Review

Moving towards the Future of Radio-Immunotherapy: Could We "Tailor" the Abscopal Effect on Head and Neck Cancer Patients?

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Abstract: The abscopal effect (AbE) is defined as radiation-induced shrinkage of distant, non-treated, neoplastic lesions and it is considered the best clinical picture of the efficient immune stimulation by irradiation. The first report about abscopal tumor regression upon radiotherapy dates back to the beginning of the 20th century. The growing preclinical and clinical synergism between radiation and immunotherapy gave birth the purpose to more easily reproduce the abscopal effect, nevertheless, it is still rare in clinical practice. In this review we summarize immunological modulation of radiotherapy, focusing on the well-balanced equilibrium of tumor microenvironment and how radio-immunotherapy combinations can perturb it, with particular attention on head and neck squamous cell cancer. Finally, we investigate future perspectives, with the aim to "tailor" the abscopal effect to the patient.

Keywords: abscopal effect; HNSCC; precision medicine; immunotherapy; radiotherapy

1. Introduction

Head and neck squamous cell cancer (HNSCC) is the sixth most common cancer worldwide, more than 90% involving oral cavity, pharynx, or larynx. Known risk factors are excessive use of tobacco and alcohol, acquiring infection with human papilloma virus, especially HPV 16, HPV 18, and Epstein–Barr virus [1]. Radiotherapy represents a cornerstone in its management, and it is clinically implemented in different regimens; for locally advanced disease, modulated intensity radiotherapy (IMRT) is classically used, with daily doses ranging from 1.8 to 2.0 Gy, either alone or in combination with concomitant chemotherapy or cetuximab [2,3]. In recurrent or metastatic disease, the standard first line is platinum-based chemotherapy with 5-fluorouracil and cetuximab [4], however, stereotactic body radiotherapy (SBRT) with fractionation between 8 and 15 Gy is widely used [5]; in fact, thanks to its high spatial precision it could achieve a better control of local symptoms reducing toxicities. Its growing use increased reports of the abscopal effect as a systemic distant response of non-irradiated tumors or metastasis, as a consequence of proinflammatory changes in tumor microenvironment (TME) against cancer antigens [6,7]. A further boost has been given by the introduction of the immunotherapy targeting Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) and Programmed Death–Ligand 1 (PD-L1)/PD-1 axis: RT stimulates a robust tumor antigen cross-presentation in nodes while CTLA-4 blockade enhanced the priming of responsive T-cells in TME, highlighting an interesting clinical synergism. [8,9]. The goal of radioimmunotherapy should be to stimulate but also improve the duration of the immune response. Among various combination strategies, SBRT is most effective in inducing the abscopal effect, although the right dose and ideal fractionation has yet to be identified [9].

