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FOCUSSED RESEARCH REVIEW

Antitumor immune responses induced by ionizing irradiation and further immune stimulation

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Abstract The therapy of cancer emerged as multimodal treatment strategy. The major mode of action of locally applied radiotherapy (RT) is the induction of DNA damage that triggers a network of events that finally leads to tumor cell cycle arrest and cell death. Along with this, RT modifies the phenotype of the tumor cells and their microenvironment. Either may contribute to the induction of specific and systemic antitumor immune responses. The latter are boosted when additional immune therapy (IT) is applied at distinct time points during RT. We will focus on therapy-induced necrotic tumor cell death that is immunogenic due to the release of damage-associated molecular patterns. Immune-mediated distant bystander (abscopal) effects of RT when combined with dendritic cell-based IT and the role of fractionation of radiation in the induction of immunogenic tumor cell death will be discussed. Autologous whole-tumor-cell-based vaccines generated by high hydrostatic pressure technology will be introduced and the influence of cytokines and the immune modulator AnnexinA5 on the ex vivo generated or in situ therapy-induced vaccine efficacy will be outlined. RT should be regarded as immune adjuvant for metastatic disease and as a tool for the generation of an in situ vaccine when applied at distinct fractionation doses or especially in combination with IT to generate immune memory against the tumor. To identify the most beneficial combination and chronology of RT with IT is presumably one of the biggest challenges of innovative tumor research and therapies.

Keywords Radiotherapy · Immune therapy · Immunogenic tumor cell death · Damage-associated molecular patterns · Dendritic cell · CITIM 2013

Introduction

During the last years, the therapy of cancer emerged as multimodal treatment strategy. Surgery, radiotherapy (RT) and chemotherapy (CT) are the main pillars of achieving local and systemic tumor control. Since recurrent tumors and metastases are still major causes of tumor-associated death, a long-lasting and specific recognition of the tumor cells should be aspired. Consequently, immune therapy (IT) is an appropriate adjunct to standard tumor therapies since it aims to activate the patient's immune system against malignant cells even outside the primary treatment regions of, e.g., RT [1]. However, tumors manipulate the myeloid system and thereby create an immune-suppressive microenvironment by suppressing T cell functions [2]. Immune-suppressive cytokines such as transforming growth factor beta (TGF-β), being the most prominent one, get released by tumor cells. Tumor-based immune suppression has therefore to be converted into immune activation by distinct combinations and chronologies of RT, CT and IT, probably the biggest challenge in the fight against most tumor entities.

This article focuses on how locally applied RT in combination with further immune activation may induce

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systemic antitumor immune responses. It is well known that the major mode of action of RT is the induction of DNA damage that triggers a network of events that finally leads to tumor cell cycle arrest or cell death. The modulation of the tumor cell phenotype by RT might render the tumor visible to the immune system and immunogenic [3], as already demonstrated for certain chemotherapeutic agents [4].

RT is capable of inducing immunogenic tumor cell death forms

The two main and best known forms of cell death are apoptosis and necrosis. While apoptotic cells usually create an immune-suppressive microenvironment [5] and contribute to immune escape of tumors [6], necrotic ones induce inflammatory events. However, it has become more and more evident that the immunological consequences emanating from dying and dead cells have to be regarded in a more detailed manner and in strong dependence of the individual phenotype of the cells after distinct stress and death stimuli. Since RT induces DNA damage and consecutively alters the phenotype of tumor cells by cell death induction, radiation bears the potential to foster dendritic cell (DC)-mediated antitumor immunity in this way [7].

Dendritic cells are potent antigen-presenting cells (APCs) critical in regulating immune responses. They present antigens and expose the appropriate stimulatory molecules to initiate an adaptive immune response. However, DCs may also induce immune tolerance. Critical for the immune-activating function of DCs is the presence of danger signals such as adenosine-5′-triphosphate (ATP), heat shock protein 70 (HSP70) and high mobility group box 1 protein (HMGB1). Endoplasmic reticulum (ER) stress and reactive oxygen species (ROS) production are prerequisites for therapy-induced immunogenic tumor cell death forms [8]. Since the major mode of action of RT is the production of ROS, this classically applied cancer treatment for local tumor control may also activate DCs by inducing the surface exposure, release or secretion of such damage-associated molecular patterns (DAMPs). In this way, stimulated DCs may indeed release tumor-promoting pro-inflammatory cytokines, but activation of toll like receptor 4 (TLR4) by HMGB1 on DCs also induces cross-presentation of tumor antigens and the generation of antigen-specific T cells [9]. An increased expression of activation markers and homing receptors on DCs was observed after contact of the immune cells with HSP70 being released from human colorectal tumor cells after RT plus further immune stimulation with hyperthermia (HT, 41.5 °C for 1 h) [10]. Just recently, it was demonstrated that DCs differentiate in an ATP-dependent manner to immune-activating inflammatory ones after migration into doxorubicin-treated tumors [11].

