Clinical Cancer Research

Translational Impact of Nanoparticle–Drug Conjugate CRLX101 with or without Bevacizumab in Advanced Ovarian Cancer

Elizabeth Pham[1], Michael J. Birrer[2], Scott Eliasof[3], Edward G. Garmey[3], Douglas Lazarus[3], Christina R. Lee[1], Shan Man[1], Ursula A. Matulonis[4], Christian G. Peters[3], Ping Xu[1], Carolyn Krasner[2], and Robert S. Kerbel[1,5]

Abstract

Purpose: Increased tumor hypoxia and hence elevated hypoxia-inducible factor-1α (HIF1α) is thought to limit the efficacy of vascular endothelial growth factor (VEGF) pathway–targeting drugs by upregulating adaptive resistance genes. One strategy to counteract this is to combine antiangiogenic drugs with agents able to suppress HIF1α. One such possibility is the investigational drug CRLX101, a nanoparticle–drug conjugate (NDC) containing the payload camptothecin, a known topoisomerase-I poison.

Experimental Design: CRLX101 was evaluated both as a monotherapy and combination with bevacizumab in a preclinical mouse model of advanced metastatic ovarian cancer. These preclinical studies contributed to the rationale for undertaking a phase II clinical study to evaluate CRLX101 monotherapy in patients with advanced platinum-resistant ovarian cancer.

Results: Preclinically, CRLX101 is highly efficacious as a monotherapy when administered at maximum-tolerated doses. Furthermore, chronic low-dose CRLX101 with bevacizumab reduced bevacizumab-induced HIF1α upregulation and resulted in synergistic efficacy, with minimal toxicity in mice. In parallel, initial data reported here from an ongoing phase II clinical study of CRLX101 monotherapy shows measurable tumor reductions in 74% of patients and a 16% RECIST response rate to date.

Conclusions: Given these preclinical and initial clinical results, further clinical studies are currently evaluating CRLX101 in combination with bevacizumab in ovarian cancer and warrant the evaluation of this therapy combination in other cancer types where HIF1α is implicated in pathogenesis, as it may potentially be able to improve the efficacy of antiangiogenic drugs. *Clin Cancer Res; 21(4); 808–18. ©2014 AACR.*

Introduction

Angiogenesis is generally considered an important contributor to the growth and metastatic dissemination of tumors with vascular endothelial growth factor (VEGF) considered a key regulator of tumor angiogenesis in most cancer types. Drugs developed to target VEGF or its receptors, including bevacizumab, an antibody that targets circulating VEGF, are designed to decrease or prevent tumor neovascularization and thereby deprive tumor cells of optimal levels of oxygen and nutrients (1, 2). Despite the approval of a number of different VEGF-targeting drugs, anti-VEGF therapy remains associated with only modest survival benefits (3, 4).

One possible reason for the limited success of anti-VEGF therapy is the emergence of adaptive or "evasive" drug resistance due to increases in tumor hypoxia and expression of hypoxia-inducible factors (HIF; refs. 3–5). Increased HIF1α and/or HIF2α can promote tumor cell survival, augment compensatory proangiogenesis pathways, increase metastasis, induce epithelial-to-mesenchymal transition, and contribute to maintaining or expanding the cancer stem-cell microenvironment (4, 6). Overexpression or upregulation of HIF1α appears associated with poor prognosis in several human cancers including ovarian carcinoma (7).

Some preclinical studies have shown that combining antiangiogenic therapy with strategies that inhibit HIF1α can lead to improved anticancer efficacy (8). For example, analogues of camptothecin, such as topotecan, have been shown to inhibit HIF1α protein accumulation and activity (9–11). Interestingly, protracted daily administration is more efficacious than intermittent treatment, resulting in sustained HIF1α inhibition (11). This daily exposure of tumor cells to topotecan increases the likelihood of targeting replicating cells in S-phase, where topoisomerase-I inhibitors have been most effective (12). Such prolonged topoisomerase-I inhibition may be required to mediate more potent HIF1α inhibition (11). To achieve sustained drug exposure, the dose and schedule of chemotherapy drugs, such as topotecan, gemcitabine, TS-1, and doxorubicin, have been modified to be

[1]Biological Sciences Platform, Sunnybrook Research Institute, Toronto, Ontario, Canada. [2]Gillette Center for Gynecologic Oncology, Massachusetts General Hospital, Boston, Massachusetts. [3]Cerulean Pharma Inc., Cambridge, Massachusetts. [4]Dana-Farber Cancer Institute, Boston, Massachusetts. [5]Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada.

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

Corresponding Authors: Robert S. Kerbel, Biological Sciences Platform, Sunnybrook Research Institute, S217-2075 Bayview Avenue, Toronto, ON, M4N 3M5, Canada. Phone: 416-480-5711; Fax: 416-480-5884; E-mail: robert.kerbel@sri.utoronto.ca; and Carolyn Krasner, Gillette Center for Gynecologic Oncology, Massachusetts General Hospital, Harvard Medical School, 55 Fruit Street, Boston, MA 02114. Phone: 617-724-4800; Fax: 617-726-1842; E-mail: cndrasner@partners.org

doi: 10.1158/1078-0432.CCR-14-2810

Translational Relevance

Currently, there are seven different vascular endothelial growth factor (VEGF) pathway–targeting antiangiogenic drugs approved globally for the treatment of 10 types of cancer. Despite therapeutic successes, intensive efforts are being pursued to improve the generally modest survival benefits associated with such drugs. Two promising strategies are improving the nature of the chemotherapy backbone partner for drugs such as bevacizumab, and preventing increase in tumor hypoxia-mediated hypoxia-inducible factor-1α (HIF1α) caused by antiangiogenic therapy. Here, we present preclinical data in a model of advanced metastatic ovarian cancer, suggesting that CRLX101, a topoisomerase-I targeting nanoparticle–drug conjugate (NDC), has potent antitumor activity and HIF1α-suppressive effects, including when it is combined with bevacizumab. Preliminary data from ongoing clinical studies of CRLX101 in patients with platinum-resistant ovarian cancer, presented here, also suggest that this agent can result in net tumor reductions. On the basis of these overall preclinical results and initial clinical findings, a phase II trial of CRLX101 and bevacizumab is now under way.

administered "metronomically" (e.g., on a daily basis at lower doses) and have been shown, as a result, to more effectively target HIF1α (11, 13–15). However, because of toxicity, metronomic administration of topotecan does not appear to be well-tolerated and therefore may not be a viable clinical strategy (16). Alternatively, sustained drug exposure can also be achieved with new drug formulations, such as pegylated, liposomal, and polymeric nanoparticles of topoisomerase inhibitors (and other drugs such as taxanes, anthracyclines, and vinca alkaloids) that intrinsically have a longer half-life (9, 17, 18). An example of the latter is the investigational drug, CRLX101, a polymeric nanoparticle–drug conjugate (NDC; refs. 19, 20).

CRLX101 is a self-assembling NDC composed of a cyclodextrin-containing polymer conjugated to camptothecin. The cyclodextrin polymer itself is well-tolerated with no observable side effects or antitumor efficacy when tested in mice with doses up to 240 mg/kg (21). CRLX101 preferentially accumulates in tumor tissue through the enhanced permeability and retention (EPR) effect, where leaky tumor vasculature and reduced lymphatic drainage accumulates nanoparticles inside tumors (17, 22, 23). Once inside tumor cells, camptothecin is released from CRLX101 over several days with a plasma half-life of approximately 17 to 20 hours compared with <2 hours for camptothecin in rats (20, 24). The slow release nature of CRLX101 provides prolonged drug exposure within tumors, sustaining topoisomerase-I inhibition, while limiting the level of freely circulating camptothecin and reducing unwanted systemic toxicity (20, 25, 26).