2. Insight into the Abscopal Effect: Modulation of the Tumor Microenvironment (TME) by Irradiation

Beneficial consequences of the abscopal effect have been known for many years, understood as a reduction in the development of metastases in patients treated with radiotherapy on the primary tumor [8]. The starting point is the stimulation of the immune response and the anti-neoplastic transition of the TME induced by radiation treatment, however, nowadays it is not known what the best radiotherapy strategy for this purpose is [9]. In fact, current preclinical evidence could be very conflicting, testifying to the difficulty in the reproducibility of the phenomenon [6,9]. Ionizing radiation damages DNA in target cells, causing DNA–DNA crosslinks, DNA–protein crosslinks, modification of the deoxyribose rings and bases, and induces release of cytokines and chemokines involved in the alteration of the tumor stromal microenvironment [10]. Radiotherapy can modify the TME from immunosuppressive or "cold" to immunostimulatory or "hot", even though the reverse phenomenon has also been observed. The relationship between TME and radiotherapy is extremely complex, and it can be compared to a well balanced scale [11–15]. Typical aspects of a "cold" TME are a high number of regulatory T (T-reg) cells and myeloid-derived suppressive cells (MDSCs), poor T-Helper 1 (T-h1), Natural Killer (NK), and CD8+ cells, few functional antigen-presenting cells (APC) and production of immunosuppressive cytokines, while "hot" TME is rich in Th1-type chemokines, NK and CD8+ lymphocytes, high PD-L1 expression, and APCs (Figure 1). The creation of an immunosuppressive TME reflects one of the hallmarks of cancer, the immune escape. Macrophages infiltrating the TME should be differentiated in two phenotypes, M1 and M2: M1 macrophages are pro-inflammatory and secrete interleukin-12 (IL-12) and Tumor Necrosis Factor α (TNF-α), that may act killing tumor cells; on the other hand, M2 macrophages work as anti-immunogenic cells, expressing anti-inflammatory cytokines such as interleukin-10 (IL-10) and Transforming Growth Factor-β (TGF-B), which subsequently inhibit the function of effector T-cells and favor tumor progression [16,17].
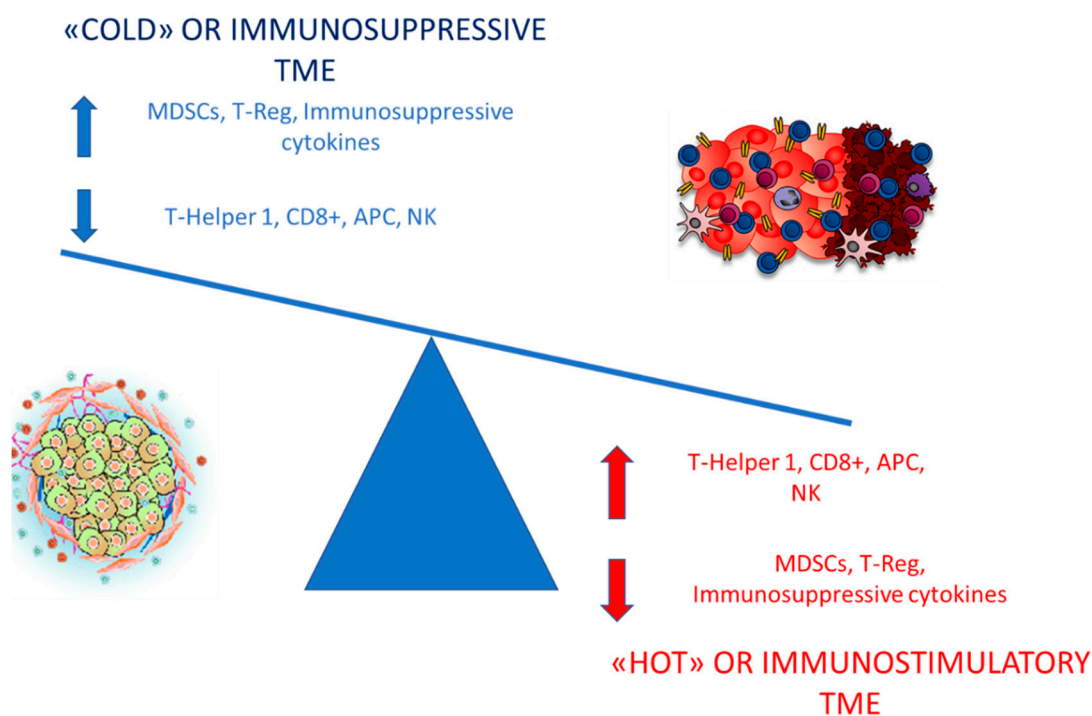
Figure 1. Differences between "cold" and "hot" TME.

Anti-inflammatory M2 macrophages polarize cold TME and antagonize the immune response induced by radiation [18]. Ideally, associations between chemotherapy, im-

munotherapy, and radiotherapy should shift the balance, promoting pro-immunogenic effects and mitigating immunosuppressive features. The immune response induced by radiation treatment is extremely articulated and is carried out by cytokines and chemokines with contrasting functions: IL-6, IL-10, and Colony Stimulating Factor-1 (CSF-1) have a pro-tumorigenic profile, stimulating the proliferation and growth of neoplastic cells [19]; Chemokine-Receptor-Type (CXCR)-4 ligand mediated chemotaxis of pro-tumorigenic CD11b+ myeloid-derived cells and modulate organ-specific metastasis of solid tumors, whereas CXCL9, CXCL10, and CXCL16 block tumor growth and boost antitumor immunity via effector T-cells [20–22].

Damage-associated molecular patterns (DAMPs), such as the non-histone chromatin-binding protein high-mobility group 1 (HMG-1) and mitochondrial DNA (mtDNA), are endogenous danger signal molecules, normally invisible to the immune system, released to the extracellular space from damaged or dying cells due to irradiation [23]. Once DAMPs are released from the cell into the TME, they promote an innate immune inflammatory response by binding to a pattern-recognition receptor, in particular Toll-Like Receptors (TLRs) [23]. This potently immunostimulatory mechanism is named immunogenic cell death [24,25]. DAMPs enable the recruitment and activation of Basic Leucine Zipper ATF-Like Transcription Factor 3 (BATF3)-dependent dendritic cells, critical for tumor-specific cross-priming of CD8+ T-cell memory [23]. BATF3-driven DC might also contribute in recruiting circulating cytotoxic T lymphocytes as well as in activating tumor-resident CD8+ T-cells, under tonic inhibition by PD-1 and TGFβ1 signaling.