The uptake of tumor cells by DCs is essential for a consecutive (cross-) presentation of tumor-derived antigens to T cells. Since phosphatidylserine (PS)-exposing stressed cells are swiftly recognized and cleared by macrophages in an anti-inflammatory manner [12], the immunogenicity of dying tumor cells can be increased by blocking this interaction, thereby indirectly fostering the uptake by DCs [13, 14]. AnnexinA5 (AnxA5) is a naturally occurring ligand for PS, and its sole injection around growing tumors comprising dying cells resulted in tumor growth retardation in preclinical ectopic mouse models. The combination of AnxA5 with RT further improved the strong tumor control effects of RT [15]. Currently, we are testing in preclinical mouse models combinations of fractionated irradiation being applied in the clinics and AnxA5 with regard to its immune-stimulating effects. First results indicate that RT induces an enhanced migration of DCs and CD8+ T cells inside the tumor, but also that of immune-suppressive myeloid-derived suppressor cells (MDSCs). The addition of AnxA5 does not affect the infiltration of the tumor by DCs and T cells, but significantly decreases the amount of MDSCs (own unpublished data). Just recently, Shurin and colleagues reported that certain chemotherapeutic agents such as paclitaxel in ultra-low non-cytotoxic doses are capable of promoting the differentiation of MDSCs into DCs [16]. Besides reducing the amount of immune-suppressive cells in the tumor microenvironment by IT, the stimulation of their differentiation into DCs by low-dose CT may contribute to improved tumor defense. Since RT fosters the migration of cytotoxic CD8+ T cells into the tumor, combinations of radiochemotherapy (RCT) and IT are promising of inducing long-lasting antitumor responses. Of note is that clinical trials have recently been set up to analyze whether RT increases the number of tumor infiltrating T cells in human tumors and thus potentially enhance antitumor immune response [17]. Certainly, each tumor treatment modality has its advantages and disadvantages when separately viewed: improved RT techniques allow a maximum local tumor control by concomitantly minimizing the normal tissue side actions, but do not primarily result in systemic antitumor responses. Chemotherapeutic agents act systemically and thereby target the primary tumor and metastases, but are also cytotoxic for most cells of the whole organism. Low dose CT is beneficial for shifting the tumor microenvironment into an activating one, but may not have the sufficient cytotoxicity to kill the tumor cells. Immune therapies induce a long-lasting antitumor response, but cannot deal with big tumor masses. However, in a concerted action, the described single treatments could synergistically contribute to killing of the tumor cells and to the induction of systemic antitumor immune responses. Figure 1 summarizes how induction of necrotic tumor cell death by multimodal treatments finally leads to antitumor immunity.

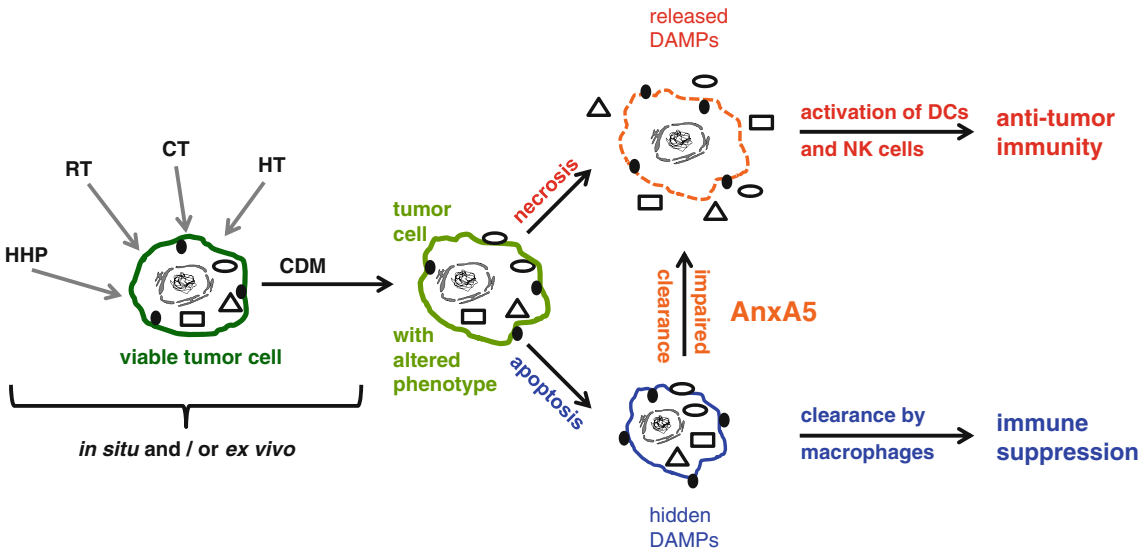

Fig. 1 Immunogenicity of therapy-induced dying and dead tumor cells. Cytotoxic agents such as chemotherapeutics (CT), ionizing radiation administered by radiotherapy (RT), hyperthermia (HT) when applied along with RT and/or CT as well as cell death modifiers (CDM) such as the pan caspase inhibitor zVAD-fmk already alter the tumor cell phenotype very early after their application. Stress proteins such as HSP70 (*white circle*) and recognition molecules for phagocytes such as phosphatidylserine (PS, *black circle*) get exposed. Later on, the cells undergo cell death via apoptosis or necrosis. The latter exists in an accidental and programmed form. While necrotic cells lose their membrane integrity resulting in the release of immune-activation-damage-associated molecular patterns (DAMPs) such as HMGB1 (*triangle*), ATP (*rectangle*), or HSP70 (*white circle*), apoptotic ones maintain their integrity and DAMPS stay hidden. Apoptotic cells get swiftly cleared and recognized via PS, and an immune-suppressive microenvironment is created by the release of anti-inflammatory cytokines by macrophages. In contrast, DAMPs mature and activate DCs and foster the cross-presentation of tumor-cell-derived antigens to T cells. A specific cellular antitumor immune response is started. Additionally, DAMPs may also directly activate cells of the innate immune systems such as natural killer (NK) cells. The necrotic immunogenic form of tumor cell death can be fostered by impairing the clearance of apoptotic cells by macrophages with AnnexinA5 (AnxA5) or by inducing massive amounts of apoptotic cells in multimodal therapy settings. The latter can then be regarded as inducers of an in situ vaccine. Immunogenic tumor cell death forms can also be induced ex vivo, by killing biopsy-derived fresh tumor cells with techniques that result in complete cell death of the tumor cells by concomitantly increasing their immunogenicity. In such a way prepared whole tumor cells by high hydrostatic pressure (HHP) technology are currently tested as tumor vaccines in preclinical mouse models

