Analysis of Antitumor Activity of an Interleukin-13 (IL-13) Receptor-Targeted Cytotoxin Composed of IL-13 Antagonist and Pseudomonas Exotoxin

Mitomu Kioi, Koji Kawakami, and Raj K. Puri
Laboratory of Molecular Tumor Biology, Division of Cellular and Gene Therapies, Center for Biologies Evaluation and Research, Food and Drug Administration, Bethesda, Maryland

ABSTRACT

We have shown previously that a chimeric fusion protein composed of human interleukin-13 (IL-13) and Pseudomonas exotoxin (PE), termed IL-13 cytotoxin (IL13-PE38), is specifically cytotoxic to various cancer cell lines and primary cell cultures derived from a variety of solid cancers. In addition, we have shown that IL-13 mutant IL13E13K, in which glutamic acid (E) residue at position 13 of IL-13 molecule was substituted by a lysine (K), is a powerful antagonist of IL-13 and binds to IL-13 receptor with a higher affinity compared with wild-type IL-13. In this study, we have generated an IL-13 cytotoxin IL13E13K-PE38, in which IL-13 antagonist is fused to PE to determine whether this molecule has improved cytotoxicity to tumor cells compared with wild type (wt)IL13-PE38. Highly purified IL13E13K-PE38 was tested in various tumor cell lines including seven glioblastoma multiforme cell lines to compare its binding to the cells, in vitro cytotoxicity, in vivo antitumor activity, and safety in mouse model with wtIL13-PE38. IL13E13K-PE38 bound to U251MG and IL-13Rx2 chain-transfected tumor cell lines with 3 to 10 times higher affinity compared with wtIL13-PE38. However, IL13E13K-PE38 did not show higher cytotoxicity compared with wtIL13-PE38 in glioblastoma multiforme or any other cell lines tested. The antitumor activity of IL13E13K-PE38, when administered intraperitoneally to nude mice bearing U251 tumors, was also similar to wtIL13-PE38. Some improvement in antitumor activity was observed when lower doses of IL13E13K-PE38 were injected intratumorally in subcutaneous tumors. These results indicate that in general, IL13E13K-PE38 mediates similar cytotoxicity and antitumor activity to wtIL13-PE38 despite its improved binding affinity to IL-13 receptors.

INTRODUCTION

Glioblastoma multiforme (GBM), malignant form of gliomas, has aggressive invasion property among all of the intracranial tumors. GBM are incurable because they present as diffuse infiltration and cytological heterogeneity (1). We have reported previously that a variety of human brain tumor cell lines and primary cell cultures derived from malignant glioma express receptors for interleukin-13 (IL-13R; ref. 2, 3). Because IL-13Rs are overexpressed in cancer cells, it is speculated that IL-13 should play a prominent role in cancer biology. In that regard, IL-13 has been shown to act as an autocrine growth factor for Hodgkin’s lymphoma cells (4). On the other hand, IL-13 can inhibit growth of human renal cell carcinoma and breast tumor cell lines in vitro (5, 6), and gene transfer of IL-13 can inhibit tumorigenicity of mastocytoma cells in animal model (7).

IL-13 binds to two receptor chains, IL-13Rx1 and IL-13Rx2. IL-13Rx1 chain is a relatively low-affinity receptor, which forms a heterodimer with IL-4Rx, and the complex internalizes into the cytosol after binding to IL-13. IL-13Rx2 chain binds to IL-13 with higher affinity and internalizes without the involvement of other chains. We have previously characterized the expression of IL-13R in various malignant tumor cell lines and tissues derived from human malignant glioma, head and neck cancer, Kaposi’s sarcoma, ovarian cancer, and renal cell carcinoma (2, 3, 8–14). These cancer types express high levels of IL-13Rx2 chain (3, 10, 12).

To target IL-13R on cancer cell surface, we generated IL-13 cytotoxin termed IL13-PE38QQR, which is composed of IL-13 and a mutated form of Pseudomonas exotoxin (PE; ref. 10, 12, 14–16). IL13-PE38QQR is highly cytotoxic to IL-13R-positive cancer cells in vitro and in vivo. Later, we generated IL13-PE38, in which COOH-terminus of PE did not contain any QQR mutation. This molecule (IL13-PE38) was found to have similar cytotoxic activity to IL-13R–expressing head and neck tumor cells in vitro compared with IL13-PE38QQR (13).

