Synergistic Inhibition with a Dual Epidermal Growth Factor Receptor/HER-2/neu Tyrosine Kinase Inhibitor and a Disintegrin and Metalloprotease Inhibitor

Lois Witters, Peggy Scherle, Jordan Friedman, Eian Caulder, Robert Newton, and Allan Lipton

1Department of Medicine, The Milton S. Hershey Medical Center/Pennsylvania State University College of Medicine, Hershey, Pennsylvania and 2Incyte Corp., Wilmington, Delaware

Abstract

The ErbB family of receptors is overexpressed in numerous human tumors. Overexpression correlates with poor prognosis and resistance to therapy. Use of ErbB-specific antibodies to the receptors (Herceptin or Erbitux) or ErbB-specific small-molecule inhibitors of the receptor tyrosine kinase activity (Iressa or Tarceva) has shown clinical efficacy in several solid tumors. An alternative method of affecting ErbB-initiated tumor growth and survival is to block sheddase activity. Sheddase activity is responsible for cleavage of multiple ErbB ligands and receptors, a necessary step in availability of the soluble, active form of the ligand and a constitutively activated ligand-independent receptor. This sheddase activity is attributed to the ADAM (a disintegrin and metalloprotease) family of proteins. ADAM 10 is the main sheddase of epidermal growth factor (EGF) and HER-2/neu cleavage, whereas ADAM17 is required for cleavage of additional EGF receptor (EGFR) ligands (transforming growth factor-α, amphiregulin, heregulin, heparin binding EGF-like ligand). This study has shown that addition of INCB3619, a potent inhibitor of ADAM10 and ADAM17, reduces in vitro HER-2/neu and amphiregulin shedding, confirming that it interferes with both HER-2/neu and EGFR ligand cleavage. Combining INCB3619 with a lapatinib-like dual inhibitor of EGFR and HER-2/neu kinases resulted in synergistic growth inhibition in MCF-7 and HER-2/neu–transfected MCF-7 human breast cancer cells. Combining the INCB7839 second-generation sheddase inhibitor with lapatinib prevented the growth of HER-2/neu–positive BT474-SC1 human breast cancer xenografts in vivo. These results suggest that there may be an additional clinical benefit of combining agents that target the ErbB pathways at multiple points.

Introduction

The ErbB family of receptors is made up of four members, the epidermal growth factor (EGF) receptor (EGFR; ErbB1/HER1), ErbB2/HER2-neu, ErbB3/HER3, and ErbB4/HER4. When their appropriate ligands, e.g., EGF, transforming growth factor-α (TGF-α), heparin binding EGF-like ligand (HB-EGF), amphiregulin, heregulin, or betacellulin (1–3), bind to the receptor ectodomain, formation of homodimers or heterodimers between family members occurs (1, 3, 4). HER-2/neu is the preferred partner for heteromerization following ligand binding (1, 5, 6). This homodimer and heterodimer formation triggers activation of numerous downstream pathways, e.g., the RAS/mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase 1/2 (ERK1/2), and phosphatidylinositol 3-kinase (PI3K)/Akt pathways (1, 4), making ErbB family members important regulators of growth and survival.

Cancer cells often produce and secrete growth factors as well as express their corresponding receptors, allowing for stimulation of cell growth through autocrine mechanisms (1, 7–10). Overexpression also often confers resistance to chemotherapies and antihormonal cancer treatments. This has made them attractive targets for the treatment of EGFR and HER-2/neu-overexpressing tumors. Current treatments consist of antibodies that directly bind to specific receptors, e.g., trastuzumab (Herceptin) and cetuximab (Erbitux), or inhibitors that interfere with signaling of the receptor by blocking receptor kinase activity, e.g., erlotinib (Tarceva) and gefitinib (Iressa).

Lapatinib is a potent reversible inhibitor of both EGFR and HER-2/neu tyrosine kinases (14). It is relatively specific for these kinases and results in cytostatic or cytotoxic effects, depending on tumor cell type (14, 15). Lapatinib exposure causes decreased phosphorylation of EGFR and HER-2/neu in vitro and in vivo (14–16). It also causes decreased phosphorylation of MAPK-ERK1/2 and Akt and decreased expression of cyclin D, downstream effectors of cell proliferation and survival pathways (14–16).

