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Authors: Sapna Lunj[1], Tim Andrew Davies Smith[2], Kimberley Jayne Reeves[3,] Fred Currel[4], Jamie Honeychurch[3,5], Peter Hoskin[3] & Ananya Choudhury[1,6]

[1]Division of Cancer Sciences, University of Manchester, Oglesby Cancer Research Building, Manchester, UK.

[2]Nuclear Futures Institute, School of Computer Science and Engineering, Bangor University, Bangor, UK.

[3]Division of Cancer Sciences, University of Manchester, Paterson Building, Manchester, UK
[4]The Dalton Cumbria Facility and the Department of Chemistry, University of Manchester, UK.

[5]Lydia Becker Institute of Immunology and Inflammation, University of Manchester, UK.
[6]The Christie NHS Foundation Trust, Manchester, UK.

Corresponding author: Sapna Lunj (sapna.lunj@manchester.ac.uk).

External beam radiotherapy (EBRT) is used for radical treatment of organ-confined prostate cancer and to treat lesions in metastatic disease whereas molecular radiotherapy with labelled prostate-specific membrane antigen ligands and radium-223 (^{223}Ra) are indicated for metastatic prostate cancer and have demonstrated substantial improvements in symptom control and overall survival (OS) compared with standard-of-care treatment. Prostate cancer is considered an immunologically cold tumour, so limited studies investigating the treatment-induced effects on the immune response have been completed. However, emerging data supports that radiotherapy induces an immune response in prostate cancer, but whether the response is an anti-tumour or pro-tumour response is dependent on the radiotherapy regime and is also cell-line dependent. In vitro data demonstrates that single-dose radiotherapy regimes induce a greater immune suppressive profile than fractionated regimes; even less is known about the immune response induced by molecular radiotherapy agents but evidence suggests that these agents might induce an immune-suppressive systemic immune response, indicated by increased expression of inhibitory checkpoint molecules such as programmed-death ligand 1 and 2 and that these changes could be associated with clinical response. Different radiotherapy modalities can induce distinct immune profiles, which can either activate or suppress immune-mediated tumour killing and the current pre-clinical models used for prostate cancer research are not yet optimal for studying the complexity of the radiotherapy induced immune response.

[H1] Introduction

Prostate cancer is the most common cancer in men in 112 countries and in 2020 it accounted for an estimated 1.4 million new cases and 359,000 deaths globally.[1,2] In the UK, ~15–30% of men diagnosed with prostate cancer will present with bone metastasises. [2,3] More than 40% of men with advanced prostate cancer will experience disease progression to metastatic castration-resistant prostate cancer (mCRPC), which is a lethal form of prostate cancer that has limited treatment options.[3,4]

Radiotherapy has an important role in the treatment of prostate cancer at all stages including those with metastatic disease.[5,6] External beam radiotherapy (EBRT) delivers high energy x-ray beams to the tumour to induce cell killing.[7] EBRT is used to treat the primary prostate tumour in men who have locally advanced (invade into the capsule that surrounds the prostate) or advanced disease (spread to lymph nodes or bones in the pelvis and spine) in combination with hormone therapy or chemotherapy.[8,9] Men with prostate cancer can also receive treatment with brachytherapy. Brachytherapy delivers either high-dose rate (HDR) or low-dose rate (LDR) γ rays and is usually administered to organ-confined (localized) prostate cancer.[10,11]

Molecular radiotherapy is used to treat metastatic prostate cancer and is delivered via intravenous injection. Radionuclides used in molecular radiotherapy include a range of α and β emitting radionuclides.[12] These radionuclides are targeted to prostate cancer cells by conjugation with prostate-specific membrane antigen (PSMA), such as ^{177}lutetium(Lu)-PSMA) [13] or are selectively taken up by bone (for example radium-223 (^{223}Ra) [14] adjacent to prostate cancer cell invasion.

The radium isotope, ^{223}Ra, is a calcium mimetic, has natural affinity to bone and is preferentially taken up in areas of osteoblast activity including adjacent to bony metastases.[14] ^{223}Ra emits high-energy α particles with a range of up to ~100µm. When incorporated in bone adjacent to invasive prostate cancer, α particles emitted by ^{223}Ra induce lethal double-stranded deoxyribonucleic acid (DNA) breaks in tumour. ^{223}Ra treatment prolongs OS in men with mCRPC (14.9 months versus 11.3 months) as well as improving symptomatic measures such as time to first symptomatic skeletal event and bone pain, which can affect the health-related quality of life of these men. [2,14,15–17] More recently, early clinical trial data suggest that ^{223}Ra might have efficacy in earlier disease settings. [18,19] Radiolabelled PSMA ligands such as ^{177}Lu-PSMA761 target PSMA present on tumour cells.[13] PSMA is overexpressed 100–1000 times higher in prostate cancer cells than in healthy prostate epithelium. [23,20] The medium-energy β particles emitted by ^{177}Lu have a maximum range of 2000µm.[3] Treatment of men with mCRPC with ^{177}Lu-PSMA671 prolongs OS (15.3 months versus 11.3 months) and improves symptomatic measures similar to ^{223}Ra.[20,21] The unique decay scheme of radionuclides might have an important role in the resulting efficacy, as the daughter emissions from the radionuclides might differ from the parent

radionuclide in terms of emission type and energy. The differences in physical properties of the parent and daughter emissions are likely to affect dosimetry and hence DNA damage and immune responses. (**Figure 1**).

The clinical success of ^{223}Ra and ^{177}Lu has paved the way for the development of other radionuclides for treating mCRPC. Currently, two additional radionuclides, ^{225}Actinium (^{225}Ac) and ^{227}Thorium (^{227}Th) are being investigated for use in prostate cancer, although based on clinical data, ^{227}Th development in prostate cancer is likely to cease and be replaced with two additional radionuclides, ^{212}Lead and ^{211}Astatine.[22] ^{225}Ac and ^{227}Th are being developed as a construct conjugated to PSMA or human epidermal growth factor receptor 2 (HER2).[22] ^{225}Ac and ^{227}Th are α-emitting radionuclides and, therefore, fall in the same emission class as ^{223}Ra, but because ^{225}Ac and ^{227}Th have different decay schemes and energy depositions from ^{223}Ra, different efficacies in inducing cell kill and immune responses might result (**Figure 1**). Comparative efficacy and toxic effect studies for ^{223}Ra, ^{177}Lu, ^{225}Ac and ^{227}Th are needed to inform treatment decisions in terms of deciding which radionuclide to use for treating prostate cancer. In addition, understanding whether differences exist between the immune response induced by the different radionuclides would help provide improve personalization of the approach to treating patients.

The primary mechanism of action for the different radiotherapy modalities used for treating prostate cancer is DNA damage to tumour cells leading to cell death.[23, 24] However, the cellular composition of tumours is complex as tumours contain a variety of cell types including immune cells, endothelial cells and fibroblasts .[25-28] How radiotherapy modulates these cell types and whether this potential modulation can influence the efficacy of radiotherapy is not fully established, but mounting evidence from pre-clinical and clinical studies demonstrate that radiotherapy can alter the presence or function of immune cells within the tumour microenvironment (TME). [29–40] Thus, understanding the immune profile of prostate cancer tumours pre-treatment could also be important in understanding the mechanism by which radiotherapy modulates the immune response and whether the pre-treatment immune profile might affect the efficacy of radiotherapy.

The immune profile of tumours is highly variable, but can be broadly defined as immunologically hot or cold depending on the extent of infiltration by lymphocytes and other immune effector cells.[41,42] Immunologically hot tumours are characterized by large numbers of infiltrating CD8-positive T lymphocytes (T cells) and the presence of pro-inflammatory cytokines such as interferon γ, and often display a high tumour mutational burden (TMB). By contrast, immunologically cold tumours are characterized by the presence of regulatory T cells (Tregs) and suppressive myeloid cells (monocytes and granulocytes), low CD8-positive T cell infiltration, and are frequently less mutated than immunologically hot tumours.[43,44] Tumours from men with prostate cancer are considered immunologically cold despite having a high TMB owing to low levels of infiltrating CD8-positive T cells and a high presence of suppressive immune cells (**Figure 2**).[28],[45,46]

This Review will focus on what is known about the interactions between conventional radiotherapy, such as EBRT and brachytherapy, and the immune system. We discuss how

that knowledge can be applied to investigate gaps regarding the immune interactions of molecular radiotherapies, specifically ^{223}Ra and ^{177}Lu and those that are being developed for prostate cancer (such as ^{225}Ac and ^{227}Th).

[H1] Radiotherapy and the immune system

To understand the interaction between the immune system and radiotherapy, and how immune effects could enhance radiotherapy efficacy, understanding the components involved in the immune response is important. The immune response can be divided into two main sub-types; innate and adaptive, and the immune response to radiotherapy involves both of arms of the immune system. [47–50] However, the specificity and cellular components involved in the two responses differ. The innate immune response involves macrophages, dendritic cells and natural killer (NK) cells and displays a rapid and broad response that lacks immune memory.[51] By contrast, the adaptive immune response involves T cells and B lymphocytes (B cells) and displays a slow antigen-specific response that results in immune memory to previously encountered antigens.[24,52]

The radiotherapy–immune system interaction is complex. Radiotherapy can affect immune function but the pre-treatment immune profile is also important for sustaining the effects of radiotherapy, especially when considering combining radiotherapy with immune modulatory agents.[29–40, 53] The immune profile can affect the initial tumour response to radiotherapy as well as induce adaptive resistance to radiotherapy, which is largely linked to the ability of radiotherapy to activate or suppress the anti-tumour immune response.[54–58] (**Figure 3**).

