Imaging, Diagnosis, Prognosis

Breast Cancer Cell Targeting by Prenylation Inhibitors Elucidated in Living Animals with a Bioluminescence Reporter

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Abstract

Purpose: Inhibitors of protein prenylation, including prenyltransferase inhibitors and aminobisphosphonates such as zoledronic acid, are being investigated intensively as therapeutics in cancer and other diseases. Determining whether prenylation inhibitors directly or indirectly target tumor and/or host cells is key to understanding therapeutic mechanisms.

Experimental Design: To determine which cell types can be targeted directly by distinct classes of prenylation inhibitors in vivo, we describe herein the development and implementation of a sensitive and pharmacologically specific bioluminescence-based imaging reporter that is inducible by prenylation inhibitors.

Results: In mouse xenograft models of breast cancer, using reporter-bearing mammary fat pad- or bone-localized tumor cells, we show that a prenyltransferase inhibitor robustly induces reporter activity in vivo. In contrast, zoledronic acid, a bone-associated aminobisphosphonate that exerts adjuvant chemotherapeutic activity in patients with breast cancer, fails to induce reporter activity in tumor cells of either model.

Conclusions: Although a prenyltransferase inhibitor can directly target breast cancer cells in vivo, zoledronic acid and related aminobisphosphonates are likely to exert antitumor activity indirectly by targeting host cells. Accordingly, these findings shift attention toward the goal of determining which host cell types are targeted directly by aminobisphosphonates to exert adjuvant chemotherapeutic activity. Clin Cancer Res; 18(15); 4136–44. ©2012 AACR.

Introduction

The mevalonate biosynthetic pathway operates in all human organs and cell types to provide precursors for synthesizing steroids and isoprenoids that maintain cell membrane structure, function as endocrine hormones, produce heme A and ubiquinone for electron transport, or modify proteins posttranslationally with isoprenoid lipids (prenylation) or N-linked oligosaccharide chains (1). Mevalonate pathway inhibitors that blunt protein prenylation are being investigated intensively for treating cancer and other diseases. For example, statins, which inhibit 3-hydroxy-3-methylglutaryl coA reductase (HMG-CoA reductase) to treat hypercholesterolemia, are under investigation in cancer and dementia (2–4). Aminobisphosphonates, which inhibit farnesylpyrophosphate (FPP) synthase in osteoclasts to reduce bone loss in osteoporosis and metastatic cancer (5–8), are being studied preclinically and clinically as adjuvant chemotherapeutics that exert antitumor effects in breast cancer (9–11). Inhibitors of farnesyltransferase or geranylgeranyltransferase enzymes (FTI and GGTI, respectively) that attach isoprenoid lipids to proteins are being explored for treating cancer (12–14), Hutchinson–Gilford progeria syndrome (15), malaria (16, 17), and other diseases.

Identification of tissues and cell types targeted therapeutically by mevalonate pathway inhibitors that block protein prenylation remains a crucial but elusive goal. An important example is aminobisphosphonate-based adjuvant chemotherapy in breast cancer. Here, whether the antitumor activity of zoledronic acid or other aminobisphosphonates occurs by direct targeting of tumor cells or indirect targeting of osteoclasts or other host cell types remains unknown despite intensive investigation (6, 7, 10, 18, 19). Such questions have persisted because surveying and quantifying drug efficacy and pharmacodynamics in tumors or various host organs, tissues, and cell types in vivo has proved difficult with biochemical methods used heretofore to assess prenylation inhibition (6, 7, 20).

