

# Plerixafor Improves Primary Tumor Response and Reduces Metastases in Cervical Cancer Treated with Radio-Chemotherapy

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## Abstract

**Purpose:** There is an important need to improve the effectiveness of radio-chemotherapy (RTCT) for cervical cancer. The CXCL12/CXCR4 pathway can influence RT response by recruiting normal myeloid cells to the tumor microenvironment that in turn can exert radioprotective effects, and may promote metastases. The objective of this study was to explore the efficacy and toxicity of combining RTCT with CXCL12/CXCR4 inhibition in cervical cancer.

**Experimental Design:** CXCR4 expression was measured in 115 patients with cervical cancer. Two primary orthotopic cervical cancer xenografts (OCICx) with different levels of CXCR4 expression were treated with RT (30 Gy: 15 daily fractions) and weekly cisplatin (4 mg/kg), with or without the CXCR4 inhibitor Plerixafor (5 mg/kg/day). The endpoints were tumor growth delay and lymph node metastases. Acute intestinal toxicity was assessed using a crypt cell assay.

**Results:** There was a fivefold variation in CXCR4 mRNA expression in the patient samples, and good correlation between the expression in patients and in the xenografts. The combination of RTCT and Plerixafor produced substantial tumor growth delay and reduced lymph node metastases compared with RTCT alone in both of the xenograft models. There was a trend toward reduced acute intestinal toxicity with the addition of Plerixafor to RTCT. There were no changes in normal organ morphology to suggest increased late toxicity.

**Conclusions:** This study demonstrates that the addition of Plerixafor to standard RTCT improves primary tumor response and reduces metastases in cervical cancer with no increase in toxicity. This combination warrants further investigation in phase I/II clinical trials. *Clin Cancer Res*; 23(5); 1242–9. ©2016 AACR.

## Introduction

Cervical cancer is a global health problem (1). It affects women at all stages of life and is associated with substantial morbidity, mortality, and, on a societal scale, social and economic disruption. Although the disease is endemic in many developing nations, it also remains a significant problem in more developed countries because of the failure of screening and early detection, the long lead-time for human papilloma virus (HPV) vaccination programs to impact incidence (2), and high rates of immigration. Many patients have locally advanced disease at diagnosis with or without lymph node metastases and are not appropriate for surgery. The current standard of care for these patients is pelvic radiotherapy (RT) and concurrent weekly cisplatin chemotherapy

(RTCT) followed by brachytherapy (3). Overall, 50% to 60% will be cured with this approach, but local recurrence and the development of metastases after treatment remain major problems, particularly in patients with bulky disease initially. There are very few effective treatments for patients with recurrent cervical cancer after RTCT, and virtually all die often with pain, bleeding, and other debilitating symptoms. Efforts are underway to improve the curative potential of RT with more precise tumor targeting and dose escalation (4), but this alone is unlikely to completely solve the problem. There is a critical need to develop new systemic treatments for use in combination with RTCT that target molecular pathways involved in both radiation treatment resistance and the development of metastases.

The CXCL12 (also known as stromal-derived factor 1 or SDF1) chemokine pathway plays a central role in bone marrow homeostasis and the trafficking of both normal myeloid and cancer cells (5). The CXCL12 receptor, CXCR4, is upregulated in cervical cancer by HPV infection (6), and both CXCL12 and CXCR4 are upregulated by tumor hypoxia (7, 8). Hypoxia is common in newly diagnosed, locally advanced cervical cancer and is associated with poor patient survival (9). RT may further increase the expression of CXCL12 in cervical cancer (10) by increasing hypoxia (11) or by other mechanisms in a hypoxia-independent manner, as described in other settings (11, 12). RT-induced tumor expression of CXCL12 in brain tumors mediates the recruitment of bone marrow derived myeloid cells (BMDC), which in turn contribute to treatment resistance by multiple mechanisms including protection of the tumor vasculature and new vessel formation (11). In cervical cancer and other malignancies, high

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### Translational Relevance

Cervical cancer is among the most common malignancies globally. Many women present with advanced disease but nevertheless are potentially curable with radiotherapy and concurrent chemotherapy (RTCT). This study explores the role of the CXCL12/CXCR4 signaling pathway in primary tumor radiation response in cervical cancer and the effect on lymph node metastases. The study was designed to mimic the clinic as closely as possible to assure relevance. Cervical cancer xenografts derived directly from patients were grown in the cervixes of mice and treated with targeted, fractionated RT and weekly cisplatin, with or without the CXCR4 inhibitor Plerixafor. The combination of RTCT and Plerixafor enhanced primary tumor response and reduced lymph node metastases compared with RTCT alone, the clinical standard of care for the treatment of cervical cancer, with no increase in toxicity. The findings provide a strong rationale for future phase I/II clinical trials.