Immune activation following radiotherapy involves the cancer immunity cycle — a multi-step process that requires interaction of the innate and the adaptive immune response and defines the processes required to elicit an immune response to cancer.[24, 49, 58] A major driver of radiotherapy-induced immune activation and the cancer immunity cycle is the induction of immunogenic cell death (ICD).[33, 54, 56–58] During ICD, radiotherapy-induced damage leads to the expression or release of damage-associated molecular patterns (DAMPs) such as high mobility group box-1 protein (HMGB1), adenosine triphosphate (ATP) and calreticulin (CALR) from or on dying tumour cells.[48, 50, 59–60] Release of HMGB1 and ATP and expression of CALR can increase the recruitment and maturation of dendritic cells, as well as enhance antigen uptake, processing and presentation following phagocytic uptake of dying tumour cells.[33, 53, 58, 61-62] Radiotherapy can also induce phenotypic changes such as upregulation of major histocompatibility complex 1 (MHC1), intracellular adhesion molecule (ICAM) and FAS that enhance immune surveillance. [53,58,63–67] In addition, the presence of cytosolic DNA occurring as a result of radiotherapy-induced cell damage can activate the cyclic GMP-AMP synthase (cGAS)– stimulator of interferon genes (STING) pathway, which stimulates the release of inflammatory cytokines including interleukin (IL) 1 β, tumour necrosis factor (TNF) and type 1 and 2 interferons.[68] Activation of cGAS–STING also forms part of the immune response to radiotherapy by promoting dendritic cell activation [33,53,69] Activation of this innate immune response orchestrates the adaptive immune response, with cross-presentation of tumour antigen by dendritic cells leading to priming and activation of tumour-antigen specific naive T cells in the presence of co-stimulation. Activated T cells then clonally expand and traffic out of the lymph nodes. These T cells can then infiltrate into the tumour where they induce antigen specific T cell-mediated tumour cell death, which might enhance the efficacy of radiotherapy.[47]

In contrast to stimulating anti-cancer immune responses, localized radiotherapy can also induce immunosuppressive effects. Radiotherapy-induced expression of immune-modulatory cytokines including IL4, IL6, IL10, and IL33, inactivates NK cells; promotes recruitment of a variety of immune suppressive myeloid cells and Tregs; and polarizes macrophages and neutrophils towards a pro-tumour phenotype.[70–72] Pre-clinical data suggest that radiotherapy leads to up-regulation of the expression of macrophage colony-stimulating factor (MCSF), which promotes macrophages to shift towards a pro-tumour phenotype.[73] These tumour-supporting macrophages secrete growth factors and anti-inflammatory cytokines such as transforming growth factor β (TGFβ) and IL10 that suppress the immune system by promoting the expansion of Tregs and inducing T cell and dendritic cell dysfunction.[74–76] In addition, radiotherapy can activate the C-C chemokine receptor type 2 (CCR2) pathway, which increases the presence of immune-suppressive myeloid cells via the STING pathway that can lead to resistance to radiotherapy.[75–76] Radiotherapy has also been shown to transiently decrease the sensitivity of tumour cells to perforin-mediated killing, suggesting that tumours might become resistant to NK and T cell lysis following radiotherapy. [38] Furthermore, radiotherapy can induce upregulation of expression of checkpoint molecules within the TME.[77–79] Inhibitory checkpoint molecules (such as programmed death-ligand 1 [PDL1], T-cell immunoglobulin and mucin-domain containing-3

[TIM3], and lymphocyte-activation gene 3 [LAG3]) suppress the immune response by inducing immune cell exhaustion and anergy. [77-79] Exhausted immune cells have a reduced proliferative and survival capacity and have dampened expression of interferon γ (IFNγ), TNF and granzymes. [77-79] Interest in understanding the relationship between soluble checkpoint molecule signalling such as soluble PD-L1 and response to radiotherapy is increasing. Emerging data from patients with either primary or recurrent glioma showed that radiotherapy increased the plasma concentration of soluble PD-L1 compared with baseline.[80] Patients with decreased plasma soluble PD-L1 post radiotherapy displayed a worse median OS compared with patients with a radiotherapy-induced increase in soluble PD-L1, but the mechanistic reason for this difference in soluble PD-L1 was not further explored. These data suggest that soluble checkpoint molecules function to counteract their membrane form and that monitoring radiotherapy-induced changes in soluble checkpoint molecules could provide much needed biomarkers.

Radiotherapy clearly modulates the immune response both within the TME and systemically. However, to add to the complexity, pre-clinical data demonstrates that different radiotherapy fraction sizes can influence the response to therapy and that expression of many immune components are context-dependent and can have opposing effects dependent on the distribution or isoform.[81–83] For example, HMGB1 exists as three distinct redox isoforms, of which only one stimulates the immune response. However, the effect of different radiotherapy schedules on the production of HMGB1 isoforms is not clear. [84,85] This observation again highlights the need to improve understanding of the immune profile that arises in response to different radiotherapy regimes and modalities. Understanding which specific radiotherapy protocols induce an anti-tumour immune response will enable treatment to be tailored to improve efficacy of radiotherapy.

To summarize, the immune system is complex and requires tight regulation of both activators and suppressors of the immune response. Radiotherapy-induced cell death can activate ICD, leading to immune-mediated tumour cell killing, improving the efficacy of radiotherapy. However, the radiotherapy-induced ICD potential differs with radiotherapy dose, and understanding these differences will be important when making clinical decisions regarding the optimum radiotherapy regime.

[H1] EBRT, brachytherapy and the immune response

Investigations in immune responses to radiotherapy in prostate cancer have largely been limited to preclinical models and clinical data from localized disease. The most commonly used mouse model of prostate cancer is the TRAMP-C model, which has a myeloid-cell rich immune profile reflective of that typically observed in men with prostate cancer. [27,86, 87] This profile is characterized by the presence of tumour-associated macrophages (TAMs; CD11b+, F480+) that have high CD206 and low inducible nitric oxide synthase (iNOS) expression. [86] This CD206:iNOS ratio is one of many phenotypes of TAMs that are polarized towards a pro-

tumour, and immune suppressive phenotype, that lead to poorer clinical outcomes in prostate cancer. [87,88]

Data from the TRAMP-C model demonstrate that fractionated radiotherapy (3 x 5 Gy) enhances the immunosuppressive TME already present in prostate cancer tumours. Fractionated radiotherapy does not reverse or enhance the pro-tumour TAM profile observed in prostate cancer tumours pre-treatment but has been shown to increase the infiltration of these TAMs, which is thought to be facilitated by the colony-stimulating factor-1 – colony-stimulating factor-1 receptor signalling pathway.[35, 40] Fractionated radiotherapy also promotes production of IL6 and IL4 by TAMs, resulting in T cell dysfunction and dampening of the anti-cancer immune response.[81] In addition, fractionated radiotherapy increases the infiltration of CD11b+ myeloid cells, which have a role in inducing radiotherapy resistance by supporting the formation of a vascular network during vasculogenesis.[29] Changes to the lymphocyte subsets are also observed with fractionated radiotherapy: increased $CD4^+$ T cell infiltration but no effect on the infiltration of cytotoxic $CD8^+$ T cells or Tregs. [29] Although, the frequency of $CD8^+$ T cells is not affected, the phenotype is altered, as demonstrated by radiotherapy-induced increases in programmed cell death protein 1(PD1) expression.[35] Phenotypic immune cell changes like those observed with PD1 expression can modulate activity or function of these cells and so are important to evaluate. For example, PD1 is a marker of T cell activation, but chronic activation can result in exhaustion, leading to reduced immune responses. [89, 90-92] Thus, investigating the duration of response is important for understanding the resulting radiotherapy response. In pre-clinical models, radiotherapy-induced immune changes occur soon after initiation of treatment (within 7 days) and are lost by the experimental end point.[35] This observation suggests that the timing of measurements is important to fully appreciate the induced immune response. Hence, protocols collecting patient samples from clinical studies need to reflect early (days), intermediate (weeks) and late (months) effects, with the early and intermediate potentially providing the key information regarding mechanism of radiotherapy-induced immune effects.