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Bioluminescence Imaging of Prenylation Inhibition

Translational Relevance

Zoledronic acid and related aminobisphosphonates are being used clinically in breast cancer as adjuvant chemotherapeutic agents that exhibit antitumor activity. Whether aminobisphosphonates exert antitumor effects by inhibiting protein prenylation in tumor cells or host cells remains unclear despite intensive investigation. The present study addresses this question in a novel way by developing a bioluminescence reporter for studying breast cancer cell targeting by aminobisphosphonates or other prenylation inhibitors in living animals. Bioluminescence imaging shows that a prenyltransferase inhibitor readily targets breast cancer cells in mouse xenograft models of breast cancer. In contrast, zoledronic acid administered at a therapeutic dose fails to induce the reporter in tumors. These findings support the hypothesis that aminobisphosphonates exert antitumor activity by targeting host rather than tumor cells. Accordingly, they focus future investigations on determining which host cell types are targeted directly by aminobisphosphonates in adjuvant chemotherapy.

To eliminate this hurdle, we describe herein the development of a noninvasive, genetically encoded, bioluminescence-based imaging reporter that specifically and quantitatively detects direct targeting of living cells by prenylation inhibitors. We investigate the use of this imaging reporter by introducing it into breast cancer cells and determining whether distinct classes of prenylation inhibitors can target tumor cells directly in mouse xenograft models of breast cancer.

Materials and Methods

Reagents

MDA-MB-231 cells were obtained from Dr. Theresa Guise (Indiana University School of Medicine, Indianapolis, IN; ref. 21). Drugs were obtained from the following sources: clodronate and GGTI-298 (Sigma-Aldrich), simvastatin (Calbiochem), and zoledronic acid (Novartis Pharma AG).

Reporter construction

The VP16 transcriptional activation domain from pVP16 (Clontech) was inserted downstream of the Gal4 DNA-binding domain coding region in pM3 (Clontech). The Gal4-VP16 coding region was inserted upstream of the GFP coding region in pEgfp-C1 (Clontech) to create a Gal4-VP16-GFP fusion. Oligonucleotides encoding the C-terminal 19 amino acids of Cdc42 with a functional (WT) or inactivated (C→S) prenylation site were used to generate plasmids encoding Gal4-VP16-GFP-Cdc42tail fusion proteins. The firefly luciferase (Fluc) coding region from pGL3 (Promega) was inserted into pcDNA6-V5-HisA (Invitrogen) with 5 copies of a consensus Gal4 DNA-binding site. The (Gal4)5-Fluc, ubiquitin C promoter/MCS/IREs/Renilla luciferase [from pRLTK (Promega)], the Gal4-VP16-GFP-Cdc42tail fragments, and a PGKneo cassette from pPGKneo-I (Genbank accession #AF335419) were inserted into plasmid FCIV for lentivirus packaging. HEK293T cells were cotransfected with the FCIV constructs, pSVSG, and D8.91 plasmids using Effectene (QIAGEN) to generate lentivirus (pSVG, D8.9 packing vector and the transfer vector FCIV were provided by J. Milbrandt; Washington University School of Medicine, St. Louis, MO). MDA-MB-231 cells were plated in 6-well dishes (2 × 10^4 cells per well) with 8 mg/mL polybrene (Sigma) and infected with 4 to 500 mL of virus-containing medium. After 24 hours, cells were transferred into 10-cm dishes. At 48 hours, 800 μg/mL G418 (Sigma) was applied to select for stably transduced cells.

Confocal microscopy

MDA-MB-231 cells were transfected (Lipofectamine 2000, Invitrogen) on coverslips in 6-well dishes with plasmids expressing prenylated or nonprenylated mutant (C→S) forms of Gal4-VP16-GFP-Cdc42tail. After 6 hours, fresh medium with vehicle (dimethyl sulfoxide; DMSO) or GGTI-298 (0.75 or 1 mmol/L) was added. Cells were fixed 18 hours later with paraformaldehyde (4%) and mounted in VECTASHIELD mounting medium (Vector Laboratories). Fluorescence images were captured on an Olympus BX52 microscope equipped with a 1.35NA × 1.5Planapo objective, spinning disc confocal scanner unit (CSU10), Picarro Cyan (488 nm) and Cobalt jive (561 nm) lasers, and a Stanford Photonics XR MEG-A-10 charge-coupled device (CCD) camera, with InVivo software (Media Cybernetics). Only brightness, contrast, and color balance were adjusted using ImageJ.

