

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

## 4,800

Open access books available

## 122,000

International authors and editors

## 135M

Downloads

Our authors are among the

## 154

Countries delivered to

## TOP 1%

most cited scientists

## 12.2%

Contributors from top 500 universities

**WEB OF SCIENCE™**Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.

For more information visit [www.intechopen.com](http://www.intechopen.com)

# Combination of Immunotherapy & Radiotherapy for the Treatment of Prostate Cancer

Zsuzsanna Tabi, Lisa K. Spary and John Staffurth  
*Department of Oncology, School of Medicine, Cardiff University, Cardiff  
United Kingdom*

## 1. Introduction

There are around 35,000 new cases of prostate carcinoma (PCa) in the UK per annum, making it the most common solid malignancy. Approximately 10,000 men die of PCa each year in the UK (<http://infocancerresearchuk.org/cancerstats/>). Disease incidence is increasing partly due to earlier detection and the increasing age of the population. Environmental causes especially dietary factors have been postulated but this is still an area of research. At presentation ~60% of patients have localised, ~30% locally advanced and 10% metastatic disease.

Radical radiotherapy (RT) can be used as part of curative therapy for both localised and locally advanced disease but has no proven role in the metastatic setting. Recently, radiation has been shown to cause immunogenic tumour cell death and to modify immunosuppression in the tumour environment. Importantly, reduction of tumour burden by RT, in an ablative setting, has been shown to depend largely on T cell responses (Lee et al., 2009). Combination of ionising radiation (IR) and immunological approaches in pre-clinical models of PCa has also proved to be synergistic. Immunotherapy offers a unique co-treatment that enables the patients' own immune cells to contribute to the success of RT. Immunological memory, developing as the result of the combination treatment, may provide long-term protection from tumour recurrence. There are however very few clinical trials addressing how immunotherapy and RT can be best combined for clinical efficiency.

## 2. Radiotherapy of prostate cancer

There are four major treatment approaches for localised prostate cancer: active surveillance, radical prostatectomy, external beam radiotherapy (EBRT) and low-dose rate (LDR) brachytherapy. RT is conventionally delivered with photons with delivery systems that have developed considerably over the past decade, leading to lower toxicity and allowing safe dose escalation. Higher doses have been demonstrated to improve tumour control outcomes in several large Phase III trials (Viani et al., 2009). Present trials are evaluating the role of intensity modulated radiotherapy (IMRT), hypofractionation (treatment in ~4 weeks) and improved imaging during treatment with image-guided radiotherapy (IGRT) (Khoo & Dearnaley, 2008). Further developments in EBRT delivery systems allow highly targeted treatment in 5-7 fractions, called stereotactic body radiotherapy (SBRT), although tumour control outcomes are not yet known (King et al., 2011; Madsen et al., 2007).

Proton therapy is the delivery of EBRT using protons instead of photons. Protons have a different pattern of dose delivery within tissue, with energy deposited in a very tightly defined area as the protons slow. This results in less radiation being delivered beyond the target, and has become the radiotherapeutic modality of choice for childhood cancers and several other tumors. The evidence base for proton therapy for prostate cancer is less established, but its use in some countries has become widespread partly due to the results of a dose escalation trial using protons (Coen & Zietman, 2009). Proton therapy has not been compared to dose-equivalent photon-RT. LDR brachytherapy, which uses multiple permanently planted radioactive seeds, can be used to deliver a very high radiation dose to a highly targeted volume in a single treatment with equivalent outcomes to EBRT and surgery.

Locally advanced disease is usually treated with a combination of EBRT and androgen deprivation therapy (ADT) (Shelley et al., 2009; Shelley et al., 2009; Warde et al., 2010). However, the outcome is still relatively poor. Recent and ongoing UK-based trials are currently exploring the potential advantage of dose escalation in either systemic therapies (James et al., 2009; Guerrero Urbano et al., 2010). High dose rate (HDR) brachytherapy, which uses a single high-intensity radiation source that is temporarily inserted into multiple positions in the prostate, may also have a role in locally advanced disease as a single agent or in combination with ADT and/or RT (Hoskin, 2008). EBRT has a proven role as adjuvant or salvage therapy after radical prostatectomy. In the adjuvant setting, it has been shown to reduce the rate of relapse in high risk patients by approximately 50% in three randomised trials (Bolla et al., 2007; Thompson et al., 2006; Wiegel et al., 2009).

The commonest site of metastases in castrate refractory metastatic PCa is bone, with 80% of patients dying with prostate cancer dying with bone metastases. They can cause one of several skeletal-related events, but pain is the predominant problem. Palliative EBRT is highly efficacious for single sites of disease. An alternative approach is the use of therapeutic bone-targeted radioisotopes. The interim results of a trial with a novel alpha-emitting isotope, Radium-223, have reported a 3-month overall survival advantage, (<http://www.algeta.com>) suggesting that these drugs will be used more widely in the future. Radioimmunotherapy (RIT) refers to the use of antibody labelled with a therapeutic radionuclide, with the aim of delivering a cytotoxic radiation dose specifically to the tumour. The concept is equivalent to bone-targeted radioisotopes, but with the targeting of tumour-associated antigens (TAA) rather than osteoblastic metastases. The same principle can be used for imaging of micrometastatic disease if radionuclides of different properties (radiation type and energy) are used. There is much research in this field over recent years (Bouchelouche et al., 2011), partly due to the increasing number of PCa-specific TAA, as discussed later in this chapter.

### 3. Immunological aspects of PCa

PCa is an immunogenic cancer, as evidenced by a positive correlation between the frequency of CD8<sup>+</sup> tumour-infiltrating T cells and prostate-specific antigen (PSA) recurrence-free survival (Kärjä et al., 2005). Immune cell behaviour towards tumour cells has been described by three stages: (1) elimination of tumour cells, (2) equilibrium between tumour, and immune cells – maintained by active immunological control of the tumour – and (3) escape of tumour cells from immunological control. Apart from evidence from animal models underpinning this theory (Teng et al., 2008), clinical observations of donor-derived melanoma developing in immunosuppressed organ transplant recipients provide

indirect evidence about the immune system's role in tumour-control (Strauss & Thomas, 2010). Overcoming anti-tumour immune responses is described as an emerging hallmark of cancer (Hanahan & Weinberg, 2011). Further observations and experiments are accumulating in order to provide firm evidence about the role of anti-tumour immune responses in the control of cancer.

### 3.1 Tumour-associated antigens in PCa

The specificity of tumour-infiltrating T cells reflects engagement with TAA. The presence of TAA-specific T cells in the TIL pool results in longer median survival compared to those patients whose TIL did not contain tumour-specific T cells, as observed in melanoma (22.5 months vs. 4.5 months) (Haanen et al., 2005). There is no such prognostic correlation for the frequency of TAA-specific T cells in the peripheral blood of patients. TAA-specific T cell infiltration is likely to be important in PCa too.

PCa-associated antigens include prostate-specific differentiation antigens, expressed both on healthy and malignant prostate epithelial cells, such as kallikrein-4, PAP (prostatic acid phosphatase) and PSA. Tumour antigens that are overexpressed on malignant cells (not all specific for PCa) compared to healthy epithelial cells are: PSMA (prostate specific membrane antigen), PSCA (prostate stem cell antigen), Her-2, MUC-1, survivin, STEAP (six transmembrane epithelial antigen of the prostate) and telomerase. Cancer-germline or cancer-testis oncofetal antigens observed in PCa are not expressed on normal cells, but may be expressed by placental trophoblasts and testicular germ cells, such as NY-ESO, MAGE-C1, MAGE-C2 and 5T4 (Chen et al., 1997; Hudolin et al., 2006; Southall et al., 1990).

More recent additions to the list of potential TAAs in PCa are; AMACR (Honma et al., 2009), WT-1 (Nakatsuka et al., 2006), ADAM-17 (Sinnathamby et al., 2011), RHAMM (CD168) (Gust et al., 2009), SIM2 (Arredouani et al., 2009), TARP (Epel et al., 2008), SH3GLB2 (Fassò et al., 2008) and the androgen receptor (Olson & McNeel, 2011). T cells specific for some of these antigens have been identified in PCa patients and T cell clones or lines killed PCa cells, confirming the suitability of most of these TAA-antigens for targeted therapies.

### 3.2 Tumour-infiltrating immune cells in PCa

Prostate cancer has a complex microenvironment which develops during the course of tumour development. Tumour cells are surrounded by endothelial cells of blood vessels, stromal fibroblasts, bone marrow-derived cells and lymphocytes. These cells produce growth factors and enzymes that enhance tumour growth and survival, aid stroma-remodelling and recruit further immune cells into the tumour. The two main immune cell types infiltrating the tumour are lymphocytes and myeloid cells.

#### 3.2.1 Lymphocytes

The presence of activated T and/or natural killer (NK) cells in the tumour tissue is a positive prognostic factor in several solid cancers, including PCa (Kärjä et al., 2005; Gannon et al., 2009). CD8<sup>+</sup> T cells are responsible for direct killing of target cells which express appropriate peptides on MHC Class I molecules, while NK cells play a role in killing tumour cells which downregulate MHC Class I molecules as an evasion mechanism from T cell recognition. Target cell killing occurs via delivering perforin and apoptosis-inducing granzyme complexes into the target cell (Thiery et al., 2011). CD4<sup>+</sup> T cells, depending on their subtype: Th1, Th2, Th17 or T regulatory cells (Treg) produce cytokines which support pro- or anti-

inflammatory responses, respectively. CD4<sup>+</sup>, CD8<sup>+</sup> and regulatory T cells are all present in PCa tumour tissue, with CD8<sup>+</sup> T cells being predominant, unlike in the peripheral blood where CD4<sup>+</sup> T cell frequencies are higher (Bronte et al., 2005). The majority of tumour-infiltrating CD8<sup>+</sup> T cells are memory or terminally differentiated cells but their ability to upregulate activation markers is impaired (Bronte et al., 2005; Drake, 2010). In radical prostatectomy specimens of PCa, IFN- $\gamma$  and perforin expression is lower than in T cells in healthy prostate tissue (Ebelt et al., 2008). Lymphocytes can mainly be observed in clusters in peritumoural areas while only a few infiltrate the tumour areas (Fig. 1).

