Plasma and Cerebrospinal Fluid Pharmacokinetics of Imatinib after Administration to Nonhuman Primates

Kathleen Neville,1 Robert A. Parise,3 Patrick Thompson,2 Alexander Aleksic,1 Merrill J. Egorin,3,4 Frank M. Balis,5 Leticia McGuffey,4 Cynthia McCully,5 Stacey L. Berg,1 and Susan M. Blaney1

1Texas Children’s Cancer Center and 2Department of Pediatrics, Baylor College of Medicine, Houston, Texas; 3Program of Molecular Therapeutics and Drug Discovery, University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania; 4Departments of Medicine and Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; and 5The Pediatric Oncology Branch, National Cancer Institute, NIH, Bethesda, Maryland

ABSTRACT

Purpose: Imatinib mesylate (Gleevec, Glivec, STI571, imatinib) is a potent tyrosine kinase inhibitor approved for the treatment of chronic myelogenous leukemia and gastrointestinal stromal tumors. The role of imatinib in the treatment of malignant gliomas and other solid tumors is being evaluated. We used a nonhuman primate model that is predictive of the cerebrospinal fluid penetration of drugs in humans to study the pharmacokinetic parameters of imatinib in plasma and cerebrospinal fluid (CSF) after i.v. and p.o. administration.

Experimental Design: Imatinib, 15 mg/kg i.v. over 30 min (n = 3) or 30 mg/kg p.o. (n = 3), was administered to nonhuman primates. Imatinib was measured in serial samples of plasma and CSF using high-pressure liquid chromatography with UV absorbance or mass spectroscopic detection. Pharmacokinetic parameters were estimated using model-independent methods.

Results: Peak plasma imatinib concentrations ranged from 6.4 to 9.5 μM after i.v. dosing and 0.8 to 2.8 μM after p.o. dosing. The mean ± SD area under the plasma concentration time curve was 2480 ± 1340 μM·min and 119 ± 146 μM·min after i.v. and p.o. dosing, respectively. The terminal half-life was 529 ± 167 min after i.v. dosing and 266 ± 88 min after p.o. dosing. After i.v. dosing the steady state volume of distribution was 5.9 ± 2.8 liter/kg, and the total body clearance was 12 ± 5 ml/min/kg. The mean peak CSF concentration was 0.25 ± 0.07 μM after i.v. dosing and 0.07 ± 0.04 μM after p.o. dosing. The mean CSF:plasma area under the plasma concentration versus time curve ratio for all of the animals was 5% ± 2%.

Conclusions: There is limited penetration of imatinib into the CSF of nonhuman primates after i.v. and p.o. administration.

INTRODUCTION

Imatinib mesylate (Gleevec, STI-571), a low-molecular weight, synthetic, 2-phenylamino-pyrimidine derivative, is a potent and selective inhibitor of the abl tyrosine kinases (1). Imatinib also inhibits other tyrosine kinases, including c-kit and the platelet-derived growth factor receptor tyrosine kinases (1). Imatinib has demonstrated marked clinical activity in Bcr/Abl-expressing chronic myelogenous leukemias (CMLs) and c-kit-expressing gastrointestinal stromal tumors (2–10). In addition, preclinical in vitro and in vivo studies have shown that imatinib effectively disrupts platelet-derived growth factor/platelet-derived growth factor receptor ligand-receptor autocrine loops that are implicated in the growth of gliomas and other malignant solid tumors (11). Although platelet-derived growth factor and other tyrosine kinases are expressed on a variety of normal cells, the antiproliferative effects of imatinib in preclinical studies are selective for neoplastic cells (12, 13).

Wolff et al. (14) evaluated recently the effect of chronic treatment with imatinib at therapeutic doses in mice reconstituted with Bcr/Abl-transduced bone marrow cells. Imatinib effectively controlled the systemic proliferation of transduced cells, but many of the mice unexpectedly developed progressive neurological deficits due to leukemic cell infiltration of the brain and leptomeninges after 2–4 months of imatinib treatment, suggesting that there was inadequate imatinib penetration of the drug into the central nervous system (CNS). Subsequent limited pharmacokinetic studies in this mouse model showed that cerebrospinal fluid (CSF) imatinib concentrations were <1% of plasma concentrations and one third of the concentrations required to achieve 50% inhibition of cellular Bcr/Abl-related tyrosine phosphorylation (0.25 μM; Ref. 13).

