 (1.21 to 2.21)#	3	144596	1.56 (1.31 to 1.86)#	2	1.74 (1.45 to 2.08)#
Brach vs no XRT	3	135716	0.65 (0.36 to 1.19)	1	95826	0.28 (0.04 to 1.99)	N/A	
Any XRT vs surgery	6	300488	1.30 (0.99 to 1.71)	2	94931	1.56 (1.26 to 1.93)#	2	1.74 (1.45 to 2.08)#
EBRT vs surgery	5	249549	1.38 (1.12 to 1.70)#	2	94931	1.56 (1.26 to 1.93)#	2	1.74 (1.45 to 2.08)#
Brach vs surgery	2	133828	0.49 (0.35 to 0.67)	N/A			N/A	

LUNG CANCER								
Any XRT vs no XRT	7	188911	1.31 (0.97 to 1.76)*	3	241498	1.55 (1.00 to 2.40)*#	2	1.45 (0.70 to 3.01)*
EBRT vs no XRT	6	187023	1.33 (0.97 to 1.82)*	3	175681	1.60 (1.06 to 2.42)*#	2	1.38 (0.74 to 2.56)
Brach vs no XRT	3	54605	0.62 (0.39 to 0.97)	1	95826	0.71 (0.43 to 1.19)	1	0.70 (0.22 to 2.23)
Any XRT vs surgery	5	173074	1.16 (0.81 to 1.68)*	2	41335	2.66 (0.52 to 13.64)*	2	1.45 (0.70 to 3.01)*
EBRT vs surgery	5	172661	1.19 (0.83 to 1.71)*	2	41335	2.66 (0.52 to 13.64)*	2	1.37 (0.71 to 2.61)
Brach vs surgery	1	1761	0.44 (0.13 to 1.48)	N/A			1	0.70 (0.22 to 2.23)
HEMATOLOGICAL CANCERS								
Any XRT vs no XRT	6	180032	1.33 (1.05 to 1.69)#	2	173232	1.30 (0.79 to 2.13)	1	1.64 (0.90 to 2.99)
EBRT vs no XRT	5	177740	1.36 (1.05 to 1.77)#	2	173232	1.30 (0.79 to 2.13)	1	2.09 (0.15 to 29.75)
Brach vs no XRT	3	54605	1.08 (0.80 to 1.46)	1	95826	0.36 (0.13 to 0.92)	1	0.50 (0.09 to 2.78)
Any XRT vs surgery	4	164195	1.16 (0.78 to 1.72)	1	32465	1.91 (0.96 to 3.83)	1	1.64 (0.90 to 2.99)
EBRT vs surgery	4	272093	1.08 (0.98 to 1.19)	1	32465	1.91 (0.96 to 3.83)	1	2.09 (0.15 to 29.75)
Brach vs surgery	1	1761	1.09 (0.39 to 3.01)	N/A			1	0.50 (0.09 to 2.78)

* = I^2 greater than 75% indicating significant heterogeneity

= statistically significant at $p<0.05$

Abbreviations: OR = Odds ratio; HR = Hazard ratio; XRT = radiotherapy; EBRT = external beam radiotherapy; Brach = brachytherapy. Notes: N/A = no data available for meta-analysis.

Table 5.5. Absolute difference in secondary cancer per 100 patients, stratified by radiotherapy modality and comparator group (95% confidence interval).

	Any lag	5-year lag	10-year lag
BLADDER CANCER			
Any XRT vs no XRT	0.4 (0.0 – 0.7)	0.1 (0.0 - 0.2)	0.6 (0.5 – 0.7)
EBRT vs no XRT	0.2 (-0.2 – 0.6)	0.1 (0.0 – 0.2)	0.6 (0.5-0.7)
Brach vs no XRT	0.0 (-0.2 – 0.3)	0.4 (0.2 – 0.6)	N/A
Any XRT vs surgery	0.3 (-0.2 – 0.7)	0.1 (-0.2 – 0.4)	0.4 (0.1 – 0.7)
EBRT vs surgery	0.3 (-0.4 – 1.0)	0.1 (-0.2 – 0.4)	0.4 (0.1 – 0.7)
Brach vs surgery	0.0 (-0.4 – 0.4)	N/A	N/A
COLORECTAL CANCER			
Any XRT vs no XRT	0.5 (0.3 – 0.8)	0.4 (0.2 – 0.6)	0.4 (0.4 – 0.5)
EBRT vs no XRT	0.7 (0.3 – 1.0)	0.4 (0.2 – 0.6)	0.4 (0.4 – 0.5)
Brach vs no XRT	0.3 (-0.5 – 1.1)	1.4 (0.8 – 2.7)	N/A
Any XRT vs surgery	0.2 (0.1 – 0.3)	0.2 (0.1 – 0.3)	0.3 (0.1 – 0.5)
EBRT vs surgery	0.2 (0.1 – 0.3)	0.2 (0.1 – 0.3)	0.3 (0.1 – 0.5)
Brach vs surgery	0.5 (-0.9 – 2.0)	N/A	N/A
RECTAL CANCER			
Any XRT vs no XRT	0.2 (0.1 – 0.3)	1.0 (-0.4 – 2.3)	0.2 (0.2 – 0.3)
EBRT vs no XRT	0.2 (0.0 – 0.4)	0.3 (0.1 – 0.4)	0.2 (0.2 – 0.3)
Brach vs no XRT	-0.1 (-0.3 – 0.0)	-0.2 (-0.2 – -0.2)	N/A
Any XRT vs surgery	0.2 (0.0 – 0.3)	0.2 (0.0 – 0.5)	0.3 (0.1 – 0.5)
EBRT vs surgery	0.2 (0.0 – 0.4)	0.2 (0.0 – 0.5)	0.3 (0.1 – 0.5)
Brach vs surgery	-0.2 (-0.2 – -0.2)	N/A	N/A
LUNG CANCER			
Any XRT vs no XRT	0.2 (-0.1 – 0.4)	0.2 (0.2 – 0.3)	1.1 (-0.5 – 2.6)
EBRT vs no XRT	0.2 (-0.1 – 0.5)	0.4 (0.1 – 0.7)	1.1 (-0.5 – 2.6)
Brach vs no XRT	-0.5 (-1.1 – 0.0)	-0.5 (-0.6 – -0.3)	N/A
Any XRT vs surgery	0.0 (-0.2 – 0.2)	0.4 (-0.4 – 1.2)	1.9 (1.5 – 2.3)
EBRT vs surgery	0.0 (-0.1 – 0.2)	0.4 (-0.4 – 1.2)	1.1 (-0.5 – 2.6)
Brach vs surgery	-0.9 (-1.2 – -0.6)	N/A	N/A
HEMATOLOGIC CANCER			
Any XRT vs no XRT	0.2 (0.0 – 0.3)	0.1 (0.0 – 0.1)	0.1 (0.0 – 0.1)
EBRT vs no XRT	0.2 (0.1 – 0.3)	0.1 (0.0 – 0.1)	0.1 (0.0 – 0.1)
Brach vs no XRT	0.0 (0.0 – 0.1)	-0.6 (-0.7 – -0.5)	N/A
Any XRT vs surgery	0.1 (-0.1 – 0.3)	0.1 (0.0 – 0.1)	N/A
EBRT vs surgery	0.1 (0.0 – 0.2)	0.1 (0.0 – 0.1)	N/A
Brach vs surgery	0.1 (-0.3 – 0.5)	N/A	N/A

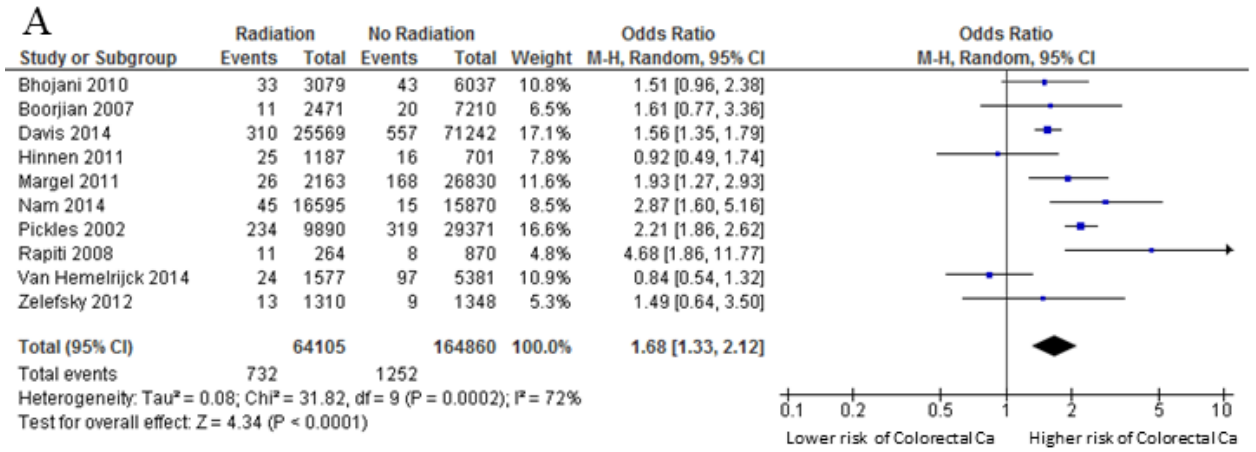
Note: N/A = no data available for meta-analysis.

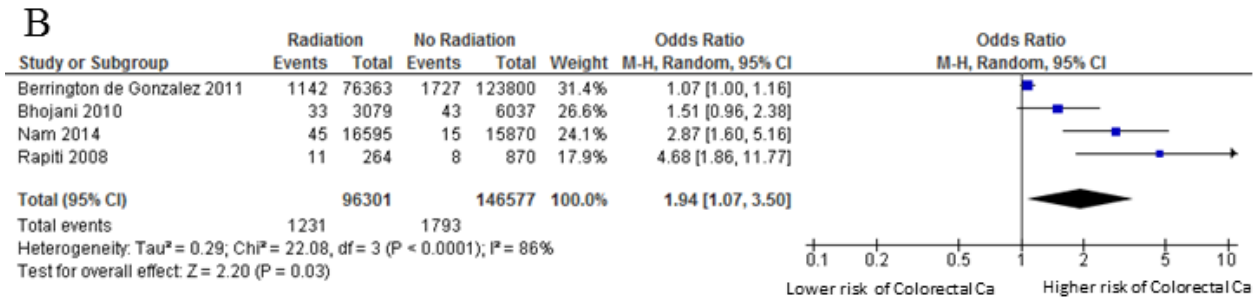
5.4.4 Colorectal Cancer

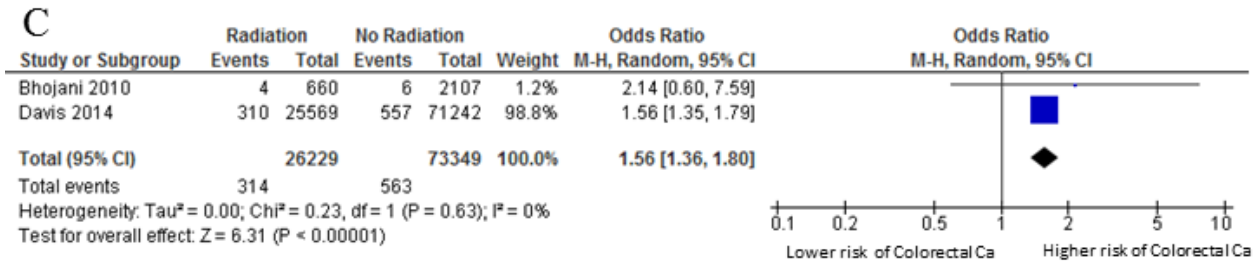
On unadjusted analysis with no lag period, we found an increased odds of colorectal cancer following any form of radiotherapy as compared with no radiation (10 studies, 228,965 participants, unadjusted OR 1.68, 95% CI 1.33 to 2.12; I^2=72%; Figure 5.3). Again, results were similar after restriction to studies employing a five-year lag period (4 studies, 242,878 patients, unadjusted OR 1.94, 95% CI 1.07 to 3.50, I^2=86%; Table 5.4) or a ten-year lag period (2 studies, 99,578 patients, unadjusted OR 1.56, 95% CI 1.36 to 1.80, I^2=0%). Pooled multivariable adjusted hazard ratios showed an increased risk for colorectal cancer in those treated with radiotherapy (3 studies, adjusted HR 1.79, 95% CI 1.34 to 2.38; I^2=28%).

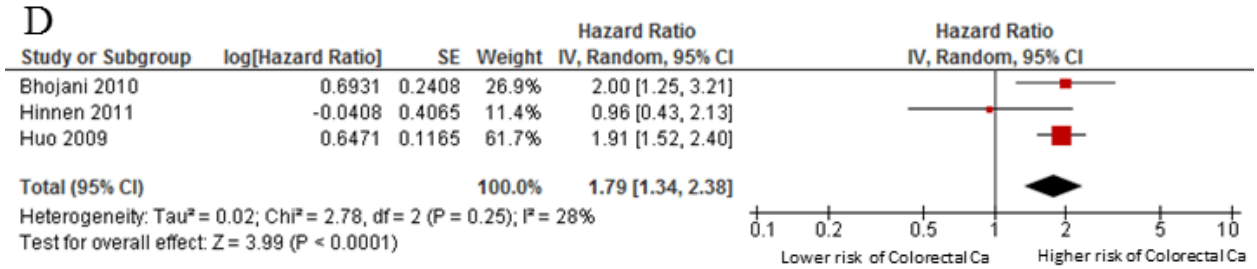
The absolute difference in colorectal cancers ranged from 0.2 to 1.4 cases per 100 patients for those treated with radiotherapy and controls, depending on radiotherapy modality, comparator and lag period (Table 5.5).

