HHS Public Access

Author manuscript

Semin Radiat Oncol. Author manuscript; available in PMC 2016 January 01.

Published in final edited form as:

Semin Radiat Oncol. 2015 January ; 25(1): 54–64. doi:10.1016/j.semradonc.2014.07.003.

Current Clinical Trials Testing Combinations of Immunotherapy and Radiation

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Abstract

Preclinical evidence of successful combinations of ionizing radiation with immunotherapy has inspired testing the translation of these results to the clinic. Interestingly, the preclinical work has consistently predicted the responses encountered in clinical trials. The first example came from a proof-of-principle trial started in 2001 that tested the concept that growth factors acting on antigen-presenting cells improve presentation of tumor antigens released by radiation and induce an abscopal effect. Granulocyte-macrophage colony-stimulating factor was administered during radiotherapy to a metastatic site in patients with metastatic solid tumors to translate evidence obtained in a murine model of syngeneic mammary carcinoma treated with cytokine FLT-3L and radiation. Subsequent clinical availability of vaccines and immune checkpoint inhibitors has triggered a wave of enthusiasm for testing them in combination with radiotherapy. Examples of ongoing clinical trials are described in this report. Importantly, these trials include careful immune monitoring of the patients enrolled and will generate important data about the proimmunogenic effects of radiation in combination with a variety of immune modulators in different disease settings. Results of these studies are building a platform of evidence for radiotherapy as an adjuvant to immunotherapy and encourage the growth of this novel field of radiation oncology.

Introduction

Although evidence for contribution of the immune system to the clinical response of radiotherapy dates as far back as the mid-1970s,[1] it is only in the past 10 years that trials have started exploring this novel approach in the clinic. For instance, there is now some evidence of tumor-specific immunity in patients following radiation. In one study, it was demonstrated that radiotherapy and antiandrogen hormone therapy induced autoantibody responses to a variety of tumor-associated antigens (Ags) in 25%-30% of patients with prostate cancer.[2] In another study, after radiation some patients with colorectal cancer or prostate cancer had T cells specific for an Ag that is overexpressed by tumors detectable by tetramer analysis.[3] The host's recruited immune response against the irradiated tumor has the potential to actively contribute to the success of the course of radiotherapy.

Moreover, if sufficiently robust, this newly acquired immune response could achieve systemic antitumor effects. In this scenario, tumor-specific effector T cells can target cancer cells at metastatic sites, achieving an abscopal effect of radiotherapy (ab scopus = away

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from the target).[4,5] Clinical abscopal effects of radiotherapy have been described, although very uncommon.[4] Their rare occurrence reflects the fact that by itself, standard radiotherapy is inadequate at subverting the existing immunosuppression or tolerance characteristic of the microenvironment of an established tumor. However, the ability of radiation to prime antitumor responses is likely to be key in obtaining a therapeutic synergy with immunotherapies that can unleash these immune responses.

The first trial testing the ability of a combination of radiation and immunotherapy to induce abscopal responses, a proof-of-principle trial that has opened this field, is described in the following sections.[4] A brief summary of the ongoing trials of immunotherapy and radiation that are currently open for patient enrollment is given. Without the ambition of representing all the ongoing research on the subject, this report is meant to offer some examples of current investigations in this field and introduce the reader to some of the challenges intrinsic to the combination of the 2 modalities.

Initial Trials of Radiotherapy and Immunotherapy

Investigators at New York University (NYU) originally hypothesized that ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated, reporting out of the field responses in a murine model of syngeneic mammary carcinoma treated with FLT-3L and radiation.[5]

The same group conducted the first “proof-of-principle” trial, exploring the combination of subcutaneous (s.c.) administration of granulocyte-macrophage colony-stimulating factor (GM-CSF) with chemoradiotherapy to 1 metastasis in patients with metastatic solid tumors and at least 3 measurable metastatic lesions.[4] The trial was based on the hypothesis that, even in a setting of established and pretreated metastatic disease, the use of GM-CSF and radiation could stimulate the patient’s immune system to overcome immune tolerance. GM-CSF increases the percentage of dendritic cells (DCs) and their maturation, facilitating cross-presentation of newly released Ags after cell death at the site of radiotherapy. In NYU 01-XX, enrollment was offered to pretreated patients with metastatic solid tumors after stable disease or progression on systemic chemotherapy; the same systemic therapy was maintained and radiotherapy was added to 1 lesion, 3.5 Gy × 10 daily fractions, for a total dose of 35 Gy for 2 weeks. Starting on day 7 (after 1 week of radiation), GM-CSF, 125 mg/m^2, was given s.c., and repeated daily for 14 days. An abscopal response was defined as a measurable response in any of the measurable lesions outside the radiation field. Assessment was performed by positron emission tomography-computed tomography (PET/CT). The results of this trial were reported at ASTRO in 2012, and a manuscript describing the long-term outcome of the treated patients is in preparation. Interestingly, patients displaying an abscopal response were also more likely to have longer survival, suggesting a better immune competence among responders. A clear weakness of this study is the lack of immune monitoring of these patients.

A different approach was aimed at optimizing available DCs to uptake and present tumor-derived Ags released by radiation in patients with hepatoma. DCs were injected intratumorally to patients with hepatoma 2 and 24 days after a single dose of 8 Gy of

external-beam radiation therapy (XRT) to the tumor. Of 14 patients, 12 showed partial responses, and most patients had increases in α-fetoprotein–specific immune responses by enzyme-linked immunospot assay.[7] These data confirm the clinical effect of radiotherapy on the host's immune system and warrant accurate monitoring of immune biomarkers during combination treatment.

