



Interaction of Radiotherapy and Hyperthermia with the Immune System: a Brief Current Overview

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Abstract

Purpose of Review This review focuses on the opposing effects on the immune system of radiotherapy (RT) and the consequences for combined cancer treatment strategies of RT with immunotherapies, including hyperthermia (HT). How RT and HT might affect cancer stem cell populations is also briefly outlined in this context.

Recent Findings RT is one of the crucial standard cancer therapies. Most patients with solid tumors receive RT for curative and palliative purposes in the course of their disease. RT achieves a local tumor control by inducing DNA damage which can lead to tumor cell death. In recent years, it has become evident that RT does not only have local effects, but also systemic effects which involves induction of anti-tumor immunity and possible alteration of the immunosuppressive properties of the tumor microenvironment. Though, often RT alone is not able to induce potent anti-tumor immune responses since the effects of RT on the immune system can be both immunostimulatory and immunosuppressive.

Summary RT with additional therapies such as HT and immune checkpoint inhibitors (ICI) are promising approaches to induce anti-tumor immunity effectively. HT is not only a potent sensitizer for RT, but it might also improve the efficacy of RT and certain chemotherapeutic agents (CT) by additionally sensitizing resistant cancer stem cells (CSCs).

Keywords Radiotherapy · Hyperthermia · Immune checkpoint inhibitors · Cancer stem cells · Immune system · Anti-tumor immunity

Introduction

Cancer management is still a huge challenge to modern medicine. There were 19.3 million new cases of cancer diagnosed in 2020 and almost 10 million deaths were caused by cancer worldwide [1]. Radiotherapy (RT) is one of the most

crucial cancer treatments following surgery and chemotherapy. Approximately 60% of patients with solid tumors receive RT in the course of their disease and the need for this classical tumor therapy tends to increase in the next years [2]. For cancer therapy, ionizing radiation is used to achieve local tumor control by inducing cancer cell death and inhibition of the cancer cell proliferation. DNA damage, such as single- and double-strand breaks, induced by ionizing radiation is either caused directly or indirectly by high oxidative stress caused by ionization of other molecules [3]. If clusters of DNA damage accumulate beyond the cells repair capabilities, it causes cancer cell death or senescence [4]. The severity of DNA damage from RT is also known to be influenced by the state of the tumor microenvironment (TME), specifically if it is hypoxic or not [4, 5]. Unfortunately, normal cells are affected by ionizing radiation as well. However, since as tumor cells have an impaired DNA repair machinery, damage to normal tissue can be minimized by applying RT in fractions of smaller doses. Furthermore, the implementation of advanced modulated beam therapies

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such as intensity-modulated RT and spatially fractionated RT improve the efficacy of RT at the same time with decreased damage to normal tissue [6].

Additionally, RT has also some non-targeted, systemic effects. For a long time, RT was known for its immunosuppressive properties thus has been used to condition the immune system before hematopoietic stem cell transplantation. In recent years, however, the immunostimulatory effects of RT are being increasingly recognized as an effective way to induce anti-tumor immune responses. In patients with advanced cancer, local RT has the ability to induce systemic anti-tumor responses [7]. Due to its immunostimulatory properties, RT is now being investigated in combination with other therapies, specifically immunotherapy (IT), in several clinical trials, and it already showed improvement of local and systemic tumor control in e.g. head and neck cancer as well as lung cancer [7, 8]. The idea to exploit the patient's own immune system to fight the cancer by using immunotherapies has always been in interest. In recent decades immunotherapies have been developed and applied to induce anti-tumor immune responses in different settings. However, the response rate of the immunotherapies is so far relatively low. Thus, applying RT with further immune modulation, e.g., by hyperthermia (HT) treatment is an ideal option. In this review, we will briefly discuss how RT affects the anti-tumor immune response and how it modulates the tumor microenvironment. Possibilities to combine RT with IT and with further immunomodulation by HT are outlined. Lastly, the effects of RT and HT on cancer stem cells (CSCs) will be addressed.