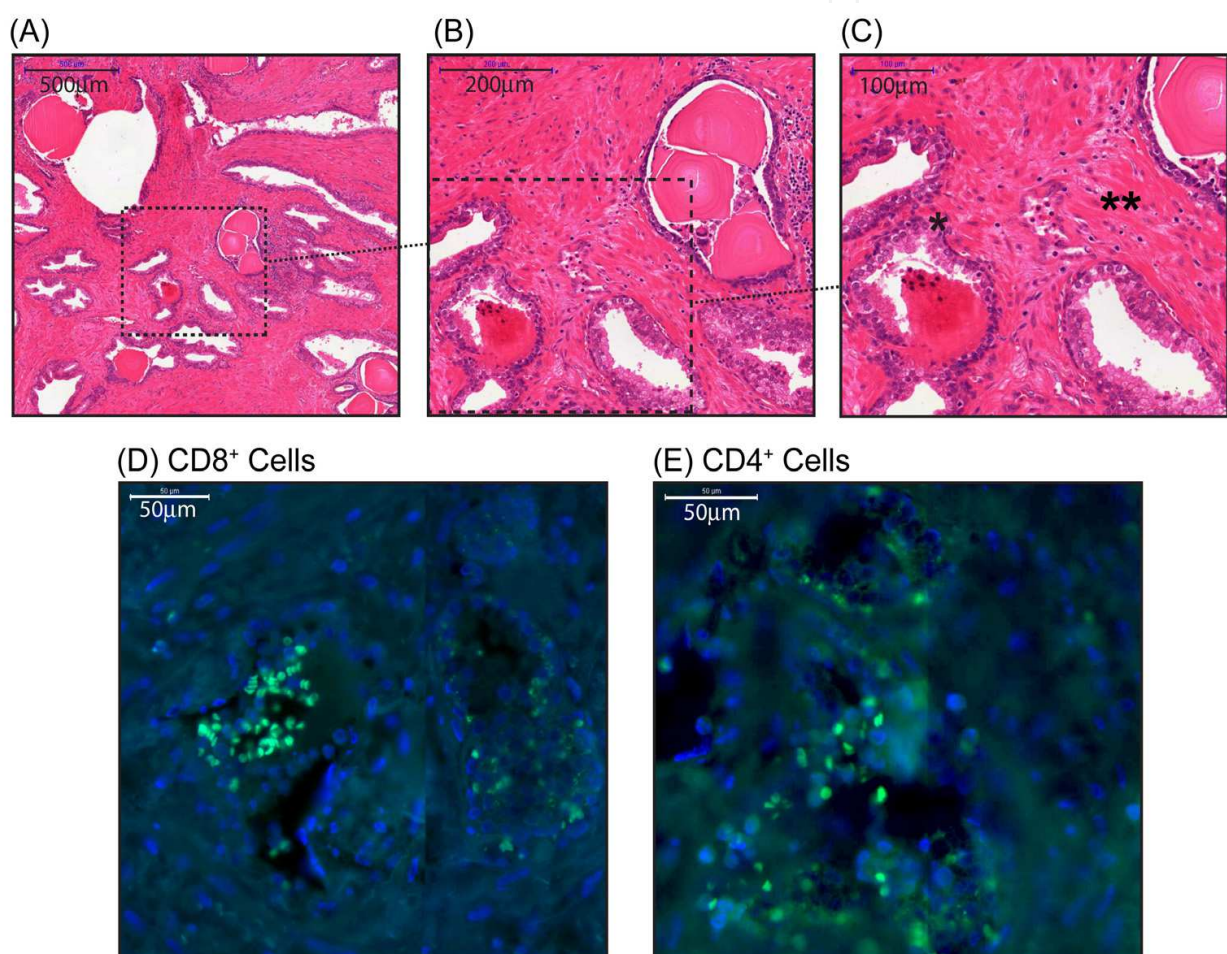


Fig. 1. (A-C) PCa prostatectomy sections stained with haematoxylin and eosin shown at increasing magnifications; (C) a region with glandular (★) and stromal areas (★★). (D) peritumoural CD8<sup>+</sup> T lymphocyte cluster, identified by fluorescence microscopy. (E) CD4<sup>+</sup> cells are also present in this region.

T cells with regulatory function (CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup>) are present at higher frequencies in PCa than in healthy tissue. They can be found mainly in T cell clusters surrounding prostate cancer lesions or in the stroma (Ebelt et al., 2009; Miller et al., 2006; Sfanos et al., 2008). Some of these Treg cells express the glucocorticoid-induced TNF-receptor (GITR) and ICOS (a CD28-superfamily costimulatory molecule) at higher levels than in blood, indicating recent

activation. They also express CCL22 which mediates Treg cell trafficking into the tumour (Miller et al., 2006). T cell clusters infiltrated by Treg cells often express high levels of PD-1 and B7-H1 markers (Ebelt et al., 2008) which indicates T cell exhaustion and functional impairment.

### 3.2.2 Myeloid cells

CD68<sup>+</sup> monocytes and macrophages have been observed at higher frequencies in PCa compared to benign prostate tissues in a Gleason-score and disease stage-associated manner (Lindholm et al., 2010). CD68<sup>+</sup> monocytes and less differentiated CD11b<sup>+</sup>CD33<sup>+</sup> myeloid cells have been shown by immunohistochemistry to be present in PCa stroma (Sorrentino et al., 2011). There is no information available as to whether these cells function as tumour-associated macrophages (TAM) or myeloid-derived suppressor cells (MDSC). Monocytic MDSC have been characterised as CD11b<sup>+</sup>, CD14<sup>+</sup>, CD15<sup>+/-</sup>, CD16<sup>-</sup>, CD33<sup>+</sup>, CD66b<sup>+</sup>, CD124<sup>+</sup>, VEGFR1<sup>-</sup> and HLA-DR<sup>low</sup> cells (Gabrilovich & Nagaraj, 2009; Marigo et al., 2008). More work is needed to establish if immunosuppressive MDSC are present in the microenvironment in PCa. Myeloid-derived dendritic cells (DC) have also been documented in PCa tissues although at relatively low frequencies and in a minimally activated state (Troy et al., 1998).

### 3.3 Immune evasion in PCa

The most common immunosuppressive factors in the tumour tissue include vascular endothelial growth factor (VEGF), transforming growth factor beta (TGF- $\beta$ ), interleukin (IL)-10 and adenosine which inhibit proliferation, differentiation and activation of T lymphocytes and DC.

VEGF supports tumour growth and metastasis by initiating endothelial cell proliferation and the formation of new blood vessels, thus providing a continuous blood supply to the tumour (Carmeliet & Jain, 2000). Increased angiogenesis has been observed in PCa tissue compared to benign prostatic hyperplasia (BPH) (Jackson et al., 1997). VEGF production by PCa cells *in vitro* is enhanced by addition of IL-1 and tumour necrosis factor alpha (TNF- $\alpha$ ), both of which are found in the tumour microenvironment in PCa (Ferrer et al., 1997). VEGF is also important in immune suppression not only by inhibiting DC maturation and T cell development but also by acting as a chemoattractant for MDSC (Gabrilovich et al., 1996; Ohm et al., 2003; Oyama et al., 1998).

TGF- $\beta$  is a pleiotropic cytokine. During PCa development it first acts as a tumour suppressor and later switches roles to become a tumour promoter and immunosuppressor in the tumour environment. TGF- $\beta$  regulates immune cells by inhibiting cytotoxic T cell function, supporting the development of Treg cells and interfering with DC differentiation (Wan & Flavell, 2007; Wrzesinski et al., 2007). PCa-derived TGF- $\beta$  has been shown to convert CD4<sup>+</sup>CD25<sup>-</sup> T cells into CD4<sup>+</sup> CD25<sup>+</sup> Foxp3<sup>+</sup> Treg cells (Liu et al., 2007).

The anti-inflammatory cytokine IL-10 secreted by tumour and stromal cells can inhibit proliferation, differentiation and activation of T lymphocytes via impaired DC (Sato et al., 2002). In the presence of IL-10, alternatively activated DC maintain an immature phenotype in the tumour microenvironment and induce tolerance rather than immune activation and support Treg cell development (H Huang et al., 2010).

Extracellular adenosine is generated from ATP or ADP via the combined action of CD39 and CD73. These are ecto-nucleoside triphosphate diphosphohydrolases which, in the tumour tissue, are predominantly expressed on Treg cells while CD73 is also expressed on CD8<sup>+</sup> T cells and tumour cells (CD73) (Stagg & Smyth, 2010). CD39 and CD73 are also expressed on

tumour-derived exosomes, thus potentially extending the immunosuppressive tumour microenvironment (Clayton et al., 2010). Adenosine, generated by CD39 (ATP to ADP) and CD73 (ADP and AMP to adenosine) engage androgen receptors on effector T cells, monocytes and DC. As a consequence, pro-inflammatory cytokine production (IL-2, IL-4, IFN $\gamma$ , TNF $\alpha$ , IL-12) and co-stimulatory molecule expression (e.g. CD86) decreases and cyclic AMP (cAMP) accumulates in these cells. cAMP further amplifies anti-inflammatory responses (Ernst et al., 2010).

Cytokines, such as monocyte chemoattractant protein-1 (MCP-1) and stromal-derived factor-1 alpha (SDF-1 $\alpha$ ) secreted by tumour cells and tumour-associated stromal cells have been associated with enhanced prostate epithelial cell proliferation and migration (Begley et al., 2005; Lu et al., 2006). They also induce the recruitment of myeloid cells at the tumour site and suppress immune responses (Allavena et al., 2008; Loberg et al., 2007). TAM also produce MCP-1 resulting in an amplification loop for further monocyte recruitment (Allavena et al., 2008).