NYU Trials of Immunotherapy and Radiation

Currently, 4 institutional review board (IRB)–approved, investigator-initiated trials combining radiotherapy and immunotherapy are ongoing at NYU (Table 1).

NYU S11-00533 "Fresolimumab and Radiotherapy in Metastatic Breast Cancer"

This clinical trial is a component of Multi-Team Award BC100481 of the breast cancer program of the Department of Defense, which was conducted in collaboration with University of California, Los Angeles. The trial tests the effect of combining transforming growth factor β (TGF-β) blockade with radiation to a metastatic site of breast cancer. It directly translates the preclinical work on this combination. The 4T1 mammary carcinomas in the flank of syngeneic Balb/c mice were used to test the potential for combinations of TGF-β inhibition and RT. TGF-β–neutralizing monoclonal antibody (mAb) 1D11.16 given 24 hours before RT augmented the response of established tumors to RT. Importantly, lung metastases were significantly reduced compared with each treatment alone ($P < 0.01$, 1-sided *t* test), which indicates that TGF-β inhibition in combination with RT promotes systemic antitumor effects. Patients are randomly assigned to either 1 or 10 mg/kg dose of fresolimumab, a neutralizing antibody against all 3 TGF-β isoforms. A baseline and serial PET/CT scans are required to monitor the response. Immunomonitoring with tetramer and enzyme-linked immunospot assay and Treg measurements are important components of this study.

NYUS11-0598 "Phase I/II Study of TLR7 Agonist Imiquimod, Cyclophosphamide, and Radiotherapy in Breast Cancer Patients With Chest Wall Recurrence or Skin Metastases"

Funded by NIH R01CA161891-01, this trial tests the combination of radiation and a toll-like receptor (TLR) agonist with and without low-dose cyclophosphamide (CTX) in women with metastatic breast cancer and skin metastases. The rationale for this study is derived by the fact that radiotherapy has been shown to induce an immunogenic cell death and promote cross-presentation of tumor Ags, that is, at least partially, dependent on TLR4 imiquimod (IMQ), a synthetic TLR7 agonist, that activates DCs and primes Th1 and cytotoxic T lymphocyte (CTL) responses to coadministered Ags, including tumor Ags. It has also been shown to be a potent adjuvant to local treatments, such as cryosurgery and intralesional IL-2. Complete tumor regressions have been observed in patients with metastatic breast cancer as well as primary skin tumors after topical IMQ treatment without additional therapies, including a 20% response rate in a prospective trial.[8]

The trial translates the findings in the tumor-specific Ag mammary tumor model that the addition of immunomodulatory CTX to IMQ and radiation improved antitumor response,

especially the development of long-term protective immunity, which was evident in successful tumor rejection after rechallenge of the animals.[9]

In addition to classical immune monitoring, serial biopsies of the treated lesions are part of the trial to study interferon (IFN)-alfa and IFN-gamma signaling, infiltrating CXCR3+ cytotoxic lymphocytes, natural killer cell activity and regulatory T-cell (Treg) numbers.

NYU S12-02746 "A Phase II Randomized Trial of Ipilimumab versus Ipilimumab With Radiation Therapy in Metastatic Melanoma"

This study is based on extensive preclinical work conducted at NYU to test the combination of CTLA-4 blockade and radiotherapy.[9-11] The design of the trial was inspired by a case report by Postow et al[12] of an abscopal response in a patient with NY-ESO-1 (cancer-testis Ag)–positive melanoma treated with local radiotherapy in combination with ipilimumab, a mAb against CTLA-4. Because ipilimumab has established activity in metastatic melanoma, this trial will randomly assign patients to ipilimumab during radiation to 1 metastatic site vs ipilimumab alone. The rationale is to define the additional contribution of radiotherapy during the ipilimumab therapy of metastatic melanoma. Immune monitoring includes serial biopsies of irradiated lesions.

NYU S14-00208 "Combining Radiotherapy and Anti–CTLA-4 Immunotherapy in Metastatic Lung Cancer"

Inspired by the preclinical work in metastatic mammary carcinoma models[9-11] and a recently reported case of an abscopal response to ipilimumab and radiation in patients with non–small cell lung cancer (NSCLC) and refractory metastatic disease (currently disease free, 22 months from the treatment),[13] this trial prospectively enrolls patients with NSCLC to ipilimumab and radiation to 1 metastatic site. The trial was enabled by a gift of the drug ipilimumab from Bristol-Myers Squibb. Currently, 5 patients have enrolled to the study, and they have shown excellent tolerability of the combination.

Immunomonitoring and serial biopsies of irradiated lesions are included in the study design.

Earle A. Chiles Research Institute, Providence Cancer Center (Portland Medical Center, Oregon), Trials of Immunotherapy and Radiation

Currently, 5 IRB-approved, investigator-initiated trials combining radiotherapy and immunotherapy are ongoing at Chiles Research Institute (Table 2).