CXCL12 expression in lymph nodes, lung, liver, and bone promotes the arrest of CXCR4-expressing tumor cells in these tissues and organs and the development of metastases (5, 13, 14). Plerixafor, an inhibitor of the CXCR4/CXCL12 interaction that is approved for harvesting bone marrow precursors prior to transplant, can mitigate these effects leading to improved radiation treatment response and reduced metastases (11, 15). Taken together, these findings suggest that adding Plerixafor to RTCT may be an effective strategy for improving local tumor control and reducing metastases in patients with cervical cancer.

The objective of this study was to derive preclinical evidence in animal models to support enhanced efficacy, without increased toxicity, of combined treatment with RTCT and Plerixafor, as a necessary precursor to phase I/II clinical trials in potentially curable patients with cervical cancer. The study was designed to mirror the clinical environment as closely as possible, with the expectation that the results would better reflect the response to be expected in patients (15, 16). To this end, efficacy testing was done using early-passage patient-derived cervical cancer xenografts (PDX) obtained directly from clinical biopsies and grown in the cervixes of mice. Fractionated, image-guided RT and concurrent cisplatin were delivered in clinically relevant doses over several weeks, similar to protocols used in patients with cervical cancer. The findings indicate that the combination of RTCT and Plerixafor is well tolerated and leads to improved tumor response and reduced metastases compared with RTCT alone, suggesting that it warrants further investigation in the clinic.

## Materials and Methods

### Mice

Six- to 8-week-old female NOD-Rag1nullIL2r $\gamma$ null (NRG) immunosuppressed female mice (radiation tolerant and impaired adaptive immunity but intact innate immunity) were bred in house at the Ontario Cancer Institute/Princess Margaret Cancer Centre (OCI/PMCC) small animal facility accredited by the Canadian Council on Animal Care. They were treated in accordance with approved animal care protocols at University Health Network (UHN). Antibiotics were administered to all of the mice in their drinking water beginning mid-

way through the growth delay experiments after oral swabs revealed infection with *Enterococcus gallinarum* and *Staphylococcus xylosum*.

### Patient-derived, orthotopic cervical cancer xenografts

The PDX models were developed from clinical cervical cancer biopsies at the Princess Margaret Cancer Centre and grown orthotopically in the cervixes of mice (Fig. 1B) as previously described (16). The PDX models have been shown to mirror the clinical and biological behavior of cervical cancer in patients, including the development of lymph node metastases (Fig. 1D), and allow efficacy and toxicity testing of clinically relevant fractionated radiotherapy and multi-dose chemotherapy regimens in combination with targeted agents in advance of phase I/II trials (16, 17).

