Published in final edited form as:

Clin Cancer Res. 2010 June 1; 16(11): 3067–3077. doi:10.1158/1078-0432.CCR-10-0054.

Two-Stage Phase I Dose-Escalation Study of Intratumoral Reovirus Type 3 Dearing and Palliative Radiotherapy in Patients with Advanced Cancers

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Abstract

Purpose—To determine the safety and feasibility of combining intratumoral reovirus and radiotherapy in patients with advanced cancer and to assess viral biodistribution, reoviral replication in tumors, and antiviral immune responses.

Experimental Design—Patients with measurable disease amenable to palliative radiotherapy were enrolled. In the first stage, patients received radiotherapy (20 Gy in five fractions) plus two intratumoral injections of RT3D at doses between 1×10^8 and 1×10^{10} TCID₅₀. In the second stage, the radiotherapy dose was increased (36 Gy in 12 fractions) and patients received two, four, or six doses of RT3D at 1×10^{10} TCID₅₀. End points were safety, viral replication, immunogenicity, and antitumoral activity.

Results—Twenty-three patients with various solid tumors were treated. Dose-limiting toxicity was not seen. The most common toxicities were grade 2 (or lower) pyrexia, influenza-like symptoms, vomiting, asymptomatic lymphopenia, and neutropenia. There was no exacerbation of the acute radiation reaction. Reverse transcription-PCR (RT-PCR) studies of blood, urine, stool, and sputum were negative for viral shedding. In the low-dose (20 Gy in five fractions) radiation group, two of seven evaluable patients had a partial response and five had stable disease. In the high-dose (36 Gy in 12 fractions) radiation group, five of seven evaluable patients had partial response and two stable disease.

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

Disclosure of Potential Conflicts of Interest

K.J. Harrington, R.G. Vile, H.S. Pandha, and A.A. Melcher: commercial research grant; Matt Coffey, employee.

Conclusions—The combination of intratumoral RT3D and radiotherapy was well tolerated. The favorable toxicity profile and lack of vector shedding means that this combination should be evaluated in newly diagnosed patients receiving radiotherapy with curative intent.

Ionizing radiation, with either curative or palliative intent, plays an important role in the treatment of a range of tumor types. In an attempt to increase therapeutic benefit, much preclinical and clinical work has focused on combining molecular oncology therapeutics with radiation. Radiation sensitization, in which cytotoxic enhancers cooperate with radiation within the radiation field, aims at producing a greater effect on the local tumor than would be expected from simple additive cell killing (reviewed in refs. 1, 2).

Reovirus type 3 Dearing (RT3D, Reolysin; Oncolytics Biotech, Inc.) is a naturally occurring nonpathogenic, double-stranded RNA virus isolated from the respiratory and gastrointestinal tracts of humans (3). Most healthy adults possess antireoviral antibodies, suggesting a high incidence of subclinical infection in early life (4). RT3D exerts selective toxicity against cells with an activated Ras pathway—either through *Ras* mutation or upregulated epidermal growth factor receptor (EGFR) signaling. It also has the ability to activate both innate and adaptive antitumor immune responses (5, 6).

Thus far, oncolytic viruses that have been administered through intratumoral injections in clinical trials have shown favorable toxicity and safety profiles, but limited efficacy (7, 8). Strategies that involve combining oncolytic virotherapy, such as RT3D, with external beam radiotherapy may allow us to exploit synergies between the two treatment modalities (9, 10). There are several potentially positive theoretical interactions between RT3D and radiotherapy. Tumor radiation resistance is, at least partly, mediated by the Ras signal transduction pathway (11). EGFR overexpression, activating *Ras* mutations, and phosphorylation of Akt and phosphoinositide-3-kinase are all associated with radioresistance *in vitro* and, in the case of EGFR and Akt, to the failure of radiotherapy in cancer patients (11–14). Inhibition of this pathway (15, 16) sensitizes cells to radiation-induced cytotoxicity. We have recently shown that combining RT3D and radiotherapy synergistically enhances cytotoxicity in a variety of tumor cell lines *in vitro* and in three different *in vivo* tumor models (17).

Therefore, building on previous clinical trial experience with RT3D (18, 19), we designed and conducted a phase I, open-label, dose-escalation study of intralesional RT3D combined with fractionated palliative radiotherapy to determine the safety and tolerability of the combination therapy and, thereby, define a recommended phase II dose.

