Synergy of Radiotherapy and a Cancer Vaccine for the Treatment of HPV-Associated Head and Neck Cancer

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Introduction

Human papillomavirus (HPV) is the most common sexually transmitted infection in Western countries (1, 2). Mucosal infection with "high-risk" HPV types (e.g., HPV16) has long been acknowledged to be a major risk factor for malignant neoplasms such as cervical, anal, penile, vaginal, and head and neck squamous cell carcinoma (HNSCC). The efficacy of the irradiation and vaccine association was tested using a model of HNSCC obtained by grafting TC-1 luciferase cells at a subcutaneous site of the inner lip of immunocompetent mice. Irradiation and the STxB-E7 vaccine acted synergistically with both single and fractionated irradiation schemes, resulting in complete tumor clearance in the majority of the treated mice. A dose threshold of 7.5 Gy was required to elicit the dramatic antitumor response. The combined treatment induced high levels of tumor-infiltrating, antigen-specific CD8⁺ T cells, which were required to trigger the antitumor activity. Treatment with STxB-E7 and irradiation induced CD8⁺ T-cell memory, which was sufficient to exert complete antitumor responses in both local recurrences and distant metastases. We also report for the first time that a combination therapy based on local irradiation and vaccination induces an increased pericyte coverage (as shown by αSMA and NG2 staining) and ICAM-1 expression on vessels. This was associated with enhanced intratumor vascular permeability that correlated with the antitumor response, suggesting that the combination therapy could also act through an increased accessibility for immune cells. The combination strategy proposed here offers a promising approach that could potentially be transferred into early-phase clinical trials. Mol Cancer Ther; 14(6); 1336–45. © 2015 AACR.

Abstract

There is growing interest in the association of radiotherapy and immunotherapy for the treatment of solid tumors. Here, we report an extremely effective combination of local irradiation (IR) and Shiga Toxin B (STxB)–based human papillomavirus (HPV) vaccination for the treatment of HPV-associated head and neck squamous cell carcinoma (HNSCC). The efficacy of the irradiation and vaccine association was tested using a model of HNSCC obtained by grafting TC-1 luciferase cells at a subcutaneous site of the inner lip of immunocompetent mice. Irradiation and the STxB-E7 vaccine acted synergistically with both single and fractionated irradiation schemes, resulting in complete tumor clearance in the majority of the treated mice. A dose threshold of 7.5 Gy was required to elicit the dramatic antitumor response. The combined treatment induced high levels of tumor-infiltrating, antigen-specific CD8⁺ T cells, which were required to trigger the antitumor activity. Treatment with STxB-E7 and irradiation induced CD8⁺ T-cell memory, which was sufficient to exert complete antitumor responses in both local recurrences and distant metastases. We also report for the first time that a combination therapy based on local irradiation and vaccination induces an increased pericyte coverage (as shown by αSMA and NG2 staining) and ICAM-1 expression on vessels. This was associated with enhanced intratumor vascular permeability that correlated with the antitumor response, suggesting that the combination therapy could also act through an increased accessibility for immune cells. The combination strategy proposed here offers a promising approach that could potentially be transferred into early-phase clinical trials. Mol Cancer Ther; 14(6); 1336–45. © 2015 AACR.


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In addition, radiotherapy could act as a vascular remodeler by promoting the recruitment of inflammatory cells and the infiltration of activated immune effectors to disrupt the tolerance related to an immunosuppressive tumor microenvironment (16).

The B subunit of the Shiga toxin (STxB) is a nonreplicative vector that targets dendritic cells (DC) in vivo (17). When coupled to various tumor antigens, it elicits a strong induction of specific CD8+ T cells, which can be further enhanced by the addition of adjuvants (18). The STxB-based vaccine used in the present study (STxB-E7) carries an epitope from the HPV16 E7 oncoprotein, which is actively expressed in HPV-related HNSCC (19). We recently showed that mucosal (intranasal) immunization with STxB-E7 elicits a sustained antitumor response in a murine model of HPV-positive HNSCC (20). However, the therapeutic efficacy of the vaccine was limited when it was administered by a systemic route.