### CXCL12 and CXCR4 expression in patient cervical tumors and PDX models

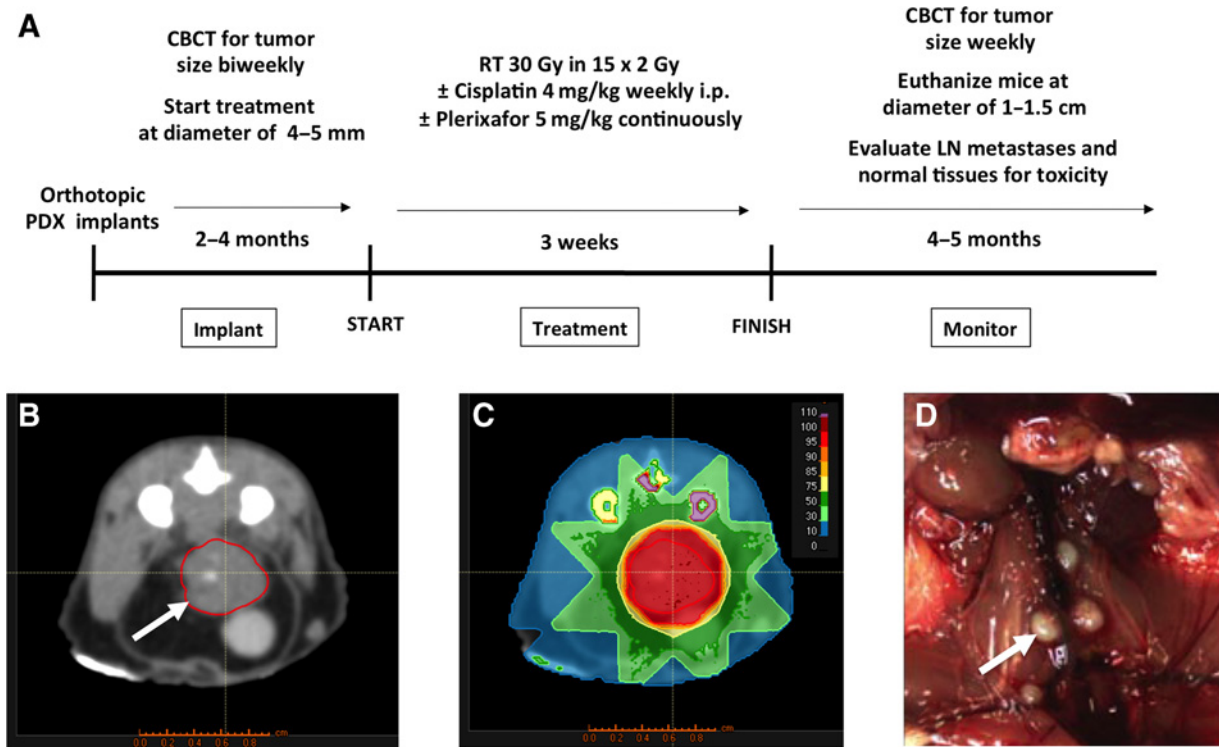
CXCR4 gene expression was evaluated in frozen biopsies obtained from the primary tumors of 115 patients with cervical cancer prior to RTCT, in 16 normal cervical biopsies and in 19 cervical cancer PDX models. The characteristics of the patients are summarized in Table 1. Total RNA was extracted from frozen tissue sections using the Qiagen RNeasy Mini Extraction Kit (Qiagen), and real-time PCR was performed according to the manufacturer's instructions as previously described (15). Human L32, a ribosomal protein, was used as an endogenous control for normalization. The CXCR4 primer used for these experiments was human-specific. Two of the 19 PDX models (OCICx 13 and 20, passage 3 in the mice) were selected for the growth delay and metastases studies based on different relative human CXCR4 expression levels, representative of the range seen in patients. Relative human CXCL12 expression was also evaluated in these two PDX models, and in the corresponding patient biopsies, using the same methodology.

### PDX CT imaging and radiation treatment

Anesthetized mice were immobilized in a lucite jig for radiation treatment and cone-beam CT (CBCT) imaging (X-Rad 225Cx, Precision X-ray; ref. 18). The X-ray tube was calibrated at 225kVp, 13mA (HVL: 0.93 mm CU, added filtration: 0.3 mm Cu) following the TG-61 protocol (19). CBCT imaging was used before treatment and at weekly intervals afterwards to evaluate primary tumor volume, and immediately prior to each radiation treatment fraction to assure reproducible tumor targeting. The imaging dose was <0.01 Gy per CBCT. Radiation treatment was delivered to the primary tumor at a dose rate of 3 Gy/minute using eight coplanar, circular (8–15 mm in diameter depending on tumor size) beams with equal 45° angular spacing around the tumor (SmART Plan; ref. 20; Fig. 1C).

### Tumor growth delay and metastases

The treatment protocol is summarized schematically in Fig. 1A. Mice were randomly assigned to one of five experimental groups containing 4 to 5 mice each when the cervical PDXs reached a size of 4 to 5 mm as determined using biweekly CBCT imaging. The tumor volume was calculated from the largest diameter in each dimensional plane (x, y, z) based on an ellipsoid model of volume ( $4/3 \cdot \pi \cdot (x/2) \cdot (y/2) \cdot (z/2)$ ). All of the CT scan measurements were made by the same observer, and a second observer confirmed these measurements. The groups were: Controls (no treatment);



**Figure 1.** The experimental design (A), a representative CBCT image of a primary orthotopic cervical tumor (B), the RT isodose distribution (C) for the tumor in (B), and para-aortic lymph node (LN) metastases (D). The CBCT image shows a tumor (arrow and red contour) measuring 0.65 cm in diameter (volume 0.14 cm<sup>3</sup>). The radiation plan with eight coplanar beams was developed using SmART-Plan, a Monte Carlo-based treatment planning system for the small animal irradiator. The red color wash corresponds to the 95% to 100% dose region, the yellow approximately 75%, and the dark green approximately 50%. Lymph node metastases (arrow) were identified grossly and confirmed histologically.

RT alone (30 Gy in 15 daily 2 Gy fractions over 3 weeks); RTCT (RT + concurrent weekly cisplatin 4 mg/kg intraperitoneally); Plerixafor alone (5 mg/kg/day by continuous subcutaneous infusion for 3 weeks, Alzet osmotic pump); and RTCT + Plerixafor. The RTCT regimen was designed to mimic the external RT beam clinical treatment of patients with cervical cancer. The radiation and cisplatin doses were optimized in earlier studies (data not shown) to balance efficacy versus toxicity. The dose of Plerixafor was chosen based on previous preclinical radiation studies (11, 21) and by extrapolation from a phase I/II trial in patients with glioblastoma multiforme (NCT01977677). The animals were weighed and examined for signs of illness weekly after treatment.

