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REVIEW ARTICLE

Tumor microenvironment, immune response and post-radiotherapy tumor clearance

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Abstract
Radiotherapy is the treatment of choice for many cancer patients. Residual tumor leads to local recurrence after a period of an equilibrium created between proliferating, quiescent and dying cancer cells. The tumor microenvironment is a main obstacle for the efficacy of radiotherapy, as impaired blood flow leads to hypoxia, acidity and reduced accessibility of radiosensitizers. Eradication of remnant disease is an intractable clinical quest. After more than a century of research, anti-tumor immunity has gained a dominant position in oncology research and therapy. Immune cells play a significant role in the eradication of tumors during and after the completion of radiotherapy. The tumor equilibrium reached in the irradiated tumor may shift towards cancer cell eradication if the immune response is appropriately modulated. In the modern immunotherapy era, clinical trials are urged to standardize immunotherapy schemes that could be safely applied to improve clearance of the post-radiotherapy remnant disease.

Keywords Radiotherapy · Immunotherapy · Residual disease · Tumor microenvironment

Introduction

The history of cancer therapy with ionizing radiation goes back to 1896 when, just two years after the discovery of X-rays, Emil Grube treated a woman with recurrent breast carcinoma with X-rays [1]. During the following 120 years, important developments in tumor and normal tissue radiobiology, clinical trials, and technological advances established radiotherapy as a principal treatment modality, directly challenging the position of surgery in many diseases, like prostate cancer [2]. The curability of early stages of skin, prostate, bladder, cervical, head-neck, or even lung cancer, offered by radiotherapy exceeds 70%. In locally advanced stages, however, the results are far lower, the curability dropping below 40% depending upon the disease. Moreover, there are certainly highly radio-resistant types of tumors, like glioblastomas, hepatobiliary, and pancreatic carcinomas, or even locally advanced lung carcinomas, where curability is rather exceptional.

The mechanisms of radiotherapy-induced cell death have been extensively explored, but there are certainly lots of biological aspects that remain obscure. The DNA has been considered as the main target of radiation damage, as single and double DNA strand breaks induced by free radicals produced by X-rays, or even directly induced by particle irradiation, lead to apoptosis or mitotic catastrophe [3]. More recently, the damage induced by radiation on cytoplasmic organelles and their function, like the damage induced to mitochondria and the endoplasmic reticulum, has focused attention of researchers [4]. In experimental studies, intense basal levels or treatment-induced autophagic flux have been recognized as obstacles for tumor eradication with radiotherapy [5]. Autophagic cell death is emerging as an additional death pathway, further to apoptosis and mitotic catastrophe, involved in radiotherapy [6].

During the past decades, a considerable body of experimental and clinical trials has been published, investigating the role of radiosensitizers as adjuncts to improve the efficacy of radiotherapy. Agents targeting the DNA damage and repair, DNA synthesis, apoptosis pathways, or cell-signaling pathways have been successfully tested in experimental studies. However, most agents have failed to prove any benefit

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in clinical trials or have never reached clinical evaluation. It is quite frustrating that after all these long decades of research, it is only cisplatin that gained an indisputable position in clinical radiotherapy. 5-fluorouracil, the oldest ever drug used for solid tumors, remains the gold standard for the radio-chemotherapy of gastrointestinal adenocarcinoma. Taxanes and anti-EGFR therapies are also used in the radio-chemotherapy of lung and head-neck cancer, respectively, but the benefit offered over cisplatin alone remains questionable.

Combination of potent radiosensitizers with radiotherapy are expected to enhance the tumor eradication rates offered by radiotherapy. Failure of radiotherapy and of radiosensitizing policies to eradicate the loco-regional disease is, however, a frequent outcome in patients with locally advanced tumors, so that residual disease is a common intractable clinical problem in the daily oncology routine. Surgery can be applied with some success in a subset of patients, further chemotherapy may delay clinical progression in a small percentage of patients but, overall, residual disease after radiotherapy is a fatal condition. Understanding the biology of the tumor tissue response to radiation and of the microenvironment established in the residual disease is an important step towards the development of post-radiotherapy policies to enhance tumor eradication. Under the new light shed by the developments in the era of modern immunotherapy, understanding the interplay between radiation, tumor microenvironment and anti-tumor immune response emerges as a pillar for the opening of a new research area aiming to enhance tumor radio-curability by enhancing clearance of the irradiated cancer tissue. The current review aims to bring forward the biological basis and rational for the development of post-radiotherapy tumor-clearance therapies.

