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MINI REVIEW

Radiation meets immunotherapy – a perfect match in the era of combination therapy?

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Abstract

Purpose: **This review focuses on recent advances in the field of combining radiation with immunotherapy for the treatment of malignant diseases, since various combinatorial cancer therapy approaches have lately proven highly successful.**

Results: **With initial case reports and anecdotes progressively converting into solid clinical data, interest in cancer immunotherapy (CIT) has risen steeply. Especially immune checkpoint blockade therapies have recently celebrated tremendous successes in the treatment of severe malignancies resistant to conventional treatment strategies. Nevertheless, the high variability of patient responses to CIT remains a major hurdle, clearly indicating an urgent need for improvement. It has been suggested that successful cancer therapy most probably involves combinatorial treatment approaches. Radiotherapy (RT) has been proposed as a powerful partner for CIT due to its broad spectrum of immune modulatory characteristics. Several preclinical studies, supported by an increasing number of clinical observations, have demonstrated synergistic interactions between RT and CIT resulting in significantly improved therapy outcomes.**

Conclusions: **Numerous reports have shown that radiation is capable of tipping the scales from tumor immune evasion to elimination in different tumor types. The next puzzle to be solved is the question of logistics – including types, schedule and dosage of combinatorial RT and CIT strategies.**

Keywords: Radiation, radiotherapy, immunotherapy, cancer, combination therapy

Introduction

For several decades, the concept of cancer immunotherapy (CIT) has been struggling to establish itself as the fourth pillar of acknowledged cancer treatment strategies alongside surgery, radiation and chemotherapy. With its nomination as Science 'Breakthrough of the Year 2013' (Couzin-Frankel 2013) and preclinical studies gradually translating into clinical data, the field of CIT has finally reached a state of acceptance among the established oncological domains. Currently, different immunotherapeutic approaches are standing their ground as powerful treatment strategies for a wide range of malignant diseases. A very prominent and recent example of an outstanding CIT success involves immune checkpoint blockade therapy by monoclonal antibodies (mAb) targeting inhibitory molecules on either immune effector T-cells or tumor cells. Interfering with co-inhibitors has been shown to unleash a powerful anti-tumor T-cell response (Pardoll 2012). Promising early-stage clinical trials have shown safety and impressive activity of mAb blocking activity of programmed cell death 1 (PD1), expressed on T-cells (Topalian et al. 2012), or one of its ligands, programmed death-ligand 1 (PD-L1) (Brahmer et al. 2012). Recently, the FDA approved lambrolizumab, a PD1-targeting mAb for treatment of advanced or unresected melanomas that no longer respond to other drugs (Hamid et al. 2013). Furthermore ipilimumab, a mAb against cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) on T-cells, was approved for the treatment of metastatic melanoma (Lipson and Drake 2011). In 2013, a combination of anti-CTLA4 and anti-PD1 mAb treatment was reported to act synergistically in increasing survival and tumor regression in advanced melanoma patients (Wolchok et al. 2013). This novel immunomodulatory approach exhibits great potential especially for the treatment of severe malignancies resistant to conventional therapies.

However, major obstacles to broad clinical applicability of CIT become more evident. Whereas significant improvements of overall and progression-free survival can be achieved in individual cancer patients, most CIT strategies fail to establish long-lasting tumor rejection in large patient groups – with many patients responding poorly to treatment (Brahmer and Pardoll 2013, Fishman 2014, Raval et al. 2014). The precise processes behind this high variability of therapeutic efficacy remain to be clarified, but most likely involve high heterogeneity of different tumor types as well as poor

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immunogenicity and evolving capability to escape immune recognition (Kalbasi et al. 2013, Kelderman et al. 2014). Based on the concept of cancer immunoediting, tumors undergo three distinct phases of interaction with the immune system: Elimination, equilibrium and ultimately escape (Schreiber et al. 2011). Progressing through these steps involves tumors actively shaping the immune system in order to circumvent immune recognition and establish a state of permanent immune evasion. Successful therapy approaches counteract this active immunosuppression and empower the immune system to regain control over tumor growth. In numerous patients, CIT by itself fails at stably reversing the immunoediting process (Kalbasi et al. 2013). Therefore, the search has begun to look for potent partners in tipping the scales back from tumor immune escape to elimination.