TGFβ1 has a key role in preserving a cold TME by promoting regulatory T-cell differentiation, reducing CD8+ cytotoxicity, suppressing CD4+ T-cell differentiation, and blocking NK-cell proliferation [26]. It is rapidly stimulated by radiation across a wide range of RT doses [19,27]. Radiation-damaged cancer cells release cancer-specific peptides, processed by DC and presented to T-cells, and enhance the pro-phagocytosis signal, the calreticulin, that mediates macrophages and dendritic cells, stimulating the presentation of antigens and the priming of T-cells [28,29]. In addition, RT-damaged DNA is released from the nucleus into the cytoplasm, activating the cGAS-STING (Stimulator of Interferon Genes) transduction cascade, which stimulates IFNγ transcription. This cytokine upregulates the class I MHC on APC, such as macrophages and dendritic cells, which interact with tumor neoantigens in nodes, where they mainly activate CD8+ T lymphocytes. As a result, effector T-cells primed in lymph nodes migrate both in the primitive tumor and in the un-irradiated metastases to exert their cytocide effect [30–33]. Such a robust response can not only drive the eradication of radioresistant cancer cells in the irradiated lesions, but also potentially attack non-irradiated metastases, commonly referred to the abscopal response [27,28]. Further evidence about the immunological nature of this mechanism are that abscopal responses could be abolished in preclinical murine models lacking CD8+ T-cells, as well as in mice in which type I IFN signaling is inhibited [34].

3. Radiotherapy and Immune-Checkpoint Inhibitors (ICIs): Potentially a Well Balanced Synergism

Radiotherapy immunogenicity is strictly related to tumor-specific characteristics, such as heterogeneity and radiosensitivity, and specific RT aspects like dose, fractionation, and timing of administration. Most of the clinical data regarding the use of radio-immunotherapy combinations are limited to either anti-CTLA-4 or anti-PD-1 agents: RT stimulates antigen cross-presentation and T-cell priming in draining nodes while CTLA-4 inhibitors enhance priming phase of effector T-cell activation induced by RT, leading to pro-immunogenic infiltrate of the TME. Blocking CTLA-4 enhances T-cell activation, increasing the CD8+/T-reg ratio, and strengthening the in situ vaccination effect [35–39].

PD-1 is expressed on T-cells, DCs, and NK cells: PD-1/PD-L1 pathway primarily inhibits T-cell proliferation by blocking cell-cycle progression, thus protecting tumor cells from T-cell attack. A recent study suggested that radiation-induced double-strand breakage of DNA results in upregulation of the expression of PD-L1 thanks to Ataxia-telangiectasia-mutated (ATM) and ataxia telangiectasia and Rad3-related (ATR) kinases [40–43]. Therefore,

PD-L1 hyperexpression on cancer cells could be associated with a higher rate of responses to specific inhibitors while radiotherapy could act as a "Trojan horse" [42,43].

An excellent murine model published by Twyman-Saint Victor et al. clarified that radiotherapy and anti-CTLA4 monoclonal antibodies (mAbs) led to upregulation of PD-L1 on melanoma cells, mediating T-cell exhaustion and explaining at least in part the limited local and abscopal responses observed [44]. Anti-CTLA4 predominantly inhibits T-reg cells, thereby increasing CD8/T-reg ratio while radiation enhances the diversity of T-cell receptor (TCR) repertoire of intratumoral T-cells [44]. Addition of PD-L1 inhibitor reverses T-cell exhaustion, mitigates depression in the CD8/Treg ratio, and further encourages oligoclonal T-cell expansion [44]. Similarly to results from mice, patients with melanoma showing high PD-L1 levels on neoplastic cells did not respond to radiotherapy plus anti-CTLA4 mAb, demonstrated persistent T-cell exhaustion and rapidly progressive disease.

Evidence regarding the optimal timing, dose, schedules, sequences, and fractionation of radiotherapy are conflicting. Pre-clinical studies highlighted that starting anti-PD-L1 treatment 7 days following RT was inferior to starting on either the first or the last day [45]; however, some data also show how radiotherapy-anti PD-L1 sequence, with late administration of the checkpoint inhibitor, reinvigorates exhausted T-cells while an early sequence favors the differentiation and initial activation of T-cells [46,47]. Nowadays, it is clear that there is a synergism between immunotherapy and RT either as a single-fraction or in fractionated courses. Following irradiation with 12 Gy on 2 consecutive days, overall leukocyte and CD8+ T-cell frequencies peak at 5 days post-RT and then gradually decline to pre-RT levels [48]. Five days post-RT also reflects the highest CD8/T-Reg ratio, probably the ideal time-point for checkpoint blockade. Moreover, Frey et al. showed that after irradiation of 5 Gy $\times$ 2 fractions, CD8+ peak at day 8 and then decline while T-regs have a bimodal peak on days 8 and 10 [49]. Knowing the kinetics of infiltration of immune cells should be correlated with the time of administration of the ICIs, in order to achieve the maximum immunostimulant effect.