PH&S IRB 11-062A "SBRT and High-dose IL-2 in Metastatic Melanoma"

This is a phase II clinical trial following up on promising data from our phase I study on metastatic melanoma and renal cell carcinoma demonstrating an increased response rate with the combination of stereotactic body radiation therapy (SBRT) and high-dose IL-2 when compared with historical controls.[1] This study is supported in part by Prometheus Pharmaceuticals, Providence Portland Medical Foundation, and NIH R21CA176705-01. This trial is a randomized study that compares treatment with high-dose IL-2 alone with SBRT plus high-dose IL-2 in patients with metastatic melanoma. SBRT at a dose of 20 Gy

or 20 Gy × 2 is delivered to 1-3 metastatic lesions on a Friday or a Wednesday and Friday schedule, respectively. This is followed by the administration of high-dose IL-2 at 600,000 IU/kg every 8 hours × 14 as planned. IL-2 administration is repeated up to 6 times with imaging obtained after 2 cycles of IL-2. Only responding patients receive additional IL-2. For patients randomized to IL-2 alone, if they have progressive disease at the first scan, they can cross over to receive additional SBRT before the third cycle of IL-2. Immune monitoring on peripheral blood, serum, and biopsies is being performed.

PH&S IRB 10-088 "Anti-OX40, Cyclophosphamide (CTX) and Radiation in Patients With Progressive Metastatic Prostate Cancer"

This is a phase Ib clinical trial supported with funds from a prostate cancer foundation creativity award. The trial tests the effect of CTX in combination with focal radiation and anti–OX40 agonist antibody in patients with progressive metastatic prostate cancer. The rationale was based on preclinical studies that demonstrated synergy of OX40 agonist antibody in combination with CTX[2] as well as the synergy of OX40 agonist antibody with radiation.[3] The rationale is that both CTX and radiation will result in tumor breakdown and Ag release, resulting in an "autovaccine," and that OX40 agonist will augment this autovaccine by enhancing effector T-cell function and number. CTX is administered on day 1. All the patients receive radiation at a dose of 8 Gy in a single fraction to up to 3 bone metastatic sites on day 4 in the morning. Anti-OX40 at 0.4 mg/kg is given intravenously (i.v.) on days 4, 6, and 8. The CTX dose is escalated in successive cohorts of 3-6 patients to ensure safety. The doses of CTX are 300, 600, and 900 mg/m^2 i.v. An additional 20 patients can enroll in the study at the maximum total dose of CTX. The primary objective of this study is to identify the safety of the combination therapy as well as to perform immune monitoring studies on peripheral blood and serum.

PHS&S IRB 12-017A "Stereotactic Body Radiation and Monoclonal Antibody to OX40 in Breast Cancer Patients With Metastatic Lesions (OX40 Breast)"

This is a phase Ib clinical trial supported with funds from the Safeway foundation and Providence Portland Medical Foundation. The study tests the combination of SBRT with the investigational antibody anti-OX40. The combination was based on preclinical studies demonstrating enhanced antitumor responses when radiation and OX40 were administered in combination and that the combination resulted in increased numbers of recently activated CD8+CD25+ T cells.[3] Patients with metastatic breast cancer that have progressed on chemotherapy and have metastatic lesions in the lung and liver are eligible. This is a dose-escalation safety study with escalating doses of radiation. Patients receive 15 Gy, 20 Gy, or 20 Gy × 2 to up to 3 metastatic lesions in the lung or liver on day 1 or days 1 and 3 for patients receiving 2 fractions. Patients are also treated with anti-OX40 at 0.4 mg/kg i.v. on days 1, 3, and 5. The primary objective is to define the maximum tolerated dose (MTD) of radiation within the specified range. Secondary objectives include estimating the response rate of combined modality treatment in both irradiated and nonirradiated lesions as well as to perform exploratory studies of circulating CD4 and CD8 T cells and study serum markers of tumor lysis and immunogenicity.

PH&S IRB 10-141B "An Exploratory Phase I Trial of Immunochemoradiotherapy in Locally Advanced and Borderline Resectable (LA/BR) Pancreatic Adenocarcinoma"

This is an exploratory trial to determine feasibility and safety of chemoradiotherapy with combinatorial immunotherapy in locally advanced and borderline resectable (LA/BR) pancreatic cancer. This study includes a vaccine, GV1001, which is a peptide-based vaccine that targets human telomerase and is administered with GM-CSF.[4] This is in combination with a phosphodiesterase 5 inhibitor, tadalafil. Phosphodiesterase 5 inhibition has been shown in preclinical models to reduce myeloid-derived suppressor cell function.[5] In this study, patients receive initial gemcitabine chemotherapy in combination with the vaccine followed by concurrent chemoradiotherapy with the standard fractionated radiation (50.4 Gy in 28 fractions) in combination with low-dose gemcitabine administered i.v. twice weekly. Patients are evaluated for respectability, and surgery is performed if feasible. Patients with stable or responsive disease or resected disease then receive an additional 2 cycles of vaccine and gemcitabine chemotherapy. Tadalafil is administered throughout the study. The primary end points are to determine the feasibility and safety of the study as well as to monitor Ag-specific antitumor immune responses. In addition, the immunohistology of the resected tumor specimens are analyzed and pathologic response rates are determined.

