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TITLE PAGE

Immune modulation in advanced radiotherapies: targeting out-of-field effects

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ABSTRACT:

By virtue of being a localized treatment modality, radiotherapy is unable to deliver a tumoricidal radiation dose to tissues outside of the irradiated field. Ionizing radiation may result in the radiation damage mediated by a bystander like effect away from the irradiated field, but this response is likely to be modest when radiotherapy is the sole treatment modality. Over the last decade there has been a re-emergence of immune modulating therapies as an anti-cancer treatment. Clinical benefits from vaccines have on the whole been largely disappointing, but greater response rates have been observed from the immune checkpoint modulators. A clinical benefit of using such agents has been shown in disease sites such as melanoma and non-small cell lung cancer. There is growing pre-clinical data and a number of case reports which suggest the presence of abscopal effects when radiotherapy is delivered during co-administration with immune checkpoint inhibitors, suggesting that this combination may lead to an enhanced tumor response outside of the primary treatment field. In this review, the mechanisms of such an enhanced out-of-field tumor response, the potential clinical utilities, the optimal radiotherapy delivery and considerations for clinical follow-up following treatment are discussed.

MAIN MANUSCRIPT

Introduction:

In the last decade technological advances have permitted more accurate delivery of radiotherapy to target sites within the body, which reduces damage to surrounding normal tissue and allows radiotherapy dose escalation to maximize curability [1]. High dose, high precision and hence highly localized radiotherapy to tumor sites outside of the brain is known as stereotactic ablative body radiotherapy (SABR) [2]. SABR has been used to treat primary lung cancer with comparable control rates to surgery [3, 4]. SABR is also used in the treatment of oligometastatic disease, where a patient has only a small number of metastatic sites are detected by conventional imaging [5]. As the name suggests, SABR delivers high dose, hypofractionated treatment and hence an ablative dose. For example, in the treatment of early stage lung cancer, regimens of 54 to 60 Gy in 3 fractions are delivered in just over a week which is in stark contrast to a standard dose of 60 to 66 Gy delivered in 30 to 33 fractions over 6 weeks [6]. When delivered with consideration for the tolerance of adjacent normal tissues SABR treatments are relatively free from significant toxicity or side-effects [6]. However, in the high dose region the ablative dose (e.g. for a patient receiving 54 Gy in 3 fractions with a maximum dose of up to 130% within the target volume, will in effect receive a biologically equivalent dose (BED) of up to 227 Gy, assuming an α/β ratio of 10) leads to intense radiation damage and hence a significant tissue response with an associated immune response [7]. This immune response can be observed clinically in lung SABR treatments as pneumonitis transforming into fibrosis in the surrounding normal lung [8]. Although this immune response to radiation is clearly undesirable, it may be possible to harness this effect and use it to target cancer cells not irradiated within the primary tumor.

In conjunction with the recent advances in radiotherapy delivery, over the last decade there has also been a greater understanding of the immune response to malignancy which has led to the development of a number of immune modulating agents [9, 10]. Agents such as the immune checkpoint modulators have been shown to lead to regression of a number of solid malignancies and in some cases, a prolonged complete response to treatment has been observed [11]. In addition to this, there is growing pre-clinical data and a growing number of case reports which suggest the presence of abscopal effects when radiotherapy is delivered during co-administration with immune checkpoint inhibitors [12-15]. In the abscopal effect, an enhanced tumor response in disease locations which are outside of the primary treatment field and which have not received any radiation dose is observed [12, 16]. It is suggested that the ablative nature of radiotherapy treatment leads to an immune response which can be augmented by immune modulating agents [17]. The underlying cellular mechanisms driving these out-of-field responses is still poorly understood but a greater mechanistic understanding could provide significant therapeutic opportunities. One area of potential clinical use of the combination of SABR and immune modulating agents is in the setting of oligometastatic disease [18]. The term oligometastatic, initially suggested by Hellman and Weichselman, describes the presence of a limited number of metastatic sites of disease, usually less than 6 in number [19]. If an oligometastatic state is in existence, the implication is that there are no viable micrometastases and that all the metastases that are present have declared themselves [20]. Hence eradication of the oligometastases should lead to long term cure. In this setting the potential of enhanced in-field may lead to an increased chance of long term control and even if a patient's disease is

not truly oligometastatic and if there are clinically undetectable metastases elsewhere, immune mediated out of field responses may prevent progression of non-irradiated undetectable metastasis and may led to an increased chance of a complete response. This review considers possible mechanisms of such an enhanced out of field tumor response, the potential clinical utilities, optimal radiotherapy delivery, the timing of immune modulation and any considerations for clinical follow-up after treatment.