Hypofractionated radiotherapy (hRT) is the delivery of fewer, larger (>2 Gy) doses of radiotherapy and is a potential strategy for improving dose intensity. hRT appears particularly immunogenic: T lymphocytes, including Tumor-infiltrating lymphocytes (TILs), have generally been considered as highly radiosensitive [50,51], that is why it could be postulated that extending hRT schedules might be less immunogenic if administered in the period in which T lymphocytes, stimulated by RT/ICI combination, migrate to TME [50,51]. Following the experience of Frey [49], Zhang et al. compared a combination of anti-PD1 treatment and hRT with different schedules and equivalent biologically effective doses in mice affected by melanoma. Anti-PD1 antibody was given weekly while primary tumor was irradiated with 3 $\times$ 9.18 Gy in 3 or 5 days or with 5 $\times$ 6.43 Gy in 10 days [52]. All the combinations inhibited growth of irradiated primary and non-irradiated secondary tumors greater than hRT and anti-PD1 monotherapy; similarly, local and systemic tumor-specific CD8+ T-cell responses and TILs were also similar across short or extended hRT schedules [52]. Zhang's experience also highlighted how regional and abscopal antineoplastic biological effects of the extended schedules are similar to the shorter ones only if the regional lymph nodes provide sufficient cancer-specific T-cells in the TME [52].

Historically, HNSCC was considered poorly responsive to high-dose hRT rather than low-dose hRT, however, low-dose schedules may induce lymphopenia and immunosuppression [53]. Morisada et al. analyzed immune correlates, primary tumor, and abscopal control rates as a result of two different radio-immunotherapy combinations of PD-1 monoclonal antibody with either daily low-dose fractionated radiotherapy (LDRT, 2 Gy $\times$ 10) or high-dose hypofractionated treatment (8 Gy $\times$ 2) in syngeneic mice. High-dose hRT did not affect peripheral and tumor-infiltrating CD8+ T-cells, reduced neoplastic accumulation of granulocyte-like myeloid suppressor cells, while T-reg lymphocytes were largely unaltered. Expression of IFN-responsive MHC class I peptides and PD-L1 were enhanced in tumors treated with 8 Gy $\times$ 2 compared to 2 Gy $\times$ 10 schedule. Functionally, tumor-specific CD8$^+$-lymphocytes responses within tumor draining lymph nodes were enhanced following 8 Gy

× 2 schedule but suppressed following 2 Gy × 10 irradiation [53]. When combined with PD-1 mAb, reversal of adaptive immune resistance was observed following 8 Gy × 2, but not following 2 Gy × 10, with subsequent enhancement of CD8+ cell-dependent primary and abscopal tumor response. These data strongly support that high-dose hRT preserves or enhances anti-tumor immunity compared to daily low-dose fractionated irradiation and, when combined with PD-1 inhibitor, reverses adaptive immune resistance, promotes anti-tumor immunity, and controls primary and distant lesions [53].

In order to determine the optimal dose for tumor and immunological response, Schaue et al. conducted a single fraction dose escalation study with doses ranging from 5 to 15 Gy and demonstrated that fractions of 7.5 Gy and above are immune-stimulatory, determining an increased number of tumor-reactive T-cells [54]. Instead a dose higher than 15 Gy in single fraction increased splenic T-reg fraction, while the same total dose fractionated boosted the number of effector T-cells in the spleen and decreased T-regs, with an optimal dose fractionation of 7.5 Gy × 2 [54]. Dewan et al. investigated three different regimens of radiotherapy (20 Gy × 1, 8 Gy × 3, or 6 Gy × 5) to obtain abscopal response in syngeneic mice models, with or without antiCTLA-4, finding that a significant AbE was only induced when RT was administered in fractionated schedules [55]. Additionally, observations by Vanpouille-Box, et al. suggest that AbE could only be achieved with hRT (8 Gy × 3) when combined with immune checkpoint inhibitors [32,33].

The systemic antitumor response of focal hRT combined with ICI may also be mitigated by the immunosuppressive properties of the non-irradiated distant tumors, that hinder T-cell entry and T-cell functioning [56,57]. In this regard, strategies that attempt to modulate the stroma of metastasis to improve T-cell infiltration are basic to invigorate the systemic antitumor response of combined hRT and immune checkpoint inhibitors. In fact, despite its very poor tumor-killing effect, LDRT is effective to T-cell recruitment [58,59]. Klug et al. have shown that LDRT (with one fraction of 2 Gy) may reprogram macrophages in TME, leading to the production of inducible nitric oxide synthase (iNOS), which normalizes tumor vasculature, thus promoting T-cell infiltration and enhancing the efficacy of adoptive T-cell therapy [58]. Moreover Yin et al. preclinically demonstrate that LDRT on established metastases, in combination with ICIs, significantly enhances the abscopal response to hRT treatment on the primary tumor compared to hRT/anti-PD1, hRT/LDRT, or LDRT/anti-PD1 combined treatments [60]. The enhanced abscopal effect was linked to an increased infiltration of CD8+ effector T-cells and an upregulated expression of T-cell-attracting chemokines, so localized LDRT to a second lesion should be indispensable for an enhanced systemic antitumor response, triggered by combined focal hRT and anti-PD1 therapy [60].