PH&S IRB 13-026A "Chemoimmunotherapy and Radiation in Pancreatic Cancer"

This is a phase I study designed to evaluate the safety of chemotherapy and hypofractionated XRT in combination with a myeloid-targeting agent tadalafil in patients with LA/BR pancreatic cancer. It is supported in part by a grant, NIH R01 CA182311-01A1. This trial is based on the observation that high-dose per fraction radiation is associated with a robust antitumor immune response[6] but also can be associated with a strong suppressive myeloid response.[7,8] The immune modulator tadalafil has been shown to reduce myeloid-derived suppressor cell function[5] and can target the suppressive myeloid response associated with hypofractionated radiation. Patients with LA/BR pancreatic cancer are eligible. The patients will be treated with tadalafil for the duration of the study. Patients will be initially treated with a 21-day cycle of gemcitabine followed by hypofractionated radiation of 30 Gy in 3 fractions delivered on alternative days to the primary tumor and grossly involved nodes. Patients are subsequently evaluated for response and resectability by imaging. Patients with resected or stable and responding disease or both receive 3 additional cycles of gemcitabine. Primary end points of this study are feasibility and safety. Secondary end points include immune monitoring on blood and serum samples as well as immunohistology of resected tumor specimens and pathologic response rates.

Stanford Trials of Immunotherapy and Radiation

In 2007, Stanford reported that in a murine model of a widely metastatic B-cell lymphoma, the combination of chemotherapy plus intratumoral injection of oligodeoxynucleotides containing unmethylated C-G motifs (CpG), a TLR9 agonist, could completely eradicate the inoculated tumor. This therapeutic effect required that the CpG be injected directly into the tumor and was dependent on CD8 T cells. Although the efficacy of CpG oligodeoxynucleotides has been thought to depend on the expression of TLR9, it was unexpectedly found that tumor rejection did not require host expression of TLR9. Using a

TLR9-deficient tumor and a TLR9KO host, they demonstrated that TLR9 expression by either the host or the tumor was required. These results indicated that activation of Ag presentation by cells within the tumor via TLR9 stimulation can be an effective form of immunotherapy.[22] When local radiotherapy was used instead of the systemic chemotherapy, the synergistic effect with local injection of TLR9 agonist was once again observed. These preclinical observations were translated in 2 phase 1/2 clinical trials reported in 2010 and 2012 by the Stanford group.

In total, 15 patients with low-grade B-cell lymphoma were treated by low-dose radiotherapy (4 Gy over 2 doses and 2 days) to a single tumor site and—at that same site—we injected 6 mg of the C-G enriched (also referred to as CpG), TLR9 agonist PF-3512676, synthetic oligodeoxynucleotide before the first dose of radiation, after the second dose, and then weekly for 8 weeks. This in situ vaccination maneuver was well tolerated with only grades 1 to 2 local or systemic reactions and no treatment-limiting adverse events were observed. One patient had a complete clinical response, 3 had partial responses, and 2 had stable but continually regressing disease for periods significantly longer than that achieved with prior therapies. Vaccination induced tumor-reactive memory CD8 T cells. Some patients' tumors were able to induce a suppressive, regulatory phenotype in autologous T cells in vitro, and these patients tended to have a shorter time to disease progression. One clinically responding patient received a second course of vaccination after relapse, resulting in a second, more rapid clinical response.[23]

Based on these findings, this trial of in situ vaccination was extended to newly diagnosed patients before any other therapy, a group that may have a more intact immune system. Patients were eligible if they had follicular lymphoma grades I-IIIa, stage III or IV, and were not in need of immediate treatment. One involved lymph node was biopsied, and viable suspensions of tumor cells were prepared and cryopreserved for use as stimulators in immune assays. A second site received low-dose XRT (2 Gy on each of 2 successive days) together with 10 weekly injections of 18 mg (3-fold higher than in our prior trial) of CpG oligonucleotide (PF-3512676) all into the same tumor site, beginning on the second day of XRT. Peripheral blood lymphocytes were obtained before each injection and 2 weeks after the last injection. These were cryopreserved and used as responder cells for assays of T-cell immune responses. The kinetics of antitumor T-cell immune responses as well as the type of T-cell response that was the most informative were investigated: tumor cells were thawed and activated for 3 days with CpG and soluble CD40L. They were then incubated for 5 days with autologous T cells. Fresh stimulator cells were then added for a final overnight culture. In response to in situ vaccination, all patients made antitumor immune responses. Some generated only CD4 responses, some only CD8 responses, and others made both CD4 and CD8 T-cell responses. For CD4 responses, the activation marker CD278 (ICOS) was particularly informative and usually restricted to the CD45RO+ memory subset. For CD8 responses, the most robust readout was intracellular expression of the combination of perforin and granzyme B. The CD8 immune responses became positive as early as 2 weeks after the start of vaccination. The CD4 responses became positive by 4 weeks of vaccination.[24] Clinical responses occurred, with regression of tumors at uninjected or untreated sites of disease, and an evaluation of the magnitude and duration of these clinical responses and their relation to immune parameters was performed.

This approach is under current investigation in 2 clinical trials testing an alternative formulation of CpG and a different patient population. As regards to low-grade non-Hodgkin's lymphoma (NHL), a trial was initiated using an alternative formulation of CpG from Dynavax in "A phase 1/2, non-randomized, open-label, multicenter, dose escalation and expansion study of intratumoral injections of SD-101 in combination with localized low-dose radiation in patients with untreated low-grade B-cell lymphoma." The treatment will be similar to the prior trial consisting of local radiation given for 2 days (day 1 and day 1; 2 Gy each day) followed by 5 weekly intratumoral injections of SD-101 starting on day 1 with doses ranging from 1-8 mg. Primary end points include assessing the following: (1) safety and tolerability of escalating doses of SD-101 in combination with localized low-dose XRT in participants with untreated low-grade B-cell lymphoma, (2) pharmacodynamic profile of IFN-inducible genes in whole blood 24 hours after intratumoral injection of SD-101, and (3) MTD or optimal dose of intratumoral SD-101 in combination with localized low-dose XRT. Secondary objectives include assessing the following: (1) preliminary response both locally and systemically, (2) tumor shrinkage of the target lesion (local), and (3) tumor shrinkage outside the target lesion (systemic). Exploratory objective is to estimate the duration of tumor response both locally and systemically.