The purpose of our study was to test whether local irradiation could boost the efficacy of the novel STxB-E7 vaccine to treat HNSCC. We thereby used an immunocompetent mouse model of HPV-related HNSCC and, by different radiation schedules, demonstrated synergistic activity of the irradiation and vaccination. To elucidate the mechanisms underlying the interaction between STxB-E7 and radiotherapy, we focused our analysis on the tumor-infiltrating immune cells and the vascularization pattern. We showed that irradiation promoted HPV-E7-specific CD8+ T-cell tumor infiltration and vascular normalization in vaccinated tumors, suggesting that the combination therapy acts both through increased levels and tumor accessibility of cytolytic CD8+ T cells.

Materials and Methods

Cells

Firefly luciferase-expressing TC1/Luc cells, generated by the HPV16 E6/E7 and c-H-ras retroviral transduction of lung epithelial cells of C57BL/6 origin, were kindly provided by T.C. Wu (Johns Hopkins Medical Institutions, Baltimore, MD) in 2009. The cells were cultured in vitro in RPMI-1640 supplemented with 10% FBS, 1% sodium pyruvate, 1% non-essential amino acids, 10 mmol/L HEPES, and 1% penicillin/streptomycin and were grown at 37°C with 5% CO2. Master stocks of the cells were prepared upon receipt and then used within 6 months after resuscitation. Expression of E6 and E7 genes was confirmed by RT-PCR as previously described (21), as well as MHC haplotype H2b by flow cytometry.

Head and neck tumor and lung metastasis models

Female C57BL/6 mice (age, 7 to 8 weeks) were purchased from Janvier CERT and housed in the Gustave Roussy animal facility. Animal procedures were performed according to the protocols approved by the Ethical Committee CEEA 26 and in accordance with recommendations for the proper use and care of laboratory animals. To establish a HNSCC implantation model, syngeneic tumor grafts were initiated at day 0 by injection of 0.1 mL of PBS suspension containing 5 × 10^5 TC1/Luc cells at a submucosal site of the right inner lip. For rechallenge experiments, mice were injected in the left inner lip. Lung metastases were established by injecting 10^6 TC1/Luc cells suspended in 0.2 mL of PBS in the tail vein. Throughout the study, the health, weight, and behavior of the mice was assessed daily; mice were humanely euthanized upon the presentation of defined criteria (tumor size, ulceration, loss of >20% of the initial weight), and survival time was recorded to perform a survival analysis for the treatment groups.

Irradiation

Mice received single-beam local irradiation to the head and neck region using a 200 kV Varian X-ray irradiator. Selective irradiation of the tumor grafts was performed by the interposition of a 4-cm thick lead shield. According to the treatment group, a schedule delivering 0, 2.5, 5, or 7.5 Gy in a single fraction or 10.4 Gy in four consecutive fractions (2.6 Gy/day) was administered at a dose rate of 1.08 Gy/min. The 10.4 Gy dose represented the biologically equivalent dose of 7.5 Gy in a single fraction, as calculated using the linear quadratic model with an α/β = 10.

Vaccination protocol

The STxB-E7 vaccine was produced by the chemical coupling of the N-bromoacetylated E743-57 peptide and the sulfhydryl group of a recombinant non-toxic Shiga toxin B-subunit variant, termed STxB/Cys, according to previously described procedures (22). Recombinant STxB/Cys was purified from bacterial periplasmic extracts as previously described (23). In brief, periplasmic extracts were loaded on a QHP column (GE Healthcare) and eluted in a linear NaCl gradient in Tris-HCl buffer. STxB/Cys containing fractions were pooled, validated for purity by SDS-PAGE, and snap frozen in liquid nitrogen before storage at −80°C. The adjuvant α-galactosylceramide (KRN7000, Funakoshi, Japan) was administered alone or together with the first dose of vaccine. Vaccine and/or KRN7000 were administered i.p. at 20 and 1 μg per mouse, respectively.

CD8+ T lymphocyte depletion

Mice received i.p. injections of 100 μg per mouse of anti-CD8 mAb (clone 2.43; BioXCell) or isotype control mAb on day 7 (one day before irradiation) to deplete CD8+ T cells, as previously reported (20).

