CD20-Directed Small Modular Immunopharmaceutical, TRU-015, Depletes Normal and Malignant B Cells

Abstract

**Purpose:** CD20-directed therapy with rituximab is effective in many patients with malignant lymphoma or follicular lymphoma. However, relapse frequently occurs within 1 year, and patients become increasingly refractory to retreatment. Our purpose was to produce a compact, single-chain CD20-targeting immunotherapeutic that could offer therapeutic advantages in the treatment of B-cell lymphoma.

**Experimental Design:** Rituximab is a chimeric antibody containing two heavy chains and two light chains. Here, we describe the properties of TRU-015, a small modular immunopharmaceutical specific for CD20, encoded by a single-chain construct containing a single-chain Fv specific for CD20 linked to human IgG1 hinge, CH2, and CH3 domains but devoid of CH1 and CL domains.

**Results:** TRU-015 mediates potent direct signaling and antibody-dependent cellular cytoxicity but has reduced size and complement-mediated cytotoxicity activity compared with rituximab. TRU-015 is a compact dimer of 104 kDa that comigrates with albumin in size exclusion chromatography and retains a long half-life in vivo. TRU-015 induced growth arrest in multiple B-lymphoma cell lines in vitro and showed effective antitumor activity against large, established subcutaneous Ramos or Daudi xenograft tumors in nude mice. TRU-015 also showed rapid, dose-dependent, and durable depletion of peripheral blood B cells following single-dose administration to nonhuman primates.

**Conclusion:** These results indicate that TRU-015 may improve CD20-directed therapy by effectively depleting embedded malignant B cells and nonmalignant pathogenic B cells and do so with reduced complement activation.

The clinical success of rituximab has shown CD20 to be an effective target for the depletion of normal or malignant B cells. The ability of CD20 to support strong antibody-mediated complement-mediated cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC) activity was recognized early (1) and a murine IgG2a monoclonal antibody (mAb) to CD20 was shown to deplete malignant B cells in non-Hodgkin’s lymphoma patients (2). A CD20 signal for B-cell growth arrest was also recognized early with the B1 mAb (3, 4), whereas another CD20 mAb, 1F5, induced proliferation of CD40-stimulated B cells (5, 6). The synergy of CD20 mAbs with cytotoxic drugs was not recognized until later (7–11), but combining rituximab with chemotherapy has had a major clinical effect in the treatment of chronic lymphocytic leukemia, follicular cell lymphoma, and aggressive lymphomas. Since approval in 1997, rituximab has become the standard of care in malignant or follicular B-cell lymphomas (12–14) and in chronic lymphocytic leukemia and aggressive lymphomas when combined with chemotherapy (15–21). In spite of the impressive activity of rituximab, most patients with indolent lymphoma still relapse and become increasingly refractory to therapy.

Efforts to increase the potency of CD20-directed therapy include conjugation of antibody with a radioisotope [Bexxar (GSK); Zevalin (Biogen IDEC)] or cytotoxics (22–25) or molecular engineering of mAbs with enhanced effector function (26–30). Molecular efforts have focused on increasing direct signaling, ADCC and CDC activity, although the importance of CDC remains speculative because complement activation has been temporally linked to infusion reactions in patients (31). Here, we describe the activity of a CD20-directed single-chain protein. The molecule exhibited potent anti-lymphoma activity in vitro, in nude mouse xenograft models, and showed rapid and sustained B-cell depletion in nonhuman primates. The CD20-directed small modular
**Translational Relevance**

Despite high initial response rates, CD20-targeting antibody therapeutics do not produce durable responses and patients with indolent disease are prone to relapse. We describe herein a compact, homogeneous single-chain immunotherapeutic, TRU-015, for the targeted elimination of CD20+ lymphoma cells. TRU-015 showed both direct, proapoptotic activity and Fc-mediated cellular cytotoxicity against B lymphoma cells. Despite reduced complement-mediated cytotoxicity, the molecule was highly active against two xenograft models of established, large volume human lymphoma. The data suggest that TRU-015 may improve access to and therapeutic activity against embedded lymphoma cells that pose a potential reservoir of residual disease. Based on these attributes, TRU-015 has entered clinical trials for treatment of lymphoma and inflammatory disease.

immunopharmaceutical protein has reduced CDC activity but retains strong ADCC and potient direct signaling activity in vivo, indicating that CDC activity can be reduced without sacrificing efficacy in animal models. TRU-015 has shown clinical efficacy and tolerability in phase IIA and IIB studies in patients with rheumatoid arthritis, and clinical development efforts for the treatment of lymphoma and inflammatory disease are ongoing.