The DVL3 cell line, a genetic model of localized prostate cancer derived from prostate tissue taken from a *Pten*$^{-/-}$/*trp53*$^{-/-}$ mouse, has been developed. The DVL3 model is radioresistant and retains the morphological and immunological characteristics of localized, high-risk prostate cancer observed in men, having an immunologically cold TME, characterized by high numbers of CD11b+ myeloid derived suppressor cells (MDSC) and low levels of CD8 T cell infiltration.[82] Development of the DVL3 model is a much needed step forward for modelling the influence of radiotherapy and other treatments on the immune response in prostate cancer, but models for assessing immune responses in the metastatic settings are still needed. For this setting, the data are limited to *in vitro* analysis using human prostate cancer cell lines. [77, 93] However, data generated from *in vitro* studies can be used to improve the design of pre-clinical and clinical studies when investigating radiotherapy combinations, as the *in vitro* data suggest that fractionation and site of metastasis are important factors in investigating radiotherapy-induced immune responses. Single-dose radiotherapy (>10Gy) induced a less favourable immune profile in prostate cancer cells, as measured by an

increased number of up-regulated immune-suppressive genes (such as PD-L1 and PD-L2) than a fractionated regime. Interestingly, the *in vitro* data suggest that radiotherapy-induced changes are cell-line dependent, as a second metastatic cell line, LNCaP demonstrated minimal changes in immune-related genes after both single-dose and fractionated radiotherapy. [77] PC3 cells are human tumour cells derived from bone metastasis, whereas LNCaP cells are derived from lymph node metastasis. This differential response across cell lines reflects the heterogeneity observed in patients with metastatic disease and suggests that the radiotherapy-induced immune responses differ across metastatic sites and, therefore, the site of metastasis might be an important factor to consider when administering radiotherapy. In another *in vitro* studying treatment induced immune checkpoint expression using PC3 and DU145 metastatic cell lines, both single-dose and fractionated radiotherapy increased the release of B7H3-enriched extracellular vesicles, decreasing the proliferative capacity of CD8+ T-cells. [93] Suggesting that radiotherapy schedule affects the expression of different immune checkpoint molecules to varying degrees. This highlights the complexity of the induced immune response and the importance of establishing the mechanism behind these responses. Given the increased interest in combining radiotherapy with immunotherapeutics, understanding the treatment induced immune response is critical for optimising radiotherapy-immunotherapy combinations.

Taken together, although limited, the *in vitro* and preclinical data show that radiotherapy does not reverse the already immune suppressive TME that is characteristic of prostate cancer tumours, but can further enhance immune suppression by increasing infiltration of other suppressive myeloid cells via changes in the cytokine profile and immune checkpoint expression.

As with the preclinical literature, the clinical data published on radiotherapy-induced immune responses is limited to localized disease. [30–34, 36, 37, 39] Comparison of the preclinical and clinical data is difficult as the sample type differs, with the majority of the pre-clinical data relating to immune changes within the TME (tumour tissue) whereas the clinical data are largely focused on systemic immune changes (blood). The reason for this discrepancy most probably relates to accessibility of samples, as post-treatment tumour biopsies from men with prostate cancer are difficult to obtain. For these reasons, a back-translational research approach is preferred as it provides a clinically relevant approach for identifying novel targets or mechanistic pathways associated with radiotherapy-induced immune responses. Back translation uses patient samples and clinical outcome data to identify potential targets for which the mechanism can be further investigated in pre-clinical models. The pre-clinical models could also be used to understand the relationship between the TME response with what is observed systemically, potentially overcoming issues that might arise between sample types.

The clinical data also demonstrates increased diversity as it compares different radiotherapy modalities and adds the complexity of combining radiotherapy with androgen deprivation and chemotherapy (**Table 1)**.[31–34,36,37,39] The clinical data highlight that different

radiotherapy doses and combinations can have opposing immune effects and, therefore, could potentially explain the conflicting data in clinical samples. For example, brachytherapy alone does not affect the $CD4^+$ subset but when combined with EBRT an overall decrease in this population is observed. [33,39] This difference might be a result of the volume of tumour that is targeted, as brachytherapy treats the prostate alone, whereas combination radiotherapy expands outside the prostate gland to include a margin and often pelvic lymph nodes. The clinical data also highlights that radiotherapy might not always induce changes in immune cell numbers, but phenotypic changes (such as proliferative capacity, checkpoint molecule expression) could be affected differently with distinct radiotherapy modalities. Understanding the differences between the induced immune response by different radiotherapy modalities is a step towards the much-needed personalized medicine approach. An improved understanding of the differences would help clinicians and patients make more informed decisions on which radiotherapy and immunotherapy combinations are likely be most effective for subsets of patients.

The lack of comparative studies investigating the effects of different radiotherapy modalities, doses and combinations on the proportion of immune subsets and the phenotype has created a gap in the knowledge pertaining to the immune response induced by radiotherapy in men with prostate cancer.

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Radiotherapy can increase expression of checkpoint molecules on T cells, so the pre-clinical and clinical data support the rationale for combining radiotherapy with immunomodulating agents, notably checkpoint inhibitors.[40,45,75] However, choosing the right combination is essential for success of such strategies. Part of the decision regarding which radiotherapy combination is best could be informed by understanding the pre-treatment immune profile of the tumours. Immunologically hot tumours might respond more effectively to ICD than immunologically cold tumours because they are immune infiltrated, less suppressive and have increased numbers of dendritic cells.[53] This activation is likely to be followed by T cell exhaustion owing to chronic immune cell activation. In these tumours, combining radiotherapy with checkpoint inhibitors (such as PD1 and cytotoxic T-lymphocyte associated protein 4 inhibitors) could have a favourable effect on patient outcome. However, in the case of prostate cancer, in which the tumours are classified as immunologically cold, the tumours are less likely to respond to ICD than immunologically hot tumours owing to the highly immune suppressive TME and reduced presence of dendritic cells. In this instance, checkpoint inhibitors alone are unlikely to improve radiotherapy, but the patient might benefit from combined cytokine treatment to switch the pre-existing immune contexture.[53] Thus, this possibility further emphasises the importance of characterizing the immune profile before radiotherapy administration.

[H2] Molecular targeted radiotherapy (MRT) and the immune response

Published data investigating the immune effects of both ^{223}Ra and ^{177}Lu are limited. The data thus far suggest that human prostate cancer cell lines (LNCaP and PC3) that survive radiotherapy-induced cell death after initial exposure to ^{223}Ra undergo immune changes, including increased expression of histocompatibility leukocyte antigens (HLAs) and CALR, which sensitize them to T cell-mediated killing.[94,95] In vivo data using mice treated with ^{223}Ra at a dose reflecting the clinical regime demonstrate that ^{223}Ra induces an initial (up to 12 days post treatment) increase in the cytotoxic capabilities of splenic NK cells This suggests that ^{223}Ra induces both direct effects on immune cell function and an alteration in the sensitivity of tumours to immune cell killing.[96] To date, no pre-clinical studies have been published in which the immune responses induced by ^{177}Lu is investigated.

Clinical studies investigating the immune effects of ^{223}Ra or ^{177}Lu in prostate cancer are also limited and consist of small cohorts. To date, four clinical studies in which the immune effects of ^{223}Ra have been investigated have been undertaken and one study on ^{177}Lu has been published (**Table 2**).[,97–103] The clinical data demonstrate that ^{223}Ra does not affect the frequency of circulating T cells or T cell cytokine production compared with baseline levels. Increases in checkpoint molecules (PDL1 and LAG3) and activation markers (inducible T-cell costimulator [ICOS] and PD1) are observed in circulating T cells, suggesting that ^{223}Ra might induce phenotypic changes on T cells. ^{223}Ra decreases the frequency of circulating CD3-positive cells that are not T cells, so^{223}Ra could possibly affect other CD3^{+} populations, such as circulating NK-like T cells. This possibility would need to be further investigated as the immune phenotyping for ^{223}Ra is currently limited to T cell analysis. In addition, other radiotherapy studies have shown that immune-suppression phenotypes associated with radiotherapy might be associated with reduced sensitivity of tumour cells to immune-mediated death as opposed to reduced immune cell function.

The influence of the ^{223}Ra-induced immune changes on OS outcome have not fully been investigated. Interim analysis suggests that men who demonstrate a decrease in the frequency of circulating PD1-expressing T cells and an increase in exosomal expression of PDL1, LAG3 and IDO after treatment with ^{223}Ra potentially have an unfavourable survival outcome **[Au: please add the data here as well, or at least some example data from one of the studies.]** (**Table 2**).[797, 98, 101–103] The potential clinical effect of monitoring changes in circulating immune cells emphasises the importance of understanding changes in both the tumour-infiltrated and the systemic immune profile, and how these two components influence the radiotherapy response. Even less is known about the immune effects of ^{177}Lu than ^{223}Ra but current evidence suggests that ^{177}Lu might induce an immunogenic priming effect that leads to durable responses to anti-PD1 therapy in patients with mCRPC without high mutational burden and microsatellite stability.[100] The observed priming effect could enable expanded use of checkpoint inhibitors in patients with prostate cancer, as currently checkpoint inhibitors are limited to patients with high tumour mutational burden or microsatellite instability **[Au: Please reference this statement.]** . In another study in which 168 patients were treated with ^{177}Lu-labelled PSMA, high expression of PD-L2 was associated with improved response to ^{177}Lu-labelled PSMA. [104] The mechanisms have not been elucidated, but the data suggest that an interaction exists between immune-related

proteins and MRT response, providing further rationale for the importance of understanding the MRT-induced immune response and how this response affects response to therapy.

In summary, both conventional radiotherapy and MRTs induce an immune response. Data for conventional radiotherapy highlights that investigating the effects of different radiation doses will be important for understanding the MRT-induced immune response to improve MRT efficacy. The lack of concordance between sample collection in pre-clinical and clinical studies and absence of reliable pre-clinical prostate cancer models is a challenge that needs to be addressed to enable clinically relevant research to be conducted.

