**Biological Puriﬁcation of Breast Cancer Cells Using an Attenuated Replication-competent Herpes Simplex Virus in Human Hematopoietic Stem Cell Transplantation**

Aiguo Wu, Amitabha Mazumder, Robert L. Martuza, Xia Liu, Myint Thein, Kenneth R. Meehan, and Samuel D. Rabkin

Molecular Neurosurgery Laboratory [A. W., R. L. M., S. D. R.]; Bone Marrow Transplantation Program [A. W., A. M., X. L., M. T., K. R. M.]; Departments of Neurosurgery [R. L. M., S. D. R.]; and Microbiology and Immunology [S. D. R.], Georgetown University Medical Center, Washington, DC 20007

**ABSTRACT**

Autologous hematopoietic stem cell transplantation after myeloablative chemotherapy is used for the treatment of high-risk breast cancer and other solid tumors. However, contamination of the autologous graft with tumor cells may adversely affect outcomes. Human hematopoietic bone marrow cells are resistant to herpes simplex virus type 1 (HSV-1) replication, whereas human breast cancer cells are sensitive to HSV-1 cytopathicity. Therefore, we examined the utility of G207, a safe replication-competent multimerized HSV-1 vector, as a biological purging agent for breast cancer in the setting of stem cell transplantation. G207 infection of human bone marrow cells had no effect on the proportion or clonogenic capacity of CD34+ cells but did enhance the proliferation of bone marrow cells in culture and the proportion of CD14+ and CD38+ cells. On the other hand, G207 at a multiplicity of infection of 0.1 was able to purge bone marrow of contaminating human breast cancer cells. Because G207 also stimulates the proliferation of human hematopoietic cells, it overcomes a limitation of other purging methods that result in delayed reconstitution of hematopoiesis. The efﬁcient infection of human bone marrow cells in the absence of detected toxicity suggests that HSV vectors may also prove useful for gene therapy to hematopoietic progenitor cells.

**INTRODUCTION**

Approximately 6–9% of white women in Europe and North America will develop breast cancer in their lifetime (1). Despite progress made over the last decades in the diagnosis and treatment of breast cancer, overall survival has not signiﬁcantly improved, with the median 10-year survival <50%. Intensive, multiagent chemotherapy, “adjuvant therapy,” has become a standard for treatment (2). Unfortunately, bone-marrow toxicity is the major confounding factor and limitation in dose escalation. Therefore, high-dose chemotherapy has been followed by autologous bone marrow or PBPC3 transplantation (3–6). Contamination of bone marrow or peripheral blood with breast cancer cells is common, ranging from approximately 30% to 80% in bone marrow and 10 to 55% in PBPC, depending on the stage of disease and detection technique (7–11), and may be a highly signifcant predictor of relapse (12, 13). There is some uncertainty about the correlation between tumor cell contamination of autologous stem cell products and survival. However, patients with signiﬁcant tumor cell contamination are often excluded from autologous bone marrow transplant protocols. Inferior outcomes occur when tumor cell-contaminated autologous bone marrow is infused after myeloablative chemotherapy (11, 14, 15). In contrast, no association between occult tumor contamination and overall survival has been found in patients receiving PBPCs (16, 17).

**Ex vivo** purging of tumor cell-contaminated bone marrow or PBPCs before transplantation may enhance the efficacy of autologous hematopoietic stem cell transplant if toxicity to stem cells can be minimized. A number of approaches have been described for purging bone marrow of breast cancer cells, including **ex vivo** chemotherapy (9, 18), CD34+ cell-enrichment (9, 19), immunotoxins (20–23), immunomagnetic removal (22, 24), and adenovirus vectors expressing wild-type p53 (25) or “suicide” genes (26, 27).