Primary tumor volume was also assessed weekly using CBCT. Mice were euthanized when the maximal primary tumor size reached 1 to 1.5 cm or they became ill. Metastatic progression to para-aortic lymph nodes was assessed at the time that the mice were euthanized based on lymph node size and hematoxylin and eosin histology.

**Intestinal toxicity**

Intestinal toxicity, which often is dose-limiting in patients receiving RTCT for cervical cancer, was assessed in two ways. Portions of the bowel and rectum in close proximity to the cervix were excised and examined by a veterinarian pathologist for evidence of acute or late toxicity. Acute toxicity was also assessed using the well-established, gut colony assay (22). Whole body, single-fraction radiation doses of 10, 11, 12, and 14 Gy were administered to C57BL/6 female mice using anterior-posterior-opposed beams with or without cisplatin (4 mg/kg as a single intraperitoneal dose immediately prior to RT) and Plerixafor (5 mg/kg/day by continuous subcutaneous infusion for 3 days prior to RT and 3 days afterwards). Mice were euthanized 3.5 days after the RT, and the jejunum was removed, flushed with PBS, fixed in formalin, and stained for Ki-67 to identify proliferating crypt cells. Aperio (Image Scope) was used to count the number of regenerating crypts (defined as crypts with >5 Ki-67-positive cells; ref. 23).

**Table 1.** Patient characteristics

Characteristic	Group	Number
FIGO stage	IB/IIA	36
	IIB/IIIA	48
	IIIB/IV	31
Histology	Squamous	97
	Adenocarcinoma or adenosquamous carcinoma	14
	Other	4
Maximum tumor size	Median and range	5 (2.3–9) cm
Pelvic lymph node status	Negative	72
	Positive	43

Abbreviation: FIGO, International Federation of Gynecologists and Obstetricians.

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### Statistical analysis

For growth delay, the times for the tumors to double relative to the start of treatment were calculated for each individual tumor and compared using the nonparametric Kruskal–Wallis test for differences among the five experimental groups and the Mann–Whitney test for differences between individual groups. For lymph node metastases, differences among experimental groups were analyzed using one-way ANOVA with the Newman–Keuls Multiple Comparison test. Results with a  $P$  value  $< 0.05$  were considered statistically different.

## Results

### CXCL12 and CXCR4 is expressed in biopsies from cervix cancer patients and in orthotopic PDX models derived from patient biopsies

Relative human CXCR4 mRNA expression was evaluated in the two orthotopic cervical cancer PDX models and compared with the expression levels in tumor biopsies obtained directly from 115 patients with cervical cancer and 16 normal cervix biopsies, as shown in Fig. 2. Given the human specificity of the CXCR4 primer, CXCR4 levels in the patient biopsies may reflect a combination of tumor and myeloid cell expression. However, in the PDX models, the CXCR4 levels are due to tumor expression alone. Relative CXCR4 expression was higher in all of the patient tumors and PDXs than in normal cervix. There was approximately a 5-fold difference in relative expression across the patient tumors, although the possibility of heterogeneity associated with the analysis of a single biopsy must be recognized. However, a similar range of expression was also seen in the PDXs. Two PDX models with high (OCICx20) and low (OCICx13) CXCR4 expression levels representative of the full range seen in patients were chosen for the growth delay and metastases experiments. Immunohistochemical staining of CXCR4 in the two xenografts (OCICx 13 and 20) is shown in the inset to Fig. 2. It is primarily in the tumor cell regions with limited staining in the stroma. OCICx 13 was derived from a patient with stage 3b HPV-negative cervical adenocarcinoma, and OCICx 20 from a patient with stage 3b squamous cell carcinoma that was associated with HPV 18 infection. CXCR4 expression was measured twice in each of these tumor models,

and the results were comparable (OCICx20: 10.3- and 12.6-fold higher relative to controls; OCICx 13: 1.2- and 2.8-fold higher relative to controls). CXCR4 expression levels were similar to the expression levels in the original patient biopsies from which the PDXs were derived (OCICx20: mean 11.5- vs. 17.6-fold higher relative to controls in the PDX and patient; OCICx13: mean 2.0 vs. 4.8 in the PDX and patient). CXCL12 expression levels were also higher in OCICx 20 than in OCICx 13 (14.9- vs. 5.0-fold higher relative to controls) and, again, roughly corresponded to the patient biopsies (OCICx20: 14.9- vs. 19.7-fold higher relative to controls in the PDX and patient; OCICx13: 5.0 vs. 8.0 in the PDX and patient).