In the second ongoing trial, a more challenging patient population has been selected to test, "Intratumoral injection of an immunostimulatory CpG, SD101, combined with local radiation for the treatment of recurrent or progressive lymphoma after allogeneic hematopoietic cell transplantation." In this trial, patients receive low-dose radiation to all bulky or symptomatic lymph nodes on days 2 and 1. SD101 is administered intratumorally to the single largest palpable node within 24 hours after completion of radiation on day 0. Two additional intratumoral SD101 injections are given on days 7 and 14. The tested doses of SD-101 include 0.3, 1, and 3 mg per injection. The primary end point is determining the MTD based on dose-limiting toxicity. Secondary end points include measuring the following: (1) cytotoxic Tcell activity changes pretreatment and posttreatment of tumor-infiltrating lymphocytes and peripheral blood lymphocytes using enzyme-linked immunosorbent assay and immunohistochemistry, (2) tumor response by PET/CT scan imaging, and (3) level of donor-specific tumor-infiltrating lymphocytes using flow cytometry and immunofluorescence.

Given the observed efficacy of this approach in low-grade NHL, clinical efficacy is being explored among patients with mycosis fungoides (MF), the most common subtype of cutaneous T-cell lymphoma, which forms pleomorphic skin lesions including patches, plaques, tumor lesions, and erythroderma. The skin-tropic malignant cells are CD3+/CD4+/CLA+/CCR4+ with loss of CD7 and CD26. In total, 15 patients were treated with the identical dose and schedule of both CpG and radiation as in the NHL trial. Clinical responses were assessed at the distant, untreated sites as a measure of systemic antitumor activity. Five clinically meaningful responses were observed. The procedure was well tolerated and adverse effects consisted mostly of mild and transient injection site or flulike symptoms. The immunized sites showed a significant reduction of CD25+, Foxp3+ T cells that could be either MF cells or tissue-regulatory T cells and a similar reduction in S100+, CD1a+ DCs. There was a trend toward greater reduction of CD25+ T cells and skin DCs in clinical responders vs nonresponders. The trial established the feasibility of an in situ

vaccination strategy in MF and the clinical responses that occurred in a subset of patients warranted further study with modifications to augment these therapeutic effects.[25]

To optimize local CpG and XRT, we returned to the mouse model of A20 lymphoma and inoculated A20 lymphoma tumor cells at 2 sites s.c. (right and left abdomen). Only 1 site was injected with CpG allowing us to evaluate the systemic antitumor response at the distant site. CpG was administered intratumorally. T-cell modulation was accomplished using systemic (intraperitoneal) administration of mAbs against T-cell targets, including regulatory T cells, which were depleted using anti–folate receptor 4 (FR4) antibody or functionally blocked using anti-GITR antibody, and effector T cells, which were stimulated using anti-OX40 antibody (to trigger their costimulatory molecule) or anti–CTLA-4 antibody (to block inhibitory signals). Treatment with intratumoral injection of CpG alone did not cure any mice. Treatment with CpG and a single antibody (anti-OX40, anti–CTLA-4, anti-FR4, or anti-GITR) cured 20%-30% of mice. Interestingly, some combinations of antibodies (anti-OX40 + anti–CTLA-4, anti-OX40 + anti-FR4, and anti–CTLA-4 + anti-GITR) potentiated T-cell modulation and further enhanced the efficacy of CpG vaccination. In particular, the combination of anti-OX40 and anti–CTLA-4 appeared to be especially potent when combined with intratumoral CpG. Indeed, this combination (CpG + anti-OX40 + anti–CTLA-4) induced antitumor T cells capable of secreting IFN gamma in response to overnight culture with A20 tumor cells. It cured more than 80% of mice bearing large and systemic lymphoma tumors without the need for chemotherapy (effective therapy required both CD4 and CD8 T cells). Importantly, this therapy produced high numbers of anti-tumor memory T cells and provided long-lasting immunity against tumor rechallenge.[26]

As these results show that antibody-mediated T-cell modulation greatly enhances the therapeutic efficacy of intratumoral vaccination with CpG, we next explored whether T-cell modulation should be delivered systemically or intratumorally. The data generated by the Stanford group, both preclinical and clinical, suggested that activation of TLR9 by direct injection of unmethylated CpG nucleotides into a tumor can induce a therapeutic immune response; however, regulatory T cells eventually inhibit the antitumor immune response and thereby limit the power of cancer immunotherapies. In tumor-bearing mice, we found that regulatory T cells within the tumor preferentially express the cell surface markers CTLA-4 and OX40. We showed that intratumoral coinjection of anti–CTLA-4 and anti-OX40 together with CpG depleted tumor-infiltrating regulatory T cells. This in situ immunomodulation, which was performed with low doses of antibodies in a single tumor, generated a systemic antitumor immune response that eradicated disseminated disease in mice. Further, this treatment modality was effective against established central nervous system lymphoma with leptomeningeal metastases, sites that are usually considered to be tumor cell sanctuaries in the context of conventional systemic therapy.[27] These results demonstrated that antitumor immune effectors elicited by local immunomodulation can eradicate tumor cells at distant sites.