Attenuated, replication-competent HSV vectors are an attractive strategy for tumor therapy because mutant viruses are available that replicate in dividing cells with consequent cell death and **in situ** viral spread but are incapable of replication in normal tissue (28, 29). We have constructed such a multimerized HSV-1 vector, termed G207, containing deletions of both g34.5 loci, the major viral determinant of neurovirulence (30), and an _E. coli lacZ_ insertion that inactivates the ICP6 gene, encoding the large subunit of ribonucleotide reductase (31, 32). G207 is efﬁcacious in the treatment of multiple human tumors in athymic mice (32–34), including breast cancer (35), and mouse tumors in syngeneic animals (36, 37). However, G207 is nonpathogenic in HSV-sensitive mice and nonhuman primates (38, 39), and no toxicity has been observed after intracerebral inoculation in a Phase I clinical trial for the treatment of recurrent malignant glioma (40). Human bone marrow cells, both proliferating and nonproliferating cells, are very resistant to HSV cytopathicity. The different susceptibilities of bone marrow and breast cancer cells to G207 prompted us to investigate the use of G207 to purge contaminating breast cancer cells from human bone marrow in the setting of autologous stem cell transplantation.

**MATERIALS AND METHODS**

Cells and Viruses. Human bone marrow was obtained by flushing the collection bags used for harvesting marrow from normal donors. The Department of Health and Human Services/NIH guidelines on protection of human subjects were followed, with the authorization of the Georgetown University Institutional Review Board. Bone marrow was diluted in PBS (Life Technologies, Inc.) and centrifuged for 30 min at 900 × g. The buffy coat was collected and washed twice with RPMI 1640 (Life Technologies, Inc.) containing 10% FCS (BioFluids Inc., Rockville, MD). The MNC were adjusted to 106 cells/ml in MyeloCult H5100 long-term culture medium (Stem Cell Technologies Inc., Vancouver, British Columbia, Canada).

Human breast cancer cell lines MDA-MB-231 (41) and MDA-MB-435 (42) and human chronic myelogenous leukemia cell line K562 were obtained from the American Type Culture Collection (Manassas, VA) and maintained in RPMI 1640 media supplemented with 10% FCS, glutamine, antibiotics, non-

---

1 **CANCER RESEARCH 61, 3009 –3015, April 1, 2001**

2 To whom requests for reprints should be addressed, at Molecular Neurosurgery Laboratory, Massachusetts General Hospital-East, 13th Street, Building 149, Box 17, Charlestown, MA 02129. Phone: (617) 726-6817; Fax: (617) 724-9610; E-mail: rabkin@helix.mgh.harvard.edu.

3 The abbreviations used are: PBPC, peripheral blood progenitor cell; HSV, herpes simplex virus; MNC, mononuclear cells; X-gal, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; MOI, multiplicity of infection; CFU, colony-forming unit; p.i., post-infection; GM-CSF, granulocyte macrophage colony-stimulating factor; IL, interleukin; GFU-Tr; CFU-tumor cells; RT-PCR, reverse transcription-PCR; FACS, ﬂuorescence-activated cell sorter; ICP, infected cell polypeptide.

4 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.

5 Supported in part by NIH Grant ROI-NS32677 to R. L. M. and Department of Defense Grant DAMD17-99-1-9202 to S. D. R., Samuel D. Rabkin and Robert L. Martuza are consultants to NeuroVir, Inc., which has a license from Georgetown University for G207.

6 To whom requests for reprints should be addressed, at Molecular Neurosurgery Laboratory, Massachusetts General Hospital-East, 13th Street, Building 149, Box 17, Charlestown, MA 02129. Phone: (617) 726-6817; Fax: (617) 724-9610; E-mail: rabkin@helix.mgh.harvard.edu.

7 The abbreviations used are: PBPC, peripheral blood progenitor cell; HSV, herpes simplex virus; GCN, monocellular cells; X-gal, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; MOI, multiplicity of infection; CFU, colony-forming unit; p.i., post-infection; GM-CSF, granulocyte macrophage colony-stimulating factor; IL, interleukin; GFU-Tr; GFU-tumor cells; RT-PCR, reverse transcription-PCR; FACS, fluorescence-activated cell sorter; ICP, infected cell polypeptide.
G207 is an HSV-1 mutant containing deletions in both copies of the γ34.5 gene and an E. coli lacZ insertion inactivating the ICP6 gene (32). Wild-type HSV-1 strain KOS was obtained from David Knipe (Harvard Medical School, Boston, MA). Virus stocks were prepared from low-multiplicity infections of Vero cells (39) and titrated by plaque assay on Vero cells. Defective HSV vector dvHCL was generated using ampicillin plasmid pHCL and helper virus HSV-1 tsK as described (43).