The tumor microenvironment

We are, inevitably, called to answer an annoying question: why the experimental evidence that many chemical and biological agents are potent radiosensitizers does not translate to a robust clinical benefit? Among many answers, we should certainly elaborate on a critical difference between experimental and clinical conditions. Radio-chemotherapy of a tumor growing in the body of a patient is not radio-chemotherapy of cancer cells but rather radio-chemotherapy of a well-organized tissue. This tissue is composed of cancer cells, most often organized in well-defined nests or glandular structures (undifferentiated tumors excluded), separated from each other by bands of fibroblasts that form the stroma of the tumor [7]. A vascular network grows in the stroma and the periphery of the tumor, and inflammatory cells infiltrate the stroma that may have anti-tumor or tumor-supporting activities; Fig. 1. A solid signaling and

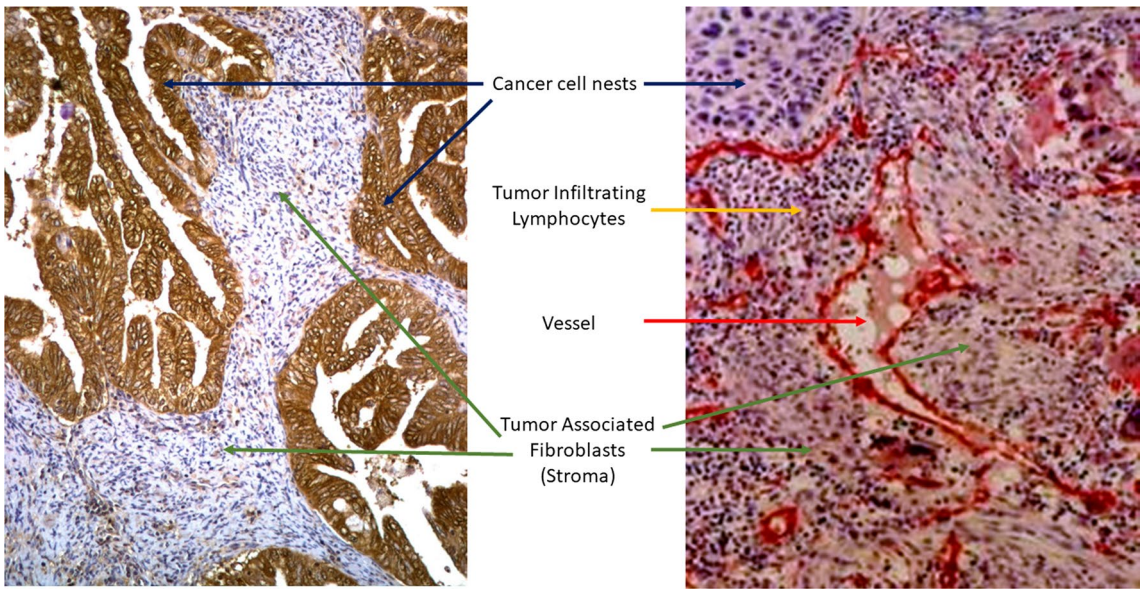

Fig. 1 The tumor tissue is composed of cancer cells organized, most often, in well-defined nests surrounded by the tumor stroma composed by fibroblasts. Within the stroma, vessels grow, bringing oxygen and nutrients to the tumor. Vessels are the only means for any drug or radiosensitizer to reach the tumor microenvironment. Similarly, immune cells, lymphocytes, and monocytes reach the tumor through the vessels and transmigrate into the stroma. The left image shows a lung adenocarcinoma where cancer cell nests are immunostained a monoclonal antibody recognizing the glucose transporter 1 on cancer cell membranes (brown staining), while the adjacent stroma stains blue (hematoxylin). The right image shows a squamous cell lung cancer immunostained for CD31 endothelial membrane antigen (red color shows vessels identified by the anti-CD31 monoclonal antibody, while blue shows cancer cells or stromal fibroblasts and lymphocytes as stained by hematoxylin)

metabolic cross-talk between all these components [8] define the growth and the response of the tumor to external stimuli and cytotoxic assaults, so that the efficacy of radiotherapy and chemotherapy does not depend only on intrinsic cancer cell resistance mechanisms but, equally, on tissue-resistance related pathways.