**Materials and Methods**

**Cells and reagents.** The murine anti-human CD20 hybridoma 2H7 has been reported previously (5). Cell lines DoHH2 (follicular lymphoma), WSIU-NHL (histiocytic lymphoma), SU-DHL-6 (non-Burkitt’s B lymphoma), and Rec-1 (mantle cell lymphoma) were obtained from DSMZ. WIL2-S (lymphoblastoid), SU-DHL-4 (diffuse large cell), RL (diffuse large cell), Ramos (Burkitt’s lymphoma), Daudi (Burkitt’s), and Raji (Burkitt’s) cell lines were from the American Type Culture Collection.

**Cloning and expression of TRU-015.** Variable domains were cloned from the 2H7 hybridoma as described previously (32, 33). PCR-amplified products were cloned into PCR 2.1-TOPO vectors (Invitrogen), sequenced using ABI Big Dye Terminator 3.1 reagents, and analyzed with an ABI 3100 DNA sequencer. The 2H7 V regions were assembled into cassettes to form the antigen-binding domain of a small modular immunopharmaceutical. Small modular immunopharmacological is a class of modified, recombinant constructs linking an antigen-binding domain such as a single-chain Fv to an effector domain such as human immunoglobulin constant region(s). To produce protein for characterization, DNA was transfected into COS cells using PEI or DEAE-dextran for transient expression as described previously (34, 35). For stable expression, CHO DG44 cells were transfected by electroporation and cultured in serum-free, Excell 302 medium (JRH Biosciences) containing 4 mmol/L glutamine, 1 mmol/L sodium pyruvate, 100 μmol/L nonessential amino acids, 0.5 μg/mL recombinant insulin, and 20 to 500 μmol/L methotrexate.

**Competition binding.** WIL2-S B cells (1.5 × 10^7 per well) were pelleted in 96-well plates and resuspended in 100 μL of 2 nmol/L monoclonal anti-human CD20/FTTC (Ancell) in FACS buffer (1% fetal bovine serum in Dulbecco’s PBS; Life Technologies/Invitrogen) and incubated on ice for 45 min in the dark. The cells were then washed by centrifugation and resuspension three times in FACS buffer. Purified anti-CD20 (Ancell), TRU-015, or anti-HER-2 mAb (Herceptin; Genentech) as an irrelevant control were diluted to 20 nmol/L in FACS buffer. Sample proteins were titrated in triplicate from 20 to 0.04 nmol/L in 2-fold dilutions in FACS buffer. Titrations were done in triplicate in two round-bottomed 96-well plates and washed cells were resuspended in 100 μL diluted proteins. Following incubation on ice for 60 min in the dark, the cells were then washed by centrifugation and resuspension twice in Dulbecco’s PBS, and resuspended cells were added to 100 μL of 2% paraformaldehyde (USB) and then read and analyzed on a FACS Calibur with CellQuest Pro software (Becton Dickinson).

**In vitro growth inhibition.** Five different CD20+ lymphoma cell lines were plated at 5 to 10,000 per well in 150 μL medium and then treated for 96 h with serial dilutions of TRU-015. Following incubation, plates were labeled with 100 μL ATPlile reagent according to the protocol product (Perkin-Elmer) and relative light units was measured on a TopCount plate reader (Perkin-Elmer). The IC50 values listed were determined as the concentration where cell growth was inhibited 50% compared with untreated wells. Data presented are the mean ± SD of three independent assays.