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[H1] Future directions

Clearly, pre-clinical models with increased robustness are needed for assessing the radiotherapy-induced immune response, especially for the metastatic setting. The use of patient-derived models would provide a potential approach for investigating the immune response in a more relevant model that closely mimics the human disease and accounts for heterogeneity that is observed in patients. [105] In addition, increased research focus needs to be applied to understanding how different radiation emissions and absorbed dose modulate the immune response, as this knowledge would enable improved understanding of the associated radiobiology, which could be applied to developing biomarkers and identifying novel combination strategies. Identification of predictive biomarkers of MRT response would avoid unnecessary treatment-associated toxic effects in patients who are unlikely to respond to MRTs. For those patients who are unlikely to respond to MRT monotherapy, understanding the MRT-induced immune response would provide novel combination strategies. For example, for patients with prostate cancer, immune checkpoint inhibitors have been limited to a small subset of patients: those with high mutational burden and microsatellite instability **[Au: Please reference this statement.]**. Early data for ^{177}Lu suggests that ^{177}Lu can prime patients with mCRPC outside of this subset to receive an anti-PD1 inhibitor and demonstrate durable response [100]. This observation suggests a potential expanded use of checkpoint inhibitors in prostate cancer. Checkpoint regulation is complex and a number of different molecules are involved that go beyond the PD1–PDL1 pathway. Despite this complexity, the majority of checkpoint inhibitors developed target the PD1–PDL1 pathway **[Au: Please reference this statement.]** , which might not be the most relevant checkpoint molecule for all tumour types. Given that both ^{223}Ra and ^{177}Lu are being investigated in combination with checkpoint molecules [106,107], understanding the interplay between MRTs and the immune response will have an important role in understanding which, if any immune checkpoint inhibitors will be best for patients with prostate cancer. Together, this knowledge would enable clinicians and patients to make improved informed decisions on the type of MRT, dose and combinations to use, which would move cancer treatment for patients with prostate cancer closer to the much-needed personalized medicine approach.

Success in the metastatic setting increases the potential of MRTs to be used in earlier disease settings. Clinical data already suggests that ^{223}Ra demonstrates potential benefit in patients with hormone-sensitive metastatic prostate cancer[97,98]. Understanding the dependency of MRT efficacy on the immune response and whether this immune context is similar in earlier metastatic disease could ultimately improve the overall prognosis of men with metastatic prostate cancer.

[H1] Conclusions

EBRT and brachytherapy are standard-of-care treatment options for men with prostate cancer at different stages of the disease and have been part of the cancer treatment pathway much longer than the approved MRT agents. Thus, that there are more publications investigating the immune effects of EBRT and brachytherapy than ^{223}Ra and ^{177}Lu is not surprising. However, this Review has highlighted that even for EBRT and brachytherapy, major gaps still exist in knowledge regarding the immune effects of these radiotherapy modalities in prostate cancer. Dampening of the immune response requires the presence of suppressive immune cell types such as pro-tumour TAMs, suppressive myeloid cells and Tregs and the published data demonstrates that different radiotherapy modalities affect the infiltration of these immune cells into the TME. Investigating changes in the proportion of immune cells alone is not sufficient to fully address the effects of radiotherapy on the immune system. Improved profiling of the cytokines and checkpoint molecules is needed to fully understand how radiotherapy can influence the immune response. In addition, the lack of comparative studies investigating the immune effects of different radiotherapy modalities adds conflict to the published data, with opposing effects on the same immune cell type being observed. Comparative studies would enable improved understanding of the individual and combined effects of different treatment options, which would facilitate informed decisions to be made on the best combinations for individual patients, especially when considering combining radiotherapy with immune-modulating agents.

Current preclinical models investigating the immune effects of radiotherapy in prostate cancer include the use of mouse models, human prostate cancer cell lines, patient-derived xenografts and explants, and organoids. Each of these models offers advantages but only mouse models at present are able to sufficiently model radiotherapy-induced immune responses.[82,105] However, mouse models are still lacking in complexity as they do not take into account the immune modulatory effects of previous therapy or radiotherapy combination often seen in advanced disease.

The immune system can enhance the efficacy of radiotherapy by the process of ICD. However, for tumours to successfully respond to ICD post radiotherapy, immune active cells (such as dendritic cells) and cytokines need to be either present within the TME or recruited.

As prostate cancer is considered an immunologically cold tumour, the pre-treatment TME has increased importance as the presence of immune-suppressive cells types such as TAMs, suppressive myeloid cells and Tregs can reduce the efficacy of radiotherapy. However, limitations in collecting matched pre-treatment and post-treatment biopsies are still limiting factors when trying to understand how the existing TME could influence radiotherapy immune response.

With respect to MRT, this Review demonstrates that data published on investigating the immune effects of ^{223}Ra or ^{177}Lu are limited. The data thus far suggest that ^{223}Ra induces a systemic immune response but the consequence of these changes on patient outcome are yet to be determined. In addition, no studies detail the effects of ^{223}Ra or ^{177}Lu on the bone TME in patient samples. However, limitations in collecting pre-treatment and post-treatment biopsies might be limiting factors in understanding the effects of radionuclides on local immune effects. For this reason, investigating the systemic immune response induced by these MRTs that can be measured using liquid biopsies (for example, plasma, serum exosomes) are an attractive option.

The data published on EBRT and brachytherapy suggest that different radiotherapy modalities have different immune effects. This observation has highlighted that comparison of the immune effects of α (^{223}Ra, ^{225}Ac, ^{227}Th) and β emitters (^{177}Lu) will be crucial to identifying how best to use these radionuclides in treating men with prostate cancer. This knowledge is important as radionuclides can have more than one emission profile (for example, α emitters can decay and emit β particles). In addition, profiling changes observed in the cytokine and checkpoint molecules will be important for identifying which MRTs are likely to induce an immune suppressive response and has not yet been fully investigated in prostate cancer for either conventional radiotherapy or MRTs.

Another gap in knowledge that comes mostly from the paucity of clinical samples and appropriate mouse models is the lack of understanding relating to how the other cells types (endothelial cells andfibroblasts) present within the TME affect the radiotherapy-induced immune response. This understanding is especially important when thinking about combination strategies. Both ^{223}Ra and ^{177}Lu are currently being investigated in combination with immune agents in clinical studies.[95, 96] Understanding the ability of MRTs to induce ICD and immune modulation is of particular importance as clinical data from a Phase Ib study in which ^{223}Ra treatment was combined with a PD-L1 inhibitor in men with mCRPC demonstrated that no clinical benefit is gained by treating these men with ^{223}Ra plus anti-PD-L1 therapy.[4]

Owing to the systemic and targeted nature, MRTs could improve the OS and quality of life of men with prostate cancer with high metastatic tumour burden. Furthermore, MRTs such as ^{223}Ra might have a role in earlier disease settings, which could ultimately improve the overall prognosis of men with prostate cancer.[18,19]

To conclude, further research is needed for investigating the role of EBRT, brachytherapy and MRT in modulating the immune response. An improved understanding of the

interaction between radiotherapy and the immune response could lead to improved efficacy of radiotherapy and improved combination strategies. Understanding the mechanism by which radiotherapy induces an anti-tumour or pro-tumour immune response could also help identify which patients are likely to respond to radiotherapy. Improved access to patient samples and accessible ways to monitor immune function and radiotherapy response are needed. Translational research from clinical studies is of upmost importance, as these samples better reflect the complexity and treatment history that is observed with men with mCRPC.

References

1. James, N.D., et al. The Lancet Commission on prostate cancer: planning for the surge in cases. Lancet. 403, 1683–1722 (2024).
2. Hague, C. & Logue, J.P. Clinical experience with radium-223 in the treatment of patients with advanced castrate-resistant prostate cancer and symptomatic bone metastases. *Therapeutic Advances in Urology*. 8, 175–180 (2016).
3. McBean, R., O'Kane, B., Parsons, R. & Wong, D. Lu277-PSMA therapy for men with advanced prostate cancer: initial 18 months experience at a single Australian tertiary institution. *Journal of Medical Imaging and Radiation Oncology*. 63, 538–545 (2019).
4. Fong, L. et al. A Phase 1b study of atezolizumab with Radium-223 Dichloride in men with metastatic castration-resistant prostate cancer. *Clinical Cancer Research*. 27, 4746–4756 (2021).
5. Wang, S., Tang, W., Luo, H., Jin, F. & Wang, Y. The role of image-guided radiotherapy in prostate cancer: A systematic review and meta-analysis. Clinical and Translational Radiation Oncology. 38, 81–89 (2023).
6. Parker, C.C., et al. Radiotherapy to the primary tumour for newly diagnosed, metastatic prostate cancer (STAMPEDE): a randomised controlled phase 3 trial. The Lancet. 392, 2353-2366 (2018).
7. Desouky, O., Ding, N. & Zhou, G. Targeted and non-targeted effects of ionizing radiation. *Journal of Radiation Research and Applied Sciences*. 8, 247-254 (2019).
8. Parker, C., et al. Prostate cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Annals of Oncology*. 31, 1119–1134 (2020).
9. Bolla, M., Henry, A., Mason, M., & Wiegel, T. The role of radiotherapy in localised and locally advanced prostate cancer. *Asian Journal of Urology*. 6, 153–161 (2019).
10. Mendez, L.C., & Morton, G.C. High dose-rate brachytherapy in the treatment of prostate cancer. *Translational Andrology and Urology*. 7, 357–370 (2018).
11. Zaorsky, N.G., et al. The evolution of brachytherapy for prostate cancer. *Nature Review Urology*. 14, 415–439 (2017).
12. Goyal, J. & Antonarakis, E.S. Bone-targeting radiopharmaceuticals for the treatment of prostate cancer with bone metastases. *Cancer Letters*. 323, 135–146 (2012).