In vitro luciferase assays and Western blotting

The Dual-Luciferase Reporter Assay System (Promega) was used. MDA-MB-231 cells stably expressing reporters driven by prenylated or nonprenylated mutant (C→S) forms of Gal4-VP16-GFP-Cdc42tail were seeded in 24-well plates (1.5 × 10^4 cells per well). Cells were treated the following day with varying doses of GGTI-298, zoledronic acid, simvastatin, or clodronate. Cells were harvested 24 hours later in passive lysis buffer and assayed according to the manufacturer’s protocol with a GLOWMAX platereader luminometer (Promega). The same lysates were resolved by SDS-PAGE, transferred to polyvinylidene difluoride (PVDF) membranes, and probed with antibodies by standard methods. Blots were analyzed using the ChemiDoc imaging system (Bio-Rad) and ImageLab software (Bio-Rad) for band intensity quantification. Antibodies used were goat anti-unprenylated Rap1A (Santa Cruz; C-17), anti-actin C4 (Millipore; MAB1501), horseradish peroxidase (HRP)-conjugated rabbit anti-goat IgG (Pierce), and goat anti-mouse IgG (Pierce).

Animals and reporter imaging

All experiments were conducted in strict accordance with the Guide for the Care and Use of Laboratory Animals of the NIH (Bethesda, MD). Mice were housed under pathogen-
free conditions according to the institutional guidelines. All procedures were approved by the Animal Studies Committee. Female 6-week-old nu/nu mice (NCRNU-F homozygous, Taconic) were used. Intratibial (1 × 10^5 MDA-MB-231 cells stably transduced with the WT or C→S mutant reporter into the right and left tibia, respectively) tumor cell injections were conducted as previously described and allowed to grow for 2 weeks (22). For subcutaneous injections, 1 × 10^6 MDA-MB-231 cells stably transduced with the WT or C→S mutant reporter into the right and left fourth dorsal mammary fat pad, respectively, were injected in a 1:1 ratio with Matrigel (BD Biosciences) and allowed to grow for 1 week. GGTI-298 (15 mg/kg twice daily in 20% DMSO), zoledronic acid (30 μg/kg/d), clodronate (20 mg/kg/d), or vehicle (20% DMSO in PBS) was administered by intraperitoneal or subcutaneous injection. Serum collagen type I was measured for each tumor with Living Image 2.6 using a CTX ELISA system (Immuno-diagnostics Systems) 3 weeks after tumor implantation. At the indicated times after drug or vehicle administration, live animal bioluminescence imaging was conducted using a CCD camera (IVIS 100, Caliper) and Living Image 3.2 software (23) as previously described (ref. 22; exposure times, 1 second–5 minutes; binning, 16; field of vision (FOV), 15 cm; f/1, no filter). Total photon flux (photons/s) was measured for each tumor with Living Image 2.6 using either a software-defined region of interest (ROI) or a fixed user-defined ROI.

Statistical analyses

Results are expressed as the mean ± SEM. In animal imaging studies, data were analyzed by using the Student t test with 2-tailed distribution and 2-sample equal variance.

Results

Imaging reporter design and characterization

We designed a bioluminescence imaging reporter (Fig. 1A) inducible by prenylation inhibitors based on the understanding that (i) isoprenoid lipids are conjugated posttranslationally to the carboxy termini of intracellular proteins, including lamin A and GTP-binding signaling proteins such as Ras, RhoA, and Cdc42, that have key roles in cancer and other diseases; (ii) isoprenoid lipid attachment drives association of otherwise soluble proteins with cell membranes; and (iii) transcriptional regulation often occurs by altering the subcellular localization of transcription factors. Accordingly, we reasoned that appending a prenylation motif to the C-terminus of a chimeric Gal4-VP16-GFP transcription factor should target the protein to membranes, thereby limiting the ability of this transactivator to drive transcription of a chromosomally integrated Fluc reporter controlled by Gal4 DNA-binding sites. Conversely, unprenylated forms of the Gal4-VP16-GFP transactivator produced by inhibiting prenylation or mutationally inactivating the prenylation motif should fail to associate with membranes. Unprenylated chimeric transactivators should accumulate in the nucleoplasm by the action of intrinsic nuclear localization sequences within the Gal4 DNA–binding domain, thereby augmenting Fluc expression. Such a system would provide a gain-of-function assay for quantifying the potency and efficacy of mevalonate pathway inhibitors.