Patients and Methods

Patients

Subjects with histologically or cytologically confirmed advanced or metastatic solid tumors whose disease was refractory to standard therapy but amenable to localized short-course palliative radiotherapy were enrolled. Acute toxic effects of prior chemotherapy, radiotherapy, or surgical procedures had to have resolved to Common Terminology Criteria for Adverse Events (CTCAE, version 3.0) grade 1, with any surgery (except localized biopsy) occurring ≥ 28 days before study enrollment. Hormone replacement was allowed for patients with prostate or breast cancer. Patients were ages ≥ 18 years; using adequate birth control; had a life expectancy of ≥ 3 months; had measurable or assessable disease; an Eastern Cooperative Oncology Group performance status of 0 to 2; adequate hepatic, renal, and bone marrow function [aspartate aminotransferase/alanine aminotransferase $\leq 2.5\times$ the institutional upper limit of normal; total bilirubin $\leq 1.5\times$ upper limit of normal; serum creatinine $\leq 1.5\times$ upper limit of normal; hemoglobin ≥ 9.0 mg/dL; absolute neutrophil count

1,500/ μ L; platelet count 100,000/ μ L]; and prothrombin time and activated partial thromboplastin time 1.5 $\times$ the institutional upper limit of normal. Ras or EGFR status of the primary or recurrent tumor was not an inclusion/exclusion criterion and was not assessed.

The main exclusion criteria were prior radiation to the treatment site; known brain metastases; pregnancy or breastfeeding; concurrent immunosuppressive therapy; any investigational therapy within the previous 4 weeks; known HIV, hepatitis B, or hepatitis C infections; clinically significant cardiac disease (New York Heart Association Class III or IV); and inability to give written informed consent. The trial protocol was approved by the independent ethics and genetically modified organism safety committees of each center and conducted in accordance with the Declaration of Helsinki.

Study design and dose escalation

This was a nonrandomized, open-label, dose-escalating, two-center phase I study of intratumoral administration of RT3D (Reolysin) in patients receiving fractionated radiotherapy. The study proceeded in two stages. In the first stage (phase Ia), patients received local tumor irradiation to a dose of 20 Gy in five consecutive daily fractions in combination with two intratumoral injections of RT3D in sequential log dose-escalating (from 1×10^8 TCID₅₀ to 1×10^{10} TCID₅₀ on days 2 and 4) cohorts of three patients. In the second stage (phase Ib), patients received local tumor irradiation to a dose of 36 Gy in 12 fractions over 16 days in combination with RT3D as two doses of 1×10^{10} TCID₅₀ (days 2 and 4 in week 1) for the first cohort, as four doses of 1×10^{10} TCID₅₀ (days 2 and 4 in week 1 and days 9 and 11 in week 2) for the second cohort, and as six doses of 1×10^{10} TCID₅₀ (days 2 and 4 in week 1, days 9 and 11 in week 2, and days 16 and 18 in week 3) for the third cohort (Supplementary Table S1). Subjects were initially enrolled in groups of three and individually assessed for safety and dose-limiting toxicity (DLT). Subjects were evaluable for dose escalation decisions if they had received at least one viral administration or had been withdrawn from the study as a result of a drug-related toxicity. Subjects withdrawn from the study without meeting these criteria were replaced. If one of three subjects in a cohort experienced a DLT, three more subjects were added to that dose group. If two or more subjects in a dose group experienced a DLT during the first cycle, the previous lower dose would be defined as the maximum tolerated dose and would be considered the recommended dose for study in the second phase of the trial (phase Ib). DLT was defined during the course of treatment as grade 3/4 radiation-induced skin or mucosal toxicity; absolute neutrophil count of $<0.5 \times 10^9$ /L lasting for >5 days, or absolute neutrophil count of $<0.5 \times 10^9$ /L with sepsis; platelet count of $<25 \times 10^9$ /L; grade 2 or greater neurotoxicity or cardiotoxicity; and any other drug-related nonhematological grade 3/4 toxicity, with the exceptions of flu-like symptoms, nausea, and vomiting if appropriate prophylactic or therapeutic measures had not been administered. Intrasubject dose escalations were not permitted.