The vasculature of the tumor is generated mainly in the tumor periphery by stimulating angiogenesis from normal adjacent vessels, or even by the migration of stem-cells in the tumor periphery [9]. Engulfment of this neo-vasculature follows as the tumor grows so that the blood flow in the inner tumor mass is subsequently defined by the ability of the tumor to sustain the survival and promote maturation of engulfed vessels, which occurs via the secretion of vascular growth and survival factors, like VEGF. In any case, the density, maturity, and spatial distribution of the vasculature and the interstitial pressure defines the blood perfusion, and thus the amount of oxygen and nutrients that reach the tumor. These also define the accessibility of any drug to the tumor. In an interesting study, Aboagye et al. [10] showed that radiolabeled 5-fluorouracil distribution in liver metastasis is often compromised by intrinsically low uptake by tumors. Studying the blood flow and 5-fluorouracil uptake in a hypovascular liver metastasis model, Burke et al. [11] showed that both parameters drop in parallel while moving from the tumor periphery to inner tumor areas. Indeed, the vascular density widely varies, up to 20-fold, among tumors even of the same histology and differentiation [12] and often decreases within the tumor body compared to outer layers [13]. The distribution of any radiosensitizer, drug, or biological agent is strongly dependent on blood perfusion. The ability of any, even optimal, radiosensitizer to provide clinically detectable improvement is, therefore, impaired by its accessibility to cancer cells.

Similarly, the oxygen availability to cancer cells depends on the blood perfusion in the tumor stroma and on the spatial distribution of the vessels in relation to the shape and dimensions of the cancer cell nests. Inevitably, many tumors suffer from a perfusion-related hypoxia [14]. This low oxygen tension prevailing in smaller or larger areas of the tumors results in increased levels of Hypoxia-Inducible Factors (HIF), as under hypoxic conditions enzymes involved in the degradation of HIFs (i.e., prolyl-hydroxylases) are deactivated [15]. HIFs enter the nuclei, where they regulate the transcription of a large number of genes involved in angiogenesis, anaerobic glycolysis, apoptosis inhibition, and others [16]. Further to this extrinsically induced tumor hypoxia, cancer cells may be intrinsically hypoxic, having an upregulated HIF-pathway due to oncogenic activation of the PI3K-AKT-mTOR pathway, resulting again to the same hypoxic cell phenotype [17]. Thus extrinsic hypoxia is linked to deficient oxygen free radical generation by radiation, reduced DNA damage, and, therefore, reduced efficacy of radiotherapy to eradicate hypoxic tumor compartments, a well-documented phenomenon since the early 30 s, the so-called 'oxygen effect' [18]. Besides, both extrinsic and intrinsic hypoxia leads to increased resistance of cancer cells to radiotherapy and chemotherapy by upregulating metabolism, DNA repair, and anti-apoptosis pathways, or even by interfering the autophagic machinery [19]. A strong association of HIF expression with poor response to radiotherapy and poor survival has been reported in many clinical studies [20, 21].

As HIFs directly regulate two essential metabolism genes, namely Lactate dehydrogenase A (LDHA) and Carbonic-anhydrase 9 (CA9), acidification of the tumor environment occurs [22, 23]. LDHA is the major LDH isoenzyme involved in the anaerobic usage of glucose, as pyruvate is transformed to lactate for ATP acquisition. Carbonic anhydrase catalyzes the transformation of carbon dioxide to carbonic acid. Both reactions result in the enrichment of the stroma with protons and reduction of the intra-tumoral stroma pH. Hypoxia and acidity are, therefore, directly interrelated and actively contribute to the creation of a tumor microenvironment barrier to the activity of radiotherapy and chemotherapy [24]. Weak base drugs, like doxorubicin or mitoxantrone, poorly penetrate an acidic tumor stroma [25]. Overexpression of LDHA and CA9 has been associated with clinical radio-resistance [20, 21, 26, 27].

A frequently met clinical dead-end

Irradiation of a tumor, with or without chemotherapy or other radiosensitizers, will kill all kinds of cells composing the tumor tissue, triggering a tissue response towards the re-organization of the structure and survival. Angiogenic regeneration and stroma re-organization is a major response that supports cancer cell survival and tumor relapse [28, 29]. The entrance of cancer cells to senescence, with an eventually secretory phenotype, and re-activated cancer stem-cells will form the cancer seeds immersed in the re-organizing stroma that will progress to tumor re-creation [30].