In vivo imaging

To monitor tumor growth, bioluminescence imaging was performed using the Xenogen In Vivo Imaging System 50 (IVIS, Caliper Life Sciences). Mice were placed in the supine position in the imaging chamber 5 minutes after i.p. injection of firefly luciferin; images were acquired whereas the mice were under sedation via the continuous administration of isoflurane. The photographic images and bioluminescence color images were superimposed, and signal quantification was performed using the LivingImage V 4.3.1 software (Caliper Life Sciences).

Tumor histopathology and vascular assessment

Immunohistochemistry for alpha smooth muscle actin (αSMA) was performed using a validated standard protocol on a Ventana Discovery Ultra autostainer (Roche Tissue Diagnostics; primary Ab ref ab5694, Abcam). Manual staining protocols on OCT frozen sections were used to detect CD31 (BD Pharmingen ref 553370), and ICAM-1 (Abcam ref ab25375). Slides were counterstained with hematoxylin. NG2 (primary Ab: Millipore ref AB5320) was detected by immunofluorescence. CD31 and αSMA staining were quantified using the algorithm for vessel detection in the Tissue Studio software package from Definiens.
(Definiens). Vessels were automatically detected according to their HIC staining spectral properties in regions of interest that had been manually selected. The percentage of the area stained for NG2 was measured using the ImageJ v1.41 software. ICAM-1 staining was determined using a semiquantitative score based on the staining area (less than 1/field = 0; 1–2/field = 1; 3 or more/field = 2) and intensity (no staining = 0; weak = 1; moderate = 2; strong = 3).

**Tumor vascular permeability by Miles assay**

To assess vascular permeability, 0.2 mL Evans Blue dye (0.5%) was administered by tail vein injection. Mice were sacrificed 30 minutes after the injection. Dissected tumor samples were eluted in formaldehyde (0.5 mL for 50 mg of tissue) at 58 °C overnight. The concentration of the accumulated dye for each aliquot was quantified by the use of a spectrophotometer by reading the absorbance at 730 nm.

**Analysis of tumor-infiltrating-specific CD8+ T cells**

To detect tumor infiltrating anti-E7+/-Db-specific CD8+ T cells, tumors were dissociated, washed twice in PBS and then incubated with the Fc receptor block CD16/CD32 (eBioscience). Tumors were then labeled with the E7+/-Db tetramer according to the manufacturer's recommendations (Beckman Coulter Immunomics) and the anti-CD8 antibody. Cells were incubated for 35 minutes at 4°C with the PE-labeled tetramer. After incubation and washes, the cells were labeled using the live/dead cell viability assay (Life Technologies) and anti-CD8 (APC-Cy7; eBioscience), PD1 (FITC; eBioscience), CTLA4 (Brilliant Violet 57/Db tetramer according to the staining area (less than 1/field = 0; 1–2/field = 1; 3 or more/field = 2) and intensity (no staining = 0; weak = 1; moderate = 2; strong = 3).

**Statistical analysis**

Survival data were analyzed using the Kaplan–Meier and log-rank tests for survival distribution. The bioluminescence signal as a measure of tumor size, the immune infiltrate, and tumor perfusion levels were analyzed using an ANOVA analysis followed by the Tukey post hoc test for multiple comparisons. Correlations were assessed using the Pearson correlation test. Statistical analyses were performed using Prism Version 6 (GraphPad).

**Results**

**The therapeutic efficacy of the STxB-E7 vaccine in an orthotopic head and neck tumor model is strongly enhanced by local irradiation**