13. Ritawidya, R., et al. Lutetium-177-Labeled Prostate-Specific Membrane Antigen-617 for Molecular Imaging and Targeted Radioligand Therapy of Prostate Cancer. *Advanced Pharmaceutical Bulletin*. 13, 701–711 (2023).
14. Marques, I.A., et al. Targeted alpha therapy using Radium-223: From physics to biological effects. *Cancer Treatment Reviews*. 68, 47-54 (2018).
15. Parker, C., et al. Alpha emitter radium 223 and survival in metastatic prostate cancer. *The New England Journal of Medicine*. 369, 213–223 (2013).
16. Badrising, S.K. et al. Integrated analysis of pain, health-related quality of life, and analgesic use in patients with metastatic castration-resistant prostate cancer treated with Radium-223. *Prostate Cancer and Prostatic Disease*. 25, 248–255 (2022).
17. Morris, M.J., et al. Radium-224 mechanism of action: implications for use in treatment combinations. *Nature Reviews Urology*. 16, 745–756 (2019).
18. O'Sullivan, J.M., et al. Results of the ADRRAD trial of pelvic IMRT plus radium-223 in men with mHSPC metastatic to bone. *Journal of Clinical Oncology*. 38 (2020).
19. Turner, P., et al. First survival data from the ADRRAD clinical trial; pelvic radiotherapy and concurrent Radium-223 in metastatic hormone sensitive prostate cancer (mHSPC). *Clinical Oncology*. 32, E130–E131 (2020).
20. Sartor, O. et al. Lutetium-177 PSMA-617 for metastatic castration-resistant prostate cancer. *New England Journal of Medicine*. 385, 1091–1103 (2021).
21. Rinne, S.S. & Vorobyeva, A. Radiometals – chemistry and radiolabling. *Nuclear Medicine and Molecular Imaging*. (2022).
22. Jang, A., Kendi, A.T., Johnson, G.B., Halfdanarson, T.R. & Sartor, O. Targeted Alpha-Particle Therapy: A Review of Current Trials. *International Journal of Molecular Sciences*. 24, 11626 (2023).
23. Abbott, E.M., Falzone, N., Lenzo, N. & Vallis, K.A. Combining external beam radiation and radionuclide therapies: rationale, radiobiology, results and roadblocks. *Clinical Oncology*. 33, 735–743 (2021).
24. Brandmaier, A. & Formenti, S. The impact of radiation therapy on innate and adaptive tumor immunity. *Seminars in Radiation Oncology*. 30, 139–144 (2019).
25. Wei, R., Liu, S., Zhang, S., Min, L. & Zhu, S. Cellular and Extracellular Components in Tumor Microenvironment and Their Application in Early Diagnosis of Cancers. *Analytical Cellular Pathology*. 2020, 6283796 (2020).
26. Pun, J., et al. Identification of cancer-associated fibroblasts subtypes in prostate cancer. *Frontiers in Immunology*. 14, 1133160 (2023).
27. Hirz, T., et al. Dissecting the immune suppressive human prostate tumor microenvironment via integrated single-cell and spatial transcriptomic analyses. *Nature Communications*. 14, 663 (2023).
28. Wu, Z., et al. The Landscape of Immune Cells Infiltrating in Prostate Cancer. *Frontiers in Oncology*. 10, 517637 (2020).
29. Brown, J.M. Vasculogeneis: a crucial player in the resistance of solid tumours to radiotherapy. *The British Journal of Radiology*. 87, 1035 (2013).
30. Eckert, F., et al. Impact of curative radiotherapy on the immune status of patients with localised prostate cancer. *Oncoimmunology*. 11, (2018).

31. Hoffman, E., et al. Radiotherapy planning parameters correlate with changes in the peripheral immune status of patients undergoing curative radiotherapy for localised prostate cancer. *Cancer Immunology, Immunotherapy*. 71, 541-552 (2022).
32. Hurwitz, M.D., Kaur, P., Nagaraja, G.M., Bausero, M.A., Manola, J. & Asea. Radiation therapy induces circulating serum Hsp72 in patients with prostate cancer. *Radiotherapy and Oncology*. 95, 350–358 (2010).
33. Finkelstein, S.E., et al. Combining Immunotherapy and Radiation for Prostate Cancer. *Clinical Genitourinary Cancer*. 13, 1-9 (2015).
34. Kubo, M., et al. Enhanced activated T cell subsets in prostate cancer patients receiving iodine-125 low-dose-rate prostate brachytherapy. *Oncology Reports*. 39, 417–424 (2018).
35. Philippou, Y., et al. Impacts of combining anti-PD-L1 immunotherapy and radiotherapy on the tumour immune microenvironment in a murine prostate cancer model. *British Journal of Cancer*. 123, 1089–1100 (2020).
36. Nesslinger, N.J., et al. Standard treatments induce antigen specific immune responses in prostate cancer. *Clinical Cancer Research*. 13, 1493–1502 (2007).
37. Schaue, D., et al. T-cell responses to surviving in cancer patients undergoing radiation therapy. *Clinical Cancer Research*. 14, 4883–4890 (2008).
38. Tuomela, K., et al. Radiotherapy transiently reduces the sensitivity of cancer cells to lymphocyte cytotoxicity. *PNAS*. 119, 3 (2022).
39. Wang, H., et al. Immune cell profiling in Gleason 9 prostate cancer patients treated with brachytherapy versus external beam radiotherapy: an exploratory study. *Radiotherapy and Oncology*. 155, 80-85 (2021).
40. Xu, J., et al. CSFIR signalling blockade stanches tumour-infiltrating myeloid cells and improves the efficacy of radiotherapy in prostate cancer. *Cancer Research*. 73, 2782–94 (2013).
41. Ren, X., Guo, S., Guan, X., Kang, Y., Liu, J., & Yang, X. Immunological classification of tumour types and advances in precision combination immunotherapy. *Frontiers in Immunology*. 13, 790113 (2022).
42. Bonaventura, P., et al. Cold tumors: a therapeutic challenge for immunotherapy. *Frontiers in Immunology.* 10, 168 (2019).
43. Stultz, J. & Fong, L. How to turn up the heat on the cold immune microenvironment of metastatic prostate cancer. *Prostate Cancer and Prostatic Disease*. 24, 697–717 (2021).
44. Zhang, J., Huang, D., Saw, P.E. & Song, E. Turning cold tumors hot: from molecular mechanisms to clinical applications. *Trends in Immunology*. 43, 523–545 (2022).
45. Keam, S.P., et al. High dose-rate brachytherapy of localized prostate cancer converts tumors from cold to hot. *Journal for ImmunoTherapy of Cancer*. 8, e000792 (2020).
46. Andersen, L.B., et al. Immune cell analyses of the tumor microenvironment in prostate cancer highlight infiltrating regulatory T cells and macrophages as adverse prognostic factors. The *Journal of Pathology*. 255, 155–165 (2021).
47. Chen, D.S & Mellman, I. Oncology meets immunology: the cancer immunity cycle. *Immunity*. 39, 1–10 (2013).

48. Apetoh, L., et al. The interaction between HMGB1 and TLR4 dictates the outcome of anticancer chemotherapy and radiotherapy. *Immunology Review*. 220, 47–59 (2007).
49. Apetoh, L., et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nature Medicine*. 13, 1050–1059 (2007).
50. Obeid, M., et al. Calreticulin exposure dictates the immunogenicity of cancer cell death. *Nature Medicine*. 13, 54–61 (2007).
51. Dar, T.B., Henson, R.M. & Shiao, S.L. Targeting innate immunity to enhance the efficacy of radiation therapy. *Frontiers in Immunology*. 9, 3077 (2018).
52. Sharma, P., Wagner, K., Wolchok, J.D. & Allison, J.P. Novel cancer immunotherapy agents with survival benefit: recent successes and next steps. *Nature Review Cancer.* 11, 805–812 (2012).
53. Ruckert, M., Flohr, A.S., Hecht, M. & Gaipl, U.S. Radiotherapy and the immune system: More than just immune suppression. *Stem cells*. 39, 1155–1165 (2021).
54. Colton, M., Cheadle, E.J., Honeychurch, J. & llidge, T.M. Reprogramming the tumour microenvironment by radiotherapy: implications for radiotherapy and immunotherapy combinations. *Radiation Oncology*. 15, 254 (2020).
55. Sharma, R.A. et al. Clinical development of new drug-radiotherapy combinations. Nature Reviews: *Clinical Oncology*. 13, 627–642 (2016).
56. Carvalho, A.H. & Villar, R.C. Radiotherapy and immune response: the systemic effects of a local treatment. *Clinics*. 73:e557s (2018).
57. Demaria, S., et al. Ionizing Radiation Inhibition Of Distant Untreated Tumors (Abscopal Effect) Is Immune Mediated. *International Journal of Radiation Oncology, Biology, Physics*. 58, 862– 870 (2004).
58. Honeychurch, J & llidge, T.M. The influence of radiation in the context of developing combination immunotherapies in cancer. *Therapeutic Advances in Vaccines and Immunotherapy*. 5, 115–122 (2017).
59. Ghiringhelli, F., et al. Activation of the NLRP3 inflammasome in dendritic cells induces IL-1beta-dependent adaptive immunity against tumours. *Nature Medicine*. 15, 1170–1178 (2009).
60. Obeid, M., et al. Calreticulin exposure is required for the immunogenicity of gamma-irradiation and UVC light-induced apoptosis. *Cell Death Differentiation*. 14:1848–1850 (2007).
61. Gameiro, S.R., Jammeh, M.L., Wattenberg, M.M.,Tsang, K.Y., Ferrone, S & Hodge, J.W. Radiation-induced immunogenic modulation of tumour enhances antigen processing and calrecticulin exposure, resulting in enhanced T-cell killing. *Oncotarget*. 5, 403–416 (2014).
62. Golden, E.B., Frances, D., Pellicciotta, I., Demaria, S., Barcellos-Hoff, M.H. & Formenti, S.C. Radiation fosters dose-dependent and chemotherapy-induced immunogenic cell death. *Oncoimmunology*. 3:e28518 (2014).
63. Chakraborty, M., et al. Irradiation of tumour cells upregulates Fas and enhances CTL lytic activity and CTL adoptive immunotherapy. *The Journal of Immunology*. 170, 6338–6347 (2003).