We have also generated a mutant of human IL-13, in which a glutamic acid (E) residue at position 13 was substituted by a lysine (K) residue (17). This mutant (termed IL13E13K) was found to act as a powerful antagonist of IL-13 in that it inhibited biological activities of IL-13 (17). This mutant was fused to a mutated form of PE (18). Because IL13E13K-PE fusion protein was found to bind IL-13Rx2–positive tumor cells with higher affinity compared with wild-type (wt)IL13-PE, it was hypothesized that IL13E13K-PE38QQR would be superior to wild-type IL13-PE38QQR in mediating cytotoxicity. Indeed, IL13E13K cytotoxins, e.g., IL13E13K-PE38QQR or IL13E13K-PE4E (containing IL-13E13K and a complete PE molecule with a mutation in the binding domain), were more cytotoxic to two
glioma cell lines in vitro and in vivo compared with unmutated IL-13 cytotoxins. In addition, because IL13E13K-PE molecule showed higher binding affinity to IL-13Rα2-expressing cell lines, it was reported that IL13E13K-PE4E would have lower cytotoxicity to normal cells lacking IL-13Rα2 chain (18). We wished to confirm this information and determine whether IL13E13K-PE38 will mediate superior antitumor activity in additional glioma tumors in vitro and in vivo. We generated highly purified IL13-PE38 and IL13E13K-PE38 and tested their cytotoxic activity on seven human brain tumor and other tumor cell lines in vitro. In addition, we examined the safety and antitumor activity against U251 GBM xenografts in vivo.

MATERIALS AND METHODS

Cell Culture and Reagents. Human GBM cell lines were cultured as described previously (11, 19). MDA-MB-231 and DU145 cells were stably transfected with IL-13Rα2 or vector cDNA as described previously (20). Recombinant wtIL-13 and IL-4 were purchased from Pepro Tech Inc. (Rocky Hill, NJ). Recombinant IL13E13K-PE38 and wtIL13-PE38 were produced and purified as described previously (17). The purity of these highly purified (> 99%) recombinant proteins was verified by SDS-PAGE.

Cell Proliferation Assay. The biological activity of fusion proteins was determined by cell proliferation and protein synthesis assays (17). Briefly, TF-1 cells (1 × 10⁵) were incubated with different concentrations (500 or 1,000 ng/ml) of IL13E13K-PE38 or wtIL13-PE38 in the presence or absence of IL-13E13K for 52 hours at 37°C and pulsed with 0.5 µCi of [¹²⁵I]Thymidine (PerkinElmer Life Sciences, Inc., Boston, MA) for an additional 12 hours.

Cytotoxicity Assay. The in vitro cytotoxic activity of IL-13 cytotoxins was measured by the inhibition of protein synthesis (21). All assays were done in quadruplicate, and the concentration of IL-13 cytotoxin at which 50% inhibition of protein synthesis occurred was calculated (IC₅₀).

Radioreceptor Binding Assay. The IL-13 equilibrium-binding studies were done as described previously (17). Briefly, 1 × 10⁶ cells were incubated for 2 hours with 200 pmol/L [¹²⁵I]IL-4 (specific activity, 16.7 µCi/µg) or 200 to 500 pmol/L [¹²⁵I]IL-13 (specific activity, 18.8 µCi/µg) with or without various concentrations (1.5 to 120 nmol/L) of unlabeled IL13E13K-PE38 or wtIL13-PE38 at 4°C. Cell-bound radioactivity was counted by a gamma counter.

Animal Studies. Athymic nude mice 6 weeks old (about 20 g of body weight) were obtained from the Frederick Cancer Center Animal Facilities (National Cancer Institute, Frederick, MD). Human malignant brain tumor xenografts were established in the nude mice by subcutaneous injection of U251 cells into flank. Palpable tumors developed within 5 days (tumor size ~25 mm³ or ~65 mm³). The mice then received injection of excipient (0.2% human serum albumin in PBS) or chimeric toxins IL13E13K-PE38 or wtIL13-PE38 by either intraperitoneal (500 µL with a 27-gauge needle) or intratumoral (30 µL with microinjection syringe) routes. For serum chemistry and organ toxicity analyses, 6 weeks old athymic nude mice and C57BL/6 mice (female) were used.