Radiation therapy (RT) comes into play as a particularly attractive partner for CIT in empowering the immune system to re-engage in tumor elimination, since recent years have shown a number of mechanisms through which RT interacts with immunity. In addition to being highly effective at reducing tumor burden by inducing irreversible DNA damage causing cancer cell death, RT has been demonstrated to contribute actively to tumor immune recognition by various means as will be outlined in the following section (Kwilas et al. 2012).

From escape back to elimination – the immune modulatory capacities of radiotherapy

Numerous lines of evidence have initiated a paradigm shift from the traditional notion of radiation causing detrimental effects on various immune cell types towards the recognition of a potent systemic immunostimulatory impact (McBride et al. 2004, Formenti and Demaria 2013). This more recent concept has moved into the center of attention due to repeated observations of so-called abscopal RT effects (Kalbasi et al. 2013), which describe the regression of tumor growth at sites distant from the primary field of irradiation (Demaria et al. 2004). Several such case reports inspired researchers to investigate how radiation can systemically induce tumor elimination which led to the discovery of various mechanisms through which RT actively induces anti-tumor immunity (Barker and Postow 2014).

Of utmost importance, radiation causes a strong increase in tumor-associated antigen (TAA) quantity and variety through induced cancer cell death (Corso et al. 2011, Burnette et al. 2012) as well as enhanced protein translation (Reits et al. 2006), permitting specialized antigen-presenting cells (APC) such as dendritic cells (DC) to effectively prime T-cells for specific recognition and efficient clearance of residual tumor cells (Ahmed et al. 2013, Frey et al. 2014). In addition, it has been shown that radiation is capable of inducing activation of DC by the release of specific damage-associated molecular patterns (DAMP) through immunogenic tumor cell death, a process critical for enabling DC to orchestrate a potent anti-tumor immune response (Roses et al. 2014). Particularly high-mobility group box 1 protein (HMGB1), released from irradiated dying tumor cells, has been demonstrated to induce sustained DC maturation through activation of the toll-like receptor (TLR) 4 pathway, which also increases efficiency of TAA processing and presentation (Apetoh et al. 2007). Persistent DC activation forms a critical part in generating a potent anti-tumor immune response since immature antigen-presenting DC induce anergy or even deletion of antigen-reactive T-cells – a mechanism which has been shown to be actively exploited by tumor cells as a means to escape T-cell recognition and cytotoxicity (Kim et al. 2006). The combined effects of radiation providing antigens along with adjuvant activating signals hinder tumors at taking the final step in the immunoediting process – escape – and have therefore led to the concept of referring to RT as an in situ anti-tumor vaccine (Demaria et al. 2014, Frey et al. 2014).

But the effects of RT on TAA detection and presentation go beyond increasing antigen availability: The induction of calreticulin translocation to the surface of tumor cells serves as a signal for recognition and phagocytosis by DC, thereby enhancing TAA processing and presentation (Obeid et al. 2007). Also trafficking of APC to regional lymph nodes, where interaction with T-cells takes place, has been described to be augmented by RT (Lugade et al. 2005). A similar effect has been reported regarding recruitment and cytotoxicity of $CD8^+$ T-cells. Lim et al. (2014) observed the induction of type I and II interferons (IFN) through radiation, leading to enhanced intratumoral numbers and cytolytic activity of effector T-cells. Also Draghiciu et al. (2014) reported enhanced recruitment of tumor-specific $CD8^+$ cells into tumors upon low-dose radiation. In addition, the production of chemokines such as CXCL16 has been shown to be upregulated following radiation, which also attracts effector T-cells to the irradiated tumor site (Matsumura and Demaria 2010). The direct recruitment of cytotoxic T-cells into the tumor provides a strong basis for the immune system to regain control over the transformed tissue.

RT has also been described to alter the phenotype of residual tumor cells surviving irradiation, mostly due to lower doses being transmitted further away from the radiation source (Garnett et al. 2004, Gameiro et al. 2014). This alteration includes upregulated expression of various surface molecules, such as major histocompatibility complex (MHC) I, co-stimulatory T-cell signaling molecules, adhesion molecules and death receptors, thus further contributing to immune recognition and elimination by rendering tumor cells more visible to the immune system (Chakraborty et al. 2004, 2008b, Bernstein et al. 2014).