The immune response to malignancy

It has been shown that the immune system can detect and respond to the presence of malignant cells [10, 21]. The immune response to cancer can be activated by the presence of antibodies to antigens on malignant cells, from tumor specific T-cells and tumor infiltrating lymphocytes [22-24]. It has been noted that in some cancers, the presence of tumor infiltrating immune cells correlates with clinical outcome [25]. Although the immune system may mount a response to cells which have undergone malignant transformation, in order to survive and proliferate tumors must develop the ability to evade surveillance and control by the host immune system [26]. Cancers use a variety of mechanisms to evade the immune system which include inhibition of tumor antigen presentation, secretion of immunosuppressive cytokines, recruitment of immunosuppressive cell types and inhibition of attack by immune cells [21, 27]. This ability to avoid immune destruction has been recently described as one of the new updated hallmarks of cancer [28].

Immunotherapeutic approaches in the treatment of cancer

Evasion of the immune system by cancer is essential to the process carcinogenesis, tumor development and metastasis, but modulation of the immune system may also be a potential therapeutic strategy. Immunotherapeutic strategies can be considered as either active or passive. Passive approaches involved the use of exogenous effectors of an immune response such as with humoral anti-tumor antibodies (e.g. Trastuzumab which targets and binds to the extra-cellular domain of the HER-2/neu receptor which interferes with receptor function and expression) or by a cellular adoptive approach such as with allogeneic transplantation [29]. Active approaches include non-specific immune modulation, therapeutic vaccines, modulation of T-cell function and oncolytic viruses. Examples of these are shown in table 1 [30-48].

Non-specific immune stimulation has been in routine clinical use for over a decade with the use of Interferon- α and interleukin-2 in the treatment of diseases such as melanoma and renal cell carcinoma. These agents act by modulating the immune system rather than by a direct cytotoxic effect [30]. However, as the name suggests, these agents are non-specific and produce significant side-effects, such as fatigue and fever, with only modest clinical benefit [31, 32, 49]. In contrast vaccine therapy is highly specific with the immune response targeted only to tumor antigens. Vaccine therapy can be directed against whole cells or directed to specific tumor antigens [50]. Despite early positive signals of response, larger studies of vaccine therapy both in the palliative or adjuvant setting have been on the whole disappointing and it is likely that vaccine therapy alone will not be efficacious strategy in anti-cancer treatment [51-53]. Oncolytic viruses have a dual mode of action. Viruses such as Talimogene laherparepvec are activated only in the malignant cell of interest [48].

When activated these viruses are directly cytotoxic to the malignant cell infected but they also trigger an immune response to the malignant cell, causing antigen presentation of components of the infected malignant cell and thus recognition of the malignant cell is changed and it becomes an immune target.

Of emerging clinical interest are agents which modulate T-cell function. It has been shown that tumors can evade and then escape the immune system by producing immunosuppressive cytokines, such as interferon- γ (IFN γ) either from the tumor cells directly or from cells within in the tumor microenvironment [54, 55]. These immunosuppressive cytokines lead to T-cell inactivation and hence a loss of immune response. A number of immune checkpoint inhibitors such as the anti-CTL antigen-4 (CTLA-4) or anti-programmed death-1 (PD-1) antibodies which inhibit these cytokines by a number of mechanisms have been used in clinical trials and some of the agents are listed in table 1 [41, 44]. CTLA-4 is crucial in maintaining tolerance to self-antigens, hence preventing auto-immune disease and is a master regulator of T-cell activation [56]. In malignancy and in the milieu of the tumor micro-environment CTLA-4 acts to reduce or inactivate the T-Cell response. In a normal immune response the T-cell receptor will bind to a presented antigen. In order for the T-cell to be activated CD28 must also bind to CD80 and CD86 on the surface of the antigen presenting cell to provide a co-stimulation of the T-cell. CTLA-4 competitively competes with the CD28/CD80/CD86 complex and in the tumor micro-environment where CD80 and CD86 are depleted, the result is T-cell inhibition mediated by CTLA-4 [56]. CTLA-4 is also expressed on CD4+ regulatory T cells which are also known as (Tregs). On Tregs CTLA-4 induces the production of the highly suppressive cytokine transforming growth factor beta which also acts to down-

regulate T-cell function [57]. Thus CTLA-4 has a key role in reducing T-cell mediated immunity. Mono-clonal antibodies against CTLA-4 such as ipilimumab have been shown to be effective as single agents, with some durable clinical responses noted [41].