Our objective was to evaluate whether radiotherapy could enhance the therapeutic potential of the STxB-E7 systemic vaccination for the treatment of HNSCC. To test this hypothesis, we used an orthotopic model of HNSCC cancer by grafting HPV16 E7-expressing TC-1/Luc cells at a submucosal site of the inner lip in C57BL/6 mice. We designed a treatment protocol combining local tumor irradiation (IR) with i.p. administration of STxB-E7 vaccine associated with the mucosal adjuvant KRN7000 (Fig. 1A). KRN7000, either alone or in combination with radiotherapy, did not impact on tumor growth (Supplementary Fig. S1A); therefore, all experiments were performed with KRN7000-treated mice as control groups. Bioluminescent in vivo imaging (Fig. 1B) showed that the mice developed fast-growing tumors (Fig. 1C) that resulted in the survival of control mice for 2 weeks after TC1/Luc cell injection (Fig. 1D). Treatment with a single dose of 7.5 Gy IR resulted in a limited tumor growth delay with no significant increase of survival (Fig. 1B–D). In line with our previous results (20), systemic immunization with STxB-E7 only partially improved tumor control, but its combination with local radiotherapy led to an effective antitumor response and 70% of the mice were still alive 70 days after the tumor engraftment (Fig. 1D, P < 0.001 vs. control, log-rank test). The tumor burden of mice treated with the combined therapy was significantly smaller compared with the single therapy (IR or STxB-E7)–treated mice (Fig. 1C, P < 0.01, one-way ANOVA). Most of the mice of the combination therapy group had complete tumor clearance that lasted until the end of the 90-day observation period (Fig. 1B). To obtain a therapeutic synergy between radiotherapy and immunization, a specific dose threshold had to be attained because irradiation doses lower than 7.5 Gy did not significantly affect tumor growth, even in combination with STxB-E7 (Supplementary Fig. S1B). Of note, when the equivalent dose of 7.5 Gy was given in fractions to mimic a clinically relevant therapeutic regimen, tumor control by the combination treatment was still effective (Fig. 1E). These data, obtained using a relevant model of HNSCC, indicate that an approach based on STxB-E7 systemic vaccination in combination with local radiotherapy represents a powerful therapeutic strategy.

**High levels of tumor-infiltrating and splenic antigen-specific CD8+ T cells are induced by combined irradiation and vaccination**

We hypothesized that the potent antitumor effect observed in mice treated with the combination of STxB-E7 and local irradiation could be linked to an enhanced immune response in this group. We thus characterized the population of tumor-infiltrating lymphocytes in mice treated by the different regimens. We first focused on the CD8+ T-cell population because these cells play a pivotal role in the tumor control of TCI cells (24). The CD8+ level was low in control tumors, and almost no E7-specific CD8+ T cells were found in this group (Fig. 2A). Irradiation alone was not sufficient to induce a CD8+ infiltrate, whereas vaccination upregulated the percentage of E7-specific CD8+ T cells, as demonstrated by the E7-tetramer analysis. When irradiation was added to STxB-E7 immunization, the infiltration of CD8+ T cells was significantly enhanced (Fig. 2A, left). Most of the CD8+ T cells were antigen-specific, as the level of E7 tetramer-positive CD8+ T lymphocytes reached a surprising 67% in this group (Fig. 2A, right). We also performed an analysis of the E7-specific CD8+ infiltrate by identifying the CTLA4−, PD1−, and Tim3-positive subpopulations, but we found no significant differences among the treatment groups (Supplementary Fig. S2A–S2C). The induction of a specific CD8+ T-cell response was not restricted to the tumor but was rather systemic. Indeed, when we analyzed the percentage of E7-specific CD8+ T cells in the spleen, the combination of irradiation and vaccination resulted in the highest antigen-directed response compared with vaccine treatment alone (Fig. 2B). Moreover, the percentage of intratumoral E7-specific CD8+ lymphocytes was inversely correlated with the tumor size (P
<0.05, Pearson’s correlation test, Fig. 2C), suggesting a central role for CD8$^+$ T lymphocytes in the antitumor response in our preclinical HNSCC tumor model. To mechanistically validate the role of CD8$^+$ T cells in this therapeutic setting, we injected mice undergoing combined irradiation and vaccination with anti-CD8 antibodies to deplete the CD8$^+$ T lymphocytes. The administration of anti-CD8 antibodies reversed the antitumor response, resulting in a complete loss of efficacy of the combined treatment on tumor size and survival, as all of the CD8$^+$-depleted mice had to be euthanized together with the controls (Fig. 2D). These data clearly indicate that CD8$^+$ T lymphocytes are required to elicit the antitumor response triggered by the combination of irradiation and vaccination.