#### **4. Immunological aspects of radiation therapy**

The effects of IR on human tissue have been studied for decades. The key therapeutic effect is thought to be via direct killing of tumour cells by initiating irreparable double-stranded DNA breaks. However, the consequences of RT are much more complex than that, as radiation also affects tumour stroma, including tumour-resident immune cells, and results in the re-modelling of the tumour microenvironment. One of the consequences is the reduction of immunosuppression in the tumour tissue.

##### **4.1 IR-mediated release of cellular tumour antigens**

The details of the multiple cellular events downstream of radiation-induced DNA damage are beyond the scope of this chapter. The damage results in activation of damage recognition pathways and proliferative arrest, which can ultimately be repaired (fully or partially), or lead to cell death. RT-induced apoptotic and necrotic tumour cell death provide a cellular source of tumour antigens. The tissue damage attracts phagocytic cells to the site of radiation. Monocytes, macrophages and dendritic cells (DC) phagocytose and process dead tumour cells and carry TAA into draining lymph nodes where antigen presentation and T cell stimulation occur. Contrary to natural cell death which occurs without generating an inflammatory response, IR-treated tumour cells express heat shock proteins, translocate antigens such as calreticulin (CR) from the endoplasmic reticulum to the cell surface and passively release high mobility group protein B1 (HMGB1). DNA, RNA and ATP release are also observed at the site of radiation damage. Phagocytosis and simultaneous signalling in DC by HMGB1 via Toll-like receptors (TLR) such as TLR4 and TLR2, or via RAGE (receptor for advanced glycan end products) trigger DC to release IL-1 $\beta$  and present TAA in an immunogenic manner to T cells and B cells (Ma et al., 2011). ATP, released by damaged cells, also contributes to DC maturation via stimulating purinergic P2RX7 receptors and driving IL-1 $\beta$  secretion (Aymeric et al., 2010). Local radiation of mouse B16 tumour has generated DC efficiently cross-present tumour antigens in a Type I interferon (IFN)-dependent manner (Burnette et al., 2011). Single nucleotide polymorphisms (SNP) of TLR4 (Asp299Gly and Thr399Ile) or P2RX7 (Glu496Ala) (Sluyter et al., 2004; Arbour et al., 2000), which affect the function of these molecules, may be associated with worse prognosis, as shown in breast cancer patients undergoing chemotherapy (Apetoh et al., 2007).

#### 4.2 Chemokine and cytokine induction by IR

Tumour cells, not killed by IR, respond to radiation by increasing the production of pro-inflammatory cytokines that include TNF $\alpha$ , IL-1 $\alpha/\beta$ , IL-6 and IL-8 (Shiao & Coussens, 2010; Formenti & Demaria, 2009; Van Der Meeren et al., 1999; Matsumura & Demaria, 2010). The main effect of these cytokines is an inflammatory response and the recruitment of activated T cells and macrophages to irradiated tumours (Formenti & Demaria, 2009). The widely used PCa cell line, LNCaP, is extremely sensitive to TNF $\alpha$  (Chopra et al., 2004). TNF $\alpha$ -treatment results in growth-arrest and apoptosis of LNCaP cells but not of normal prostate epithelial cells, suggesting that IR-induced TNF $\alpha$  expression may selectively induce apoptosis of tumour cells without affecting normal prostate epithelial cells. Immune cell recruitment is further enhanced by the production of the chemokine CXCL16 which is induced by IL-1 $\beta$  and TNF $\alpha$ , both upregulated by IR (Lu et al., 2008). PCa cell lines (LNCaP, DU145, C4-2B and PC3) produce CXCL16 in culture without IR treatment (Lu et al., 2008).

#### 4.3 Irradiated tumour cells become sensitive to immune cell attack

In tumour cells, not killed by IR, surface molecules such as MHC, the death receptor Fas and heat-shock proteins become upregulated (Shiao & Coussens, 2010; Garnett et al., 2004; Lugade et al., 2008). IR-induced upregulation of MHC Class I molecules, on both tumour cells and APC, improves antigen presentation and may enhance tumour cell recognition by activated CD8<sup>+</sup> cells that infiltrate the tumour at an enhanced rate following radiation (Reits et al., 2006; Lugade et al., 2005).

Adhesion molecules, such as intracellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1 and platelet endothelial cell adhesion molecule (PECAM)-1, along with integrins, selectins and cadherins are also upregulated in the tumour tissue by IR (Baluna et al., 2006; Lugade et al., 2008). ICAM-1 is known to be upregulated by inflammatory cytokines such as TNF $\alpha$ , IL-1 $\alpha/\beta$  and IL-6 thus resulting in lymphocyte and macrophage accumulation in inflamed tissues. As previously discussed, these cytokines are upregulated in the tumour tissue as a response to IR. ICAM-1 has an important role in enhancing T cell killing via cell-cell adhesion to lymphocyte function-associated antigen (LFA)-1 and by directly co-stimulating activated T cells (Garnett et al., 2004; Baluna et al., 2006). PCa cells express ICAM-1 and VCAM-1 and in tissue areas of high lymphocyte and neutrophil accumulation the expression of ICAM-1 is significantly elevated (Fujita et al., 2008; Rokhlin & Cohen, 1995). These data suggest that ICAM-1 upregulation by IR may facilitate immune responses by recruiting lymphocytes and macrophages to the tumour site. Increased adhesion between tumour cells with upregulated ICAM-1 and activated CD8<sup>+</sup> T cells expressing LFA-1<sup>+</sup> may result in more powerful cytotoxic T cell activity.

#### 4.4 Direct effect of IR on immune cells

Immune cells are highly susceptible to IR-induced damage and readily undergo apoptosis. Therapeutic doses of RT often result in lymphopenia. One of the potential immunologically positive effects of direct IR on tumour-infiltrating immune cells is the depletion of Treg cells. However, there is some controversy regarding this question. Cao et al. observed that the proportion of Foxp3<sup>+</sup> cells within purified CD4<sup>+</sup>CD25<sup>+</sup> T cell population decreased significantly (48.2% to 23.3%) following irradiation with 1.8 Gy *in vitro* and was abolished (1.2%) by 30 Gy. The suppressive function of these Treg cells was also impaired by IR



probably due to loss of membrane-bound TGF- $\beta$  (Cao et al., 2009). Tregs were especially sensitive to low-dose radiation compared to effector T cells (Cao et al., 2011). Another study, in TRAMP mice, had the opposite findings: Treg cell numbers increased in immune organs after local or whole body irradiation without changes to their functional activity (Kachikwu et al., 2010), indicating relative resistance to 0-20 Gy radiation. It remains to be seen what happens to Tregs in situ during RT of PCa.

We showed recently that lymphopenia following prostate and pelvic RT causes preferential death of naïve or unstimulated T-cells (Tabi et al., 2010). Elevated frequencies of Treg cells were observed in the circulation following 44 Gy radiation in 20 fractions to the pelvic nodes and 55 Gy to the prostate (Fig.2). T cell proliferative function was also impaired (Tabi et al., 2010) but it was restored in vitro with exogenous IL-2 without increasing Treg frequencies (Fig. 2). This indicates that IL-2 maybe used to support T cell function after patients completed standard RT.

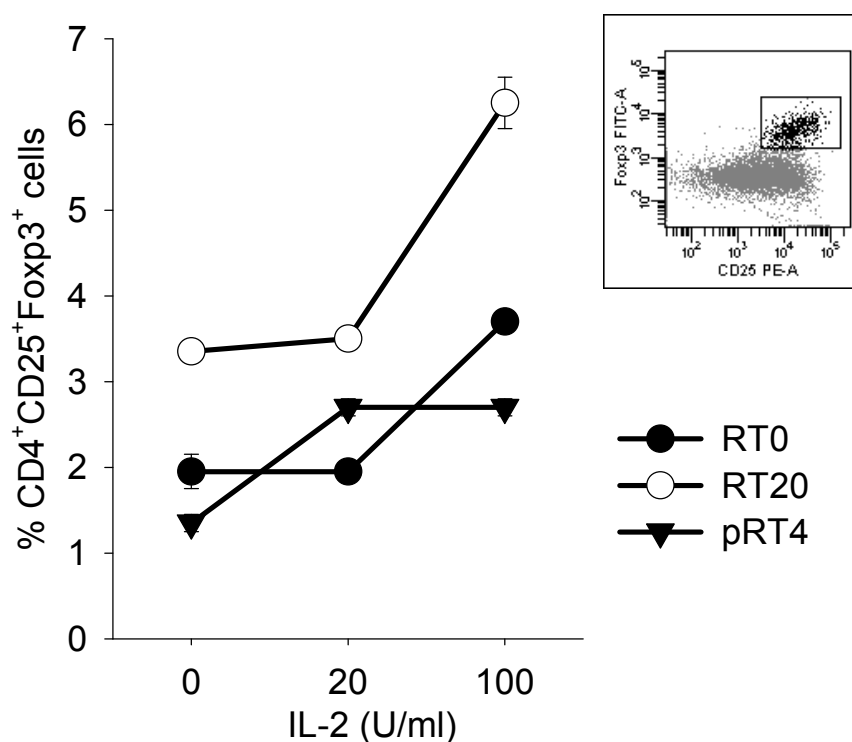


Fig. 2. IL-2 response of Treg cells from the peripheral blood of PCa patients undergoing standard RT. Frequencies of CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> T cells (see gate in insert) were measured before (RT0), immediately after radical radiation in 20 fractions (RT20) and 4 weeks after the last fraction (pRT4). Means and SD of triplicates are shown from a representative patient. Frequencies of Tregs were elevated at RT20, but returned to pre-radiation (RT0) level at pRT4. Unlike at RT0 or RT20, exogenous IL-2, added to the cells in vitro, did not increase Treg frequencies at pRT4.

Most importantly, we identified novel TAA-specific T-cell responses post-RT (Tabi et al., 2010), which were not present before RT. Similar findings were observed by others (Nesslinger et al., 2007; Schaeue et al., 2008), indicating the ability of radiation to shift the balance between tumour-specific regulatory and effector immune mechanisms.