Based on the prior clinical trials and preclinical testing, the Stanford group proposed that, rather than using immunomodulation to target cancer cells systemically, treatment could be used to target the tumor-infiltrative immune cells locally, thereby eliciting a systemic immune response and reducing systemic toxicity. A clinical trial is ongoing testing this

general hypothesis, “A phase 1/2 clinical trial of ipilimumab and local radiation therapy in treating patients with recurrent melanoma, nonHodgkin’s lymphoma, colon, or rectal cancer.” Treatment will include 25 mg of ipilimumab intratumorally on day 1 and local XRT initiated within 48 hours for at least 3 fractions of approximately 2-10 Gy each depending on histology. The primary end point is assessing the safety of combining intratumoral anti–CTLA-4 immunotherapy with local XRT with a monotherapy ipilimumab safety lead in. Secondary end points include assessing the following: (1) induction of an antitumor immune response, (2) tumor response rates and duration of response at unirradiated tumor sites, and (3) putative immunologic biomarkers of tumor response.

Finally, building on the observations that tumor irradiation induces innate and adaptive immune responses which, rarely, lead to tumor regression at distant sites, the abscopal effect, in 2014 the Stanford group first report of both a preclinical murine model and patient case series following local radiation and systemic anti–PD-L1 (NCT01375842). In a 2-tumor, syngenic, A20, lymphoma BALB/c model, fractionated single tumor radiation and systemic (intraperitoneal) anti–PD-L1 were tested. Fractionated radiation delayed tumor growth at the treated site only, and systemic anti–PD-L1 reduced tumor growth rate at both sites. However, despite prolonged survival all mice died by day 38 following either monotherapy (radiation or anti–PD-L1). By contrast, combination local fractionated radiation and systemic anti–PD-L1 flattened tumor growth at both the irradiated and unirradiated site, and prolonged survival with 50% survival at day 48 posttumor inoculation. Modulation of PD-L1 expression postradiation and tumor-specific augmentation of IFN-gamma secretion correlated with the enhanced antitumor activity. This work was translated clinically with patients receiving MPDL3280A, a human mAb containing an engineered Fc-domain, as part of the phase 1 clinical trial with mixed responses or asymptomatic progression of disease eligible for the addition of local XRT. Five patients including 4 with solid tumors received fractionated, nondefinitive dose radiation with at least stabilization of systemic progression in all patients and a Response Evaluation Criteria in Solid Tumors partial response at systemic sites in 1 patient, notably with a synovial sarcoma. Transient, grade 1-2 inflammatory adverse events (fevers and flulike symptoms) occurred with no dose-limiting toxicities or serious immunerelated toxicities. Human immune responses including cell phenotype and function were investigated, specifically assessing expression of PD-L1 and production of IFN gamma by standard flow cytometry and time-of-flight mass cytometry, identical to the techniques performed in the murine models. Modulation of PD-L1 expression, T-cell phenotype, and IFN-gamma secretion was observed in all patients.[28] The magnitude of the immune response and abscopal response rate in mice and humans provides proof of concept that anti–PD-L1 may be a more potent combination immunotherapy with radiation compared with the experience with CpG or anti–CTLA-4 or both (Table 3).

NCI Radiation and Immunotherapy Trials

NCT01496131/NIH 11-C-0247 "Deprivation Therapy and Radiation Therapy for Untreated, Intermediate and High-risk Prostate Cancer Patients"

NCT01496131 is a phase II trial designed to determine the effect of L-BLP25/tecemotide, a vaccine that is designed to elicit immune responses to MUC-1 on tumor cells, in addition to standard treatment on the MUC1-specific systemic immune response in patients with newly diagnosed high- or intermediate-risk prostate cancer L-BLP25 vaccine in combination with androgen-deprivation therapy and XRT. Prior studies with this immunotherapy for stage III NSCLC (Butts et al Lancet Oncol, 2014) showed an apparent improvement in efficacy when given concurrently with chemotherapy and XRT, rather than sequentially. NCT01496131 is following immune end points to determine whether the vaccine, given concurrently with radiation and androgen-deprivation therapy, produces significant increases in immune responses to the tumor-associated Ag, MUC-1.

(NCT and NIH Numbers Pending) "A Pilot Study of AMP-224—a PD-1 Inhibitor—In Combination With Stereotactic Body Radiation Therapy (SBRT) in Patients With Metastatic Colorectal Cancer"

This is a pilot study whereby all patients will receive SBRT to 1 liver lesion and concomitant AMP-224. A single treatment of low-dose CTX will be administered in conjunction with the SBRT therapy before the first AMP-224 treatment. Hypofractionated radiation will be administered to a metastatic disease site at a dose and schedule of 8 Gy × 1 or 8 Gy × 3. Secondary end points will include characterization of the pharmacokinetic parameters of AMP-224 in combination with SBRT in patients with metastatic colorectal cancer, and evaluation of the response rate as measured by progression-free survival and overall survival in patients with colorectal cancer during and following treatment with AMP-224 in combination with SBRT. Exploratory end points will include measurement of immune parameters in the peripheral blood and tumors in patients with metastatic colorectal cancer during and following treatment with AMP-224 in combination with SBRT. Patients must have at least 1 metastatic lesion in the liver that is amenable to SBRT and not amenable to potentially curative resection. Additionally, patients must have progressed on or been intolerant of at least 1 prior oxaliplatin- or irinotecan-containing chemotherapeutic regimen and have disease that is not amenable to potentially curative resection. This study is pending final IRB approval (Table 4).