Compared with monoclonal antibodies (mAbs), which target epitopes on the extracellular domain (ECD), small molecules, such as lapatinib, exert effects intracellularly. mAbs have limited effects in the presence of mutated and/or truncated forms of the receptors. In addition, constitutively activated receptors cannot be affected by mAbs.

ErbB ligands must be cleaved to be released in the soluble form capable of activating ErbB signaling (1). ErbB receptors are also subject to cleavage, resulting in constitutively activated ligand-independent signaling (3, 17). Cleavage of the HER-2/neu receptor liberates the ectodomain (ECD), leaving the remaining, constitutively active p95 membrane bound portion (3, 18). As with overexpression of tumor HER-2/neu levels, elevated HER-2/neu ECD in serum has been shown to correlate with poor prognosis and reduced effectiveness of some therapies in cancer patients (3, 18, 19).

Sheddase is an enzymatic activity attributed to the ADAM (a disintegrin and metalloprotease) family of proteins that is involved in the ectodomain cleavage of ErbB ligands and receptors.
(3, 20, 21). ADAM17 has been suggested to be the major ErbB ligand sheddase, with a role in the cleavage of multiple ligands, e.g., TGF-α, HB-EGF, and amphiregulin (1, 3, 21, 22). ADAM17 has been found to be up-regulated in a number of tumor types, including breast and non–small cell lung cancer, where it is required for HER3 and EGFR signaling (1, 23). ADAM10, however, is believed to be the main sheddase of EGF and betacellulin in mouse embryonic fibroblasts (1, 3, 21) and is at least one of the sheddases responsible for HER-2/neu cleavage (3). Small interfering RNAs that selectively inhibit ADAM10 expression have been shown to reduce HER-2/neu shedding (3).

Sheddase inhibitors are potent small-molecule inhibitors of ADAM10 and ADAM17 that prevent ErbB ligand and receptor cleavage, thereby preventing availability of multiple ligands, activation of the ligand/receptor complex, and subsequent cellular growth and survival. The purpose of this study was to evaluate the combination of a sheddase inhibitor and HER-2/neu–directed therapies on the growth of human breast cancer cells.

Materials and Methods

Materials. The HER-2/neu–transfected MCF-7 cell line, MCF/18, was supplied by Genentech, Inc. Human breast cancer cell line BT-474 was obtained from the American Type Tissue Culture Collection. INCB3619 was a gift from Incyte Corporation. The HER-2/neu ELISA kit was a gift from Oncogene Science. The HER-2/neu ELISA is a sandwich enzyme immunoassay that uses a mouse monoclonal antibody for capture and a different biotinylated mouse monoclonal antibody that specifically detects the extracellular domain of human HER-2/neu protein. GW2974, the dual lapatinib-like inhibitor of EGFR and HER-2/neu, was produced by GlaxoSmithKline and purchased from Sigma Aldrich. Lapatinib used for animal studies was synthesized by the Incyte Chemistry Department.

Cell growth experiments. The MCF-7 cells were grown in high glucose DMEM and MCF/18 cells were maintained in a 50:50 mix of Ham’s medium and high glucose DMEM supplemented with l-glutamine. Both cell lines were supplemented with 10% fetal bovine serum and maintained in 5% CO2 at 37°C. G418 was added to the MCF/18 cell medium to support continued growth and survival. The MCF-7 cells were seeded at 750,000 cells per 75-cm² flask. After an overnight incubation to allow attachment, INCB3619 or the corresponding amount of DMSO (control) was added and an additional 3 d incubation was done. The supernatant was then centrifuged to remove cellular debris and measurement of HER-2/neu shedding was done using a HER-2/neu ELISA kit specific for the ectodomain of human HER-2/neu (Oncogene Science). The supernatant was incubated in microwell plates to allow binding of the antigen by the capture antibody immobilized on the surface of the wells. The immobilized antigen reacted with a detector antibody. The antigen/detector antibody was measured by binding it with a streptavidin/horseradish peroxidase conjugate, which catalyzed the conversion of the chromogenic substrate o-phenylenediamine into a colored product. Quantitation of HER-2/neu is done by reading the absorbance at 490 nm on a microplate reader.