64. Chakraborty, M., Abrams, S.I., Coleman, C.N., Camphausen, K, Schlom, J. & Hodge, J.W. External beam radiation of tumors alters phenotype of tumour cells to render them susceptible to vaccine-mediated T cell killing. *Cancer Research*. 64, 4328–4337 (2004).
65. Garnett, C.T., Palena, C., Chakraborty, M., Tsang, K.Y., Schlom, J. & Hodge, J.W. Sublethal irradiation of human tumour cells modulates phenotype resulting in enhanced killing by cytotoxic T lymphocytes. *Cancer Research*. 64, 7985–7994 (2004).
66. Ifeadi, V. & Garnett-Benson, C. Sub-lethal irradiation of human colorectal tumour cells imparts enhanced and sustained susceptibility to multiple death receptors signalling pathways. *PLoS ONE*. 7, e31762 (2012).
67. Reits, E.A., et al. Radiation modulates the peptide repertoire enhances MHC class I expression, and induces successful antitumour immunotherapy. *The Journal of Experimental Medicine*. 203, 1259–1271 (2006).
68. Deng, L., et al. STING-dependent cytosolic DNA sensing promotes radiation-induced type I interferon-dependent antitumour immunity in immunogenic tumours. *Immunity*. 41, 843–852 (2014).
69. Diamond, J.M., et al. Exosome shuttle TREX1-sensitive IFN-stimulatory dsDNA from irradiated cancer cells to DCs. *Cancer Immunology Research*. 6, 910–920 (2018).
70. Carvalho, A.H. & Villar, RC. Radiotherapy and immune response: the systemic effects of a local treatment. *Clinics*. 73:e557s (2018).
71. Najafi, M., et al. Macrophage polarity in cancer: A review. *The Journal of Cellular Biochemistry*. 120, 2756–2765 (2019).
72. Shevtsov, M., Sato, H., Multhoff, G. & Shibata, A. Novel approaches to improve the efficacy of immune-radiotherapy. *Frontiers in Oncology.* 9,156 (2019).
73. Seifert, L., et al. Radiation therapy induces macrophages to suppress T-cell responses against pancreatic tumors in mice. *Gastroenterology*. 150, 1659–1672 (2016).
74. Batlle, E. & Massague, J. Transforming Growth Factor-β Signaling in Immunity and Cancer. *Immunity*. 50, 924-940 (2019).
75. Ko, V.M., et al. Radiotherapy and cGAS/STING signaling: Impact on MDSCs in the tumor microenvironment. *Cellular Immunology*. 362, 104298 (2021).
76. Liang, H., et al. Host STING-dependent MDSC mobilisation drives extrinsic radiation resistance. *Nature Communications*. 8, 1736 (2017).
77. Eke, I., et al. Long-term expression changes of immune-related genes in prostate cancer after radiotherapy. *Cancer Immunology, Immunotherapy*. 71, 839-850 (2022).
78. Derer, A., et al. Chemoradiation increases PD-L1 expression in certain melanoma and glioblastoma cells. *Frontiers in Immunology*. 7,610 (2016).
79. Wan, X. et al. The mechanism of low-dose radiation-induced upregulation of immune checkpoint molecule expression in lung cancer. *Biochemical and Biophysical Research Communications*. 607, 102–107 (2022).
80. Ding, X.C., et al. The change of soluble programmed cell death-ligand 1 in glioma patients receiving radiotherapy and its impact on clinical outcome. *Frontiers in Immunology*. 11 (2020).
81. Lin, Y., Xu, J. & Lan, H. Tumour-associated macrophages in tumour metastasis: biological roles and clinical therapeutic applications. *Journal of Hematology & Oncology*. 12 (2019).

82. Haughey, C.M., et al. Investigating Radiotherapy Response in a Novel Syngeneic Model of Prostate Cancer. *Cancers*. 12, 2804 (2020).
83. Dovedi, S.J., et al. Fractionated Radiation Therapy Stimulates Antitumor Immunity Mediated by Both Resident and Infiltrating Polyclonal T-cell Populations when Combined with PD-1 Blockade. *Clinical Cancer Research*. 23, 5514–26 (2017).
84. Chen,R., Kang, R. & Tang, D. The mechanism of HMGB1 secretion and release. *Experimental & Molecular Medicine*. 54, 91–102 (2022).
85. Kwak, M.S.,et al. Peroxiredoxin-mediated disulfide bond formation is required for nucleocytoplasmic translocation and secretion of HMGB1 in response to inflammatory stimuli. *Redox Biology*. 24, 1012023 (2019).
86. De Groot, A. E., et al. Characterisation of tumour-associated macrophages in prostate cancer transgenic mouse model. *Prostate*. 81, 629–647 (2021).
87. Boibesset, C., et al. Subversion of infiltrating prostate macrophages to a mixed immunosuppressive tumor-associated macrophage phenotype. *Clinical and Translational Medicine*. 12, e581 (2022).
88. Viola, A., Munari, F., Sánchez-Rodríguez, R., Scolaro, T. & Castegna, A. The metabolic signature of macrophage responses. *Frontiers in Immunology*. 10, 1462 (2019).
89. Jubel, J.M., Barbati, Z.R., Burger, C., Dieter C. Wirtz, D.C & Schildberg, F.A. The role of PD-1 in acute and chronic infection. Frontiers in Immunology. 11, 487 (2020).
90. Li, K., et al. PD-1 suppresses TCR-CD8 cooperativity during T-cell antigen recognition. *Nature Communications*. 12, 2746 (2021).
91. Wartewig, T., et al. PD-1 instructs a tumor-suppressive metabolic program that restricts glycolysis and restrains AP-1 activity in T cell lymphoma. *Nature Cancer*. 4, 1508–1525 (2023).
92. Mizuno, R., et al. PD-1 primarily targets TCR signal in the inhibition of functional T-cell activation. *Frontiers in Immunology*. 10, 630 (2019).
93. Kim, Y., Lavoie, R.R., Dong, H., Park, S., & Lucien-Matteoni, F. Radiotherapy inhibits the antitumour immune response through release of immunosuppressive tumor-derived extracellular vesicles in prostate cancer. *Cancer Research*. 81, abstract 675 (2021).
94. Cursano, M.C., et al. Combination radium-223 therapies in patients with bone metastases from castration-resistant prostate cancer: A review. *Critical Reviews in Oncology/Hematology*. 146 (2020).
95. Malamas, A.S., Gameiro, S.R., Knudson, K.M. & Hodge, J.W. Sublethal exposure to alpha radiation (223Ra dichloride) enhances various carcinomas' sensitivity to lysis by antigen-specific cytotoxic T lymphocytes through calreticulin-mediated immunogenic modulation. *Oncotarget*. 7, 86937-86947 (2016).
96. Leung, C.N., Howell, D.M. & Howell, R.W. Radium-223 dichloride causes transient changes in natural killer cell population and cytotoxic function. *International Journal of Radiation Biology*. 97, 1417–1424 (2021).
97. Kim, J.W., et al. Immune Analysis of Radium-223 in Patients with Metastatic Prostate Cancer. *Clinical Genitourinary Cancer*. 16, e469–e476 (2018).
98. Kim, J.W., et al. Survival and immune analysis of radium-223 in patients with metastatic prostate cancer. *American Society of Clinical Oncology*. Abstract number: e24144 (2018).