The primary end point was to determine the feasibility and safety of intratumoral administration of RT3D to patients with advanced cancers during fractionated radiotherapy and to recommend a phase II combined dose schedule. Secondary end points were assessment of the antitumor activity of the combination of RT3D and radiotherapy, evaluation of intratumoral viral replication, and measurement of antireoviral immune responses.

Treatment

Virus administration—Reolysin was supplied by Oncolytics Biotech, Inc. in single-use 1-mL glass vials containing a frozen viral suspension in PBS. Stock was stored at -70°C , thawed rapidly, and the appropriate TCID₅₀ dose was diluted to 250 mL in 0.9% sodium

chloride. Tumors received injection volumes of between 2 and 10 mL per tumor. Tumors with a volume of 20 cm³ or less received the minimum injectate volume of 2 mL; tumors with volumes of >20 cm³ received injectate volumes of 10% of the tumor volume to a maximum of 10 mL. The injected volume was divided in to as many as four separate aliquots and injected along straight tracks throughout the tumor at equally spaced intervals to allow the maximum area of tumor to be injected with the virus. Patients were injected in a side room and the site was covered with an occlusive dressing. Patients were observed closely (including blood pressure, temperature, and heart rate monitoring) during and for 1 hour after RT3D administration. Thereafter, they attended the Radiotherapy Department to receive treatment and were then able to go home.

Radiation—Recruitment was limited to patients with superficial tumors that were accessible to superficial irradiation and virus injection. For the majority of patients, radiation was delivered with electrons or orthovoltage X-rays of sufficient energy to treat the deep extent of the tumor with a margin of 5 to 10 mm. In one case, a patient with a massive parotid tumor was treated with a direct 1.25 MV photon field delivered using a ⁶⁰Co source prescribed to 100% at 2-cm depth. The gross tumor volume was delineated in three dimensions by clinical and/or radiological examination. A circumferential margin of 1 cm was added to comprise the planning target volume. A lead cutout of appropriate thickness was used to encompass this planning target volume and to shield adjacent normal tissues where necessary.

Baseline, treatment, and follow-up studies

Safety was assessed by evaluating the type, frequency, and severity of adverse events; changes in physical examination, clinical laboratory tests (including hematology, clinical chemistry, coagulation studies, and urinalysis), and immunogenicity. Interval medical history and physical assessment were done on each day that virus was administered, weekly thereafter for four consecutive weeks, and at 8 and 12 weeks postradiotherapy.

Analysis of viral shedding by reverse transcription-PCR

Patients enrolled on to the study had biological samples (blood, urine, feces, and sputum) collected for the detection of viral titers at baseline and after treatment (day 8, 31, 66, and 95). Blood was collected into EDTA tubes, centrifuged at 1,200 rpm for 10 minutes at 4°C, and stored at -70°C. Urine, sputum, and fecal swab (after PBS elution) samples were also stored at -70°. Samples were analyzed using the OneStep RT-PCR Enzyme Mix kit (QIAGEN Ltd.). Sample processing and reverse transcription-PCR (RT-PCR) methods has been previously described (18).

Detection of neutralizing antireoviral antibodies

A modified neutralizing antibody assay was used to detect antibody titers by measuring the effect of patient serum samples on the ability of a reovirus to kill a monolayer of target mouse L929 cells (20, 21). The neutralizing antireoviral antibody (NARA) titer of serum samples was expressed as the last dilution causing <80% cell killing (21). NARA titers were measured at baseline, during treatment (days 8, 33, 61, and 95 for phase Ia and days 8, 29, 57, and 108 for phase Ib), and at the end of treatment.

Estimation of virus titer from tumor samples

Paired pretreatment and posttreatment tumor biopsy samples for RT3D detection were thawed and macerated in 500 µL of DMEM and centrifuged at 1,200 rpm for 5 minutes. Supernatant was taken, serially diluted (1:10), and placed on to L929 cells in quadruplicates in a 96-well plate. Titer was calculated using the Kärber statistical method for a standard

TCID₅₀ assay. In addition, photo-micrographs were taken of the plates at the time of calculating the viral titer.

Response evaluation

Response was measured clinically (for superficial lesions) and radiologically by Response Evaluation Criteria in Solid Tumors every 8 weeks until progressive disease or study withdraw. Only the injected and irradiated lesion was considered as the target lesion. All other tumors were considered to be nontarget lesions. Both target and non-target lesions were assessed.

