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Combining Heavy-Ion Therapy with Immunotherapy: An Update on Recent Developments

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Abstract

Clinical trials and case reports of cancer therapies combining radiation therapy with immunotherapy have at times demonstrated total reduction or elimination of metastatic disease. While virtually all trials focus on the use of immunotherapy combined with conventional photon irradiation, the dose-distributive benefits of particles, in particular the distinct biological effects of heavy ions, have unknown potential vis-a-vis systemic disease response. Here, we review recent developments and evidence with a focus on the potential for heavy-ion combination therapy.

Keywords: combination immunotherapy-radiotherapy; heavy-ion radiotherapy; radiation-induced immunogenic cell death; abscopal effect

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Review

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Introduction

Cancer therapy today delineates into 2 paradigms: control of local disease and control of distant disease. Local control combines surgical expertise, that is, attempting to physically remove as much tumorous tissue from the body as possible, with radiation therapy (RT), in which tumor cells are destroyed. Depending on disease staging, these are paired with distant disease control modalities consisting of chemotherapy, immunotherapy (IT), and, depending on disease type, hormonal therapy. In particular, IT is an increasingly broad category incorporating techniques ranging from checkpoint inhibitors to ex vivo modulation and reimplantation of immunoactivating cells [1–3].

By convention, late-stage metastatic disease requiring significant systemic treatment is thought of as palliative, life-extending care with the goal of creating an environmental balance in which patients continue their life indefinitely with cancer, but cancer elimination is not thought of as possibility. However, for decades clinical reports have been published showing total-remission cases [4], and in the case of RT, this destruction of systemic disease following local-focused irradiation has come to be known as the abscopal effect. In light of proposed immunologic mechanisms driving the abscopal effect, and following the advent of regular IT use in the patient setting, the idea that metastatic disease may be cured, as opposed to slowed, has started to