**Fc-dependent cellular cytotoxicity.** BJAB Burkitt’s lymphoma cells (1 × 10^5/mL) were labeled with 500 μCi/mL 51Cr sodium chromate for 2 h at 37°C in IMDM with 10% fetal bovine serum. The 51Cr-loaded BJAB cells were then washed three times in RPMI with 10% fetal bovine serum (complete medium) and resuspended to 4 × 10^4/mL. Peripheral blood mononuclear cells from in-house donors were isolated from heparinized whole blood via centrifugation over lymphocyte separation medium, washed twice, and resuspended at a density of 5 × 10^7/mL in complete medium. Reagent samples were prepared to complete medium and serial dilutions for each reagent were prepared and then added to 96-well U-bottomed plates at 50 μL/well for the indicated final concentrations. The 51Cr-labeled BJAB cells were then added to the plates at 50 μL/well (2 × 10^4 per well). Peripheral blood mononuclear cells were added to the plates at 100 μL/well (5 × 10^5 per well) for a final ratio of 25:1 effectors (peripheral blood mononuclear cells):target (BJAB). Effectors and targets were added to medium alone to measure background killing. The 51Cr-labeled BJAB cells were added to medium alone to measure spontaneous release of 51Cr and to medium with 5% NP-40 to measure maximal release of 51Cr. The plates were incubated for 6 h at 37°C in 5% CO2. Fifty microliters of supernatant from each well were then transferred to a Lumaplate-96 and dried overnight at room temperature. cpm was read on a Packard TopCount-NXT. Percent specific killing was calculated as (cpm of sample) - (cpm spontaneous release) / (cpm maximal release) - (cpm spontaneous release) × 100.

**CDC.** Ramos B cells (5 × 10^5) were added to 96-well V-bottomed plates in 50 μL IMDM (Life Technologies/Invitrogen). TRU-015 (Trubion Pharmaceuticals) or rituximab (Genentech/IDEC) in IMDM, or IMDM alone (control), was added to the wells in 50 μL at two times the indicated final concentration. The cells were then incubated in medium for 45 min at 37°C. The cells were washed two times in IMDM with no fetal bovine serum and resuspended in IMDM with normal human serum (Quidel) at the indicated concentrations. The cells were then incubated for 60 min at 37°C, washed, and resuspended in 125 μL PBS with 2% fetal bovine serum (Life Technologies/Invitrogen; staining medium). The cells were transferred to FACS cluster tubes (Costar) and 125 μL staining medium with 5 μg/mL propidium iodide (Molecular Probes) was added. The cells were incubated with the propidium iodide for 15 min at room temperature in the dark and then read and analyzed on a FACS Calibur with CellQuest software (Becton Dickinson). Spontaneous background lysis of control samples was subtracted from TRU-015 or rituximab-mediated cell lysis to yield specific cell lysis. Percent specific lysis was calculated as specific lysis signal / maximal lysis signal × 100.

**Established human tumor xenografts in nude mice: Ramos xenograft.** Five million Ramos cells were injected subcutaneously into the flank of female athymic nu/nu mice. When palpable tumors were apparent, 7 to 8 days post-tumor inoculation (day 0), mice were sorted into groups (n = 10) with equivalent mean tumor volumes and injected
intravenously with either 100 µg TRU-015 or rituximab. Human IgG (HulG; 100 µg) was administered to mice in the control group. Mice were dosed on days 0, 2, 4, 6, and 8 of the studies (every other day x 5). Tumors were measured with calipers three times per week by an observer blinded to the treatment groups. Tumor volumes were calculated using the formula: \( V = \frac{1}{2} \times \text{length} \times \text{width}^2 \). This report summarizes pooled data from four separate experiments. The pooled baseline mean tumor volume for these experiments was 397 mm³, the median baseline tumor size was 394 mm³, and the range was 276 to 507 mm³. Survival data were analyzed for statistically significant differences between groups using the log-rank test.

**Daudi xenografts.** Daudi lymphoma model was established as described for the Ramos model (above) and groups (n = 10 animals per group) with mean tumor volume of 228 mm³ at baseline were injected intravenously with either 100 µg TRU-015, rituximab, or HulG (control). Dosing, tumor volume determination, and analysis of survival data were as described for the Ramos model.

**B-cell depletion in nonhuman primate.** A multiple-dose toxicology study in cynomolgus monkeys, conducted according to good laboratory practices, was done at a contract laboratory. The multiple-dose toxicity study evaluated 12 naive monkeys (6 male and 6 female) at each dose level of TRU-015 (0.1, 5, and 50 mg/kg) and with a PBS control. Animals were dosed intravenously once weekly for 4 weeks and evaluated for adverse effects, pharmacodynamics (B-cell depletion), pharmacokinetics, and immunogenicity. Three animals per sex per group were sacrificed at the end of the dosing period (day 29), and the remaining animals were followed and sacrificed after 6 full months of recovery, anticipating a long duration of B-cell depletion. A fifth cohort of 4 animals (2 male and 2 female) received 50 mg/kg rituximab (also weekly for 4 weeks) and were followed with clinical observations and pharmacodynamics only.