Results

Patients

Between September 2005 and January 2008, 25 patients with a variety of tumor types were enrolled in the study. Two patients were never treated due to physical deterioration attributed to disease progression. Patient demographics, tumor diagnoses, and target/nontarget lesions are displayed in Table 1. Details of primary tumor diagnosis and prior treatments are shown in Table 2. In the phase Ia part, three patients were treated in cohort 1 (1×10^8 TCID₅₀ days 2 and 4), four patients in cohort 2 (1×10^9 TCID₅₀ days 2 and 4), and four patients in cohort 3 (1×10^{10} TCID₅₀ days 2 and 4). In the phase Ib, three patients were treated in cohort 4 (1×10^{10} TCID₅₀ days 2 and 4), five in cohort 5 (1×10^{10} TCID₅₀ days 2, 4, 9, and 11), and four in cohort 6 (1×10^{10} TCID₅₀ days 2, 4, 9, 11, 16, and 18; Supplementary Table S1). Of the 23 evaluable patients, 18 patients (3 from each cohort) completed the entire course of treatment. Four patients did not complete treatment: three (one each from cohorts 2, 5, and 6) due to patient refusal and one patient from cohort 5 due to disease progression. One patient from cohort 2 was mistakenly treated as for cohort 1 (with 1×10^8 TCID₅₀) and was replaced.

Safety and toxicity

Treatment was well tolerated in all cohorts. All adverse events possibly or probably attributable to the study treatment are listed in Table 3. The most common toxicities observed were grade 2 (or lower) pyrexia, influenza-like symptoms, vomiting, asymptomatic lymphopenia, and neutropenia. Influenza-like symptoms including fever, fatigue, nausea/vomiting, and chills were fully resolved with paracetamol and/or nonsteroidal anti-inflammatory therapy. Severe grade >2 toxicities were asymptomatic lymphopenia in one patient (4.3%) and infection without neutropenia in two patients (8.7%). With the exception of pyrexia, which occurred in all four patients at the highest dose, there was no apparent relationship between the RT3D or radiotherapy dose level and the incidence and grade of these symptoms.

No evidence of exacerbation of radiation-induced skin toxicity was observed in this combination of RT3D and radiation. Only one patient with head and neck cancer presented with grade 3 ulceration of her treated lesion, but this was judged to be attributable to direct fungation of the tumor through the skin. Three patients presented with mild to moderate pain or erythematous reactions at the injection site. Six serious adverse events were reported and found to be unrelated to the study combination. Four patients died from complications due to tumor progression. No DLT was observed, and therefore, the maximum tolerated dose was not reached. The recommended dose schedules for phase II evaluation are 20 Gy in five fractions with two intratumoral injections of RT3D on days 2 and 4 for patients suitable for short course palliative radiotherapy and 36 Gy in 12 fractions combined with six intratumoral injections of RT3D on days 2, 4, 9, 11, 16, and 18 for patients suitable for a more prolonged palliative radiotherapy regimen.

Viral biodistribution

All pretreatment and posttreatment blood, urine, saliva, and rectal swab samples were negative for RT3D detection using RT-PCR screening based on 25 cycles of amplification (data not shown).

NARA response

Pretreatment and peak end point NARA titers are summarized for the whole study population in Fig. 1A. Data were not available for the fold increase in neutralizing antibodies titers for all of the patients. In patients for whom fold increase data were available, there was an increase in detectable NARA titers to a maximum at 4 weeks. Fold increases between pretreatment and the day 30 end point ranged from 243- to 2187-fold. Representative NARA curves are shown for the patients in cohort 1 (Fig. 1B) and were similar in cohorts 2 to 6 (data not shown).

Viral replication in tumor biopsies

Biopsy analysis of the target lesions (pretreatment and posttreatment) for the presence of viable virus in posttreatment biopsies was done in three patients. One of three supernatants from freeze-thawed and macerated tumor biopsies that were taken after the end of treatment caused cytopathic effect in L929 target cells (Fig. 1C). None of the lysates from the pretreatment samples caused cytopathic effect. The presence of reovirus in the posttreatment sample was confirmed by RT-PCR (Fig. 1D).