Immune Modulatory Effects of RT

RT has been long time known only for its local effects. However, there are also non-targeted effects (NTE) [4, 9] of RT that are caused by the release of danger signals and cytokines from the RT-treated area into the surrounding tissue [10, 11]. There are two key types of NTE, a) “bystander effects” which determine the tumor regression of non-irradiated area surrounding tumor sites after local RT and b) “abscopal effects” which is tumor regression in distant tumor sites [12]. The latter are known from clinical observations of tumor regression outside the irradiation field in tumor masses elsewhere in the body. This was the very first evidence that RT stimulates the immune system. These findings were later supported by preclinical studies and proof-of-principle clinical studies [13–15] which confirmed that these effects are immune-mediated, strongly via the induction of immunogenic tumor cell death (ICD) [16, 17]. Unfortunately, these NTE occur rarely after RT alone, thus indicating that RT as single therapy is not enough to induce an anti-tumor immune response [18]. Recently,

immunotherapies (IT) in combination with RT are currently tested in clinical trials to improve the response rate of IT and improve the immune activating effects of RT and in the ideal case the overall patient survival [19].

Moreover, after RT, the expression of MHC molecules, stress ligands, and death receptors increase on the surface of tumor cells [20, 21••]. Ionizing radiation also modifies the tumor immune phenotype and its microenvironment [22–24]. When tumor cells escape from host anti-tumor immune response, they develop their own microenvironment to support the tumor cell growth and to protect them from the host's immune system by harnessing immune cells and avoid cancer immune surveillance [25]. This self-sustaining TME is a very complex system with different cellular components that each have their distinct role. The TME includes supporting cells, such as fibroblasts and endothelial cells, and cells of the adaptive and innate immune system [26]. Furthermore, it is enriched with immunosuppressive chemokines and cytokines, which together with the tumor-associated immune cells, resulting in further tumor progression and immune suppression, including a reduced function of cytotoxic T-lymphocytes (CTL) and natural killer (NK) cells [27]. Furthermore, tumor cells express immune inhibitory checkpoint molecules (ICMs) on their surface (e.g., programmed death ligand 1 and 2 (PD-L1, PD-L2), and herpesvirus entry mediator (HVEM)) to inhibit T cell responses against tumor cells. These inhibitory ICMs are normally used by antigen-presenting cells (APCs) physiologically to downregulate the T cell response after a pathogen is eliminated to prevent T cells to attack their own body cells [28]. Thus, tumor cells mask themselves with these inhibitory ICMs to successfully escape from immune surveillance.

However, RT can alter this immunosuppressive TME by inducing immunogenic tumor cell death (ICD). ICDs is cell death forms that induce the release of damage-associated molecular pattern (DAMPs) which happens typically when cells lose their integrity after necrosis and necroptosis [29]. DAMPs are normally present only inside of the cells, but when they are released outside of the cell, they attract and activate cells of the innate and adaptive immune system. The well-known DAMPs that are induced by RT includes chromatin-binding protein high mobility group box 1 (HMGB1), calreticulin, adenosine triphosphate (ATP), and heat shock proteins (HSPs) [18, 30•, 31]. Each of these DAMPs have different effects, for example, HMGB1 facilitates tumor antigen presentation and type I interferon-mediated dendritic cell (DC) maturation [32]. Type I interferons (IFN α and IFN β) stimulate DCs to cross-present the antigen to T cells and further facilitate T cell responses [33]. HSPs (preferably HSP70, HSP90) and calreticulin act as an “eat me” signal to APCs leading to the release of proinflammatory cytokines, such as tumor necrosis factor alpha (TNF- α) and IL-6, which further lead to

the activation of other immune cells [31, 34]. Additionally, HSPs activate NK cells [35] and facilitate the APC's cross presentation of antigen to CD8+ T cells, ideally leading to their activation and subsequent T cell-mediated eradication of tumor cells [36, 37] (Fig. 1). ATP attracts DCs into the tumor and promotes phagocytic clearance of tumor cells [38]. Moreover, RT modulates the immunogenicity of the tumor cells by increasing the expression of stimulatory molecules, such as cluster of differentiation 80 (CD80), and major histocompatibility complex class 1 (MHC I) [39–41].

Besides its immunostimulatory effects, RT is also known for its immunosuppressive properties [42]. Unlike necrosis, apoptosis is a non- or even anti-inflammatory cell death form (Fig. 1), as the cell membrane remains intact, and thus danger signals are not released [43–45]. Furthermore, when cells undergo apoptosis, they expose phosphatidylserine on the outer membrane leaflet, which serves as phagocytic signal for particularly macrophages.