RESULTS

Biological Activity of wtIL13-PE38 and IL13E13K-PE38. To determine whether mutation exists in IL13E13K-PE38 and whether IL-13 portion of wtIL13-PE38 molecule maintains biological activities similar to wtIL-13, we tested the effects of these highly purified proteins (> 99% purity, gel not shown) on proliferation and protein synthesis in TF-1 cells. wtIL-13 can stimulate proliferation and protein synthesis of TF-1 cells, and we have reported that IL-13E13K protein does not mediate TF-1 cell proliferation (17). Additionally, we have...
reported that IL-13E13K can inhibit the growth stimulatory activity of wtIL-13 in TF-1 cells. As shown in Fig. 1A, wtIL13-PE38 was able to induce proliferation of TF-1 cells in a dose-dependent manner. At the highest concentration (1,000 ng/ml), a 4-fold induction in thymidine uptake was observed. In contrast, IL13E13K-PE38 failed to induce proliferation of TF-1 cells. Interestingly, approximately 8-fold molar excess of IL-13 antagonist IL-13E13K reversed the proliferation of TF-1 cells to some extent whereas 16-fold excess completely neutralized the growth stimulatory activity of wtIL13-PE38. Similar to thymidine uptake, wtIL13-PE38 also induced protein synthesis of TF-1 cells, whereas IL13E13K-PE38 showed no effect (Fig. 1B). Again, similar to proliferation assays, IL-13E13K blocked the effect of wtIL13-PE38 on protein synthesis in a dose-dependent manner. These results suggest that IL-13 portion in wtIL13-PE38 molecule is biologically active, and IL-13E13K can block the effect of wtIL13-PE38 as well.

IL13E13K-PE38 Shows Higher Binding Avidity to IL-13Rα2 Chain–Expressing Cells Compared with wtIL13-PE38. We have demonstrated previously that IL-13E13K possesses 4- to 8-fold higher binding avidity compared with wtIL-13 in displacing 125I-IL-13 binding to U251 and PM-RCC cells, which naturally express IL-13Rα2 chain (17). IL13E13K-PE4E also showed eight to ten times better affinity to glioma...
cells compared with wtIL-13-PE4E (18). However, the competitive effect of fusion proteins (IL13E13K-PE38 and wtIL-13-PE38) on the binding avidity of IL-13 in IL-13Rα2–positive and IL-13Rα2–negative cells has not been reported. To address this, we used IL13E13K-PE38 and wtIL13-PE38 as competitor in radiolabeled IL-13 binding assays, using PM-RCC and U251 cell lines. Both fusion proteins inhibited the binding of radiolabeled IL-13 to these cells in a dose-dependent manner. Interestingly, IL13E13K-PE38 was superior to wtIL13-PE38 in competing 125I-IL-13 binding (Fig. 2A). These observations were consistent with our previous results (17), and we speculated that it is because both PM-RCC and U251 cell lines overexpress IL-13Rα2 chain, which is a high-affinity–binding component of the IL-13R system. We next determined the contribution of IL-13Rα2 chain in binding of IL13E13K-PE38 to tumor cells. For this, MDA-MB-231 breast cancer cells stably transfected with IL-13Rα2 (MDA-MB-231/α2) were used to perform receptor-binding assays. As shown in Fig. 2B, IL13E13K-PE38 seemed to be superior to wtIL13-PE38 in displacing 125I-IL-13 binding to MDA-MB-231/α2 cells whereas no significant difference was observed in MDA-MB-231/mock control cells. We also competed binding of 125I-IL-4 by both IL13E13K-PE38 and wtIL13-PE38. As shown in Fig. 3B, the inhibitory effect of IL13E13K-PE38 in displacing radiolabeled IL-4 binding was modest compared with wtIL-13PE38. These results suggest that the effect of IL13E13K-PE38 on cancer cells is predominantly mediated through the IL-13Rα2 chain, because this chain is not involved in the IL-4R system.