### Plerixafor improves primary tumor response to RTCT and reduces metastases

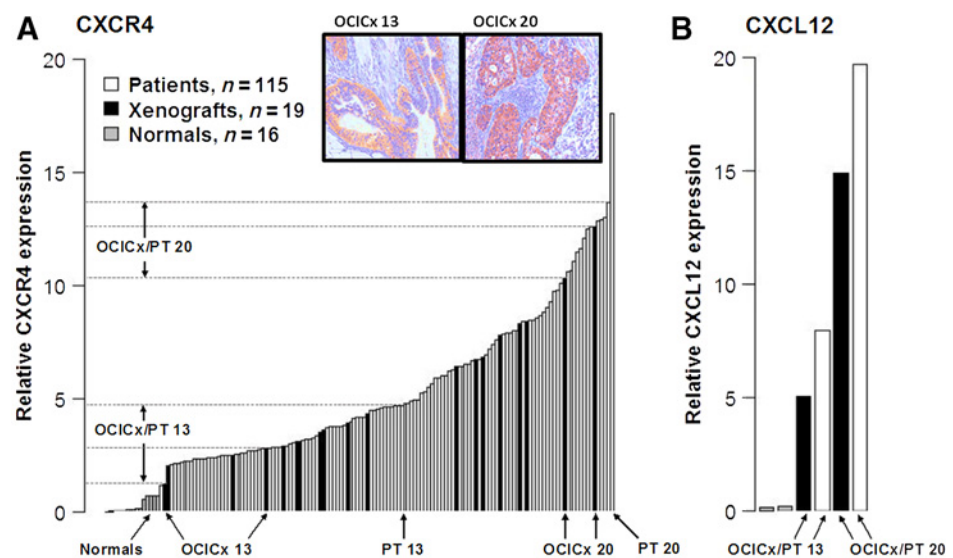
Plerixafor, administered as a single agent, had no significant effect on tumor growth relative to untreated controls in either OCICx 13 or OCICx 20, as illustrated in Fig. 3 and Supplementary Table S1. However, Plerixafor significantly delayed primary tumor regrowth when combined with RTCT compared with RTCT alone (OCICx 13: median tumor doubling time 183 vs. 147 days,  $P = 0.016$ ; OCICx 20: median doubling time 148 vs. 52 days,  $P = 0.008$ ; the doubling times were calculated for each individual tumor and a median was determined for each group). Para-aortic lymph node metastases were evaluated when the mice were euthanized. There was no significant effect of Plerixafor alone on metastatic burden in either tumor model compared with controls (Fig. 4). There were fewer lymph node metastases in the RT alone group than in controls possibly because some of the lower lymph node regions were close to the primary tumor and within the irradiated volume, and even fewer metastases with RTCT. However, Plerixafor, when added to RTCT, further reduced metastases in both tumor models relative to RTCT ( $P < 0.001$ ).

### Plerixafor is well tolerated when added to RTCT with no increase in intestinal toxicity

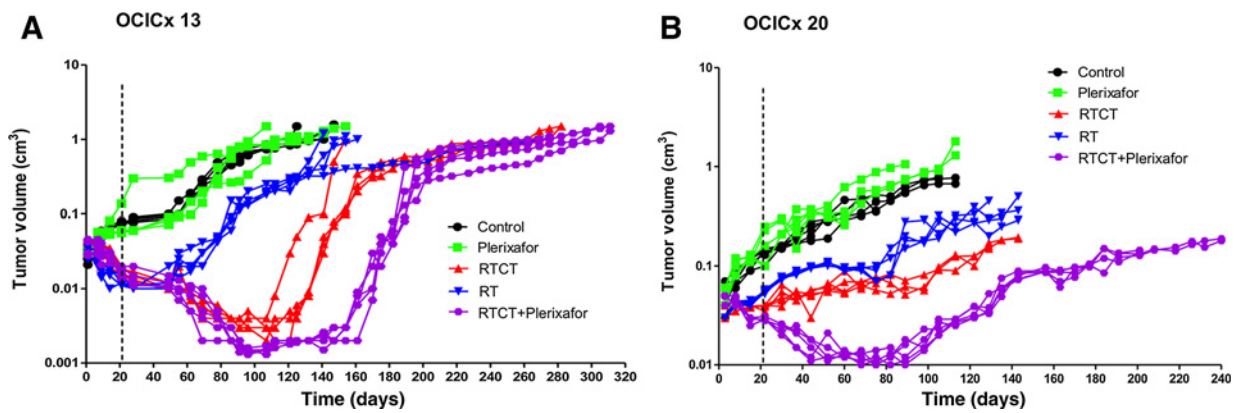
The mice were assessed daily during treatment and weekly thereafter. There were no observable systemic manifestations of toxicity. The mean mouse weight was similar in the five