Response assessment

Fourteen patients were considered evaluable for treatment response. In the phase 1a low-dose (20 Gy in five fractions) radiation group, two of seven evaluable patients had a partial response (PR) and five had stable disease (SD). In the phase 1b high-dose (36 Gy in 12 fractions) radiation group, five of seven evaluable patients had a PR and two had SD. Detailed data on the assessment of tumor response in these 14 evaluable patients are presented on Table 4. The best response in nontarget lesions on days 29 to 33 was SD in 26 and PD in 9 lesions. Patient 01-0101 with metastatic esophageal adenocarcinoma who received intralesional reovirus and radiation to a supraclavicular lymph node achieved a PR that lasted 8 months until his death due to cerebral metastasis. In addition, he had a volume reduction of 15% in nonirradiated mediastinal disease for >7 months (Fig. 2A–D). Patient 01-0203 presented with a large tumoral mass affecting his parotid gland not amenable to surgery. Following radiation and intralesional RT3D, the tumor showed extensive necrosis and became operable (Supplementary Fig. S1). Patient 0602 with metastatic malignant melanoma, who received intralesional injections of reovirus alongside radiotherapy to submandibular mass, developed vitiligo within the irradiated area (Fig. 2E) and he was still alive 17 months posttreatment.

Discussion

We have previously shown that oncolytic reovirus synergistically enhances the effect of single-dose irradiation both *in vitro* and *in vivo* in a range of tumor cell lines (including melanoma, head and neck, and colorectal cancer; ref. 17). We have also shown that the virus itself is not inactivated by high doses of radiation and acts in a manner that is independent of treatment schedule (17). An earlier phase I study confirmed the safety of intratumoral injections of RT3D and provided guidance on the dose schedules used in this study (19). The principal aim of the current study was to determine the safety of combining RT3D with ionizing radiation in patients with advanced cancers representing histologic subtypes that we had previously assessed in pre-clinical studies. Eligible patients had tumors that could be

appropriately treated with palliative irradiation and that were accessible to direct intratumoral injection of RT3D. No DLT was seen during the study and the maximum tolerated dose of RT3D plus radiotherapy was not defined. Therefore, on the basis of these data, it was possible to recommend a dose of up to six injections of 1×10^{10} TCID₅₀ RT3D for future phase II evaluation with palliative radiotherapy. Although it might have been possible to increase the dose of RT3D beyond the 1×10^{10} TCID₅₀ dose level, we do not believe that this was warranted given the fact that our ultimate intention is to evaluate this agent in combination with full-dose curative radiotherapy or chemoradiotherapy.

However, rather than trying to establish a regimen for use as a means of enhancing palliative radiotherapy, one of the main aims of this research approach was to evaluate the safety of combining RT3D with radiotherapy as a prelude to clinical assessment of RT3D given during radical irradiation in patients with newly diagnosed, potentially radiocurable disease. Therefore, we designed a two-part study that allowed us first (phase Ia) to escalate the dose of virus administered with short-course palliative radiation (20 Gy in five fractions) and then to increase the radiation dose to a more protracted high-dose palliative regimen (36 Gy in 12 fractions) during which an escalating number of viral injections were given. The absence of significant virus-related toxicity or evidence of exacerbation of normal tissue radiation reactions, even with the use of nonconventional fractionation schedules (3 and 4 Gy per fraction), is extremely reassuring for future studies in patients receiving radiotherapy with curative intent. Such studies are currently in development and will involve standard radiotherapy fractionation regimens (2 Gy per fraction) and multiple doses of virus. The lack of viral excretion (as for the previous study of intravenous RT3D; ref. 18) provides further evidence that this approach is appropriate in an outpatient setting.