### Results

**Construction and expression of TRU-015.** TRU-015 is encoded by a single-chain construct and is expressed as a homodimer of ~104 kDa (Fig. 1). TRU-015 was constructed using a single-chain Fv made from the heavy and light chain variable regions from the anti-human CD20 hybridoma 2H7 fused to HulG1 CH2 and CH3 domains through a modified HulG1 hinge sequence as described in Materials and Methods. The molecular ribbon diagram shown in Fig. 1 was constructed based on homology modeling of the primary amino acid sequence of TRU-015.

**Competition binding and direct activity of TRU-015.** Binding of TRU-015 was compared with that of the parent mAb 2H7 by assessing their relative abilities to compete a commercially available, FITC-labeled 2H7 mAb from the surface of CD20⁺ Wil-2S cells. Figure 2A shows that the competition binding of TRU-015 was slightly diminished compared with that of the parent 2H7 mAb. An irrelevant (nonbinding) mAb showed no ability to compete labeled anti-CD20 from target cells within the concentration range tested.

**Direct effects of TRU-015 on lymphoma cell lines.** To assess the direct effect of TRU-015 on lymphoma cell proliferation, five different CD20⁺ B lymphoma cell lines were treated for 96 h with serial dilutions of TRU-015. ATP release assays were used to compare the percentage of viable cells remaining in treated or untreated (control) wells as described in Materials and Methods. Figure 2B shows IC50 values, defined as concentrations where cell growth was inhibited 50% compared with cells in untreated wells, ranged from 8 to 40 nmol/L TRU-015 on four of five lines. Although sensitive to TRU-015, IC50 for WSU-NHL cells was not reached within the concentration range tested. These data indicated that TRU-015 could directly deplete target cells independent of effector cells or complement.

**Effector-mediated activities of TRU-015**

**Fc-dependent cellular cytotoxicity.** To assess whether the IgG1 Fc region of TRU-015 could interact with Fcy receptors found on natural killer cells and mediate target cell lysis, in vitro assays were conducted to determine the ADCC activity of TRU-015 or rituximab against CD20⁺ BJAB cells using peripheral blood mononuclear cells from healthy donors as the source of natural killer effectors. Figure 3A illustrates the ability of TRU-015 to induce lysis of CD20⁺ target cells compared with that of rituximab. ADCC mediated by rituximab was increased relative to TRU-015 at concentrations <0.5 µg/mL, whereas ADCC mediated by TRU-015 was comparable with rituximab at concentrations >0.5 µg/mL.

**CDC.** The role of complement in CD20-directed therapy is controversial, contributing to antitumor activity (37), yet potentially linked to untoward infusion site reactions in some patients (31). CDC is initiated by the binding of C1q, a primary component of the complement cascade, to the CH2 domain of antibodies bound to their cognate antigen. This is followed by complement complex assembly and cytolysis. To evaluate CDC activity, TRU-015 or rituximab was incubated with target...
Ramos cells in the presence of normal human serum as a source of complement. Figure 3B shows that both TRU-015 and rituximab mediated Ramos cell killing in a dose-dependent manner. Under these conditions, TRU-015 appeared to be attenuated in CDC activity by 50-fold compared with rituximab.

Activity of TRU-015 against established human tumor xenografts in nude mice. Subcutaneous Ramos tumors were established in nude mice and allowed to reach a mean tumor volume of 397 mm$^3$ before treatment with TRU-015, rituximab, or control HuIgG as described in Materials and Methods. Established, large volume, solid tumors can present a significant challenge to intervention by antibody-based therapies. Reduction in tumor volume was clearly observed during the active dosing phase with TRU-015 (data not shown). Survival data analyzed using the log-rank test showed TRU-015-treated mice had a statistically significant survival advantage ($P = 0.01$) over animals treated with equivalent doses (mg/kg) of control immunoglobulin or rituximab (Fig. 4A). Median survival time was 28 days for TRU-015-treated mice and 13 and 8 days for rituximab or HuIgG (control)-treated animals respectively. Both TRU-015- and rituximab-treated animals showed a statistically significant survival benefit versus HuIgG-treated controls in this setting (Table 1). Twenty-nine percent of mice treated with TRU-015 experienced complete tumor ablation without relapse for the 90-day duration of the study (Fig. 4B), representing a 49% increase in complete tumor regressions compared with rituximab-treated mice. No signs of dose-related toxicity or weight loss were observed in any of the treatment groups.