The latter thereby release anti-inflammatory cytokines, such as TGF-β [44, 46]. Additionally, they increase the expression of immune inhibitory checkpoint molecules like PD-L1 to make the tumor microenvironment immune suppressive and these effects can even be amplified by RT [47–49]. Furthermore, RT induces the production of chemokines that attract immune cells into the tumor to sustain the immune suppressive TME [50]. CCL2 is one of those chemokines attracting monocytes into the tumor, which then differentiate into tumor-associated macrophages (TAMs) and further promote tumor growth [51].

Based on these opposing effects of RT with immunosuppression counteracting the stimulation by ICD induction, RT alone often does not induce anti-tumor responses effectively. Fortunately, recently preclinical and clinical studies have shown that by complementing RT with additional immunotherapies, RT-induced anti-tumor immune responses can be augmented and the immune suppressive effects of RT can be reduced [30, 52, 53].

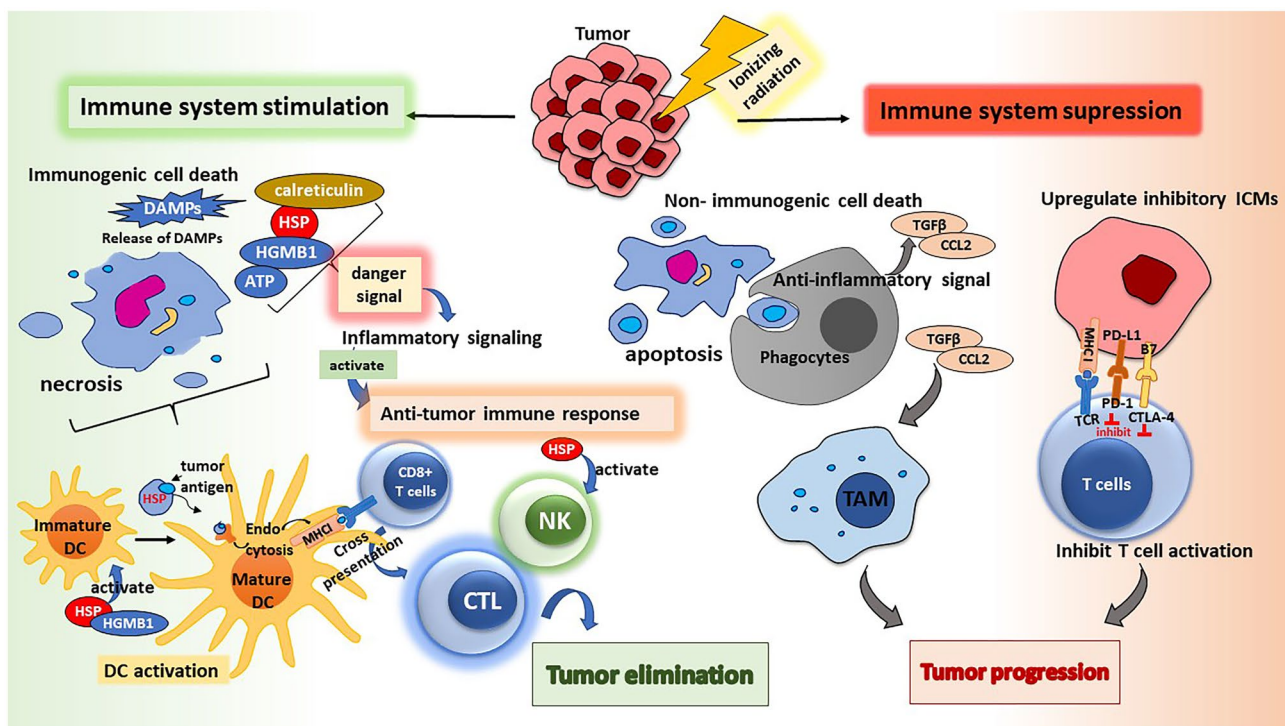


Fig. 1 The effects of ionizing radiation on the immune system. Radiotherapy (RT) has both immune stimulatory and immune inhibitory effects. After RT, tumor cells also die of necrosis, resulting in the release of damage associated molecular patterns (DAMPs). DAMPs including HSPs and HGMB1 activate dendritic cells. HSPs bound to tumor antigen facilitate antigen presentation by e.g. DCs and initiate cytotoxic T cell responses against tumor cells. However, on the other hand when tumor cells are treated with RT, it can also lead to immunosuppression. When tumor cells die of apoptosis, DAMPs are not released, instead apoptotic cells are taken up by phagocytes which release anti-inflammatory signals, such as TGF-β and CCL-2. Latter induces differentiation of tumor-associated macrophages