PD-1 is an inhibitory receptor expressed on T-cells whose normal function is crucial for preventing the development of autoimmune disease [58]. PD-1 may not have an overarching role in the normal immune response but it is thought that the function of PD-1 is to limit normal tissue damage in the presence of inflammation [59]. Two main ligands of PD-1 (PD-L1 and PD-L2) have been identified and these bind to PD-1 to inhibit T-cell function and up-regulation of these, within tumours, is common and is associated with a poor prognosis [60]. A number of PD-L1 and PD-L2 inhibitors have been shown to be effective across a range of tumor sites [44, 45]. This mechanism is illustrated in figure 1 and a summary of the currently available PD-1 inhibitors is shown in table 2 [28, 45-47, 61-63].

Why the combination of immunotherapy with radiotherapy may be synergistic.

Out of field effects following radiotherapy treatment, mediated by a number of different mechanisms, have been previously described [64]. One of the clinical manifestations of this, the abscopal effect, has been described in a number of case reports [65-68]. As stated earlier, abscopal effects have been reported when radiotherapy has been delivered to patients on immune modulating agents [12, 15, 69]. In a seminal experiment by Demaria et al, radiation responses were compared between immunocompetent and T-cell deficient (nude) mice each with two tumors. Both sets of mice received irradiation to one tumor only, both sets demonstrated a

retardation of growth in the irradiated tumor but only the immunocompetent mice had growth retardation in the non-irradiated tumor [70]. The authors went onto hypothesize that the abscopal effect must be in some part immune mediated and is T-cell dependent [71].

So might the combination of ionizing radiation and immune modulation be synergistic? The accepted key mechanism of cell death following radiotherapy is that radiation induced damage mediated by free radicals to cellular structures, in particular the DNA and the production of DNA double-strand breaks, which in turn leads to apoptosis when the damage is not repaired [72, 73]. However there is longstanding evidence that radiation exposure can lead to non-targeted effects, specifically bystander signalling mediated by cytokine release [64]. These have been classified into three distinct categories [74]. Firstly, classical bystander effects where irradiated cells signal with non-irradiated cells within a tissue, for example under conditions of localised irradiation including very low dose exposures. Secondly, abscopal effects, as described above, where responses at sites outside the treatment field are observed. A third category have been termed cohort effects where variable dose regions are observed typically within tumours receiving advanced radiotherapies where intercellular signalling leading to out-of-field effects can also play a role [75, 76]. However, in all three of these categories, it is also thought that radiotherapy, through direct cellular damage, leads to antigen presentation and may trigger an immune response [17]. Key to this process is the involvement of the tumor micro-environment [77, 78]. Ionizing radiation induces key chemokines, such as IFN- γ , CXCL9, CXCL10, and CXCL16, which attract effector T cells to the tumor by increasing T-cell motility and vascular permeability to T-cells

[79-81]. Furthermore, following radiation, tumor cells may up-regulate many key cell surface ligands that increase the probability of antigen presentation to cytotoxic T-cells in a process known as immunogenic modulation [82]. Other elements of immunogenic modulation include changes in the mechanism of antigen presentation and translocation of calreticulin to the cell surface [82-85]. It has also been suggested that modulated radiation induced immune responses may show a long duration of effect [86]. Thus the combination of radiotherapy and immune modulation represents a possible new paradigm shift in the management of advanced malignancy [87].