The primary purpose of this study was to assess the combination of CRLX101 with bevacizumab in the treatment of advanced ovarian cancer. Specifically, we sought to evaluate whether CRLX101 combined with bevacizumab shows improved or even synergistic efficacy preclinically and whether this combination could offer an increased clinical benefit to patients over CRLX101 monotherapy. In preclinical studies, we evaluated the antitumor efficacy of CRLX101 in several models of ovarian cancer, including subcutaneously implanted A2780 or SKOV3 tumors, as well as advanced intraperitoneally (i.p.) implanted metastatic SKOV3-13, an aggressive variant of SKOV3 tagged with luciferase. Both high and low doses of CRLX101 were evaluated as monotherapies or when combined with bevacizumab. On the basis of the experiments described above, the decision was made to clinically evaluate CRLX101 in settings of HIF1α overexpression. In particular, a noncomparative two-arm clinical trial was developed to evaluate both CRLX101 monotherapy and the combination of CRLX101 with bevacizumab in patients with relapsed platinum-resistant ovarian, tubal, and peritoneal cancer. Patients received 15 mg/m^2 CRLX101 administered intravenously on days 1 and 15 of a 28-day cycle. To minimize the number of patients treated in the eventuality of poor CRLX101 activity in this setting, a Simon two-stage optimum design was used. Here, we report our detailed preclinical results and initial clinical findings.

Materials and Methods

Cell culture

A2780 was maintained in DMEM, SKOV3 and SKOV3-13 were maintained in RPMI, both supplemented with 5% to 10% FBS. A2780 was obtained from the NCI-Frederick Cancer DCI Tumor Repository in 2009 and SKOV3 was obtained from Cell Biolabs in 2011. SKOV3-13 (last authenticated in 2013) is a highly aggressive clonal variant of SKOV3 (from the ATCC) selected *in vivo* after i.p. implantation (27) and luciferase-tagged for bioluminescence imaging.

Cell authentication

SKOV3-13 cells were processed by Genetica DNA Laboratories (a LabCorp Specialty Testing Group) for authentication testing using analytic procedures for DNA extraction, polymerase chain reaction (PCR), and capillary electrophoresis on a 3130xl genetic analyzer (Applied Biosystems). The 13-core CODIS short-tandem repeat (STR) loci plus PENTA E and PENTA D, and the gender-determining locus, amelogenin, were analyzed using the commercially available PowerPlex 16HS amplification Kit (Promega Corporation) and GeneMapper ID v3.2.1 software (Applied Biosystems). Appropriate positive and negative controls were used concurrently throughout the analysis. Authentication of cell lines is confirmed by entering the STR DNA profile of each tested cell line into known repository cell line databases (i.e., ATCC, DSMZ, etc.); authentication is defined as having a percentage match with the reference STR profile at or above 80% when using the ANSI/ATCC guidelines (ASN-0002-2011) OR having a "unique" STR DNA profile (no matches found) for "in-house" cell lines not distributed by any cell line repository.

Subcutaneously implanted ovarian tumors

A2780 and SKOV-3 human ovarian cells (10×10^6) were injected subcutaneously into the right flank of 8- to 10-week-old nude mice purchased from Taconic Farms or Harlan Laboratories. Therapy was initiated when tumor volumes were approximately 100 mm^3. Tumor weights were measured by calipers twice weekly. All experimental work was approved by the Institutional Animal Care and Use Committee before initiation.

Intraperitoneally implanted ovarian tumors to mimic advanced disease in patients

Six- to 8-week-old female CB-17 SCID mice were purchased from Charles River Canada and 8-week-old female YFP-SCID

mice were bred in-house from breeding pairs generously provided by Dr. Janusz Rak (McGill University, Montreal, QC, Canada; ref. 28). All mice were housed in microisolator cages with vented racks and were manipulated using aseptic techniques. Procedures involving animals and their care were conducted in strict conformity with the animal care guidelines of Sunnybrook Health Science Centre (Toronto, ON, Canada) and the Canadian Council of Animal Care. SKOV3-13 cells (3×10^6) were injected i.p. into CB-17 SCID mice. Treatment was initiated 2 weeks later when the presence of advanced disease can be detected by imaging (27). Drug tolerability and clinical symptoms, including body weight loss and overall appearance, were monitored at least twice weekly. Mice were euthanized when showing more than 20% body weight loss or when moribund. In this model of advanced ovarian cancer, at the time of treatment initiation, tumor masses are disseminated throughout the peritoneal cavity including the mesentery and omentum surfaces, as well as invasion into the pancreas, diaphragm, and abdominal wall (27). The endpoint was thus defined as distended abdomen due to ascites and overall tumor load, body weight loss of >20% or signs of jaundice (indicating a tumor mass causing bile duct blockage).

Drug dosages and schedules

CRLX101 was supplied by Cerulean Pharma Inc. and reconstituted into PBS as per the manufacturer's instructions. Bevacizumab (Roche) was purchased from the institutional pharmacy as a 25 mg/mL solution. Topotecan was purchased from Sigma-Aldrich. All doses of CRLX101 were administered once a week by i.v. injection in the subcutaneous studies or i.p. injection in the orthotopic studies. Bevacizumab was administered twice a week by i.p. injection. Topotecan was administered every 4 days by i.p. injection. In all experiments, vehicle-treated mice were administered PBS by i.p. injection.

Total body bioluminescence imaging to detect tumor growth and response to therapy

Tumor growth in mice implanted with SKOV3-13, which stably expresses firefly luciferase, was monitored by weekly total body bioluminescence imaging. Mice were administered luciferin (150 mg/kg) and imaged in an IVIS200 Xenogen under isoflurane anesthesia, as previously described (27). Bioluminescence images were analyzed using the Living Image software and bioluminescence is reported as photons/second.

Statistical analysis

Results were reported as the mean ± SD or SEM, as indicated. Bioluminescence measurements were reported as the mean ± SD. Survival curves were plotted by the method of Kaplan–Meier and tested for survival differences with the log-rank test. Statistical significance was assessed by one-way analysis of variance (ANOVA; Kruskal–Wallis test with Dunn *post hoc* test) or *t* test (Mann–Whitney, two-tailed) using GraphPad Prism 4 ($P < 0.05$ was used as the threshold of statistical significance).

Results

CRLX101 treatment inhibits both HIF1α and HIF2α protein levels in subcutaneously grown tumors

Given prior published results showing suppression of HIF1α by topotecan, SN38-conjugates and irinotecan, using metronomic-like protocols (9, 11), we asked whether CRLX101 is capable of mediating a similar effect, which could result in a less toxic alternative for combination with an antiangiogenic drug such as bevacizumab. A single dose of either CRLX101 or topotecan was administered to nude mice bearing A2780 tumors. CRLX101 was able to inhibit HIF1α and HIF2α in a sustained manner, whereas topotecan was not (Fig. 1A and B). CRLX101 was also significantly more potent in suppressing tumor growth compared with topotecan. Notably, beyond a week after CRLX101 administration, both HIF1α and HIF2α levels gradually increased, corresponding to tumor regrowth, consistent with the need for prolonged drug exposure to maintain antitumor efficacy as well as HIF inhibition (11, 12). Additional markers known to be regulated by HIF1α were also downregulated by CRLX101 (Supplementary Fig. S1; ref. 4).

CRLX101 monotherapy treatment of subcutaneously implanted A2780 and SKOV3 ovarian tumors

CRLX101 administered once weekly at 12 mg/kg, near the maximum-tolerated dose (MTD) in nude mice (20, 29), was used to treat established subcutaneous tumors of ovarian xenografts of the A2780 and SKOV3 cell lines. For comparison, the efficacy of topotecan administered near the MTD at 12 mg/kg every 4 days was also evaluated. Each drug was administered for 3 weeks. For both models, topotecan was only minimally effective at delaying tumor growth, as shown in Fig. 1C and D. The limited efficacy of topotecan is consistent with our previously published studies using a preclinical model of advanced metastatic ovarian cancer when using topotecan at its MTD (27). In contrast, CRLX101 was efficacious in both models. Growth of the A2780 tumors remained completely suppressed for >8 weeks while SKOV3 tumor growth was potently suppressed for approximately 7 weeks before progression, resulting in extended survival of CRLX101-treated mice, compared with both vehicle- and topotecan-treated mice.