**Figure 2.**

Relative human CXCR4 expression (A) was measured by qRT-PCR in 16 normal cervix biopsies (gray bars), 115 cervical cancer biopsies (open bars), and 19 orthotopic cervical cancer PDXs (black bars). The OCICx 13 and 20 PDXs were chosen for the growth delay and metastases experiments. CXCR4 was measured twice in each of the PDX models and once in the patient biopsies. Inset (20x magnification): CXCR4 immunohistochemistry in OCICx 13 and OCICx 20 showing expression predominantly in tumor rather than stroma (Santa Cruz anti-goat; 1/800 overnight—this antibody is not human specific). Relative human CXCL12 expression (B) was measured in two normal cervix biopsies (gray bars), in the OCICx 13 and 20 PDXs (black bars), and in the original patient biopsies (open bars).







**Figure 3.** Tumor growth delay plots for OCICx 13 (A) and OCICx 20 (B). There were 4 to 5 mice in each experimental arm of the study. The vertical dashed lines indicate the treatment window. Tumor volume was monitored after treatment using weekly CBCT. Tumor volume was calculated using orthogonal tumor dimensions, assuming an elliptical shape.

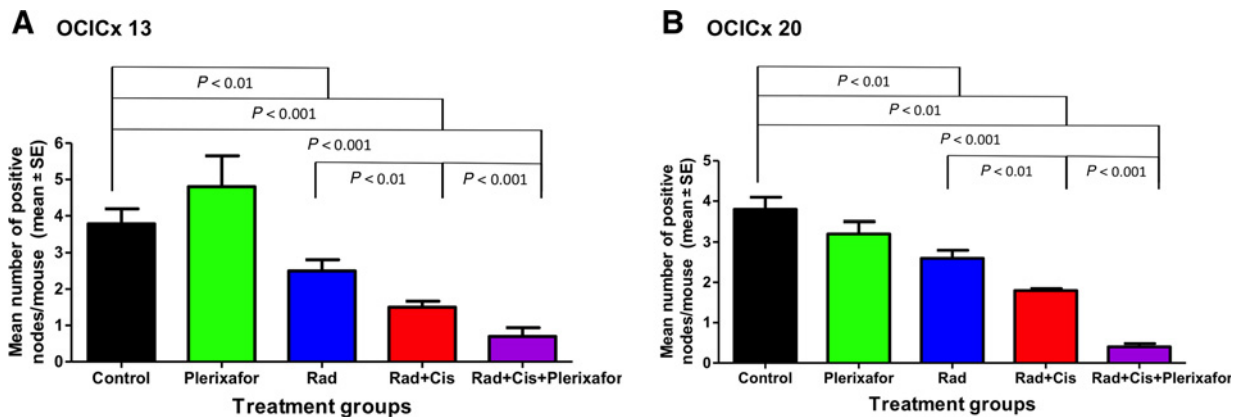
experimental groups over the course of the study (data not shown). There were no differences in hemoglobin, neutrophil, or creatinine levels (data not shown) among the experimental groups at the time that the mice were euthanized. The small bowel and rectum in close proximity to the irradiated volume were excised and examined for evidence of injury. There were no morphologic or histologic changes to indicate acute or late intestinal toxicity in any of the experimental groups.

Acute intestinal toxicity was also evaluated by counting the number of regenerating jejunal crypts following treatment, and the results are shown in Fig. 5. RTCT produced significantly greater crypt cell depletion than RT alone at a dose of 14 Gy. The addition of Plerixafor enhanced crypt cell survival after a single RT dose of 14 Gy regardless of whether or not cisplatin was used (RT+Plerixafor vs. RT alone,  $P < 0.05$ ; RTCT+Plerixafor vs. RTCT alone,  $P < 0.05$ ).