Figure 5.3. Forest plots of studies assessing the risk of colorectal cancer following any radiotherapy compared with no radiation by (a) no restriction to lag period; (b) restriction to studies using a 5-year lag period; (c) restriction to studies using a 10-year lag period; and (d) studies reporting adjusted hazard ratios.









5.4.5 Rectal Cancer

When we limited to only cases of rectal cancer following radiotherapy, we identified an increased odds of rectal cancer associated with radiotherapy in unadjusted analysis without restriction to lag period (8 studies, 157,239 participants, unadjusted OR 1.62, 95% CI 1.26 to 2.08; I^2=33%; Figure 5.4). Restriction to those studies employing a five-year lag period showed no significant association (3 studies, 204,064 patients, unadjusted OR 1.68, 95% CI 0.90 to 3.15, I^2=76%; Table 5.4) while restriction to those studies using a ten-year lag period showed an association similar to the primary analysis (2 studies, 99,578 patients, unadjusted OR 2.20, 95% CI 1.72 to 2.81, I^2=0%). Pooling of multivariable adjusted hazard ratios demonstrated an increased risk similar to our primary analysis (3 studies, adjusted HR 1.79, 95% CI 1.34 to 2.38; I^2=28%).

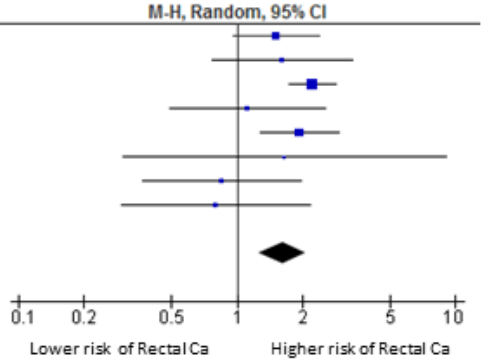
The absolute difference in risk between radiotherapy exposed and unexposed patients ranged between -0.2 and 1.0 cases of rectal cancer per 100 patients (Table 5.5).

Figure 5.4. Forest plots of studies assessing the risk of rectal cancer following any radiotherapy compared with no radiation by (a) no restriction to lag period; (b) restriction to studies using a 5-year lag period; (c) restriction to studies using a 10-year lag period; and (d) studies reporting adjusted hazard ratios.

A

Study or Subgroup	Radiation Events	Radiation Total	No Radiation Events	No Radiation Total	Weight	Odds Ratio M-H, Random, 95% CI
Bhojani 2010	33	3079	43	6037	17.8%	1.51 [0.96, 2.38]
Boorjian 2007	11	2471	20	7210	9.1%	1.61 [0.77, 3.36]
Davis 2014	112	25569	142	71242	30.3%	2.20 [1.72, 2.82]
Hinnen 2011	17	1187	9	701	7.8%	1.12 [0.50, 2.52]
Margel 2011	26	2163	168	26830	19.8%	1.93 [1.27, 2.93]
Rapiti 2008	2	264	4	870	2.1%	1.65 [0.30, 9.07]
Van Hemelrijck 2014	7	1577	28	5381	7.5%	0.85 [0.37, 1.96]
Zelefsky 2012	7	1310	9	1348	5.6%	0.80 [0.30, 2.15]
Total (95% CI)		**37620**		**119619**	**100.0%**	**1.62 [1.26, 2.08]**
Total events	215		423			

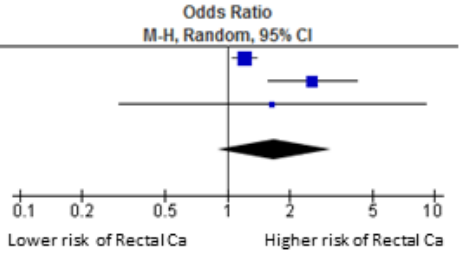
Heterogeneity: Tau² = 0.04; Chi² = 10.38, df = 7 (P = 0.17); I² = 33%
Test for overall effect: Z = 3.75 (P = 0.0002)



B

Study or Subgroup	Radiation Events	Radiation Total	No Radiation Events	No Radiation Total	Weight	Odds Ratio M-H, Random, 95% CI
Berrington de Gonzalez 2011	371	76363	495	123800	50.3%	1.22 [1.06, 1.39]
Bhojani 2010	29	660	37	2107	39.0%	2.57 [1.57, 4.21]
Rapiti 2008	2	264	4	870	10.7%	1.65 [0.30, 9.07]
Total (95% CI)		**77287**		**126777**	**100.0%**	**1.68 [0.90, 3.15]**
Total events	402		536			

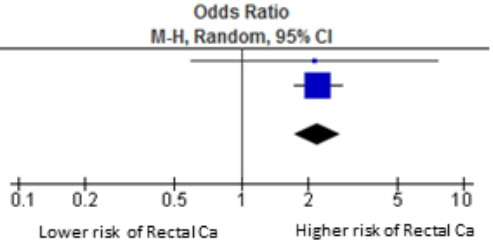
Heterogeneity: Tau² = 0.20; Chi² = 8.30, df = 2 (P = 0.02); I² = 76%
Test for overall effect: Z = 1.63 (P = 0.10)



C

Study or Subgroup	Radiation Events	Radiation Total	No Radiation Events	No Radiation Total	Weight	Odds Ratio M-H, Random, 95% CI
Bhojani 2010	4	660	6	2107	3.7%	2.14 [0.60, 7.59]
Davis 2014	112	25569	142	71242	96.3%	2.20 [1.72, 2.82]
Total (95% CI)		**26229**		**73349**	**100.0%**	**2.20 [1.72, 2.81]**
Total events	116		148			

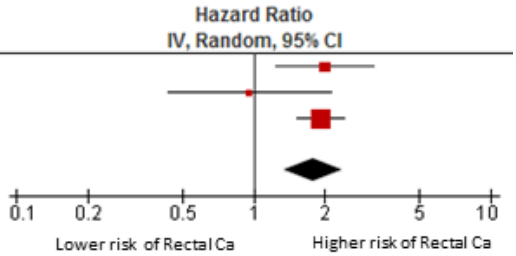
Heterogeneity: Tau² = 0.00; Chi² = 0.00, df = 1 (P = 0.96); I² = 0%
Test for overall effect: Z = 6.35 (P < 0.00001)



D

Study or Subgroup	log[Hazard Ratio]	SE	Weight	Hazard Ratio IV, Random, 95% CI
Bhojani 2010	0.6931	0.2408	26.9%	2.00 [1.25, 3.21]
Hinnen 2011	-0.0408	0.4065	11.4%	0.96 [0.43, 2.13]
Huo 2009	0.6471	0.1165	61.7%	1.91 [1.52, 2.40]
Total (95% CI)			**100.0%**	**1.79 [1.34, 2.38]**

Heterogeneity: Tau² = 0.02; Chi² = 2.78, df = 2 (P = 0.25); I² = 28%
Test for overall effect: Z = 3.99 (P < 0.0001)



5.4.6 Lung Cancer

Unadjusted analysis of studies without restriction to lag period demonstrated no association between radiotherapy and lung cancer (7 studies, 188,911 participants, unadjusted OR 1.31, 95% CI 0.97 to 1.76; I^2=84%; Figure 5.5). Restriction to studies using a five-year lag period showed marginal significance (3 studies, 241,298 participants, unadjusted OR 1.55, 95% CI 1.00 to 2.40; I^2=88%; Table 5.4) while there was no association in those studies using a ten-year lag period (2 studies, 99,478 patients, unadjusted OR 1.58, 95% CI 0.89 to 2.83, I^2=76%). There was no association after pooling of multivariable adjusted hazard ratios (2 studies, adjusted HR 1.45, 95% CI 0.70 to 3.01; I^2=86%).

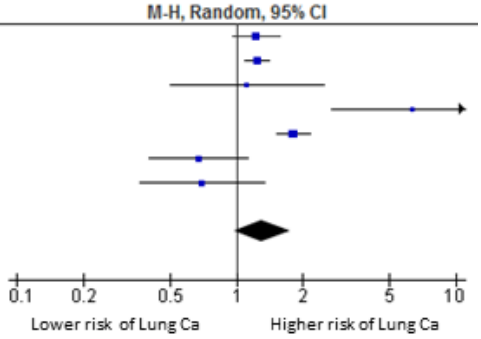
For lung cancers, the absolute difference between patients treated with radiotherapy and those not exposed ranged from -0.9 to 1.1 cancers per 100 patients (Table 5.5).

Figure 5.5. Forest plots of studies assessing the risk of lung cancer following any radiotherapy compared with no radiation by (a) no restriction to lag period; (b) restriction to studies using a 5-year lag period; (c) restriction to studies using a 10-year lag period; and (d) studies reporting adjusted hazard ratios.

A

Study or Subgroup	Radiation Events	Radiation Total	No Radiation Events	No Radiation Total	Weight	Odds Ratio M-H, Random, 95% CI
Bhojani 2010	104	2937	172	5933	19.0%	1.23 [0.96, 1.57]
Davis 2014	344	25569	770	71242	21.1%	1.25 [1.10, 1.42]
Hinnen 2011	17	1187	9	701	8.2%	1.12 [0.50, 2.52]
Nam 2014	40	16595	6	15870	7.7%	6.39 [2.71, 15.07]
Pickles 2002	209	9890	346	29371	20.4%	1.81 [1.52, 2.15]
Van Hemelrijck 2014	17	1577	86	5381	13.0%	0.67 [0.40, 1.13]
Zelefsky 2012	15	1310	22	1348	10.5%	0.70 [0.36, 1.35]
Total (95% CI)		**59065**		**129846**	**100.0%**	**1.31 [0.97, 1.76]**
Total events	746		1411			

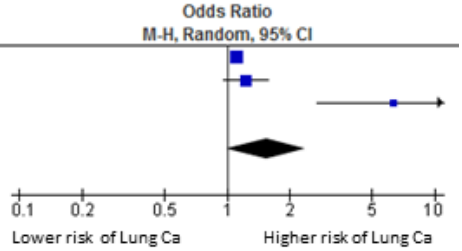
Heterogeneity: Tau² = 0.10; Chi² = 36.48, df = 6 (P < 0.00001); I² = 84%
Test for overall effect: Z = 1.79 (P = 0.07)



B

Study or Subgroup	Radiation Events	Radiation Total	No Radiation Events	No Radiation Total	Weight	Odds Ratio M-H, Random, 95% CI
Berrington de Gonzalez 2011	1450	76363	2115	123800	44.2%	1.11 [1.04, 1.19]
Bhojani 2010	104	2937	172	5933	39.2%	1.23 [0.96, 1.57]
Nam 2014	40	16595	6	15870	16.6%	6.39 [2.71, 15.07]
Total (95% CI)		**95895**		**145603**	**100.0%**	**1.55 [1.00, 2.40]**
Total events	1594		2293			

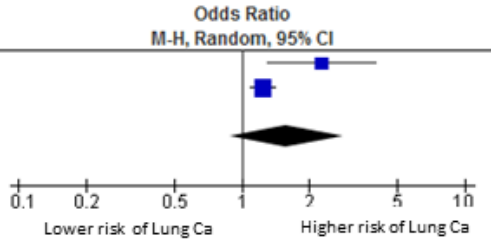
Heterogeneity: Tau² = 0.11; Chi² = 16.31, df = 2 (P = 0.0003); I² = 88%
Test for overall effect: Z = 1.94 (P = 0.05)



C

Study or Subgroup	Radiation Events	Radiation Total	No Radiation Events	No Radiation Total	Weight	Odds Ratio M-H, Random, 95% CI
Bhojani 2010	21	619	31	2048	39.4%	2.28 [1.30, 4.01]
Davis 2014	344	25569	770	71242	60.6%	1.25 [1.10, 1.42]
Total (95% CI)		**26188**		**73290**	**100.0%**	**1.58 [0.89, 2.83]**
Total events	365		801			

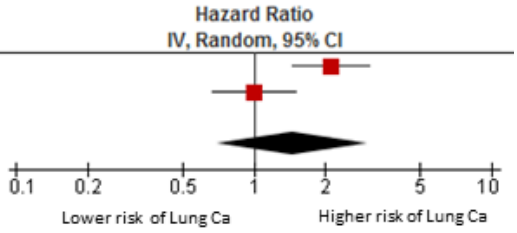
Heterogeneity: Tau² = 0.14; Chi² = 4.24, df = 1 (P = 0.04); I² = 76%
Test for overall effect: Z = 1.56 (P = 0.12)



D

Study or Subgroup	log[Hazard Ratio]	SE	Weight	Hazard Ratio IV, Random, 95% CI
Bhojani 2010	0.7419	0.1907	50.5%	2.10 [1.45, 3.05]
Huang 2011	0	0.2043	49.5%	1.00 [0.67, 1.49]
Total (95% CI)			**100.0%**	**1.45 [0.70, 3.01]**

Heterogeneity: Tau² = 0.24; Chi² = 7.05, df = 1 (P = 0.008); I² = 86%
Test for overall effect: Z = 1.01 (P = 0.31)



5.4.7 Hematologic Cancers

We found an increased odds of hematologic cancers following radiotherapy in studies without restriction to lag period (6 studies, 180,032 participants, unadjusted OR 1.33, 95% CI 1.05 to 1.69; I^2=50%; Figure 5.6) but this was not confirmed in studies using a five-year lag period (2 study, 172,232 patients, unadjusted OR 1.30, 95% CI 0.79 to 2.13; I^2=57%; Table 5.4), a ten-year lag period (1 study, 96,811 patients, unadjusted OR 1.09, 95% CI 0.94 to 1.27) or multivariable adjusted hazard ratios (1 study, adjusted HR 1.64, 95% CI 0.90 to 2.99).