CRLX101 combined with bevacizumab in subcutaneous tumor models

Given the potent efficacy of CRLX101 even as a monotherapy, a lower dose of 5 mg/kg (equivalent to 15 mg/m^2, the clinical MTD) was combined with bevacizumab to determine whether the combination would show improved efficacy. Nude mice with subcutaneously implanted A2780 tumors were treated with vehicle, bevacizumab, CRLX101, or CRLX101 + bevacizumab, for 3 weeks. CRLX101 monotherapy resulted in a tumor-growth delay of approximately 20 days while the combination showed a significantly longer growth delay of approximately 70 days despite bevacizumab monotherapy showing minimal antitumor efficacy (Fig. 2). Tumors were collected 3 days after 8 days of treatment and whole-tumor lysates were used for Western blot analyses of HIF1α protein levels. Although bevacizumab increased HIF1α compared with vehicle, CRLX101 was able to inhibit HIF1α protein levels and prevent bevacizumab-induced HIF1α upregulation (Fig. 2C). This blunting of an induced increase in HIF1α when CRLX101 is combined with bevacizumab is not specific to bevacizumab. A similar improved antitumor efficacy and HIF1α inhibition was observed when treating tumors with the VEGF trap drug, aflibercept, and the small-molecule, oral tyrosine kinase inhibitor (TKI), pazopanib (Supplementary Figs. S2 and S3).

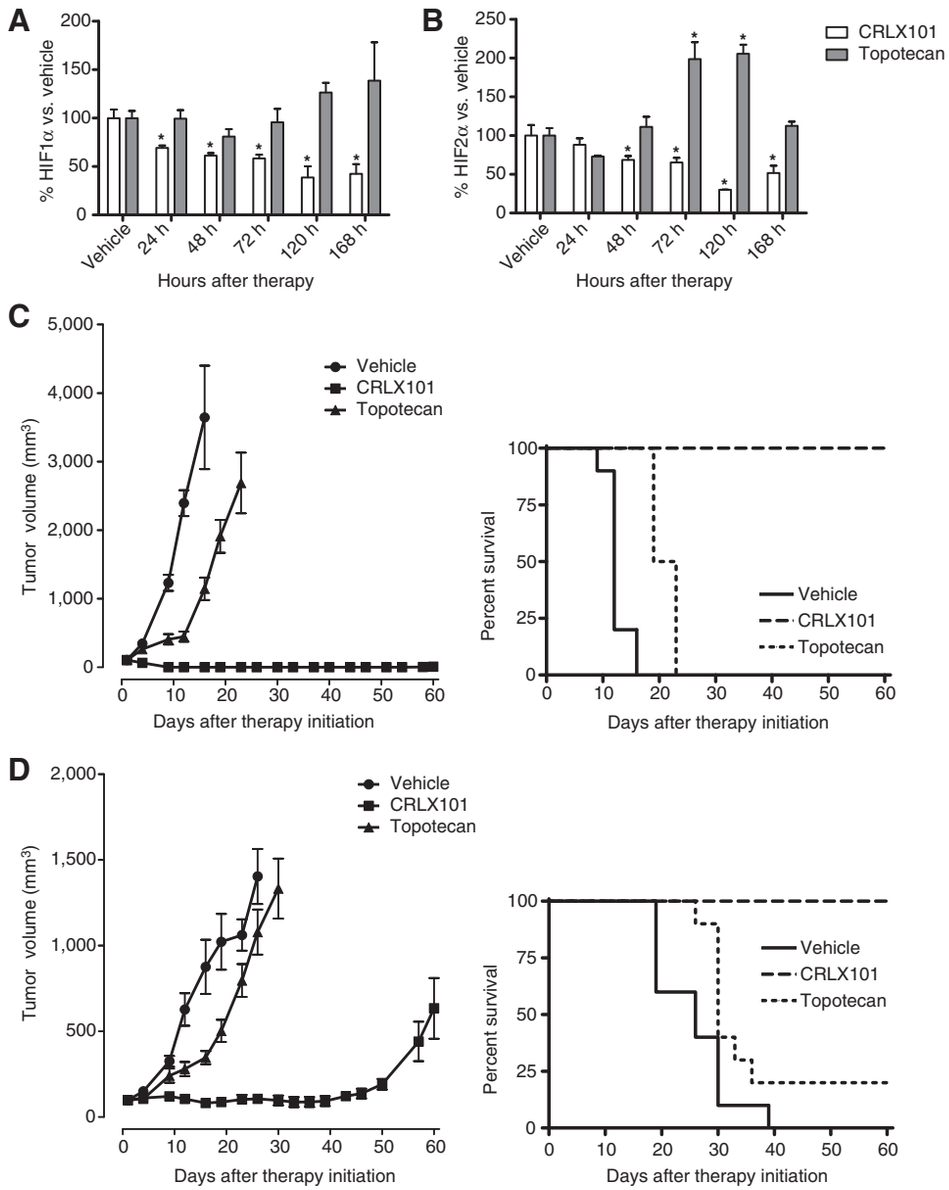

Figure 1.
CRLX101 is better able to inhibit HIF1α, suppress tumor growth, and extend survival in mice compared with topotecan. Nude mice bearing subcutaneous A2780 tumors were administered CRLX101 (6 mg/kg) or topotecan (10 mg/kg). Tumors were analyzed for HIF1α (A) and HIF2α (B) protein levels at various timepoints. Protein levels are reported as percentage difference compared with vehicle (tumors isolated at 72 hours after vehicle injection). Error bars are SD (*, $P < 0.05$ vs. vehicle, 3 tumors/timepoint). Tumor growth delay curves and corresponding survival curves for mice bearing A2780 (C) and SKOV3 (D) subcutaneously grown tumors. Mice were treated with vehicle, CRLX101 (12 mg/kg) once weekly, and topotecan (12 mg/kg) every 4 days, for 3 weeks. ($P < 0.05$, CRLX101 vs. vehicle, 10 mice/group).

Efficacy of CRLX101 with bevacizumab against i.p. implanted advanced ovarian cancer

We reported previously that while antiangiogenic and chemotherapy drugs may be active when treating subcutaneously grown primary tumors or established orthotopic primary tumors, they may not be efficacious when treating mice with the more clinically relevant circumstance of advanced metastatic disease (30). Thus, to more accurately mimic ovarian cancer progression in the clinic, CRLX101 was evaluated in mice i.p. implanted with metastatic luciferase-tagged and highly aggressive SKOV3-13 cells. Mice were treated with 0, 2, 4, 8, or 12 mg/kg CRLX101 once weekly i.p. for 3 weeks to determine the MTD in this model. A dose of 8 mg/kg was determined to be near the MTD for CRLX101 in CB-17 SCID mice (Supplementary Fig. S4). Notably, this is the most potent chemotherapy drug we have tested in this model thus far; other drugs previously tested include cyclophosphamide, paclitaxel, irinotecan, and topotecan (unpublished observations and refs. 27, 31).

We next evaluated the efficacy of long-term CRLX101 therapy combined with bevacizumab. SCID mice implanted with SKOV3-13 cells were treated with vehicle, bevacizumab, MTD CRLX101 (8 mg/kg, once weekly), and MTD CRLX101 + bevacizumab. Unlike our preliminary experiments and previously reported studies in which CRLX101 was administered upfront for 2 to 3 weeks only (21, 32), here tumor-bearing mice were treated with CRLX101 long term (up to 8 months). Data show that the drug was well tolerated and tumor burden was significantly suppressed. As evident from Fig. 3A, MTD CRLX101 greatly reduced tumor burden as measured by bioluminescence imaging. This reduction in tumor burden and inhibition of disease progression persisted for >1 year. Both MTD CRLX101 monotherapy and MTD