99. Vardaki, I., et al. Radium-223 treatment increase immune checkpoint expression in extracellular vesicles from the metastatic prostate cancer bone microenvironment. *Clinical Cancer Research*. 27, 3253–3264 (2021).
100. Aggarwal RR et al. Immunogenic priming with 177Lu-PSMA-617 plus pembrolizumab in metastatic castration resistant prostate cancer (mCRPC): A phase 1b study (2021). *Journal of Clinical Oncology*. 39, 15.
101. Marshall, C.F., et al. Randomized phase II trial of sipuleucel-T with or without radium-223 in men with bone-metastatic castration-resistant prostate cancer. *Clinical Cancer Research*. 27, 1623–1630 (2021).
102. Creemers JHA et al. Immunophenotyping reveals longitudinal changes in circulating immune cells during radium-223 therapy in patients with metastatic castration-resistant prostate cancer. *Frontiers in Oncology*. 18, 667658 (2021).
103. Vardaki I et al. Radium-223 treatment increase immune checkpoint expression in extracellular vesicles from the metastatic prostate cancer bone microenvironment. *Clinical Cancer Research*. 27, 3253–3264 (2021).
104. Handke, A., et al. Analysing the tumor transcriptome of prostate cancer to predict efficacy of Lu-PSMA therapy. *Journal for ImmunoTherapy of Cancer*. 11, e007354 (2023).
105. Risbridger, G.P., et al. Preclinical models of prostate cancer: Patient-derived xenografts, organoids and other explant models (2018).
106. Dorff, T.B., Stein, C., Kortylewski, M., Posadas, E., Synold, T. & Quinn, D. Evaluating Changes in Immune Function and Bone Microenvironment during Radium-223 Treatment of Patients with Castration-Resistant Prostate Cancer. *Cancer Biotherapy and Radiopharmaceuticals*. 35, 485-489 (2020).
107. Kgatle, M.M., et al. Immune checkpoints, inhibitors and radionuclides in prostate cancer: promising combinatorial therapy approach. *International Journal of Molecular Sciences*. 22, 4109 (2021).

[Au: Tables formatted to journal style]

Table 1: Clinical studies on the immune effects of EBRT and brachytherapy on prostate cancer

Stage of prostate cancer	Type of radiotherapy	Dose	Sample type	Effects of radiotherapy (compared with baseline)	Reference
Localized	Not specified (n = 18)	70–78 Gy in 35–39 fractions.	PBMCs	• Decreases B cells (4.1% to 1.8%, p<0.001). • Increases proportion of NK cells (31.1% to 38.8%,	30

				p<0.01) and proliferative capacity. • Increases proportion of Tregs (4.2% to 6.4%, p<0.05) but not proliferative capacity. • Decreases proportion of CD8 T cells (32.9% to 24.6%, p<0.05) but increases the proliferative capacity of these CD8 T cells. • No change to the proportion of CD4 T cells but the proliferative capacity is increased.	
Localized	Not specified (n =18)	70–78 Gy in 35–39 fractions.	PBMCs	• Decreases T cells and B cells. • Increases NK and Treg. • Age of patient affects immune cell population	31

				s.	
Localized	EBRT (n = 13)	Treated for 8 weeks, dose not stated	Serum, plasma and PBMCs	• Increases HSP72 (9.8pg/ml to 34.0pg/ml, *p*=0.0002), CD8 T cells (4.1% to 8.5%, *p*=0.005) and NK (2.3% to 7.4%, *p*=0.001) cells. • Increases in TNF (10pg/ml to 17.9pg/ml, *p*=0.01) and IL6 (9.8pg/ml to 32.3pg/ml, *p*=0.01).	32
Localized	Iodine-125 BT (n = 36)	LDR – dose not specified.	Whole blood	• Increases granulocytes. • Decreases B cells. • No effect on the proportion of CD4 or CD8 T cells and NK cells. • Increases activation status of CD4 and CD8 T cells. • Decreases suppressive myeloid cells. • Increase in	34

				proportion of Tregs.	
Localized	EBRT or brachytherapy (n = 64)	EBRT: 74 Gy in 37 fractions	Serum	• Induces antigen-specific immune response.	36
Localized	Chemoradiotherapy (n = 20)	45 Gy in 25 fractions	PBMCs	• Increases tumour-specific CD8 T cells.	37
High-risk disease (n = 11)	EBRT or EBRT plus brachytherapy	46 Gy in 23 fractions followed by 32 Gy in 16 fractions or a single 15 Gy HDR brachytherapy	PBMCs	Comparing EBRT alone with EBRT plus brachytherapy • No changes in B cells, dendritic cells, monocytes and NKs. • Ratio of CD4:CD8 T cells decreased in EBRT plus brachytherapy arm. • Proportion of CD4 T cells decreased in EBRT plus brachytherapy. • Proportion of CD4 T cells expressing PD1 increased in EBRT plus brachytherapy arm. • Proportion	39

				of CD8 T increases in EBRT plus brachytherapy. • IL2 and Granzyme B expression on CD8 T cells increased with EBRT plus brachytherapy.	

EBRT = external beam radiotherapy; HDR = high dose rate; HSP72 = heat shock protein 72; LDR = low dose rate; NK = natural killer; PBMCs = peripheral mononuclear blood lymphocytes; PD1 = programmed cell death protein 1 TNF = tumour necrosis factor; Treg = regulatory T cells.

Table 2: The immune effects of Ra-223 or Lu-177-PSMA in prostate cancer from clinical studies

Radionuclide	Stage of prostate cancer	Effects of MRT (compared with baseline)	Reference
^{223}Ra	mCRPC (n = 15)	<u>Circulating immune cells:</u> • No change in central memory, effector memory or effector T cells. • Decreases PD-1 expression on effector CD8+ memory T cells. • No change to CD27 or CD28 expression on effector CD8+ memory T cells. • No change in CD8 effector memory T cells that express IL-7α receptor. However, PD-1 expression in this subset significantly decreased (20.6% to 14.6%, p = 0.020) • No change in production of IFN-γ TNF, and IL13 by CD8 T cells.	97, 98
^{223}Ra	mCRPC (n = 32)	• Changes in peripheral antigen PA2024-specific T cell responses.	101
^{223}Ra	mCRPC	<u>Circulating immune cells:</u>	102

	(n = 30)	• Decrease in CD3-positive cells (~6-month decrease of 20.3% [CI -31.8% - -8.8%]). • Increase in ICOS, PD1 and PDL1 on CD4 and CD8 T cells.	
^{223}Ra	mCRPC (n = 25)	<u>Circulating exosomes:</u> • Significantly increased levels of PDL1 (p = 0.049), LAG3 and IDO in patients who demonstrated unfavourable prognosis after treatment.	103
^{177}Lu	mCRPC (n =18)	• Immunogenic priming effect.	100
^{177}Lu	mCRPC (n =168)	• A high PD-L2 signature expression in tumour cells associated with better prognosis after treatment (median OS 17.2 vs 5.7 months in higher vs lower PD-L2).	104

ICOS = inducible T-cell costimulatory; IDO = indoleamine 2,3-dioxygenase; IFNγ = interferon gamma; IL13 = interleukin 13; LAG3 = lymphocyte activation gene 3; mCRPC = metastatic castration-resistant prostate cancer; PD1 = programmed cell death protein 1; PDL1 = Programmed death ligand 1; TNF = tumour necrosis factor.

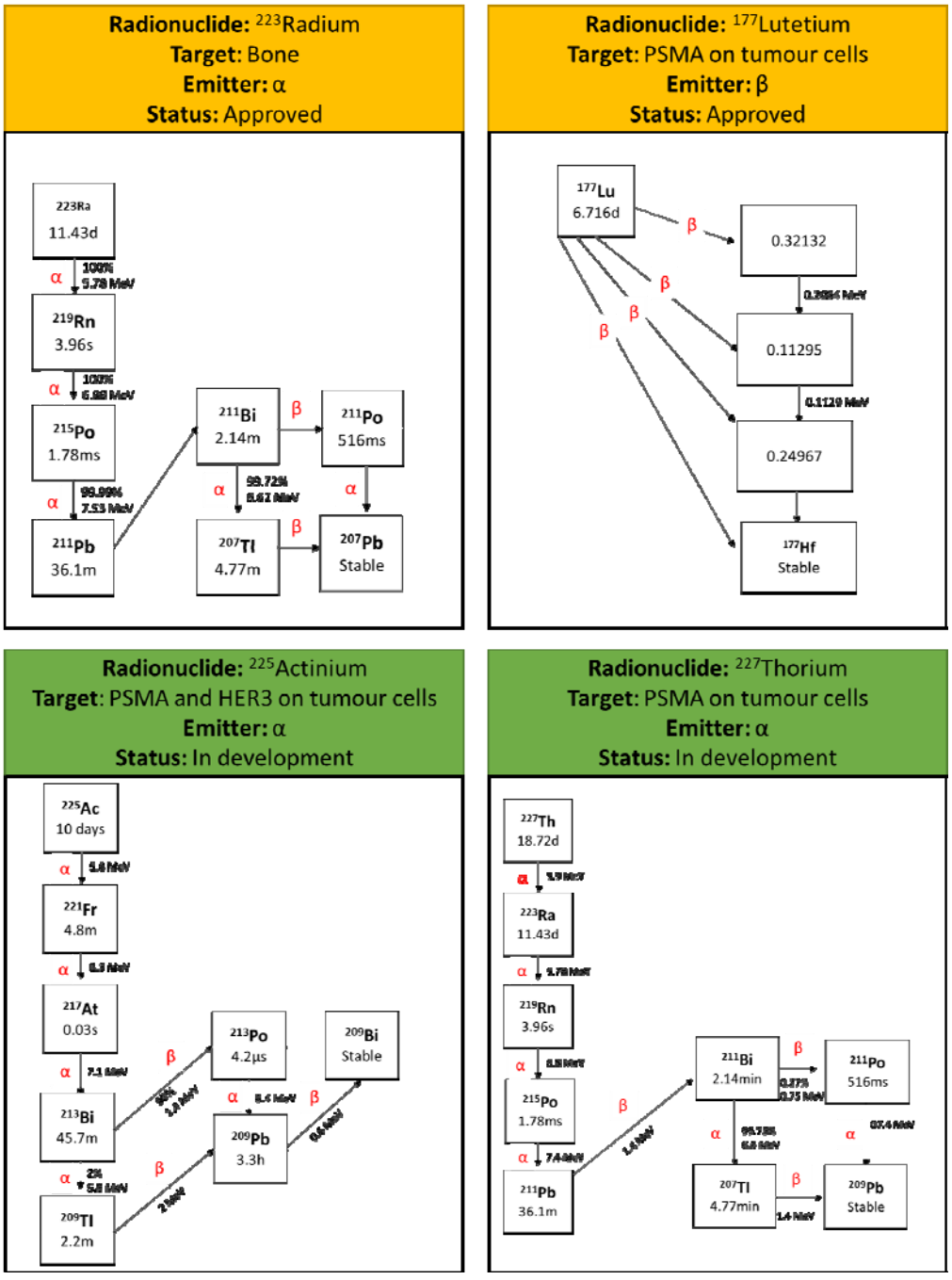

Figure 1: Radionuclides approved by the FDA or currently under investigation for prostate cancer