As with our previous study of i.v. infused RT3D (18), intralesional administration of RT3D resulted in a significant increase in NARA levels in all patients who gave samples at 1 month. We have previously shown in immunocompetent mouse models that the NARA response acts as a significant obstacle to viral delivery to tumors after i.v. delivery, but as a useful protection against virus-mediated systemic toxicity (22). Therefore, in the setting of disease that can be encompassed by a localized radiotherapy field and which is suitable for direct injection of RT3D, the problem of the systemic neutralization of viral vectors may be avoided without losing protection against systemic dissemination and toxicity. This approach, therefore, has a significant potential advantage over systemic administration. It will be important to continue to analyze data on the NARA response in patients treated in future curative radiotherapy or chemoradiotherapy protocols because these patients will receive viral injections over a more prolonged period and this may alter the kinetics of the antibody response. In addition, we have reported that RT3D can prime innate and adaptive antitumor immunity through mechanisms that include activation of dendritic cells as well as supporting natural killer and T-cell cytotoxicity (5, 6, 23–25). Interestingly, during this clinical study, two patients showed responses that may have involved immune-mediated mechanisms, although no laboratory studies were done to investigate this issue. Patient 0101 with esophageal cancer received radiotherapy to a supraclavicular nodal mass and achieved a durable PR (Fig. 2A and B) but also showed a long-standing response in a mediastinal mass that was outside the radiation portals (Fig. 2C and D). Patient 0602 with malignant melanoma developed vitiligo in the treated area after reovirus intratumoral injections (Fig. 2E) and was still alive 17 months after treatment. Future studies of RT3D and radiation should include measurement of antitumor immunity wherever possible.

This study was not designed to evaluate the antitumor activity of the combined strategy, but patients were monitored for evidence of antitumor activity. From the 23 enrolled patients, 14 were evaluable for response. All 14 patients showed objective evidence of either SD or PR by Response Evaluation Criteria in Solid Tumors criteria. In this short-term palliative study,

it is not possible to draw any conclusions on the contribution of RT3D to the therapeutic responses. In a limited number of patients, it was also possible to obtain paired pretreatment and posttreatment biopsies, and one of these samples confirmed persistence of viable oncolytic RT3D (as measured by TCID₅₀ assay and RT-PCR (Fig. 1C and D).

In conclusion, this study confirms the safety of combining oncolytic RT3D with radiotherapy in patients with advanced cancer. The novel trial design allowed us to study viral dose escalation (in phase Ia) and an escalated radiation dose, and increasing number of virus administrations (in phase Ib). These data will serve as essential background information for developing protocols involving radical dose radiotherapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work is supported by grants from the National Institute of Health (R01CA107082 and R01CA130878).

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Translational Relevance

We have recently reported *in vitro* and *in vivo* data demonstrating synergy between oncolytic reovirus and external beam radiotherapy in a range of tumour cell lines. We have now completed a phase I dose-escalation study of this combination strategy in patients receiving two different dose schedules of palliative radiotherapy, confirming the safety and tolerability of this approach. The study showed that virus is not shed after administration and this opens the way for outpatient treatment regimens. Most importantly, the ease of virus administration and the fact that there was no exacerbation of radiation-induced toxicity strongly support development of this treatment combination in patients with newly diagnosed, radiocurable cancers.

Fig. 1.

A, pretreatment and posttreatment NARA titers. Blue, pretreatment antibody samples; red, peak titers. Patients without a peak sample are patients who did not have a day 30 assessment. Patient 0303 did not have baseline assessments due to deteriorating performance status. B, representative NARA profiles from patients in cohort 1. All patients showed a right shift in the NARA curves by the end of the first month. Control antibody 3054 was a goat polyclonal antireoviral serum. C, inguinal lymph node biopsy sample from patient 0102. Serial dilutions of supernatants from freeze-thawed and macerated pretreatment and posttreatment biopsies showing the presence of cytopathic effect in the posttreatment samples at a dilution up to 1×10^{-4} . D, reverse transcription PCR from pretreatment and

posttreatment biopsy specimens from patient 0102. Left, a strong PCR signal at ~300 bp from the posttreatment biopsy sample, consistent with the signal seen in the positive control lane. Right, RT-PCR results from RNA preparations taken from L929 cells infected with reovirus at multiplicities of infection (MOIs) between 0.1 and 5.

Fig. 2.

Pretreatment and posttreatment computed tomography imaging data for target (A and B) and nontarget (C and D) lesions of patient 0101. A and B, pretreatment and posttreatment imaging of left supraclavicular fossa mass (red arrows) showing significant volume reduction maintained at 7 months (B). C, D, pre- and posttreatment imaging of mediastinal disease (white arrows) showing a maintained response at 7 months (D). E, Vitiligo arising in the irradiated field of patient 0602. Note the sharp demarcation between the normal pigmentation in the hair-bearing skin and the depigmented, nonhair-bearing skin at the superior border of the radiation field (black arrow). Focal loss of hair pigmentation is also seen (white arrows).