Effective combination therapy induces T-cell memory

Because most of the mice that received the combination of STxB-E7 and IR showed a complete remission of the tumor (Fig. 1B), we wondered whether the sustained CD8$^+$ T-cell response observed in this group resulted in the development of an effective T-cell memory. To test this hypothesis, 4 mice that were still tumor free 90 days after tumor grafting, as observed by in vivo imaging (Fig. 3A and B), were challenged again with TC1/Luc cells in the submucosa of the opposite inner lip. Four days after the second injection, bioluminescence imaging showed that all mice were correctly engrafted, with tumor cells localized on left inner lip (see representative Fig. 3A, D$''$4). Five days later (day 8, D$''$8), without any additional treatment, the luciferase activity was completely absent, indicating a spontaneous clearance of the tumor cells. In contrast, treatment-naïve mice used as positive controls displayed a tumor growth curve similar to those depicted in Fig. 1C (not shown). When mice were euthanized to collect the spleens (14 days after the second TC1/Luc challenge), the same results were observed (Fig. 3A, D$''$14). Cytolymphometric analysis showed that rechallenged mice displayed high percentages of E7-specific CD8$^+$ T cells at similar levels to those of mice at the end of the combined treatment (Fig. 3C), indicating that the spontaneous antitumor response was maintained.

Combination treatment by local radiation and therapeutic STxB-E7 vaccine elicits an effective antitumor response in an orthotopic head and neck cancer model.

At day 0, C57BL/6 mice were injected with TC1/Luc cells in the inner lip. Mice were then subjected to the therapeutic treatments as depicted in the schematic diagram (A; single 7.5 Gy irradiation, 10 mice per group) and (E; left, fractionated irradiation four times 2.6 Gy, 5 mice/group). In vivo bioluminescent imaging was performed to follow tumor growth (B), and quantification of the bioluminescent signal was performed (C and E, center). **, \( P < 0.01 \); ***, \( P < 0.001 \), one-way ANOVA followed by the Tukey post-test of tumor sizes at day 14. Kaplan–Meier survival curves for single dose (D) or fractionated (E, right) irradiation experiments are shown. **, \( P < 0.01 \); ***, \( P < 0.001 \) versus the control group, the log-rank test. One of two representative experiments is shown.

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activity observed was linked to a persistent CD8+ response. A similar experiment was performed on three mice that were rechallenged with TC1/Luc cells 18 months after the first treatment, again resulting in the complete spontaneous rejection of tumor cells (not shown). It was also important to evaluate whether the immune response elicited from effective combined therapy provided protection against potential metastasis formation. We tested this by i.v. injecting TC1/Luc cells to induce the development of lung metastases. As shown by in vivo imaging analysis, in treatment-naïve mice, a bioluminescent signal localized to the lungs was already detectable four days after the injection of TC1/Luc cells (Fig. 3D, left). The size of the lung metastases increased quickly until day 15, when the mice had to be euthanized. In mice that had already experienced a complete remission of the head and neck tumor upon administration of the combination of STxB-E7 and IR (rechallenge group), the tumor signal in the lungs was already smaller (or absent) at day 4 when compared with treatment-naïve mice. TC1/Luc cells were spontaneously cleared starting at day 8 (Fig. 3D, right) and all four tested mice remained metastasis-free until the end of the 60-day observation period (not shown). We repeated the same experiment by injecting TC1/Luc cells in 5 mice 9 months after the first
treatment, obtaining similar results (Supplementary Fig. S3). These data indicate that a combination of local irradiation and immunization elicits a CD8<sup>+</sup>T-cell memory that is sufficient to exert a complete antitumor response in both local recurrences and distant metastases.

Combined therapy promotes tumor vessel normalization

The increased efficacy of STxB-E7 vaccination observed upon irradiation can be at least partly linked to an increased accessibility of the tumor stroma by circulating leukocytes (25). We therefore analyzed the vascular structure in the different treatment groups. Immunohistochemical staining by the endothelial cell (EC) marker CD31 indicated that there were no significant differences in tumor vessel surface and density at either 24 hours (day 9) or 6 days after irradiation (day 14) that were elicited by the various treatments (Supplementary Fig. S4A and S4B). In contrast, when we analyzed the levels of pericytes by performing αSMA staining, we observed a significant increase in the stained surface in the tumors of mice treated with both STxB-E7 and IR at day 14 (Fig. 4A). Because pericytes are recognized to be key regulators of the vascular structure and because the extent of their coverage on tumor vessels is typically diminished compared with normal tissues (26), these data suggest that the combined treatment induces a restoration of vascular functionality. This was further confirmed by the increase in NG2 staining (an alternative pericyte marker) observed at day 14 in mice treated with both local irradiation and vaccination (Fig. 4B and representative images in Supplementary Fig. S5A). These observations were supported by the Miles assay, a functional test that was performed to evaluate tumor vascular permeability (27). At 24 hours after irradiation (day 9), the permeability of tumor vessels was not yet affected by the treatments (Fig. 4C, left); however, at day 14 after tumor challenge, the permeability was significantly increased by the combined treatment compared with both control and single therapies (Fig. 4C, right). The observed increase was inversely correlated with the bioluminescent signal (P < 0.05, Pearson