This preclinical model has been later confirmed by a post-hoc analysis of a 3 immuno-radiation trial by Menon et al. in which patients, that received LDRT (1–20 Gy), either as scatter from high-dose radiation or from intentional treatment of a second isocenter with low-dose radiation, in association with high-dose radiation and immunotherapy, were evaluated for response [61]. The LDRT lesions were compared to those that received no radiation (<1 Gy total). They assessed that LDRT may increase systemic response rates of metastatic disease treated with high-dose radiation and ICI combinations, offering a clinical proof-of-principle of the ability of LDRT to polarize tumor macrophage in M1 subtype and shift TME from "cold" to "hot" [61,62].

Summarizing, although CD8+ T lymphocytes are present between neoplastic cells, they could be exhausted and could not recognize cancer cells that they were primed to because of an immune-suppressive TME. An ideal radiation dose and fractionation may induce immunogenic cancer cell death and activate anti-tumor T-cell response via APC maturation. Radiation doses > 12 Gy per fraction may attenuate immunogenicity while hypofractionated regimens (i.e., 8 Gy × 3) should be more effective in combination with ICIs for better local response rate and abscopal effect [32].

4. The Abscopal Effect in Clinical Practice: What about HNSCC?

The abscopal effect is a rare phenomenon predominantly described in highly immunogenic tumors like renal cell cancer, melanoma, and hepatocellular carcinoma. In their proof of principle trial, Golden et al. demonstrated objective abscopal responses in 11 patients with metastatic tumors, mainly breast and non-small cell lung cancer (NSCLC), treated with a combination of local radiotherapy with granulocyte-macrophage colony stimulating factor (G-CSF), as a prototype of immunotherapy [62]. Trommer et al. retrospectively collected metastatic patients, treated simultaneously with pembrolizumab or nivolumab and radiotherapy, with at least one lesion not locally targeted; they described AbE in seven patients between melanoma, NSCLC, and renal cell cancer [63]. In the PEMBRO-RT trial, Theelen et al. investigated objective response rate and progression free survival about the sequence SABR 8 Gy × 3-pembrolizumab versus pembrolizumab alone in the metastatic setting. SABR was administered 7 days before ICI. ORR and median PFS were significantly increased in SABR-pembrolizumab group, respectively, 36% vs. 18% and 6.6 months vs. 1.9 months [64]. Moreover, secondary analysis of KEYNOTE-001 also revealed that RT-pembrolizumab sequence showed longer progression free survival versus pembrolizumab alone in advanced NSCLC; however, the correct sequence is still far from being established considering that previous systematic analyses suggest that ICI-RT may be associated with superior outcomes [65].

The RT-induced abscopal effect is anecdotal for HNSCC. Definitive radiotherapy for stage III or IV HNSCC was delivered in 1.8–2 Gy in 35 daily fractions, with a median whole dose of 70 Gy, and simultaneous chemotherapy, mostly cisplatin (with or without 5-Fluorouracil). Palliative RT ranges in 20–30 Gy in five fractions even though different regimens have been established. A split course of two cycles of 25 Gy in 10 fractions was introduced by Stevens et al., with total dose of 50 Gy, leading to tumor response in 82% of patients [66]. The "QUAD SHOT regimen", that consisted of 3.7 Gy in four fractions given twice a day for 2 consecutive days in order to achieve a total of 44.4 Gy in 12 fractions, impacted positively on clinical outcomes, overall tumor response rate, and symptom relief in 50–70% of patients, with minimal toxicity [67]. Moreover Agarwal et al. proposed a dose-intensive palliative RT regimen of 40 Gy in 16 fractions, obtaining a treatment response rate and symptom relief of about 75%; therefore, as a palliative treatment, a higher dose of hRT may be proposed with positive outcomes [68].

Clinical data evaluating ICI-RT combination other than metastatic melanoma or NSCLC are mostly limited to case reports, especially for head and neck squamous cell cancer (Table 1). Interestingly Menon post-hoc analysis did not collect immune-irradiation abscopal responses in HNSCC patients. This result seems in part due to its genetics [61]: molecularly HNSCC, particularly HPV-, is considered a "loss of function" cancer, and inactivating mutations of TP53 and CDKN2A dominate the genetic landscape [69]. This may affect the mode of RT-induced cell death, as well as its immunogenicity. In fact, the best abscopal responses upon RT have always been achieved with "gain of function" cancer models driven by activating mutations in RAS, such as melanoma, and there seems to be a link between RAS status and PD-L1 expression [70,71].

Table 1. Case reports about the abscopal effect in HNSCC.