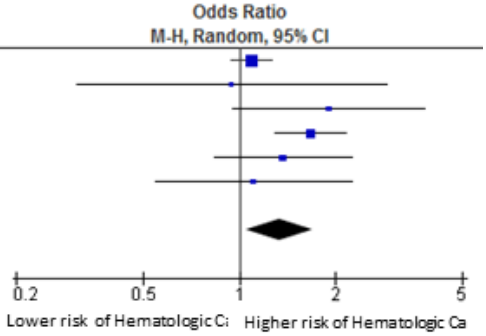
The absolute difference in risk ranged between -0.6 and 0.2 cases per 100 patients for patients treated with radiotherapy and controls, depending on radiotherapy modality, comparator, and lag period (Table 5.5).

Figure 5.6. Forest plots of studies assessing the risk of hematologic cancer following any radiotherapy compared with no radiation by (a) no restriction to lag period; (b) restriction to studies using a 5-year lag period; (c) restriction to studies using a 10-year lag period; and (d) studies reporting adjusted hazard ratios.

A

Study or Subgroup	Radiation Events	Radiation Total	No Radiation Events	No Radiation Total	Weight	Odds Ratio M-H, Random, 95% CI
Davis 2014	246	25569	628	71242	35.4%	1.09 [0.94, 1.27]
Hinnen 2011	8	1187	5	701	4.1%	0.94 [0.31, 2.90]
Nam 2014	24	16595	12	15870	9.2%	1.91 [0.96, 3.83]
Pickles 2002	91	9890	162	29371	27.7%	1.67 [1.29, 2.17]
Van Hemelrijck 2014	22	1577	55	5381	14.7%	1.37 [0.83, 2.25]
Zelefsky 2012	16	1301	15	1348	8.9%	1.11 [0.54, 2.25]
Total (95% CI)		**56119**		**123913**	**100.0%**	**1.33 [1.05, 1.69]**
Total events	407		877			

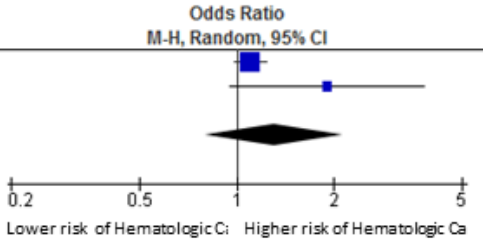
Heterogeneity: Tau² = 0.04; Chi² = 10.01, df = 5 (P = 0.07); I² = 50%
Test for overall effect: Z = 2.36 (P = 0.02)



B

Study or Subgroup	Radiation Events	Radiation Total	No Radiation Events	No Radiation Total	Weight	Odds Ratio M-H, Random, 95% CI
Moon 2006	457	46226	847	94541	70.1%	1.10 [0.99, 1.24]
Nam 2014	24	16595	12	15870	29.9%	1.91 [0.96, 3.83]
Total (95% CI)		**62821**		**110411**	**100.0%**	**1.30 [0.79, 2.13]**
Total events	481		859			

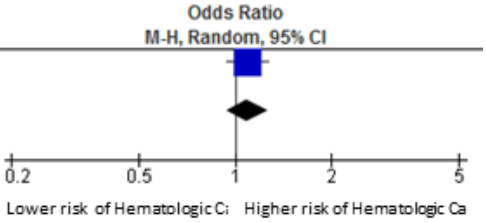
Heterogeneity: Tau² = 0.09; Chi² = 2.35, df = 1 (P = 0.13); I² = 57%
Test for overall effect: Z = 1.05 (P = 0.29)



C

Study or Subgroup	Radiation Events	Radiation Total	No Radiation Events	No Radiation Total	Weight	Odds Ratio M-H, Random, 95% CI
Davis 2014	246	25569	628	71242	100.0%	1.09 [0.94, 1.27]
Total (95% CI)		**25569**		**71242**	**100.0%**	**1.09 [0.94, 1.27]**
Total events	246		628			

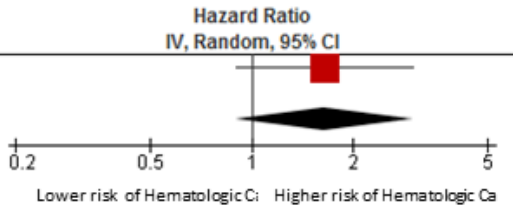
Heterogeneity: Not applicable
Test for overall effect: Z = 1.17 (P = 0.24)



D

Study or Subgroup	log[Hazard Ratio]	SE	Weight	Hazard Ratio IV, Random, 95% CI
Huang 2011	0.4947	0.3062	100.0%	1.64 [0.90, 2.99]
Total (95% CI)			**100.0%**	**1.64 [0.90, 2.99]**

Heterogeneity: Not applicable
Test for overall effect: Z = 1.62 (P = 0.11)



5.4.8 Subgroup Analysis

Studies limited to EBRT predominately reported an increased odds of secondary malignancy following radiotherapy whereas those limited to brachytherapy did not demonstrate this association (Figures 5.7 and 5.8; Table 5.2).

Figure 5.7. Forest plots of studies assessing the risk of secondary cancers associated with external beam radiotherapy (EBRT) without a lag period for (a) bladder cancer; (b) colorectal cancer; (c) rectal cancer; (d) lung cancer; and (e) hematologic cancer.

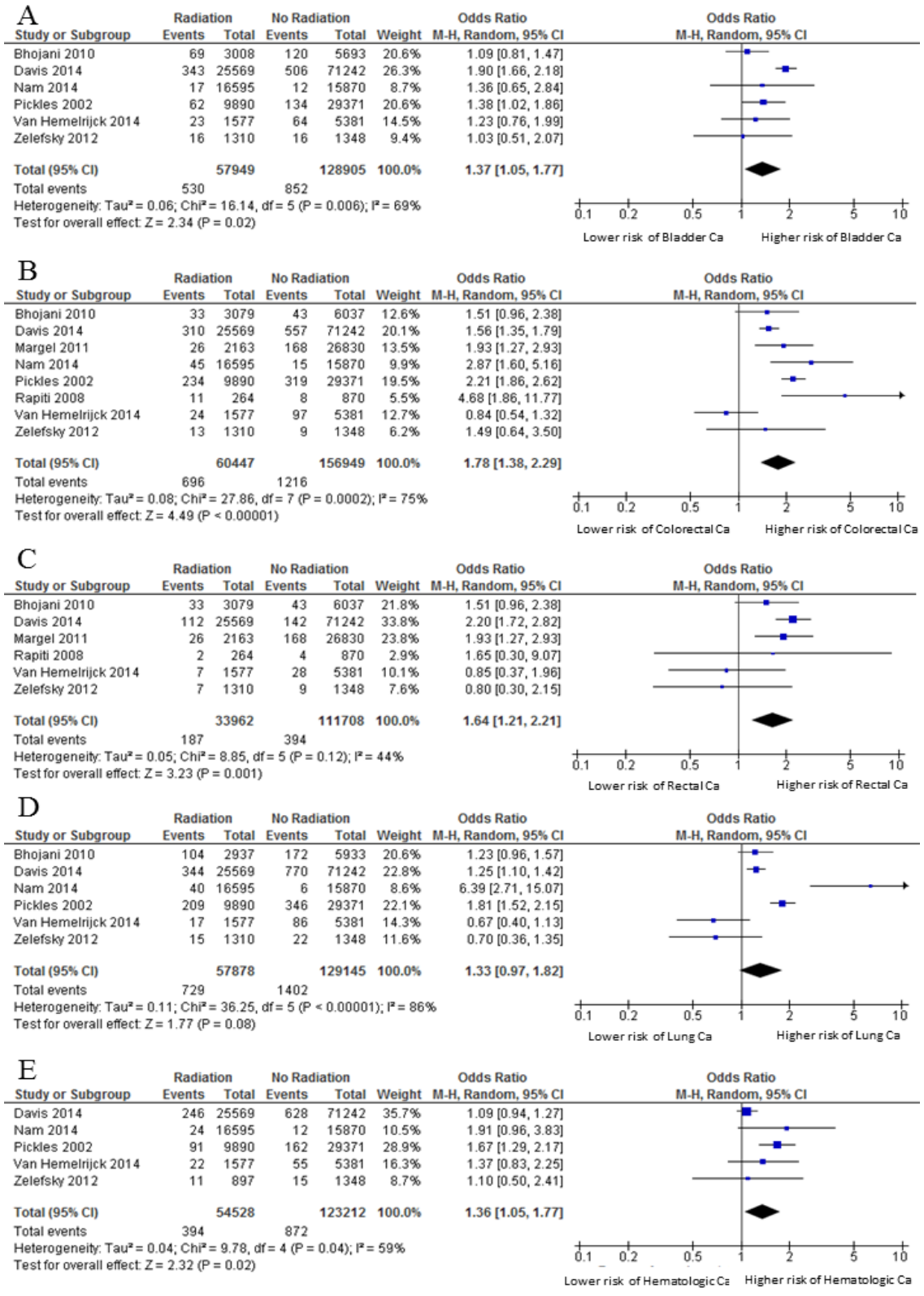
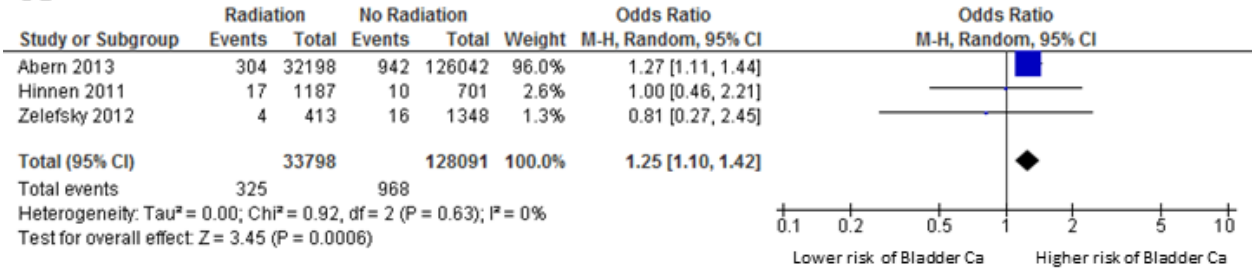
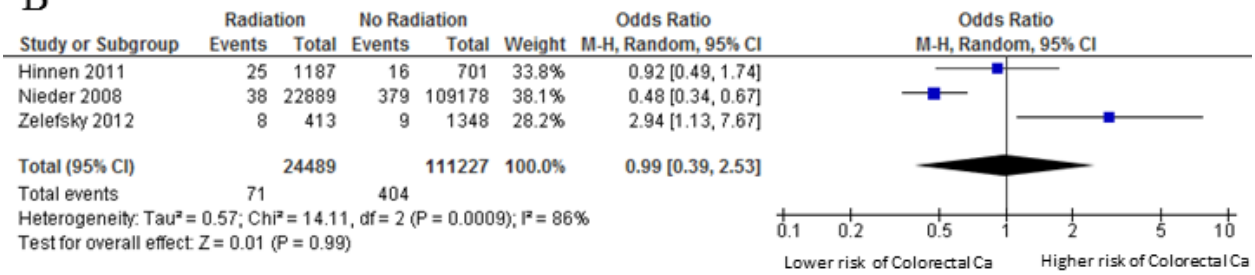


Figure 5.8. Forest plots of studies assessing the risk of secondary cancers associated with brachytherapy without a lag period for (a) bladder cancer; (b) colorectal cancer; (c) rectal cancer; (d) lung cancer; and (e) hematologic cancer.