We tested these predictions by analyzing cells expressing chimeric Gal4-VP16-GFP transactivators bearing wild type (WT) or unprenylated mutant (C→S) forms of the Cdc42 prenylation site at their C-termini (Gal4-VP16-GFP-WT Cdc42tail and Gal4-VP16-GFP-(C→S)Cdc42tail, respectively). In transiently transfected MDA-MB-231 breast cancer cells, the WT transactivator localized mainly to the nuclear envelope, which is contiguous with the endoplasmic reticulum (ER) membrane where enzymes responsible for Cdc42 processing reside (24). In contrast, the mutant prenylation site transactivator localized in the nucleoplasm (Fig. 1B and C). Similarly, treating cells with the geranylgeranylation inhibitor GGTI-298 caused the WT transactivator to accumulate in the nucleoplasm (Fig. 1B and C). Thus, the expected consequences of genetically or pharmacologically inhibiting posttranslational prenylation of the chimeric transactivator were readily observed in living cells.

We next determined whether the prenylation state of Gal4-VP16-GFP-Cdc42tail transactivators affected expression of a Gal4-driven Fluc reporter. These experiments used human MDA-MB-231 breast cancer cells stably transduced with a lentivirus that contained a Gal4 DNA-binding site–driven Fluc reporter, expressed a Gal4-VP16-GFP transactivator possessing a WT or mutant (C→S) Cdc42 prenylation site at its C-terminus, and expressed Renilla luciferase (Rluc) from an internal ribosome entry site (IRES) for concurrent normalization of Fluc reporter expression. Results indicated that basal Fluc expression was approximately 2-fold greater in cells expressing the mutant transactivator relative to those expressing the WT transactivator (Fig. 2A), indicating that the prenylated, nuclear envelope–localized WT transactivator activated the chromosomally integrated Gal4-driven Fluc reporter less effectively than the unprenylated mutant, nucleoplasm–localized transactivator. Basal expression of the reporter driven by the WT transactivator may be due in part to the occurrence of some cells in which the fusion protein localizes to the nucleoplasm (Fig. 1B and C). Inhibiting prenylation at various enzymatic steps with GGTI-298, simvastatin, or zoledronic acid augmented Fluc expression approximately 2-fold in cells expressing the WT transactivator relative to untreated controls (Fig. 2A). All compounds, including zoledronic acid, induced Fluc expression in cellulo with apparent potencies similar to those determined by measuring accumulation of unprenylated Rap1A (Fig. 2B and C and Table 1), an established biochemical marker of prenylation blockade (6, 7, 20). These inhibitors failed to affect Fluc expression driven by the mutant prenylation site transactivator showing that drug effects on reporter activity did not occur by prenylation-independent mechanisms. Moreover, clodronate (a bisphosphonate that does not inhibit FPP synthase) failed to induce Fluc expression driven by either the WT or mutant transactivator, or cause accumulation of unprenylated Rap1A (Fig. 2). Likewise, a farnesyltransferase inhibitor
FTI-277 failed to induce the reporter or Rap1A deprenylation when used at a concentration that inhibits H-Ras farnesylation (100 nmol/L; ref. 25; data not shown), as expected because Cdc42 and Rap1A are geranylgeranylated rather than farnesylated. Therefore, our bioluminescence reporter displayed pharmacologic sensitivity and specificity required to serve as a reliable indicator of prenylation inhibition.