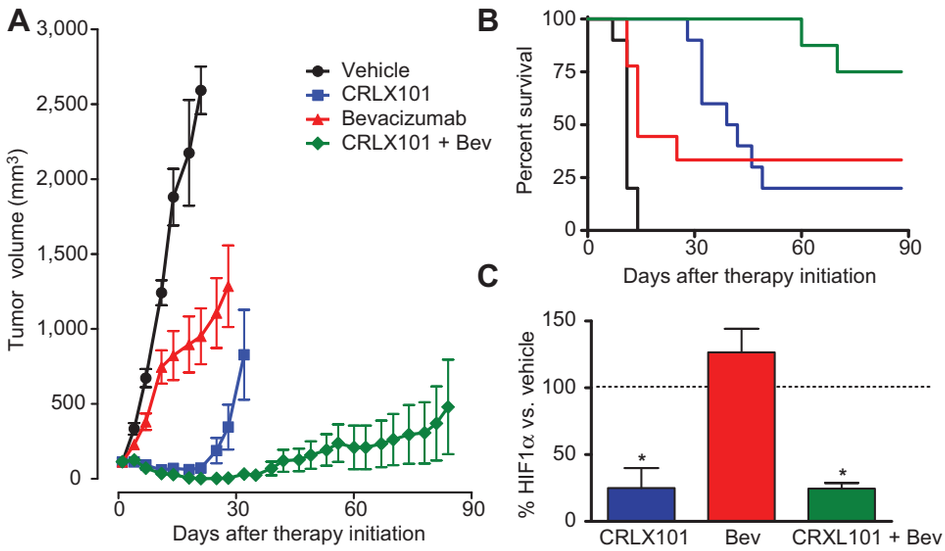

Figure 2.
CRLX101 combined with bevacizumab shows improved antitumor efficacy in subcutaneous A2780 tumors. A, tumor growth of subcutaneously implanted A2780 tumors in mice treated with vehicle, bevacizumab monotherapy (5 mg/kg, twice weekly), CRLX101 (5 mg/kg weekly) monotherapy, and CRLX101 + bevacizumab. Mice were treated for 3 weeks (3 treatments of CRLX101 and 6 treatments of bevacizumab administered). B, median survival (in days): vehicle (11), bevacizumab (14), CRLX101 (39; $P < 0.001$ vs. vehicle) and CRLX101 + bevacizumab (>90; $P < 0.001$ vs. vehicle; 10 mice/group). C, tumors were isolated three days after 8 days of treatment for subsequent Western blot analyses of whole tumor lysates for HIF1α protein levels (error bars are SD, 3–4 tumors/group; *, $P < 0.05$ vs. bevacizumab monotherapy).

combination groups underwent 8 months of continuous therapy. In both groups, bioluminescence signals have remained low with very minimal persistent residual disease that did not relapse even 4 months after terminating all treatments. Because of the significant efficacy of CRLX101 at 8 mg/kg, it was not possible to show synergy or improved efficacy with bevacizumab (Fig. 3B).

A subset of tumors was isolated after 2 weeks of therapy for analysis. Although tumor weights of bevacizumab-treated tumors were comparable with vehicle, both MTD CRLX101 monotherapy and combination treatment tumors were significantly smaller (Fig. 4A, left). This reduction in tumor weight when mice were treated with CRLX101 correlates with an increase in apoptosis, based on immunohistochemistry analysis of cleaved caspase-3 (Fig. 4A, right). CRLX101 treatment, whether alone or combined with bevacizumab, also resulted in reduced cell proliferation, as determined by Ki67 staining (Fig. 4B, left). CD31 staining was used to determine any differences in microvessel density (MVD) between the different therapies. Despite its lack of antitumor activity as a monotherapy, bevacizumab greatly reduced the number of blood vessels compared with vehicle-treated tumors. The combination resulted in a slight reduction in MVD in SKOV3-13 tumors (not statistically significant), while CRLX101 itself had no effect on MVD (Fig. 4C, left). CRLX101-treated tumors showed significantly reduced tumor necrosis and carbonic anhydrase IX (CAIX) staining, used as a marker for tumor hypoxia and downstream HIF1α activity (33). When combined with bevacizumab, CRLX101 also slightly reduced CAIX staining (not statistically significant; Fig. 4B, right and Fig. 4C, right).

As noted, 8 mg/kg CRLX101 showed significant efficacy even as a monotherapy, and thus it was not possible to show improved activity with bevacizumab. Therefore, a less toxic, lower dose of 1.5 mg/kg was chosen to determine whether combining CRLX101 with bevacizumab would show synergistic antitumor efficacy. SCID mice i.p. implanted with SKOV3-13 cells were treated with vehicle, bevacizumab monotherapy, CRLX101, or CRLX101 + bevacizumab. On the basis of bioluminescence imaging, both low-dose CRLX101 monotherapy and the combination were able to significantly inhibit disease progression compared with vehicle- and bevacizumab-treated mice (Fig. 5A and B). This reduction in tumor burden did not translate into a survival benefit with low-dose CRLX101 monotherapy. However, despite bevacizumab not showing any antitumor efficacy or survival benefit, the combination of low-dose CRLX101 and bevacizumab showed a statistically significant survival benefit, with a median survival difference of 47 days compared with vehicle ($P < 0.001$). HIF1α and CAIX staining on tumor sections harvested after 3 weeks of therapy confirm that even low-dose CRLX101 was able to inhibit both HIF1α and downstream CAIX expression compared with vehicle, even when combined with bevacizumab (Fig. 5C and D).

Preliminary clinical experience with CRLX101 in ovarian cancer patients

A total of 29 patients with relapsed platinum-resistant ovarian, tubal, and peritoneal cancer were enrolled and treated with CRLX101 monotherapy (ClinicalTrials.gov Identifier #NCT01652079; ref. 34). Of these, 7 patients completed ≥6 cycles with 2 patients remaining on-study as of September 1, 2014 with 15 and 16 cycles of therapy completed, respectively. Of the 29 enrolled monotherapy patients, 22 were classified as having platinum-resistant disease and, of these patients, 19 were evaluable for efficacy with follow-up computerized tomography (CT) scans performed every two cycles. Of these 19 evaluable patients, 14 (74%) achieved net tumor reductions and 3 (16%) achieved durable RECIST partial responses

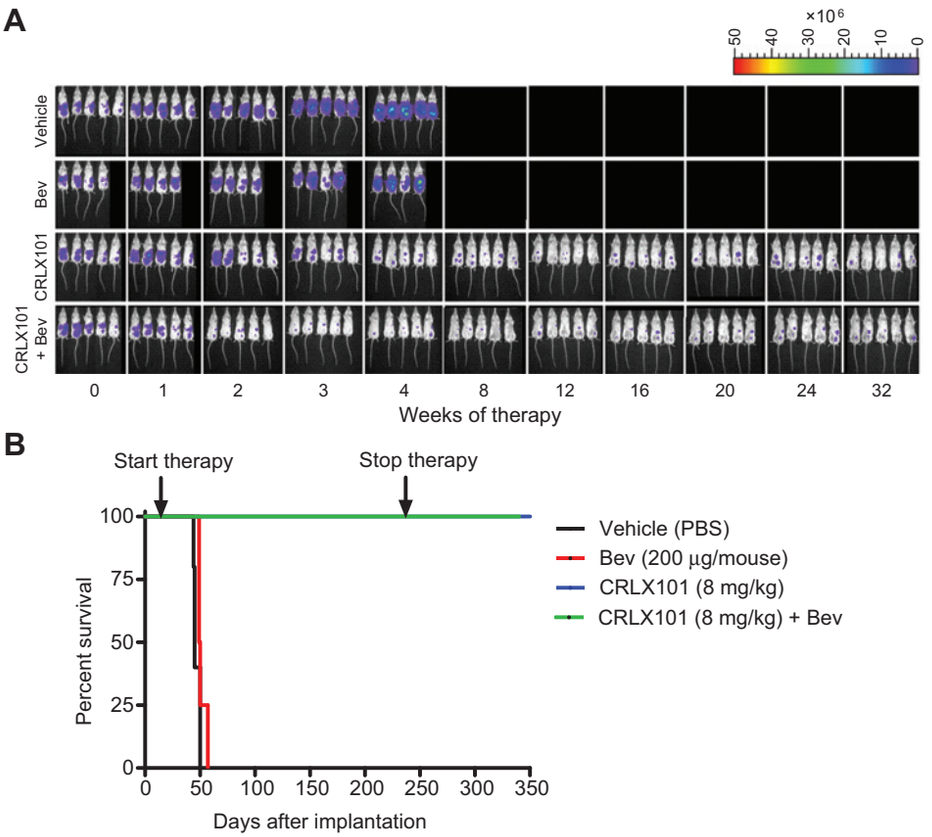

Figure 3.
CRLX101 administered at MTD significantly reduces metastatic tumor burden, extending survival in mice. Bioluminescence images showing change in tumor burden (A) and survival (B) of SKOV3-13 tumor-bearing SCID mice treated with vehicle, bevacizumab monotherapy (200 μg/mouse, twice weekly), MTD CRLX101 (8 mg/kg) monotherapy, and CRLX101 + bevacizumab (4–5 mice/group). Treatments were initiated on day 14 after tumor cell implantation into the peritoneal cavity. Median survival (in days): vehicle (45), bevacizumab (50), MTD CRLX101 (>8 months), and CRLX101 + bevacizumab (>8 months).