Following the US Food and Drug Administration approval of Sipuleucel-T for the treatment of advanced hormone resistant prostate cancer there is has been a re-emergence of interest in vaccine therapy [35]. A number of pre-clinical and clinical studies have examined the effects of combining radiotherapy with vaccines, the rationale behind this combination is that the immune response mediated by radiotherapy may enhance the efficacy of the vaccine or vice versa. A range of vaccine types have been used in combination with radiotherapy and examples of the human studies are listed in table 3 [88-93]. At present vaccine therapy with radiotherapy is confined to early phase studies across a range of tumor types such as lung cancer [94]. Furthermore, the optimal radiotherapy dose and scheduling of vaccine delivery around radiotherapy will likely depend on the vaccine and tumor type and this will require further clinical investigation [95].

A number of case reports of abscopal effects with the combination of CTLA-4 inhibitors and radiotherapy have led to interest in this combination [12, 69]. However

there are also case reports of immune mediated abscopal responses in patients who have not responded to CTLA-4 inhibitors, hence the mechanism of this interaction is not clear [15]. Agents such as Ipilumimab in these settings used as monotherapy may not be entirely effective in triggering an immune response against the cancer. But, when radiotherapy is delivered, the subsequent immune response in addition to the CTLA-4 inhibitor is sufficient to trigger a T-cell mediated response to the malignant cells. Of note, both intra and inter-personal factors, both in terms of tumor heterogeneity and immune programming most probably will be determinants of differential responses. Of particular interest is the combination of anti-PD-1 antibodies with radiotherapy. The unique role of PD-1 in down-regulating the immune response to inflammation suggests that turning off this pathway in combination with radiotherapy delivery may lead to an enhanced response beyond that seen with anti-PD-1 therapy alone [59]. In a mouse glioma model, the combination of a constructed anti-PD-1 antibody and stereotactic radiotherapy leads to long term survival supporting the efficacy of this combination [13]. In another murine model of breast and colorectal carcinoma, enhanced tumor control was seen with the combination of radiotherapy and an anti-PD-1 antibody [14].

Is radiation dose and fractionation important?

Considering the variables in radiotherapy delivery along with immune modulating agents, it may be hypothesized that higher doses of radiotherapy may lead to increased DNA damage and hence an enhanced immune response. In preclinical studies, low dose irradiation has been shown to increase T-cell migration into the irradiated field [96]. It has also been suggested that radiotherapy doses from as low as 2 Gy but up to 20 Gy may be sufficient to trigger immunogenic cell death [17]. In

the case reports of an abscopal effect with the combination of radiotherapy and ipilumimab, hypofractionated doses of 30 Gy delivered in 5 fractions and 28.5 Gy delivered in 3 fractions were used but these do not represent as high a BED as to those seen in SABR (BED of 48 Gy, assuming an α/β ratio of 10 for the tumor as compared of a BED of 151 Gy with a SABR type dose of 54 Gy delivered in 3 fractions) [12, 15]. Given the high local and distant control rates in these reports, might immunotherapy provide some compensation for the lower radiation doses delivered? However, in the era of high local control with typical SABR doses, maintaining good local control is still a key goal and future clinical studies of radiotherapy combinations with immunotherapy in oligometastatic disease are likely to use SABR type doses to avoid compromise on local control. It has been shown that fractionated but not single dose radiotherapy induces an immune mediated abscopal effect; hence if SABR doses are to be used, they should be fractionated to some extent [97]. What is almost certain is that the immune effect of radiotherapy is very likely to depend on the tumor type and immune modulation used. Although differences in radiotherapy dose, fractionation regimens used and the nature of radiation delivery (photons as comparable to particle beams) will be factors in engendering an abscopal response, where possible and for the purposes of designing high quality controlled studies, radiotherapy delivery should be standardized where possible [98].

Issues for clinical follow-up in the setting of immune modulation

Single agent immune modulating studies have poor clinical response rates. For example in a study of ipilumimab in melanoma the best overall response (complete or partial response) rate was 10.9% [41]. However, a minority of patients achieve

long-term disease control often requiring several months to demonstrate an objective response. This slow to emerge, but prolonged and durable effect of immunotherapy is a feature of many of the clinical studies [11]. Hence in any studies examined the combination of immune modulating agents must undertake careful and prolonged follow-up. Furthermore, early termination of such studies on the grounds of futility must not be at the expense of missing an important late effect.