In the successful event where, after irradiation, all cancer cells are depleted, tumor tissue will gradually regress, leaving back (or not) a remnant scar fibrotic tissue. This is indeed achievable in 70–90% of early stages of tumors like head-neck, prostate, and other carcinomas. The complete radiological response does not always indicate complete cancer cell depletion, as tumor foci of up to 10^8 cells are radiologically undetectable. Gross remnant tumor is expected either to disappear gradually within 2–4 months, enter a status of tumor dormancy/non-progression or a phase of slow or rapid re-growth, months, or even years after the documentation of partial or complete radiological remission. Confirmation of viable residual tumor either by endoscopy and biopsy or PET-CT imaging, a quite frequent event in the

clinical practice, brings oncologists in the awkward position to follow a 'wait and see' practice. Most often, patients have already received the maximum tolerable dose of radiation, further chemotherapy is unlikely to produce a sustainable benefit, and surgery is seldom feasible.

A residual tumor after radiotherapy contains dying cancer cells, proliferating cells, stem-cells, and quiescent or senescent cells. The balance between these different compartments will keep the tumor at a non-progressing state, a fragile equilibrium that will shift, at some time point, to progression. All clinical and experimental research conducted until recently has focused on the development of methods that would increase the percentage of irradiated tumors that reach total cancer cell depletion. Knowing the individual microenvironmental conditions before the onset of radiotherapy would better define the radiotherapy and radiosensitization policy that would have a better chance to reach eradication. For example, hypovascular hypoxic tumors would be the best candidates to receive proton or heavy particle radiotherapy. The oxygen effect applies only to sparsely ionizing photon or electron radiation, while high LET radiation kills equally hypoxic and euoxic cells [31]. Inhibitors of HIFs and downstream genes like LDHA and CA9 may also prove of value for the eradication of such tumors with photon therapy [32]. Research on the development of biomarkers and in vivo imaging of microenvironmental conditions is of importance [33].

Immunity and post-radiotherapy tumor equilibrium

Once, however, the overall radiotherapy/radiosensitizing approach has ended to incomplete tumor response, the only apparent feasible way to shift this balance towards tumor disappearance is to handle another essential component of the tumor microenvironment, namely the immune infiltrating cells. Lymphocytes and macrophages transmigrate through the tumor vessels and populate the tumor stroma (Fig. 1). Cytotoxic T-cells, NK-cells, and M1-type macrophages can readily kill cancer cells. Immunogenic death is a crucial component of the equilibrium established in the post-radiotherapy residual tumor [34]. This type of cancer cell death, unlike apoptosis, necrosis and autophagic death that faint rapidly through time, remains active and sustains the status of non-progression for weeks, months or years after therapy, till the time point where intensification of cancer cell proliferation or compromised immune activity will end to clinical relapse. The importance of immunogenic death has been well known for many decades, from in vivo animal experiments. The dose of radiotherapy demanded to eradicate a tumor growing on an immunocompromised mouse is 2–3 fold higher than the dose demanded if the same tumor grows on an immunocompetent mouse [35].

Given the importance of the immune system, two main questions are raised: (1) why does immunity not eradicate the remnant tumor? Moreover, (2) are there any methods to boost this immune-related rejection effect? There are, indeed, many well-known reasons that explain this failure of anti-tumor immunity and provide a basis for therapeutic manipulations. These can be summarized as follows:

1. The tumor microenvironment can become a significant obstacle to anti-tumor immunity. Low vascular density and reduced blood flow impair the accessibility of lymphocytes to the tumor stroma. In such cases, immune attack can only occur in the tumor periphery that is proximal to adjacent normal tissue. Besides, the expression of inflammation-inducing proteins by the cancer endothelium, like the sialic acid-binding lectins, may prevent the attachment and transmigration of immune cells [36], and this is an exciting area of research attempting to enhance activation and migration of cytotoxic cells that reach the tumor [37].
2. Once immune cells have reached the tumor stroma, they become exposed to hypoxia and acidity. Such conditions block the cytotoxic activity of CD8+ T-cells and NK-cells, block macrophage activity, and also repress the secretion of important cytokines like TNF-α [38, 39]. Overexpression of LDHA and glycolytic enzymes by cancer cells has been linked with poor infiltration of tumor stroma by tumor-infiltrating lymphocytes (TILs) [40], while CA9 overexpression is linked with the prevalence of immune-suppressing FOXP3+ regulatory T-cells [41]. Targeting hypoxia, glycolysis, or CA9 may be essential to restore anti-tumor immunity.
3. An additional adverse microenvironmental condition is the abundance of immune blocking metabolites. Under cytotoxic and hypoxic stress, cancer cells release ATP in the extracellular milieu that binds to type-2 purinergic receptors on the immune cell surface, promoting innate and adaptive immunity. However, overexpression of ectonucleotidases transforms ATP to adenosine that has intense immunosuppressive activity by blocking the proliferation of T-cells and by promoting the prevalence of FOXP3+ regulatory TIL-presence [42, 43]. Anthraquinone derivatives and other molecules are under investigation as blockers of ectonucleotidases to overcome microenvironmental immuno-suppression. Kynurenine is another cancer cell metabolite with similar immunosuppressive properties. Indoleamine dioxygenase (IDO1) is the key enzyme catalyzing the oxidation of tryptophan to formylkynurenine. Indeed, overexpression of IDO1 has been linked with poor outcomes after radiochemotherapy for head-neck cancer [44]. Molecules like indoximod and navoximod are experimentally tested to restore kynurenine related immune suppression [45].

Depletion of the semi-essential amino acid arginine from the tumor environment, due to overexpression of arginase and iNOS is also a significant obstacle for anti-tumor immunity, as lymphocytes demand arginine for their proliferation and activation [46]. Arginase blockers may also prove of therapeutic value.

4. A vital feature blocking cytotoxic immune cell activity is the overexpression of immune checkpoint inhibitory molecules like PD-L1, CTLA4, and CD47. The entrance of immune checkpoint inhibitors in clinical practice during the last decade has revolutionized the oncology practice. A clinical paradigm regarding the importance of handling immunogenic death in the outcome of radiotherapy is shown in Fig. 2. The phase 3 PACIFIC study in stage III non-small cell lung cancer confirmed that immunotherapy with anti-PD-L1 antibodies after incomplete remission to radio-chemotherapy offers an important survival advantage. Durvalumab is the first-ever immunotherapy agent approved to treat cancer patients immediately after the completion of radiotherapy, to enhance immunogenic cancer cell death [47].
5. Finally, exhaustion of the T-cells and NK-cells is an important issue that emerges gradually during the long period of months before the diagnosis of cancer. T-cells and NK-cells may have entered an inactive state, which is further aggravated by the killing effect of radiotherapy exerted both on local tumor and regional nodes, on bone marrow and the blood passing through the irradiated body area. Indeed, cytotoxic T-cell counts drop by 2–3 fold during radiotherapy, and of interest, the radioprotective agent amifostine protects and stimulates the expansion of lymphocytes during radiotherapy [48, 49]. Administration of 'old-era' immunotherapy agents, like IL2 or of GM-CSF and IFNα that expand and re-activate exhausted lymphocytes, may prove of importance in the new immunotherapy era of immune checkpoint inhibitors to help elimination of the remnant tumor after radiotherapy [50, 51]. Immune exhaustion should be diagnosed at tissue level with immunohistochemistry markers detecting lymphocyte and monocyte subpopulations, or at the peripheral blood level with flow cytometry for specific lymphocytic or monocytic markers or with the detection of specific proteins/cytokines in the plasma.

An unexpected ally: the irradiated tumor tissue itself

Significant changes induced by irradiation on cancer cell antigenicity but, also, on stroma cell secretory activity play, eventually, an important role in the rejection of the remnant tumor. Irradiation induces phenotypic changes in both cancer and stromal cells, promoting the INF type-I response, secretion of TNFα, and cytokines that activate cytotoxic immune cells [52, 53]. Exposure to ionizing radiation also restores HLA-class I molecule expression and antigen presentation to dendritic and cytotoxic T-cells [54]. Transmigration of immune cells into the tumor stroma is facilitated by the induction of endothelial adhesion molecules [55]. Overall, an important body of evidence supports the notion that