(Fig. 6A). One additional partial response occurred in a platinum-sensitive patient. The primary efficacy endpoint was met with median progression-free survival (PFS) of 161 days and 6 patients achieving PFS ≥6 months.

Of the 27 discontinued patients, 17 discontinued because of disease progression, 2 due to toxicity (1 patient with prolonged neutropenia and 1 patient with unrelated worsening of preexisting bladder symptoms), and 8 patients discontinued for other reasons. In total, 21 patients experienced treatment emergent adverse events (TEAEs), which were generally low grade. The most frequent TEAEs reported in at least 30% of patients were nausea (69%), fatigue (55%), anemia (31%), and constipation (31%). Ten patients experienced TEAEs considered by investigators to be at least possibly related to CRLX101 treatment with nausea, fatigue, and anemia being the most common. Eight patients experienced at least one grade 3 or higher TEAE, including pulmonary embolism, hyperglycemia, hyponatremia, hypophosphatemia, nausea, vomiting, anemia, thromboembolic event, elevated liver function tests [aspartate aminotransferase (AST)], abdominal pain, decreased neutrophil count, and increased alkaline phosphatase level. In addition, hypersensitivity reactions were reported in 4 patients and all 4 patients continued treatment following a desensitization protocol. Four patients experienced serious adverse events (SAE), including hyponatremia and dyspnea (1 patient), ileal perforation and sepsis (1 patient), and partial bowel obstruction and pulmonary embolism (1 patient each).

Following completion of enrollment in the monotherapy arm, new patients are enrolled in a second arm evaluating CRLX101 in combination with bevacizumab (10 mg/kg) administered on days 1 and 15 of a 28-day cycle. Patients in this combination arm are being restricted to those with platinum-resistant disease and receipt of one to two prior therapy regimens. Among the first 5 combination patients with evaluable data, no new drug-related SAEs, treatment discontinuations, or deaths have been observed and all 5 patients remained active on combination therapy as of September 1, 2014. In addition, all of these patients experienced CA-125 reductions and 4 of those patients have experienced net tumor reductions of 10% to 36% including one RECIST partial response (Fig. 6B). Initial robust activity observed among these combination patients builds on above-referenced preclinical data, suggesting that CRLX101-mediated suppression of HIF1α may overcome a key pathway of resistance to antiangiogenic drugs. Global randomized clinical trials are currently being designed. A more detailed report on both monotherapy and combination clinical data will follow upon study completion.

Discussion

Camptothecin is a naturally occurring cytotoxic alkaloid with a broad range of antitumor activity. Despite early reports of promising clinical antitumor activity, camptothecin had a number of limitations, including (i) an unstable lactone ring that readily hydrolyzed at physiologic pH to yield an inactive carboxylate form of the drug, (ii) low water solubility, (iii) dependence on passive diffusion into cells and potential susceptibility to resistance mediated by multidrug transport

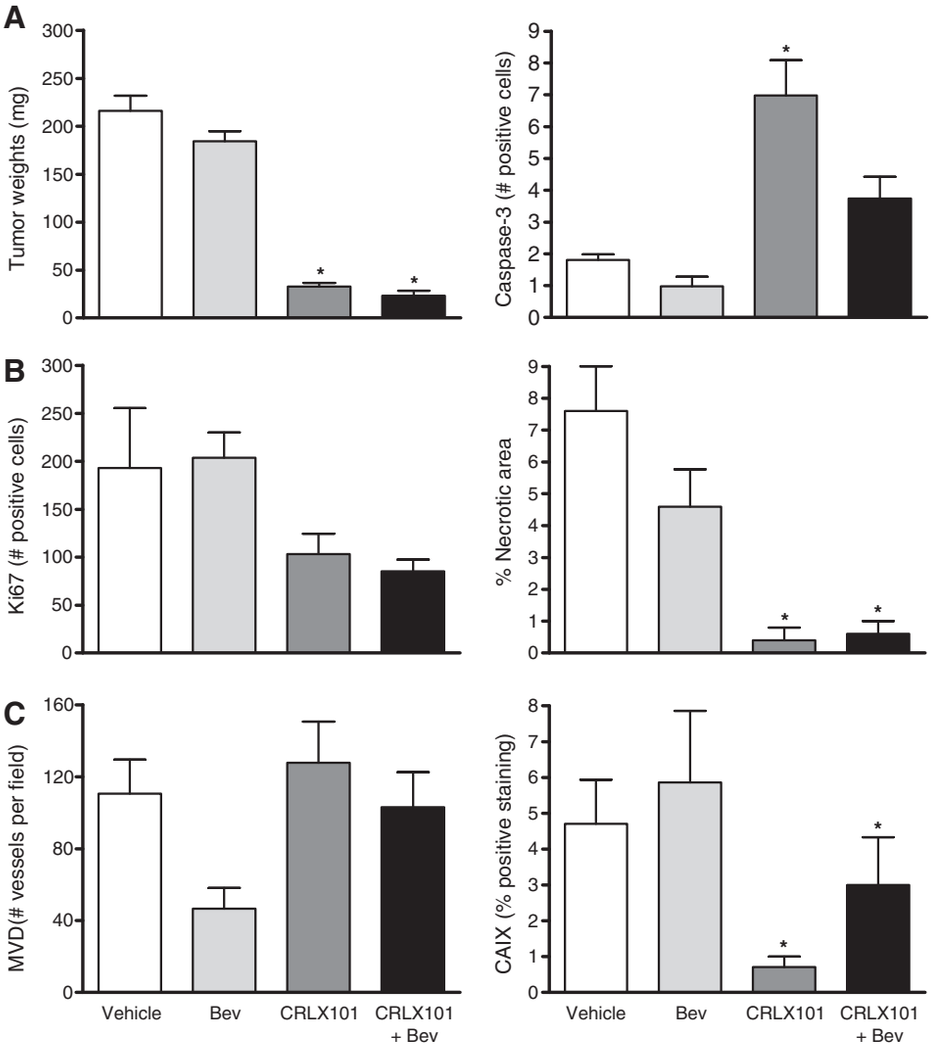

Figure 4.
Immunohistochemistry analysis of tumor metastases treated with CRLX101, with and without bevacizumab. A, differences in tumor weights and cleaved caspase-3 staining for apoptosis in SKOV3-13 tumors after 2 weeks of therapy (2 doses of CRLX101 and 4 doses of bevacizumab). All tumor masses were collected from the peritoneal cavity and pooled to determine total tumor weight. B, Ki67 staining for cell proliferation and H&E staining for tumor necrosis assessment. C, CD31 staining for MVD and CAIX staining as a downstream marker for HIF1α. Error bars are SD for tumor weights and SEM for all stainings; *, $P < 0.05$ vs. vehicle; 5 tumors/group.

proteins and most importantly, and (iv) severe systemic toxicity (35, 36). A number of camptothecin analogues have been developed to address these limitations, with topotecan (Hycamtin; GlaxoSmithKline) currently approved as a second-line therapy for ovarian, cervical, and small cell lung cancer, and irinotecan (Camptosar; Pfizer) approved as first- and second-line therapy for colorectal cancer (37). Topotecan and irinotecan show similar potency as camptothecin but still exhibit suboptimal pharmacokinetics and dose-limiting toxicities (12, 19). CRLX101 was designed with the intention of addressing a number of limitations associated with camptothecin: the conjugation stably maintains camptothecin in its active form, CRLX101 is more soluble compared with camptothecin by three orders of magnitude, and preclinical studies have shown improved tumor biodistribution, thus limiting systemic exposure and toxicity (20, 24, 32).