Table 1

Patient demographics and clinical data

Patient characteristics	No. of patients
Total number	23*
Median age (range), y	58.6 (38–75)
Male/female (%)	13 (56.5)/10 (43.5)
PS	
0	4
1	18
2	1
Tumor type	
Melanoma	8
Head and neck	3
SCC of the skin	3
Lung	2
Ovarian	2
Colorectal	2
Esophagus	1
Pancreas	1
Unknown primary	1
Months elapsed since diagnosis (mean $\pm$ SD, range in parentheses)	45.8 $\pm$ 60.3 (1.6–239.6)
Mean tumor target lesion size (mm; mean $\pm$ SD, range in parentheses)	62.9 $\pm$ 69.8 (20–350)
Patients with nontarget lesions	23
Median number of nontarget lesions (range)	2 (0–8)

Abbreviation: SCC, squamous cell carcinoma.

* Twenty-five patients were enrolled but two were never treated; PS, Eastern Cooperative Oncology Group Performance Status.

Table 2

Details of primary tumor diagnoses and prior treatments (surgery, radiotherapy, and chemotherapy) for each patient

Patient	Diagnosis	Surgery		Radiotherapy		Chemotherapy	
0101	Esophageal adenocarcinoma	Nil		Pelvis (P)		1	Cisplatin/capecitabine (P)
						2	Irinotecan/capecitabine (P)
						3	PXD (P)
0102	Malignant melanoma	1	WLE	Nil		1	DTIC (P)
		2	Reexcision and inguinal LN dissection				
0103	Pancreatic adenocarcinoma	1	Pancreatico-duodenectomy	Nil		1	5-FU (A)
						2	Gemcitabine (P)
0201	SCC skin (upper limb)	1	WLE and radical axillary LN dissection	Nil		1	Cisplatin/Capecitabine (P)
0202	Ovarian adenocarcinoma	1	Bilateral salpingo-oophorectomy, omentectomy, pelvic and para-aortic LN dissection	1	Left supraclavicular fossa (P)	1	CPE (P)
		2	Supraclavicular LN excision	2	Right supraclavicular fossa (P)	2	Carboplatin (P)
		3	Excision of skin nodules (back, scalp, leg, and chest wall)	3	Mediastinum (P)	3	Paclitaxel (P)
		4	Partial parotidectomy	4	Chest wall (P)	4	Doxil/caelyx (P)
						5	Sorafenib (P)
						6	Paclitaxel (P)
						7	Capecitabine (P)
						8	Gemcitabine (P)
						9	Letrozole [#] (P)
0203	SCC skin (head and neck)	1	Multiple resections of primary tumor	1	PORT head and neck (A)	1	Carboplatin/capecitabine (P)
		2	Selective neck dissection			2	Docetaxel (P)
		3	Axillary LN dissection				
0204	Small-cell lung cancer	Nil		1	Left lung (P)	1	Carboplatin/etoposide
				2	Left lung (P)	2	CAV
0301	Undifferentiated carcinoma of unknown primary origin	Nil		Nil		1	ECF (P)
						2	Docetaxel (P)
						3	VEGF/CDK inhibitor (P)
0302	SCC skin (head and neck)	Nil		1	Left mastoid (P)	1	Cisplatin/5FU (N)
						2	Cisplatin/5FU (P)
0304	Colorectal adenocarcinoma	1	Right hemicolectomy	1	Chest wall (P)	1	5-FU (A)
						2	Oxali/5-FU/Cetuximab (P)
						3	Capecitabine (P)