Figure 3.
Combination treatment induces a long-term antitumor immune response. Mice were treated with a combination of STxB-E7 vaccine and localized irradiation, as described in Fig. 1. Four mice that showed complete regression 3 months after the first TC1/Luc challenge were subjected to a second graft in the left inner lip. IVIS in vivo imaging was performed to follow tumor growth (A), and a quantification of the signal was performed (B). Mice were then euthanized 14 days after the second TC1/Luc challenge (D''14), and spleens were collected. C, splenic E7-specific CD8<sup>+</sup>T cells were detected using a tetramer assay (Rechallenge) and compared with those of mice that were subjected to combination treatment for the first time (STxB-E7 + IR), as in Fig. 2B. D, IVIS in vivo imaging following i.v. injection of TC1/Luc in 4 treatment-naïve mice (left) and in 4 mice that showed complete regression 3 months after the first TC1/Luc challenge (right).
correlation test, Fig. 4D), indicating that vascular permeability was linked to the tumor size at this time point. Finally, we evaluated vessel activation by detecting ICAM-1 expression in the tumors. ICAM-1, a transmembrane protein implied in the arrest and transmigration of leukocytes from blood vessels (28), was increased in tumors from mice undergoing the different treatments when compared with control mice; the highest score was observed in the combination group (Fig. 4E and representative images in Supplementary Fig. S5B). Taken together, these data indicate that combination treatment promotes tumor vessel normalization, which can favor the recruitment and infiltration of leukocytes in the tumor tissue.

Discussion

There is currently a growing interest in combination approaches based on radiotherapy and immunotherapy for the treatment of solid tumors, and cancer vaccines could play an important role in this setting (12). Peptide vaccines coupled to the non-toxic STxB have been successfully tested in several preclinical settings and represent a promising tool for vaccine-based cancer therapy (20, 29–31). Nevertheless, their use in association with radiotherapy has not been previously reported. Here, we report a novel, extremely effective combination of STxB-based vaccination (STxB-E7 vaccine) and local irradiation for the treatment of HPV-associated HNSCC. In our murine model, this novel therapeutic approach resulted in the complete clearance of the majority of treated tumors. The new STxB-based vaccination strategy acted synergistically with either single dose or fractionated irradiation, which is of particular relevance because fractionated radiotherapy is a mainstay for the treatment of HNSCC (32).

In agreement with previous observations (11, 20), the antitumor response in our preclinical model was dependent on CD8+ T-cell activity (Fig. 2D). Previously, we have shown that the homing of T cells to mucosal tumors is improved by intranasal immunization (20). Here, we demonstrate that irradiation overcomes the necessity of intranasal (mucosal) immunization with STxB-E7, because high levels of tumor-infiltrating, antigen-specific CD8+ T lymphocytes are obtained after i.p. administration (Fig. 2A and
2B). The high number of CD8+ T cells observed upon combined treatment was required to trigger the potent antitumor response, since the low CD8+ levels detected upon systemic vaccination alone were not sufficient to induce tumor control and regression (Fig. 1B). Our data, thus, suggest that a threshold level of antigen-specific CD8+ lymphocytes should be reached to overtake the tumor growth, resulting in tumor clearance, and that irradiation enhances the vaccine‐induced CD8+ response above this required level.