Furthermore, effects of RT on vascular normalization and density within tumors have been observed. As a result of excessive production of pro-angiogenic factors, tumors establish an abnormal vascular structure, which creates a hypoxic microenvironment that polarizes inflammatory immune cells towards immunosuppressive activity and hinders immune cells at effectively entering into tumor tissue (Huang et al. 2013). In CIT, increasing evidence indicates that a normalized tumor vasculature substantially enhances immunotherapeutic success as it reverses the hypoxic microenvironment and enables immune effector cell infiltration (Huang et al. 2013). RT was shown to induce normalization of tumor vasculature by increasing expression of chemokines CXCL9 and CXCL10, leading to vessel remodeling, as well as vascular cell adhesion protein 1 (VCAM-1),

facilitating T-cell migration into tumors (Kershaw et al. 2013). A study by Ganss et al. (2002) showed that RT led to enhanced vessel density and diameter within tumors, which facilitated access of T-cells by enabling them to adhere to the endothelium, ultimately leading to tumor regression (Ganss et al. 2002). Effects of radiation on vessel remodeling therefore provide another promising rationale for its combination with CIT by actively contributing to tumor elimination.

Another branch of the immune system affected by RT is humoral immunity. In melanoma patients, increases in tumor-specific antibody levels have been observed following radiation (Postow et al. 2012). Demaria et al. (2005a) reported RT to induce production of pro-inflammatory cytokines, resulting in the activation and migration of various immune cell subsets. Nevertheless, the actual contribution of a heavily pro-inflammatory cytokine milieu on tumor progression or rejection has become a matter of debate among immunologists and has to be considered carefully in a context-dependent manner (Grivennikov et al. 2010).

Nevertheless, when dissecting mechanisms of RT impacting on immunity, its effects on immunosuppression also have to be taken into account. Such mechanisms include proportionally increasing regulatory T-cell (T_{reg}) incidence, which can be attributed to an inherently higher radioresistance of these cells (Formenti and Demaria 2013), as well as induction of transforming growth factor (TGF) β secretion, which was shown to inhibit systemic immune-activating effects of RT (Diamond et al. 2013). Blockade of TGFβ was proven not only to induce abscopal RT effects, but also to overcome local immunosuppression (Diamond et al. 2013). In addition to these observations, expression of co-inhibitory molecules such as PD-L1 was shown to be induced in tumor cells after local high-dose irradiation (Deng et al. 2014). This consequence provides a clear example of the strong rationale for combining RT with immune checkpoint blockade. Hence, it has been implicated that radiation may promote immunosuppression by different means in a dose-dependent manner (Kwilas et al. 2012).

The importance of gaining deeper understanding of RT-mediated effects and the resulting cellular interactions becomes apparent when taking the increased recognition of the tumor stroma into account. It has become well-established that various types of cells ranging from cancer cells themselves (including cancer stem and bulk cells), local and bone marrow-derived stromal stem and progenitor cells, endothelial cells, pericytes and cancer-associated fibroblasts to immune cells, contribute to the formation of a unique tumor microenvironment (Hanahan and Weinberg 2011). In order to achieve sustained therapy success, this entire tumor niche has to be considered and numerous groups currently focus on gaining deeper understanding of the predominant processes and cellular interactions.

Finding the perfect RT-CIT match – preclinical and clinical observations

Several groups have embarked on the mission of actively evaluating synergisms between individual RT-CIT combinations and first results showed robust improvements in therapy outcome. At a preclinical state, external beam RT (EBRT) in combination with adoptive tumor-specific $CD8^+$ T-cell therapy (Chakraborty et al. 2003, Reits et al. 2006) and vaccination approaches including recombinant virus strategies and TLR ligand administration (Chakraborty et al. 2004, Demaria et al. 2013, Witek et al. 2014) was demonstrated to result in drastically enhanced tumor regression by increased $CD4^+$ and $CD8^+$ T-cell responses. Similar results were obtained with radiolabeled mAb (Chakraborty et al. 2008a) as well as brachytherapy and vaccine-mediated CIT (Hodge et al. 2012). As indicated earlier, combining local radiation with antibodies targeting immune checkpoint blockade molecules such as CTLA4 or PD-L1 also yielded highly synergistic effects on therapy outcome (Demaria et al. 2005b, Dewan et al. 2009, Deng et al. 2014). Another immunotherapeutic agent, which has been investigated in search of beneficial combinatorial strategies, is IL-2. Given its limitations in establishing long-term tumor rejection, relatively low response rates and association with severe side effects (Siegel and Puri 1991, Atkins et al. 1999, Schwartz et al. 2002, McDermott 2007, Seung et al. 2012a), several preclinical studies have focused on evaluating the potential of combining IL-2 with RT for improved therapy successes (Cameron et al. 1990, Safwat et al. 2003, 2004). Importantly, these studies showed varying results based on RT dose and target with promising synergistic observations for the administration of focal radiation prior to IL-2 injection in a mouse model of liver metastases (Cameron et al. 1990). These are just few examples of the manifold preclinical investigations conducted thus far, which have been paving the way for a clinical evaluation of combination treatments.