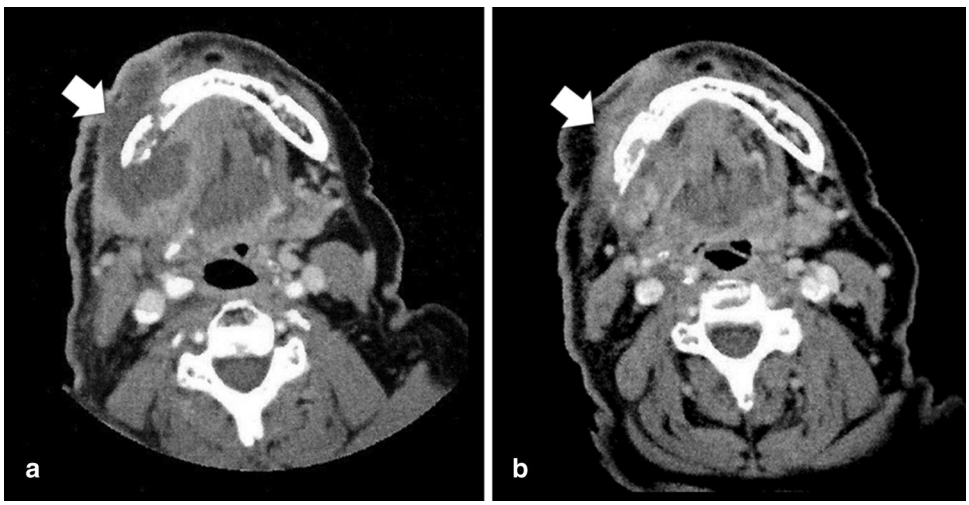

Fig. 2 Following radical chemo-radiotherapy for a T3N0 staged squamous cell carcinoma of the floor of the mouth, the tumor regressed and remained in an equilibrium for a period of 1 year. A rapid re-growth with central necrosis was, subsequently, documented invading the mandible (**a**) (arrow). Anti-PD-1 immunotherapy resulted in a dramatic response, leading to almost complete regression of the tumor and bone healing, four months after the onset of immunotherapy (**b**) (arrow). The question raised is whether immunotherapy delivered after the completion of radiotherapy would have eliminated the far more depopulated viable remnant cancer tissue and would have protected the patient from an eventually incurable recurrence

the irradiated tumor becomes easy prey for immunotherapy. This unveiled face of the tumor to the host immune system induces the so-called 'radio-vaccination effect'. Under certain conditions, e.g. suppression of immune check point molecules on cancer cell membranes by novel immunotherapies, radio-vaccination would enhance cytotoxic attack to the tumor.

Concluding the unfinished task and beyond

It is essential to offer radiotherapy the chance to express its maximum efficacy, to keep minimum the percentage of patients with an incomplete response. Sensitization policies with chemotherapy and/or agents targeting cancer cell molecular pathways may enhance DNA damage and/or prevent repair of the DNA damage induced by radiotherapy. The microenvironment of the tumor should also be taken into account. Assessment of intra-tumoral hypoxia, either by PET-CT and MRI imaging or by immunohistochemical markers of hypoxia (like HIF1α, CA9), will allow the identification of subgroups of patients who would benefit from proton therapy, hypoxic sensitizers, carbogen breathing, and nicotinamide or, hopefully, with novel molecules targeting hypoxia and anaerobic metabolism-related genes [20, 56].

Once, however, the treatment fails to eradicate the tumor, at a clinical or subclinical level, the remnant disease will inevitably progress and kill our patients. Surgery can be of help in a minority of patients. Further or second-line chemotherapy is unlikely to provide any sustainable benefit. Immunity is the only ally that remains and should always be considered to shift the equilibrium towards tumor eradication. The blockage of immune checkpoint inhibitory molecules with antibodies that have become available in the current novel immunotherapy era is an essential tool. We should, however, not forget the era of the '90 s when clinical trials with IL2, IFNα, and GM-CSF ended in a relative frustration. Such potent immuno-stimulatory agents can unblock immune exhaustion, promote anti-tumor cytotoxicity, and should be thoroughly re-evaluated in conjunction with immune checkpoint inhibitors for tumor persisting after radiotherapy. Adoptive immunotherapy to harness T-cell response may also be critical [57]. Also, targeting microenvironmental conditions may unleash the potency of immunity by immuno-fertilizing the tumor stroma [58].