Similarly, CNS relapses have been reported recently in CML patients receiving chronic p.o. imatinib after successful attainment and maintenance of systemic remission (15–17). In a recent report, 12% of patients with relapsed or refractory Philadelphia-positive leukemias receiving single agent imatinib developed CNS leukemia (18). Anecdotal determinations of plasma and CSF imatinib concentrations in several patients who experienced a CNS leukemia relapse have shown that CSF imatinib concentrations were <1% to 3% of the simultaneous plasma concentrations (15–17).

Because imatinib has a demonstrated role in the treatment of CML and may play a role in the treatment of a variety of solid tumors with the potential for leptomeningeal dissemination, we studied the CSF penetration of imatinib in a nonhuman primate model-independent methods. 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.

Grant support: In part by NICHD U01 HD37242, U01 HD 37242-03S, and NCT 2P30 CA 47904.

Requests for reprints: Susan Blaney, Texas Children’s Cancer Center, 6621 Fannin Street, CC1410.00, Houston, TX 77030. Phone: (832) 822-4215; Fax: (832) 825-4299; E-mail: sblaney@txccc.org.
model that is highly predictive of CSF penetration in humans (19–21). Our initial studies, performed before the commercial availability of p.o. imatinib, characterized the plasma and CSF profile of imatinib after i.v. dosing; subsequent studies were performed with p.o. imatinib.

MATERIALS AND METHODS

Drug. Imatinib for the i.v. dosing studies was obtained from Novartis Pharmaceutical Corporation (East Hanover, NJ). The dose of imatinib was dissolved in 5% dextrose solution to a final concentration of 2.3–2.7 mg/ml. This solution was filtered through a Millex-GV 0.22-μm filter unit (Millipore Products Division, Bedford, MA) before administration. Commercially available imatinib (Novartis Pharmaceuticals Corporation), 100 mg capsules, was purchased for the p.o. dosing studies. The contents of the capsule were dissolved in saline before administration and administered to each animal via a nasogastric tube.

Animals. Adult, male Rhesus monkeys (Macaca mulatta), weighing 11.0–14.4 kg, were studied. They were fed Purina Monkey Chow twice daily and were socially housed in small groups in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Center, National Academy Press, Washington, DC, 1996). Experiments were approved by either the Baylor College of Medicine Animal Protocol Review Committee (i.v. dosing studies) or the National Cancer Institute Animal Care and Use Committee (p.o. dosing studies).

Drug Administration and Sampling. Imatinib was administered i.v. over 30 min (15 mg/kg) or p.o. (30 mg/kg, with rounding to the nearest 100 mg). Three animals received imatinib by the p.o. route and 5 by the i.v. route. Although a total of 5 animals were dosed with i.v. imatinib, pharmacokinetic data from another animal.

Blood samples were drawn through a catheter placed in a femoral or saphenous vein contralateral to the site of drug infusion. Blood samples associated with i.v. dosing were collected in heparinized tubes before dosing, at 15 min into the infusion, at the end of the infusion, at 5, 15, and 30 min, and at 1, 2, 4, 7, 8, 10, 12, 18, and 24 h after the end of the infusion.

Blood samples associated with p.o. dosing were collected in heparinized tubes before dosing and at 0.5, 1, 3, 4, 6, 8, 10, and 24 h after dosing. Plasma was separated immediately by centrifugation at ambient temperature at 1000 × g and was frozen at −70°C until analysis.

CSF samples were drawn from a s.c. Ommaya reservoir attached to an indwelling Pudenz catheter, with its tip located in the fourth ventricle (19). The reservoir was pumped three or four times before and after each CSF sample collection to ensure adequate mixing with ventricular CSF. Ventricular CSF samples associated with i.v. dosing were collected in polypropylene tubes before the infusion, at 15 min into the infusion, at the end of the infusion, and at 5, 15, and 30 min, and 1, 2, 4, 7, 10, 12, 18, and 24 h after the end of the infusion. Ventricular CSF samples associated with p.o. dosing were collected in polypropylene tubes at 1, 2, 3, 4, 6, 8, 10, 24, and 48 h after drug administration. CSF was frozen and stored at −70°C until analysis.