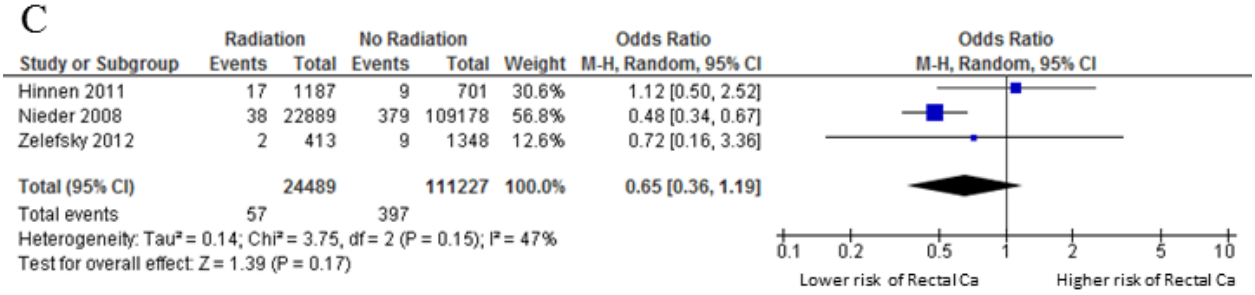
A



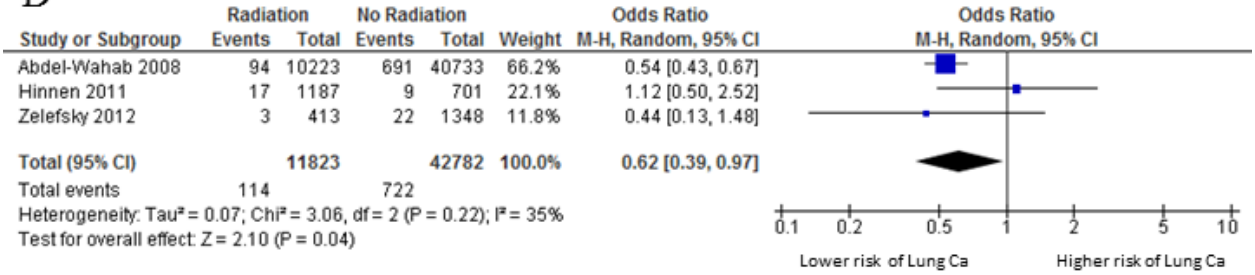
B



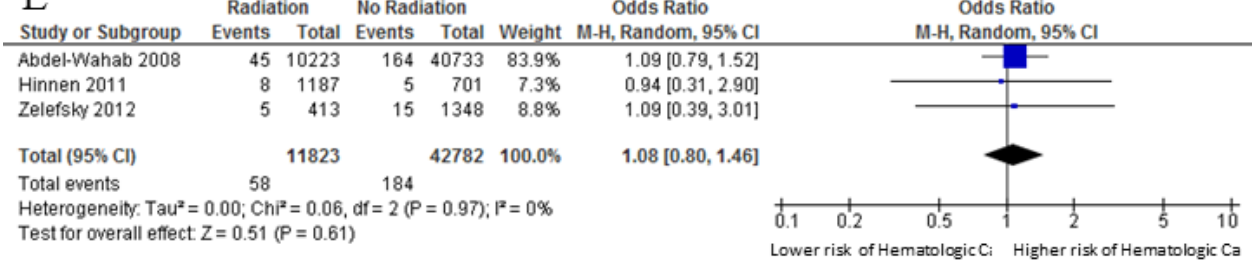
C



D



E



5.5 Discussion

In this comprehensive review and meta-analysis of 21 studies with moderate risk of bias, we identified an association between radiotherapy for prostate cancer and the development of secondary cancers of the bladder, colorectal tract, and rectum, compared to no radiotherapy or surgery. The absolute risks for the development for these cancers were very low. These results were consistent when we pooled multivariable adjusted hazard ratios and performed restriction to studies using five- or ten-year lag periods between treatment and outcome ascertainment. In particular, it is notable in our analysis that ORs for bladder and rectal cancer increased with a longer lag time (OR at 5-year lag vs. 10-year lag: 1.3 vs.1.89 for bladder cancer; 1.68 vs. 2.2 for rectal cancer). It is important to note that the differences in absolute risks between cases and controls were low (Table 5.1). In *post-hoc* analyses, the absolute risk difference for patients treated with radiotherapy compared to non-radiated patients ranged from -0.9 to 1.9 cancers per 100 patients with differences observed based on radiotherapy modality, comparator group, and lag time duration indicating that absolute risk for these secondary cancer is very low.

Given the current understanding that the risk of radiation-related second malignancy increases over time, a progressive increase in OR over time in our study supports a potential association between radiotherapy and the development of a secondary malignancy of the bladder and rectum. We did not find an association between radiotherapy and lung or hematologic cancers. It must be noted that many of the results were obtained from a very small number of studies (varied between 2 and 10 in each analyses) and the absolute risk of secondary malignancy remains low. Variation in the crude incidence of secondary cancers is, at least in part, due to differences in follow-up between the included studies.

There was a trend across all analyses for lower odds ratios or hazard ratios in the pooled analysis resulting from studies using surgically treated patients as the control group rather than those using patients unexposed to radiotherapy. This may reflect a selection bias with lower outcome ascertainment in those patients not treated with a definitive local therapy. Similarly, as patients treated with radiotherapy may experience increased bowel urgency and other rectal-anal symptoms including bleeding as a result of their treatment[7], there is a potential for detection bias for colorectal and rectal cancers.

There was significant between-study heterogeneity for many of our outcomes, likely in large part due to the differences in control groups used and specific types of radiation that was delivered. Some studies used a surgery group[20,413,430,434,435] while others used a "no radiation" group[17,429,440,442] and yet others used a "no radiation, no surgery" group[431]. We sought to diminish the influence of these differences by providing stratified sub-group analysis. Further, within the external beam radiotherapy category, there was heterogeneity in radiotherapy techniques utilized (2-[413,446] and 3-D[413] conformational radiotherapy, external beam radiotherapy without further specification[17,429-431,434,435,442], and intensity modulated radiotherapy[20]. Differences in the length of follow-up may contribute further to the heterogeneity. We further explored the role of study risk of bias in the observed heterogeneity. However, meta-regression failed to demonstrate significant effect of risk of bias on the observed estimates.

To our knowledge, there exists only one other meta-analysis on this subject in addition to non-systematic reviews of the literature[251-254,414]. Our review differs from previous meta-analysis[414] on this topic which included only 4 studies. We identified significantly more studies and even amongst them we had to select studies from SEER cohort. In addition, the majority of their analyses relied on a single publication[437]. Further, they did not assess different

radiotherapy techniques separately. Ours is the first attempt to quantify available knowledge on the subject in the most comprehensive, reproducible and methodologically appropriate fashion.

Secondary primary cancers may arise due to common etiologic factors, including genetic predispositions, or due to treatment-related effects. Further, there may be issues of diagnostic bias when comparisons are made between treated patients and the general population. However, considering that the comparison between patients treated with radiotherapy and those treated with surgery showed similar results to the main analysis, our data suggest that secondary cancers are largely due to treatment-related effects.

Several studies have demonstrated differences in risk of secondary cancer by specific radiation treatment modality. In our analysis, the association between radiotherapy and secondary cancers was much weaker for patients treated with brachytherapy than those treated with external beam radiotherapy in keeping with others' work[447]. Notably, when we assessed crude absolute incidence rates, patients treated with brachytherapy not infrequently had secondary cancer rates lower than the control group – this likely represents selection of younger and healthier patients for brachytherapy. In a comparison of a single centre case series to the SEER population registry, Huang et al. showed no difference in rates of secondary malignancies between those men treated with brachytherapy and radical prostatectomy[438]. Moon et al. found that treatment with brachytherapy was associated with an increased odds of bladder cancer than treatment with surgery, but that this risk did not apply to other tumor sites[440]. It can be speculated that brachytherapy may pose a less risk of radiation-related secondary malignancy than external beam radiotherapy, because it delivers much less integral radiation dose to normal tissues (outside the prostate) than external beam radiotherapy. While we did not examine IMRT separately from other EBRT modalities, it has supplanted conformal EBRT in many

jurisdictions[448]. Only a single study to date by Zelefsky et al. has independently examined the effect of IMRT treatment on secondary cancers and reported no increased risk[20].

Major strengths of our review include comprehensive search, careful selection of studies, critical appraisal of studies, planned subgroup analyses, analyses accounting for time-lag in different methods (dichotomous and time-incorporated hazards) and inclusion of adjusted estimates for hazard ratio. However, we must acknowledge limitations. First, given the number of studies derived from the SEER registry, we had to select a representative study. We used an explicit and transparent method; however, in order to ascertain whether study selection affected the results, we undertook a sensitivity analysis using the Newcastle-Ottawa risk of bias score as the primary determinant and sample size as the second. This resulted in the selection of a different study in 24 out of 83 comparisons (28.9%; Appendix 4). For these 24 comparisons, the change in selected study resulted in an average change of -3.5% in the odds ratio estimate. As a result, the use of a different selection criteria resulted in an average change in odds ratio estimate of -1.02% when all 83 comparisons were considered. Therefore, we consider the study selection was robust. Second, we lacked important information on confounders and comorbidities and other risk factors associated with cancers other than prostate cancer which may be higher in the patients who are treated with radiotherapy. Of particular note is the lack of information on smoking for the ascertainment of lung and bladder cancer risk, and obesity, which may predispose patients to colon [449] and prostate cancer[450]. This may bias the increased risk attributed to radiotherapy. Third, small number of studies in individual subgroup analyses limited power in our conclusions. Finally, studies included had moderate risk of bias and there is an ongoing need for high quality and minimally biased studies.

In view of limited number of studies and limited assessment adjusting for confounders, we identify a significant need for future studies assessing risk of secondary malignancy following prostate cancer treatment with radiotherapy. This can be undertaken either as large prospective cohort studies or multinational prospective registries. Further studies are required before conclusive implication of the association between radiotherapy and secondary malignancy in these patients.

5.6 Conclusion

In conclusion, we identified that radiotherapy is likely associated with increased odds of secondary cancers compared to no radiotherapy or surgery. We identified consistent evidence of an increased risk of bladder, colorectal, and rectal cancers in men treated with radiotherapy. We did not find consistent evidence for an association between radiotherapy and secondary lung and hematologic cancers. Although there was an increase in risk, the absolute rates of these secondary cancers remain very low, particularly compared to other rates of complications associated with prostate cancer treatment. This information may be helpful in the decision making process regarding prostate cancer treatment.

CHAPTER 6: SUMMARY AND DISCUSSION

The screening for and treatment of clinically-localized prostate cancer remain controversial. As in any health care decision, these choices depend on a balance of benefits and risks. Necessary to inform such a decision therefore is a nuanced understanding of benefit and risk. There is level 1 evidence for a survival benefit to prostate cancer screening[163] and the treatment of intermediate and high-risk disease in men with reasonable life expectancy[177]. Further, there is level 2a evidence that surgical therapy confers a survival benefit when compared to radiotherapy[15]. Further nuances of these issues are presented in *Chapter 2: Background* of this dissertation (*Section 2.4.1 Prostate cancer screening*; *Section 2.4.3 Prostate cancer treatment*; *Section 2.5.1.1 Comparative oncologic outcomes following prostate cancer treatment*). There are many, well described risks to both prostate cancer screening and treatment. With respect to prostate cancer screening, risks include patient anxiety, harms of prostate biopsy, and the overtreatment with its incumbent toxicity[170].

As discussed in *Section 2.4.1 Prostate cancer screening*, primary care guidelines in Canada[171] and the United States[170] have highlighted these risks when recommending against PSA screening. While the best characterized and most frequently discussed complications of prostate cancer treatment are urinary incontinence and erectile dysfunction[7], we have recently focused on a more fulsome characterization of the long-term toxicity of surgery and radiotherapy[8-10,207,249]. In order to allow physicians to provide more accurate counselling and patients to make more informed choices, this dissertation has sought to further explore issues which may arising following local, prostate cancer directed therapy among men with clinically-localized prostate cancer.

Issues following prostate cancer treatment

In an era of shared decision making, it is critical that patients' wishes and preferences are paramount in the final selection of prostate cancer treatment. Unfortunately, recent data indicate that patients' final treatment decisions are not significantly associated with their initial treatment preferences, but are rather driven by physician recommendations[451]. The selection of prostate cancer treatments is a quintessential preference sensitive health care decision[452]. It is in weighing the acceptability of various oncological and functional states and their likelihood follow prostate cancer treatment that patients may truly make an educated choice. The first barrier to such an outcome is the appropriate provision of impartial information to patients: previous work has identified that both urologists and radiation oncologists have a "speciality bias" towards the treatment that they themselves administer[453]. Even with appropriate information on the risks and benefits of treatment, patients often have unrealistic expectations of treatment[454]. Further, behavioural, demographic and health factors, such as current sexual and a family history of cancer death, may significant affect patients' perceptions of the tolerability of various health states (such as erectile dysfunction and metastatic disease, respectively)[455]. Therefore, decision-support tools, informed by data such as that presented in this dissertation, may enable patients and physician to select prostate cancer treatments which are most appropriate for the values and priorities of each patient[455].

6.1 Research synopsis

In this dissertation, we employed a variety of epidemiologic techniques in order to explore outcomes of importance for physicians who treat prostate cancer and patients who are diagnosed with prostate cancer. We drew upon a variety of data sources including institutional

datasets with fine granularity, linked population-based administrative databases, and previously published data representing institutional, registry, and population-based work from across the globe.

In the first component of this dissertation, *Chapter 3*, we utilized a matched case-control design in conjunction with bootstrapped automated variable selection in order to identify a number of microRNA which were associated with the development of radiographic metastatic disease following radical prostatectomy for clinically-localized prostate cancer. We identified a panel of five miRNAs (miR200a, miR375, miR376b, miR17, and miR27a) which was significantly associated with prostate cancer metastasis (area under the receiver operating curve 89.5%, 95% CI 79.5-99.5%). Each of these miRNAs have previously been associated with carcinogenesis, though many have not yet been associated with prostate cancer pathogenesis.