Viral Infection of Human Bone Marrow and Tumor Cells. Bone marrow or K562 cells were pelleted in 15-ml Falcon tubes (Becton Dickinson Labware, Franklin Lakes, NJ) and then mixed with G207 or KOS in 0.2 ml of PBS/1% heat-inactivated FCS at the MOI indicated or with vehicle alone (mock). The tubes were intermittently rocked for 45 min and incubated for an additional 60 min at 37°C. The viral supernatants were removed by centrifugation. Infected bone marrow cells were resuspended in MyeloCult medium supplemented with 20 ng/ml GM-CSF, 10 ng/ml IL-3, and 10 ng/ml stem cell factor (R&D Systems, Inc., Minneapolis, MN) at 10⁵ cells/ml or RPMI 1640 media supplemented with 10% FCS and cultured at 37°C. Only colonies containing more than 40 cells were counted. Hemoglobinized (10⁵ cells) were set up in 35-mm Petri dishes in duplicate and incubated at 37°C for 60 min followed by 94°C for 5 min. Two μl of the cDNA was amplified in 50 μl of 20 μM Tris (pH 8.4), 50 mM KCl, 2 mM MgCl₂, 0.1 mM deoxyribonucleotide triphosphates, 0.5 μM primers, and 2.5 μl of Taq DNA polymerase (Life Technologies, Inc.). The keratin-19 primer sequences were 5’-ATCTTCCTGTCCCCCGGCA-3’ (sense) and 5’-AGGTTGATTC- CGCGCGGGGCA-3’ (antisense). The reaction was started at 94°C for 4 min, followed by 35 cycles of 45 s at 94°C, 45 s at 60°C, and 2 min at 72°C, and finished at 72°C for 7 min. The PCR products were electrophoresed on a 2% agarose gel, stained with ethidium bromide, and visualized on a UV transilluminator.

RESULTS

G207 Enhances the Proliferation of Human Bone Marrow Cells. Infection of human bone marrow cells by G207 or wild-type HSV-1 KOS did not lead to any detectable decrease in the cell number in the first week after infection, even at an MOI of 3 (Fig. 1). Interestingly, by 2 weeks p.i. the number of viable cells in the G207-infected cultures was significantly greater than in the mock or KOS-infected cultures (Fig. 1; P < 0.001; Student’s t test). The proliferative effect was somewhat dose dependent, because there was a larger increase in cell number in the cultures infected with a 10-fold higher dose of G207 (Fig. 1; MOI, 3). A similar 2-3-fold increase in cell number was seen when human bone marrow cells were cultured in conditioned media obtained from G207-infected bone marrow cultures at day 14 p.i. (data not shown).

The proportion of mitotically active cells in S + G2-M phases was determined by flow cytometry (Table 1). The average proportion of mitotically active cells in six fresh human bone marrows was 15.3%, which decreased to 9.9% after 14 days in culture. In the HSV-infected cultures, the proportion did not decrease, whereas the G207-infected cells actually increased to 19.8% (Table 1; P < 0.001 compared with mock; Student’s t test). The increase in S + G2-M phase cells further illustrates the proliferative effect of G207 infection.
HSV Infection of Human Bone Marrow Cells. To determine whether HSV was able to actually infect bone marrow cells, cells were infected with a defective HSV vector expressing lacZ driven by the CMV immediate-early promoter (dvHCL). Numerous X-gal positive cells were seen, some with very intense staining (Fig. 2; dvHCL*) and others with low levels of staining (Fig. 2, dvHCL<). After G207 infection, only a small proportion of cells were stained with X-gal (Fig. 2; G207). G207 contains the lacZ gene driven by the ICP6 promoter, a “leaky” early promoter (45), transactivated by ICP0 (31). We were unable to detect infectious virus in G207-infected bone marrow (MOI, 1 or 0.1) at day 6 p.i. (limit of detection was 10^2 plaque-forming units/ml). This suggests that the block to G207 replication occurs at an early step after infection.