## 5. RT and immunotherapy combination modalities

### 5.1 Pre-clinical models

The use of IL-2 as a monotherapy in cancer has been extensively researched but due to issues with toxicity its clinical use has been limited. In the murine renal adenocarcinoma model, IR was found to augment the response of pulmonary metastases to IL-2 therapy (Younes et al., 1995). Following IR to one lung, plus systemic IL-2 treatment, a reduction in tumour size was observed in both lungs. The effect is dose-dependent and immunohistological studies show significant infiltration of T cells and macrophages at the tumour site. IL-2 is capable of rescuing T cells from IR-induced apoptosis and restores T cell proliferation after RT (Tabi et al., 2010). Its use in combination with RT may minimise the immunosuppressive effects of RT and enhance tumour cell killing via T cells (Mor & Cohen, 1996). In PCa, the combination of IL-2 and radiation in a mouse bone metastases model demonstrated a ~50% inhibition of tumour growth (Hillman et al., 2003). There was a greater degree of tumour destruction in IL-2-treated irradiated tumours than in irradiated tumours alone and the histology revealed increased fibrosis and elevated numbers of infiltrating inflammatory immune cells.

Antitumour effects were also observed in a model utilising IL-12 and RT. IL-12 is secreted by mature DC and macrophages and required for IFN $\gamma$  and TNF $\alpha$  production from T cells and mediates a Th1-type immune response. Adenovirus-derived IL-12 plus RT significantly increased local antitumour and systemic antimetastatic effects in a preclinical model of metastatic PCa when compared to either treatment alone (Fujita et al., 2008). This antimetastatic activity is due to the antitumoural activities of natural killer (NK) cells. These results were also observed in the 4T1 mammary carcinoma. The combination of RT and an adenoviral vector encoding IL-12 and the co-stimulatory molecule CD80 resulted in a significant reduction in tumour growth (Lohr et al., 2000). The antitumour effect observed in the combination therapy group was far superior if the IL-12 and CD80-expressing adenovirus was administered after the final fraction of radiation.

Further cytokine studies have evaluated the combined effect of IL-3 and RT. IL-3 differentiates haematopoietic stem cells into myeloid progenitor cells and stimulates the proliferation of myeloid-derived cells such as DC and monocytes. In mouse models of fibrosarcoma and PCa, IL-3 was found to increase the tumour response to radiation. Combining adenoviral-IL-3 and radiation in the TRAMP-C1 mouse prostate model caused significant delays in tumour growth. Further reports indicated that adenoviral-IL-3 plus radiation enhanced IFN $\gamma$ -producing CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses in the spleen (Oh et al., 2004). This shifted the immune response to a Th1-type response from a suppressive Th2-type response (Tsai et al., 2006).

The combination of the pro-inflammatory cytokine TNF $\alpha$  and RT in mouse mammary carcinoma delayed tumour growth at a greater extent than either treatment alone (Nishiguchi et al., 1990). Similar synergistic effects have also been observed in mouse melanoma, lung adenocarcinoma and brain tumours. The combined effects were attributed to increased recruitment and enhanced activation of lymphocytes and neutrophils (Gridley et al., 1996; Gridley et al., 2002; Gridley et al., 2000; Jin et al., 2005; Li et al., 1998).

Adoptive-cell-transfer (ACT) therapy is the passive transfer of tumour-specific T cells that have been expanded *ex vivo*. Local tumour irradiation can enhance the therapeutic efficacy of ACT therapy (Teitz-Tennenbaum et al., 2009). Combination of RT with ACT of carcinoembryonic antigen (CEA)-specific CD8<sup>+</sup> T cells in a mouse colon carcinoma

demonstrated increased tumour rejection, that could be attributed to the upregulation of the death receptor Fas on the surface of irradiated tumour cells (Chakraborty et al., 2003).

The anti-tumour effect can be further enhanced by intratumoural administration of DC. In a murine metastatic melanoma model reduction in the size of tumour and extent of spontaneous metastasis and prolonged survival were observed after irradiation and intratumoural DC administration. This was associated with an increase in proliferation, accumulation and cytokine production of CD4<sup>+</sup> cells. Similar results were observed in DC plus irradiation in melanoma and sarcoma models (J. Huang et al., 2007). Kjaergaard et al. (2005) reported a method of fusing DC with tumour cells via an electric field resulting in a TAA-DC primed vaccine. These DC/tumour cell fusions induce a potent immune response in combination with local cranial RT in mouse glioma. Both CD4<sup>+</sup> and CD8<sup>+</sup> T cells infiltrate the tumours, leading to complete tumour regression. Tumour rejection was also observed after subsequent tumour challenge, indicating the presence of immunological memory.

Vaccines containing either modified tumour cells that are more immunogenic than the “native” tumour cells or TAA-vaccines have been tested extensively in preclinical models. Combination of cytokine-producing vaccines with local RT in mouse glioma demonstrated that IL-4 and GM-CSF vaccines alone were capable of curing 20-40% of mice but in combination with local RT 80-100% of the mice were cured. The brain tumours were heavily infiltrated with CD4<sup>+</sup> T cells (Lumniczky et al., 2002). The increased anti-tumour effect of GM-CSF and RT was also demonstrated in mouse glioma using a vaccine which contained GM-CSF-secreting tumour cells (Newcomb et al., 2006).

Strategies using RT in combination with monoclonal antibodies that are specific for TAA are now commonly used in clinical oncology (reviewed by Drake, 2010). In a mouse lung cancer model, monoclonal antibody to OX40 (a secondary co-stimulatory molecule expressed on activated CD4<sup>+</sup> and CD8<sup>+</sup> T cells) and RT resulted in a synergistic effect on survival compared to either treatment alone (Yokouchi et al., 2008). The effect was CD8<sup>+</sup> T cell dependent. Antibody-based immunotherapy strategies aiming to neutralise molecules implied in immune tolerance have also been examined. Antibodies for the cytotoxic T lymphocyte antigen (CTLA)-4 (a CD28-superfamily molecule causing T cell functional inhibition) have been shown to induce effective anti-tumour responses via lowering the threshold of tumour-specific T cell activation (reviewed by Drake, 2010). Based on the preclinical findings, CTLA-4 inhibition by the antibody Ipilimumab is now an FDA approved treatment of metastatic melanoma (Chambers et al., 2001). Its combination with RT is being tested in animal models (Dewan et al., 2009) and in clinical trials (see next section).

## 5.2 Clinical trials

There is huge potential for augmentation of the radiation response with the use of immunotherapy but as yet there are only a few clinical trials published. These were carried out in PCa patients at the National Cancer Institute, using a recombinant viral vaccine consisting of recombinant vaccinia virus (rV) encoding PSA, admixed with rV encoding the co-stimulatory molecule B7.1, followed by booster vaccinations with recombinant fowlpox (rFP) vector expressing PSA prior to RT. The product has been further developed and is presently marketed as Prostavac® which encodes ICAM-1 and LFA-3 in addition to B7.1 (PSA-TRICOM). This agent has been shown to improve median overall survival from 16.6 months to 25.1 months in a phase II multi-centre randomized controlled trial in 125 men with asymptomatic or minimally symptomatic metastatic castrate refractory prostate cancer (Kantoff et al., 2010). There was similar progression-free survival in the two arms of the trial,

but the hazard ratio for death was 0.56 (95% CI 0.37 to 0.85) in the PSA-TRICOM arm and the treatment was generally well tolerated.

The initial results of the phase II trial in combination with radiation were highly encouraging. Thirty patients were randomised in a 2:1 ratio to receive vaccine plus EBRT or EBRT alone. In this trial the vaccine consisted of a priming vaccine with rV-PSA plus rV-B7.1 followed by monthly booster vaccines with rFP-PSA. The immunological adjuvants used were GMCSF and high dose IL-2. PSA-specific T cell responses generated prior to RT were not adversely affected by RT, confirming our observation (Tabi et al., 2010). In total, 13 of the 17 patients in the combination arm had increases in PSA-specific T cells and epitope spreading to 4 other prostate cancer TAA (PSMA, PAP, PSCA and MUC-1) was noted (Gulley et al., 2005), possibly due to cross-presentation of a mix of TAA from dying tumour cells by DC (Obeid et al., 2007). There was some IL-2 toxicity, which was reduced in a single-arm follow-up study using lower doses but longer durations of IL-2; immunological effects were equivalent (Gulley et al., 2005; Lechleider et al., 2008).

There is one other reported ongoing work of immunotherapy-radiotherapy combination with intraprostatic injections of autologous DC. The first report confirms the safety of this approach in 5 HLA-A2<sup>+</sup> patients with high risk, localised disease, also treated with ADT, EBRT to 45 Gy and LDR brachytherapy. Autologous intraprostatic DC injections were given at four timepoints during EBRT. Measurable, induced increases in TAA-specific T cell frequencies in peripheral blood using ELISPOT were observed in some patients. The pattern of distribution of CD8<sup>+</sup> cells in tissue was consistent with PCa TAA-targeting, rather than non-specific organ infiltration (Finkelstein et al., 2011).

There are no other published studies in PCa patients of EBRT in combination with cytokines, antibodies, immune modulators or immunologically relevant gene therapy. However, there are a few prostate cancer clinical trials combining RT and immunotherapy currently recruiting according to the ClinicalTrials.gov website, such as anti-CTLA-4 (Ipilimumab) antibody therapy in castration-resistant prostate cancer following RT (Phase III trial, Bristol-Myers Squibb; NCT00861614) and treatment with anti-OX40 and cyclophosphamide in combination with RT in metastatic prostate cancer (Providence Health & Services, Oregon USA, Phase I/II trial; NCT01303705).