Thomas Jefferson University Radiation and Immunotherapy Trials

Investigators at Thomas Jefferson University (TJU) are currently testing the combination of ipilimumab (anti–CTLA-4) with radiation in brain metastases from melanoma.

Based on the hypothesis that treatment with high doses of XRT would result in tumor cell death, releasing tumor debris and liberating potential tumor Ags, investigators at TJU designed a trial that combines XRT with ipilimumab. The idea is that ipilimumab will facilitate immune recognition of these novel tumor-specific Ags, yielding a synergistic effect. An additional advantage of combining immune therapy with radiation is that by focusing the immune system on tumor Ags, it may minimize aberrant immune activation of

normal tissues, consequently reducing the incidence of immunerelated adverse effects. Combination of radiation treatment with ipilimumab will likely result in better local control, decrease the risk of developing new brain metastases, and improved overall survival. As the safety profile and toxicities of combining ipilimumab with brain radiation treatment are unknown, the current phase I study will assess the safety profile of combining different doses of ipilimumab with standard dose radiation treatment either with whole-brain radiation therapy or stereotactic radiosurgery. The study will determine the MTD, as well as a recommended phase II trial dose of ipilimumab (Table 5).

Conclusions

The listed ongoing trials in 7 distinct tumor sites, at different stages of disease, represent a snapshot of some of the ongoing research testing in patients with cancer the feasibility and efficacy of combining radiotherapy and immunotherapy. These trials are initial explorations and represent the beginning of a new era of research. Importantly, they often test the combination in a metastatic setting, a stage where radiotherapy is traditionally reserved for palliation of symptoms. If these tests prove to be effective, they could open the way to a larger use of this modality, with a therapeutic instead of a palliative intent.

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Table 1

Clinical Trials of Immunotherapy and Radiation Currently Open at NYU

Institution/Study ID	Tumor Site/Stage (Planned Accrual)	Study Aims	RT Dose/fx	Key Inclusion Criteria
NYULMC S11-00533, phase I-II	Breast cancer/metastatic (28)	Assess the safety and feasibility of combining TGF-β–neutralizing antibody (GC1008, fresolimumab) and local radiotherapy in patients with metastatic breast cancer Determine whether treatment with fresolimumab and localized RT achieves an abscopal tumor regression Examine whether treatment is associated with immunologic changes in patients with metastatic breast cancer	7.5 Gy × 3	At least 2 distinct measurable metastatic sites, with 1 of at least 1 cm or larger in its largest diameter.
NYU S11-00598, phase I-II	Breast cancer/metastatic (42)	Assess the safety and feasibility of combining a topical toll-like receptor agonist (imiquimod) and local radiotherapy ± low-dose cyclophosphamide in patients with metastatic breast cancer Determine whether treatment with imiquimod and localized RT and ± low-dose cyclophosphamide achieves an abscopal tumor regression Examine whether treatment is associated with immunologic changes in patients with metastatic breast cancer	6 Gy × 5	At least 1 measurable skin metastasis and distant, measurable metastases (outside of skin), or At least 2 distinct measurable metastatic sites, with 1 of at least 1 cm or larger in its largest diameter.
NYU S12-02746, phase II randomized	Melanoma/metastatic (100)	Evaluate the safety and feasibility of anti–CTLA-4 mAb and concurrent local radiotherapy to a metastatic site Compare systemic response to ipilimumab in patients randomly assigned to radiation to a measurable lesion or not	6 Gy × 5	At least 2 distinct measurable metastatic sites, with 1 of at least 1 cm or larger in its largest diameter and may have additional nonmeasurable but established metastatic lesions (ie, bone metastases).
NYU S14-00208, phase I-II	NSCLC/metastatic (30)	Evaluate the safety and therapeutic efficacy of anti–CTLA-4 mAb and concurrent local radiotherapy to a metastatic site	6 Gy × 5	At least 2 distinct measurable metastatic sites. Patients may have additional nonmeasurable metastatic lesions (eg, bone metastases).

Table 2

Clinical Trials of Immunotherapy and Radiation Currently Open at Earle A. Chiles Research Institute (EACRI), Providence Cancer Center