Authors	Diagnosis	Treatment-Induced Abscopal Effect
Ellerin et al. [72]	Poorly differentiated non-small cell carcinoma of the right parotid as metastatic pulmonary disease	Palliative radiotherapy to the primary site
Forner et al. [73]	Advanced sinonasal squamous cell carcinoma with lung metastases	Nivolumab with palliative radiotherapy to single lung metastasis
Mazzaschi et al. [74]	Hypopharyngeal carcinoma with a single bone metastasis	Definitive chemoradiotherapy on the primary site
Choi et al. [75]	Cutaneous squamous cell carcinoma and larynx tumor	Immunotherapy plus stereotactic radiotherapy
Shinde et al. [76]	Squamous cell carcinoma of the hypopharynx and oropharynx	Nivolumab plus ipilimumab plus Quad Shot radiotherapy

Nevertheless, Ellerin et al. reported that an 84-year-old woman, affected by stage IV poorly differentiated non-small cell carcinoma of the right parotid and lung metastases, underwent only palliative radiotherapy to the primary site, with no chemotherapy or immunotherapy: parotid cancer regressed while several non-irradiated lung lesions completely resolved [72]. Forner et al. presented a case of a man that underwent craniofacial resection and adjuvant chemoradiotherapy for advanced sinonasal squamous cell carcinoma, and rapidly progressed with lung metastases. Nivolumab was initiated, even with oligometastatic progression as the best response. Integrating palliative radiotherapy to single metastasis, the abscopal effect was observed with all distant sites of metastatic disease shrinking [73]. Mazzaschi et al. described a case of a hypopharyngeal carcinoma with a single bone metastasis, treated with definitive chemoradiotherapy omitting local treatment of the bone lesion, who remained relapse-free for 6 years [74]. Choi et al. presented two patients with recurrent metastatic head and neck squamous cell carcinoma, a cutaneous squamous cell carcinoma, and a larynx tumor, that developed a complete response via the abscopal effect following combined immunotherapy and SBRT, the former with a total of 45 Gy in five fractions, the latter with a total of 21 Gy in three fractions of 7 Gy [75]. The abscopal effect in HNSCC could be triggered even in the case of immune-resistance progressive disease too. Shinde et al. reported a patient affected by squamous cell carcinoma of the left hypopharynx and oropharynx with bulky left-neck lymphadenopathy, with local and lung progression after ipilimumab and nivolumab, infused concurrently every three weeks for four cycles. Palliative QUAD SHOT regimen was given with 3.7 Gy BID for 2 days to the pharynx and 3.3 Gy BID for 2 days to the microscopic areas at high risk of disease, continuing nivolumab plus ipilimumab beyond progression. RT was not administered to the lung. Fourteen days after irradiation, left-neck adenopathy decreased, but lung metastases too. QUAD SHOT RT was repeated twice at one-month intervals, with excellent response and tolerability [76]. Notably, QUAD SHOT regimen without immune-checkpoint inhibitors did not lead to the abscopal effect [77].

In a phase I/II trial, Bahig et al. assessed the safety and efficacy of triple treatment combination (TTC), durvalumab plus tremelimumab plus SBRT (up to 15 Gy per fraction for 2–5 metastases), in metastatic HNSCC. No evidence of AbE was shown, however, a positive trend of overall survival (OS) and progression-free survival (PFS) was demonstrated, maybe thanks to a synergism between local SBRT and ICIs [78]. Ongoing trials could provide assistance for future observations about the abscopal effect in radio-immunotherapy combinations (Table 2): proton SBRT with PD-1 blockade immunotherapy in both the locoregionally recurrent and metastatic settings (ClinicalTrials.gov Identifier: NCT03539198); radiotherapy with concurrent and adjuvant Pembrolizumab versus concurrent chemotherapy in patients with advanced/intermediate-risk p16+ head and neck squamous cell carcinoma (ClinicalTrials.gov Identifier: NCT03383094).

Table 2. Ongoing trials about radioimmunotherapy combinations in HNSCC. SBRT, stereotactic body radiotherapy; RT, radiotherapy; HNSCC, head and neck squamous cell cancer; SCC, squamous cell cancer.

Study	Phase	Interventional Arm	Patient Population	Status
NCT03283605	I/II	Durvalumab + Tremelimumab + SBRT	Metastatic HNSCC	Recruiting
NCT03383094	II	Pembrolizumab + RT	Intermediate/high-risk p16-positive locoregionally advanced HNSCC	Recruiting
NCT03539198	II	Nivolumab + Proton SBRT	Recurrent/Progressive Locoregional or Metastatic HNSCC	Not recruiting
NCT04454489	II	Pembrolizumab + QUAD SHOT RT	Advanced/Recurrent/Metastatic HNSCC	Recruiting
NCT02999087	III	Cetuximab + Avelumab + RT	Locally Advanced HNSCC	Not recruiting
NCT03426657	II	Durvalumab + Tremelimumab + RT	Locally Advanced HNSCC	Not recruiting
NCT03799445	II	Nivolumab + Ipilimumab + IMRT	Low-Intermediate Volume, Local-Regionally Advanced HPV-Positive Oropharyngeal SCC	Recruiting