Abscopal antitumor effects of RT

Of special interest in preclinical and clinical research and in clinical practice is whether only the therapy-modified tumors are attacked by the immune system or whether tumor masses such as metastases outside the irradiation field get then also recognized and killed by immune cells. Just recently, Postow and colleagues reported about regression of a metastatic lesion outside the irradiation field of a patient with malignant melanoma who received RT in combination with Ipilimumab, an antibody inhibiting an immune-suppressive checkpoint on T cells [18]. Since antibody responses against various antigens after RT were found, as well as an increase in MHCII-positive immune cells and a decrease in MDSCs were observed in the peripheral blood, one can assume that the abscopal effects (distant bystander effects) are, at least in part, immune mediated. Adjuvant RT after breast-conserving surgery resulted in significant prolonged long-term survival of the patients, also suggesting that systemic tumor control of small tumor masses was induced by ionizing radiation [19].

Preclinical models clearly demonstrated that irradiation combined with IT that activates DCs result in tumor shrinkage of tumors outside the irradiation field, but just in immune-competent, and not in T cell-deficient mice [20]. We observed that adaptive and innate immune cells are involved in tumor growth retardation in the irradiated tumors themselves [21], suggesting that innovative radiation protocols should take into account such immune reactions and radiation-free days may be expanded to allow the immune system to react [3]. Nevertheless, it is even unclear whether immune cells in the tumor and in its microenvironment are as radiosensitive as it is always speculated. While murine CD8+ T cells are sensitive to a high dose of 2Gy, NK cells are very resistant with regard to apoptosis induction [22]. Macrophages are also not affected in their viability and phagocytic function by irradiation with a single dose up to clinical relevant 2Gy [23]. Like macrophages, DCs have been shown to be very resistant to

cancer chemotherapy [24] and exposure to ROS that are induced during RT [25].

RT in combination with immune therapy induces antitumor immunity

The essential role of CD8+ T cells in radiation therapy and of DCs in priming T cell-mediated specific antitumor immune responses has been mainly proven in preclinical models [4, 26, 27]. We observed in the CT26 colorectal syngeneic Balb/c mouse tumor model that especially CD8+ T cells migrate into the tumors in a narrow time slot, between days 3 and 5 after the last irradiation [28]. Regarding treatments with anthracyclines, an enhanced CD8+ T cell infiltration into the tumor was observed after 7 days [29]. Such observations strongly suggest that IT has to be administered in multimodal therapy settings with RT, CT or RCT at clearly defined time points during or after the classical treatments and in dependence of the death stimuli.

In contrast to certain classes of chemotherapeutic agents, under most circumstances, RT alone is a poor inducer of immune-mediated local and abscopal effects. But combinations of RT with further immune stimulants are more effective, since the immune-suppressive tumor microenvironment can thereby be overcome. The combination of RT with T helper cell type 1 (Th1) therapy resulted in the induction of CD8+ tumor-specific cytotoxic T cells (CTLs), both at the tumor tissue and at the tumor-draining lymph nodes [30]. Also high linear energy transfer (LET) radiation, primarily aiming to increase local control of primary tumors, has recently been shown to be capable of inducing antitumor immunity. A significant reduction in tumor formation after secondary tumor challenge was observed in a murine squamous cell carcinoma model, and the antitumor immune effects were again most beneficial when combing radiation with DC treatment [31]. The latter can be administrated from the outside, or DCs are directly activated in situ. The strategy of whole tumor cell vaccines is based on such an in vivo activation of DCs and consecutively of CTLs by providing not a single tumor-associated antigen (TAA), but multiple ones.