Chronic administration of anticancer agents, achieved by metronomic administration of either standard chemotherapy drugs at lower doses or reformulated drugs with long half-lives, has been demonstrated to inhibit HIF1α (9, 11, 13–15). In this study, CRLX101, given in a metronomic-like manner of weekly injections over long periods of time, showed potent antitumor efficacy and was an effective inhibitor of HIF1α in our preclinical models of advanced ovarian cancer. When administered at the MTD, CRLX101 significantly reduced tumor burden in mice bearing SKOV3-13, with all treated mice alive at the end of an 8-month treatment. Notably, CRLX101 administered at its MTD was sufficiently efficacious that it was not possible to show synergy or improved activity with bevacizumab. In contrast, when CRLX101 was administered at a lower, less efficacious dose, an obvious synergy when combined with bevacizumab was observed both in terms of metastatic tumor burden and mice survival. These results using the lower dose of CRLX101 are notable because of differences in camptothecin activity between mice and humans. Camptothecin contains a lactone ring that exists in both a closed active form and an open inactive form. In mouse plasma, the open and closed forms exist in a 50:50 ratio but in human plasma the ratio shifts to 90:10 (38). This shift of 50% activity in mice to 10% or less in humans may lead to the large discrepancies in the efficacy of camptothecin, and thus CRLX101, observed when treating tumors in mice compared

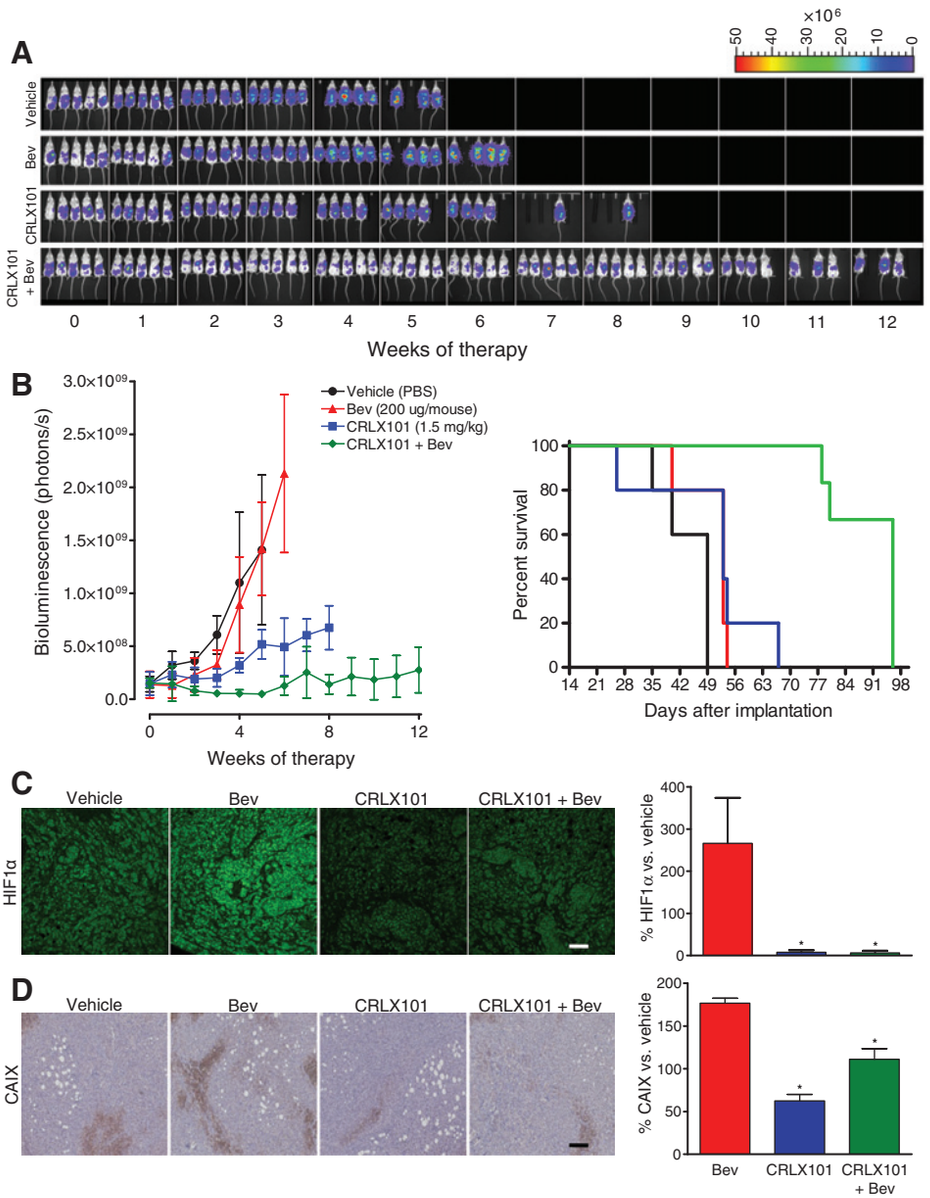

Figure 5.
Bevacizumab combined with a lower dose of CRLX101 shows improved antitumor efficacy in mice with advanced, metastatic disease. A and B, bioluminescence images showing change in tumor burden of SKOV3-13 tumor-bearing mice treated with vehicle, bevacizumab monotherapy (200 μg/mouse), CRLX101 (1.5 mg/kg) monotherapy ($P < 0.001$ vs. vehicle), and CRLX101 + bevacizumab ($P < 0.001$ vs. vehicle and $P < 0.05$ vs. CRLX101 monotherapy) and corresponding survival curves for all groups. Treatments were initiated on day 14 after tumor cell implantation into the peritoneal cavity. Median survival (in days): vehicle (49), bevacizumab (53), CRLX101 (53) and CRLX101 + bevacizumab (96; $P < 0.001$ vs. vehicle; error bars are SD, 5 mice/group). C, representative images of HIF1α immunofluorescence staining (left), and average staining from 4 mice (right). D, representative images of CAIX immunohistochemistry staining (left), and average staining from 4 mice (right). All images taken at ×10 objective, scale bars are 200 μm. Results are reported as percentage of tumor area with positive staining compared with vehicle-treated tumors. Error bars are SEM, 4 mice/group; *, $P < 0.05$ vs. bevacizumab monotherapy. All tumors were isolated after three weeks of therapy (3 doses of CRLX101 and 6 doses of bevacizumab).

with humans. It is, therefore, not surprising that the dramatic effects seen preclinically, such as the complete tumor eradication after only three doses of CRLX101, are not observed in the clinic. In this regard, the synergy we observed between the less efficacious lower dose of CRLX101 with bevacizumab is encouraging. Our results suggest the potential for improved efficacy when CRLX101 is combined upfront with bevacizumab in the clinic.

There are several theories proposed to explain the effects of combining antiangiogenic agents with chemotherapy on drug delivery. The vessel "normalization" hypothesis suggests that superior antitumor efficacy is observed when combining bevacizumab with chemotherapy because the antiangiogenic agent structurally and functionally normalizes some of the remaining abnormal tumor vasculature, leading to improved intratumoral drug delivery and distribution, thus enhancing chemotherapy efficacy (39). However, some recent studies have shown the opposite, that chemotherapy drug delivery is impaired as a result of bevacizumab therapy causing vascular damage and tumor necrosis (40, 41). Because the delivery of CRLX101 to tumor cells relies in part on the leaky nature of tumor vasculature, we evaluated whether combining CRLX101 with bevacizumab would result in improved, impaired, or unaffected CRLX101 drug delivery to tumors. Our results demonstrated that CRLX101 uptake into tumors was reduced both when administered concomitantly with bevacizumab and 7 days later (Supplementary Fig. S5). Nevertheless, even with such reductions in intratumoral chemotherapy drug delivery, overall efficacy of the combination treatment remains superior, or at least equivalent to chemotherapy alone, highlighting the controversy regarding how such combinations improve overall efficacy (2, 42).

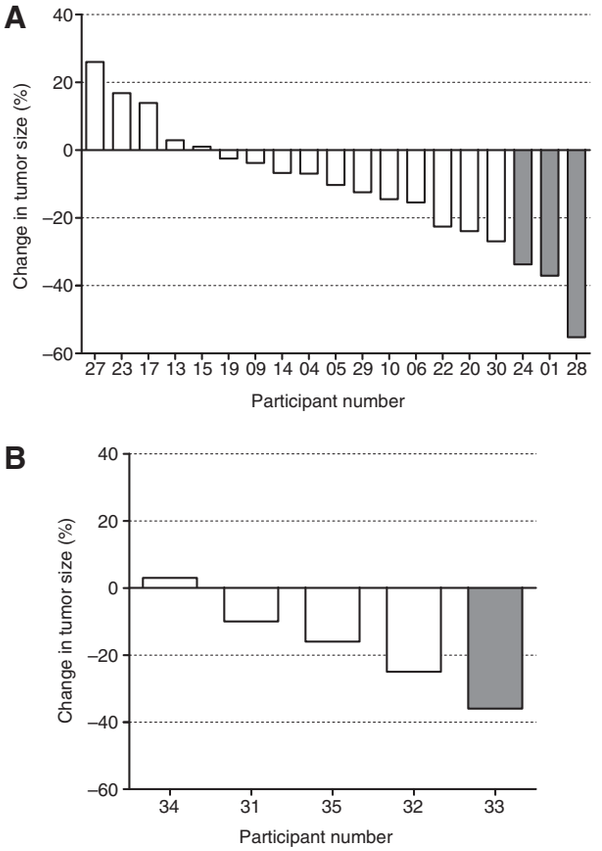

Figure 6.
Waterfall plots of best response in patients with RECIST-evaluable disease in the 19 platinum-resistant ovarian, tubal, and peritoneal cancer patients treated with CRLX101 monotherapy (A) and in the 5 platinum-resistant ovarian, tubal, and peritoneal cancer patients treated with CRLX101 in combination with bevacizumab (B). Gray bars indicate RECIST responses with tumor reductions of greater than or equal to 30%.