**IL13E13K-PE38 and wtIL13-PE38 Show Identical Cytotoxic Activity against Tumor Cells.** We compared the cytotoxic activity of wtIL-13- and IL-13E13K–cytotoxins on glioblastoma cell lines by protein synthesis inhibition assays. It has been shown that IL-13Rα2 chain plays a critical role in ligand binding and internalization, and higher affinity of IL13E13K-PE38 to IL-13Rα2–expressing cells would exert improved cytotoxic activity to IL-13Rα2–expressing cancer cells compared with wtIL13-PE38 (20). To our surprise, the cytotoxic activity of both fusion proteins (IL13E13K-PE38 and wtIL13-PE38) was identical in all seven GBM cell lines examined including U251, A172, SF295, U373MG, SN19, T98G, and U87MG (Fig. A/B).
cells engineered to overexpress IL-13Rα2 chain (Fig. 2A) or transfected with IL-13Rα2 chain. To address this issue, DU145 prostate carcinoma cells stably transfected with IL-13Rα2 chain were mixed with vector-only transfected (mock control) cells in various ratios, and the cytotoxic activity of IL-13 cytotoxins was evaluated by protein synthesis inhibition assays. As shown in Fig. 3C, as the concentration of IL-13Rα2 chain-positive cells increased, the cytotoxic effect of both IL13E13K-PE38 and wtIL13-PE38 increased. Interestingly, wtIL13-PE38 mediated some baseline cytotoxicity even when IL-13Rα2-transfected cells were not mixed. These results indicate that target cells overexpressing IL-13Rα2 chain were much more susceptible to the cytotoxic effects of IL13E13K-PE38 compared with IL-13Rα2-negative cells, and IL-13α2 chain is indeed involved in mediating cytotoxicity.

**Antitumor Activity of IL13E13K-PE38 against Human Glioblastoma Tumor Xenograft in Nude Mice.** To compare antitumor activities of IL13E13K-PE38 and wtIL13-PE38 against U251 glioblastoma xenografted in nude mice, IL-13 fusion proteins were injected either intraperitoneally (25 or 50 μg/kg; twice a day for 5 days) or intratumorally [25 or 125 μg/kg; three alternate days (day 5, 7, and 9)] when subcutaneous tumors were completely established. As shown in Fig. 4A, U251 tumors treated with excipient control grew linearly, and mean tumor size reached 300 ± 30 mm² by day 26. On the other hand, mice treated by intraperitoneal route with IL-13 cytotoxins showed suppressed tumor growth during the treatment schedule (day 5–9 after implantation). Although animal groups treated with each cytotoxin showed significant tumor regression by day 26 (P < 0.05; IP 25 or 50 μg/kg dose versus control), there was no significant difference in tumor size between the 25 or 50 μg/kg dosages of IL13E13K-PE38 and wtIL13-PE38.

We next examined the efficacy of these cytotoxins when injected by intratumoral route. As shown in Fig. 4B, tumors in excipient-only injected control mice again grew aggressively (238 ± 30 mm² by day 38). After injections with 125 μg/kg dosages of either IL13E13K-PE38 or wtIL13-PE38, four of five mice tumors completely disappeared. Mean tumor sizes by day 38 were 22 ± 44 mm² (wtIL13-PE38; P < 0.05 versus control) and 7 ± 16 mm² (IL13E13K-PE38; P < 0.05 versus control). There was no statistically significant difference in tumor size between both cytotoxins. Interestingly, at a lower dose (25 μg/kg) IL13E13K-PE38 also showed a potent antitumor activity against U251 tumor. As a result of IL13E13K-PE38 treatment,
Antitumor Activity of a Mutated IL-13 Cytotoxin

mean tumor size by day 38 was $27 \pm 50 \text{ mm}^2$, and four of five mice showed complete regression of tumors throughout the experimental period. Although wtIL13-PE38 at the dosage of 25 μg/kg showed a statistically significant antitumor activity ($P < 0.05$ versus control), the mean tumor size by day 38 was $111 \pm 32 \text{ mm}^2$, and no complete response was observed. Significant difference in tumor size was observed at day 38 between IL13E13K-PE38 and wtIL13-PE38 ($P < 0.05$); however, no statistically significant difference was observed at earlier time points.