Sample Analysis. Plasma and CSF samples obtained after p.o. imatinib dosing were analyzed using a previously described, validated high performance liquid chromatography (HPLC)/mass spectrometry assay (22). Briefly, the assay used deuterated imatinib as the internal standard, acetonitrile deproteinization; a Luna C18 (Ref. 2: 5 μm, 50 × 4.6 mm; Phenomenex, Torrance, CA) reverse-phase, analytical column; a gradient mobile phase of 0.1% formic acid in methanol and water; and electrospray, positive-ion mode mass spectrometric detection. The lower limit of quantitation for imatinib in plasma was 30 ng/ml (0.05 μM), and the assay was linear between 30 ng/ml (0.05 μM) and 3000 ng/ml (5 μM). The lower limit of quantitation for CSF was 3 ng/ml (0.005 μM), and the assay was linear between 3 ng/ml (0.005 μM) and 30 ng/ml (0.05 μM). The correlation coefficient was >0.992. The intraday coefficient of variation imatinib triplicate standards was <7.2% at all of the concentrations and the between-day coefficient of variation of the slopes associated with the standard curves was 1.63%.

Imatinib concentrations in plasma and CSF after i.v. dosing were measured using HPLC. Plasma samples (1.0 ml) were loaded onto Varian Bond Elut C8 100 mg/1.0 ml solid phase extraction columns (Varian Incorporated, Harbor City, CA) that had been conditioned with 1 ml of methanol and rinsed with 1 ml of water. Columns were then washed with 1 ml of water followed by 1 ml 5% methanol in water and dried for 30 s with vacuum. Recovery was 99% ± 1%. Imatinib was then eluted with 2 ml of acetonitrile. Eluates were dried under nitrogen stream at 38°C. Recovery was 73.3% ± 5.4%. Before injection onto the HPLC system, samples were reconstituted with 500 μl of mobile phase. CSF samples were directly injected onto the HPLC column.

The HPLC system (Waters Associates, Inc., Milford, MA) consisted of a 600E multisolvent delivery system, a 717 plus autosampler, and a 996 photodiode array detector. Samples were injected onto a C18 Xterra (3.5 μm; 3 mm × 150 mm column) with a C18 Xterra (3.5 μm; 3 mm × 20 mm) guard column (Waters) and eluted with a mobile phase of acetonitrile:3 mM triethylamine (33:67, v/v) at a flow rate of 0.35 ml/min. Column eluate was monitored at 264 nm. Under these conditions imatinib had a retention time of ~9 min. Standard curves for plasma and CSF were prepared for each experiment by the addition of known amounts of imatinib to plasma and mobile phase, respectively. Standard curves were linear (r2 > 0.995) over the range of 0.09 μM to 17 μM. The intraday and interday variation of imatinib standards was 1.63% and 7.34%, respectively.

Pharmacokinetic Analysis. The areas under the plasma and CSF concentration versus time curves (AUC) were calculated using the linear trapezoidal method (23) and extrapolated to infinity by adding the quotient of the final plasma concentration divided by the terminal rate constant. Total body clearance was determined by dividing the dose by the AUC. The volume of distribution at steady state was calculated by using the area under the moment curve (24). The fraction of imatinib penetrating into the CSF was calculated from the ratio of the AUCs in CSF and plasma. The half-lives were calculated by dividing 0.693 by the rate constant. These noncompartmental
analyses were performed using Hypercard.2.0 v2 (Apple Computer, Inc.).