At its MTD, CRLX101 appears potent even as a monotherapy. In this regard, clinical results in patients with ovarian cancer treated with CRLX101 monotherapy demonstrated an overall response rate of 16% in 19 evaluable patients with platinum-resistant disease, a patient population with poor prognosis. Additionally, when a lower dose of CRLX101 was combined with bevacizumab, the combination appeared superior to either drug alone. These data compare favorably with the response rate for FDA-approved topotecan, which was 3% in second- and third-line platinum-resistant ovarian cancer (43). Although the response rate for topotecan is 12% in patients with second-line platinum-resistant ovarian cancer (according to the FDA label for topotecan), 85% of patients in the CRLX101 trial were third-line or higher, making the former study population a more accurate comparison. Preliminary data for the combination of CRLX101 plus bevacizumab in ovarian cancer are promising but it is too early to conclude whether they are superior to CRLX101 monotherapy. Emerging clinical results of a phase Ib/IIa study of CRLX101 plus bevacizumab in patients with refractory metastatic renal cell carcinoma (mRCC) have been reported elsewhere and suggest promising activity. In that setting, the combination of CRLX101 and bevacizumab appears to be well tolerated with no dose-limiting toxicities reported to date, and in the first 9 evaluable patients, 3 patients (33%) achieved confirmed partial responses with an additional 4 patients demonstrating stable disease (44, 45). All patients had refractory disease that was previously treated with multiple lines of therapy. These data are in contrast to the complete absence of responses observed in 14 evaluable mRCC patients treated with topotecan (46), and a 4% response rate in mRCC patients treated with bevacizumab (47).

In aggregate, the positive results observed in our preclinical model of advanced ovarian cancer and preliminary response rates observed for CRLX101 in patients with ovarian cancer (along with encouraging results observed in highly refractory mRCC patients) suggest that CRLX101 combined with bevacizumab can potentially provide a new standard of care in settings where HIF1α contributes to resistance to current standards of care. These data support the rationale for undertaking the phase II study that is currently evaluating this combination therapy regimen in patients with recurrent ovarian, tubal, and peritoneal cancer. This multiprong approach of combining targeted antiangiogenic agents with potent cytotoxic drugs, delivered metronomically, and in ways that secondarily counteract adaptive HIF1α responses, may lead to more effective use of antiangiogenic drugs in the clinic.

Disclosure of Potential Conflicts of Interest

S. Eliasof has ownership interest (including patents) in Cerulean Pharma. E. Garmey is an employee of and has ownership interest (including patents) in Cerulean Pharma. R.S. Kerbel reports receiving a research grant from and is a consultant/advisory board member for Cerulean Pharma. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: E. Pham, S. Eliasof, E. Garmey, S. Man, C. Krasner, R.S. Kerbel

Development of methodology: E. Pham, E. Garmey, S. Man

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): E. Pham, M.J. Birrer, D. Lazarus, C.R. Lee, S. Man, C.G. Peters, P. Xu, C. Krasner

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): E. Pham, M.J. Birrer, S. Eliasof, E. Garmey, D. Lazarus, C.G. Peters, P. Xu

Writing, review, and/or revision of the manuscript: E. Pham, M.J. Birrer, S. Eliasof, E. Garmey, U.A. Matulonis, C. Krasner, R.S. Kerbel

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M.J. Birrer, E. Garmey, S. Man

Study supervision: M.J. Birrer, E. Garmey, D. Lazarus, C. Krasner, R.S. Kerbel

Acknowledgments

The authors thank Cassandra Cheng for outstanding administrative assistance.

Grant Support

This work was supported by grants to R.S. Kerbel from the Canadian Institutes of Health (CIHR, grant number MOP-119499), the Canadian Breast Cancer Foundation (CBCF, grant number not applicable), and a donation from Dr. Rena Buckstein administered through the Israel Cancer Research Fund (ICRF, grant number not applicable). Financial support was also provided by Cerulean Pharma Inc.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received November 2, 2014; revised December 9, 2014; accepted December 12, 2014; published OnlineFirst December 18, 2014.