The efficacy of TRU-015 was similarly assessed against established, moderate size (mean tumor volume, 228 mm$^3$) xenograft tumors of Daudi human lymphoma cells in nude mice. Figure 4C and D show the percent survival and percent of tumor-free animals following treatments. The control (HuIgG)

---

**Fig. 2.** Competition binding and direct in vitro activity of TRU-015. 
A, competition binding of TRU-015 was compared with that of the parent mAb 2H7 and a nonbinding (control) small modular immunopharmaceutical. CD20$^+$ Wil-2S lymphoma cells, labeled with anti-CD20 mAb 2H7-FITC, were combined with increasing concentrations of TRU-015 or mAb 2H7 and washed to remove unbound mAbs and loss of fluorescent signal was determined by flow cytometry. Representative of triplicate determinations. B, direct effect of TRU-015 on cell proliferation. CD20$^+$ lymphoma cell lines were treated for 96 h with serial dilutions of TRU-015. Following incubation, plates were labeled with 100 μL ATP-lite reagent. Columns, mean of at least three independent experiments; bars, SE.

**Fig. 3.** TRU-015 mediates ADCC and CDC activity. 
A, ADCC-mediated specific lysis of TRU-015-treated (●), rituximab-treated (□), or medium (control)-treated (○) target cells was determined by $^{51}$Cr release assay. Target cells were mixed with natural killer-enriched peripheral blood mononuclear cells at an effector/target ratio of 25:1. B, TRU-015-treated (●) or rituximab-treated (□) target cells were incubated at 37°C in 10% human serum. The percentage of nonviable cells was identified by flow cytometry after staining with propidium iodide. Representative of triplicate experiments. Bars, SD.
treatment group had a median survival time of 14 days, whereas those groups treated with 100 μg TRU-015 showed median survival times of 56 days and groups treated with 100 μg rituximab showed a median survival times of 29 days. Survival data analyzed by log-rank test showed TRU-015-treated mice had a statistically significant survival advantage (P = 0.0001) over control treated animals and over those treated with rituximab (Table 1; P = 0.03). Median survival time was 40

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Median survival time (d)*</th>
<th>P (log-rank test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ramos HuIgG</td>
<td>8</td>
<td>—</td>
</tr>
<tr>
<td>TRU-015</td>
<td>28</td>
<td>0.0001</td>
</tr>
<tr>
<td>Rituximab</td>
<td>13</td>
<td>0.0001</td>
</tr>
<tr>
<td>TRU-015 vs rituximab</td>
<td>—</td>
<td>0.0107</td>
</tr>
<tr>
<td>Daudi HuIgG</td>
<td>14</td>
<td>—</td>
</tr>
<tr>
<td>TRU-015</td>
<td>56</td>
<td>0.0001</td>
</tr>
<tr>
<td>Rituximab</td>
<td>29</td>
<td>0.0001</td>
</tr>
<tr>
<td>TRU-015 vs rituximab</td>
<td>—</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*Time for tumors to progress to a maximum burden of 1,500 mm³, requiring sacrifice of the animal as mandated by ADCU guidelines. P values refer to differences between TRU-015 and HuIgG or between rituximab and HuIgG.
Depletion of circulating B cells in NHP. TRU-015 was administered to cynomolgus monkeys to evaluate tolerability and effect of TRU-015 on circulating B cells. Doses (10 mg/kg; 94 μmol/kg) of either TRU-015 or rituximab (10 mg/kg; 60 μmol/kg) effectively depleted B cells in peripheral blood with comparable recovery compared with control animals treated with PBS. B-cell depletion was determined as either the percent of CD40+ (shown) or CD20+ lymphocytes (data not shown) before, during, and after treatment with comparable results for either detection method in all treatment groups.