In *Chapter 4*, we made use of a rich variety of population-based data that are available in Ontario, Canada through the Institute of Clinical Evaluative Sciences (ICES) to conduct a population-based, propensity-score matched retrospective cohort study among patients treated for clinically-localized prostate cancer. Utilizing Fine and Gray competing risks models, we examined the risk of non-prostate cancer mortality, cardiovascular mortality, and ischemic cardiovascular events associated with primary treatment modality (surgery or radiotherapy) and the use of androgen-deprivation therapy. We identified an increased risk of non-prostate cancer mortality (aHR 1.57, 95% CI 1.35 – 1.83), cardiovascular mortality (aHR 1.74, 95% CI 1.27 – 2.37), and ischemic cardiac events (aHR 1.13, 95% CI 1.03 – 1.24) among patients who received radiotherapy, rather than surgery, as their initial treatment modality. We did not demonstrate an association between androgen deprivation therapy and any of these outcomes.

Finally, in *Chapter 5*, we conducted a systematic review and stratified meta-analysis utilizing random effects models to assess the association between radiotherapy in the treatment of prostate cancer and secondary malignancies. We comprehensively reviewed the published literature and meticulously reviewed all available studies in order to include information from all eligible patients without duplication. As many studies have been published utilizing the Surveillance, Epidemiology, and End-Results (SEER) registry, we scrutinized these reports in order to include those of the highest methodologic quality with the most relevant outcomes. Patients treated with radiotherapy were more likely to be subsequently diagnosed with bladder cancer (OR 1.39, 95% CI 1.12 – 1.71), colorectal cancer (OR 1.68, 95% CI 1.33 – 2.12) and rectal cancer (OR 1.62, 95% CI 1.26 – 2.08) but not hematologic or lung cancers. We conducted a number sensitivity analyses to verify the robustness of these findings. First, we examined the effect of a "lag-period" between the date of prostate cancer treatment and diagnosis of the secondary cancer. These results were consistent across analyses employing a five- or ten-year lag period. Second, we considered only studies which utilized a time-to-event analysis and adjusted for important demographic features. Again, the results were consistent. We conducted further subgroup analyses to explore the effect of varying radiotherapy modalities. While treatment with external beam radiotherapy was consistently associated with an increased risk of secondary malignancies, treatment with brachytherapy was not.

In addition to diversity in the research methodology and study outcome, the studies included in this dissertation span the full spectrum of clinical applicability. The results of the meta-analysis presented in *Chapter 5* have already been advocated to form a key component of the consent process for prostate cancer radiotherapy[456]. In contrast, the panel of miRNA

identified in *Chapter 3* represents an initial discovery requiring validation followed by commercialization prior to clinical impact.

6.2 Implications of the present work

Sir Austin Bradford Hill established nine criteria by which the evidence for a causal association between a putative etiology and an effect could be assessed. These include the strength of association; its reproducibility; its specificity; the temporality; a biologic gradient; plausibility; coherence between epidemiologic and biologic data; analogy; and demonstration through experimentation[457]. We will highlight some of these criteria to assess the implications of this dissertation work on clinical practice and further research endeavours.

6.2.1 Reproducibility

A consistent, reproducible effect demonstrated by different investigators, among different patient populations, in different geographic locations strengthens the likelihood that an observed relationship represents a true effect[457]. Related to the issue of reproducibility is that of generalizability – how well does a study represent the population of patients to which we would seek to apply its conclusions. The recently published American Cancer Society (ACS) Prostate Cancer Survivorship guidelines[458] have emphasized the important of consistent, replicated research in order to inform the development of treatment and follow-up guidelines. Thus, the reproducibility of study conclusions is critical for their clinical applicability.

As with the spectrum of clinical applicability mentioned previous, the work in this dissertation spans the spectrum of epidemiologic consistency. At one extreme, the panel of miRNA described in *Chapter 3* represents a novel discovery. Prior to any clinical utility, these

findings require extensive validation. There is a vast library of studies detailing biomarkers which have demonstrated a remarkable strength of association within small, single institutional cohorts which have failed to show meaningful associations among independent cohorts. In the field of prostate cancer biomarkers, even among those which have undergone significant laboratory investigation and commercialization[129,459-461], there are significant concerns regarding their validity and clinical applicability. The 4Kscore is a panel of 4 kallikreins which is designed to reduce the utilization of biopsy among men who are considered at elevated risk for prostate cancer[459]. Following initial derivation (training) and internal validation, this panel underwent external and prospective validation[129,459-461]. However, the American Centers for Medicare & Medicaid Services (CMS) concluded that 4Kscore testing is "not reasonable and necessary and is [therefore] not covered by Medicare" due to a lack of clear definition of the exposure population, lack of a generalizability of results, and methodologic limitations in the supporting studies[462]. Thus, much work remains to be done prior to clinical utility of the newly described miRNA panel. Currently, *in silico* validation is underway using data derived from The Cancer Genome Atlas project[347] and collaborations are currently underway to undertake external validation at other Canadian institutions. In this setting, the utility of a novel biomarker depends in large part on the generalizability of the cohort in which it was developed. This is, in fact, one of the main criticisms the CMS had in their rejection of the 4Kscore panel[462]. As our miRNA discovery occurred in a dataset derived from a single institution among patients treated by two surgeons, the generalizability of these findings will be critical component of the planned validation.

Further along the spectrum of reproducibility is the finding from *Chapter 4* that initial local treatment with radiotherapy is associated with an increased risk of non-prostate cancer

mortality and cardiovascular mortality. While strictly speaking, this is a novel finding, it is in keeping with a reasonable interpretation of the published literature. First, there is consistent, evidence among observational studies that patients who receive radiotherapy have significantly shorter overall survival than those who undergo surgery[15]. Further, we have recently demonstrated that radiotherapy is associated with an increased risk of coronary artery disease, myocardial infarction and sudden cardiac death[226]. However, further study will be required to identify and measure unknown confounding that could bias these results.

However, the other finding from *Chapter 4*, that androgen deprivation therapy is not associated with non-prostate cancer mortality or cardiovascular mortality, is somewhat less clear. There is a well-established relationship between androgen deprivation therapy and cardiovascular events[260], and we have recently shown that this holds true among patients treated for clinically-localized disease[226]. The difference between the present study and this previous work may arise for a number of reasons. First, the additional risk adjustment which was possible in this cohort may have accounted for residual biases which drove an observed effect in the prior studies. In our recent manuscript, patients receiving androgen deprivation therapy were older and had higher levels of comorbidity as measured by the Charlson score. Given the known limitations of the Charlson score, including within-category heterogeneity, it is plausible that there were significant unmeasured baseline differences that confounded the previously observed relationship which we were able to quantify in this analysis. Potential such confounders include validated diagnosis of hypertension and diabetes, history of myocardial infarction and other cardiovascular risk factors. Additionally, there may be a biologic relationship with cardiovascular events which does not translate to cardiovascular mortality. This hypothesis is supported by previous work based on secondary analyses of randomized trials which has shown

no increase in cardiovascular mortality following androgen deprivation therapy[381]. The present study validates these findings and demonstrates that they apply at the population-level. This is important given the known efficacy-effectiveness gap[463,464] which may result in overly optimistic conclusions due to the strict patient selection and follow-up involved in randomized trials.

With respect to generalizability, the matched cohort study presented in *Chapter 4* was limited to patients over the age of 66 due to the lack of available data on androgen deprivation therapy prescriptions among younger patients. This preferentially includes patients treated with radiotherapy as younger men are more likely to undergo surgery and restricted the number of patients available for matching. Further, as age is an independent risk factor for cardiovascular events[14], these results may not be generalizable to younger men. However, over half of prostate cancer cases are diagnosed in men over 65 years old and these results will be informative to their care. In addition, we limited our analysis to patients with non-metastatic disease. Therefore, these results should not be considered applicable to patient with metastatic disease for whom androgen deprivation therapy has previously been identified as a risk factor for cardiovascular disease[260].

Further, while concerns regarding generalizability typically focus on the representativeness of the study sample, one may be concerned that methodological limitations may limit the applicability of study findings to a wider population. As there is a significant potential for residual confounding in propensity-score matched observational cohort studies, this a concern for the work presented in *Chapter 4*. Currently, the lead author of the ProtecT study is examining how treatment outcomes differ between patients who are willing to be randomized and those who select their treatment modality. This work will lend significant insights into the

potential effects of unmeasurable preferences. Further, additional analyses may be undertaken to assess for the influence of residual confounding including the use of tracer analyses[465]. One such example would be to examine rates of mortality due to chronic obstructive pulmonary disease or pneumonia which ought not to differ based on prostate cancer treatment modality, but may be affected by unmeasured underlying differences in the study cohorts.

Finally, in *Chapter 5*, we explored the association between radiotherapy in the treatment of prostate cancer and secondary malignancies. The very nature of a systematic review and meta-analysis is that the reproducibility of prior publications is assessed and integrated into the analysis and interpretation. In our analysis, we calculated the statistical heterogeneity among studies using I^2 values[428] and used random-effects models to account for clinical heterogeneity. In a quantifiable manner, we demonstrated variable heterogeneity among the secondary cancer tumor site specific comparisons, ranging from 0% to 88%. This analysis drew up on large population- and registry-based cohorts from the United States; British Columbia, Ontario and Quebec, Canada; Israel; Geneva and Zurich, Switzerland as well as single institution series from the United States and the Netherlands. Further, it encompassed nearly two decades of potential inclusion and a variety of different methods of delivering radiotherapy (2-dimensional conformal radiotherapy, 3-dimensional conformal radiotherapy, intensity-modulated radiotherapy, brachytherapy, and brachytherapy with external beam boost). Thus, the conclusions derived from these analyses are likely applicable to most patients undergoing radiotherapy for prostate cancer.

6.2.2 Plausibility and coherence

Biologic plausibility and the coherence between epidemiologic findings and laboratory evidence are intuitively very important to clinicians and researchers. However, as Bradford Hill points out, these are the most prone to the fallibility of our current knowledge[457]: our current lack of knowledge of a biologic mechanism certainly doesn't preclude its presence. For this reason, Bradford Hill noted that "lack of such evidence cannot nullify the epidemiological effect on associations"[457]. However, there are strong biologic correlates to each of the epidemiologic observations described in this dissertation.

Within our research program examining miRNA in prostate cancer, we have used epidemiologic research in order to inform our biologic, mechanistic studies. Prior work assessing miRNA which are associated with biochemical recurrence led to the identification of 5 seemingly important miRNAs[153]. We have subsequently undertaken a number of investigations to understand the biologic function of these miRNA in prostate cancer progression and metastasis including overexpression of these miRNA in prostate cancer cell lines and mouse xenograft models; transcriptome sequencing and Western blotting; and Luciferase assays[95,348]. Thus, rather than relying on biologic mechanisms to support epidemiologic findings, we believe that these epidemiologic findings may allow for more efficient and targeted future biologic research. As a result, in addition to use a prognostic biomarker, these findings offer to potential for the development of novel therapeutics[466]. Anatagomirs are a class of chemically engineered molecules which serve to silence endogenous miRNA function[467]. These antagomirs may be administered intravenously, with specific, efficient and long-lasting effect[467]. As each miRNA has many biologic targets, significant future research is required in order to understand and mitigate unwanted effects of this therapeutic approach[69].

With respect to the observed association between radiotherapy and cardiovascular disease demonstrated in *Chapter 4*, there is a rationale biologic mechanism for this relationship. As outlined in *Section 4.5 Discussion*, prostate-directed radiotherapy may induce a systemic inflammatory response and direct vascular endothelial injury, both of which have established relationships with cardiovascular mortality[298-302,387-390]. Thus, the findings of the present study serve to demonstrate the clinical applicability of previously identified biological risk factors.

Similarly, recent work from the Cancer Genome Project and the International Cancer Genome Consortium (ICGC) has elucidated a number of mutational signatures among radiotherapy-induced secondary cancers[468]. This work demonstrated significant genomic differences in radiotherapy-associated tumors as compared to those which were radiotherapy-naïve, particularly with enrichment of extra small (1-100 base pair) deletions and an increase in balanced inversions[468]. Thus, recent evidence has corroborated the carcinogenic potential of radiotherapy which we epidemiologically demonstrated in *Chapter 5*.

6.2.3 Analogy

The criterion of analogy is most relevant and interesting with respect to *Chapter 3* in which we have documented the discovery of a novel panel of miRNA for prostate cancer prognostication. There are many lessons which can be learned from both prior work in prostate cancer, as well as other malignancies.

As described in *Chapter 2: Background* (*Section 2.3.2.2 Novel molecular-based markers*), there are a number of prostate cancer biomarkers which have recently been commercialized and entered mainstream use. As highlighted both in that section and above in *Section 6.2.1 Reproducibility*, there are significant limitations to the use of these biomarkers

including systematic errors in the design and conduct of the discovery studies[147]. Previous work, including our own[95,153,348], has demonstrated that miRNA expression may allow for prognostication among patients with localized prostate cancer.

Beyond classifying patients who are likely to experience disease recurrence, physicians would like to identify those patients who are likely to benefit from adjuvant therapies. Recently, a multi-institutional collaboration of American investigators developed a genomic expression score called the Post-Operative Radiation Therapy Outcomes Score (PORTOS) in order to identify patients from whom post-operative radiotherapy could decrease the risk of distant metastasis[469]. Future clinical applications will likely rely on a combination of prognostic and predictive markers in order to identify patients who are at risk for an adverse oncologic outcome who can benefit for adjuvant therapies.