References

1. Ferrara N, Kerbel RS. Angiogenesis as a therapeutic target. Nature 2005;438:967–74.
2. Kerbel RS. Tumor angiogenesis. N Engl J Med 2008;358:2039–49.
3. Ebos JM, Kerbel RS. Antiangiogenic therapy: impact on invasion, disease progression, and metastasis. Nat Rev Clin Oncol 2011;8:210–21.
4. Rapisarda A, Melillo G. Overcoming disappointing results with antiangiogenic therapy by targeting hypoxia. Nat Rev Clin Oncol 2012;9:378–90.
5. Bergers G, Hanahan D. Modes of resistance to anti-angiogenic therapy. Nat Rev 2008;8:592–603.
6. Semenza GL. Cancer–stromal cell interactions mediated by hypoxia-inducible factors promote angiogenesis, lymphangiogenesis, and metastasis. Oncogene 2013;32:4057–63.
7. Daponte A, Ioannou M, Mylonis I, Simos G, Minas M, Messinis IE, et al. Prognostic significance of hypoxia-inducible factor 1 alpha(HIF-1 alpha) expression in serous ovarian cancer: an immunohistochemical study. BMC Cancer 2008;8:335.
8. Rapisarda A, Hollingshead M, Uranchimeg B, Bonomi CA, Borgel SD, Carter JP, et al. Increased antitumor activity of bevacizumab in combination with hypoxia inducible factor-1 inhibition. Mol Cancer Ther 2009;8:1867–77.
9. Sapra P, Kraft P, Pastorino F, Ribatti D, Dumble M, Mehlig M, et al. Potent and sustained inhibition of HIF-1alpha and downstream genes by a polyethyleneglycol-SN38 conjugate, EZN-2208, results in anti-angiogenic effects. Angiogenesis 2011;14:245–53.
10. Merritt WM, Danes CG, Shahzad MM, Lin YG, Kamat AA, Han LY, et al. Anti-angiogenic properties of metronomic topotecan in ovarian carcinoma. Cancer Biol Ther 2009;8:1596–603.
11. Rapisarda A, Zalek J, Hollingshead M, Braunschweig T, Uranchimeg B, Bonomi CA, et al. Schedule-dependent inhibition of hypoxia-inducible factor-1alpha protein accumulation, angiogenesis, and tumor growth by topotecan in U251-HRE glioblastoma xenografts. Cancer Res 2004;64:6845–8.
12. Houghton PJ, Cheshire PJ, Hallman JD II, Lutz L, Friedman HS, Danks MK, et al. Efficacy of topoisomerase I inhibitors, topotecan and irinotecan, administered at low dose levels in protracted schedules to mice bearing xenografts of human tumors. Cancer Chemother Pharmacol 1995;36:393–403.
13. Khan N, Hou H, Hodge S, Kuppusamy M, Chen EY, Eastman A, et al. Recurrent low-dose chemotherapy to inhibit and oxygenate head and neck tumors. Adv Exp Med Biol 2014;812:105–11.
14. Lee K, Qian DZ, Rey S, Wei H, Liu JO, Semenza GL. Anthracycline chemotherapy inhibits HIF-1 transcriptional activity and tumor-induced mobilization of circulating angiogenic cells. Proc Natl Acad Sci U S A 2009;106:2353–8.
15. Zeng L, Ou G, Itasaka S, Harada H, Xie X, Shibuya K, et al. TS-1 enhances the effect of radiotherapy by suppressing radiation-induced hypoxia-inducible factor-1 activation and inducing endothelial cell apoptosis. Cancer Sci 2008;99:2327–35.
16. Kummar S, Raffeld M, Juwara L, Horneffer Y, Strassberger A, Allen D, et al. Multihistology, target-driven pilot trial of oral topotecan as an inhibitor of hypoxia-inducible factor-1alpha in advanced solid tumors. Clin Cancer Res 2011;17:5123–31.
17. Svenson S. What nanomedicine in the clinic right now really forms nanoparticles? Wiley Interdiscip Rev 2014;6:125–35.
18. Allen TM, Cullis PR. Liposomal drug delivery systems: from concept to clinical applications. Adv Drug Deliv Rev 2013;65:36–48.
19. Svenson S, Wolfgang M, Hwang J, Ryan J, Eliasof S. Preclinical to clinical development of the novel camptothecin nanopharmaceutical CRLX101. J Control Release 2011;153:49–55.
20. Schluep T, Cheng J, Khin KT, Davis ME. Pharmacokinetics and biodistribution of the camptothecin-polymer conjugate IT-101 in rats and tumor-bearing mice. Cancer Chemother Pharmacol 2006;57:654–62.
21. Cheng J, Khin KT, Davis ME. Antitumor activity of beta-cyclodextrin polymer-camptothecin conjugates. Mol Pharm 2004;1:183–93.
22. Davis ME, Chen ZG, Shin DM. Nanoparticle therapeutics: an emerging treatment modality for cancer. Nat Rev Drug Discov 2008;7:771–82.
23. Maeda H, Nakamura H, Fang J. The EPR effect for macromolecular drug delivery to solid tumors: improvement of tumor uptake, lowering of systemic toxicity, and distinct tumor imaging *in vivo*. Adv Drug Deliv Rev 2013;65:71–9.
24. Schluep T, Hwang J, Hildebrandt IJ, Czernin J, Choi CH, Alabi CA, et al. Pharmacokinetics and tumor dynamics of the nanoparticle IT-101 from PET imaging and tumor histological measurements. Proc Natl Acad Sci U S A 2009;106:11394–9.
25. Gaur S, Wang Y, Kretzner L, Chen L, Yen T, Wu X, et al. Pharmacodynamic and pharmacogenomic study of the nanoparticle conjugate of camptothecin CRLX101 for the treatment of cancer. Nanomedicine 2014;10:1477–86.
26. Eliasof S, Lazarus D, Peters CG, Case RI, Cole RO, Hwang J, et al. Correlating preclinical animal studies and human clinical trials of a multifunctional, polymeric nanoparticle. Proc Natl Acad Sci U S A 2013;110:15127–32.
27. Hashimoto K, Man S, Xu P, Cruz-Munoz W, Tang T, Kumar R, et al. Potent preclinical impact of metronomic low-dose oral topotecan combined with the antiangiogenic drug pazopanib for the treatment of ovarian cancer. Mol Cancer Ther 2010;9:996–1006.
28. Tait LR, Pauley RJ, Santner SJ, Heppner GH, Heng HH, Rak JW, et al. Dynamic stromal-epithelial interactions during progression of MCF10DCIS.com xenografts. Int J Cancer 2007;120:2127–34.
29. Young C, Schluep T, Hwang J, Eliasof S. CRLX101 (formerly IT-101)-A novel nanopharmaceutical of camptothecin in clinical development. Curr Bioact Compd 2011;7:8–14.
30. Guerin E, Man S, Xu P, Kerbel RS. A model of postsurgical advanced metastatic breast cancer more accurately replicates the clinical efficacy of antiangiogenic drugs. Cancer Res 2013;73:2743–8.
31. Cruz-Munoz W, Di Desidero T, Man S, Xu P, Jaramillo ML, Hashimoto K, et al. Analysis of acquired resistance to metronomic oral topotecan chemotherapy plus pazopanib after prolonged preclinical potent responsiveness in advanced ovarian cancer. Angiogenesis 2014;17:661–73.
32. Schluep T, Hwang J, Cheng J, Heidel JD, Bartlett DW, Hollister B, et al. Preclinical efficacy of the camptothecin-polymer conjugate IT-101 in multiple cancer models. Clin Cancer Res 2006;12:1606–14.
33. Hynninen P, Vaskivuo L, Saarnio J, Haapasalo H, Kivela J, Pastorekova S, et al. Expression of transmembrane carbonic anhydrases IX and XII in ovarian tumours. Histopathology 2006;49:594–602.
34. Krasner CN, Birrer MJ, Berlin ST, Horowitz NS, Buss MK, Eliasof S, et al. Phase II clinical trial evaluating CRLX101 in recurrent ovarian, tubal, and peritoneal cancer. J Clin Oncol 32, 2014 (Suppl 5; abstr 5581).
35. Scott DO, Bindra DS, Stella VJ. Plasma pharmacokinetics of lactone and carboxylate forms of 20(S)-camptothecin in anesthetized rats. Pharm Res 1993;10:1451–7.
36. Schaeppi U, Fleischman RW, Cooney DA. Toxicity of camptothecin (NSC-100880). Cancer Chemother Rep 1974;5:25–36.
37. Zunino F, Pratesi G. Camptothecins in clinical development. Expert Opin Investig Drugs 2004;13:269–84.
38. Giovanella BC, Harris N, Mendoza J, Cao Z, Liehr J, Stehlin JS. Dependence of anticancer activity of camptothecins on maintaining their lactone function. Ann N Y Acad Sci 2000;922:27–35.
39. Chauhan VP, Stylianopoulos T, Martin JD, Popovic Z, Chen O, Kamoun WS, et al. Normalization of tumour blood vessels improves the delivery of nanomedicines in a size-dependent manner. Nat Nanotechnol 2012;7:383–8.
40. Cesca M, Bizzaro F, Zucchetti M, Giavazzi R. Tumor delivery of chemotherapy combined with inhibitors of angiogenesis and vascular targeting agents. Front Oncol 2013;3:259.
41. Arjaans M, Oude Munnink TH, Oosting SF, Terwisscha van Scheltinga AG, Gietema JA, Garbacik ET, et al. Bevacizumab-induced normalization of blood vessels in tumors hampers antibody uptake. Cancer Res 2013;73:3347–55.
42. Kerbel RS. Antiangiogenic therapy: a universal chemosensitization strategy for cancer? Science 2006;312:1171–5.
43. Poveda AM, Selle F, Hilpert F, Reuss A, Pasic A, Savarese A, et al.Weekly paclitaxel (PAC), pegylated liposomal doxorubicin (PLD) or topotecan (TOP) ± bevacizumab (BEV) in platinum (PT)-resistant recurrent ovarian cancer (OC): analysis by chemotherapy (CT) cohort in the GCIG AURELIA randomised phase III trial. Ann Oncol 23, 2012 (suppl 9; abstr LBA26).
44. Keefe SM, Heitjan D, Hennessey M, Robinson J, Mykulowicz K, Marshall A, et al. Interim results of a phase 1b/2a study evaluating the nano pharmaceutical CRLX101 with bevacizumab (bev) in the treatment of patients (pts) with refractory metastatic renal cell carcinoma (mRCC). J Clin Oncol 32, 2014 (suppl 4; abstr 412).

45. Keefe SM, Hennessey M, Gunnarsson O, Mamtani R, Vaughn DJ, Hoffman-Censits JH, et al.Phase 1b/2a study of the nanopharmaceutical CRLX101 with bevacizumab (bev) in the treatment of patients (pts) with refractory metastatic renal cell carcinoma (mRCC): results from the planned interim analysis [abstract]. In: Proceedings of the 105th Annual Meeting of the American Association for Cancer Research; 2014 Apr 5–9; San Diego (CA)/ Philadelphia (PA): AACR; 2014. (abstr nr CT340).
46. Law TM, Ilson DH, Motzer RJ. Phase II trial of topotecan in patients with advanced renal cell carcinoma. Invest New Drugs 1994;12:143–5.
47. Hainsworth JD, Shipley DL, Reeves J Jr, Arrowsmith ER, Barnes EK, Waterhouse DM. High-dose bevacizumab in the treatment of patients with advanced clear cell renal carcinoma: a phase II trial of the Sarah Cannon Oncology Research Consortium. Clin Genitourin Cancer 2013;11:283–9 e1.