(TAM) which promotes tumor progression. Also, tumor cells inhibit T cell activation by upregulating the expression of immune inhibitory checkpoint molecules, thus escaping from the T cell-mediated tumor clearance. DAMPs, damage-associated molecular patterns; DC, dendritic cell; HSP, heat shock protein; HMGB1, high mobility group protein 1; ATP, adenosine triphosphate; MHC I, major histocompatibility complex class 1; TGF-β, tumor growth factor β; TAM, tumor-associated macrophages; CCL2, monocyte chemoattractant protein-1; PD-1, programmed cell death protein 1; PD-L1, programmed death ligand 1; CTLA-4, cytotoxic T-lymphocyte-associated protein 4

The immunological effects of RT are highly influenced by radiation dose and fractionation. However, no “perfect” immune-stimulating radiation dose and fractionation scheme has been identified to date, as it also depends on the size and stage of the tumor and numerous other factors. But generally speaking, in most tumor entities, RT of 1.8–2.0 Gray (Gy) per day, 5 times a week, for 5–6 weeks is used for solid tumors. Hypofractionation with a single dose higher than 2.0 Gy per day was reported to have a better outcome and modulates the immune response by causing more immunogenic cell death compared to lower dose radiation [12, 54, 55]. In preclinical studies, hypo-fractionated RT of 8 Gy \times 3 was more effective to induce abscopal effects compared to a single fractionation of 20 Gy when it is combined with ICIs [56, 57], but 8 Gy \times 3 is also less immunogenic as 8 Gy \times 2 when being combined with an autologous tumor cell–ased vaccine [58]. It has already also clinically been proven that locally delivered RT induces systemic modulation of the immune system in tumor patients, but different RT approaches appear to specifically affect distinct immune components [59].

Combination of Radiotherapy and Immunotherapies

Cancer immunotherapy is increasingly becoming one of the cornerstones of cancer treatments. The ability of the immune system to recognize and to eliminate tumors was discovered more than a century ago and ever since broadly studied and clinically applied in the form of immunotherapy successfully in recent years. Consequently, (re-)inducing anti-tumor immune responses by inactivating inhibitory immune receptors (a.k.a., immune checkpoint molecules (ICMs)) has been developed and initially used as a monotherapy and showed great success [60–62]. Immune checkpoint inhibitors (ICIs) directed against anti-cytotoxic T-lymphocyte–associated protein a (CTLA-4) and the PD-1/PD-L1 axis successfully (re)activates the immune response and improves the survival of advanced stage cancer patients [60, 62, 63]. ICIs are clinically approved for many tumor entities but despite the promising results the response rate of ICIs is still lower than 15% [64]. It has become clear that when RT is used in combination with IT, local tumor control is improved and even systemic anti-tumor immune response can be induced. ITs that are used in combination with RT include cytokines (e.g., IL-12, IFN), growth factors (e.g., Flt3-L), and immune checkpoint inhibitors (ICIs) (anti-CTLA-4, anti-PD1, anti-HVEM). IT improves the clinical outcome and local tumor control [65–67]. The efficacy of RT is more prominent when it is combined with IT. There are several factors that affect the effectiveness of RT and IT combinations such as tumor burden, immunotolerance, and scheduling of RT and IT [56,

61]. Nevertheless, there has been extensive studies tried to explore the responsible mechanisms and predictive markers and combination treatment options to increase the efficacy of ICIs. One major predictive marker of the response of PD-L1 inhibitor is the amount of cytotoxic CD8+ effector T cells infiltrating the tumor site [68•, 69]. Therefore, it has been suggested that therapies which increase the infiltration of CTLs into tumor sites like RT would increase response to IT.

It has been further observed that the expression of PD-L1 increases on tumor cells following irradiation [70, 71], thus suppressing antigen presentation of APCs and dampen CTLs in effector phase [63]. Thus, using RT in combination with anti-PD-1/anti-PD-L1 will improve the number of responders and also at the same time effectiveness of both therapies [21••, 30•]. Recently, the number of clinical trials that study the efficacy and the safety of the ICIs are increasing and even some clinical trials reported that RT does not increase the ICI-related side effects [72, 73]. There are also other novel strategies of RT and IT combination being developed, such as the combination of RT with agonists of immune costimulatory molecules like OX-40L and CD40 [65, 74–76]. However, combining these treatments is a complex matter and several factors should be considered when planning the treatments. The timing of the RT and ICIs is one of the most important factors to consider when these treatments are combined. For example, it has been reported that giving CTLA-4 inhibitors 7 days prior to irradiation is much more effective than giving it 1 or 7 days after the treatment in a CT26 murine model [77]. PD-1 inhibitors showed best results when they are given simultaneously with RT compared to 7 days after the irradiation [68•, 78]. In contrast, the agonistic OX40 antibody is better used right after RT [77]. Each ICI has different functions and thus RT should be matched with the individual property of each ICI.