Amphiregulin shedding experiments. MCF-7 cells were plated in 75-cm² flasks at a concentration of 750,000 cells per flask. After an overnight incubation to allow attachment, cells were treated with corresponding concentrations of DMSO, the solvent for both agents. The cells were incubated for an additional 3 d, and the cell number was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Each single dose and combination was done in triplicate in each assay. Percent of control was normalized to control (untreated) cells (100% growth).

Statistical analysis. Synergism of the growth-inhibitory effects was determined using Biosoft CalcuSyn software. This program calculated the combination index (CI) equation based on the equation of Chou-Talalay (24, 25).

HER-2/neu shedding experiments. HER-2/neu–transfected MCF-7 cells were plated in 75-cm² flasks at a concentration of 750,000 cells per flask. After an overnight incubation to allow attachment, INCB3619 or the corresponding amount of DMSO (control) was added and an additional 3 d incubation was done. The supernatant was then centrifuged to remove cellular debris and measurement of HER-2/neu shedding was done using a HER-2/neu ELISA kit specific for the ectodomain of human HER-2/neu (Oncogene Science). The supernatant was incubated in microwell plates to allow binding of the antigen by the capture antibody immobilized on the surface of the wells. The immobilized antigen reacted with a detector antibody. The antigen/detector antibody was measured by binding it with a streptavidin/horseradish peroxidase conjugate, which catalyzed the conversion of the chromogenic substrate o-phenylenediamine into a colored product. Quantitation of HER-2/neu is done by reading the absorbance at 490 nm on a microplate reader.
**Animal Studies**

**Animals.** Female athymic mice (CD-1 nu/nu, 8–12 wk old) were obtained from Charles River Breeding Laboratories. Animals were housed in a barrier facility fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International. All of the procedures were conducted in accordance with the USPHS Policy on Humane Care and Use of Laboratory Animals and with Incyte Animal Care and Use Committee guidelines.

**Efficacy study.** When mice were 7 to 8 wk old, each mouse was inoculated with $1 \times 10^7$ BT-474 cells in 0.2 mL of medium s.c. The treatments were started when the tumor size reached $\approx 200 \text{ mm}^3$. INCB007839 was dosed with mini-osmotic pumps (Alzet) implanted s.c. and lapatinib was administered orally twice daily. Lapatinib was administered by oral gavage to ensure that the animals received the compound. Tumor sizes were measured twice weekly in two dimensions using a caliper, and the volume is presented in mm$^3$ using the formula: $V = 0.5ab^2$, where $a$ and $b$ are the long and short diameters of the tumor, respectively. Tumor growth delay was measured as time (days) for the treated group to reach an arbitrary tumor size of 1,000 mm$^3$. Responses were designated as complete remission when tumor volume decreased in size to the point of being undetectable ($<3 \text{ mm } \times 3 \text{ mm}$) and as partial remission when tumor volume decreased to $<50\%$ of its starting volume.

**Results**

Combination of a sheddase inhibitor and a dual EGFR/HER-2/neu tyrosine kinase inhibitor on the *in vitro* growth of human breast cancer cells. Because overexpression of HER-2/neu confers poor prognosis and resistance to chemotherapy and hormonal therapy, and because sheddase inhibitors interfere with the cleavage of the HER-2/neu receptor, we chose to examine the effects of the sheddase inhibitor in combination with the dual EGFR/HER-2/neu inhibitor on the MCF-7 human breast cancer cell line (low HER-2/neu expression) and the HER-2/neu–transfected MCF-7 cell line (MCF/18). Using both cell lines enabled us to determine if the sheddase inhibitor alone or in combination had a differential effect depending on HER-2/neu expression. The addition of the lapatinib-like dual EGFR/HER-2/neu inhibitor (GW2974; 0.5–1 μmol/L) to MCF-7 and HER-2/neu–transfected