The most analogous and informative clinical scenario for prostate cancer researchers is that of breast cancer. As described in *Section 2.3.1 General considerations regarding prognostic factors in oncology*, breast oncologists were historically limited to tumor grade and stage upon which to base treatment decisions and prognostication[101]. Recently, the introduction of genetic tests has revolutionized breast cancer treatment, including targeted provisioning of chemotherapy and endocrine treatments[103]. The use of a 21-gene recurrence score assay has recently been shown to significantly affect the use of chemotherapy[470]. While patients identified at higher risk of recurrence based on the assay were significantly more likely to receive chemotherapy, more notable is the observation that those patients who did not undergoing assay testing were much more likely to receive chemotherapy[470]. Therefore, the use of a recurrence assay may help to reduce the burden of adjuvant therapies on patients with little to benefit while targeting treatment to those with the most to gain.

Issues following prostate cancer treatment

In addition to their potential use of prognostic biomarkers, the newly identified miRNA offer the potential for the development of new therapeutics using antagomirs, as discuss above. While this field is in its nascence and yet to reach clinical applicability, there is ongoing work examining the potential of miRNA inhibition or replacement to treat lymphoma, breast cancer, liver cancer, colorectal cancer, lung cancer, and brain cancer[471]. In oncology, there is a history of such a developmental pipeline. The study of chronic myeloid leukemia (CML) is perhaps the best example. The so-called "Philadelphia chromosome", a reciprocal translocation between the long arms of chromosomes 9 and 22 (t(9:22)(q34;q11)), was the chromosomal abnormality linked to a specific malignancy[472,473]. Subsequent work identified that this chromosomal translocation resulted in the translocation of c-*ABL* (an oncogene normally found on chromosome 9) to a region of chromosome 22 which came to be known as the breakpoint cluster region (*BCR*)[474-476]. This fusion gene product, *BCR-ABL,* was subsequently shown to be sufficient to induce leukemia in animal models[477,478]. Subsequent work elucidated the molecular mechanism of action of *BCR-ABL*. This understanding of the molecular mechanism of leukemia resulted in the identification of a tyrosine-kinase imatinib (Gleevac)[479] which was approved by the United States Food and Drug Administration within 3 years of the start of the first human phase 1 studies[480].

The breast cancer literature provides a similar example. HER-2/*neu* is an oncogenic epidermal growth factor receptor, the overexpression of which has been associated with disease recurrence, metastasis and shortened survival among women with breast cancer[481]. It is present in among 25-30% of women with breast cancer[482]. Further, HER-2/*neu* expression has been associated with resistance to chemotherapy and hormonal therapies[481]. Due to its oncogenic role in the progression of HER-2/*neu*-positive breast cancer, this receptor was a focus of targeted

drug discovery leading to the development of Trastuzumab (Herceptin), a monoclonal antibody which binds to the extracellular portion of Her-2/*neu*. Treatment with trastuzumab, in combination with chemotherapy, significantly prolonged disease-free survival, objective response, and survival among women with HER-2/*neu*-positive breast cancer[483,484].

These analogies offer hope that further research beginning with the identification of novel biomarkers associated with prostate cancer prognosis may culminate in not only clinically relevant tests but novel targeted therapeutics.

Analogy also helps to support the findings presented in *Chapters 4* and *5*. The National Council on Radiation Protection and Measurements (NCRP) considers that cardiovascular disease and secondary cancers "are among the most serious and life-threatening late adverse effects" following cancer treatment and "are due in part to radiotherapy"[485]. Among patients receiving thoracic and mediastinal radiotherapy, an increased risk of cardiovascular disease is well-established[292]. Further, among patients treated with abdominal radiotherapy for testis cancer, radiotherapy was also associated with an increased risk of cardiovascular disease[293]. There are many analogous clinical scenarios relating to the induction of secondary cancers from radiotherapy treatments. While urologists will be most aware of the risk of secondary cancers following radiotherapy for testis cancer[486,487], the data is perhaps even more compelling for Hodgkin lymphoma[488], non-Hodgkin lymphoma[489,490], breast cancer[491], and cervical cancer[492]. These include both sites within the radiotherapy field and those outside.

6.2.4 Summary of clinical implications

Thus, as shown through reproducibility, coherence and analogy, we have demonstrated a key role for the investigation and discovery of novel biomarkers for prostate cancer

prognostication as well as the role of surgery and radiotherapy in long-term toxicity of prostate cancer treatment.

6.3 Methodologic considerations

Methodologic considerations for each chapter have been mentioned previously in the *Discussion* sections of each of these chapters. Here, we will review some of the most pertinent considerations.

First, in *Chapter 2*, our discovery analysis is based on a relatively small sample of 19 pairs of patients (38 patients). As outlined in *Figure 3.1 Cohort derivation*, we initially examined all 32 patients who developed metastasis following radical prostatectomy in our institutional cohort. Of these, a total of 13 were excluded due to an inability to find a suitable control or insufficient sample for analysis. While this small sample size imposed restrictions on our study design, it is worth noting that The Cancer Genome Atlas includes only 8 patients (of approximately 400 with miRNA data) who subsequently developed metastasis. However, we were limited in our ability to account for known prognostic factors during our biomarker selection due to these power considerations.

Secondly, while we used a variable selection strategy (bootstrapping with automated backward selection) which has been previously described[307], its use in this setting is novel. This technique seeks to imitate the concept of the central-limit theorem but sub-sampling the study population in order to derive normally-distributed distributions of predictors. This technique mitigates some of the concerns regarding Type I error associated with other variable selection strategies and identifies models which predict outcomes well. However, it may not perform as well in identifying individual predictors of importance. Further, there are no strict criteria by

which variables are selected for inclusion. We used an frequency of greater than 100 samples (of 1000 bootstrapped repeats) for inclusion.

In *Chapter 4*, we employed propensity-score matching to balance the baseline risks among patients treated with surgery and radiotherapy and then competing risks models to examine the association of primary treatment modality with mortality. The potential limitations of the use of propensity-scores to account for treatment selection bias, as well as the use of instrumental variable analysis in its place, are extensively discussed in *Section 4.5 Discussion*. In short, due to their reliance on observed covariates propensity-score matched analyses have been shown to be more prone to residual confounding than instrumental variable analyses[407]. However, there are significant limitations to the use of instrumental variable analyses in medical research, and prostate cancer research in particular. Most notably, there are no well accepted instruments in this field and the interpretation of the results is not intuitive or informative for most physicians. Thus, while instrumental variable analyses are most helpful for policy making[407,411], propensity-score matched analyses continue to be used for answering specific clinical questions.

Patients diagnosed with prostate cancer are at significant risk of competing risks of death. In fact, in this analysis, non-prostate cancer deaths were the outcome of interest. In our primary analysis, we examined the cumulative incidence of non-prostate cancer mortality while accounting for prostate cancer mortality; in our secondary analysis, we examined cardiovascular mortality while accounting for prostate cancer and non-cardiac non-prostate cancer mortality. Competing risks analyses were employed as one of the assumptions underlying Kaplan Meier survival analysis and traditional Cox proportional hazards models is non-informative censoring. This means that it is assumed that, for all patients who are censored, continued monitoring

would demonstrate a comparable risk of an event of interest as those patients who are not censored[493]. However, this is clearly not true in our study design – patients who die of prostate cancer cannot possibly have non-prostate cancer mortality. Taken to its logical extreme, a therapy which increased the risk of prostate cancer mortality without affecting non-prostate cancer mortality would appear to be associated with a decreased risk of non-prostate cancer mortality in an analysis which failed to account for competing events. We employed sub-distribution hazards using Fine and Gray models[377]. The resulting hazard ratios represent the instantaneous risk of experiencing an event at a given time, conditional on not already having experienced the event. Therefore, patients who die of competing causes are retained in the denominator, in contrast to their removal due to censoring in a standard analysis. In contrast with cause-specific hazard ratios, sub-distribution hazard ratios are directly related to observed differences in cumulative incidence. Further, they are more helpful in quantifying a patient's risk of experience an event[494].

Finally, due to our matched study design, there is inherent clustering within the pairs. One of the foremost assumptions underlying survival analysis is the independence of observations[495]. This is violated in the case of matched data and, as a result, measures of variance may be under-estimated. There are two accepted methods to account for this non-independence: marginal models or conditional models[495,496]. We employed a marginal model approach in which we calculated a population-averaged estimate of the effect of each covariate using a robust variance estimator to account for within-cluster correlations. This approach does not explicitly model the between-cluster variation, unlike a conditional approach.

The validity of meta-analyses, such as that presented in *Chapter 5*, depends on the quality of the evidence synthesized. We employed random effects models in order to account for

the observed clinical and statistical heterogeneity. However, methodologic manipulations are unable to account for any deficiencies in the included studies. As the included studies were all observational in nature, the potential for selection bias or residual confounding remains. The only contemporary randomized controlled trial comparing radiotherapy, surgery and active monitoring among patients with localized prostate, the ProtecT trial[236], accrued 1643 patients. Given the low absolute risk of secondary malignancies, this study cohort is unlikely to be informative on this question.

6.4 Future directions

We are currently undertaking validation of the miRNA panel through multiple collaborating institutions and *in silico* validation with The Cancer Genome Atlas dataset[347]. Further, we are working to assess whether these findings may be translated earlier in the disease process. To this end, we are undertaking correlative studies to assess whether genetic abnormalities in the primary prostate tumor from radical prostatectomy can be identified in serum. This may allow the use of a miRNA prognostic test much earlier in the disease process. This would also allow assessment of risk among men prior to surgical therapy, allowing for more nuanced risk assessment prior to initiating active surveillance.

Recent advances in radiotherapy have sought to diminish the effect of treatment on adjacent tissues in order to decrease the risk of well-characterised complications including bladder and bowel toxicity[497]. It is our hope that a better appreciation of these complications, recognized by the National Council on Radiation Protection and Measurements (NCRP) to be "among the most serious and life-threatening late adverse effects" following cancer treatment, will make further refinements in radiotherapy delivery. Notably, while we found no increased

risk in cardiovascular disease or secondary malignancies among patients who received brachytherapy, the use of this modality has declined[498,499].

6.5 Conclusions

There are many issues which may arise and significantly affect the quality and quantity of life for patients following prostate cancer treatment. A better understanding of these offers physicians the opportunity to optimize the care they provide patients and allows patients to be more fully informed during the consent process. In this dissertation, we have identified a novel panel of miRNA which were associated with metastasis following radical prostatectomy for clinically-localized prostate cancer. Should these results prove robust in validation studies, the use of such a panel offers both the opportunity to tailor adjuvant therapies to patients at highest risk of disease progression and the potential for the development of novel, targeted therapeutics. Further, we have identified a possible association for radiotherapy as a risk factor for non-prostate cancer mortality; cardiovascular mortality; and secondary cancers of the bladder, colorectal tract, and rectum. These findings will require further study to account and identify for further unmeasured confounding.

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APPENDICES

Appendix 4.1: ADT exposure definitions

Therapy	Trade name	Drug Identification Number
Buserelin acetate	Suprefact	01989677 (Suprefact 1mg/mL), 02225166 (Suprefact 1mg), 02225158 (Suprafact 1mg), 02228955 (Suprefact depot 2mo 6.3mg), 02240749 (Suprefact depot 3mo 9.45mg)
Leuprolide acetate	Lupron, Eligard	00727695(Lupron 5mg) 00884502 (Lupron depot 3.75mg), 00836273 (Lupron depot 7.5mg), 02239834 (Lupron depot 11.25mg), 02230248 (Lupron depot 22.5mg), 02239833 (Lupron depot 30mg), 02248239 (Eligard 7.5mg). 02248240 (Eligard 22.5mg) 02248999 (Eligard 30mg). 02268892 (Eligard 45mg)
Goserelin acetate	Zoladex	00857599 (Zoladex 3.6mg), 02049325 (Zoladex 3.6mg), 02225905 (Zoladex 10.8mg)
Triptorelin pamoate	Trelstar	09857199 (Trelstar 3.75mg/mL), 02240000 (Trelstar 3.75mg), 02243856 (Trelstar 11.25mg), 09857200 (Trelstar LA 11.25mg/mL), 02412322 (Trelstar 22.5mg)
Degarelix acetate	Firmagon	02337029 (Firmagon 80mg), 02337037 (Firmagon 120mg)

Appendix 4.2: Ischemic cardiovascular event outcome definitions

Diagnosis	ICD-10 code / OHIP billing code
Myocardial infarction	I21.x, I22.x (and OMID)
Intermediate coronary syndrome	I24.0, I24.8, I24.9
Angina pectoris	I20.x
Coronary atherosclerosis	I25.x
Angiography	G297, Z442, G263
Angioplasty	Z434, G262, G298
Coronary artery bypass grafting	R742, R743