Patient	Diagnosis	Surgery		Radiotherapy		Chemotherapy	
0305	SCC larynx	1	Total laryngectomy and neck dissection	1	Larynx (R)	Nil	
0401	Malignant melanoma (unknown primary)	1	Internal fixation of left humerus	Nil		Nil	
0402	Lung adenocarcinoma	Nil		1	Popliteal mass (P)	1	Cisplatin/gemcitabine (P)
						2	Docetaxel (P)
0403	Colorectal adenocarcinoma	1	Left hemicolectomy	1	Para-aortic mass (P)	1	Oxaliplatin/5-FU (P)
		2	Right hemihepatectomy and cholecystectomy	2	Mediastinum (P)	2	5-FU (P)
						3	Oxaliplatin/capecitabine (P)
						4	Capecitabine (P)
						5	MVA-5T4 (P)
0501	1 SCC hypopharynx	1	WLE (for skin cancer)	1	Hypopharynx and neck (R)	1	Cisplatin (C)
	2 SCC skin			2	Skin (A)		
0502	Malignant melanoma (lower limb)	1	WLE	1	Left pelvis (P)	1	DTIC (P)
0503	SCC piriform fossa	Nil		Nil		Nil	
0504	Malignant melanoma (lower limb)	1	WLE	Nil		DTIC (P)	
		2	WLE of recurrence				
		3	Inguinal LN dissection				
		4	Resection of in transit disease				
0505	Ovarian adenocarcinoma	1	Interval debulking	1	Axillary LN (P)	1	Carboplatin/paclitaxel (N)
						2	Carboplatin/paclitaxel (A)
						3	Carboplatin (P)
0602	Malignant melanoma (trunk)	1	WLE	Nil		Nil	
		2	Axillary LN dissection				
		3	Radical neck dissection				
0603	Malignant melanoma (upper limb)	1	WLE	Nil		Nil	
		2	WLE of local recurrence				
		3	Axillary LN dissection				
		4	Forequarter amputation				
0604	Malignant melanoma (upper limb)	1	WLE	Nil		Nil	
		2	Neck dissection				
		3	Axillary LN dissection				
		4	WLE of local recurrence				
0605	Malignant melanoma (lower limb)	1	WLE	Nil		Nil	
		2	Multiple excisions of local recurrences				

Patient	Diagnosis	Surgery	Radiotherapy	Chemotherapy
		3	Inguinal LN dissection	
		4	Laparotomy (×2)	

NOTE: The patient numbers indicate the cohort and the order in which they were recruited and treated (e.g., 0101, first cohort, first patient; 0202, second cohort, second patient). Patients 0303 and patient 0601 were replaced before receiving treatment due to physical deterioration attributed to disease progression. The intent of treatment is indicated by letters in parentheses: (A), adjuvant; (N), neoadjuvant/induction; (P), palliative; (R), radical.

Abbreviations: PXD, phenoxodiol; WLE, wide local excision; DTIC, dacarbazine; LN, lymph node; 5-FU, 5-fluorouracil; carboplatin, paclitaxel, etoposide (CPE); PORT, postoperative radiotherapy; CAV, cyclophosphamide, doxorubicin (adriamycin), vincristine; ECF, epirubicin, cisplatin, 5-fluorouracil; MVA-5T4, modified vaccinia Ankara virus-expressing 5T4 antigen (Trovax); Oxali, oxaliplatin; VEGF/CDK inhibitor, vascular endothelial growth factor and cyclin-dependent kinase inhibitor.

Most common adverse events related to the combination treatment

Clin Cancer Res. Author manuscript; available in PMC 2014 January 31.

Table 4
Assessment of response in target lesions in 14 evaluable patients up to 3 months posttreatment

Patient	Diagnosis	Target lesion	Baseline (mm)	1 mo (mm)	2 mo (mm)	3 mo (mm)	Best response
0101	Esophageal adenocarcinoma	Supraclavicular mass	42	30	15	15	PR
0102	Malignant melanoma	Groin: subcutaneous nodule	30	30	N/A	N/A	SD
0103	Pancreatic adenocarcinoma	Anterior abdominal wall	70	80	N/A	N/A	SD
0201	SCC skin	Skin: left clavicle	20	15	N/A	N/A	PR
0203	SCC skin	Parotid mass	62	64	64	N/E	SD
0302	SCC skin	Cervical LN	39	65	40	N/A	SD
0305	SCC larynx	Submental lesion	40	44	44	43	SD
0401	Malignant melanoma	Chest	130	120	120	N/A	SD
0402	Lung adenocarcinoma	Right axilla LN	32	29	25	N/A	PR
0403	Colorectal adenocarcinoma	Left supraclavicular mass	37	18	19	N/A	PR
0504	Malignant melanoma	Right axilla LN	56	40	N/A	N/A	PR
0505	Ovarian adenocarcinoma	Chest wall	48	40	31	45	PR
0604	Malignant melanoma	Left axillary LN	42	N/A	10	N/A	PR
0605	Malignant melanoma	Right inguinal LN	30	32	31	N/A	SD

Abbreviations: N/A, nonavailable; N/E, not evaluable (lesion resected).