We introduce high hydrostatic pressure technology as an innovative method for the preparation of whole-tumor-cell-based vaccines [1]. It is a well reproducible technology, since pressure force vectors act orthogonal with equal absolute value on the cell surface. Further, pressure propagation is homogenous and quasi not delayed. Our own work has demonstrated that high hydrostatic pressure (HHP) totally inactivates tumor cells and induces immunogenic forms of cell death characterized by the release of danger signals such as HSP70 and HMGB1 [32] (Fig. 1). First in vivo experiments with the CT26 colorectal carcinoma mouse model give hints that HHP vaccination harnesses the immunogenic features of RT since the most beneficial tumor growth retardation was achieved when RT was combined with HHP-generated autologous whole cell tumor vaccine and additional application of IL-12. This cytokine plays a central role in regulating both innate and adaptive immune responses and can by itself induce potent anticancer effects [33]. It favors the differentiation of naïve CD4+ T cells to Th1 cells. The interaction of Th1 cells with DCs fosters the activation of naïve CD8+ T cells by DCs via CD40/CD40-ligand interaction. IL-12 further sensitizes bone marrow-derived tumor stromal cells and thereby enhances the effects of CD8+ T cells [34] and leads in combination with further cytokines and irradiation to the activation of NK cells [35]. Future studies are now needed where vaccines prepared with HHP technology in combination with IL-12 and clinically feasible RT schemes are tested. The combination of tumor cell vaccines with RT may help to overcome weak responses against poor immunogenic tumor antigens, since RT can even promote an antigenic cascade, meaning that T cell clones reactive against different antigens than those used for vaccination occur [36, 37]. Other innovative approaches combined RT with HSP70-peptide complexes obtained from radioresistant tumor cells and proved that specific immunity to radioresistant populations of tumor cells can be induced [38]. Whole-tumor-cell-based vaccines have the great advantage that the vaccination is not only designed for one TAA. Many tumor antigens, non-immunogenic and immunogenic ones, are delivered by the killed tumor cells. The latter can be provided by vaccines or be in situ induced by RT alone or in combination with IT. Therefore, even tumors located at immune-privileged sites in the body may become beneficial targets for immune-mediated tumor cell killing.

Immune reactions at immune-privileged sites after RT

The poor prognosis of patients with malignant brain tumors is mainly caused by an extensive spread of tumor cells into surrounding regions of the brain, by low immune surveillance and an increased resistance to RT and/or CT. Since glioblastoma multiforme (GBM), being the most malignant and aggressive form of brain tumor, is a highly variable and infiltrative tumor, a personalized and systemic treatment approach should be promising. For this purpose, the immune system becomes an ideal antitumor defense instrument. First aspiring results from pilot studies and phase I and II trials are currently coming up indicating that vaccination with DCs improves immune functions and results in longer survival of patients with malignant gliomas. The authors conclude that DC vaccination in combination with RT and CT with temozolomide in patients with GBM is safe, feasible and is

capable to induce tumor-specific immune responses [39, 40]. However, ex vivo differentiation of DCs is complex and time-consuming. Our approach aims to complement the current concepts and to reduce the complex ex vivo cultivation and differentiation of DCs by inducing in situ antigen loading of stimulated DC. This should be achieved by blocking the phagocytosis of RCT-induced dead tumor cells with AnxA5 [15], by co-stimulation of DCs with heat-induced danger signals [10] and by delivering a plethora of tumor antigens with HHP-killed autologous tumor cells [32]. Analyses of danger signals in biopsies of patients with GBM before and after RT and cell culture assays revealed that fractionated RT is the main trigger to induce the release of the danger signal HSP70 by glioblastoma cells [41, 42]. Even immune responses in the peripheral blood do not always correlate with success of cancer therapies, future research should shed more light into whether immune monitoring of the peripheral blood of tumor patients, which is easy and closely meshed to perform, has added prognostic and predictive value to analyses of immune cells and danger signals present in tumor biopsies. The latter are understandably available at limited time points before, during, and after therapy.

Fractionation of RT affects the tumor's immunogenicity

The immunostimulatory potential of RT can basically be classified as effects on the tumor cell surface and on the tumor microenvironment (Fig. 2). The first make the tumor cells more sensitive for cytotoxic T cell responses, due to an increased expression of immunogenic surface molecules like adhesion molecules, death receptors, stress-induced ligands, and classical stimulatory molecules such as MHC I and CD80 [37]. As concerns the tumor microenvironment, RT triggers the timely restricted release of many pro-inflammatory molecules such as chemokines, cytokines, and immune-activating danger signals (DAMPs) as well as exosomes [43] (Fig. 2).

We originally suggested that combination of hypofractionated RT with IT is beneficial to give the immune system time to act and react [3]. Hypofractionated RT schemes (higher single dose, lower fraction numbers) have been enormously extended due to highly improved radiation devices and techniques [44]. However, the differences in immune modulation by single high doses of RT compared to distinct fractionation schemes are far away from being revealed. In preclinical mouse models, Dewan and colleagues demonstrated that only fractionated and not RT with a single high dose induces antitumor immunity when combined with anti-CTLA-4 antibody immune therapy [45]. Fractionated RT with medium-size radiation doses resulted in the B16 melanoma mouse model in the best tumor control

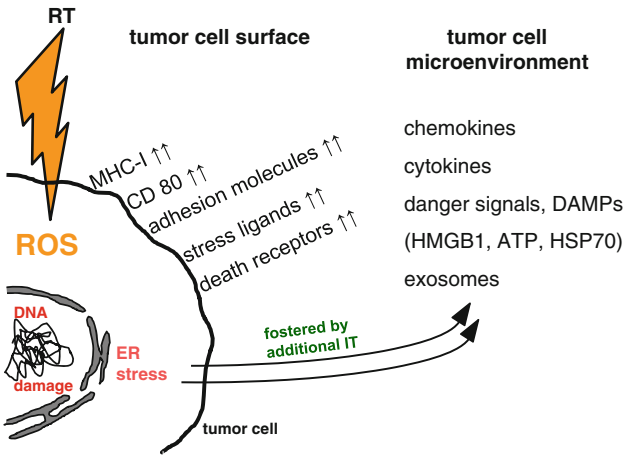

Fig. 2 Immune-activating properties of radiotherapy. The medical use of ionizing radiation in radiotherapy (RT) primarily aims to induce DNA damage in tumor cells (local tumor control), but additionally alters the tumor cell surface and its microenvironment. Via the generation of reactive oxygen species (ROS) and provoking endoplasmic reticulum (ER) stress, immune-activating cytokines and chemokines, exosomes as well as damage-associated molecular patterns (DAMPs) get released. This is strongly fostered by additional immune therapy (IT). On the tumor cell surface, molecules, ligands, and receptors that promote lysis of the tumor cells by cytotoxic T lymphocytes and contribute to immune stimulation get exposed after RT. Therefore, RT acts both, targeted on the tumor cell and non-targeted on the microenvironment perceived as the immune-mediated distant bystander (abscopal) effects of this classical local tumor treatment