HSV Infection Does Not Affect Hematopoietic Progenitors. The percentage of CD34^+ cells in fresh normal bone marrow was 2.1% as determined by flow cytometry. There was no expansion of CD34^+ cells in the HSV-infected bone marrow and no difference between G207- and mock-infected cultures in the proportion of CD34^+ cells.

![Figure 2](image_url)

**Table 1  Cytokinetic analysis of human bone marrow cells**

Bone marrow was mock-, G207-, or KOS-infected (MOI = 0.1), and the stage in the cell cycle was determined by flow cytometry on the days p.i. indicated. Values are the mean percentage of cells in S + G2/M phases ± SE (n = 3). On day 14, the mean percentage of S + G2/M phase cells after G207 infection is significantly greater than for KOS (P < 0.02; Student’s t test).

<table>
<thead>
<tr>
<th>Days p.i.</th>
<th>Virus</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mock</td>
<td>G207</td>
</tr>
<tr>
<td>0</td>
<td>15.2 ± 1.2</td>
<td>16.4 ± 2.4</td>
</tr>
<tr>
<td>3</td>
<td>16.6 ± 3.2</td>
<td>18.9 ± 1.6</td>
</tr>
<tr>
<td>7</td>
<td>18.9 ± 3.1</td>
<td>17.1 ± 2.2</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2  Phenotype of human bone marrow cells after 14 days in culture**

Cells were infected with G207 or KOS at a MOI of 0.1. At day 14, they were analyzed by FACS after staining for the following cell surface markers: CD34, a marker for hematopoietic precursor cells; CD19, pan B cell marker; CD3, T-lymphocyte receptor; CD14, a monocyte marker, and CD38, a nonlineage-restricted marker expressed on progenitor cells, plasma cells, B lymphocytes, and activated T lymphocytes. Values are the mean percentage of positive cells after gating ± SE (n ≥ 6).

<table>
<thead>
<tr>
<th></th>
<th>Mock</th>
<th>G207</th>
<th>KOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34</td>
<td>0.9 ± 0.2</td>
<td>0.8 ± 0.1</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>CD19</td>
<td>0.7 ± 0.1</td>
<td>0.9 ± 0.2</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>CD3</td>
<td>1.3 ± 0.1</td>
<td>2.0 ± 0.6</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>CD14</td>
<td>10.3 ± 1.1</td>
<td>55.7 ± 5.2</td>
<td>62.6 ± 4.8</td>
</tr>
<tr>
<td>CD38</td>
<td>30.1 ± 2.1</td>
<td>73.6 ± 5.9</td>
<td>71.0 ± 6.2</td>
</tr>
</tbody>
</table>

We were unable to detect infectious virus in G207-infected bone marrow (MOI, 1 or 0.1) at day 6 p.i. (limit of detection was 10^2 plaque-forming units/ml). This suggests that the block to G207 replication occurs at an early step after infection.
after 14 days in culture (Table 2). To further determine the health of the progenitor cells, we performed a clonogenic assay. The number of colonies from fresh normal bone marrow was 73 ± 6.7/10^5 MNCs. The clonogenic capacity of the marrow cells decreases with time in culture to 42 and 31 at days 7 and 14, respectively (Table 3). There was no difference in the clonogenic capacity of G207-infected cells. Colonies in both mock and HSV-infected groups were dominated by CFU-granulocyte/monocyte.

**Phenotypic Alterations of Human Bone Marrow Cells after HSV Infection.** There was a significant change in the phenotype of cells present in the HSV-infected cultures at 14 days p.i., consistent with the proliferation of bone marrow cells. In particular, there was a significant increase in the monocyte population (CD14+; Table 2). The expression of CD38, a nonlineage specific marker of early or activated phenotypes (46), was also elevated in the infected cells. There was no significant difference in CD37+ or CD19+ cells after HSV infection (Table 2).