**Protein Binding.** Five chamber equilibrium dialysis cells (Bel-Art Products, Pequannock, NJ) with Spectrapor-2 dialysis membranes (Spectrum Medical Industries Inc., Los Angeles, CA), 12,000–14,000 molecular weight cutoff, were used to quantify the protein-binding of imatinib, 5000 ng/ml (8.5 μM), in control nonhuman primate plasma (n = 4). After PBS (900 μl) was placed into one side of each dialysis chamber and plasma (900 μl) was placed into the other, the dialysis cells were incubated at 37°C for 24 h, and the contents of each side of the dialysis chamber were removed and assayed for imatinib concentrations using HPLC/mass spectroscopy. Imatinib, 5000 ng/ml in PBS, was incubated in the fifth chamber of each equilibrium dialysis apparatus to confirm membrane integrity and to validate that 24 h was sufficient for equilibrium to be achieved between the two sides of each dialysis chamber.

**RESULTS**

**Plasma Pharmacokinetics.** Peak (end of infusion) plasma imatinib concentrations ranged from 6.4 to 9.5 μM (n = 3) after i.v. dosing (Fig. 1). Plasma concentrations peaked between 240 and 360 min after the p.o. dose, and peak concentrations ranged from 0.8 to 2.8 μM (n = 3; Fig. 2). The mean plasma AUC (± SD) was 2480 ± 1340 μM·min after i.v. dosing and 1191 ± 146 μM·min after p.o. dosing. The elimination half-life was 529 ± 167 min after i.v. dosing and 266 ± 88 min after p.o. dosing. Tables 1 and 2 list the plasma pharmacokinetic parameters for imatinib for individual animals.

**CSF Pharmacokinetics.** After i.v. drug administration, imatinib was detected in the CSF at the first measured time point (5 min), and the concentration peaked at 30 min (Fig. 1). CSF imatinib was quantifiable until ~8 h in all 3 of the animals that received i.v. drug. Imatinib was detectable, but not quantifiable (< 0.09 μM) 24 h after the infusion in 1 animal and 18 h after the infusion in the other 2 animals. The mean peak (±SD) imatinib CSF concentration and AUC after i.v. administration were 0.25 ± 0.07 μM and 117 ± 25 μM·min, respectively. After i.v. dosing, the mean CSF penetration of imatinib, as represented by the ratio of AUCCSF to AUCPPlasma was 5% ± 2%.

After p.o. administration, imatinib was detected in the CSF at the first measured time point (30 min) and peaked between 240 and 480 min (Fig. 2). In all 3 of the animals imatinib was quantifiable in CSF for at least 6 h. Imatinib was quantifiable in 1 animal at 48 h and detectable, but not quantifiable at 24 h after the dose in the other 2 animals. The mean peak CSF imatinib concentration was 0.07 ± 0.04 μM. The mean (± SD) AUC of imatinib in the CSF was 62 ± 27 μM·min. After p.o. dosing, the penetration of imatinib into the CSF as represented by the ratio of AUCCSF to AUCPPlasma was 5 ± 2%.

**Adverse Events.** One animal experienced an episode of lethargy and unresponsiveness ~90 min after i.v. imatinib, but the animal recovered without complications and had no physical sequelae or abnormal laboratory parameters. The other two animals tolerated i.v. imatinib without clinical toxicity except for erythematous, urticarial rashes that were observed during the infusion. The rashes were most prominent on the face and resolved within 15 min after i.v. diphenhydramine (25 mg) administration. There were no clinical toxicities after a single p.o. dose of imatinib.

**Protein Binding.** Imatinib (8.5 μM) in control nonhuman primate plasma was 88.2–89.4% protein bound (10.6–11.8% free).

**DISCUSSION**

Imatinib is a molecularly targeted agent that plays an important role in the treatment of Philadelphia chromosome-positive leukemias through potent and selective inhibition of the Bcr/Abl tyrosine kinase (1). Recent preclinical studies in mice reconstituted with Bcr/Abl-transduced bone marrow cells have demonstrated the occurrence of Bcr/Abl-expressing CNS dis-
Pharmacokinetics of Imatinib in Nonhuman Primates

Table 1  Pharmacokinetic parameters after administration of imatinib 15 mg/kg i.v. over 30 min to 3 nonhuman primates