and tumor immunity while maintaining the numbers of immune-suppressive Treg low [46]. Other data based on preclinical model systems suggest that especially RT with higher single dose promotes the priming of antigen-specific DCs [26]. We analyzed the influence of different fractionation schemes on the activation of human monocyte-derived DCs with preclinical in vitro assays and found that fractionated and hypofractionated RT, compared to oligofractionation (high single-dose application), results in a significant increased activation of human DCs, as monitored by the expression of maturation markers, secretion of immune-activating cytokines, and capacity to specifically stimulate T cells ([47] and own unpublished data).

Future perspectives and needs for implementation of optimized combinations of RT and immune therapies

A paradigm shift has taken place during the last years: local irradiation delivered by RT does not only lead to DNA damage in the tumor cells, but also modifies their phenotype by inducing distinct forms of tumor cell death. Non-targeted and immune-mediated reactions result, mainly when further immune stimulation with additional IT is ensured [37]. Focus should be set on how conventional

cytotoxic therapies in conjunction with targeted therapies and IT modulate immune responses [48] and consecutively contribute to the improvement of the patient's survival and quality of life. A big challenge in tumor therapy is to identify the most beneficial combination and chronology of multiple treatment options available today (Fig. 3). One general ambition should be to generate immune-activating forms of tumor cell death such as necroptosis [49]. Such programmed forms of tumor necrosis could be induced by application of death modifiers such as the pan caspase inhibitor zVAD-fmk in adjunct to RCT and IT [50] (Fig. 1).

In summary, the immune-suppressive mode of action of RT being in the mind of many oncologists mainly refers to the immune suppression when RT is applied as whole body treatment for conditioning patients for bone marrow transplantation. In contrast, locally applied RT in the therapy of solid tumors can modify the tumor cells in a way that they get immunogenic (Fig. 2). RT is most beneficial in induction of antitumor immunity when combined with IT to overcome the general immune-suppressive microenvironment of most tumors. Radical lymph node dissection should be reassessed in the light to reduce toxicity [51] and to increase the tumors immunogenicity by allowing DCs to prime T cells in the draining lymph nodes (LNs).

The following questions have still to be answered: what is the best timing of combining RT with IT? How multimodal (RT, CT, IT, targeted therapies, and tumor cell death modulations) radio-oncologists should design their therapies to overcome the immune-suppressive properties of tumors? Which fractionation strategy *per se* is the most immunogenic without the need of further IT? How sensitive are immune cells inside the tumor for re-irradiations? Should we exclude some LNs from radiation for a distinct time during multimodal therapy to allow the immune system to react? Which cells of the innate and adaptive immune system are primarily involved in therapy-induced antitumor immune responses and how do they interact?

We conclude that numerous and convincing preclinical data about the immunogenicity of certain chemotherapeutic agents exist, while the most data about the immunogenicity of RT are based on single high-radiation doses which are mostly not used in clinical practice. Current and future work should focus on the immunogenicity of distinct fractionated RT schemes and result in the determination of innovative combinatory treatments consisting of RCT and IT (Fig. 3). Immune mechanisms leading to efficient antitumor immune response after RT have to be examined in detail, and the interplay between the innate (NK cells) and adaptive immune system (CTLs) by radio(chemo)immunotherapy-induced specific and long-lasting antitumor