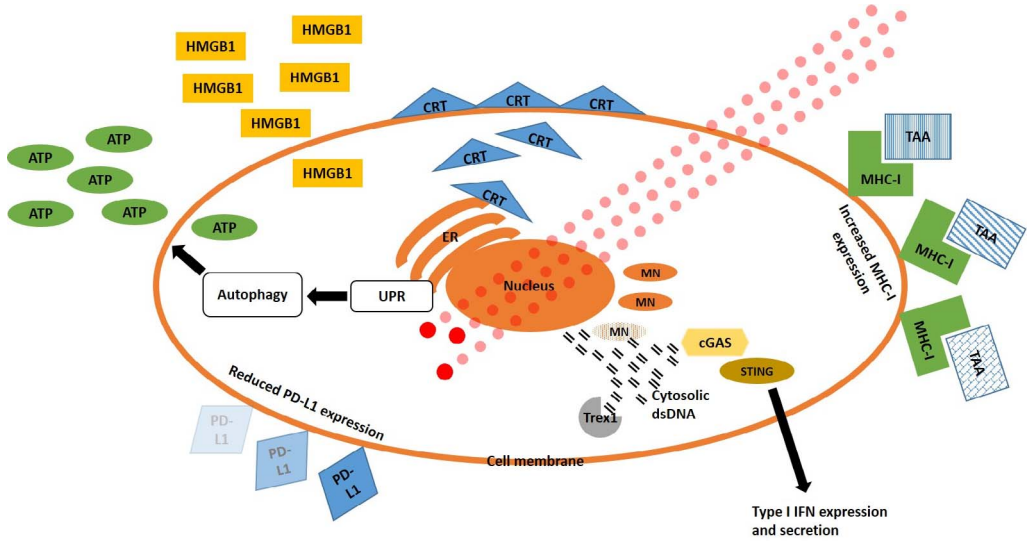

Figure. Overview of mechanisms involved in the immunogenicity of (particle) radiation exposure. Immunogenicity of radiation and the related immunogenic cell death generally depend on antigenicity and adjuvanticity, both important for an effective immune response. Antigenicity of radiation comprises increased expression of various tumor-associated antigens (TAAs) and major histocompatibility complex-I (MHC-I), thus enhancing presentation of neo-antigens. Adjuvanticity in this context describes a variety of molecular processes, predominantly the release of damage-associated molecular patterns (DAMPs), including high-mobility group protein 1 (HMGB1) and adenosine triphosphate (ATP) as well as the translocation of calreticulin (CRT) from the endoplasmatic reticulum (ER) to the cell membrane. The release of ATP seems to be related with radiation-induced autophagy, which may be triggered via unfolded protein response (UPR) stimulation. Particle radiation has further been associated with a decreased expression of programmed death-ligand 1 (PD-L1), possibly indicating a chance for greater T-cell activity. The dosing seems to play a major role in the activation of an effective immunogenic response upon radiation exposure and a dualism of cyclic GMP-AMP synthase/stimulator of interferon genes (cGAS/STING) on the one hand and three prime repair exonuclease 1 (Trex1) on the other hand has been reported to guide such activation. The underlying common factor of this dualism seem to be cytosolic double-stranded DNA (dsDNA) fragments, whose rising concentration results in the activation of STING via dsDNA sensing by cGAS and a subsequent increased expression and secretion of type I interferon (type I IFN). Nonetheless, a certain threshold concentration of cytosolic dsDNA is assumed, after which Trex1 activation leads to the degradation of dsDNA, hence resulting in a lower type I IFN expression. This suggests a minimum dose as well as an upper threshold dose that guides effective immune responses and remains to be investigated for particle radiation. Ways for dsDNA to reach the cytoplasm upon radiation stress have recently been discussed and may involve nuclear envelope rupture of the cell nucleus or the involvement of micronuclei (MN) formation and the subsequent rupture of their envelopes. Particle radiation, which features an increased formation of MN in many cell lines may hence be considered particularly effective in the induction of an immune response.

gain traction, with combination immunotherapy-radiation therapy (CIR) forming a potential therapy regimen [5]. Here, we provide a recent update on CIR with a focus on heavy-ion therapy.

The Abscopal Effect

The abscopal effect does not necessarily describe total disease resolution. Animal studies and human clinical case reports that have shown systemic disease response following RT have demonstrated variable results, with patients experiencing disease stasis, disease retardation, total disease elimination, or disease progression; duration and durability of the abscopal effect appear to vary, with any disease retardation or regression falling under the abscopal umbrella [6, 7].

The mechanism by which the abscopal effect operates remains unknown, and disease responses may belie multiple mechanistic pathways of response to disease challenge, owing to genetic, environmental, or other confounders. Similar episodes of sudden, unexplained disease response have been suggested to occur spontaneously, particularly with melanoma [8]; with RT alone or in a variety of diseases [7, 9]; and with combination therapy, suggesting the potential activation of an innate capability in the body. Our group has previously proposed a system for classifying degrees of abscopal effect and potentially assisting with classifying results [10]. To date, the proposed mechanistic thought of the abscopal effect appears to involve a local inflammatory and/or immunologic response following RT, with disruption of the local tumor microenvironment. This subsequently leads to a cascading immunoactivation that spreads to become a full-system response through an as-yet unclear mechanism, with immunologic response detectable in distant, unirradiated tumors [11]. In a complete abscopal effect

response, secondary disease appears to be immunologically eliminated [12]. However, the precise mechanisms involved in each step of this process remain to be elucidated.

Some elements of systemic response to CIR do increasingly appear clear: the inclusion of IT to local irradiation may improve outcome and increase likelihood of distant effect, above the use of either IT or radiation alone [13]. At worst, adding radiation to IT appears to have no effect on systemic outcome, with no such cases noted at time of writing, though notably no randomized controlled trials have evaluated this specific question. At this time, it may be thought that radiation disrupts the tumor microenvironment, and IT assists in amplifying and directing that local immune response into something global; the immunogenicity and efficiency of radiation may then be thought of as critical.