Beyond local tumor eradication, significant additional effects of immunotherapy are expected in patients carrying a radio-vaccinated tumor. These are the so-called 'abscopal effects' of radiotherapy. It is postulated, that having enforced, through the preceded irradiation, the presentation of tumor-specific antigens by cancer cells, effector lymphocytes, and monocytes learn how to attack any offspring of the cancer cell in the body, whether located in the irradiated area or metastasis in distant organs. Elimination of micro-metastasis or even visible metastasis by immunotherapy becomes easier, and the chances of curability increase. Further to many interesting in vivo experimental studies confirming the abscopal effects induced by Radiotherapy followed by immunotherapy [59], clinical studies have also confirmed this effect. An example is the analysis of the Keynote-001 phase 1 trial, where patients with non-small cell lung cancer treated with anti-PD-1 antibodies had improved survival when they had received any radiotherapy before the first cycle of immunotherapy [60].

Unveiling the qualities of residual disease

The importance of developing diagnostic tools to characterize each tumor as for the quality of the microenvironment established after radiotherapy, and the expression of immune-related molecules by cancer cells, stromal fibroblasts and immune cells infiltrating the tumor, is self-evident. Individualization of the demanded post-radiotherapy interventions is important, given the multitude of immune checkpoint inhibitory molecules that can be expressed by cancer cells and the different phenotypes of the tumor-infiltrating lymphocytes and macrophages that sustain a regulatory or effector microenvironmental immune response. Histopathological and immunohistochemical evaluation of biopsies from residual tumors can provide the expression status of immune checkpoint molecules, like PD-L1 or CD80/86, and characterize the prevailing lymphocytic population infiltrating the tumor stroma. For example, the abundance of CD25+ or FOXP3+ lymphocytes describes an immunosuppressive environment, while the prevalence of CD8+/CD4+ lymphocytes may suggest an activated cytotoxic response [61]. Moreover, the pathologist can provide quite accurately the hypoxia and acidity status of the tumor microenvironment by examining the expression of HIFs, CA9, and LDH5 expression [40, 41]. All the above information builds the microenvironmental and immunological profile of the residual disease, allowing the choice of appropriate targeting agents [62].

As biopsies from the residual tumor are feasible just in a small subset of patients, the development of liquid biopsies (plasma or exosomes), detecting the expression levels of immune checkpoint molecules and molecules characterizing the microenvironmental metabolic status would prove an essential tool [58, 63]. The development of radiological metabolic and immunologic imaging of tumors would also be of great value. Immuno-PET-CT imaging could be applied to detect immune checkpoint molecules or specific tumor-infiltrating immune cells [64, 65]. Glucose metabolism and hypoxia can also be assessed with specific PET-CT and MRI techniques [66, 67].

An additional important diagnostic approach should aim to the evaluation of the immune state of the patient,