### 5.3 Designs of combination clinical trials

Clinical trial design, investigating the benefit of immunotherapy in addition to EBRT is challenging in PCa, as long-term tumour control outcome is already good in both localised (assuming dose escalated image-guided IMRT) and locally advanced disease (assuming combination of long term ADT and EBRT). Phase III trials, adequately powered to show a clinically relevant improvement, would need to address biochemical relapse-free survival or overall survival, both of which require prolonged follow-up of many hundreds and perhaps thousands of patients. Before such trials were undertaken, it is important to optimise the immunotherapy-RT schedules. Reliable biomarkers of treatment-efficacy are needed and this is difficult, especially if neoadjuvant or adjuvant ADT is used, as changes in PSA-kinetics become redundant in these patients. Therefore, development of reliable immunological biomarkers is crucial. We believe that the presence of systemic TAA-specific T cell responses is likely to be the most reliable and easily detectable indicator of a sustainable immunological effect. It may also assist patient selection for optimised treatment of those patients who are most likely to benefit from combination therapies.

The successful result of the IMPACT trial showing the survival advantage with Sipuleucel-T is expected to lead to a dramatic increase in the use of systemic immunotherapy for prostate cancer (Sonpavde et al., 2011). Presently there are only production facilities within the USA and the cost is likely to remain prohibitive for many healthcare systems. Radiation has great potential to augment the effect of systemic immunotherapy and we can expect many combination trials in the future. Clinical trial design in this setting will remain challenging as immunotherapy appears to improve overall survival but demonstrable tumour or biochemical responses are often delayed. Augmenting immunotherapy with radiation may improve overall advantage further.

## 6. Conclusions

PCa has become a huge burden on the health and wealth of Western societies. It is now the commonest solid malignancy and in spite of an excellent general outcome it is expected to become the main cause of cancer-related death in men. There is therefore an urgent need for improved therapies. Sipuleucel-T for PCa was the first immunotherapy to show a survival advantage in cancer, and it has been quickly followed by ipilimumab in melanoma. Radiotherapy has many applications for PCa and it is clear that there are many interactions between immunotherapy and radiation. In general these are positive such that immunotherapy may improve outcomes for those being irradiated and vice versa. The challenge is to move the science into the clinical setting, optimising combinations and sequences, identification of appropriate biomarkers and designing appropriate trials.

## 7. Acknowledgements

Experimental work presented in this chapter was supported by Cancer Research Wales (Cardiff UK) to ZT, LKS and JS and by Velindre NHS Trust R&D (Cardiff, UK) to JS.

## 8. References

- Allavena, P., Sica, A., Solinas, G., Porta, C. & Mantovani, A. (2008). The inflammatory micro-environment in tumor progression: the role of tumor-associated macrophages. *Critical Reviews in Oncology/Hematology* 66, 1, (Apr), 1-9.
- Apetoh, L., Ghiringhelli, F., Tesniere, A., Criollo, A., Ortiz, C., Lidereau, R., Mariette, C., Chaput, N., Mira, J.P., Delaloge, S., Andre, F., Tursz, T., Kroemer, G. & Zitvogel, L. (2007). The interaction between HMGB1 and TLR4 dictates the outcome of anticancer chemotherapy and radiotherapy. *Immunol Rev* 220, (Dec), 47-59.
- Arbour, N., Lorenz, E., Schutte, B., Zabner, J., Kline, J., Jones, M., Frees, K., Watt, J. & Schwartz, D. (2000). TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet* 25, 2, 187-191.
- Arredouani, M., Lu, B., Bhasin, M., Eljanne, M., Yue, W., Mosquera, J., Buble, G., Li, V., Rubin, M., Libermann, T. & Sanda, M. (2009). Identification of the transcription factor single-minded homologue 2 as a potential biomarker and immunotherapy target in prostate cancer. *Clin Cancer Res* 15, 18, 5794-5802.
- Aymeric, L., Apetoh, L., Ghiringhelli, F., Tesniere, A., Martins, I., Kroemer, G., Smyth, M. & Zitvogel, L. (2010). Tumor cell death and ATP release prime dendritic cells and efficient anticancer immunity. *Cancer Res* 70, 3, 855-858.

- Baluna, R.G., Eng, T.Y. & Thomas, C.R. (2006). Adhesion molecules in radiotherapy. *Radiat Res* 166, 6, (Dec), 819-831.
- Begley, L., Monteleon, C., Shah, R.B., Macdonald, J.W. & Macoska, J.A. (2005). CXCL12 overexpression and secretion by aging fibroblasts enhance human prostate epithelial proliferation in vitro. *Aging Cell* 4, 6, (Dec), 291-298.
- Bolla, M., Van Poppel, H. & Collette, L. (2007). [Preliminary results for EORTC trial 22911: radical prostatectomy followed by postoperative radiotherapy in prostate cancers with a high risk of progression]. *Cancer Radiother* 11, 6-7, (Nov), 363-369.
- Bouchelouche, K., Tagawa, S.T., Goldsmith, S.J., Turkbey, B., Capala, J. & Choyke, P. (2011). PET/CT imaging and radioimmunotherapy of prostate cancer. *Semin Nucl Med* 41, 1, (Jan), 29-44.
- Bronte, V., Kasic, T., Gri, G., Gallana, K., Borsellino, G., Marigo, I., Battistini, L., Iafrate, M., Prayer-Galetti, T., Pagano, F. & Viola, A. (2005). Boosting antitumor responses of T lymphocytes infiltrating human prostate cancers. *J Exp Med* 201, 8, 1257-1268.
- Burnette, B.C., Liang, H., Lee, Y., Chlewicki, L., Khodarev, N.N., Weichselbaum, R.R., Fu, Y.X. & Auh, S.L. (2011). The efficacy of radiotherapy relies upon induction of type I interferon -dependent innate and adaptive immunity. *Cancer Res* 71, 7, (Apr 1), 2488-2496.
- Cao, M., Cabrera, R., Xu, Y., Liu, C. & Nelson, D. (2009). Gamma irradiation alters the phenotype and function of CD4+CD25+ regulatory T cells. *Cell Biol Int* 33, 5, (May), 565-571.
- Cao, M., Cabrera, R., Xu, Y., Liu, C. & Nelson, D. (2011). Different radiosensitivity of CD4+CD25+ regulatory T cells and effector T cells to low dose gamma irradiation in vitro. *Int J Rad Biol* 87, 1, 71-80.
- Carmeliet, P. & Jain, R.K. (2000). Angiogenesis in cancer and other diseases. *Nature* 407, 6801, (Sep 14), 249-257.
- Chakraborty, M., Abrams, S.I., Camphausen, K., Liu, K., Scott, T., Coleman, C.N. & Hodge, J.W. (2003). Irradiation of tumor cells up-regulates Fas and enhances CTL lytic activity and CTL adoptive immunotherapy. *J Immunol* 170, 12, (Jun 15), 6338-6347.
- Chambers, C., Kuhns, M., Egen, J. & Allison, J. (2001). CTLA-4-mediated inhibition in regulation of T cell responses: mechanisms and manipulation in tumor immunotherapy. *Ann Rev Immunol* 19, 565-594.
- Chen, Y., Scanlan, M., Sahin, U., Türeci, O., Gure, A., Tsang, S., Williamson, B., Stockert, E., Pfreundschuh, M. & Old, L. (1997). A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. *Proc Natl Acad Sci USA* 94,5,1914-1918.
- Chopra, D.P., Menard, R.E., Januszewski, J. & Mattingly, R.R. (2004). TNF-alpha-mediated apoptosis in normal human prostate epithelial cells and tumor cell lines. *Cancer Lett* 203, 2, (Jan 20), 145-154.
- Clayton, A., Al-Taei, S., Webber, J., Mason, M.D. & Tabi, Z. (2011). Cancer exosomes express CD39 and CD73, which suppress T cells through adenosine production. *J Immunol* 187, 2, (July 15) 676-683.
- Coen, J. & Zietman, A. (2009). Proton radiation for localized prostate cancer. *Nat Rev Urol* 6, 6, 324-330.
- Dewan, M., Galloway, A., Kawashima, N., Dewyngaert, J., Babb, J., Formenti, S. & Demaria, S. (2009). Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody. *Clin Cancer Res* 15, 17, 5379-5388.