---

**Figure 2.** Percentage of growth inhibition in (A) MCF-7 and (B) HER-2/neu–transfected MCF-7 human breast cancer cells exposed to the combination of INCB3619 and GW2974.
MCF-7 human breast cancer cells (MCF/18) resulted in dose-dependent growth inhibition (15–47% and 33–48% inhibition, respectively). INCB3619 is a potent sheddase inhibitor of ADAM10 and ADAM17 (Fig. 1). Exposure to INCB3619 alone at concentrations of 10 to 30 μmol/L resulted in minimal growth inhibition in the MCF-7 and MCF/18 cell lines (21% and 0–14%, respectively). Exposure to the combination of GW2974 and INCB3619 resulted in synergistic growth inhibition in the MCF-7 and HER-2/MCF-18 cells (Fig. 2) as confirmed by isobologram analysis (Table 1). Sequencing INCB3619 and GW2974 dosages (either agent given 6 hours, 24 hours, or 3 days before exposure to the other agent) did not increase the synergy beyond what was observed when the agents were added simultaneously (data not shown).

The combination was tested further on the MCF/18 cells at lower concentrations of INCB3619 (0.25–10 μmol/L). At these doses, INCB3619 did not produce growth inhibition as a single agent. When this low dose was combined with GW2974, synergism of growth inhibition was again observed (data not shown).

**Combination of a sheddase inhibitor and a dual EGFR/HER-2/neu tyrosine kinase inhibitor on the in vitro signaling of human breast cancer cells.** Because we could show that INCB3619 and GW2974 showed synergistic growth inhibition of MCF/18 cells, we examined their effects alone and in combination on several signaling pathways, including ERK, AKT, and HER-3. MCF/18 cells were treated with the compounds for 3 days as described above. INCB3619 is stable for at least 3 days with no evidence of breakdown under these cell culture conditions (data not shown). Cell extracts were then prepared and analyzed by Western blot for levels of phosphorylated ERK, AKT, and HER-3. As shown in Fig. 3, INCB3619, when tested alone, showed no significant inhibition of signaling at the concentrations tested whereas GW2974 treatment resulted in a partial decrease in the phosphorylated levels of all three proteins. However, exposure to the combination of GW2974 and INCB3619 resulted in a marked decrease in the levels of phosphorylated ERK, AKT, and HER-3 beyond what occurred following treatment with GW2974 alone. No effects of compound treatment on total protein levels were observed. Similar effects on ERK, AKT, and HER-3 signaling were also observed in BT-474 cells exposed to INCB3619 and lapatinib (data not shown).

**Effects of INCB3619 on in vitro HER-2/neu and amphiregulin shedding.** To assess whether the INCB3619 sheddase inhibitor has a direct effect on cleavage of the HER-2/neu receptor, the quantity of HER-2/neu shed from breast cancer cells after exposure to the sheddase inhibitor was measured. Supernatant from HER-2/neu-overexpressing MCF/18 cells exposed to 10 μmol/L INCB3619 for 24 hours or 3 days was collected and tested in a HER-2/neu–specific ELISA for quantitation of HER-2/neu protein (ng/mL). At 24 hours, there was no reduction in the percentage of HER-2/shedding from cells exposed to 10 μmol/L of INCB3619 compared with cells exposed to DMSO (control). At 3 days, a 41% reduction in HER-2/neu shedding was observed. Increasing the dose of INCB3619 to 25 μmol/L did not result in a greater reduction in HER-2/neu shedding (Fig. 4A) after 3 days of exposure.

Because we had previously shown that INCB3619 could inhibit EGFR ligand shedding in a non–small cell lung cell line (1), we also examined the effects of the compound on EGFR ligand shedding in the MCF-7 breast cell line. In agreement with previous reports, MCF7 cells were shown to shed the EGFR ligand, amphiregulin, into the medium following PMA stimulation (Fig. 4B). Treatment of cells with various concentrations of INCB3619 dose dependently blocked amphiregulin shedding with >80% inhibition observed at the 10 μmol/L concentration. Therefore, consistent with what has been observed in other cell lines, INCB3619 can reduce shedding of both HER2/neu and EGFR ligands in the MCF7 cell system.