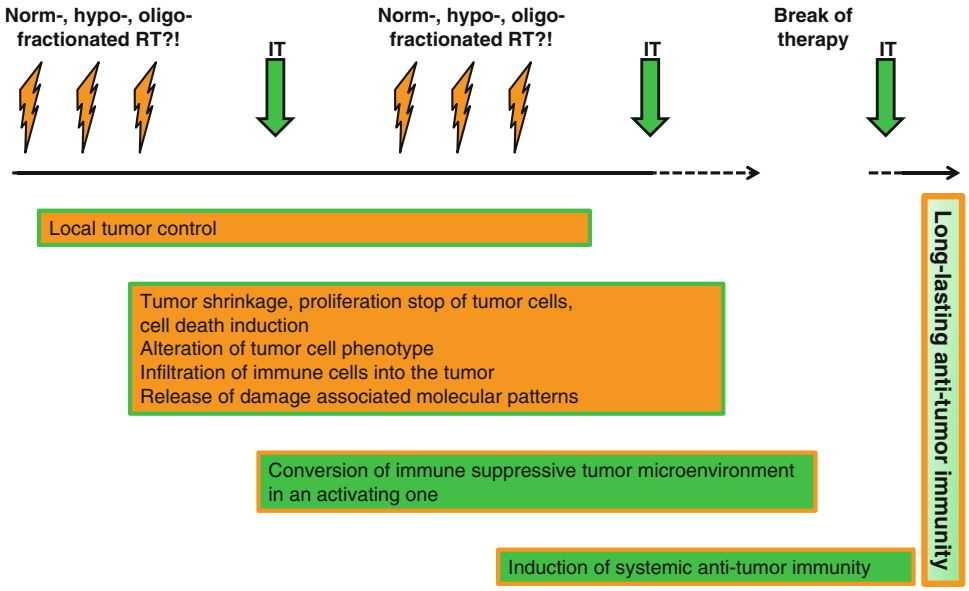

Fig. 3 Crucial aims when combining distinct fractionation schemes of radiotherapy (RT) with immune therapy (IT). The presumably biggest challenge of innovative multimodal tumor therapies is to identify the most beneficial combination and chronology of distinct chemotherapeutic and radiation protocols with immune therapies (IT). Besides local tumor control, systemic antitumor immunity should be induced. To provide the immune cells time to act and react after radiotherapy (RT), longer breaks between the single radiations might be beneficial and could be further utilized for application of IT that converts the immune-suppressive tumor microenvironment into a more activating one. Urgent need exists of further identifying the immunogenic properties of RT alone given in norm-, hypo-, or oligo-fractionated doses. To achieve long-lasting antitumor immunity, characterized by immunological memory against the individual tumor including its metastases, the immune system should be boosted again at the end of the classical therapy after a yet-to-be defined time window

immunity has to be revealed with immune-deficient mouse models. Potential synergy between antigen-specific and non-specific CD8+ T cell responses have already been described [52]. RT should be in the mind of all oncologists as immune adjuvant for metastatic disease and as a tool for the generation of an in situ vaccine when applied at distinct fractionation doses or especially in combination with IT to generate immunological memory against the tumor.

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Conflict of interest The authors declare that they have no conflict of interest.

References

1. Weiss EM, Wunderlich R, Ebel N et al (2012) Selected anti-tumor vaccines merit a place in multimodal tumor therapies. Front Oncol 2:132. doi:10.3389/fonc.2012.00132
2. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V (2012) Coordinated regulation of myeloid cells by tumours. Nat Rev Immunol 12:253–268. doi:10.1038/nri3175
3. Frey B, Rubner Y, Wunderlich R, Weiss EM, Pockley AG, Fietkau R, Gaipl US (2012) Induction of abscopal anti-tumor immunity and immunogenic tumor cell death by ionizing irradiation: implications for cancer therapies. Curr Med Chem 19:1751–1764
4. Apetoh L, Ghiringhelli F, Tesniere A et al (2007) Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat Med 13:1050–1059. doi:10.1038/nm1622
5. Voll RE, Herrmann M, Roth EA, Stach C, Kalden JR, Girkontaite I (1997) Immunosuppressive effects of apoptotic cells. Nature 390:350–351. doi:10.1038/37022
6. Gregory CD, Pound JD (2010) Microenvironmental influences of apoptosis in vivo and in vitro. Apoptosis 15:1029–1049. doi:10.1007/s10495-010-0485-9
7. Lauber K, Ernst A, Orth M, Herrmann M, Belka C (2012) Dying cell clearance and its impact on the outcome of tumor radiotherapy. Front Oncol 2:116. doi:10.3389/fonc.2012.00116
8. Krysko DV, Garg AD, Kaczmarek A, Krysko O, Agostinis P, Vandenabeele P (2012) Immunogenic cell death and DAMPs in cancer therapy. Nat Rev Cancer 12:860–875. doi:10.1038/nrc3380
9. Hagemann T, Balkwill F, Lawrence T (2007) Inflammation and cancer: a double-edged sword. Cancer Cell 12:300–301. doi:10.1016/j.ccr.2007.10.005
10. Schildkopf P, Frey B, Ott OJ, Rubner Y, Multhoff G, Sauer R, Fietkau R, Gaipl US (2011) Radiation combined with hyperthermia induces HSP70-dependent maturation of dendritic cells and release of pro-inflammatory cytokines by dendritic cells and macrophages. Radiother Oncol 101:109–115. doi:10.1016/j.radonc.2011.05.056
11. Ma Y, Adjemian S, Mattarollo SR et al (2013) Anticancer chemotherapy-induced intratumoral recruitment and differentiation of antigen-presenting cells. Immunity 38:729–741. doi:10.1016/j.immuni.2013.03.003