Another factor to consider is the tumor burden; high tumor burden is negatively correlated with the efficacy of ICI [79]. In preclinical data, a smaller tumor volume is correlated with better response of PD-1 inhibitors in non-small cell lung cancer (NSCLC) [80], and vice versa large and advanced stage tumors are resistant to PD-1 inhibitors [81]. This negative correlation was also observed in patients with metastatic melanoma [79, 82]. Higher tumor volume was predictive of poor response to PD-1 inhibitors [79, 82]. NSCLC patients with high metabolic tumor volume had poor response to PD-1 inhibitors and overall survival [83]. However, the exact threshold to describe low and high tumor burden is not determined yet, thus other measures which are more dynamic than the tumor size, for example, Huang et.al suggested the ratio of exhausted T cell subpopulation to pretreatment tumor burden is more suitable to predict which patients will benefit more from ICIs [84, 85]. Also, high tumor mutation burden was an effective predictor of anti-PD-1 response [86]. Taken together, there is increasing

evidence in preclinical and clinical settings that a combination of RT and HT improves the immunogenicity of the tumor and increases the efficacy of both treatments. To conclude, several factors have to be considered to achieve the most synergistic effects and high response rates. The efficacy of RT can further be optimized, especially regarding ways to reduce radioresistance. Tumor hypoxia is one of the main reasons of radiotherapy resistance. This can be addressed by additionally applying hyperthermia treatment.

Hyperthermia as an Additional Immunomodulator

HT is an anti-cancer treatment which locally heats the tumor to supraphysiological temperatures (39–45 °C), and is a potent sensitizer for radio- and chemotherapies (CT). HT is commonly used in clinical settings as a combination with RT and CT in various cancer entities and has shown

to significantly improve local tumor control and survival of the patients without inducing severe side effects [87–92].

Regarding the key modes of actions, HT can improve the delivery of oxygen into predisposed hypoxic tumor tissue [93]. However, one should keep in mind that due to chaotic tumor vasculature the relative increase in blood flow is only about two-folds in tumor tissue and exhibits pronounced heterogeneity compared to normal tissues [94]. Furthermore, heating tissues aggravates the protein aggregation and DNA damage which makes the tumor more sensitive to RT and CT [95, 96]. HT has direct killing effects on tumor cells, starting with temperatures of about 43 °C [97] and has been proven to modulate the innate and adaptive immune system by inducing anti-tumor immune responses in direct and indirect ways [95, 98] (Fig. 2). The local effects on tumor cells are mediated by following key mechanisms: cause of membrane dysfunction, protein denaturation and aggregation, inhibition of DNA repair mechanisms, and induction of cancer cell death by necrosis and apoptosis [99]. By increasing the perfusion, HT can increase the immune cell infiltration into