**Discussion**

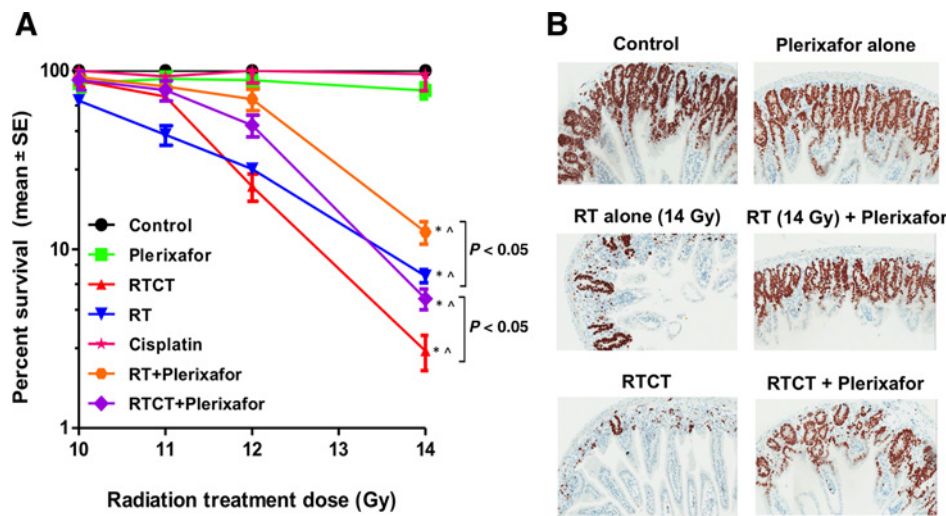
The main treatment for patients with locally advanced cervical cancer is RTCT (3). Although this offers the possibility of cure,

many patients develop progressive disease after treatment either locally in the pelvis or at metastatic sites. Salvage treatments for these patients are very limited, and most eventually die of their disease. This highlights the importance of improving front-line treatments. It is becoming increasingly evident that chemokine signaling via the CXCL12 pathway and the mobilization and activation of neutrophils and other BMDCs play important roles in cervical cancer progression and treatment response (10, 13, 14, 24, 25). In this study, we investigated the efficacy and toxicity of the combination of the CXCR4 inhibitor Plerixafor and RTCT in two OCICx xenograft cervical cancer models derived directly from patients, examining both primary tumor response and metastasis. The experimental design was unique in that tumors were grown orthotopically in the cervixes of mice and treated with conformal, image-guided fractionated RT and concurrent cisplatin to mimic the clinic as closely as possible. This is in contrast to many other preclinical mouse studies involving RT that have typically employed extensively passaged tumor models implanted subcutaneously or intramuscularly and treated with large doses of RT. The approach used in this study was adopted despite greater



**Figure 4.** Para-aortic lymph node metastases in OCICx 13 (A) and OCICx 20 (B) at the time that the mice were euthanized. The number of enlarged lymph nodes was counted in each mouse, and the nodes were then excised and examined histologically to confirm metastatic disease. The results are presented as the mean (± standard error) number of positive nodes per mouse for the individual treatment groups.

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**Figure 5.**

Jejunum crypt cell survival as a function of radiation dose and treatment arm from the gut colony assay (A) and representative images showing Ki-67 staining of proliferating crypt cells (B). Whole body RT was administered as single doses of 10, 11, 12, and 14 Gy. Cisplatin (4 mg/kg) was given as a single intraperitoneal injection immediately prior to RT and Plerixafor as a continuous infusion (5 mg/kg/day) for 3 days prior to RT and 3 days afterwards. The mice were euthanized 3.5 days after RT. The results are shown as the mean ( $\pm$  standard error) percentage of proliferating crypts ( $>5$  Ki-67-positive cells) per mouse (4–5 mice per experimental group). \* indicates significance ( $P < 0.05$ ) relative to controls within each radiation dose, ^ indicates significance between the 14 Gy and 10–12 Gy doses.

complexity because it is anticipated to yield more relevant results that are more readily translated to early phase clinical trials (26, 27).

Our findings indicate enhanced primary tumor response with the combination of RTCT and concurrent Plerixafor relative to RTCT alone (Fig. 3), which is the current standard of care for patients with cervical cancer. There was also a reduction in the number of lymph node metastases (Fig. 4) in keeping with our previous studies (15). Because the experiments were labor-intensive involving daily irradiation of the mice-bearing xenografts over several weeks, it was only possible to include a relatively small number of animals per group (4, 5). However, the findings show a high degree of consistency both within each tumor model and between the two models. A repeat study in the OCICx 20 model yielded similar results (data not shown).

The effect of adding Plerixafor to RTCT on primary tumor response could be due to a number of different factors (28). Plerixafor inhibits binding of the potent chemokine CXCL12 to one of its receptor CXCR4, which is expressed at high levels on normal myeloid cells (29). High levels of CXCL12 are seen in cervical cancer as illustrated in Fig. 2, and may contribute to the recruitment and activation of BMDCs that, in turn, induce immune suppression, invasion, angiogenesis, and other tumor-promoting effects (30, 31). In addition, RT-induced accumulation of BMDCs in cervical cancer and other tumors has been shown to stimulate new vessel formation and recovery of the vasculature after RT (30, 31). Inhibition of CXCL12 signaling using Plerixafor or other approaches can reduce BMDC accumulation and offset radioresistance due to this mechanism (11, 21, 32). This is the most likely explanation for the improved tumor response seen in our study. Any effects on T-cell-derived immunity would not be seen in our studies as we used immune-suppressed mice. Further experiments in which the mice are euthanized immediately after completing treatment (rather than at the time of tumor regrowth), with analysis of tumor CXCL12/CXCR4 expression and BMDC

accumulation, are required to confirm the effects of CXCR4 inhibition at the molecular level. Additional experiments are also needed to identify the optimal sequencing of RTCT and Plerixafor in cervical cancer, because other studies in other tumors have shown a benefit to continuing Plerixafor beyond the end of RT (11). Treatment after RTCT is also attractive from the perspective of avoiding possible Plerixafor-induced increases in tumor hypoxia (33) that could adversely influence radiation response.