Toxicity of IL13E13K-PE38 and wtIL13-PE38. To assess cytotoxin-related organ toxicities in normal tissues, blood serum chemistry was performed after intraperitoneal administration of three different doses of IL-13 cytotoxins. Both C57BL/6 and athymic nude female mice were used for these studies. Both blood samples were collected one day after the completion of treatment schedule. As shown in Table 1, most serum chemistry values remained within the normal ranges up to 150 μg/kg/day intraperitoneal administration of IL13E13K-PE38 and wtIL13-PE38 except aspartate aminotransferase and alanine aminotransferase levels were slightly increased in wtIL13-PE38–treated groups. These values were less than twice the upper limits of normal reference range. Animals in both groups showed modest elevation of lactate dehydrogenase whereas creatinine phosphokinase level was slightly increased in IL13E13K-PE38–treated (150 μg/kg) mice.

**DISCUSSION**

We show that an antagonist of IL-13 when fused to a mutated form of PE binds to IL-13R–positive tumor cells at high avidity compared with wtIL-13 fused to PE. As expected, this fusion protein (IL13E13K-PE38) did not induce thymidine uptake or enhance protein synthesis in TF-1 cell line. On the other hand, wtIL13-PE38 induced both protein synthesis and thymidine uptake in the same cell line. Despite the 3- to 10-fold–improved binding to target cells, IL13E13K-PE38 did not show higher cytotoxicity to IL-13R-positive glioma cell lines compared with wtIL13-PE38. In seven GBM cell lines, the IC50 by both mutated or wt cytotoxins remained similar. In addition, both cytotoxins did not show detectable cytotoxicity to normal cells, e.g., COS-7 fibroblast or human umbilical vein endothelial cells. Previous studies have shown that increased binding affinity of immunotoxins to their specific cell surface receptor may lead to increased cytotoxic activities (18, 22, 23). Our current results do not completely support previous conclusions. It is possible that increased binding affinity of IL13E13K-PE38 to IL-13R-expressing cells may not be sufficient to cause enhanced cytotoxicity as wtIL13-PE38 is a highly cytotoxic molecule by itself. Because ligand receptor complexes are rapidly internalized, 3- to 10-fold affinity difference may not lead to improved cytotoxicity by highly cytotoxic molecules.

Similar to in vitro results, IL13E13K-PE38 did not mediate higher antitumor activity compared with wtIL13-PE38 in vivo nude mouse model of human brain tumors when the cytotoxins were administrated by intraperitoneal route to subcutaneous tumor-bearing mice. Both cytotoxins mediated statistically significant regression of established tumors. There was no significant difference in antitumor activity of IL13E13K-PE38 and wtIL13-PE38 at two doses studied. Similarly, both cytotoxins at high doses (125 μg/kg) also mediated remarkable but similar antitumor activity when injected directly into subcutaneous tumors. Interestingly, at a lower dose, IL13E13K-PE38 mediated better antitumor activity than wtIL13-PE38 at the last day of monitoring for efficacy. The mechanism of this higher antitumor activity by IL13E13K-PE38 compared with wtIL13-PE38 at a lower dose is not known. It is possible that in these sets of experiments, IL13E13K-PE38 was able to retain more efficiently at injected tumor site compared with wtIL13-PE38 by virtue of higher binding affinity whereas wtIL13-PE38 with lower binding affinity was able to distribute deeper into tumors, resulting in dilution of wtIL13-PE38 concentration. This hypothesis is supported by the literature of Weinstein et al. (24). These authors reported that lower affinity conjugates distribute deeper into tumors avoiding antigen sinks. Future studies will examine these possibilities. Nevertheless, IL13E13K-PE38 may be potentially effective when only limited amounts of drug can be administrated. For example, lower concentration of drug would be desirable when IL-13 cytotoxins are administrated in normal brain to target infiltrating tumors to decrease recurrence of disease. In that regard, lower concentration of wtIL13-PE38QQR has been administrated into the normal brain with infiltrative disease without any observed toxicity.3, 4 Future studies will further