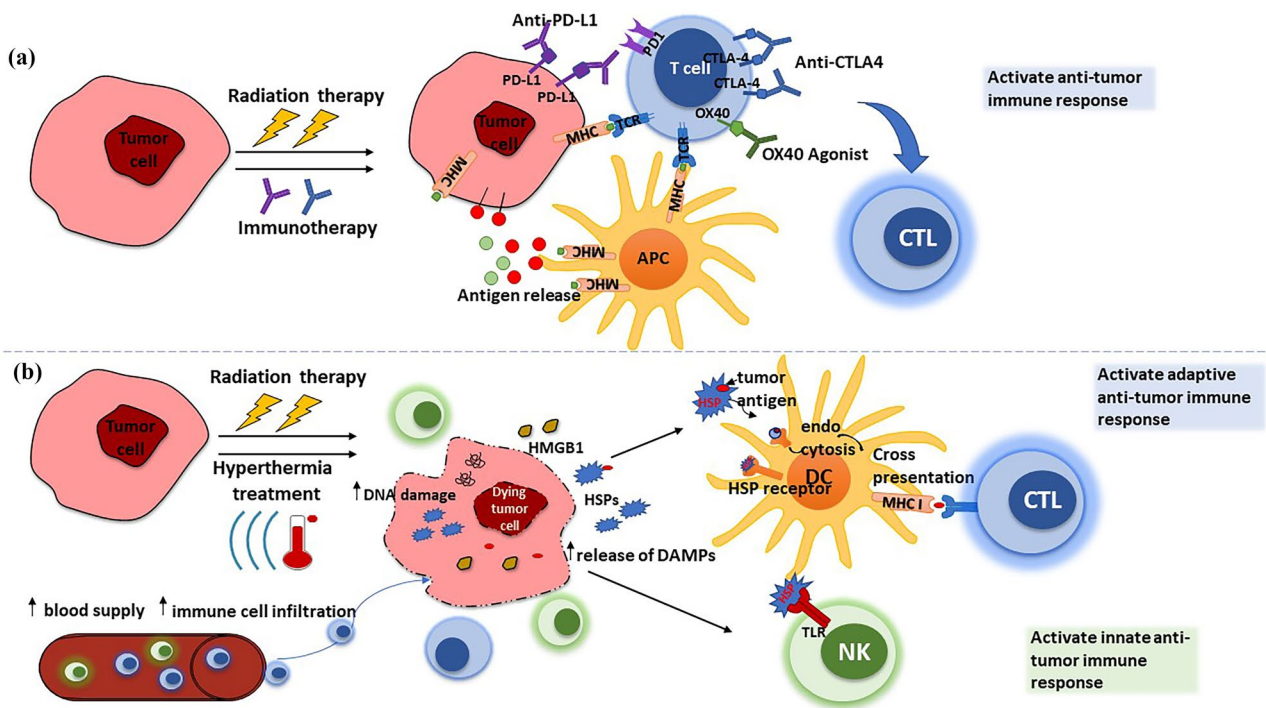


Fig. 2 The effect of radiotherapy (RT) in combination with immune checkpoint inhibitors and hyperthermia treatment on the immune system. **a** After RT certain inhibitory immune checkpoint molecules (ICMs) are upregulated on tumor cells. ICIs (anti-PD-L1, anti-CTLA-4) that inhibit these inhibitory ICMs will diminish the effects of these inhibitory ICMs on T cell activation and further activate anti-tumor immune response. DC’s cross presentation of tumor antigen with MHC I molecule leads to activation of CTLs which eliminate tumor cells. **b** HT sensitizes the tumor for RT. It boosts the effect of RT by increasing tumor cell death and release of DAMPs. DAMPs that are released from tumor cells (HSPs, HMGB1, DNA, RNA frag-

ments, and other antigens) are taken up by a DC which further activates CTLs leading to the activation of adaptive anti-tumor immune responses. HSPs also activate NK cells. HT increases tumor blood supply and immune cell infiltration into the tumor site. All these effects are leading to the activation of innate and adaptive anti-tumor immune responses. DAMPs, damage-associated molecular patterns; HSP, heat shock protein; HMGB1, high mobility group box protein 1; MHC I, major histocompatibility complex class 1; PD-1, programmed cell death protein 1; PD-L1, programmed death ligand 1; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; TLR, toll-like receptor; OX40, CD134; OX40-L, OX40 ligand

the tumor tissue [100]. HT-induced cancer cell death causes release of DAMPs, such as HMGB1, HSPs, and DNA as well as RNA fragments of cancer cells which are highly immunogenic [101, 102] (Fig. 2). Once these DAMPs are taken up by dendritic cells and macrophages, it further triggers the anti-tumor immune response [95, 98, 103]. Specifically, HSPs released by HT promotes antigen presentation and activates NK and CTLs, thus providing a potent anti-tumor immune response [104–106]. Also, when HT is combined with RT, it significantly induces more necrosis which leads to increased release of DAMPs, further inducing the anti-tumor immune response more effectively [107].

Besides the positive effects of adding HT to RT, CT also benefits from additional HT. HT improves the perfusion and membrane permeability of the tumor cells, and thus augments the effects of certain chemotherapeutic agents (summarized in [108•]). Due to these beneficial properties, HT is implemented in multimodal treatment settings. The initial clinical trials, such as HYCAN trial (ClinicalTrials.gov Identifier: NCT02369939) of anal carcinoma reports that when HT is added to radiochemotherapy the cells of the innate immune system recover faster in peripheral blood [21••].