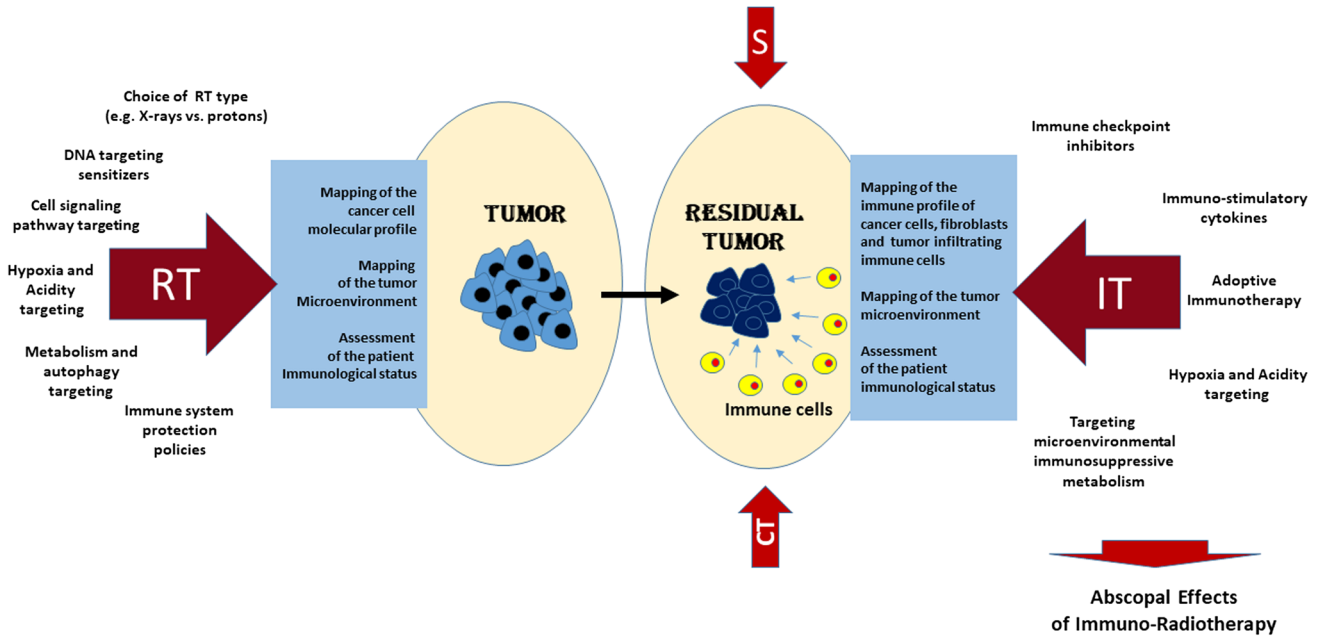

Fig. 3 The choice of the type of radiotherapy is important to maximize the effect on a hypoxic and intrinsically radio-resistant tumor by proton or heavy-ion irradiation. Combination of radiotherapy (RT) with DNA targeting or cell-signaling targeting radiosensitizers and with agents targeting hypoxia and acidity or tumor metabolism allows the maximization of the cytotoxic effect on tumors. Tumor eradication is achievable depending upon intrinsic cancer cell biology and microenvironmental conditions. The residual tumor should always be subject to further therapy. Surgery (S) should be considered when feasible, while further or second-line chemotherapy (CT) is most likely to prove ineffective or provide, at best, a short-lasting effect. Immunotherapy (IT) with immune checkpoint inhibitors would block cancer or stroma cell surface molecules that suppress the activity of cytotoxic lymphocytes or macrophages. Immune exhaustion should be diagnosed and treated with immuno-stimulatory cytokines or adoptive immunotherapy. Correction of intra-tumoral microenvironmental conditions (like hypoxia and acidity) and blockage of enzyme activity that fuels the tumor environment with immunosuppressive metabolites, is imperative. Individualization of the post-radiotherapy approach is essential and demands the development of diagnostic tools to map the microenvironment and the immune profile of the tumor tissue. Immunotherapy, empowered by the radio-vaccination effect, further to eliminating the residual irradiated tumor, may also contribute to the eradication of distant micro- or macro-metastasis (abscopal effects of radio-immunotherapy)

as immune exhaustion should be corrected before and carefully monitored during immunotherapy. Individualization of the immunotherapy policy is imperative. Figure 3 schematically shows our options to treat residual disease after radiotherapy. This is, certainly, a theoretical model that highlights an overall approach. Its feasibility remains questionable under the lack of easily-applicable and accurate pathology/radiology tests, the rather incomplete knowledge we have regarding the anti-tumor immune response and its interactions with the tumor microenvironment and, of course, the shortage, as yet, of effective therapeutic agents to target immunity and microenvironmental conditions. Nevertheless, the rapid progress achieved in the field of immunotherapy allows clinical application and clinical/translational research to ameliorate our knowledge and armory and enhance cancer curability through radiotherapy.

Conclusions

Immunotherapy emerges as a reasonable approach to treat patients with residual disease after radical radiotherapy. In the modern era of immunotherapy, clinical trials are urged to standardize immunotherapy schemes that could be safely applied, anticipating elimination of the residual tumor or even of clinically undetectable tumor micro-foci. Further to immune checkpoint inhibitors, old-era or novel immunostimulatory agents may prove of importance. Agents that transform the tumor microenvironment to a fertile ground for cytotoxic immune cells may prove essential for the clinical substantiation of the activity of immunotherapy.

Author contributions MIK: conception and writing of the paper. AG: conception and writing of the paper.

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Availability of data and material Presented data are available in the data archives of our Department.

Compliance with ethical standards

Conflict of interest The authors declare no potential conflicts of interest.

Ethical approval The patient CT-scan shown in Fig. 2 has been treated according to a protocol approved by the local Ethics and Scientific Committee (DS34/28-9-2006).

Informed consent Written informed consent was obtained from the patient. Consent for publication: There are no individual person's data included in the paper.

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