- Drake, C.G. (2010). Prostate cancer as a model for tumour immunotherapy. *Nat Rev Immunol* 10, 8, (Aug), 580-593.
- Ebelt, K., Babaryka, G., Figel, A., Pohla, H., Buchner, A., Stief, C., Eisenmenger, W., Kirchner, T., Schendel, D. & Noessner, E. (2008). Dominance of CD4+ lymphocytic infiltrates with disturbed effector cell characteristics in the tumor microenvironment of prostate carcinoma. *Prostate* 68, 1, 1-10.
- Ebelt, K., Babaryka, G., Frankenberger, B., Stief, C., Eisenmenger, W., Kirchner, T., Schendel, D. & Noessner, E. (2009). Prostate cancer lesions are surrounded by FOXP3+, PD-1+ and B7-H1+ lymphocyte clusters. *Eur J Cancer* 45, 9, 1664-1672.
- Epel, M., Carmi, I., Soueid-Baumgarten, S., Oh, S., Bera, T., Pastan, I., Berzofsky, J. & Reiter, Y. (2008). Targeting TARP, a novel breast and prostate tumor-associated antigen, with T cell receptor-like human recombinant antibodies. *Eur J Immunol* 38, 6, 1706-1720.
- Ernst, P.B., Garrison, J.C. & Thompson, L.F. (2010). Much ado about adenosine: adenosine synthesis and function in regulatory T cell biology. *J Immunol* 185, 4, (Aug 15) 1993-1998.
- Fassò, M., Waitz, R., Hou, Y., Rim, T., Greenberg, N., Shastri, N., Fong, L. & Allison, J. (2008). SPAS-1 (stimulator of prostatic adenocarcinoma-specific T cells)/SH3GLB2: A prostate tumor antigen identified by CTLA-4 blockade. *Proc Natl Acad Sci USA* 105, 9, 3509-3514.
- Ferrer, F.A., Miller, L.J., Andrawis, R.I., Kurtzman, S.H., Albertsen, P.C., Laudone, V.P. & Kreutzer, D.L. (1997). Vascular endothelial growth factor (VEGF) expression in human prostate cancer: in situ and in vitro expression of VEGF by human prostate cancer cells. *J Urol* 157, 6, (Jun), 2329-2333.
- Finkelstein, S.E., Gabrilovich, D., Rodriguez, F.A., Chuang, T., Kang, L., Torres-Roca, J., Heysek, R., Pow-Sang, J.M., Antonia, S., Fishman, M.N. & Immunotherapy Research Group. (2011). Know when to say when: Serial assessment of apoptosis and lymphocyte infiltrates with combined intraprostatic autologous dendritic cell immunotherapy radiation therapy in high-risk prostate cancer. *ASCO Meeting Abstracts* 29, 7S, 149.
- Formenti, S.C. & Demaria, S. (2009). Systemic effects of local radiotherapy. *Lancet Oncol* 10, 7, (Jul), 718-726.
- Fujita, K., Ewing, C.M., Sokoll, L.J., Elliott, D.J., Cunningham, M., De Marzo, A.M., Isaacs, W.B. & Pavlovich, C.P. (2008). Cytokine profiling of prostatic fluid from cancerous prostate glands identifies cytokines associated with extent of tumor and inflammation. *Prostate* 68, 8, (Jun 1), 872-882.
- Gabrilovich, D.I., Chen, H.L., Girgis, K.R., Cunningham, H.T., Meny, G.M., Nadaf, S., Kavanaugh, D. & Carbone, D.P. (1996). Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. *Nat Med* 2, 10, (Oct), 1096-1103.
- Gabrilovich, D.I. & Nagaraj, S. (2009). Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol* 9, 3, (Mar), 162-174.
- Gannon, P., Poisson, A., Delvoeye, N., Lapointe, R., Mes-Masson, A. & Saad, F. (2009). Characterization of the intra-prostatic immune cell infiltration in androgen-deprived prostate cancer patients. *J Immunol Methods* 348, 1-2, 9-17.
- Garnett, C.T., Palena, C., Chakraborty, M., Tsang, K.Y., Schlom, J. & Hodge, J.W. (2004). Sublethal irradiation of human tumor cells modulates phenotype resulting in enhanced killing by cytotoxic T lymphocytes. *Cancer Res* 64, 21, (Nov 1), 7985-7994.

- Gridley, D.S., Andres, M.L., Garner, C., Mao, X.W. & Slater, J.M. (1996). Evaluation of TNF-alpha effects on radiation efficacy in a human lung adenocarcinoma model. *Oncol Res* 8, 12, 485-495.
- Gridley, D.S., Li, J., Kajioka, E.H., Andres, M.L., Moyers, M.F. & Slater, J.M. (2000). Combination of pGL1-TNF-alpha gene and radiation (proton and gamma-ray) therapy against brain tumor. *Anticancer Res* 20, 6B, (Nov-Dec), 4195-4203.
- Gridley, D.S., Baer, J.R., Cao, J.D., Miller, G.M., Kim, D.W., Timiryasova, T.M., Fodor, I. & Slater, J.M. (2002). TNF-alpha gene and proton radiotherapy in an orthotopic brain tumor model. *Int J Oncol* 21, 2, (Aug), 251-259.
- Guerrero Urbano, T., Khoo, V., Staffurth, J., Norman, A., Buffa, F., Jackson, A., Adams, E., Hansen, V., Clark, C., Miles, E., McNair, H., Nutting, C., Parker, C., Eeles, R., Huddart, R., Horwich, A. & Dearnaley, D.P. (2010). Intensity-modulated Radiotherapy Allows Escalation of the Radiation Dose to the Pelvic Lymph Nodes in Patients with Locally Advanced Prostate Cancer: Preliminary Results of a Phase I Dose Escalation Study. *Clin Oncol* 22, 3, 236-244.
- Gulley, J.L., Arlen, P.M., Bastian, A., Morin, S., Marte, J., Beetham, P., Tsang, K.Y., Yokokawa, J., Hodge, J.W., Menard, C., Camphausen, K., Coleman, C.N., Sullivan, F., Steinberg, S.M., Schlom, J. & Dahut, W. (2005). Combining a recombinant cancer vaccine with standard definitive radiotherapy in patients with localized prostate cancer. *Clin Cancer Res* 11, 9, (May 1), 3353-3362.
- Gust, K., Hofer, M., Perner, S., Kim, R., Chinnaiyan, A., Varambally, S., Moller, P., Rinnab, L., Rubin, M., Greiner, J., Schmitt, M., Kuefer, R. & Ringhoffer, M. (2009). RHAMM (CD168) is overexpressed at the protein level and may constitute an immunogenic antigen in advanced prostate cancer disease. *Neoplasia* 11, 9, 956-963.
- Haanen, J., Baars, A., Gomez, R., Weder, P., Smits, M., de Gruijl, T., von Blomberg, B., Bloemena, E., Scheper, R., van Ham, S., Pinedo, H. & van den Eertwegh, A. (2005). Melanoma-specific tumor-infiltrating lymphocytes but not circulating melanoma-specific T cells may predict survival in resected advanced-stage melanoma patients. *Cancer Immunol Immunother* 1-8.
- Hanahan, D. & Weinberg, R. (2011). Hallmarks of cancer: the next generation. *Cell* 144, 5, 646-674.
- Hillman, G.G., Maughan, R.L., Grignon, D.J., Yudelev, M., Che, M., Abrams, J., Wang, Y., Layer, A., Wright, J.L., Rubio, J. & Forman, J.D. (2003). Responsiveness of experimental prostate carcinoma bone tumors to neutron or photon radiation combined with cytokine therapy. *Int J Radiat Oncol Biol Phys* 56, 5, (Aug 1), 1426-1437.
- Honma, I., Torigoe, T., Hirohashi, Y., Kitamura, H., Sato, E., Masumori, N., Tamura, Y., Tsukamoto, T. & Sato, N. (2009). Aberrant expression and potency as a cancer immunotherapy target of alpha-methylacyl-coenzyme A racemase in prostate cancer. *J Transl Med* 7, 103.
- Hoskin, P. (2008). High dose rate brachytherapy for prostate cancer. *Cancer Radiother* 12, 6-7, (Nov), 512-514.
- Huang, H., Dawicki, W., Zhang, X., Town, J. & Gordon, J. (2010). Tolerogenic dendritic cells induce CD4<sup>+</sup>CD25<sup>hi</sup>Foxp3<sup>+</sup> regulatory T cell differentiation from CD4<sup>+</sup>CD25<sup>-</sup>/loFoxp3<sup>-</sup> effector T cells. *J Immunol* 185, 9, 5003-5010.
- Huang, J., Wang, Y., Guo, J., Lu, H., Lin, X., Ma, L., Teitz-Tennenbaum, S., Chang, A.E. & Li, Q. (2007). Radiation-induced apoptosis along with local and systemic cytokine elaboration is associated with DC plus radiotherapy-mediated renal cell tumor regression. *Clin Immunol* 123, 3, (Jun), 298-310.



- Hudolin, T., Juretic, A., Spagnoli, G., Pasini, J., Bandic, D., Heberer, M., Kosicek, M. & Cacic, M. (2006). Immunohistochemical expression of tumor antigens MAGE-A1, MAGE-A3/4, and NY-ESO-1 in cancerous and benign prostatic tissue. *Prostate* 66, 1, 13 - 18.
- Jackson, M.W., Bentel, J.M. & Tilley, W.D. (1997). Vascular endothelial growth factor (VEGF) expression in prostate cancer and benign prostatic hyperplasia. *J Urol* 157, 6, (Jun), 2323-2328.
- James, N.D., Sydes, M.R., Clarke, N.W., Mason, M.D., Dearnaley, D.P., Anderson, J., Popert, R.J., Sanders, K., Morgan, R.C., Stansfeld, J., Dwyer, J., Masters, J. & Parmar, M.K.B. (2009). Systemic therapy for advancing or metastatic prostate cancer (STAMPEDE): a multi-arm, multistage randomized controlled trial. *BJU Internatl* 103, 4, 464-469.
- Jin, G.H., Jin, S.Z., Liu, Y., Xu, R.M., Yang, J.Z., Pan, X.N. & Liu, S.Z. (2005). Therapeutic effect of gene-therapy in combination with local X-irradiation in a mouse malignant melanoma model. *Biochem Biophys Res Commun* 330, 3, (May 13), 975-981.
- Kachikwu, E., Iwamoto, K., Liao, Y., Demarco, J., Agazaryan, N., Economou, J., McBride, W. & Schaefer, D. (2010). Radiation enhances regulatory T Cell representation. *Int J Radiat Oncol Biol Phys* Epub ahead of print Nov 17.
- Kantoff, P.W., Higano, C.S., Shore, N.D., Berger, E.R., Small, E.J., Penson, D.F., Redfern, C.H., Ferrari, A.C., Dreicer, R., Sims, R.B., Xu, Y., Frohlich, M.W. & Schellhammer, P.F. (2010). Sipuleucel-T Immunotherapy for castration-resistant prostate cancer. *N Engl J Med* 363, 5, 411-422.
- Kärjä, V., Aaltomaa, S., Lipponen, P., Isotalo, T., Talja, M. & Mokka, R. (2005). Tumour-infiltrating lymphocytes: A prognostic factor of PSA-free survival in patients with local prostate carcinoma treated by radical prostatectomy. *Anticancer Res* 25, 6C, 4435-4438.
- Khoo, V.S. & Dearnaley, D.P. (2008). Question of dose, fractionation and technique: ingredients for testing hypofractionation in prostate cancer--the CHHiP trial. *Clin Oncol* 20, 1, (Feb), 12-14.
- King, C.R., Brooks, J.D., Gill, H. & Presti, J.C., Jr. (2011). Long-Term Outcomes from a Prospective Trial of Stereotactic Body Radiotherapy for Low-Risk Prostate Cancer. *Int J Radiat Oncol Biol Phys*, (Feb 5).
- Kjaergaard, J., Wang, L.X., Kuriyama, H., Shu, S. & Plautz, G.E. (2005). Active immunotherapy for advanced intracranial murine tumors by using dendritic cell-tumor cell fusion vaccines. *J Neurosurg* 103, 1, (Jul), 156-164.
- Lechleider, R., Arlen, P., Tsang, K., Steinberg, S., Yokokawa, J., Cereda, V., Camphausen, K., Schlom, J., Dahut, W. & Gulley, J. (2008). Safety and immunologic response of a viral vaccine to prostate-specific antigen in combination with radiation therapy when metronomic-dose interleukin 2 is used as an adjuvant. *Clin Cancer Res* 14, 16, 5284-5291.
- Lee, Y., Auh, S.L., Wang, Y., Burnette, B., Wang, Y., Meng, Y., Beckett, M., Sharma, R., Chin, R., Tu, T., Weichselbaum, R.R. & Fu, Y.-X. (2009). Therapeutic effects of ablative radiation on local tumor require CD8+ T cells: changing strategies for cancer treatment. *Blood* 114, 3, (July 16), 589-595.
- Li, J., Andres, M.L., Fodor, I., Nelson, G.A. & Gridley, D.S. (1998). Evaluation of pGL1-TNF-alpha therapy in combination with radiation. *Oncol Res* 10, 7, 379-387.
- Lindholm, P., Lu, Y., Adley, B., Vladislav, T., Jovanovic, B., Sivapurapu, N., Yang, X. & Kajdacsy-Balla, A. (2010). Role of monocyte-lineage cells in prostate cancer cell invasion and tissue factor expression. *Prostate* 70, 15, 1672-1682.