Appendix 4.3: Statin medication exposure definitions

Generic medication name	Drug identification number (trade name)
Atorvastatin	02310899 (Actavis Atorvastatin 10mg), 02310902 (Actavis Atorvastatin 20mg), 02310910 (Actavis Atorvastatin 40mg), 02310929 (Actavis Atorvastatin 80mg), 02295261 (Apotex Atorvastatin 10mg), 02295288 (Apotex Atorvastatin 20mg), 02295296 (Apotex Atorvastatin 40mg), 02295318 (Apotex Atorvastatin 80mg), 02346486 (Pro Doc Atorvastatin 10mg), 02346494 (Pro Doc Atorvastatin 20mg), 02346508 (Pro Doc Atorvastatin 40mg), 02346516 (Pro Doc Atorvastatin 80mg), 02348705 (Sanis Atorvastatin 10mg), 02348713 (Sanis Atorvastatin 20mg), 02348721 (Sanis Atorvastatin 40mg), 02348748 (Sanis Atorvastatin 80mg), 02387891 (Sivem Atorvastatin 10mg)
Fluvastatin	02061562 (Lescol 20mg), 02061570 (Lescol 40mg), 02250527 (Lescol XL 80mg), 02400235 (Sandoz fluvastatin 20mg), 02400243 (Sandoz fluvastatin 40mg), 02299224 (Teva fluvastatin 20mg), 02299232 (Sandoz fluvastatin 40mg)
Lovastatin	02220172 (Apotex Lovastatin 20mg), 02220180 (Apotex Lovastatin 40mg), 02248572 (Cobalt Lovastatin 20mg), 02248573 (Cobalt Lovastatin 40mg), 02247231 (Dominion Lovastatin 20mg), 02247232 (Dominion Lovastatin 40mg), 02353229 (Sanis Lovastatin 20mg), 02353237 (Sanis Lovastatin 40mg), 02243127 (Mylan Lovastatin 20mg), 02243129 (Mylan Lovastatin 40mg), 02246989 (Pharmel Lovastatin 20mg), 02246990 (Pharmel Lovastatin 40mg), 02246013 (Pharmascience Lovastatin 20mg), 02246014 (Pharmascience Lovastatin 40mg), 02312670 (Pro Doc Lovastatin 20mg), 02312689 (Pro Doc Lovastatin 40mg), 02272288 (Labratoire Lovastatin 20mg), 02272296 (Labratoire Lovastatin 40mg), 02247056 (Sandoz Lovastatin 20mg), 02247057 (Sandoz Lovastatin 40mg),

	02246542 (Teva Lovastatin 20mg), 02246543 (Teva Lovastatin 40mg)
Pravastatin	02248182 (Actavis pravastatin 10mg), 02248183 (Actavis pravastatin 20mg), 02248184 (Actavis pravastatin 40mg), 02243506 (Apotex pravastatin 10mg), 02243507 (Apotex pravastatin 20mg), 02243508 (Apotex pravastatin 40mg), 02249723 (Dominion pravastatin 10mg), 02249731 (Dominion pravastatin 20mg), 02249758 (Dominion pravastatin 40mg), 02330954 (Jamp pravastatin 10mg), 02330962 (Jamp pravastatin 20mg), 02330970 (Jamp pravastatin 40mg), 02317451 (Mint pravastatin 10mg), 02317478 (Mint pravastatin 20mg), 02317486 (Mint pravastatin 40mg), 02257092 (Mylan pravastatin 10mg), 02257106 (Mylan pravastatin 20mg), 02257114 (Mylan pravastatin 40mg), 02272415 (Paladin pravastatin 10mg), 02272423 (Paladin pravastatin 20mg), 02272431 (Paladin pravastatin 40mg), 02247655 (Pharmascience pravastatin 10mg), 02247656 (Pharmascience pravastatin 20mg), 02247657 (Pharmascience pravastatin 40mg), 00893749 (Pravachol 10mg)
Simvastatin	02248103 (Avtavis simvastatin 5mg), 02248104 (Avtavis simvastatin 10mg), 02248105 (Avtavis simvastatin 20mg), 02248106 (Avtavis simvastatin 40mg), 02248107 (Avtavis simvastatin 80mg), 02247011 (Apotex simvastatin 5mg), 02247012 (Apotex simvastatin 10mg), 02247013 (Apotex simvastatin 20mg), 02247014 (Apotex simvastatin 40mg), 02247015 (Apotex simvastatin 80mg), 02405148 (Auro simvastatin 5mg), 02405156 (Auro simvastatin 10mg), 02405164 (Auro simvastatin 20mg), 02405172 (Auro simvastatin 40mg), 02405180 (Auro simvastatin 80mg), 02253747 (Dominion simvastatin 5mg), 02253755 (Dominion simvastatin 10mg), 02253763 (Dominion simvastatin 20mg), 02253771 (Dominion simvastatin 40mg),

	02253798 (Dominion simvastatin 80mg), 02281619 (Dominion simvastatin 5mg), 02281627 (Dominion simvastatin 10mg), 02281635 (Dominion simvastatin 20mg), 02281643 (Dominion simvastatin 40mg), 02281651 (Dominion simvastatin 80mg)

Appendix 5.1: Selection of representative SEER study for each outcome, highlighted in bold.

Comparison: Any radiation vs no radiation

	Any lag	5-year lag	10-year lag	Hazard ratio
Bladder cancer	Abdel-Wahab 2008 Abern 2013 Anderson 2013 Berrington 2011 Brenner 2000 Chrouser 2008 **Davis 2014** Huang 2011 Moon 2006 Movsas 1998 Neugut 1997 Nieder 2008 Pawlish 1997 Singh 2010	Berrington 2011 Moon 2006 Pawlish 1997 **Singh 2010**	**Davis 2014** Singh 2010	**Abern 2013** Singh 2010 Huang 2011
Colorectal cancer	Abdel-Wahab 2008 Berrington 2011 Baxter 2005 Brenner 2000 **Davis 2014** Huang 2011 Huo 2009 Kendal 2006 Moon 2006 Movsas 1998 Nieder 2008	**Berrington 2011** Baxter 2005 Moon 2006	**Davis 2014**	Baxter 2005 Huang 2011 **Huo 2009**
Rectal cancer	Abdel-Wahab 2008 Berrington 2011 Baxter 2005 Brenner 2000 **Davis 2014** Huang 2011 Huo 2009 Kendal 2006 Moon 2006 Movsas 1998 Neugut 1997 Nieder 2008	**Berrington 2011** Baxter 2005 Moon 2006	**Davis 2014**	Baxter 2005 Huang 2011 **Huo 2009**
Lung cancer	Abdel-Wahab 2008 Berrington 2011 **Davis 2014** Huang 2011 Moon 2006	**Berrington 2011** Moon 2006	**Davis 2014**	**Huang 2011**

	Movsas 1998			
Hematologic cancer	Abdel-Wahab 2008 Brenner 2000 **Davis 2014** Huang 2011 Moon 2006 Movsas 1998 Neugut 1997	**Moon 2006**	**Davis 2014**	**Huang 2011**

Comparison: Any radiation vs surgery

	Any lag	5-year lag	Hazard ratio
Bladder cancer	**Abern 2013** Brenner 2000 Huang 2011 Nieder 2008		**Abern 2013** Huang 2011
Colorectal cancer	Baxter 2005 Brenner 2000 Huang 2011 Kendal 2006 **Nieder 2008**	**Baxter 2005**	**Baxter 2005** Huang 2011
Rectal cancer	Baxter 2005 Brenner 2000 Huang 2011 Kendal 2006 **Nieder 2008**	**Baxter 2005**	**Baxter 2005** Huang 2011
Lung cancer	**Brenner 2000** Huang 2011		**Huang 2011**
Hematologic cancer	**Brenner 2000** Huang 2011 Neugut 1997		**Huang 2011**

Comparison: EBRT vs no radiation

	Any lag	5-year lag	10-year lag	Hazard ratio
Bladder cancer	Abdel-Wahab 2008 Abern 2013 Anderson 2013 Brenner 2000 Chrouser 2008 **Davis 2014** Huang 2011 Moon 2006 Movsas 1998 Neugut 1997 Nieder 2008 Pawlish 1997 Singh 2010	Moon 2006 Pawlish 1997 **Singh 2010**	**Davis 2014** Singh 2010	Abern 2013 **Singh 2010** Huang 2011
Colorectal cancer	Abdel-Wahab 2008 Baxter 2005 Brenner 2000 **Davis 2014** Huang 2011 Huo 2009 Kendal 2006 Moon 2006 Movsas 1998	Baxter 2005 **Moon 2006**	**Davis 2014**	**Baxter 2005** Huang 2011
Rectal cancer	Abdel-Wahab 2008 Baxter 2005 Brenner 2000 **Davis 2014** Huang 2011 Kendal 2006 Moon 2006 Movsas 1998 Neugut 1997 Nieder 2008	Baxter 2005 **Moon 2006**	**Davis 2014**	**Baxter 2005** Huang 2011
Lung cancer	Abdel-Wahab 2008 Brenner 2000 **Davis 2014** Huang 2011 Moon 2006 Movsas 1998	**Moon 2006**	**Davis 2014**	**Huang 2011**
Hematologic cancer	Abdel-Wahab 2008 Brenner 2000 **Davis 2014** Huang 2011 Moon 2006 Movsas 1998	**Moon 2006**	**Davis 2014**	**Huang 2011**

	Neugut 1997			

Comparison: EBRT vs surgery

	Any lag	5-year lag	Hazard ratio
Bladder cancer	**Abern 2013** Brenner 2000 Huang 2011 Nieder 2008		**Huang 2011**
Colorectal cancer	Baxter 2005 Brenner 2000 Huang 2011 Kendal 2006 **Nieder 2008**	**Baxter 2005**	**Baxter 2005** Huang 2011
Rectal cancer	Baxter 2005 Brenner 2000 Huang 2011 Kendal 2006 **Nieder 2008**	**Baxter 2005**	**Baxter 2005** Huang 2011
Lung cancer	**Brenner 2000** Huang 2011		**Huang 2011**
Hematologic cancer	**Brenner 2000** Huang 2011 Neugut 1997		**Huang 2011**

Comparison: Brachytherapy vs no radiation

	Any lag	5-year lag	Hazard ratio
Bladder cancer	Abdel-Wahab 2008 **Abern 2013** Moon 2006 Nieder 2008	**Moon 2006**	**Abern 2013** Huang 2011
Colorectal cancer	Abdel-Wahab 2008 Moon 2006 **Nieder 2008**	**Moon 2006**	
Rectal cancer	Abdel-Wahab 2008 Moon 2006 **Nieder 2008**	**Moon 2006**	
Lung cancer	**Abdel-Wahab 2008** Moon 2006	**Moon 2006**	**Huang 2011**
Hematologic cancer	**Abdel-Wahab 2008** Moon 2006 Neugut 1997	**Moon 2006**	**Huang 2011**

Comparison: Brachytherapy vs surgery

	Any lag	5-year lag	Hazard ratio
Bladder cancer	**Abern 2013** Nieder 2008		**Abern 2013** Huang 2011
Colorectal cancer	**Nieder 2008**		
Rectal cancer	**Nieder 2008**		
Lung cancer			**Huang 2011**
Hematologic cancer			**Huang 2011**

Appendix 5.2: Literature search strategy

The searches were run using the OvidSP search platform in the following databases: MEDLINE and EMBASE, to include articles indexed as of April 6, 2015. All references were saved in an EndNote library used to identify the duplicates. The search strategy retrieved a total of **3,048** references. There were **470** duplicates. The remaining **2,578** remaining unique references from are included for review against the inclusion criteria.

The following tables record the search strategies and terms used in each of the databases. The search strategy is limited to the prognosis search filter which includes the cohort studies design. The search strategy includes all age groups, languages and publication years contained in each database.

MEDLINE:

The search strategy for OvidSP MEDLINE (<1946 to March Week 5 2015>) retrieved 780 references of which **760** were unique and not duplicated in our other searches. I used a combination of MeSH and free text terms for

Set	History	Results	Comments
1	prostatic neoplasms/ or prostatic neoplasms, castration-resistant/	95990	Prostate Cancer Subject Terms
2	((prostate* or prostatic*) adj5 (neoplas* or cancer* or oncolog* or tumour* or tumor* or adenocarcinoma* or malignan*)).au,ti,ab	88071	Prostate Cancer text Terms
3	(Adenocarcinoma/ or genital neoplasms, male/) and (Prostate/ or prostat*.au,ti,ab.)	12161	Previous indexing and histologic type subject and text Terms
4	Or/1-3	111812	Prostate cancer search results
5	Prostate/ or prostat*.au,ti,ab.	148176	Prostate subject and text terms
6	(((multicentric* or multifocal or second* or (multiple adj2 primary)) adj2 (neoplas* or cancer* or oncolog* or tumour* or tumor* or	21232	Secondary malignancy text terms

	adenocarcinoma* or malignan*)) or ((protean or proteus) adj2 syndrome*)).au,ti,ab.		
7	5 and 6	1041	Secondary malignancy text results
8	neoplasms, multiple primary/ or hamartoma syndrome, multiple/ or proteus syndrome/ or multiple endocrine neoplasia/ or multiple endocrine neoplasia type 1/ or multiple endocrine neoplasia type 2a/ or multiple endocrine neoplasia type 2b/ or tuberous sclerosis/ or neoplasms, radiation-induced/ or leukemia, radiation-induced/ or neoplasms, second primary/	58865	Secondary neoplasms subject terms
9	4 and 8	1183	Secondary malignancy subject term results
10	**7 or 9**	**1936**	**Base Clinical Set - Secondary neoplasms results - all forms of therapy**
11	radiation dosage/ or dose-response relationship, radiation/ or radiotherapy/ or chemoradiotherapy/ or chemoradiotherapy, adjuvant/ or radiotherapy, adjuvant/ or radiotherapy, computer-assisted/ or radiotherapy, conformal/ or radiotherapy, intensity-modulated/ or radiotherapy dosage/ or dose fractionation/ or exp radiotherapy, high-energy/ or radiotherapy, image-guided/ or rt.fs.	262780	Radiotherapy Subject or SubheadingTerms
12	**4 and (6 or 8) and 11**	**325**	**Base Clinical Set - Secondary neoplasms with radiation therapy results**
13	**10 or 12**	**1938**	**Final results - radiotherapy and secondary prostatic neoplasms**
14	cohort studies/ or longitudinal studies/ or follow-up studies/ or prospective studies/ (or case-control studies/ or retrospective studies/ or observational study.pt. or observational study as topic/ or (observational adj2 stud*).au,ti,ab. or registries/ or seer program/ or (cancer adj2 (registry or registries)).au,ti,ab. or (seer adj2 program*).au,ti,ab. Or prognosis/ or disease-free survival/ or treatment outcome/ or treatment failure/ or medical futility/ or pregnancy outcome/ or disease progression/ or morbidity/ or incidence/ or prevalence/ or mortality/ or "cause of death"/ or	2754837	MEDLINE Prognosis Sensitive Filter

	child mortality/ or fatal outcome/ or fetal mortality/ or hospital mortality/ or infant mortality/ or maternal mortality/ or perinatal mortality/ or survival rate/ or survival analysis/ or disease-free survival/ or treatment outcome/ or "early termination of clinical trials"/ or treatment failure/ or watchful waiting/ natural history.mp.		
15	**13 and 14**	**780**	FINAL Review results limited to prognosis filter terms