12. Frey B, Gaipl US (2011) The immune functions of phosphatidylserine in membranes of dying cells and microvesicles. Semin Immunopathol 33:497–516. doi:10.1007/s00281-010-0228-6
13. Bondanza A, Zimmermann VS, Rovere-Querini P et al (2004) Inhibition of phosphatidylserine recognition heightens the immunogenicity of irradiated lymphoma cells in vivo. J Exp Med 200:1157–1165. doi:10.1084/jem.20040327
14. Gaipl US, Beyer TD, Baumann I, Voll RE, Stach CM, Heyder P, Kalden JR, Manfredi A, Herrmann M (2003) Exposure of anionic phospholipids serves as anti-inflammatory and immunosuppressive signal–implications for antiphospholipid syndrome and systemic lupus erythematosus. Immunobiology 207:73–81. doi:10.1078/0171-2985-00217
15. Frey B, Schildkopf P, Rodel F, Weiss EM, Munoz LE, Herrmann M, Fietkau R, Gaipl US (2009) AnnexinA5 renders dead tumor cells immunogenic–implications for multimodal cancer therapies. J Immunotoxicol 6:209–216. doi:10.3109/15476910903204058
16. Michels T, Shurin GV, Naiditch H, Sevko A, Umansky V, Shurin MR (2012) Paclitaxel promotes differentiation of myeloid-derived suppressor cells into dendritic cells in vitro in a TLR4-independent manner. J Immunotoxicol 9:292–300. doi:10.3109/1547691x.2011.642418
17. Timke C, Winnenthal HS, Klug F et al (2011) Randomized controlled phase I/II study to investigate immune stimulatory effects by low dose radiotherapy in primarily operable pancreatic cancer. BMC Cancer 11:134. doi:10.1186/1471-2407-11-134
18. Postow MA, Callahan MK, Barker CA et al (2012) Immunologic correlates of the abscopal effect in a patient with melanoma. N Engl J Med 366:925–931. doi:10.1056/NEJMoa1112824
19. Darby S, McGale P, Correa C et al (2011) Effect of radiotherapy after breast-conserving surgery on 10-year recurrence and 15-year breast cancer death: meta-analysis of individual patient data for 10,801 women in 17 randomised trials. Lancet 378:1707–1716. doi:10.1016/s0140-6736(11)61629-2
20. Demaria S, Ng B, Devitt ML, Babb JS, Kawashima N, Liebes L, Formenti SC (2004) Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated. Int J Radiat Oncol Biol Phys 58:862–870. doi:10.1016/j.ijrobp.2003.09.012
21. Frey B, Lotter M, Stangl S, Rodel F, Fietkau R, Gaipl US (2012) Adaptive and innate immune cells contribute to reduced tumor progression after local irradiation of CT26 tumors in Balb/c mice. Strahlenther Onkol 188 [Abstractband zur 18. Jahrestagung der Deutschen Gesellschaft für Radioonkologie (DEGRO)]:132
22. Bogdandi EN, Balogh A, Felgyinszki N, Szatmari T, Persa E, Hildebrandt G, Safrany G, Lumniczky K (2010) Effects of low-dose radiation on the immune system of mice after total-body irradiation. Radiat Res 174:480–489. doi:10.1667/rr2160.1
23. Wunderlich R, Frischholz B, Roedel F, Fietkau R, Frey B, Gaipl US (2013) Ionising radiation with single doses up to clinical relevant 2Gy does not influence viability and phagocytosis capability of single activated macrophages of radiosensitive BALB/c mice. Strahlenther Onkol 189 [Abstractband zur 19. Jahrestagung der Deutschen Gesellschaft für Radioonkologie (DEGRO)]:122
24. Bauer M, Goldstein M, Heylmann D, Kaina B (2012) Human monocytes undergo excessive apoptosis following temozolomide activating the ATM/ATR pathway while dendritic cells and macrophages are resistant. PLoS One 7:e39956. doi:10.1371/journal.pone.0039956
25. Bauer M, Goldstein M, Christmann M, Becker H, Heylmann D, Kaina B (2011) Human monocytes are severely impaired in base and DNA double-strand break repair that renders them vulnerable to oxidative stress. Proc Natl Acad Sci USA 108:21105–21110. doi:10.1073/pnas.1111919109
26. Lee Y, Auh SL, Wang Y et al (2009) Therapeutic effects of ablative radiation on local tumor require CD8+ T cells: changing