The Effects of Radiotherapy and Hyperthermia on Cancer Stem Cells

Cancer stem cells (CSCs) are a small population of tumor cells that has the ability to regenerate and differentiate into tumor cells when transplanted into another animal host. It is believed that tumor cells originated from these CSCs, and CSCs are responsible for tumor metastasis [99]. CSCs have important clinical relevance because CSCs are highly resistant to standard RT and CT, and they are an important target to anti-cancer therapies [109]. CSCs are also thought to be responsible for the drug resistance of tumors [110]. CSCs activate the DNA damage checkpoints in response to irradiation making the DNA repair system of tumor cells more precise [111]. Furthermore, they upregulate the expression of ROS scavenger proteins to decrease the amount of DNA damage caused by RT [99]. Also the environment where CSCs reside plays an important role in its resistance to RT and CT [112]. They reside in hypoxic areas without rich blood vessels, thus exposure to chemotherapeutic agents is decreased. The low level of ROS in these hypoxic areas also results in decreased DNA damage caused by free radicals after RT. Additionally, CSCs can upregulate BCL2 family proteins to protect themselves from the cytotoxic effects of CT [113]. Likewise, CSCs have the ability to protect themselves from the effects of RT and CT in many ways, and the CSCs that remain after the treatments are believed to cause cancer recurrence [114].

The origin of the CSCs is still controversial. Whether cancer cells gained the abilities of stem cells or distinct CSCs exist from the beginning is still not clear, and there is currently no specific biomarker to successfully distinguish CSCs from normal stem cells [115]. This lack of specific biomarkers is a current obstacle in CSC research and the development for treatments that target CSCs [30•, 115]. CSCs evade from immune system in various ways, such as inhibiting the antigen presentation and T cell activation [44] and by actively suppressing the immune system. Because of all these functions of CSCs, they are highly resistant to single cancer therapy, thus in order to eradicate cancer cells it is necessary to combine RT and CT with additional treatments, such as HT and IT [114].

HT has the potential to eliminate CSCs and sensitize them for RT and CT [99, 116]. It can improve the efficacy of RT and CT in many mechanisms. First, HT can specifically affect the hypoxic tumor areas with poor blood supply by increasing the perfusion, thus increasing the oxygen supply and ROS in that area. Second, HT can inhibit multiple DNA repair pathways that CSCs upregulate to protect themselves. Third, HT can change the immunosuppressive microenvironment of the tumor and enhance anti-tumor immune response to eliminate CSCs. Finally, HT can improve CSC's low immunogenicity by increasing cancer cell death, revealing CSC's antigen to APCs. However, these effects of HT can vary depending on whether it is used in combination with RT and CT, and also from the tumor entity itself [117]. In some tumor entities including breast cancer [117] and glioblastoma [118], HT alone did not have significant effect on CSCs, though when combined with RT it effectively reduced CSCs regeneration and proliferation. Based on all these facts, targeting CSCs with HT in combination with other treatment strategies shows huge promise and may lead to the eradication of CSCs, preventing the recurrence of the tumor.

Conclusions

RT does not only have local cell killing effects on tumor cells, but it also has a systemic effect which is caused by the activation of anti-tumor immune responses. Recently, it has been shown that RT can elicit anti-tumor immunity by inducing ICD and reshaping the tumor microenvironment. However, RT has both immune suppressive and stimulatory effects, and thus RT alone often is not able to induce anti-tumor immunity effectively. This is partly due to the fact that after RT tumor cells suppress the immune system by upregulating immune suppressive ICMs. Thus, it is beneficial to use RT with additional ITs, which further boost RT's effects and enhance the anti-tumor immune response. Furthermore, when using RT in combinational settings, RT

planning should be strongly considered. Additionally, to promote RT's effects, HT can be combined with RT to sensitize cancer cells to RT and counteract the radioresistance of cancer cells. HT is not only a potent sensitizer for RT and CT, but it can also effectively contribute to induce anti-tumor immune responses, increasing the efficacy of RT and CT. Furthermore, multimodal treatments of radiochemotherapy together with HT can target CSCs, which are resistant to RT and CT alone and further prevent tumor metastases and recurrence.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no competing interests.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- radio- and chemosensitizing effects of hyperthermia are summarized and the need of randomized trials with hyperthermia in multimodal settings is stressed.**
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