- Liu, V.C., Wong, L.Y., Jang, T., Shah, A.H., Park, I., Yang, X., Zhang, Q., Lonning, S., Teicher, B.A. & Lee, C. (2007). Tumor evasion of the immune system by converting CD4+CD25- T cells into CD4+CD25+ T regulatory cells: role of tumor-derived TGF-beta. *J Immunol* 178, 5, (Mar 1), 2883-2892.
- Loberg, R.D., Ying, C., Craig, M., Yan, L., Snyder, L.A. & Pienta, K.J. (2007). CCL2 as an important mediator of prostate cancer growth in vivo through the regulation of macrophage infiltration. *Neoplasia* 9, 7, (Jul), 556-562.
- Lohr, F., Hu, K., Haroon, Z., Samulski, T.V., Huang, Q., Beaty, J., Dewhirst, M.W. & Li, C.Y. (2000). Combination treatment of murine tumors by adenovirus-mediated local B7/IL12 immunotherapy and radiotherapy. *Mol Ther* 2, 3, (Sep), 195-203.
- Lu, Y., Cai, Z., Galson, D.L., Xiao, G., Liu, Y., George, D.E., Melhem, M.F., Yao, Z. & Zhang, J. (2006). Monocyte chemotactic protein-1 (MCP-1) acts as a paracrine and autocrine factor for prostate cancer growth and invasion. *Prostate* 66, 12, (Sep 1), 1311-1318.
- Lu, Y., Wang, J., Xu, Y., Koch, A.E., Cai, Z., Chen, X., Galson, D.L., Taichman, R.S. & Zhang, J. (2008). CXCL16 functions as a novel chemotactic factor for prostate cancer cells in vitro. *Mol Cancer Res* 6, 4, (Apr), 546-554.
- Lugade, A., Moran, J., Gerber, S., Rose, R., Frelinger, J. & Lord, E. (2005). Local radiation therapy of B16 melanoma tumors increases the generation of tumor antigen-specific effector cells that traffic to the tumor. *J Immunol* 174, 12, 7516-7523.
- Lugade, A., Sorensen, E., Gerber, S., Moran, J., Frelinger, J. & Lord, E. (2008). Radiation-induced IFN-gamma production within the tumor microenvironment influences antitumor immunity. *J Immunol* 180, 5, 3132-3139.
- Lumniczky, K., Desaknai, S., Mangel, L., Szende, B., Hamada, H., Hidvegi, E.J. & Safrany, G. (2002). Local tumor irradiation augments the antitumor effect of cytokine-producing autologous cancer cell vaccines in a murine glioma model. *Cancer Gene Therapy* 9, 1, (Jan), 44-52.
- Ma, Y., Aymeric, L., Locher, C., Kroemer, G. & Zitvogel, L. (2011). The dendritic cell-tumor cross-talk in cancer. *Current Op Immunol* 23, 1, 146 - 152.
- Madsen, B.L., Hsi, R.A., Pham, H.T., Fowler, J.F., Esagui, L. & Corman, J. (2007). Stereotactic hypofractionated accurate radiotherapy of the prostate (SHARP), 33.5 Gy in five fractions for localized disease: first clinical trial results. *Int J Radiat Oncol Biol Phys* 67, 4, (Mar 15), 1099-1105.
- Marigo, I., Dolcetti, L., Serafini, P., Zanovello, P. & Bronte, V. (2008). Tumor-induced tolerance and immune suppression by myeloid derived suppressor cells. *Immunol Rev* 222, (Apr), 162-179.
- Matsumura, S. & Demaria, S. (2010). Up-regulation of the pro-inflammatory chemokine CXCL16 is a common response of tumor cells to ionizing radiation. *Radiat Res* 173, 4, (Apr), 418-425.
- Miller, A., Lundberg, K., Ozenci, V., Banham, A., Hellström, M., Egevad, L. & Pisa, P. (2006). CD4+CD25high T cells are enriched in the tumor and peripheral blood of prostate cancer patients. *J Immunol* 177, 10, 7398-7405.
- Mor, F. & Cohen, I.R. (1996). IL-2 rescues antigen-specific T cells from radiation or dexamethasone-induced apoptosis. Correlation with induction of Bcl-2. *J Immunol* 156, 2, (Jan 15), 515-522.
- Nakatsuka, S., Oji, Y., Horiuchi, T., Kanda, T., Kitagawa, M., Takeuchi, T., Kawano, K., Kuwae, Y., Yamauchi, A., Okumura, M., Kitamura, Y., Oka, Y., Kawase, I., Sugiyama, H. & Aozasa, K. (2006). Immunohistochemical detection of WT1 protein in a variety of cancer cells. *Mod Pathol* 19, 6, 804-814.

- Nesslinger, N.J., Sahota, R.A., Stone, B., Johnson, K., Chima, N., King, C., Rasmussen, D., Bishop, D., Rennie, P.S., Gleave, M., Blood, P., Pai, H., Ludgate, C. & Nelson, B.H. (2007). Standard treatments induce antigen-specific immune responses in prostate cancer. *Clin Cancer Res* 13, 5, (March 1, 2007), 1493-1502.
- Newcomb, E.W., Demaria, S., Lukyanov, Y., Shao, Y., Schnee, T., Kawashima, N., Lan, L., Dewyngaert, J.K., Zagzag, D., McBride, W.H. & Formenti, S.C. (2006). The combination of ionizing radiation and peripheral vaccination produces long-term survival of mice bearing established invasive GL261 gliomas. *Clin Cancer Res* 12, 15, (Aug 1), 4730-4737.
- Nishiguchi, I., Willingham, V. & Milas, L. (1990). Tumor necrosis factor as an adjunct to fractionated radiotherapy in the treatment of murine tumors. *Int J Radiat Oncol Biol Phys* 18, 3, (Mar), 555-558.
- Obeid, M., Tesniere, A., Ghiringhelli, F., Fimia, G.M., Apetoh, L., Perfettini, J.L., Castedo, M., Mignot, G., Panaretakis, T., Casares, N., Métivier, D., Larochette, N., van Endert, P., Ciccocanti, F., Piacentini, M., Zitvogel, L. & Kroemer, G. (2007). Calreticulin exposure dictates the immunogenicity of cancer cell death. *Nat Med* 13, 1, 54-61.
- Oh, Y.T., Chen, D.W., Dougherty, G.J. & McBride, W.H. (2004). Adenoviral interleukin-3 gene-radiation therapy for prostate cancer in mouse model. *Int J Radiat Oncol Biol Phys* 59, 2, (Jun 1), 579-583.
- Ohm, J.E., Gabrilovich, D.I., Sempowski, G.D., Kisseleva, E., Parman, K.S., Nadaf, S. & Carbone, D.P. (2003). VEGF inhibits T-cell development and may contribute to tumor-induced immune suppression. *Blood* 101, 12, (Jun 15), 4878-4886.
- Olson, B. & McNeel, D. (2011). CD8+ T cells specific for the androgen receptor are common in patients with prostate cancer and are able to lyse prostate tumor cells. *Cancer Immunol Immunother* 60, 6, 781-792.
- Oyama, T., Ran, S., Ishida, T., Nadaf, S., Kerr, L., Carbone, D.P. & Gabrilovich, D.I. (1998). Vascular endothelial growth factor affects dendritic cell maturation through the inhibition of nuclear factor-kappa B activation in hemopoietic progenitor cells. *J Immunol* 160, 3, (Feb 1), 1224-1232.
- Reits, E., Hodge, J., Herberts, C., Groothuis, T., Chakraborty, M., Wansley, E., Camphausen, K., Luiten, R., de Ru, A., Neijssen, J., Griekspoor, A., Mesman, E., Verreck, F., Spits, H., Schlom, J., van Veelen, P. & Neefjes, J. (2006). Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. *J Exp Med* 203, 5, 1259-1271.
- Rokhlin, O.W. & Cohen, M.B. (1995). Expression of cellular adhesion molecules on human prostate tumor cell lines. *Prostate* 26, 4, (Apr), 205-212.
- Sato, K., Yamashita, N. & Matsuyama, T. (2002). Human peripheral blood monocyte-derived interleukin-10-induced semi-mature dendritic cells induce anergic CD4(+) and CD8(+) T cells via presentation of the internalized soluble antigen and cross-presentation of the phagocytosed necrotic cellular fragments. *Cell Immunol* 215, 2, (Feb), 186-194.
- Schaue, D., Comin-Anduix, B., Ribas, A., Zhang, L., Goodglick, L., Sayre, J., Debucquoy, A., Haustermans, K. & McBride, W. (2008). T-cell responses to survivin in cancer patients undergoing radiation therapy. *Clin Cancer Res* 14, 15, 4883-4890.
- Sfanos, K., Bruno, T., Maris, C., Xu, L., Thoburn, C., DeMarzo, A., Meeker, A., Isaacs, W. & Drake, C. (2008). Phenotypic analysis of prostate-infiltrating lymphocytes reveals TH17 and Treg skewing. *Clin Cancer Res* 14, 11, 3254-3261.