Clinical data on the synergism of RT and CIT are not as extensive yet. Nevertheless, various case reports strengthen pre-clinical observations and in a number of early-stage clinical trials, combinations of mostly local low-dose RT with different immunotherapeutic strategies have been proven safe and well-tolerated and exhibit great potential of synergistically improving therapy outcomes, with various trials ongoing (reviewed extensively by e.g., Kwilas et al. 2012, Formenti and Demaria 2013, Kalbasi et al. 2013, Barker and Postow 2014, Demaria et al. 2014, Wattenberg et al. 2014).

In a series of pilot trials, Gulley et al. evaluated the safety of combining EBRT with low- or metronomic-dose IL-2 and a recombinant vaccinia virus-based vaccination strategy in prostate and rectal cancer (Gulley et al. 2005, 2011, Lechleider et al. 2008). These studies revealed safety and tolerance of the therapy combinations as well as efficacy in generating tumor-specific immune responses. Another RT strategy, which is currently being evaluated in combination with recombinant vaccinia virus-based vaccination, is Samarium-153-ethylene diamine tetramethylene phosphonate (^{153}Sm-EDTMP). In an interim analysis, 29.4% of metastatic castration-resistant prostate cancer (CRPC) patients receiving the combined therapies were found to remain progression-free after 4 months as compared to 11.8% receiving RT alone (Heery et al. 2012). A more recent study, in which different regimens of stereotactic body RT (SBRT) were combined with high-dose IL-2 in metastatic

melanoma and renal cell carcinoma patients, revealed a promising 71% response rate in patients treated with 1 or 2 fractions of 20 Gy as compared to a response rate of 16% for IL-2 monotherapy, with responding patients showing enhanced immune activation (Seung et al. 2012a, 2012b).

Barker et al. and Postow et al. focus on dissecting combinatorial effects of RT and ipilimumab in melanoma patients regarding safety and preliminary efficacy. In a retrospective study, they reported a 39-month median overall survival in patients receiving RT during maintenance phase of ipilimumab administration (>16 weeks after starting ipilimumab) as compared to 9 months in patients who received RT during the induction phase (≤ 16 weeks of starting ipilimumab), which underlines the importance of future research regarding treatment schedules. Importantly, they also found the combinatorial treatment to be as safe and feasible as each individual therapy alone, which is in accordance with numerous case reports at different study centers (Postow et al. 2012, Barker et al. 2013, Barker and Postow 2014). As in melanoma, the combination of ipilimumab with RT for the treatment of CRPC was also found to be well-tolerated – nevertheless, it did not reveal significant improvements in therapy outcome as compared to ipilimumab administration alone, but further clinical trials are currently under way (Slovin et al. 2009, 2013).

Further examples involve immunotherapeutic strategies aimed at DC-mediated tumor immune recognition (Formenti and Demaria 2013). In patients bearing metastatic solid tumors, the combination of granulocyte macrophage colony-stimulating factor (GM-CSF) administration with local radiotherapy was shown to induce an abscopal response in 30% of patients (Formenti and Demaria 2009). Limited success was achieved in a different phase I trial, in which advanced hepatocellular carcinoma patients were injected intratumorally with autologous DC following a single fraction of radiotherapy, with eight of 14 patients showing enhanced tumor-specific immune responses (Chi et al. 2005). Another approach involved intratumoral autologous DC injection during fractionated EBRT in soft-tissue sarcoma patients, which led to remarkable tumor-specific immune responses and one-year progression-free survival in 12 of 17 patients (Finkelstein et al. 2012). Furthermore, a retrospective multivariate regression analysis by Dillman et al. (2011) revealed RT as one of six features correlating with survival in metastatic melanoma patients receiving vaccinations of autologous DC loaded with tumor antigens. Brody et al. (2010) conducted a phase I/II trial for the combination of low-dose RT with intratumoral injection of a DC-activating TLR9 agonist in 15 patients with low-grade B cell lymphoma and reported one complete and three partial responses. Finally, Dohnal et al. (2007) demonstrated safety and feasibility of an autologous tumor-lysate-loaded DC therapy approach in combination with RT in pediatric sarcoma patients.