Immunogenicity of Radiation Therapy

The various detailed mechanisms thought to be involved in immunogenicity of radiation and radiation-induced immunogenic cell death (ICD) are beyond the scope of this article and are reviewed elsewhere [14, 15]. A brief overview of immunogenic mechanisms with respect to particle radiation is depicted in figure 1. Immunogenicity of cell death generally depends on antigenicity and adjuvanticity, both important for an effective immune response [16]. The antigenicity of radiation is characterized by an increased expression of TAAs and has been reported to modulate the peptide repertoire of tumor cells, hence presenting neo-antigens by which cancer immunoediting may be challenged. Additionally, it enhances the MHC-I expression, thus upregulating antigen presentation [14, 17, 18]. On the other hand, the adjuvanticity of ionizing radiation is related to the release of DAMPs, which, together with cells displaying increased antigenicity, can initiate an adaptive immune response [16]. Briefly, DAMPs represent a variety of molecular changes that trigger ICD not only following radiation exposure. The release of ATP and HMGB1, as well as the translocation of CRT to the plasma membrane, are considered central hallmarks of ICD [19]. Both photon and particle radiation have been reported as bona fide inducers of ICD [16, 20–22]. Radiation-induced ICD, however, features differential changes in molecular processes that are associated with an efficient immune response as elegantly reviewed by Galluzzi and colleagues. These processes comprise unfolded protein response, autophagy, and differential expression of type I IFN, among others [16].

Particle Radiation and Immunogenicity

To date, the majority of clinical reports describing abscopal effects have been incidental in the setting of photon irradiation with or without IT, though case reports describing abscopal-type reactions sans IT have been published, including with carbon-ion therapy [7]. In the absence of dedicated clinical trials evaluating combination therapy, it remains unknown how CIR improves clinical outcomes compared with conventional therapy, particularly with regard to particle therapy.

However, both experimental and clinical evidence suggest that particle therapy, and in particular high linear energy transfer (LET) carbon-ion therapy, demonstrate improvement in metastasis rate and a reduction in local recurrence [2, 7, 23, 24].

With respect to CIR, particles have been hypothesized to be advantageous, owing to distinct cell death patterns and damage response pathway induction compared with conventional photon therapy, putatively increasing the immunogenicity of induced cell death [24]. Particle radiation, in the form of α-particles, has been reported to be a bona fide ICD inducer in vaccination experiments, with subsequent immune responses in immune-competent hosts [16, 22].

Photons and protons were shown comparable with regard to increase of CRT translocation to the cancer cell surface and the exposure of molecules important for immune recognition, with proton therapy resulting in a decrease of PD-L1 in cancer cells, possibly indicating a chance for greater T-cell activity [25]. In mouse studies, carbon-ion irradiation correlated with stronger immune activation when paired with dendritic cell injection. Combined carbon-ion therapy with IT demonstrates increased antitumor immunity and reduced the number of metastases compared with RT or IT alone, or in combination with photons [26–28]. Mouse model studies involving carbon-ion RT and IT continue, with our group working on one direction of study. To date, no clinical trials have evaluated particle irradiation in conjunction with IT.

While the majority of current clinical research is focused on conventional irradiation and IT, particle technology continues to progress. For example, in coming years irradiation with oxygen beams may open the door to multi-ion LET tumor painting [29–31], further complicating the picture of how particle irradiation may potentiate systemic effects.

It has been suggested that higher per-fraction dose, such as in stereotactic body radiation therapy (SBRT) or hypofractionation, may preferentially generate immune responses [14]. The enhanced dose distribution of ion therapy, owing to utilization of the Bragg peak seen with particle irradiation as well as the sharp lateral edges of the irradiation field proffered

by heavy-ion therapy, has enabled both higher doses than conventional treatment with generally fewer side effects, along with hypofractionated delivery of those doses, yielding perhaps the highest per-fraction doses in all of RT [32, 33].

The mechanism by which hypofractionated treatment appears to result in equivalent to superior treatment results remains hypothetical, but this has led to great interest in the potential for high-LET near-monofractional treatment of disease, and the potential immunogenicity theorized therein. Further considerations for particle CIR are discussed below.

Considerations for Dosing with Respect to Immunogenicity

Hypofractionation is a dominant trend in RT, particularly in particle RT [34], with advantages hypothesized with respect to the induction of ICD [14]. In studies using photon irradiation, increased dose has also recently been shown to depend on further factors influencing the efficiency of the immune response [35].

The threshold dosing for efficient induction of an immune response involves the activation of the cyclic GMP-AMP synthase (cGAS)/stimulator of interferon genes (STING) pathway, which has been shown to increase antitumor immune responses triggered by irradiation [35, 36]. cGAS is a sensor of cytosolic DNA; after binding double-stranded DNA (dsDNA), cGAS activates the endoplasmatic reticulum adaptor protein STING, among other cytokines. In particular, type I interferon expression is induced by STING [37, 38], which plays an important role in the recruitment of BATF-3 dendritic cells, which are necessary for tumor-associated antigen cross presentation to CD8+ CTLs and hence for an effective immune response [39–41]. cGAS appears to increase in proportion to the amount of dsDNA present in the cell and is hence dose dependent.

On the other hand, a maximal dose has been suggested in the work of Vanpouille-Box and colleagues [35], defined by the expression of Trex1, a key regulator of radiation-induced immunogenicity, which degrades cytosolic dsDNA. Trex1 has been found to be expressed at doses around 12 to 18 Gy of photons in different mouse and human carcinoma cell lines [35, 42] and, thus, counters the activation of STING and influences type I interferon expression. This suggests that there is a limit of the benefit provided by dose escalation or hypofractionation with respect to the generated immune response.

To the best of our knowledge, no data on the response of STING or Trex1 activation to particle radiation exist; however, its potential should be investigated with respect to the dualisms of STING and Trex1. It remains to be clarified whether particle irradiation immunogenicity operates by similar or different mechanisms.

DNA Damage Pattern and Micronuclei after High-LET Radiation May Influence the Immunogenic Response

Radiation may influence the mechanism by which dsDNA is released in the cytoplasm, via micronuclei (MN), which forms the basis of a recent proposal by Durante and Formenti [43]. MN consist of missegregated chromosomes and chromosome fragments that appear, for example, following cell exposure to ionizing radiation [44, 45]. High-LET radiation, such as protons or carbon ions, has been found to lead to efficient MN formation [46–49].

Per the proposed mechanism by Durante and Formenti [43], cytosolic DNA in MN may lead to activation of cGAS/STING. The nuclear envelope of MN in cancer cells may collapse during interphase and, furthermore, relocalization of cGAS to MN has been reported and has been associated with inflammatory signaling and interferon-stimulated gene expression [50, 51]. In addition, a number of cancer histologies have been found to feature increased background formation of MN [52], and p53 deficiency, a feature of many cancers, has been tied to an increased transmittance and hence persistence of MN to daughter cells as they proceed through mitosis, despite radiation-induced damage. As such, following exposure of a given cancer cell to RT and defective repair within the MN, the DNA in the MN is pulverized, and, following envelope rupture, high activation of cGAS may occur [43, 53–56]. This forms a proposed basis for the favorable results expected with radiation combination therapy, particularly with particles.

The authors further present an alternative mechanism, wherein dsDNA is released in the cytoplasm following nuclear envelope rupture regardless of MN formation and the required mitosis [43]. When irradiated, particularly with high-LET radiation, a high fraction of small DNA fragments is produced [57–59]. Furthermore, in cancer cells lacking p53 or Rb genes in particular, nuclear rupture is enhanced [43, 60].

Interestingly, cell-free circulating dsDNA fragments in body fluids of animals as well as in the plasma of patients undergoing RT has been described and linked with immunostimulation via various mechanisms [61–63]. This may be another distinct mechanism of action for particle therapy, if the aforementioned induction of very small dsDNA fragments following high-LET exposure is considered [57, 64, 65].

Induction of Autophagy after High-LET Radiation and Immunogenic Cell Death

Macroautophagy, generally also termed autophagy is a mechanism of homeostasis and stress response, as it can occur following exposure to radiation; it comprises the degradation of cellular organelles or proteins to sustain energy needs and de novo biosynthesis [66–68]. Autophagy was reported to have cell autonomous as well as cell non-autonomous effects, influencing, for example, radiosensitivity and the outcome of therapy [69]. However, it may function beyond energy generation in stressed cells. Autophagy has recently been shown to play a major role in triggering ICD and a subsequent immune response [16, 69, 70] since it leads to ATP release. ATP release by stressed or dying cells is one requirement for an efficient immune response, and further autophagy appears to be a principle mechanism [71, 72]. Autophagy appears in virtually all types of tumor cells upon irradiation [73]. In this vein, RT-induced autophagy may potentiate IT; this has been shown in a melanoma mouse model with photon irradiation [74].

Autophagy has been identified as an important type of cell death following high-LET radiation exposure, as shown with fast neutrons applied to hepatocellular carcinoma cells [75]. Altmeyer and colleagues [75] found that considerable autophagy took place in tumor xenografts following high-LET irradiation and hence suggest that autophagy may act as a predominant mode of cell death in the efficacy of high-LET radiation. This research has been extended by Jin and colleagues to carbon-ions as well [76], seeming to induce autophagy via unfolded protein response stimulation [77]. Though research is still in the initial stages across the board, these findings may provide another clue as to the mechanism behind how heavy-ion radiation effectively induces immunogenic cell death and subsequent immune response.

Further Considerations of Potential Advantages of Heavy Ion Therapy as a Partner to Immunotherapy

Although studies using photon beams have suggested several potential molecular mechanisms for the abscopal effect, evidence of abscopal effect induction by particle beams is still limited. For the induction of post-irradiation cell death, the tumor microenvironment, including factors such as hypoxia, cellular genetics, and cell cycle status, appears to have a strong impact. Generally, hypoxic cells, cells with mutations in TP53, and G1/G0-phase cells are resistant to photon irradiation; factors extend even so far as to the time of day cells are treated [78]. Furthermore, cancer stem-like cells are resistant to photon irradiation [79]. Due to this multitudinous list of factors underlying the cell status within a tumor, uniform irradiation may induce different phenomena depending on cellular condition and state.

It has been shown that high-LET radiation is able to induce cell death at lower doses against resistant cells. Furthermore, it has been reported that high-LET radiation is able to induce cell death on cancer stem-like cells or p53 mutant cells [80]. Cell death independent of p53 following radiation exposure has further been described via the ceramide pathway [81]. The ceramide pathway in turn is associated with MHC-I enhancement [82], and high-LET particles were reported to efficiently induce the ceramide pathway in tumor cells [81, 83], indicating another advantageous feature of particle radiation with respect to its immunogenicity.

In addition, high-LET radiation induced immune cell death at lower doses than photon irradiation [28]. For optimized treatment in the future, understanding how these factors contribute to post-irradiation immune response is critical.

On the other hand, the effect of irradiation on intratumoral immune cells, or locally in the radiation field at time of treatment, must also be considered when evaluating local immune stimulation. Durante and colleagues [84] have shown that carbon-ion therapy generates lower blood toxicity than photon RT, perhaps owing to a reduction in healthy tissue irradiated. Alternatively, studies of photoimmunotherapy have shown that removing regulatory T cells from a tumor causes drastic regression, including in distant tumors containing regulatory T cells [85]. This suggests that regulation of immunosuppression might contribute significantly to the induction of the abscopal effect. Because immune-related cells are generally highly radiosensitive [86], a systemic effect may be more reliably generated through targeting of intratumor inhibitory immune cells, in theory leading to induction of the abscopal effect through irradiation of a very limited amount of the distant tumor. Studies to this end are underway.

Conclusions

Widespread research efforts currently focus on elucidating the mechanisms behind cellular irradiation, local immune response, and the means by which this local response leads to systemic response. Ultimately, mechanistic understanding of distant immune response to local radiation may enable controlled induction of abscopal effects, opening the door to curative treatment of late-stage metastatic disease. Much work remains, however: a wealth of immunotherapeutic interventions are available,

with more reaching testing and market each quarter, and a variety of radiation therapies, with demonstrated differences in biological mechanisms of damage and end-stage effect, are currently available. The limited availability of heavy-ion therapy centers, today clinically limited to carbon-ion and perhaps soon oxygen-ion therapies, further complicates the study of combination therapy. Cell and animal models, in conjunction with successful early-stage clinical case reports, have suggested technical and biological superiority of heavy-ion therapy in establishing local disease control and reducing tumor metastasis. Comprehensive trials from in vitro to in vivo are necessary to evaluate the potential of CIR in treating cancer.

ADDITIONAL INFORMATION AND DECLARATIONS

Conflicts of Interest: The authors have no conflicts of interest to disclose.

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