Patients with locally advanced cervical cancer frequently develop lymph node or distant metastases after RTCT, even though the primary tumor may be controlled. Therefore, new therapies that, in combination with RTCT, target not only the primary tumor but also metastases are important. CXCL12 is expressed at high levels in lymph nodes, lung, liver, and bone and may promote the trafficking of CXCR4-expressing tumor cells to those sites (5). In cervical cancer, high tumor CXCR4 expression has been associated with lymph node metastases at diagnosis (34), and downregulation of CXCR4 expression has been shown to reduce metastases (35). We have previously reported that Plerixafor alone inhibits the development of nodal metastases in ME180 cervical cancer xenografts (15). In the present study, we demonstrate elevated CXCR4 expression in biopsies from a large cohort of patients with cervical cancer (Fig. 2), comparable CXCR4 expression in two OCICx PDX models (Fig. 2), and a reduction in metastases with the addition of Plerixafor to standard RTCT compared with RTCT alone (Fig. 4). The reduction in metastases may be due to reduced trafficking of CXCR4-positive tumor cells to lymph nodes. Alternatively, because some of the lymph nodes received at least part of the prescribed RT dose, it may be due to the same interaction between RT and Plerixafor that underlies improved primary tumor control. Regardless, this observation could have important implications for patients with cervical cancer, in whom lymph node failure remains a major clinical challenge.

There was no indication in this study of greater toxicity with the addition of Plerixafor to RTCT. In fact, the gut colony assay results

suggest greater survival of jejunal crypt cells with Plerixafor (Fig. 5) in keeping with less acute intestinal toxicity. The reason(s) for this are unclear. Mobilized mesenchymal stem cells may play a role in protection of the intestinal tract. The injection of mesenchymal stem cells has been reported to reduce inflammation and partially protect the intestinal tract from irradiation (36–39), although this has not been universally observed (40). Whether Plerixafor-induced mesenchymal stem cell mobilization and trafficking played a role in the intestinal protection observed in this study needs further investigation, as do the issues of whether the effect is observed following fractionated irradiation and also extends to protection against late intestinal damage. However, consistent with our findings, previous studies have reported that treatment with Plerixafor can mitigate against radiation damage to mouse skin following a large single radiation dose and that radioprotection is observed in mouse skin by inhibiting the influx of CD11b<sup>+</sup> myeloid cells, which is one effect of Plerixafor treatment (41, 42). In addition, inhibiting the CXCL12/CXCR4 pathway has been found to alleviate potential radiation-induced lung injury (43 and unpublished data).

In conclusion, this study using clinically relevant tumor models and treatment scenarios provides compelling evidence to indicate improved tumor response and reduced metastases without additional toxicity when Plerixafor is added to standard RTCT for cervical cancer. Further investigation is needed to better understand the interactive effects between Plerixafor and RTCT on primary tumor response and the mechanisms underlying the reduction in lymph node metastases and intestinal toxicity. Nevertheless, the study provides a strong rationale for future phase I/II clinical trials aimed at improving the efficacy of front-line curative therapy for patients with this disease.

#### Disclosure of Potential Conflicts of Interest

P. Lindsay and R. Hill are listed as inventors of the small animal image guide microirradiator system described herein. This system has been licensed

to Precision X-Ray Inc. for commercial development. No potential conflicts of interest were disclosed by the other authors.

#### Authors' Contributions

**Conception and design:** N. Chaudary, R.P. Hill, M. Milosevic

**Development of methodology:** N. Chaudary, S. Jelveh, P. Lindsay, R.P. Hill, M. Milosevic

**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** N. Chaudary, M. Milosevic

**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** N. Chaudary, M. Pintilie, P. Lindsay, R.P. Hill, M. Milosevic

**Writing, review, and/or revision of the manuscript:** N. Chaudary, M. Pintilie, P. Lindsay, R.P. Hill, M. Milosevic

**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** N. Chaudary, P. Lindsay

**Study supervision:** N. Chaudary, R.P. Hill, M. Milosevic

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