Conclusions

Radiotherapy has powerful effect on the tumor micro-environment with the potential to reverse the immunosuppressive state present in malignancy. Combinations of immune modulating agents and radiotherapy look promising both in over-coming local resistance to radiotherapy and in generating an out of field or abscopal effect. Future studies of the many possible combinations of immune modulating agents and radiotherapy are urgently needed to explore the potentially powerful abscopal effects reported in animal models and case reports to date. If the early promise from these studies is translated into a larger scale clinical benefit, then the combination of radiotherapy and immunotherapy may truly represent a paradigm shift in oligometastatic disease.

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Table 1: Examples of Active Immunotherapeutic Approaches

Table 2: Summary of PD-1 and PD-L1 checkpoint inhibitors in clinical development

Table 3: Vaccines which have been delivered in conjunction with radiotherapy in human subjects

FIGURE CAPTIONS

Figure 1:

This schema illustrates the 3 various scenarios in a T-cell response to a malignant cell:

A. This represents the normal activated T-cell response to antigen presenting cell or in the case of malignancy, a tumor cell. Following antigen presentation, the binding of CD-28 with CD-80 /CD-86 leads to T-cell activation and which in turn leads to increased T-Cell proliferation and cytokine production.

B. This represent immune escape by the tumor in which the tumor derived PD-L1 binds to PD-D on the T-cell leading to a suppressed T-cell Response with decreased T-cell Proliferation and cytokine production.

C. Illustrates the impact of PD-1 ligand inhibition and thus restored T-cell function.

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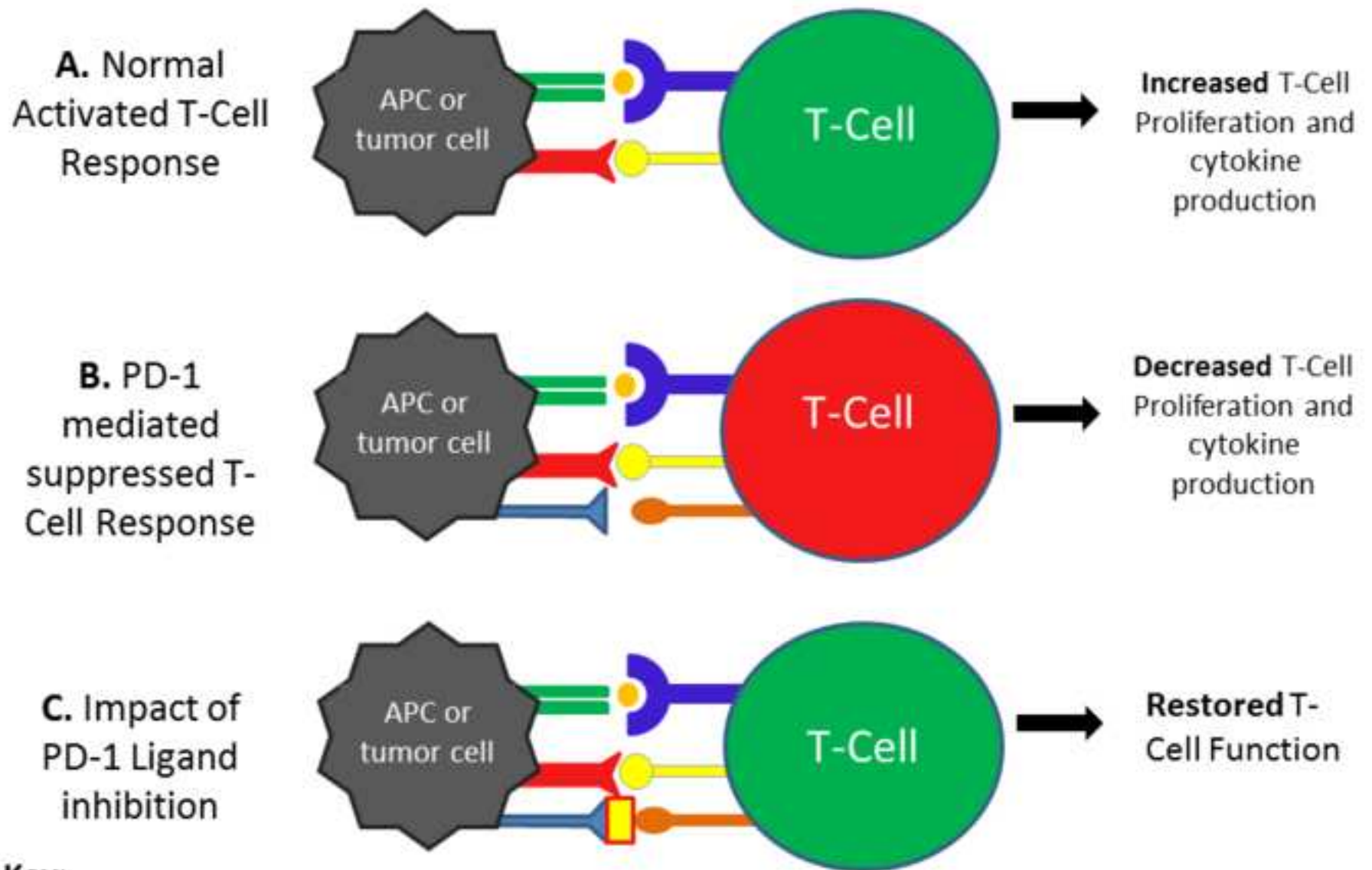
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Immune modulation in advanced radiotherapies: targeting out-of-field effects