5. Future Perspectives: Tailored Treatment to Reach the Abscopal Effect

Research to elucidate predictive factors of the abscopal effect should be focused on the model of patient-tailored treatment. Poleszczuk et al. generated a mathematical model that incorporates physiological information about T-cell trafficking to estimate the distribution of focal therapy activated-cells between metastatic lesions. They found that the dissemination of activated T-cells among multiple metastatic sites is intuitively predictable, even though they showed that not all metastatic sites participate in systemic immune surveillance equally. They considered how migration of T-cells and the initial imprinting mechanism by tumor antigen-presenting dendritic cells could be facilitated by the blood flow that physiologically sprays the affected organ, assessing that the success in triggering the abscopal effect depends, at least in part, on which metastatic site is selected for localized therapy. Collectively, their elegant work provided the framework to prospectively identify anatomically defined focal-therapy targets that should most likely trigger an immune-mediated abscopal response and therefore may inform personalized treatment strategies in patients with metastatic disease [79].

Modern techniques such as volumetric modulated arc therapy (VMAT) induce larger volumes of healthy tissues receiving low doses of ionizing radiation, that could affect circulating lymphocytes and decrease the adaptive immune response [80]. In fact, lymphopenia measured post-RT may affect the occurrence of abscopal responses, influencing the prognosis of patients treated with RT and immunotherapy in HNSCC [81]. Therefore, the best volume of irradiation in patients receiving ICI may be different from who did not receive immunotherapy, in particular concerning the need to perform elective node irradiation. Irradiation in great vessels and draining lymph nodes (main location of T-cell cross-priming by DCs) could affect immune cell functions and migration [82]. Conventional radiotherapy schedules are generally formulated to deliver a full dose (50–70 Gy) to the tumor and prophylactic coverage to draining lymph nodes (45–50 Gy), as established by multiple clinical trials in various indications (e.g., HNSCC, cervical cancer, NSCLC) [83]. This approach might not be particularly appropriate when radiotherapy is delivered in doses and fractionations that support the activation of anti-cancer immunity, and even so less in the context of radioimmunotherapy. Although draining lymph nodes are not the only sites of T-cell cross-priming by dendritic cells, which can also occur in intratumoral tertiary lymphoid structures, they constitute the major site for the initiation of local and systemic anti-tumor immune responses; that is why it might be detrimental to perturb draining nodes with radiotherapy [84]. Patients with nasopharyngeal carcinoma with tumor-free draining nodes and treated with lymph node-sparing IMRT rarely exhibited locoregional

relapse [85]. Similarly, clinical data on partial tumor irradiation sparing draining lymph nodes, combined with pembrolizumab, compare positively with previous results involving draining nodes irradiation [86].

Luke et al. reported results of a study that tested safety and feasibility of pembrolizumab and multisite SBRT in patients with metastatic solid tumors progressing on standard treatment. SBRT schedules varied by site: 15 Gy × 3 (45 Gy) for peripheral lung, liver and abdominal/pelvic; 10 Gy × 5 (50 Gy) for central lung and mediastinal/cervical; 10 Gy × 3 (30 Gy) for osseous and spinal/paraspinal. Pembrolizumab was infused within 7 days after SBRT. In order to quantify interferon-γ-induced gene expression, pre- and post-SBRT biopsy were analyzed: no significant changes have been reported from pre- to post-SBRT biopsy, however, post-SBRT IFN-γ-related immune score positively correlated to distant metastatic responses [86], with an abscopal response rate of 27% comparable to the analysis by Golden et al. [65]. Nevertheless, these results should be cautiously interpreted given that many patients in the trial were potentially sensitive to pembrolizumab alone [87].

Tumor radiosensitivity is often dose-related and associated with immune activation in solid tumors and in HNSCC too. First of all, Michna et al. identified specific networks of HNSCC clones radiosensitivity, with particular attention to senescence-associated secretory phenotype [88]; then, Reynders et al. developed the genomic-adjusted radiation dose (GARD), that provides the opportunity to adjust the dose of radiation to match the individual radiosensitivity of the tumor [89]; finally Strom et al. propose two distinct gene expression signatures (GES), the radiosensitivity index (RSI) and the 12-chemokine (CK) signature, to determine if radiosensitivity is associated with activation of the immune system [90]. RSI-low tumors had a significantly higher median 12-CK GES value compared with RSI-high tumors, indicating that radiosensitivity is associated with immune activation and radiosensitive tumors more frequently exhibit a phenotype of immune-activation. Combining these two signatures could be clinically useful to improve the identification of patients susceptible to the abscopal effect with integrated treatments, especially for non-oropharyngeal HNSCC [90]. However, HNSCC radiation resistance is intensely regulated by intrinsic cell-signaling pathways and mutational landscape. TP53 mutations are significantly associated with shorter OS and tumor resistance to radiotherapy and chemotherapy, which makes p53 status a potentially useful molecular prognostic and predictive factor for clinicians. Likewise, overexpression of epidermal growth factor receptor (EGFR) highlighted higher rates of post-radiation locoregional failure, suggesting that EGFR contributes to radiation resistance in about 40–70% of the cases [91].