Combined therapy can promote the infiltration by leukocytes by affecting the tumor stroma, specifically the vessels. Vascular normalization has been proposed to be a key mechanism in mediating antitumor responses (33, 34), enhancing cancer immunotherapy (16, 35). Moreover, endothelial cells within tumors often display a downregulation of ICAM-1/2, VCAM-1, and CD34, a phenomenon defined as "EC energy" (36). Restoration of the expression of such adhesion molecules can facilitate the interaction of effector T cells with ECs, promoting their migration through blood vessels. Here, we show for the first time that a combination therapy based on irradiation and vaccination induces increased tumor vascular permeability (Fig. 4C), which is accompanied by augmented pericyte coverage (Fig. 4A and 4B) and ICAM-1 expression (Fig. 4D). We also observed a threshold dose for synergy between irradiation and vaccine in the range of 7.5 Gy. Accordingly, it has been shown that vascular-induced changes occur in a dose‐dependent manner after a single dose of irradiation (37). In their recent work, Klug and colleagues (38) reported a reduction in vessels area and density (which we did not observe in a preclinically favorable model upon low‐dose irradiation and T‐cell transfer. They also observed a macropage repolarization toward M1, whereas our preliminary results did not suggest this repolarization (data not shown), thus highlighting the specificities of different tumor settings. In addition, in contrast with other studies (11, 39), the combination of irradiation and the cancer vaccine used here did not result in an increase in immunosuppressive cells in the tumor microenvironment (data not shown). The use of a mucosal tumor model and some specific features of each vaccine may explain these differences.

In addition to vascular effects, the synergy between IR and STxB-E7 could be also linked to the induction of immunologic cell death by radiotherapy. Immunologic cell death, when dying cells display immunologically favorable features, has indeed been suggested as a major mechanism of synergy between immunomodulation and irradiation (40). Because STxB-E7 directly targets DCs, another potential mechanism of interplay between irradiation and vaccination could be through the modulation of the function of DCs by ionizing radiation, as reported in in vitro settings (41). Experimental work is currently ongoing to elucidate these mechanisms.

Recently, two vaccines targeting HPV antigens have been tested in association with radiotherapy in murine models (11, 39). However, though HPV-associated cancers are located in mucosal sites, both studies were conducted using subcutaneous tumors. Our recent data indicate that the site of tumor implantation affects the response to cancer vaccine and the homing of induced CD8+ cells (20). It was, therefore, important to test the efficacy of irradiation combined with vaccination in a model more closely reproducing the natural lesions in humans, such as the mucosal murine model of HNSCC used here. Moreover, we were able to demonstrate the powerful antitumor efficacy of the combination treatment at much lower irradiation doses (7.5 Gy compared with the dose of 14 Gy used in the previous studies). Radiotherapy dose reduction can limit the risk of late complications, and is a lively area of investigation (42). The possibility to use low irradiation doses could be a valuable tool for treatment deintensification strategies in the clinic. Wu and colleagues (11), Draghiciu and colleagues (39) performed radiotherapy before vaccination, whereas we administered STxB-E7 before irradiation. This approach might facilitate the implementation of vaccinations in future clinical strategies because patients with a confirmed diagnosis of HPV-positive HNSCC would immediately benefit from the vaccination administration while waiting for radiotherapy treatment.

The combination of local irradiation and immunotherapy has recently been presented as a means to exert better local tumor control; however, in specific settings, it also triggers a systemic antitumor effect, the so-called "abscopal" effect (43). In our study, when the combination treatment resulted in complete tumor clearance, which occurred in 70% of treated mice, no recurrence or metastasis was found, even after a 18-month observation period. This is a consequence of the induction of an effective T-cell memory, with a protective effect both locally and systemically, as demonstrated by our rechallenge experiments (Fig. 3 and Supplementary Fig. S3). This represents a promising clinical tool, because a patient treated with this combination therapy who undergoes tumor remission will likely be protected against any local or distant relapse.

There is a need for specific therapies to manage HPV-related tumors because HPV status is a biomarker of outcome and response to radiotherapy. Currently, there are no specific therapies that increase the therapeutic ratio for these tumors. The radiovaccination strategy proposed here is a promising approach that can likely be transferred into early‐phase clinical trials.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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References


Molecular Cancer Therapeutics

Synergy of Radiotherapy and a Cancer Vaccine for the Treatment of HPV-Associated Head and Neck Cancer

Michele Mondini, Mevyn Nizard, Thi Tran, et al.

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