strategies for cancer treatment. Blood 114:589–595. doi:10.1182/blood-2009-02-206870
27. Gupta A, Probst HC, Vuong V et al (2012) Radiotherapy promotes tumor-specific effector CD8+ T cells via dendritic cell activation. J Immunol 189:558–566. doi:10.4049/jimmunol.1200563
28. Frey B, Weber J, Kohn K, Lotter M, Stangl S, Rodel F, Fietkau R, Gaipl US (2013) Hypofractionated local radiotherapy induces a timely restricted infiltration of immune cells in CT26 colon cancer tumors of BALB/c mice. Strahlenther Onkol 189 [Abstractband zur 19. Jahrestagung der Deutschen Gesellschaft für Radioonkologie (DEGRO)]:116
29. Ma Y, Aymeric L, Locher C et al (2011) Contribution of IL-17-producing gamma delta T cells to the efficacy of anticancer chemotherapy. J Exp Med 208:491–503. doi:10.1084/jem.20100269
30. Takeshima T, Chamoto K, Wakita D, Ohkuri T, Togashi Y, Shirato H, Kitamura H, Nishimura T (2010) Local radiation therapy inhibits tumor growth through the generation of tumor-specific CTL: its potentiation by combination with Th1 cell therapy. Cancer Res 70:2697–2706. doi:10.1158/0008-5472.can-09-2982
31. Matsunaga A, Ueda Y, Yamada S, Harada Y, Shimada H, Hasegawa M, Tsujii H, Ochiai T, Yonemitsu Y (2010) Carbon-ion beam treatment induces systemic antitumor immunity against murine squamous cell carcinoma. Cancer 116:3740–3748. doi:10.1002/cncr.25134
32. Weiss EM, Meister S, Janko C et al (2010) High hydrostatic pressure treatment generates inactivated mammalian tumor cells with immunogeneic features. J Immunotoxicol 7:194–204. doi:10.3109/15476911003657414
33. Weiss JM, Subleski JJ, Wigginton JM, Wiltrout RH (2007) Immunotherapy of cancer by IL-12-based cytokine combinations. Expert Opin Biol Ther 7:1705–1721. doi:10.1517/14712598.7.11.1705
34. Kerkar SP, Goldszmid RS, Muranski P et al (2011) IL-12 triggers a programmatic change in dysfunctional myeloid-derived cells within mouse tumors. J Clin Invest 121:4746–4757. doi:10.1172/jci58814
35. Ni J, Miller M, Stojanovic A, Garbi N, Cerwenka A (2012) Sustained effector function of IL-12/15/18-preactivated NK cells against established tumors. J Exp Med 209:2351–2365. doi:10.1084/jem.20120944
36. Hodge JW, Sharp HJ, Gameiro SR (2012) Abscopal regression of antigen disparate tumors by antigen cascade after systemic tumor vaccination in combination with local tumor radiation. Cancer Biother Radiopharm 27:12–22. doi:10.1089/cbr.2012.1202
37. Formenti SC, Demaria S (2013) Combining radiotherapy and cancer immunotherapy: a paradigm shift. J Natl Cancer Inst 105:256–265. doi:10.1093/jnci/djs629
38. Weng D, Song B, Koido S, Calderwood SK, Gong J (2013) Immunotherapy of radioresistant mammary tumors with early metastasis using molecular chaperone vaccines combined with ionizing radiation. J Immunol 191:755–763. doi:10.4049/jimmunol.1203286
39. Wheeler CJ, Black KL, Liu G et al (2008) Vaccination elicits correlated immune and clinical responses in glioblastoma multiforme patients. Cancer Res 68:5955–5964. doi:10.1158/0008-5472.can-07-5973
40. Van Gool S, Maes W, Ardon H, Verschuere T, Van Cauter S, De Vleeschouwer S (2009) Dendritic cell therapy of high-grade gliomas. Brain Pathol 19:694–712. doi:10.1111/j.1750-3639.2009.00316.x
41. Muth C, Schmidt V, Rubner Y, Fietkau R, Semrau S, Buslei R, Gaipl US (2013) Comparative analysis of the danger signals HMGB1, Hsp70, and Calreticulin in primary tumors and relapses of glioblastoma patients. Strahlenther Onkol 189 [Abstractband zur 19. Jahrestagung der Deutschen Gesellschaft für Radioonkologie (DEGRO)]:124
42. Rubner Y, Sieber R, Buslei R, Fietkau R, Frey B, Gaipl US (2013) Fractionated radiotherapy and not chemotherapy with temozolomide and/or valproic acid is the main stimulus for the induction of immunogenic glioma cell death in p53 mutated glioma cell lines. Strahlenther Onkol 189 [Abstractband zur 19. Jahrestagung der Deutschen Gesellschaft für Radioonkologie (DEGRO)]: 115–116
43. Gastpar R, Gehrmann M, Bausero MA, Asea A, Gross C, Schroeder JA, Multhoff G (2005) Heat shock protein 70 surface-positive tumor exosomes stimulate migratory and cytolytic activity of natural killer cells. Cancer Res 65:5238–5247. doi:10.1158/0008-5472.can-04-3804
44. Durante M, Reppingen N, Held KD (2013) Immunologically augmented cancer treatment using modern radiotherapy. Trends Mol Med. doi:10.1016/j.molmed.2013.05.007
45. Dewan MZ, Galloway AE, Kawashima N, Dewyngaert JK, Babb JS, Formenti SC, 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 15:5379–5388. doi:10.1158/1078-0432.ccr-09-0265
46. Schaue D, Ratikan JA, Iwamoto KS, McBride WH (2012) Maximizing tumor immunity with fractionated radiation. Int J Radiat Oncol Biol Phys 83:1306–1310. doi:10.1016/j.ijrobp.2011.09.049
47. Kulzer L, Schaft N, Dorrie J, Rubner Y, Frey B, Gaipel U, Fietkau R (2013) Influence of fractionated and hypofractionated radiotherapy on the activation of human dendritic cells. Strahlenther Onkol 189 [Abstractband zur 19. Jahrestagung der Deutschen Gesellschaft für Radioonkologie (DEGRO)]:119
48. Vanneman M, Dranoff G (2012) Combining immunotherapy and targeted therapies in cancer treatment. Nat Rev Cancer 12:237–251. doi:10.1038/nrc3237
49. Mantel F, Frey B, Haslinger S et al (2010) Combination of ionising irradiation and hyperthermia activates programmed apoptotic and necrotic cell death pathways in human colorectal carcinoma cells. Strahlenther Onkol 186:587–599. doi:10.1007/s00066-010-2154-x
50. Werthmoller N, Frey B, Fietkau R, Gaipl US (2013) The influence of standard combinatory tumor treatments and the apoptosis inhibitor zVAD-fmk on B16 melanoma cell death forms and consecutively on macrophages and dendritic cells. Strahlenther Onkol 189 [Abstractband zur 19. Jahrestagung der Deutschen Gesellschaft für Radioonkologie (DEGRO)]:120
51. Scheithauer HR, Schulz DS, Belka C (2011) Endometrial cancer: reduce to the minimum. A new paradigm for adjuvant treatments? Radiat Oncol 6:164. doi:10.1186/1748-717x-6-164
52. Sckisel GD, Murphy WJ (2012) Specificity may be overrated in cancer immunotherapy: getting to know the nonspecific side of memory T Cells. Oncoimmunology 1:1208–1210. doi:10.4161/onci.20779