EMBASE

The search strategy for OvidSP Embase Classic+Embase <1947 to 2015 Week 14> retrieved 2268 references of which **1818** were unique and not duplicated in our other searches. I used a combination of MeSH and free text terms for

Set	History	Results	Comments
1	prostate cancer/ or prostate tumor/ or castration resistant prostate cancer/ or prostate adenocarcinoma/ or prostate carcinoma/ or prostatic intraepithelial neoplasia/	167488	Prostate Cancer Subject Terms
2	((prostate* or prostatic*) adj5 (neoplas* or cancer* or oncolog* or tumour* or tumor* or adenocarcinoma* or malignan*)).au,ti,ab.	135024	Prostate Cancer text Terms
3	(adenocarcinoma/ or male genital tract cancer/ or male genital tract tumor/) and (Prostate/ or prostate epithelium/ or prostate fluid/ or prostate ventral lobe/ or prostat*.au,ti,ab.)	5103	Previous indexing and histologic type subject and text Terms
4	or/1-3/	183865	Prostate cancer search results
5	Prostate/ or prostate epithelium/ or prostate fluid/ or prostate ventral lobe/ or prostat*.au,ti,ab.	226010	Prostate subject and text terms
6	(((multicentric* or multifocal or second* or (multiple adj2 primary)) adj2 (neoplas* or cancer* or oncolog* or tumour* or tumor* or adenocarcinoma* or malignan*)) or ((protean or proteus) adj2 syndrome*)).au,ti,ab.	33418	Secondary malignancy text terms
7	5 and 6	1865	Secondary malignancy text results
8	cancer infiltration/ or multiple cancer/ or primary tumor/ or second cancer/ or congenital tumor/ or congenital cancer/ or (multiple adj2 hamartoma).au,ti,ab. or multiple endocrine neoplasia/ or tuberous sclerosis/ or radiation induced neoplasm/ or radiation mutagenesis/ or	72663	Secondary neoplasms subject terms

	(radiation adj2 induc* adj2 (leukem* or leukae*)).au,ti,ab.		
9	4 and 8	2826	Secondary malignancy subject term results
10	7 or 9	4215	**Base Clinical Set - Secondary neoplasms results - all forms of therapy**
11	radiological parameters/ or dose response/ or dose kidney function relation/ or dose liver function relation/ or dose time effect relation/ or hormesis/ or radiation depth dose/ or radiation dose/ or radiation dose distribution/ or radiotherapy/ or beam therapy/ or chemoradiotherapy/ or adjuvant chemoradiotherapy/ or cobalt therapy/ or cobalt teletherapy/ or computer assisted radiotherapy/ or external beam radiotherapy/ or fast electron therapy/ or fast neutron therapy/ or image guided radiotherapy/ or intensity modulated radiation therapy/ or intraoperative radiotherapy/ or radiation depth dose/ or radiation dose/ or radiation dose fractionation/ or radiation dose reduction/ or radiation response/ or rt.fs.	850641	Radiotherapy Subject or Subheading Terms
12	4 and (6 or 8) and 11	867	**Base Clinical Set - Secondary neoplasms with radiation therapy results**
13	10 or 12	4236	**Final results - radiotherapy and secondary prostatic neoplasms**
14	cohort analysis/ or longitudinal study/ or prospective study/ or follow up/ or case control study/ or hospital based case control study/ or population based case control study/ or retrospective study/ or observational study/ or (observational adj2 stud*).au,ti,ab. or cancer registry/ or disease registry/ or register/ or (seer adj2 program*).au,ti,ab. or cancer recurrence/ or cancer regression/ or cancer relapse/ or disease duration/ or disease exacerbation/ or prognosis/ or recurrent disease/ or reinfection/ or relapse/ or remission/ or tumor recurrence/ or tumor regression/ or survival/ or cancer survival/ or disease free survival/ or overall survival/ or survival rate/ or survival time/ or incidence/ or	4329417	MEDLINE Prognosis Sensitive Filter

	cancer incidence/ or familial incidence/ or morbidity/ or maternal morbidity/ or perinatal morbidity/ or newborn morbidity/ or mortality/ or cancer mortality/ or childhood mortality/ or embryo mortality/ or fetus mortality/ or infant mortality/ or maternal mortality/ or prenatal mortality/ or surgical mortality/ or perinatal mortality/ or newborn mortality/ or death/ or "cause of death"/ or dying/ or heart death/ or sudden death/ or child death/ or newborn death/ or prevalence/ or treatment outcome/ or disease free interval/ or treatment failure/ or drug treatment failure/ or death/ or "cause of death"/ or dying/ or heart death/ or sudden death/ or child death/ or newborn death/		
15	13 and 14	2268	FINAL Review results limited to prognosis filter terms

Appendix 5.3: Studies included for each outcome.

Comparison: Any radiation vs no radiation

	Any lag	5-year lag	10-year lag	Hazard ratio
Bladder cancer	Bhojani 2010 Boorjian 2007 Davis 2014 Hinnen 2011 Nam 2014 Pickles 2002 Singh 2008 Van Hemelrijck 2014 Zelefsky 2012b	Bhojani 2010 Nam 2014 Singh 2010	Bhojani 2010 Davis 2014	Abern 2013 Bhojani 2010 Boorjian 2007 Hinnen 2011
Colorectal cancer	Bhojani 2010 Boorjian 2007 Davis 2014 Hinnen 2011 Margel 2011 Nam 2014 Pickles 2002 Rapiti 2008 Van Hemelrijck 2014 Zelefsky 2012b	Berrington 2011 Bhojani 2010 Nam 2014 Rapiti 2008	Bhojani 2010 Davis 2014	Baxter 2005 Bhojani 2010 Hinnen 2011
Rectal cancer	Bhojani 2010 Boorjian 2007 Davis 2014 Hinnen 2011 Margel 2011 Rapiti 2008 Van Hemelrijck 2014 Zelefsky 2012b	Berrington 2011 Bhojani 2010 Rapiti 2008	Bhojani 2010 Davis 2014	Baxter 2005 Bhojani 2010 Hinnen 2011
Lung cancer	Bhojani 2010 Davis 2014 Hinnen 2011 Nam 2014 Pickles 2002 Van Hemelrijck 2014 Zelefsky 2012b	Berrington 2011 Bhojani 2010 Nam 2014	Bhojani 2010 Davis 2014	Bhojani 2010 Huang 2011
Hematologic cancer	Davis 2014 Hinnen 2011 Nam 2014 Pickles 2002 Van Hemelrijck 2014 Zelefsky 2012b	Moon 2006 Nam 2014	Davis 2014	Huang 2011

Comparison: Any radiation vs surgery

	Any lag	5-year lag	10-year lag	Hazard ratio
Bladder cancer	Abern 2013 Bhojani 2010 Boorjian 2007 Nam 2014 Van Hemelrijck 2014 Zelefsky 2012b	Bhojani 2010 Nam 2014	Bhojani 2010	Abern 2013 Bhojani 2010
Colorectal cancer	Bhojani 2010 Boorjian 2007 Margel 2011 Nam 2014 Nieder 2008 Van Hemelrijck 2014 Zelefsky 2012b	Baxter 2005 Bhojani 2010 Nam 2014	Bhojani 2010	Baxter 2005 Bhojani 2010
Rectal cancer	Bhojani 2010 Boorjian 2007 Margel 2011 Nieder 2008 Van Hemelrijck 2014 Zelefsky 2012b	Baxter 2005 Bhojani 2010	Bhojani 2010	Baxter 2005 Bhojani 2010
Lung cancer	Bhojani 2010 Brenner 2000 Nam 2014 Van Hemelrijck 2014 Zelefsky 2012b	Nam 2014 Bhojani 2010	Bhojani 2010	Huang 2011
Hematologic cancer	Brenner 2000 Nam 2014 Van Hemelrijck 2014 Zelefsky 2012b	Nam 2014		Huang 2011

Comparison: EBRT vs no radiation

	Any lag	5-year lag	10-year lag	Hazard ratio
Bladder cancer	Bhojani 2010 Davis 2014 Nam 2014 Pickles 2002 Van Hemelrijck 2014 Zelefsky 2012b	Bhojani 2010 Nam 2014 Singh 2010	Bhojani 2010 Davis 2014	Bhojani 2010 Singh 2010
Colorectal cancer	Bhojani 2010 Davis 2014 Margel 2011 Nam 2014 Pickles 2002 Rapiti 2008 Van Hemelrijck 2014 Zelefsky 2012b	Bhojani 2010 Nam 2014 Moon 2006 Rapiti 2008	Bhojani 2010 Davis 2014	Baxter 2005 Bhojani 2010
Rectal cancer	Bhojani 2010 Davis 2014 Margel 2011 Rapiti 2008 Van Hemelrijck 2014 Zelefsky 2012b	Bhojani 2010 Moon 2006 Rapiti 2008	Bhojani 2010 Davis 2014	Baxter 2005 Bhojani 2010
Lung cancer	Bhojani 2010 Davis 2014 Nam 2014 Pickles 2002 Van Hemelrijck 2014 Zelefsky 2012b	Bhojani 2010 Moon 2006 Nam 2014	Bhojani 2010 Davis 2014	Bhojani 2010 Huang 2011
Hematologic cancer	Davis 2014 Nam 2014 Pickles 2002 Van Hemelrijck 2014 Zelefsky 2012b	Moon 2006 Nam 2014	Davis 2014	Huang 2011

Comparison: EBRT vs surgery

	Any lag	5-year lag	10-year lag	Hazard ratio
Bladder cancer	Abern 2013 Bhojani 2010 Nam 2014 Van Hemelrijck 2014 Zelefsky 2012b	Bhojani 2010 Nam 2014	Bhojani 2010	Huang 2011
Colorectal cancer	Bhojani 2010 Margel 2011 Nam 2014 Nieder 2008 Van Hemelrijck 2014 Zelefsky 2012b	Baxter 2005 Bhojani 2010 Nam 2014	Bhojani 2010	Baxter 2005
Rectal cancer	Bhojani 2010 Margel 2011 Nieder 2008 Van Hemelrijck 2014 Zelefsky 2012b	Baxter 2005 Bhojani 2010	Bhojani 2010	Baxter 2005
Lung cancer	Bhojani 2010 Brenner 2000 Nam 2014 Van Hemelrijck 2014 Zelefsky 2012b	Nam 2014 Bhojani 2010	Bhojani 2010	Huang 2011
Hematologic cancer	Brenner 2000 Nam 2014 Van Hemelrijck 2014 Zelefsky 2012b	Nam 2014		Huang 2011

Comparison: Brachytherapy vs no radiation

	Any lag	5-year lag	Hazard ratio
Bladder cancer	Abern 2013 Hinnen 2011 Zelefsky 2012b	Moon 2006	Abern 2013 Hinnen 2011
Colorectal cancer	Hinnen 2011 Nieder 2008 Zelefsky 2012b	Moon 2006	Hinnen 2011
Rectal cancer	Hinnen 2011 Nieder 2008 Zelefsky 2012b	Moon 2006	
Lung cancer	Abdel-Wahab 2008 Hinnen 2011 Zelefsky 2012b	Moon 2006	Huang 2011
Hematologic cancer	Abdel-Wahab 2008 Hinnen 2011 Zelefsky 2012b	Moon 2006	Huang 2011

Comparison: Brachytherapy vs surgery

	Any lag	5-year lag	Hazard ratio
Bladder cancer	Abern 2013 Zelefsky 2012b		Abern 2013
Colorectal cancer	Nieder 2008 Zelefsky 2012b		
Rectal cancer	Nieder 2008 Zelefsky 2012b		
Lung cancer	Zelefsky 2012b		Huang 2011
Hematologic cancer	Zelefsky 2012b		Huang 2011