6. Conclusions

The efficacy and toxicity of radiotherapy is closely related to the immunological balance of the host: the composition of the intestinal microbiota, the presence of active infection, autoimmune diseases, concomitant or recent exposure to immunosuppressive drugs may reduce radiosensitivity and consequently the development of a robust abscopal response [92]. Additionally, immunonutrition could contribute to stimulate immune functions and antioxidant defense capacities of leukocytes in head and neck cancer patients, as evidenced by Talvas et al. [93].

In conclusion, there is still an important gap between clinical and preclinical experiences, nevertheless, we should "tailor" the abscopal effect on the patient as shown (Figure 2). It is initially necessary to identify the most radiosensitive and potentially immune-stimulable primary tumor and metastatic sites too, with particular attention to draining nodal stations; then the dose, the fractionation, and the right volume of irradiation must be analyzed, integrating radiation at low doses with high doses, preferring hRT schedules, remembering that parenchymal diseases respond differently to each other and compared to lymph node disease. Finally, the right radiotherapy-immunotherapy sequence should be established, possibly associating ICIs 5–10 days after starting radiotherapy. Optimal radioimmunotherapy combinations should be critically designed comparing the effects of associations with immunotherapy alone, as exemplified in the IMPORTANCE

trial (NCT03386357) for the treatment of recurrent or metastatic HNSCC. Finally, stronger conclusions should only be addressed with clinical trials comparing sparing lymph node RT versus draining node irradiation. This will make it easier to "tailor" the abscopal effect and dramatically improve the overall survival and quality of life of HNSCC patients.
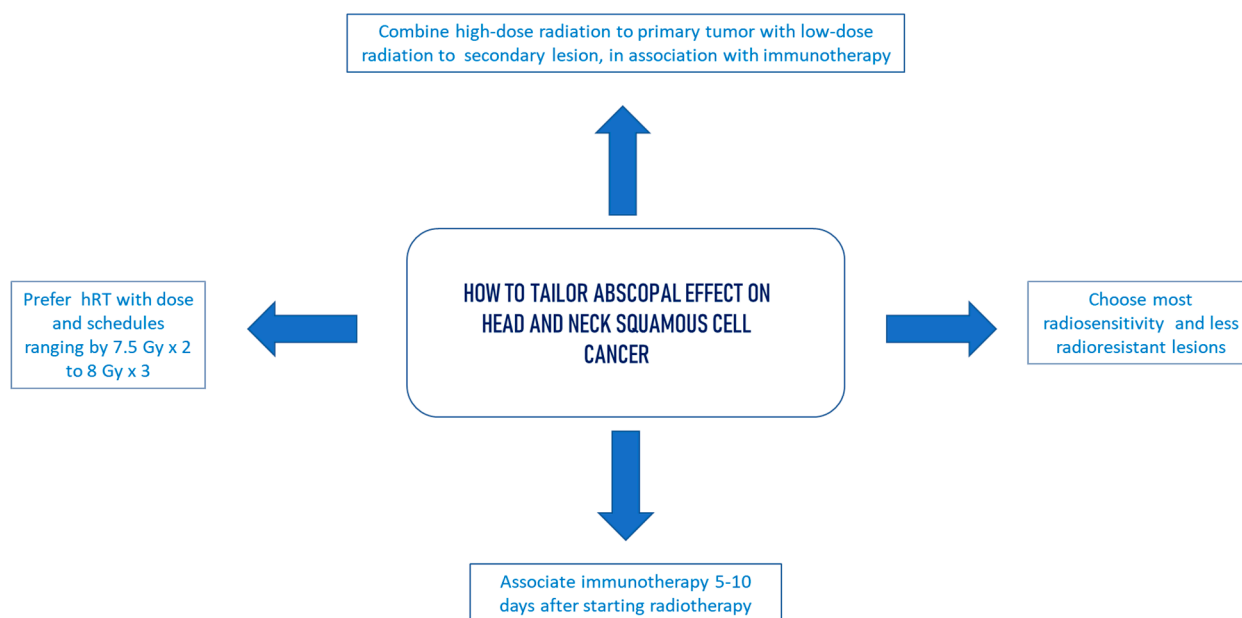
Figure 2. Clinical approaches to reproducing the abscopal effect in head and neck cancer.

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