<table>
<thead>
<tr>
<th>Animal</th>
<th>$\text{Cl}_{\text{TB}}^a$ (ml/min/kg)</th>
<th>$\text{V}_{\text{DS}}$ (liter/kg)</th>
<th>Plasma $t_{1/2}$ $\beta$ (min)</th>
<th>$\text{AUC}_{\text{Plasma}}$ (µg/min)</th>
<th>$\text{AUC}_{\text{CSF}}$ (µg/min)</th>
<th>CSF:Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV 1</td>
<td>14</td>
<td>4.6</td>
<td>350</td>
<td>1802</td>
<td>142</td>
<td>0.08</td>
</tr>
<tr>
<td>IV 2</td>
<td>6</td>
<td>4.1</td>
<td>554</td>
<td>4023</td>
<td>116</td>
<td>0.03</td>
</tr>
<tr>
<td>IV 3</td>
<td>16</td>
<td>9.1</td>
<td>682</td>
<td>1614</td>
<td>93</td>
<td>0.06</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>12 ± 5</td>
<td>5.9 ± 2.8</td>
<td>529 ± 167</td>
<td>2480 ± 1340</td>
<td>117 ± 25</td>
<td>0.05 ± 0.02</td>
</tr>
</tbody>
</table>

$a$ $\text{Cl}_{\text{TB}}$, total body clearance; $\text{V}_{\text{DS}}$, volume of distribution at steady state; $t_{1/2} \beta$, terminal half-life; $\text{AUC}_{\text{Plasma}}$, area under the concentration versus time curve in plasma; $\text{AUC}_{\text{CSF}}$, area under the concentration versus time curve in CSF; CSF:Plasma ratio of the $\text{AUC}_{\text{CSF}}$ to $\text{AUC}_{\text{Plasma}}$.

ease despite successful control of systemic disease by imatinib (14). Subsequent studies evaluating the CSF penetration of imatinib in this murine model demonstrated that there was minimal imatinib penetration into the CNS with CSF imatinib concentrations <1% of concomitant plasma concentrations. There are similar anecdotal reports of CNS relapses in CML patients who were receiving chronic treatment with imatinib (15–17), including a recent report of CNS leukemic relapse in 13% of patients receiving imatinib as monotherapy for relapse, or refractory Philadelphia chromosome-positive acute lymphoblastic leukemia or CML (18). The CSF concentrations of imatinib in patients experiencing a CNS relapse have ranged from <1% to 3% of concomitant plasma concentrations (15–18).

We used a nonhuman primate model that has been shown previously to be predictive of CSF drug penetration in humans (19–21) to characterize more precisely the CSF penetration of imatinib. Our studies confirm that there is limited CSF penetration of imatinib after i.v. and p.o. dosing. The mean CSF:plasma ratio was 5% if CSF AUC is compared with the plasma AUC derived from total (protein-bound plus free) drug concentrations. CSF protein concentrations are substantially lower than plasma protein concentrations, and as a result there is a higher fraction of free drug in CSF than plasma. The CSF imatinib AUC was 45–50% of the plasma AUC of free imatinib.

CSF imatinib concentrations in humans with a blast crisis and concomitant CNS relapse while receiving chronic imatinib therapy are reported to range from <1% to 3% of simultaneous plasma total drug concentrations (15–17). This difference in CSF exposure between the patients in blast crisis who experience a CNS relapse and the exposures measured in our model may in part be due to differences in plasma $\alpha_1$-acid glycoprotein concentrations. $\alpha_1$-Acid glycoprotein has been shown to bind to imatinib and substantially alter its pharmacokinetics (25, 26). $\alpha_1$-Acid glycoprotein concentrations are significantly higher during all stages of CML, particularly during blast crisis, when compared with normal controls (27). Therefore, it is theoretically possible that the CSF imatinib exposure for patients with CML during remission is slightly higher than during a blast crisis. It is also possible that there are interspecies differences in $\alpha_1$-acid glycoprotein levels that may account for the minimally higher CSF penetration of imatinib in nonhuman primates versus humans. The imatinib plasma protein binding in nonhuman primates observed in our experiments was 89% versus 95% in humans (28).