**Susceptibility of Human Cancer Cells to HSV Cytotoxicity.** As a model for stem cell transplantation purging, we tested two human breast cancer cell lines, MDA-MB-231 and MDA-MB-435, and a human leukemic cell line, K562. Among human breast cancer cell lines, MDA-MB-435 cells are very sensitive to G207 cytotoxicity (Fig. 3A), whereas MDA-MB-231 is poorly sensitive, with only about 40% of tumor cells killed at a MOI of 0.1, as determined by trypan blue dye exclusion (Fig. 3B). MDA-MB-231 is also less sensitive to killing by wild-type HSV-1 KOS. Similar results were reported previously (35). K562 cells are resistant to the cytopathic activity of HSV-1, even to wild-type KOS (Fig. 3C).

**Purging of Breast Cancer Cells in Human Bone Marrow.** Human breast cancer cells MDA-MB-435 and MDA-MB-231 were mixed with human bone marrow cells in vitro and infected with G207 at a MOI of 0.1. A colony-forming assay in methylcellulose was performed to detect contaminating tumor cells after infection. There was a significant decrease in the number of colonies (CFU-Tr) observed after G207 infection with either MDA-MB-231- or MDA-MB-435-contaminated bone marrow (Table 4). No MDA-MB-435 colonies were observed 6 days p.i., even with 10% contaminating tumor cells in the initial culture, whereas the number of CFU-Trs in mock-infected cultures doubled from days 3 to 6 (Table 4). Colony formation of MDA-MB-231 was inhibited by G207 infection, but a few colonies were still observed at day 6 p.i. in the 10% contaminating cell cultures (Table 4), indicative of the decreased susceptibility of these cells to G207 replication (Fig. 3B). There were no colonies formed when bone marrow cells alone were plated.

A more sensitive assay for contaminating tumor cells is RT-PCR. Keratin-19 mRNA is highly expressed in epithelial cells and most breast cancer cells and has been used to detect micrometastases of breast cancer in lymph nodes (47). RNA was extracted from G207- and mock-infected bone marrow cultures contaminated with breast cancer cells, and keratin-19 expression was characterized. Human bone marrow was negative for keratin-19 (Fig. 4). Keratin-19 mRNA detected in the bone marrow was not detected in the 10% MDA-MB-231-contaminated bone marrow (Fig. 4).

**DISCUSSION**

A concern in autologous stem cell transplantation is contamination of the graft with tumor cells. For patients with breast cancer, contamination of bone marrow or PBPCs occurs frequently, even in lymph...
node-negative patients (12, 48). The demonstration in gene marker studies of transduced bone marrow that tumor cells in relapsed patients contained the marker gene suggests that tumor cell contamination at transplant is associated with relapse (49, 50). We describe a novel approach to purging occult breast cancer cells from bone marrow in the context of autologous stem cell transplantation using an attenuated, replication-competent HSV-1 vector, G207. G207 was developed for the treatment of brain tumors (32), but it has a number of features that make it attractive for purging of bone marrow. It replicates in dividing cells, undergoing a lytic infection with consequent cell death, whereas replication in nondividing cells is restricted, targeting viral spread and cytotoxicity to tumor cells. Among its safety features are hypersensitivity to antiviral drugs such as acyclovir and ganciclovir, multiple mutations that make reversion negligible, limited reactivation from latency (51, 52), and, most importantly, lack of neuropathogenicity (38, 39). The HSV thymidine kinase gene has been used as a “suicide” gene after allogeneic bone marrow transplantation to delete transduced lymphocytes during graft-versus-host disease (53). G207 contains a functional thymidine kinase gene that would permit deletion of G207-infected bone marrow cells after transplantation.

The susceptibility of tumor cell lines to G207 replication and cytotoxicity can vary (33, 35). Among the human breast cancer cell lines we have tested, MDA-MB-435 is the most susceptible and MDA-MB-231 the least susceptible to G207 and wild-type strain F (35). As a model for \textit{ex vivo} bone marrow purging, human bone marrow was mixed with varying amounts of human breast cancer cells. G207 was able to eliminate all of the detectable contaminating breast cancer cells, both MDA-MB-435 and MDA-MB-231, from human bone marrow at a rather low viral dose (MOI, 0.1), with the exception of MDA-MB-231 at the highest ratio (10%). This level of tumor cell contamination (10%) is much higher than would be seen in the clinic, where it is frequently <0.01% (17), and patients are usually excluded from transplant when they have tumor cell contamination <1%. It is possible that all of the MDA-MB-231 cells might have been destroyed if the incubation period was continued for longer than 6 days or a higher MOI was used. Among other tumors that are treated with high-dose chemotherapy and autologous stem cell transplantation (54), human neuroblastoma cells are similarly sensitive to G207 \textit{in vitro}.\footnote{P. Hermaiz-Driever and S. Rabkin, unpublished data.}