These effects of INCB3619 on soluble ligand and receptor levels seem to result from sheddase inhibition rather than to a change in cell number in the assay systems used due to compound toxicity. Amphiregulin shedding was measured in a 2-hour assay in which any change in cell number would not be expected. For the

### Table 1. Antiproliferative activity of the combination of GW2974 and INCB3619 in human breast cancer cells using the multiple drug-effect equation of the Chou-Talalay method of isobologram analysis

<table>
<thead>
<tr>
<th></th>
<th>INCB3619 (μmol/L)</th>
<th>GW2974 (μmol/L)</th>
<th>CI (from median)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF-7 cells</td>
<td>10</td>
<td>0.5</td>
<td>0.625</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.5</td>
<td>0.708</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.5</td>
<td>0.637</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.75</td>
<td>0.633</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1.0</td>
<td>0.654</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.5</td>
<td>0.562</td>
</tr>
<tr>
<td>HER-2 MCF-7 cells</td>
<td>10</td>
<td>0.5</td>
<td>0.832</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.75</td>
<td>0.880</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.0</td>
<td>0.756</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0.5</td>
<td>0.947</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0.75</td>
<td>1.063</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1.0</td>
<td>0.928</td>
</tr>
</tbody>
</table>

NOTE: CI > 1, antagonistic effect; CI = 1, additive effect; CI < 1, synergistic effect.
HER-2/neu shedding experiments, cells were exposed to 10 μmol/L of INCB3619, a dose shown above not to affect cell number in HER-2/neu–transfected MCF-7 cells. It should also be noted that although the HER-2/neu ELISA measures full-length receptor (185 kDa) as well as the ectodomain (~105 kDa) in cell supernatants, any contribution of full-length HER-2/neu being released from toxicity-induced cell lysis would, if anything, increase the total HER-2/neu measured, masking the reduction of cleaved HER-2/neu observed with INCB3619 and resulting in an underestimate of the effects of the compound. In addition, we confirmed by Western blot analysis in BT-474 cells that INCB3619 only blocks release of the cleaved form of HER-2/neu (data not shown).

**Combination of a sheddase inhibitor and HER-2/neu-targeted therapies for the treatment of breast cancer xenografts.** To determine whether the antiproliferative effects that result from combining a sheddase inhibitor with a dual EGFR/HER2 TKI in vitro are also observed in vivo, HER-2/neu+ BT474-SC1 human breast cancer xenografts were established as described in Materials and Methods. We chose this cell line for the in vivo experiments because it is easily established in animal models, it is a HER-2/neu–positive cell line, and, as noted earlier, HER-3 and other downstream proteins are affected in a manner similar to that observed in the HER-2/neu–transfected MCF-7 cells. Because the clinical candidate ADAM protease inhibitor, INCB7839, has better pharmacokinetics than INCB3619 (data not shown) and because they have identical specificity profiles and behave identically in multiple in vitro systems, we chose to use INCB7839 for our in vivo assays. Lapatinib was used at a dose shown to block HER-2/neu phosphorylation but below the maximum tolerated dose. INCB7839 was administered at a dose capable of achieving steady-state levels of 500 nmol/L to 1 μmol/L in plasma. This concentration was previously determined to be above the IC₅₀ for HER-2/neu and EGFR ligand shedding. Treatment with (a) the INCB7839 sheddase inhibitor or (b) lapatinib alone resulted in a decrease in mean tumor volume compared with animals treated with the vehicle. Treatment with the combination of INCB7839 and lapatinib, however, resulted in complete prevention of the increase in mean tumor volume (Fig. 5). Together, these results suggest that

![Figure 4](https://example.com/figure4.png)