### Discussion

Given the activity of rituximab in therapy of B-cell malignancies and autoimmune diseases, there is interest in improving the durability of CD20-directed therapy in future molecules. However, there is no consensus on which rituximab function(s) would enhance therapeutic efficacy with minimal side effects or which design enhancements in the molecular structure of CD20-targeting molecules would improve the durability of response following treatment. Several groups have expressed new CD20 mAbs, including some with enhanced ADCC or CDC activity. The TRU-015 small modular immunopharmaceutical molecule described herein is a recombinant non-antibody structure, constructed as a single peptide chain from antibody domains to yield a compact and homogeneous agent. At equivalent dose levels, TRU-015 was more effective than rituximab in vivo against large volume Ramos and moderate volume Daudi lymphoma xenograft models and was comparable with rituximab in B-cell depletion in nonhuman primates following single-dose administration. Figure 4 shows pooled data from multiple experiments clearly showing that administration of TRU-015 to mice with large, established Ramos tumors and moderate size, established Daudi tumors resulted in a reduction in tumor volumes, statistically significant improvement in survival times, and increases in long-term complete tumor regressions compared with both control and rituximab-treated animals. It is possible that the smaller particle size or moderate avidity of TRU-015 compared with rituximab contributes to improved tumor penetration and improved activity in the xenograft model, because the difference between TRU-015 and rituximab was pronounced in models using large, established tumors before initiation of therapy. Both particle size and binding affinity are factors that are known to influence the diffusion rate of proteins through tissues (38, 39) and these differences may contribute to the superior activity of TRU-015 in these models.

An altered HulgG1 hinge was used in TRU-015 to reduce its ability to bind C1q and mediate CDC without impairment of ADCC activity. Whereas ADCC is recognized as a primary mediator of rituximab efficacy, and Fcγ receptor III (CD16) polymorphisms have effects on clinical response (40), the role of CDC in CD20-directed therapy is still controversial. Chronic lymphocytic leukemia cells from rituximab-refractory patients often have increased expression of complement inhibitors, indicating that CDC contributes to tumor cell lysis. CDC activity was required for rituximab activity in a CD20+ EL4 model in vivo (41). In addition, the complement cascade is activated within 2 h of rituximab infusion (42) and...
depletion of complement component C2 has been described in chronic lymphocytic leukemia patients during rituximab therapy (37). However, rapid complement activation during rituximab therapy has also been associated with infusion reactions (31). Rituximab binds C1q at least 2-fold better than TRU-015 as assessed by flow cytometry with CD20+ target cells (data not shown). Consistent with this, CDC assays show that complement-mediated target cell lysis by TRU-015 is reduced 10- to 50-fold compared with that of rituximab. This slower rate of complement activation/consumption compared with rituximab may reduce the occurrence of infusion reactions while retaining and sustaining some benefits of CDC activity. The molecule has shown efficacy in patients with rheumatoid arthritis (43), and in phase I clinical studies evaluating the safety, pharmacokinetics, and pharmacodynamics, no serious adverse events, serious infections, or grade 4 events were reported with TRU-015 (43, 44). Additional safety and efficacy advantages of TRU-015 may become apparent from clinical studies in lymphoma. TRU-015 was designed as a compact single-chain molecule with potent direct and ADCC-mediated activity and reduced size and CDC activity. Its significant efficacy against established solid lymphoma xenografts suggested the molecule be evaluated in treating human disease. TRU-015 is currently in clinical development for the treatment of B-cell malignancies and inflammatory diseases.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

32. Kennedy AD, Beum PV, Solga MD, et al. Rituximab infusion promotes rapid complement depletion and

www.aacrjournals.org 2745
Downloaded from clinicanres.aacrjournals.org on April 14, 2017. © 2009 American Association for Cancer Research.


Clinical Cancer Research

CD20-Directed Small Modular Immunopharmaceutical, TRU-015, Depletes Normal and Malignant B Cells


Updated version Access the most recent version of this article at:  
http://clincancerres.aacrjournals.org/content/15/8/2739

Cited articles This article cites 44 articles, 24 of which you can access for free at:  
http://clincancerres.aacrjournals.org/content/15/8/2739.full.html#ref-list-1

Citing articles This article has been cited by 4 HighWire-hosted articles. Access the articles at:  
/content/15/8/2739.full.html#related-urls

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.