Conflict of interest statement:

None of the authors have any conflict of interest to declare



Key:



Tables: Immune modulation in advanced radiotherapies: targeting out-of-field effects.

Table 1: Examples of Active Immunotherapeutic Approaches

<p>Non-specific immune stimulation</p>	<ul style="list-style-type: none"> • Cytokines (e.g. Interleukin-2, Interferon-α) [30-32] • Killer-cell immunoglobulin-like receptors antagonists [33] • Indoleamine 2,3-dioxygenase (IDO) pathway inhibitors [34]
<p>Therapeutic vaccines</p>	<ul style="list-style-type: none"> • Sipuleucel-T (prostate carcinoma) [35] • Anti-MAGE-A3 vaccine (non-small cell lung cancer and others) [36] • Racotumomab – anti N-glycolil (lung carcinoma, breast carcinoma, melanoma) [37] • Tergenpumatucel-L – (lung carcinoma) [38]
<p>Modulate T-Cell function</p>	<ul style="list-style-type: none"> • CD137 agonism [39] • CD40 agonism [40] • CTLA-4 inhibition [41] • LAG-3 inhibition [42] • OX-40 agonism [43] • PD-1 inhibition [44] • PD-L1 inhibition [45] • PD-L2 inhibition [46, 47]
<p>Oncolytic viruses</p>	<ul style="list-style-type: none"> • Talimogene laherparepvec (T-VEC, melanoma) [48]

Table 2: Summary of PD-1 and PD-L1 checkpoint inhibitors in clinical development

Agent	Classification	Mode of action
AMP-225 [61]	Recombinant Fusion Protein	Blocks interaction between PD-1 and B7-H1
BMS-936559 [45]	Humanised anti-PD-L1 monoclonal antibody	Blocks PD-L1 binding to PD-1 and CD80
MEDI4736 [61]	Humanised anti-PD-L1 monoclonal antibody	Blocks PD-L1 binding to PD-1 and CD80
MPDL3280A [63]	Humanised anti-PD-L1 monoclonal antibody	Blocks PD-L1 binding to PD-1 and CD80
Nivolumab [47]	Humanised anti-PD-1 monoclonal antibody	Blocks PD-1 binding to PD-L1 and PD-L2
Pembrolizumab [46]	Humanised anti PD-1 monoclonal antibody	Blocks PD-1 binding to PD-L1 and PD-L2
Pidilizumab [62]	Humanised anti-PD-1 monoclonal antibody	Blocks PD-1 binding to PD-L1 and PD-L2

Table 3: Vaccines which have been delivered in conjunction with radiotherapy in human subjects

Vaccine Class	Agent	Primary Tumor Type
Dendritic	Autologous immature dendritic cells	Hepatoma [88]
	Autologous dendritic cells	Numerous [89]
Viral	Pox-based vaccine	Prostate [90]
	Smallpox and fowlpox vaccine targeting carcinoembryonic antigen (CEA)	Colorectal [91]
Protein	Multiple peptide based vaccine	Esophageal [92]
Whole Cell	Pancreatic Tumor Cell Vaccine (GVAX)	Pancreas [93]
Nucleic Acid	None at present	N/A