Institution/Study ID/ clinical trials.gov Identifier	Tumor Site/Stage (Planned Accrual)	Study Aims	RT Dose/fx	Key Inclusion Criteria
PH&S IRB 11-062A NCT01416831/phase II randomized	Metastatic/melanoma (44)	Compare response rate of high-dose IL-2 to SBRT and IL-2. Measure the response of SBRT and IL-2 in crossover patients with melanoma who have disease progression after high-dose IL-2 alone. Evaluate markers of tumor lysis, inflammation, and immune activation in the blood of patients receiving combined treatment compared with patients receiving high-dose IL-2 alone	20 Gy × 1 and 20 Gy × 2	At least 2 distinct measurable metastatic sites, with at least 1 metastatic lesion amenable to SBRT in the lung mediastinum or liver.
PH&S IRB 10-088 NCT01303705/phase Ib	Metastatic/prostate cancer (37)	Determine the maximum tolerated dose of cyclophosphamide administered in combination with radiation and anti-OX40 in men with metastatic castration- and chemotherapy-resistant prostate cancer. Determine the effect of therapy on circulating numbers and phenotypes of CD4 and CD8 T cells. Measure the proliferation and activity of effector and memory T cells following therapy. Perform exploratory studies of cellular and humoral immune responses against prostate cancer cell lines. Estimate the response rate of the regimen that includes the highest dose of CTX determined to be safe.	8 Gy × 1	At least 1 bone metastatic lesion amenable to radiation and measurable or evaluable metastatic adenocarcinoma of the prostate. Patients must have confirmed progression after at least 1 androgen ablation and administration of docetaxel.
PH&S IRB 12-017A NCT01862900/phase I-II	Metastatic/breast cancer (40)	Determine the maximum tolerated dose and safety profile of radiation administered in combination with anti-OX40 Estimate the response rate of combined modality treatment in both irradiated and nonirradiated tumors Determine the influence of combined treatment on immune parameters.	Cohort 1: 15 Gy × 1 Cohort 2: 20 Gy × 1 Cohort 3: 20 Gy × 2	At least 1 site in the lung or liver that is amenable to SBRT. Evaluable disease that will not receive radiation.
PH&S IRB 10-141B	Locally advanced and borderline resectable pancreatic cancer (11)	Evaluate the safety of combination gemcitabine, tadalafil, telomerase vaccine and GM-CSF, and standard fractionated radiation.	1.8 Gy × 28	Locally advanced unresectable pancreatic cancer in the absence of distant metastatic disease or borderline resectable pancreatic adenocarcinoma.

Institution/Study ID/ clinical trials.gov Identifier	Tumor Site/Stage (Planned Accrual)	Study Aims	RT Dose/fx	Key Inclusion Criteria
NCT01342224/phase I		Determine the response rate of combined therapy. Determine the frequency of telomerase-specific T-cell responses and perform exploratory studies of immune response in the blood and resected tumors.		
PH&S IRB 13-026A	Locally advanced and borderline resectable pancreatic cancer (10)	Evaluate the safety of combination gemcitabine, tadalafil, and hypofractionated radiation	8-10 Gy × 3	Locally advanced unresectable pancreatic cancer in the absence of distant metastatic disease or borderline resectable pancreatic adenocarcinoma.
NCT01903083/phase I		Assess immune infiltrate in resected tumors. Determine the influence of combined therapy on immune parameters.		

Table 3

Clinical Trials of Immunotherapy and Radiation Currently Open at Stanford

Stanford, phase I-II	NHL	Evaluate the safety of intratumoral injection of an immunostimulatory CpG, SD101, combined with local radiation for the treatment of recurrent or progressive lymphoma after allogeneic hematopoietic cell transplantation	2 Gy × 2	At least 2 distinct measurable metastatic sites following allogeneic HCT
Stanford, phase I-II	Low-grade NHL	Evaluate the safety of dose escalation and expansion study of intratumoral injections of SD-101 in combination with localized low-dose radiation in patients with untreated low-grade B-cell lymphoma.	2 Gy × 2	At least 2 distinct measurable metastatic sites
Stanford, phase I-II	Melanoma, NHL, and CRC	Evaluate the safety of combining intratumoral anti–CTLA-4 immunotherapy with local radiation therapy with a monotherapy ipilimumab safety lead in	2-10 Gy × 2	At least 2 distinct measurable metastatic sites.

Abbreviations: CRC, colorectal cancer; HCT, hematopoietic cell transplantation.

Table 4

Clinical Trials of Immunotherapy and Radiation Currently Open at National Institutes of Health/National Cancer Institute

NIH/NCI 11-C-0247 NCT01496131 (phase II)	High- or intermediate-risk prostate cancer (48)	Evaluate the effect of the MUC1-specific vaccine (stimuvax/L-BLP25/ tecemotide) on systemic immune responses when given in combination with standard radiation and androgren-deprivation therapy.	Conventional dose and fractionation	Must have no evidence of metastatic disease, based on CT findings, and must have HLA-A2 or HLA-A3 for immune monitoring.
NIH/NCI # pending	Metastatic colorectal cancer (15)	Evaluate the safety of AMP-224—a PD-1 inhibitor—in combination with stereotactic body radiation therapy (SBRT) in patients with metastatic colorectal cancer.	8 Gy × 1 or 8 Gy × 3	Must have at least 1 site of disease in the liver that is amenable to SBRT.

Abbreviation: CT, computed tomography.

Table 5

Clinical Trial of Immunotherapy and Radiation Currently Open at Thomas Jefferson University

Institution/Study ID	Tumor Site/ Stage (Planned Accrual)	Study Aims	RT Dose/fx	Key Inclusion Criteria
TJU-NC T01703507	Metastatic melanoma to brain	Determine the maximum tolerated dose (MTD) of ipilimumab when combined with whole-brain radiation therapy (WBRT) or stereotactic radiosurgery (SRS)	SRS doses: 24, 21, 18, and 15 Gy.	Histologically confirmed patients with melanoma using imaging confirmed brain metastases. Age is 18 years or older. ECOG performance status 0 or 1.
		Secondary objectives:	Whole-brain radiation dose: 37.5 Gy	
		Determine local control rate of the brain metastases		
		Determine the rate of developing of new brain metastases		
		Determine the response of extracranial disease		
		Determine the overall survival rate and progression-free survival rate		

Abbreviation: ECOG, Eastern Cooperative Oncology Group.