Currently two radionuclides, ^{223}Raduim (^{223}Ra) and ^{177}Lutetium (^{177}Lu) are approved for treating men with metastatic castration-resistant prostate cancer. The radionuclides ^{225}Actinium (^{225}Ac) and ^{227}Thorium (^{227}Th) conjugated to either prostate-specific membrane antigen (PSMA) or human epidermal growth factor receptor 3 (HER3) are currently in development for men with prostate cancer. All four radionuclides (^{223}Ra, ^{177}Lu, ^{225}Ac and ^{227}Th) have multi-step decay schemes with varying energy depositions. This energy deposition might result in different efficacies in terms of causing DNA damage and any subsequent immune response.

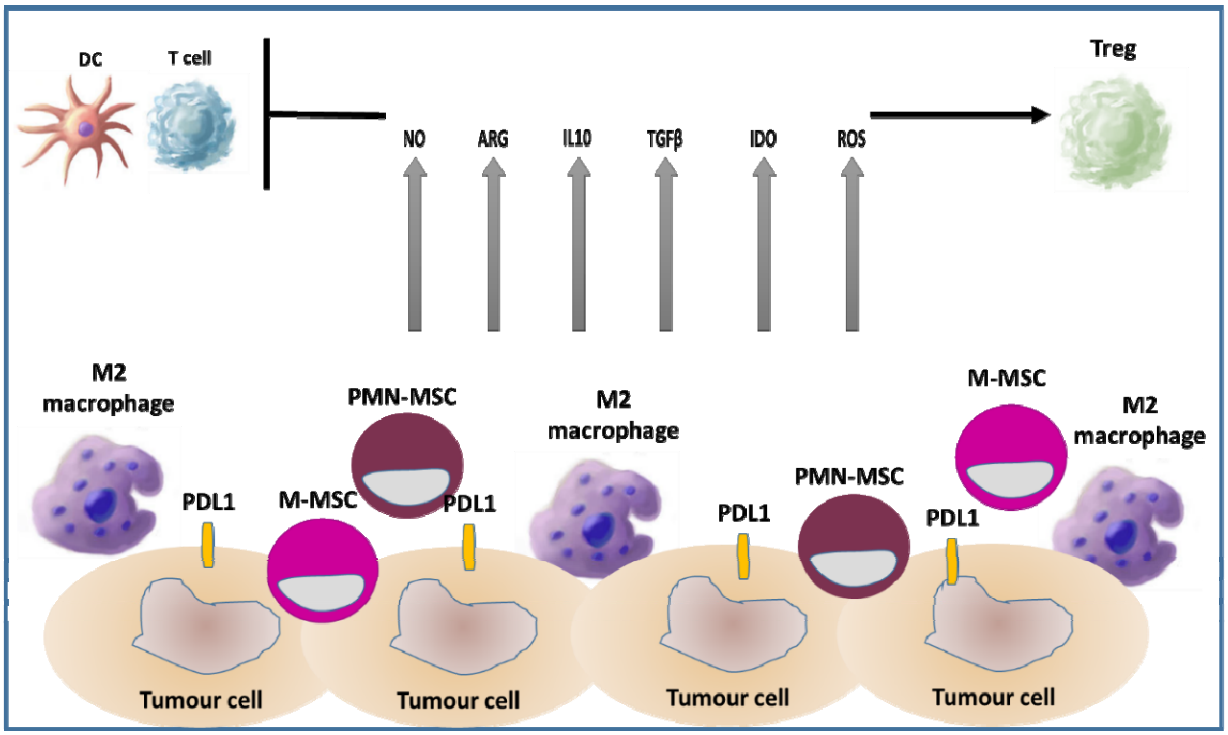

Figure 2. Immune cell composition of prostate cancer.
The tumour immune microenvironment in prostate cancer is rich in myeloid immune cells such as macrophages, myeloid-suppressor cells (M-MSC) and PMN-myeloid suppressor cells (PMN-MSC). These immune cells (most notably the macrophages) are characterised by reduced expression of NO, and increased expression of checkpoint molecules such as PDL1 and secretion of enzymes such as Arg and IDO, cytokines such as IL-10 and TGFβ and reactive species such as ROS. The expression or secretion of these molecules results in reduced antigen presentation capacity of dendritic cells, increased T cell exhaustion and regulatory T cells (Tregs) recruitment. Collectively, this creates an immunosuppressive microenvironment that is characteristic of prostate tumours.). *Arg = arginases; IDO = indoleamine 2,3-dioxygenase; IL10 = interleukin 10; NO = nitric oxide; PDL1 = programme death ligand 1; ROS = reactive oxygen species; TGFβ = transforming growth factor beta.*

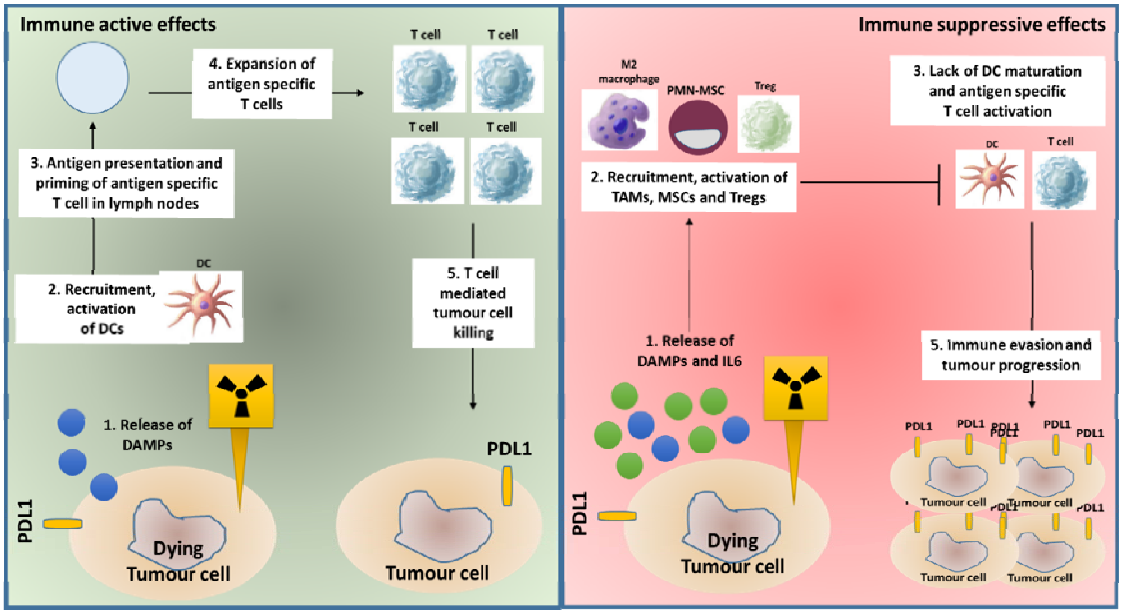

Figure 3. Opposing immune activation and immune suppressive effects of radiotherapy. Radiotherapy can have opposing immune effects, which can subsequently lead to either enhanced tumour-specific immune killing or immune evasion. A | Immune-mediated tumour killing occurs when radiotherapy-induced damage causes dying tumour cells to release damage associated molecular patterns (DAMPs) (1). Release of DAMPs results in activation of dendritic cells (2) and presentation of tumour-specific antigens (3). These dendritic cells then move to the lymph nodes and prime naive T cells (3), resulting in expansion of antigen-specific T cell clones (4). These T cells traffic to the tumour and induce T cell-mediated tumour cell death (5). B| In the suppressive setting, radiotherapy fails to induce immunogenic cell death owing to the high presence of suppressive immune cells. Radiotherapy induces release of DAMPs and cytokines such as interleukin 6 (IL6) (1). Release of IL6 recruits and activates tumour associated macrophages (TAMs), suppressive myeloid-cells (M-MSC)such as PMN-myeloid suppressor cells (PMN-MSC) and regulatory T cells (Tregs) (2). The presence of these suppressive cell types hinders the activation of dendritic cells and T cells (3) and leads to subsequent immune evasion and tumour progression (4)