Interestingly, the clearance observed in nonhuman primates after i.v. dosing was ~3-fold higher than the reported clearance rates in adults (~300 ml/min/m² in nonhuman primates versus approximately 80–90 ml/min/m² in adults; Ref. 28). This difference, which may in part be due to the interspecies difference in protein binding, may explain the shorter imatinib half-life in nonhuman primates versus adult humans (4–10 h in nonhuman primates versus up to 18 h in adult patients). Another difference between nonhuman primates and adults appears to be the fraction of imatinib that is absorbed after p.o. administration. Although this study did not directly evaluate bioavailability after p.o. imatinib dosing, the overall lower mean plasma AUC in animals receiving a 2-fold higher dose of p.o. versus i.v. imatinib suggests that the bioavailability of imatinib in nonhuman primates is <50% versus ~98% in humans (28).

The 3 animals receiving i.v. imatinib had transient cutaneous reactions characterized by an urticarial rash that was most prominent on the face. Similar reactions have been reported in a clinical trial of adult patients receiving 800 mg/day of p.o. imatinib (29). The cutaneous reaction in the adult patients was comprised of macular and/or urticarial lesions that emanated from the face and trunk, and subsequently afflicted the extremities. The reactions resolved with dose reduction and recurred in 2 of the 3 patients with an attempt to re-escalate the dose (29). Cutaneous reactions were not observed in any of the nonhuman primates receiving p.o. imatinib. This is likely due to the overall lower imatinib exposure in this group of animals.

Table 2  Pharmacokinetic parameters after p.o. administration of p.o. imatinib (30 mg/kg) to 3 nonhuman primates

<table>
<thead>
<tr>
<th>Animal</th>
<th>$\text{Cl}_{\text{TB}}^a$ (ml/min/kg)</th>
<th>Plasma $t_{1/2} \beta$ (min)</th>
<th>$\text{AUC}_{\text{Plasma}}$ (µg/min)</th>
<th>$\text{AUC}_{\text{CSF}}$ (µg/min)</th>
<th>CSF:Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO 1</td>
<td>38</td>
<td>273</td>
<td>1356</td>
<td>93</td>
<td>0.07</td>
</tr>
<tr>
<td>PO 2</td>
<td>47</td>
<td>350</td>
<td>1078</td>
<td>50</td>
<td>0.05</td>
</tr>
<tr>
<td>PO 3</td>
<td>45</td>
<td>174</td>
<td>1139</td>
<td>42</td>
<td>0.04</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>43 ± 5</td>
<td>266 ± 88</td>
<td>1191 ± 146</td>
<td>62 ± 27</td>
<td>0.05 ± 0.02</td>
</tr>
</tbody>
</table>

$a$ $\text{Cl}_{\text{TB}}$, total body clearance; $\text{V}_{\text{DS}}$, volume of distribution at steady state; $t_{1/2} \beta$, terminal half-life; $\text{AUC}_{\text{Plasma}}$, area under the concentration versus time curve in plasma; $\text{AUC}_{\text{CSF}}$, area under the concentration versus time curve in CSF; CSF:Plasma ratio of the $\text{AUC}_{\text{CSF}}$ to $\text{AUC}_{\text{Plasma}}$. 

Downloaded from clincancerres.aacrjournals.org on April 12, 2017. © 2004 American Association for Cancer Research.
In summary, our studies confirm that there is limited penetration of imatinib across the blood-CSF barrier after both p.o. and i.v. dosing. Thus, consideration must be given to the development of appropriate strategies to prevent and treat CNS relapses that may occur in CML patients with Bcr/Ab1 expressing leukemias, especially those in blast crisis or Philadelphia chromosome-positive acute lymphoblastic leukemia. Likewise, if imatinib has clinical activity in CNS and other solid tumors, the ability to achieve therapeutic imatinib concentrations in the CSF may have important implications for those tumors with a predilection for leptomeningeal dissemination. Clinical trials to define the spectrum of antitumor activity for CNS and solid tumors imatinib are in progress.

REFERENCES

Plasma and Cerebrospinal Fluid Pharmacokinetics of Imatinib after Administration to Nonhuman Primates

Kathleen Neville, Robert A. Parise, Patrick Thompson, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/10/7/2525

Cited articles
This article cites 27 articles, 12 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/10/7/2525.full.html#ref-list-1

Citing articles
This article has been cited by 8 HighWire-hosted articles. Access the articles at:
/content/10/7/2525.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.