- Shelley, M.D., Kumar, S., Coles, B., Wilt, T., Staffurth, J. & Mason, M.D. (2009). Adjuvant hormone therapy for localised and locally advanced prostate carcinoma: A systematic review and meta-analysis of randomised trials. *Cancer Treatment Reviews* 35, 7, 540-546.
- Shelley, M.D., Kumar, S., Wilt, T., Staffurth, J., Coles, B. & Mason, M.D. (2009). A systematic review and meta-analysis of randomised trials of neo-adjuvant hormone therapy for localised and locally advanced prostate carcinoma. *Cancer Treatment Reviews* 35, 1, 9-17.
- Shiao, S.L. & Coussens, L.M. (2010). The tumor-immune microenvironment and response to radiation therapy. *J Mammary Gland Biol Neoplasia* 15, 4, (Dec), 411-421.
- Sinnathamby, G., Zerfass, J., Hafner, J., Block, P., Nickens, Z., Hobeika, A., Secord, A., Lyerly, H., Morse, M. & Philip, R. (2011). ADAM metallopeptidase domain 17 (ADAM17) is naturally processed through major histocompatibility complex (MHC) class I molecules and is a potential immunotherapeutic target in breast, ovarian and prostate cancers. *Clin Exp Immunol* 163, 3, 324-332.
- Sluyter, R., Shemon, A. & Wiley, J. (2004). Glu496 to Ala polymorphism in the P2X7 receptor impairs ATP-induced IL-1 beta release from human monocytes. *J Immunol* 172, 6, 3399-3405.
- Sonpavde, G., Agarwal, N., Choueiri, T. & Kantoff, P. (2011). Recent advances in immunotherapy for the treatment of prostate cancer. *Expert Opin Biol Ther* 11, 8, 997-1009.
- Sorrentino, C., Musiani, P., Pompa, P., Cipollone, G. & Di Carlo, E. (2011). Androgen deprivation boosts prostatic infiltration of cytotoxic and regulatory T lymphocytes and has no effect on disease-free survival in prostate cancer patients. *Clin Cancer Res* 17, 6, 1571-1581.
- Southall, P., Boxer, G., Bagshawe, K., Hole, N., Bromley, M. & Stern, P. (1990). Immunohistological distribution of 5T4 antigen in normal and malignant tissues. *Br J Cancer* 61, 1, 89-95.
- Stagg, J. & Smyth, M.J. (2010). Extracellular adenosine triphosphate and adenosine in cancer. *Oncogene* 29, (Sept 30), 5346-5358.
- Strauss, D. & Thomas, J. (2010). Transmission of donor melanoma by organ transplantation. *Lancet Oncol* 11, 8, 790-796.
- Tabi, Z., Spary, L., Coleman, S., Clayton, A., Mason, M.D. & Staffurth, J. (2010). Resistance of CD45RA<sup>+</sup> T cells to apoptosis and functional impairment, and generation of tumor-antigen specific T cell responses during radiation therapy of prostate cancer. *J Immunol* 185, 2, (Jul 15), 1330-1339.
- Teitz-Tennenbaum, S., Li, Q., Davis, M.A., Wilder-Romans, K., Hoff, J., Li, M. & Chang, A.E. (2009). Radiotherapy combined with intratumoral dendritic cell vaccination enhances the therapeutic efficacy of adoptive T-cell transfer. *J Immunother* 32, 6, (Jul), 602-612.
- Teng, M., Swann, J., Koebel, C., Schreiber, R. & Smyth, M. (2008). Immune-mediated dormancy: an equilibrium with cancer. *J Leukocyte Biol* 84, 4, 988-993.
- Thiery, J., Keefe, D., Boulant, S., Boucrot, E., Walch, M., Martinvalet, D., Goping, I., Bleackley, R., Kirchhausen, T. & Lieberman, J. (2011). Perforin pores in the endosomal membrane trigger the release of endocytosed granzyme B into the cytosol of target cells. *Nat Immunol*, 19 Jun, Epub ahead of print.
- Thompson, I.M., Jr., Tangen, C.M., Paradelo, J., Lucia, M.S., Miller, G., Troyer, D., Messing, E., Forman, J., Chin, J., Swanson, G., Canby-Hagino, E. & Crawford, E.D. (2006).

- Adjuvant radiotherapy for pathologically advanced prostate cancer: a randomized clinical trial. *Jama* 296, 19, (Nov 15), 2329-2335.
- Troy, A., Davidson, P., Atkinson, C. & Hart, D. (1998). Phenotypic characterisation of the dendritic cell infiltrate in prostate cancer. *J Urol* 160, 1, (Jul), 214-219.
- Tsai, C.H., Hong, J.H., Hsieh, K.F., Hsiao, H.W., Chuang, W.L., Lee, C.C., McBride, W.H. & Chiang, C.S. (2006). Tetracycline-regulated intratumoral expression of interleukin-3 enhances the efficacy of radiation therapy for murine prostate cancer. *Cancer Gene Therapy* 13, 12, (Dec), 1082-1092.
- Van Der Meeren, A., Squiban, C., Gourmelon, P., Lafont, H. & Gaugler, M.H. (1999). Differential regulation by IL-4 and IL-10 of radiation-induced IL-6 and IL-8 production and ICAM-1 expression by human endothelial cells. *Cytokine* 11, 11, (Nov), 831-838.
- Viani, G., Stefano, E. & Afonso, S. (2009). Higher-than-conventional radiation doses in localized prostate cancer treatment: a meta-analysis of randomized, controlled trials. *Int J Radiat Oncol Biol Phys* 74, 5, 1405-1418.
- Wan, Y. & Flavell, R. (2007). 'Yin-Yang' functions of transforming growth factor-beta and T regulatory cells in immune regulation. *Immunol Rev* 220, 199-213.
- Warde, P., Mason, M., Sydes, M., Gospodarowicz, M., Swanson, G., Kirkbride, P., Kostashuk, E. & al., e. (2010). Intergroup randomised phase III study of androgen deprivation therapy (ADT) plus radiation therapy (RT) in locally advanced prostate cancer (CaP) (NCIC-CTG, SWOG, MRC-UK, INY: T94-0110; NCT00002633). *J Clin Oncol* 28, 18s, CRA 4504.
- Wiegel, T., Bottke, D., Steiner, U., Siegmann, A., Golz, R., Storkel, S., Willich, N., Semjonow, A., Souchon, R., Stockle, M., Rube, C., Weissbach, L., Althaus, P., Rebmann, U., Kalble, T., Feldmann, H.J., Wirth, M., Hinke, A., Hinkelbein, W. & Miller, K. (2009). Phase III postoperative adjuvant radiotherapy after radical prostatectomy compared with radical prostatectomy alone in pT3 prostate cancer with postoperative undetectable prostate-specific antigen: ARO 96-02/AUO AP 09/95. *J Clin Oncol* 27, 18, (Jun 20), 2924-2930.
- Wrzesinski, S., Wan, Y. & Flavell, R. (2007). Transforming growth factor-beta and the immune response: implications for anticancer therapy. *Clin Cancer Res* 13, 18 Pt 1, 5262-5270.
- Yokouchi, H., Yamazaki, K., Chamoto, K., Kikuchi, E., Shinagawa, N., Oizumi, S., Hommura, F., Nishimura, T. & Nishimura, M. (2008). Anti-OX40 monoclonal antibody therapy in combination with radiotherapy results in therapeutic antitumor immunity to murine lung cancer. *Cancer Sci* 99, 2, (Feb), 361-367.
- Younes, E., Haas, G.P., Dezso, B., Ali, E., Maughan, R.L., Kukuruga, M.A., Montecillo, E., Pontes, J.E. & Hillman, G.G. (1995). Local tumor irradiation augments the response to IL-2 therapy in a murine renal adenocarcinoma. *Cell Immunol* 165, 2, (Oct 15), 243-251.



## **Prostate Cancer - Diagnostic and Therapeutic Advances**

Edited by Dr. Philippe E. Spiess

ISBN 978-953-307-319-4

Hard cover, 378 pages

**Publisher** InTech

**Published online** 25, November, 2011

**Published in print edition** November, 2011

In this book entitled "Prostate Cancer - Diagnostic and Therapeutic Advances", we highlight many of the significant advances made in our treatment armamentarium of prostate cancer. The book is subdivided into four sections termed: 1) novel diagnostic approaches, 2) surgical treatments options, 3) radiation therapy and its potential sequelae, and 4) medical management and its treatment complications. After reading the present book, readers will be very familiar with the major clinical advances made in our multifaceted treatment approach to prostate cancer over the past decade. This book is a tribute to our pioneering urologists and allied healthcare professionals who have continually pushed forward our traditional therapeutic envelope.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Zsuzsanna Tabi, Lisa K. Spary and John Staffurth (2011). Combination of Immunotherapy & Radiotherapy for the Treatment of Prostate Cancer, Prostate Cancer - Diagnostic and Therapeutic Advances, Dr. Philippe E. Spiess (Ed.), ISBN: 978-953-307-319-4, InTech, Available from: <http://www.intechopen.com/books/prostate-cancer-diagnostic-and-therapeutic-advances/combination-of-immunotherapy-radiotherapy-for-the-treatment-of-prostate-cancer>

**INTECH**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen