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Immune mechanisms mediating abscopal effects in radioimmunotherapy

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Abstract

Radiotherapy of cancer has been traditionally considered as a local therapy without noticeable effects outside the irradiated fields. However, ionizing radiation exerts multiple biological effects on both malignant and stromal cells that account for a complex spectrum of mechanisms beyond simple termination of cancer cells. In the era of immunotherapy, interest in radiation-induced inflammation and cell death has considerably risen, since these mechanisms lead to profound changes in the systemic immune response against cancer antigens. Immunotherapies such as immunomodulatory monoclonal antibodies (anti-PD-1, anti-CTLA-4, anti-CD137, anti-OX40, anti-CD40, anti-TGFβ), TLR-agonists, and adoptive T-cell therapy have been synergistically combined with radiotherapy in mouse models. Importantly, radiation and immunotherapy combinations do not only act against the irradiated tumor but also against distant non-irradiated metastases (abscopal effects). A series of clinical trials are exploring the beneficial effects of radioimmunotherapy combinations. The concepts of crosspriming of tumor neoantigens and immunogenic cell death are key elements underlying this combination efficacy. Proinflamatory changes in the vasculature of the irradiated lesions and in the cellular composition of the leukocyte infiltrates in the tumor microenvironment contribute to raise or dampen cancer immunogenicity. It should be stressed that not all effects of radiotherapy favor antitumor immunity as there are counterbalancing mechanisms such as TGFβ, and VEGFs that inhibit the efficacy of the antitumor immune response, hence offering additional therapeutic targets to suppress. All in all, radiotherapy and immunotherapy are compatible and often synergistic approaches against cancer that jointly target irradiated and non-irradiated malignant lesions in the same patient.

Keywords: Abscopal effects, Radiotherapy, Immunotherapy, Radioimmunotherapy, monoclonal antibodies, crosspriming, immunogenic cell death.

Abbreviations:

RT: radiotherapy

SABR: Stereoatactic ablative radiation therapy

ICD: immunogenic cell death

DC: dendritic cells

CALR or CRT: calreticulin

HMGB1: high mobility group box 1

Trex; exonuclease that degrades cytosolic dsDNA

mAbs: monoclonal antibodies

TDLN: tumor draining lymph node

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Introduction

Radiotherapy is known to be a pillar treatment of early and metastatic cancer. The initial observations that suggested that the immune system contributes to the beneficial effects of radiotherapy were made in thymectomized mice as early as in 1979, but remained largely ignored during several decades (1). Traditionally radiotherapy has been considered to exert its clinically meaningful effects exclusively within the irradiated field, dose-limited by adverse effects attributed to irradiation of surrounding tissue. Due to advances in computer-assisted methods the tendency over the last two decades is to minimize irradiation fields using image-guided dosimetry and new technologies that exploit spatial distribution of external beams to attain selective irradiation and higher local doses with less fractions (namely, stereotactic ablative radiation therapy (SABR), volumetric modulated arc therapy (VMAT), intensity modulated radiation therapy (IMRT), proton therapy or carbon ion radiotherapy). Despite therapeutic advances to achieve better local control, there remains a significant need to improve systemic disease control, extending the use of radiotherapy to treat both localized lesions and metastatic disease. Over the years shrinkage of tumors lesions outside the irradiated field in absence of any systemic therapy have been anecdotally reported in various malignant diseases (2). Recent evidence in human cancer patients has demonstrated that RT induces immunomodulatory effects in the local tumor microenvironment, supporting a synergistic combination with radiotherapy and immunotherapy agents to improve systemic control. (3)

Immunotherapy of cancer is advancing fast on the shoulders of knowledge on T-cell co-stimulation and co-inhibition (4). Availability of monoclonal antibodies blocking the coinhibitory interactions of CTLA-4 or PD-1 with their ligands has meant a revolution in oncology, attaining excellent efficacy results in a small fraction of patients with

malignant diseases (5). Over the last years, radiotherapy (RT) has been used to turn patients' tumors into an *in situ* vaccine to generate anti-tumor T cells in patients who lack sufficient antitumor immunity. Indeed, when RT is combined with systemic immunomodulatory monoclonal antibodies (mAbs), such as anti-CTLA-4, anti-PD-1 or anti-CD137 mAbs, or cytokines such as IL2, it is possible to reduce distant lesions outside the irradiation field (abscopal effects) in mice, as well as in some instances already reported in humans. At present pharma companies, basic researchers and clinicians are convinced that synergistic combinations constitute the most promising route to further gain immunotherapeutic efficacy (6). In this scenario of rapidly evolving combinations for immunomodulation is where radiotherapy can potentially contribute to further tilt the cancer immunity balance towards tumor rejection. Although the biological mechanisms underlying the abscopal effect of radiotherapy are yet to be understood, several preclinical studies have helped to elucidate how combining radiotherapy plus immunotherapy would potentiate the systemic beneficial effect of radiotherapy. Here, we review the immunological mechanisms that are responsible for radiation-induced abscopal effects.

Abscopal response mechanisms:

Ionizing radiation is a "wide-spectrum" cancer treatment with ability to generate both an unrepairable DNA damage in the tumor and an inflammatory microenvironment (7, 8). When a tumor is irradiated, intratumoral cell stress can lead a cascade of pro-immunogenic effects that span: antigen release from dying tumor cells (TAA), expression of Natural killer receptor G2D (NKG2D) ligands, production of Type I Interferon (IFN), increased surface major histocompatibility complex molecules (MHC), and neoantigen expression (9). Some of these molecular events give rise to a form of immunogenic cell death (ICD). In other words, these "danger" and

inflammatory effects can convert the irradiated site into an immunogenic hub, by engaging both the innate and adaptive immune response (10). Here, we will describe the mechanisms modulated by radiotherapy that either promote or slow down antitumor immune responses.

1. Radiotherapy-induced Immunogenic cell death and cross-priming of tumor antigens.

Radiation-induced immunogenic death of cancer cells is related to the release of warning signals or alarmins that are essential for recruiting and activating dendritic cells (DCs), in a dose-dependent fashion (11, 12). According to Kroemer and Zitvogel, the immunogenicity of cell death (13) must encompass: i) Translocation of calreticulin to the outer layer of the plasma membrane (CALR, better known as CRT). This protein is normally an endoplasmic reticulum (ER) resident chaperone but, when exposed on the membrane, acts as an "eat me" signal for DC; ii) the release to the extracellular milieu of high mobility group box 1 (HMGB1), a nuclear DNA-binding protein which has been shown to act as toll-like receptor 4 (TLR-4) agonist (14) to elicited DC activation via both TLR-4 and the myeloid receptor RAGE; and iii) release of adenosine-5′-triphosphate (ATP), which binds to P2X7 purinergic receptors on DCs and triggers activation of the inflammasome leading to IL-1β secretion. (15). The cumulative effects of these molecular signals promote DC phagocytosis of tumor cells (16), thereby facilitating DC processing of tumor-derived antigens and subsequent DC-mediated cross-presentation to CD8+ cytotoxic T lymphocytes (iv) release or induction of type I IFN, triggered by mitochondrial and tumoral dsDNA acting on cGAS-STING (17). In this regard, double stranded DNA derived from cancer cells (dsDNA) is considered as a new danger signal or DAMP.

A key role of type I IFN in the recruitment of BATF3-dependent dendritic cells (conventional type 1 DC or cDC-1) to tumors and priming of anti-tumor CD8 T cells has been identified (18, 19). This is important because type I IFN (IFN-I) is necessary for T-cell cross-priming (20). Crosspriming is defined as the ability to take up antigens from other third party cells and present their antigens to CD8 T cells in such a fashion that a cytotoxic T cell response ensues. cDC-1 constitute the dendritic cell subset specialized in this function (21). Cross-priming and IFN-I release depend on the enhancement of cytoplasmic double-stranded DNA sensed by the cGAS-STING machinery in DCs (17, 19). Radiation-induced massive cell death likely results in the release of antigens that surpass the threshold necessary for cross-presentation by DCs, since it is probably beyond macrophage clearance capability. The release of antigens at sufficient levels for cross-presentation is likely dependent on total dose of irradiation, its fractionation and dose per fraction. Several groups have suggested that fraction of 7.5 Gy or higher are convenient to facilitate antigen cross-presentation (22). In a recently published series of experiments, a mechanism has been found to justify more prominent antitumor abscopal effects with ablative hypofractionated radiation such as 8Gy x3, in comparison to single 20Gy dose, (23) (24). The mechanism was found to be dependent on dsDNA presence in the cytosol of irradiated tumor cells themselves, and the difference between dose regimens comes from the fact that high acute doses induce the expression of Trex, an exonuclease that degrades cytosolic dsDNA. With repeated lower doses, Trex was not induced and, as a consequence, dsDNA becomes capable of activating the cGAS-STING axis thereby leading to IFNβ production by tumor cells (figure 2). Such molecular events were found critical to induce antitumor cytotoxic T cells able to mediate abscopal effects that were observed when tumor-irradiated mice were co-treated with anti-CTLA4 or anti-PD1 mAbs (25).

Regarding the role of cGAS-STING there are some issues unresolved. For instance, it is unclear if this dsDNA recognition machinery works primarily on irradiated tumor cells or in crosspriming dendritic cells. It is likely that a contribution from both sides is important. Another important unresolved question is how DNA from cancer cells is transferred to the cytoplasm of DCs. In this regard, it has been reported that tumor derived exosomes (TEX) produced by irradiated TSA breast cancer cells (RT-TEX) transfer dsDNA to DCs and stimulate DC upregulation of co-stimulatory molecules and STING-dependent activation of IFN-I (figure 1 and 2). Exosomes are membrane microvesicles secreted from all cell types, which provide sophisticated means of local and distal intracellular communication (26).

A form of cell death termed necroptosis has been discovered when inducing apoptosis with TNFα under inhibition of caspases. It is featured is phosphorylation of RIPK in dying cells and the phosphorylation/polymerization of MLKL that executes death forming pores in the plasma membrane (27). Whether irradiation induces this type of cell death known as necroptosis (28) remains to be seen, but it appears to be specially effective at permitting crosspresentation and crosspriming.

Once DCs uptake antigen, these antigen-presenting cells must undergo maturation to acquire chemokine receptors to traffic to the DLNs and to initiate T-cell immune responses there. Several groups have demonstrated that hypofractionated doses of radiotherapy caused DC maturation that was necessary for CD8+ T cell priming (29). As mentioned, a subset of dendritic cells excels at cross-priming CD8-mediated CTL responses. These DC, now termed cDC1, represent a minority subpopulation. Its ontogeny from committed precursors in the bone marrow is favored by sFLT-3L and absolutely depends on the transcription factors BAFT3 and IRF8 (30, 31). Accordingly, BAFT3-/- and IRF8-/- mice are devoid of this DC population. To perform their function

cDC-1 are equipped with XCR1 to chemotactically respond to XCL1 as produced by CD8 T cells and NK cells. Critically, these cDC-1 can redirect endocytosed antigenic material to the MHC-I antigen presenting pathway (22). The mechanisms behind such a function are not well understood and involve delayed endosomal protein degradation, translocation of antigens to the cytosol, and redirected vesicle trafficking to the endoplasmic reticulum. Upon maturation cDC-1 very abundantly produce IL-12 probably in order to elicit Th1 and CTL responses. Figure 1 schematically represents the crosspriming associated phenomena related to radiotherapy.

cDC-1 exist in two subsets characterized as CD103+ and CD103-. The former is deployed in peripheral tissues, while the latter resides in lymphoid organs. It has been demonstrated that the performance of cDC-1 is absolutely necessary in animal models for the immunotherapeutic success of anti-PD1/PD-L1 mAbs, anti-CD137 mAbs (32) and adoptive T-cell therapy (33). With regard to radiotherapy, cDC-1 functions are crucial for the abscopal effects mediated by the immune system upon combination of radiotherapy and immunostimulatory mAbs (34). It remains to be seem if migratory or lymph node-resident DC-1 (or both) are the absolutely required components for the efficacy of immunostimulatory monoclonal antibodies. In humans the abundance of cDC-1 cells in the tumor microenvironment correlates with the level of infiltration by CD8 T lymphocytes and favors overall prognosis (35). The presence of cDC-1 in the tumor has been recently reported to be a consequence of the number of NK cells expressing FLT-3L in the leukocyte infiltrate (36). It will be very important to determine if the benefit from radiotherapy also correlates with the baseline presence of cDC-1 in the tumor.

2. The cumbersome roles of TGF-β and Hypoxia in radioimmunoncology

There are several ways to escape immune-mediated control, often by creating an increasingly immunosuppressive microenvironment. Radiotherapy is a double-edge sword for immunotherapy since aside of its proimmune effects, it can be immunosuppressive. Here we will discuss some signaling pathways modulated by RT that govern the suppressive nature of the TME. (37, 38)

Transforming growth factor beta (TGF-β) is a pleiotropic cytokine that controls both initiation and resolution of inflammatory responses through the regulation of chemotaxis and limiting activation of leukocytes, including lymphocytes, natural killer cells, dendritic cells, macrophages, mast cells, and granulocytes (39). As mentioned, TGF-β controls T-cell effector function (40), since it inhibits T-cell proliferation (41) and contributes to immunosuppression by promoting the generation of Tregs. It has been reported that Tregs lacking the TGF-βRII developed normally in the thymus but were poorly maintained in the periphery. These results suggest that TGF-β signaling is required to promote peripheral Treg survival independent of their proliferative potential (37). Mice transgenic for a dominant negative variant of TGF-βRII in effector CD8 T-cells control tumor growth very efficiently and mount more robust cytotoxic responses (42).

TGFβ is a powerful immunosuppressive cytokine that hinders cross-priming of T cells by impairing the antigen-presenting function of dendritic cells and the functional differentiation of T cells into effectors (43). For this reason, several groups have considered that TGFβ is an actionable target. As a result of its multiple protumoral actions, TGFβ blockers have been developed preclinically and in the clinic (44). Monoclonal antibodies, SMAD inhibitors and peptides are among these agents (45). Overall, these drugs are active but the pleotropic housekeeping effectors of TGFβ lead

to compromised safety profiles. There is much interest in confining such TGFβ inhibitory effects to immune system cells and to the tumor tissue microenvironment. In radiotherapy, counteracting the effects of TGFβ has the advantage of reducing collagen deposition and fibrosis, a common and worrisome side effect in patients. Radiotherapy induces reactive oxygen species (ROS) and acidic pHs that cause a conformational change of the latency-associated peptide (LAP)-TGFβ complex releasing active TGFβ (46)_(47). Previous data have shown that activated TGFβ reduces radiosensitivity of tumor cells by promoting the DNA damage response_(48). Blocking active TGFβ during and after RT leads to a significant improvement in intratumoral DC activation and in mice results in efficient cross-priming of CD8 T cells, specific for endogenous tumor-associated antigens in the draining LN (49). In preclinical models, T cells primed by radiotherapy in the presence of systemic blockade of TGFβ more prominently induce regression of the primary irradiated tumor and non-irradiated lung metastases (50). However, in such models, tumor rejection was hampered by upregulation of PD-L1 on the cancer cells and by myeloid cells infiltrating the tumor. Consequently, combination with anti-PD-1 mAb in this setting delays tumor recurrence and significantly improves survival (49). Thus, PD-1/PD-L1 axis may represent an important obstacle to RT-induced tumor rejection, a hypothesis that is currently being tested in several clinical trials (51).

Hypoxia is a deleterious factor in cancer therapies that compromises radiotherapy response and drives to malignant progression. Hypoxia is mainly sensed by transcription factors (HIF-1α and HIF-1 β). When oxygen levels drop, these factors become stabilized since they are no longer hydroxylated in critical prolines that control proteasomal degradation. Crucially, HIF-1α stimulates the transcription and release of VEGF-A. Extracellular VEGF-A binds to VEGF receptors (VEGFR1 and VEGFR2) on

endothelial cells, triggering a tyrosine kinase-initiated pathway leading to angiogenesis. Radiation-induced hypoxia can intensify the angiogenic processes (52) and contribute to the direct up-regulation of the VEGF expression in cancer cells (53).

Although mainly studied as a chief proangiogenic moiety, VEGF is a powerful inhibitory factor for immune responses against cancer. Such functions include promotion of myeloid cells and M2-type macrophages. In this line VEGFs reportedly inhibit antigen-presenting functions of DC and their maturation/activation. Therefore, VEGF directly and indirectly reduces T and NK cell-mediated antitumor immunity. Moreover, VEGF effects on endothelial cells reduce expression of inflammatory molecules rendering them less prone to recruit lymphatic infiltrates into tumors (54). Importantly, there are multiple agents in the clinic that tamper with the VEGF pathway targeting VEGF or the signaling receptors.(55)

3. Interplay between radiotherapy and healthy tissue

Importantly, it is not only malignant tissue what becomes irradiated but also surrounding healthy tissue that receives variable doses. In these areas much orchestration of radiotherapy-related inflammation takes place and its mechanisms are poorly understood yet. Pro-inflammatory changes also occur in lymphatic endothelial cells (56) and these vessels are also disrupted by irradiation.

Radiotherapy damages the tumor vasculature that becomes disrupted because endothelial cells are highly susceptible to cytotoxic effect of ionizing radiation (57, 58). Once this happens, healing and neoangiogenesis are turned in an effort to repair the tissue. Angiogenesis is mainly mediated by VEGFs with the already commented immunesuppressive effects, perhaps reflecting an attempt to unplug healing from immune inflammations in the tissue. The remodeling endothelial cells are different from those presented before radiotherapy and are perceived by circulating leukocytes as an

inflamed territory towards which they can extravasate. This is mainly due to the endothelial surface expression of ICAM-1 and VCAM as well as leukocyte-attracting chemokines (59). Multiple white cell types are thereby invited to enter into the irradiated tissue. In general, myeloid cells probably contribute to dampen antitumor immunity as shown for neutrophils, M2 macrophages and myeloid-derived suppressor cells (60). On the contrary, attraction of lymphoid cells would contribute to the antitumor effects of irradiation.

In radiotherapy, tumor draining lymph nodes (TDLNs) are often within the irradiation fields due to proximity and this might dampen abscopal effects by elimination of tumor-reactive T cells able to recirculate and professional antigen-presenting cells. Thus, it needs to be studied if sparing TDLN is an advantage or a disadvantage in the context of radioimmunotherapy. Recently, a preclinical study has been reported to examine the immunological differences in the TME between RT techniques that spare or irradiate TDLN. In this system, the irradiation of TDLN plus tumor disrupts the chemokine driven orchestration of effector T-cell recruitment into the TME as well as to an unfavorable balance between tumoricidal and immunosuppressive intratumoral immune cells suggesting that elective irradiation of the TDLN should be avoided whenever possible in order to enhance the synergy with immunotherapy. In this context, another issue is how to reduce the radiation induced lymphopenia, that correlates with a worse clinical outcome in a variety of malignant diseases. Clinical evidence has shown that the degree of lymphopenia caused by radiotherapy was dependent on the size of the radiation field and the number of fractions. Fewer fractions in high dose regimens and smaller target volumes avoiding rich lymphatic areas give rise to less immune suppression and better clinical outcome (61). The consequences for leukocyte

trafficking for the drainage LN after RT should be explored in future clinical trials but must affect cancer immunity to a great extent (62).

From a practical point of view, these effects on the tumor microenvironment offer opportunities for combinations of agents to enhance abscopal effects of radiotherapy. These include not only anti-TGFβ and anti-VEGF agents as mentioned, but also agents diminishing myeloid cells and their negative functions on antitumor adaptive immunity. For instance, counteracting GM-CSF, PGE_2, oxygen free radicals and myeloid-cell attracting chemokines. In human radiation-elicited changes in the contexture of the tumor microenvironment remain to be defined in series of pre and on treatment biopsies in the context of radiotherapy alone and, more importantly, in radioimmunotherapy combinations. Multiplex tissue immunofluorescence, RNA expression arrays and multiparameter flow cytometry constitute the tool box to dissect such phenomena. The precision of irradiation and the scattered dose given to non-tumoral surrounding tissue are factors of utmost importance, since they can be conducive to both beneficial and detrimental effects.

Clinical Trial research combining radiotherapy and immunotherapy

Harnessing the patient's immune system against their established cancer has proven to be a successful strategy. During the last years several antibodies blocking critical "checkpoints" that control T-cell activation have been FDA and EMA approved for their use in multiple tumor types. Unfortunately, despite the enthusiasm surrounding treatment with immune checkpoints blockade, the responder patients remain a minority. For this reason, new strategies to extent ICB benefits are in development to enhance systemic response. In this context, radiotherapy has emerged as a promise partner due to the ability of RT to elicit an immune response that can exert its effects at distant sites that are not irradiated.

Preclinical evidence

Several preclinical studies have supported this marriage demonstrating the direct connection between the radiotherapy and the immune system. Figure 2 represents a time line of the key discoveries in the field of immune and abscopal effects of radiotherapy.

In 1953, R.H. Mole proposed the terms of abscopal as "out-site effects of radiotherapy". Two decades later, new preclinical data demonstrated that the immune system contributes to the beneficial effects of radiotherapy in a syngenic mouse model of fibrosarcoma (1). The authors found that the radiation dose required to control the tumor in 50% of the mice (TCD50) was lower for immunocompetent mice than for thymectomized mice (1). During many years, this curious phenomenon did not attract the interest of researchers. In 1999, the abscopal effects were revisited by a preclinical study in which a role for T cells in the tumor response to radiotherapy was found. In these experiments a syngenic metastatic mouse model of lung carcinoma was treated with a combination of local radiotherapy and systemic delivery of the immunoadjuvant FMS-like tyrosine kinase receptor 3 ligand (sFLT3L) reducing pulmonary metastases and significantly improving survival in mice with established tumors. This effect disappeared in immunodeficient athymic mice (63). Subsequent studies studies published in the same period supported dependency on the immune competence of the animals (64). In the era of checkpoint inhibitors, the first attempts to combine anti-CTLA-4 mAb and radiotherapy in the clinic have come from mouse experiments in which remarkable synergy and enhances systemic antitumor responses in a poorly immunogenic carcinoma refractory to anti-CTLA-4 monotherapy (64). In 2009, Dewan et al explored the ability of induce abscopal effects using three fraction radiotherapy regimens. They demonstrated that hypofractionated regimens (8Gy/3fx or 6Gy/5frx) caused less immune suppression and achieved superior survival benefit as compared

with a single dose of 20Gy. .In this line, one study demonstrated that radiotherapy plus PD1 blockade may reduce myeloid-derived suppressor cells, activate cytotoxic T lymphocytes and induce abscopal responses (65). Other strategies to enhance the abscopal effects are based on the combination of several immunotherapy agents. In this line, we have demonstrated that the tumor response after the triple combinations of radiotherapy with anti-PD1 plus anti-CD137 (34). Recently, we have found that TGFβ blockade in combination with the above radioimmunotherapy regimen induces further enhances immune-mediated responses and abscopal effects (ME Rodriguez-Ruiz, Molecular cancer therapeutics, submitted). Another approach that enhances T-cell cross-priming in response to RT is to activate intratumoral or peritumoral DCs using TLR agonists such as Imiquimod. A preclinical study, have shown that RT in combination with imiquimod improves survival and induce significantly decrease in tumor volumes at both primary and secondary tumor sites (66).

Clinical evidence

The number and complexity of clinical trials in this field of radioimmunotherapy is growing data fast pace. Unfortunately, in the absence of more preclinical details, the design of new immunoradiotherapy trials is done using empirical considerations and therefore, results may be inconclusive or fail to demonstrate the ability of radiation to synergize with immunotherapy. Here, we will focus just on early phase clinical trials (phase I and II) that have been completed and provide results in favor of further developing radioimmunoncology approaches. Table 1 shows a summary of completed trials combining immunotherapy and radiation. However, definitive evidence must come from relatively large randomized clinical trials recruiting homogenous series of patients. None has been performed yet.

Clinical immunoradiotherapy combinations were pioneered by the group of Ron Levy, taking advantage of TLR stimulation. In light of powerful synergistic effects in mouse models (67), these investigators combined in lymphoma patients local low-dose irradiation and intratumor injection of CpG oligonucleotides. These non-methylated dsDNA molecules are detected by TLR9 in endosomes with ensuing proinflammatory activity. Evidence for abscopal effects in a small fraction of patients was observed in indolent lymphoma as well as in mycosis fungoides (68). In the same vein, a pilot study has shown immune associated activity and signs of preliminary clinical efficacy after intratumoral injections of Poly-ICLC (dsRNA analogues that mimic viral RNA as a TLR-3 agonist, such as Hiltonol) in combination with dendritic-cell vaccines and multisite SABR (stereoatactic ablative radiotherapy) in heavily pretreated advanced cancer patients (69). A remarkable example is a patient with advanced castration-resistant prostate carcinoma presenting a prostatic mass, lung, mediastinal and inguinal lymph-node metastases and bone marrow infiltration. Hiltonol was injected into metastatic inguinal lymph nodes and SABR was administered to the prostatic tumor and the inguinal lymph nodes. A drastic reduction in the size of mediastinal and retroperitoneal lymph nodes was observed in a CT-SCAN carried out 6 months following treatment onset. However, the authors reported an out-of-field response rate of 16,6% (1 of 6 patients).

GM-CSF is a cytokine used as a vaccine adjuvant for its effects on antigen-presenting dendritic cells. Even if currently seen as a double-edge sword since it also promotes differentiation of myeloid derived suppressor cells. In a trial combining chemoradiotherapy with subcutaneous GM-CSF there was evidence for metastasis shrinkage outside of irradiation fields in a variety of solid tumor patients. Indeed, 26% of patients experienced abscopal responses.(70) Other interesting cytokines has been

studies such as IL2. This cytokine has the ability to stimulate the proliferation of cytotoxic T cells, NK cells and regulatory T cells, providing a balance between antiinflamatory and proinflamatory immune response (71). Interleukin-2 (IL2) has been used for decades to treat patients with cancer. Il-2 have been demonstrated efficacy in melanoma and renal carcinoma. In the last years, several preclinical and clinical studies have show promising results of this cytokine when combined with another agent or modatily such as radiotherapy. A phase 1 study has shown immune associated activity and signs of clinical efficacy after systemic IL2 plus single or hipofractionated doses of SABR (20Gy per fraction) in patients with metastatic melanoma or RCC (72).

In the era of immunostimulatory monoclonal antibodies, the first attempts in prostate cancer patients sequential single dose 8Gy of radiotherapy to a bone lesion alone and Ipilimumab treatment rendered negative results as compared to radiotherapy alone (73). Negative evidence for extracranial or intracraneal benefit has also been reported in a trial for melanoma with irradiated brain metastases combined with ipilimumab (74).

Moreover, a phase II, non randomized study in 22 melanoma patients receiving radiotherapy and Ipilimumab has shown similar evidence in terms of clinical activity of the combination of CTLA-4 blockade and radiotherapy to a single index lesion (18% of patients showed SD in non-irradiated lesion) as compared with ipilimumab alone (75).

In this article, a series of mouse experiments strongly advocate that PD-L1 induction in the tumor constitutes an actionable resistance mechanism to further promote efficacy in terms of abscopal effects. In the animal model radioimmunotherapy with PD-1 or PD-L1 inhibitor produced a superior response than with anti-CTLA-4 in intracranial disease. Immunotherapy plus radiation might also find a role in brain tumors by means of achieving tumor control without resorting to high radiation doses and thereby avoiding the risk of radionecrosis and minimizing other complications.

In the context of no apparent efficacy of anti-CTLA4 in NSCLC (76), one study analyzed 21 of 39 patients with NSCLC treated with Ipilimumab and radiotherapy and found 33% ORR of evaluable patients (7 out of 21 patients). Importantly, they identified that T cell activation inducing IFN beta and early dynamic changes of T cell clones were the strongest response predictors. Consistent with this data, three recent trials of radiotherapy and anti-CTLA4 reporting distant control outcomes, demonstrated abscopal response rates in unirradiated lesion were 10-27% and an additional 13-23% having stable disease for an overall progression disease of 23-50%. In addition, this combination was well tolerated without dose limiting toxicity (see table 1).(77)

PD-1/PD-L1 blockade has been FDA-approved for a number of malignant indications. Prompted by solid evidence for synergy in mouse models (22, 34, 65, 75, 78, 79). Mechanisms potentially involved include radiotherapy induction of PD-1 and PD-L1 (75); T-cell trafficking (80), immunogenic cell death (81), type I interferon production (82) and crosspriming by specialized DC (34). The first attempts in urothelial cancer patients sequential dose 8Gy by 3 fractions of radiotherapy and Ipilimumab treatment have demonstrated combination was well tolerated without evidence for benefit (83). On the contrary, recently, a phase I/II has been reported using multisite SABR in combinations with pembrolizumab (anti-PD-1) in patients with advanced solid tumors. They speculate that delivering SABR to portions of tumors > 65mL could provide cytoreduction while inducing effector T cell traffic into tumor lesions that will then eliminate the tumor. Furthermore, a modest overall systemic response rate of 13,5%, defined as a 30% reduction of the sum of the largest diameter for all of the non-irradiated RECIST target metastases, was observed. However, other groups have defined an abscopal response to immunotherapy as a 30% reduction in the size of any single nonirradiated lesion (10). The abscopal RR was of 26,9%, considered according

to the latter definition. Therefore, a consensus radiological definition of abscopal effects is yet missing and remains debatable. Most importantly its putative correlation with overall survival benefit remains to be seen. (84)

Conclusion

In conclusion, accumulating results suggest that radioimmunotherapy may lead to increased therapeutic effects whose immune mechanisms of action are only beginning to be understood. Much is to be learnt about response and side effects. For instance the possibility of radiological pseudoprogressions due to inflammation and of eliciting hyperprogession in some patients needs to be studied. Furthermore clinical development of predictive and follow-up biomarkers for radioimmunotherapy is needed.

At present a good number of rigorous clinical studies are open testing combinations of radiotherapy and immunotherapy. Results are eagerly expected. Predictive biomarkers are needed to select suitable patients who would benefit from the radioimmunotherapy combinations. Detailed immune studies need to be included in this sort of trials. Extensive investigations on biopsy and peripheral blood samples will be essential to better understand the synergistic mechanisms when they occur or when they fail to take place. Expert consensus is needed in the evaluation of clinical results and larger trials are required. a bidirectional crosstalk between immunologists and radiation oncologists is essential to make the most of such a wealth of opportunity. This opportunity can be summarized in the sense that an intricate set of immune mechanisms may turn radiotherapy from a local into a systemic treatment of cancer beyond its merely palliative interventions. The key words to achieve so are “radioimmunotherapy combinations”.

Figures and tables:

TABLE 1: Published clinical trials with evidence for abscopal effects in combination with immunotherapy.

Figure legends:

FIGURE 1. Schematic representation of the mechanisms underlying immunotherapy synergy with radiotherapy, emphasizing the processes and factors involved in tumor antigen cross-presentation to T-cells.

Figure 2. Timeline of preclinical discoveries providing evidence for abscopal effects of radiotherapy and its elicitation by radioimmunotehrapy combinations.

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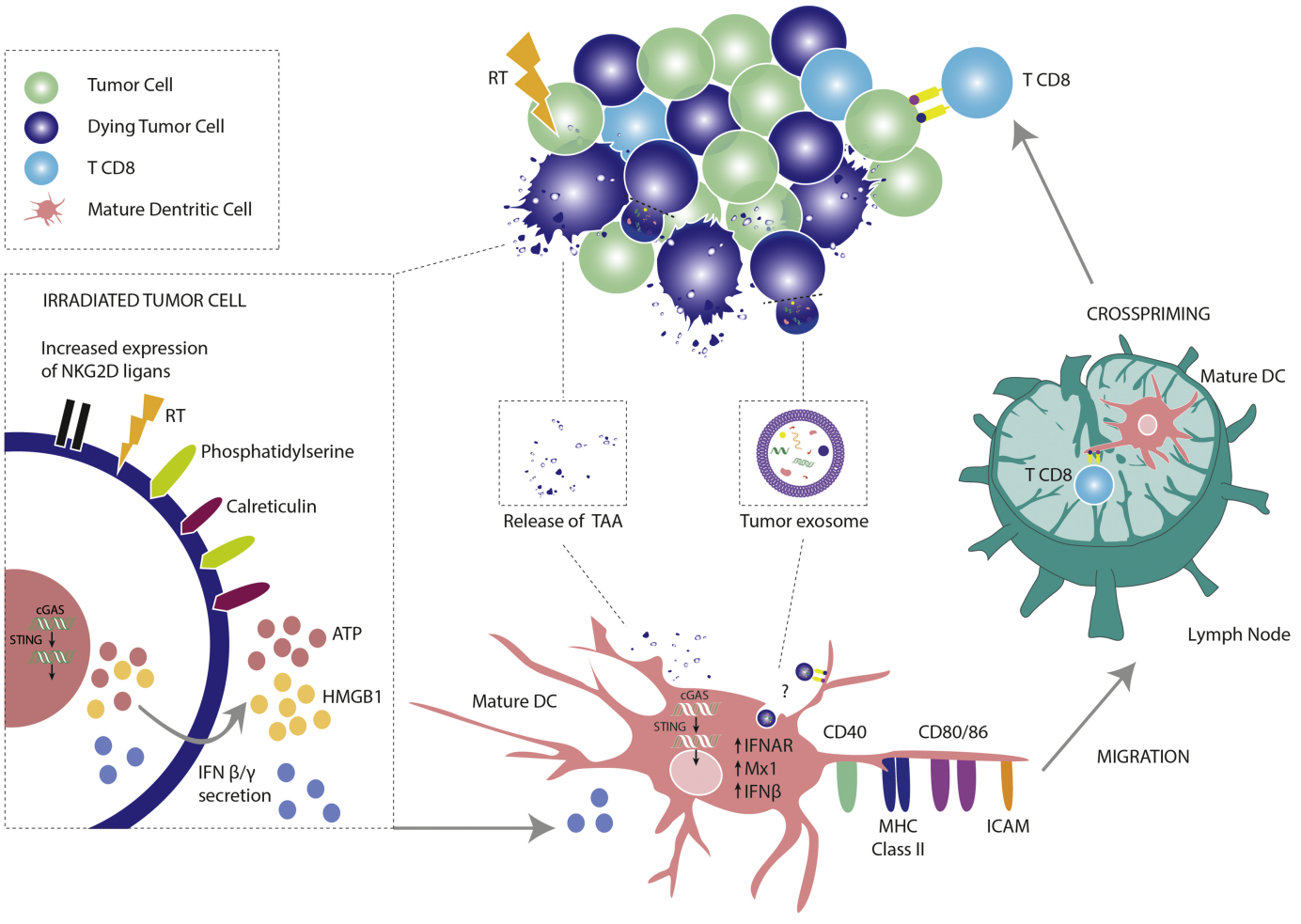
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Figure 1

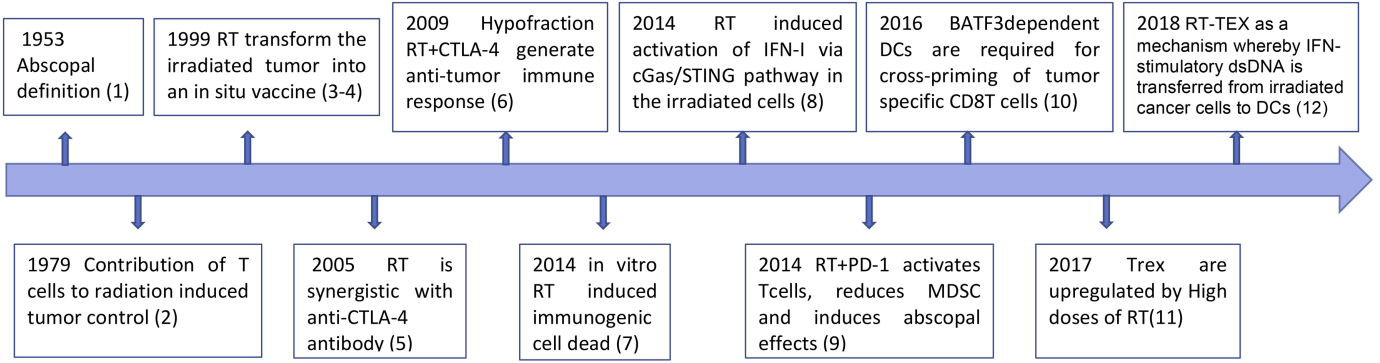

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Figure 2