Figure 4. Percentage of HER-2/neu ectodomain (A) measured in the supernatant of HER-2/neu–transfected MCF-7 human breast cancer cells exposed to INCB3619. Amphiregulin levels (B) measured in the supernatant of MCF-7 human breast cancer cells exposed to INCB3619.
interferes with the cleavage of both the HER-2/neu receptor and HER-2/neu shedding. This finding confirms that INCB3619 by >80%. Increasing the dose to 25 nmol/L did not further decrease HER-2/neu shedding reported here may result from MCF18 cells being a HER-2/neu–transfected cell line. It is possible that additional enzymes may be involved in the processing of transfected proteins that are not normally responsible for the shedding of endogenous protein.

Functionally, the combination of a sheddase inhibitor (INCB3619) and a lapatinib-like compound (GW2974) resulted in synergistic growth inhibition in both MCF-7 and HER-2/neu–transfected MCF-7 breast cancer cells \textit{in vitro}. Synergy was observed at the lowest doses of INCB3619 tested (250 nmol/L–10 μmol/L). The combination of INCB3619 and GW2974 also resulted in decreased levels of phosphorylated ERK, AKT, and HER-3, signaling pathways known to be important for tumor cell growth. In addition, the combination of a sheddase inhibitor with a dual EGFR/HER-2/neu–targeted therapy (lapatinib) resulted in complete growth inhibition in a BT-474-SC1 human breast cancer xenograft model. This observation confirms Liu’s finding in the same xenograft model that INCB3619 potentiates the effect of subsaturating concentrations of trastuzumab (Herceptin) on cell growth (18). In combination with trastuzumab, it also augments the proapoptotic and antiproliferative \textit{in vitro} effects of paclitaxel, a chemotherapeutic taxane, in HER-2/neu–overexpressing breast cancer cells (18).

A possible explanation for these results is that addition of a sheddase inhibitor to another HER-2/neu–targeted therapy may result in more complete inhibition of the HER-2/neu pathway than either alone. A second possible reason for the ability of a sheddase inhibitor to potentiate the effects of other HER-2/neu inhibitors is because of its ability to affect all members of the ErbB family, including HER3. The P3K/Akt pathway is driven primarily through transphosphorylation of the kinase-inactive HER3 (27–29). HER3 signaling persists despite continued HER family–targeted therapy and suppression of EGFR and HER-2/neu (27). This resistance to HER3 signaling and subsequent P3K/Akt effects on tumor growth and survival may explain resistance to therapies like trastuzumab (Herceptin), gefitinib, and lapatinib. It has already been reported that INCB3619 inhibits gefitinib-resistant HER3 signaling in non-small cell lung cancer (1).

In summary, sheddase inhibition prevents the cleavage of EGFR ligands and HER-2/neu receptors, thereby preventing availability of the ligand and receptors for activation of the ligand/receptor complexes and subsequent activity of downstream growth and survival pathways. Addition of a sheddase inhibitor to lapatinib or Herceptin results in synergistic antitumor activity and should be explored as a possible therapeutic approach to enhancing current ErbB-targeted therapies.

**Disclosure of Potential Conflicts of Interest**

P. Scherle, S. Friedman, J. Fridman, E. Caulder, and R. Newton: Stock, Incyte Corp.
A. Lipton: Commercial research grant and speakers bureau/honoraria, Incyte Corp.
L. Wittes disclosed no potential conflicts of interest.

**Acknowledgments**

Received 2/28/2008; revised 4/24/2008; accepted 4/25/2008.

Grant support: Incyte Corp.

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.
Synergistic Inhibition with a Dual Epidermal Growth Factor Receptor/HER-2/neu Tyrosine Kinase Inhibitor and a Disintegrin and Metalloprotease Inhibitor

Lois Witters, Peggy Scherle, Steven Friedman, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/68/17/7083

Cited articles
This article cites 29 articles, 10 of which you can access for free at:
http://cancerres.aacrjournals.org/content/68/17/7083.full.html#ref-list-1

Citing articles
This article has been cited by 9 HighWire-hosted articles. Access the articles at:
/content/68/17/7083.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.