The era of combination therapy – hurdles to be taken in the future

In the final section we want to highlight the main challenges that have to be addressed in the future to exploit the full potential of RT-CIT combination. The repertoire is sheer endless – ranging from different RT strategies including EBRT, SBRT, bone-seeking radionuclides, radiolabeled antibodies, brachytherapy and proton therapy, to numerous CIT approaches such as immune checkpoint blockade, unspecific stimulation, different vaccine-based concepts, adoptive effector cell transfer or targeted immunotherapeutics like antibodies – and not to forget the matters of dosage, timing, and choosing the right patient population as well as a reasonable stage of disease.

One major challenge remaining is to identify the most promising strategies and treatment schedules which will result in maximum efficacy by taking advantage of the strengths of each single therapy. Considering the broad spectrum of mechanisms by which RT impacts on the immune system, the balance between immunosuppression and activation ultimately determines whether a certain approach will result in successful tumor elimination. This underlines the importance to evaluate the predominant immunomodulatory effects of different RT regimens. Various publications have shown that dose, mode of delivery and schedule of RT can cause substantially different effects on the tumor immune response – with the most vital question remaining 'to fractionate or not to fractionate?' (Formenti and Demaria 2013, Barker and Postow 2014). Several groups have reported low-dose irradiation (LDI) to induce immune-activating effects by altering tumor and immune cell surface molecule expression (Ina and Sakai 2005, Kwilas et al. 2012), promoting T-cell-stimulatory capacities of DC (Shigematsu et al. 2007), or an anti-tumor macrophage phenotype (Klug et al. 2013). Simultaneously, ablative high-dose irradiation (HDI) was reported to stimulate potent anti-tumor cytotoxic T-cell responses, mediated primarily through DC activation (Lee et al. 2009, Gupta et al. 2012) whereas Schaue et al. (2012) reported beneficial effects of medium-dose fractionated versus single dose radiation. A study by Shen et al. (1988) revealed higher natural killer cell activity and superior survival in tumor-bearing mice treated with hypofractionated RT as compared to conventionally fractionated RT. All these observations indicate that to date, no conclusive explanation could be given as to which strategy will provide the best platform for combination with CIT approaches.

Another major obstacle to precisely evaluating effects of RT and CIT combinations on tumor progression is posed by the still limited available imaging modalities especially in the clinical setting (Kalbasi et al. 2013). Monitoring the successful administration of immunotherapeutic agents and their ability to interact with tumor cells often requires tracking of individual cell populations and therefore asks for labeling techniques in order to distinguish specific immune effector subsets. The optimal characteristics for labeling agents include visualization in a non-invasive manner, minimal toxicity, possibility of serial imaging over longer time periods, specificity, as well as quantitative localization (Akins and Dubey 2008). For this purpose, several molecular imaging agents have been developed – including radioisotopic, fluorescent, bioluminescent, and magnetic resonance imaging (MRI) agents (Youn and Hong 2012). However, choosing an appropriate imaging technique for a given

combinatorial treatment strategy has to take into account limitations of each technique. As an example, optical fluorescence or bioluminescence imaging offers high sensitivity, but shows poor penetration in deep tissues, which limits its clinical applicability (Liu and Li 2014). MRI on the other hand has high resolution and contrast while lacking sensitivity (Akins and Dubey 2008, Liu and Li 2014). Positron emission tomography (PET) again offers high sensitivity as well as deep penetration, but suffers from a short half-life of labeling radioisotopes (Liu and Li 2014). Furthermore, powerful combination treatments most likely induce strong inflammatory responses resulting in temporary tissue swelling which can be hard to distinguish from persistent disease if imaging techniques lack high resolution, contrast or sensitivity (Kalbasi et al. 2013). Taken together, the tracking of tumor-immune system interactions on cellular level remains challenging, especially in a clinical setting.

Based on the extensive number of advantages that both RT and CIT treatment strategies offer, it seems obvious why there is growing interest in finding the right design of combining these two approaches. As outlined, each individual therapy concept struggles with establishing potent and long-lasting tumor rejection in a large number of patients (Fishman 2014, Kelderman et al. 2014, Raval et al. 2014). The devil seems to lie in the details of overcoming the tumor's ability to suppress and manipulate the immune system in order to maintain a state of immune evasion. The manifold pathways through which RT has now been shown to interact with immunological mechanisms provide a particularly strong rationale as to why these two forces might represent specifically powerful allies in the ongoing war on cancer.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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