---

**Table 1 Changes in blood serum chemistry after injection of wtIL13-PE38 or IL13E13K-PE38**

<table>
<thead>
<tr>
<th>Profile</th>
<th>Reference range (unit)</th>
<th>Control</th>
<th>wtIL13-PE38 (μg/kg)</th>
<th>IL13E13K-PE38 (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>AST</td>
<td>72–288 (units/L)</td>
<td>45</td>
<td>63</td>
<td>42.7</td>
</tr>
<tr>
<td>ALT</td>
<td>24–140 (units/L)</td>
<td>43</td>
<td>32</td>
<td>30</td>
</tr>
<tr>
<td>CPK</td>
<td>0–800 (units/L)</td>
<td>338</td>
<td>319</td>
<td>280</td>
</tr>
<tr>
<td>LDH</td>
<td>260–680 (units/L)</td>
<td>731</td>
<td>608</td>
<td>385</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.2–0.7 (mg/dl)</td>
<td>0.5</td>
<td>0.37</td>
<td>0.3</td>
</tr>
</tbody>
</table>

NOTE. Data represent the mean of blood samples from two or three mice in each group.

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; CPK, creatinine phosphokinase; LDH, lactate dehydrogenase.
examine the role of both IL-13 cytotoxins at various concentrations in infiltrating brain tumor models in small animals.

Our current study focused on xenografted brain tumor model in which tumor cells expressed IL-13Rα2 chain. Brain tumor models with different genetic backgrounds will be of great interest in determining the antitumor activity of IL-13 cytotoxins and in addressing tumor heterogeneity. Recent studies have shown development of such brain tumor models (25). Our future studies are planned to examine the effect of IL-13 cytotoxins in these models.

As with cytotoxicity and antitumor activity, IL13E13K-PE38 and wtIL13-PE38 mediated similar toxicity when administered intraperitoneally in nude mice and C57BL/6 mice. At 50 and 100 μg/kg daily doses there was no difference in serum chemical changes by both drugs. At a higher dose (150 μg/kg) wtIL13-PE38 showed minor elevation of hepatic enzymes (aspartate aminotransferase and alanine aminotransferase) whereas IL13E13K-PE38 mildly induced creatine phosphokinase enzyme. Immunotoxins and cytotoxins based on bacterial toxins are known to cause liver toxicity, which may limit their therapeutic utility (23, 26, 27). However, up to 150 μg/kg doses, only small difference in toxicity profile was observed by both agents. Thus, either agent is useful for cancer therapy. Interestingly, both agents did not show any kidney toxicity even at high doses. This is in contrast to what has been observed in clinical trial. In patients with advanced renal cell carcinoma wtIL13-PE38QQR has been shown to cause irreversible acute tubular necrosis leading to the elevation of serum creatinine at 8 μg/kg daily intravenous doses.

It is possible that in human kidney cells, particularly in cancer-bearing hosts, IL-13Rα2 chain is amplified leading to cell damage by IL-13 cytotoxin. Future studies will examine the mechanism of differential toxicity by IL-13 cytotoxin between animals and human.

In conclusion, both IL13E13K-PE38 and wtIL13-PE38 are effective against IL-13Rα2–expressing tumors, and both molecules have a similar efficacy and safety profile. Therefore wtIL13-PE38 is a useful agent for the therapy of IL-13R–expressing tumors including malignant glioma. Ongoing phase III clinical trial using wtIL13-PE38QQR will unravel its safety and efficacy in patients with recurrent glioma.

ACKNOWLEDGMENTS

We are grateful to Drs. Bharat H. Joshi, Syed R. Husain, and Ms. Pamela Dover for general help and technical support. We also thank Dr. Saraswathy Seetharam for critical reading of this report.

REFERENCES


Analysis of Antitumor Activity of an Interleukin-13 (IL-13) Receptor-Targeted Cytotoxin Composed of IL-13 Antagonist and Pseudomonas Exotoxin

Mitomu Kioi, Koji Kawakami and Raj K. Puri


Updated version  Access the most recent version of this article at:  
http://clincancerres.aacrjournals.org/content/10/18/6231

Cited articles  This article cites 24 articles, 14 of which you can access for free at:  
http://clincancerres.aacrjournals.org/content/10/18/6231.full#ref-list-1

Citing articles  This article has been cited by 6 HighWire-hosted articles. Access the articles at:  
http://clincancerres.aacrjournals.org/content/10/18/6231.full#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.