\textit{In vivo}, in solid tumor models in nude mice, a single intratumoral inoculation of G207 can significantly inhibit tumor growth and cure established tumors (33, 35). However, s.c. or intracerebral tumor cell implants of MDA-MB-231 are not inhibited by G207, presumably because viral replication is not sufficient (35). In addition to the direct cytopathic effects of G207 on tumor cells, inoculation of syngeneic tumors in immune-competent mice induces a systemic antitumor immune response (36, 37). This immune response involves tumor cell specific CD8$^{+}$ CTLs and can inhibit the growth of noninoculated established tumors (36, 37). If such induction were to occur during bone marrow purging, this might facilitate elimination of residual or drug-resistant tumor cells remaining after high-dose chemotherapy. This also provides an incentive to combine this approach with post-transplantation immunotherapy, such as IL-2 infusions and others (55–57).

Human bone marrow cells were very resistant to HSV-1 replication and cytotoxicity, even to wild-type virus. However, they were efficiently transduced by dvHCL, suggesting that defective HSV vectors might be useful for gene therapy applications involving hematopoietic stem cells. Earlier studies showed that most human monocytes and lymphocytes are resistant to HSV infection \textit{in vitro}, with replication blocked after adsorption but before expression of immediate-early proteins (58, 59). Culture of these cells in \textit{in vitro} and/or mitogen-stimulation led to increased susceptibility to HSV-1 replication (59–61). Interestingly, it was found that nonneuroinvasive strains of HSV-2 replicate poorly in stimulated human monocytes and that rescue of the neuroinvasive phenotype also rescued the ability to replicate in stimulated monocytes (62). G207 infection of human bone marrow cells resulted in a 2–3-fold increase in viable cells when compared with controls after 2 weeks in culture, even in the presence of growth factors. This could be attributable to the increased proportion of mitotically active cells. Alternatively or in addition, there might be decreased apoptosis, because infection with KOS had only a minimal effect on the number of viable cells, although the proportion of mitotically active cells increased. It has been reported that HSV-1 infection of peripheral blood MNCs induces apoptosis in a portion of the infected cells (63). The increased cell number could also be induced by conditioned media, indicating that G207 infection likely stimulated the secretion of soluble growth factors likely to be beneficial for the engraftment of progenitors after transplantation.

These studies suggest that attenuated replication-competent HSV-1 vectors such as G207 have potential for purging occult tumor cells from human bone marrow. No toxicity has been seen after i.v. injection of G207 in mice, athymic or immune-competent (38, 64). If infusion of G207 were a concern in this patient population, antiviral drugs such as acyclovir could be used. Expression of lacZ from the ICP6 viral promotor was detected in a small proportion of the G207-infected human bone marrow cells within the first few days p.i. It is possible that use of other promoter constructs might have led to expression in larger numbers of cells, as seen with dvHCL. The use of oncolytic viruses for tumor cell purging could be augmented by the expression of cytokines or immune-modulatory genes that would enhance the engraftment of stem cells or facilitate antitumor immune responses (65). Such genes could be recombined into the G207 backbone (\textit{i.e.}, in place of lacZ) or defective vectors expressing such genes generated in combination with G207 (66).

REFERENCES

BONE MARROW PURGING OF BREAST CANCER BY HSV


Biological Purging of Breast Cancer Cells Using an Attenuated Replication-competent Herpes Simplex Virus in Human Hematopoietic Stem Cell Transplantation


Cancer Res 2001;61:3009-3015.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/61/7/3009

Cited articles
This article cites 62 articles, 32 of which you can access for free at:
http://cancerres.aacrjournals.org/content/61/7/3009.full.html#ref-list-1

Citing articles
This article has been cited by 3 HighWire-hosted articles. Access the articles at:
/content/61/7/3009.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.