**Imaging prenyltransferase inhibitor action in breast cancer cells in living animals**

To determine whether reporter induction by prenylation inhibitors can be imaged quantitatively in vivo, we used breast- and bone-localized mouse xenograft models of breast cancer. MDA-MB-231 breast cancer cells stably transduced with the reporter-bearing lentiviruses described earlier were implanted bilaterally in mammary fat pads or tibias of immunodeficient (nu/nu) mice. In each animal, one site received cells expressing the reporter driven by the WT transactivator, and the contralateral site received cells expressing the reporter driven by the mutant transactivator to control for potential effects of drug pharmacokinetics, tumor cell growth/survival, or processes that affect reporter expression independent of prenylation. One to 2 weeks after tumor cell implantation, we acquired baseline bioluminescence images to establish the initial ratio of ipsilateral (WT-driven) to contralateral (C→S mutant-driven)
reporter activity. We then used established regimens for administration of GGTI-298, clodronate at a clinically relevant dose, or vehicle and collected serial bioluminescence images of the same mice over time. For quantifying images, Fluc expression ratios [ipsilateral (WT)/contralateral (C→S)] obtained over time were normalized to the predrug ratio and expressed as fold change over the initial value. In mice treated with the geranylgeranyltransferase inhibitor GGTI-298, Fluc expression ratios in mammary fat pad-localized tumor cells increased over time up to

Figure 2. Bioluminescence reporter induction by prenylation inhibitors in cultured breast cancer cells. A, reporter induction by prenylation inhibitors in MDA-MB-231 cells stably transduced with lentiviruses carrying the reporter system driven by Gal4-VP16-GFP-Cdc42tail bearing a functional (WT; closed circles) or mutant (C→S; open circles) prenylation site or lacking a Gal4-containing transcription factor (triangles). Reporter expression (Fluc/Rluc ratio) was measured in lysates from cells (n = 4–12) treated for 24 hours with the indicated compounds or vehicle. B, blockade of Rap1A prenylation by mevalonate pathway inhibitors in breast cancer cells. Cells treated as in A were analyzed by Western blotting with an antibody recognizing unprenylated Rap1A. C, quantification of Rap1A deprenylation by mevalonate pathway inhibitors. Results (n = 4) are expressed as a percentage of the maximal ratio of unprenylated Rap1A:actin observed with a given inhibitor.
approximately 2.5-fold relative to predrug values (Fig. 3). Similarly, in tibia-localized tumor cells, GGTI-298 induced Fluc reporter ratios up to approximately 2.3-fold relative to predrug controls (Fig. 4). Importantly, vehicle (Figs. 3 and 4) and an off-target control drug (clodronate; Fig. 4) had undetectable effects on the reporter expression ratio, indicating that the reporter exhibited the appropriate pharmacologic specificity in vivo. Therefore, the pharmacodynamics of GGTI-298 was imaged readily in tumor cells in living animals.

Assessing the direct action of zoledronic acid on breast cancer cells in living animals

Having established the use of our system for in vivo imaging, we used it to address whether zoledronic acid, a bone matrix–associated aminobisphosphonate, can inhibit prenylation in tumor cells in vivo. Unresolved is whether zoledronic acid and related aminobisphosphonates exert antitumor activity directly within the tumor cell compartment or indirectly by targeting host tissues, a question that has been the subject of considerable investigation and debate in the breast cancer field given its importance for understanding drug mechanisms and potential for improving patient outcomes in aminobisphosphonate-based adjuvant chemotherapy (6, 18, 26, 27).

Table 1. Mevalonate pathway inhibitor potencies indicated by bioluminescence reporter and biochemical assays (apparent EC50; nmol/L)

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>fLuc reporter</th>
<th>Unprenylated Rap1A</th>
</tr>
</thead>
<tbody>
<tr>
<td>GGTI-298</td>
<td>35</td>
<td>75</td>
</tr>
<tr>
<td>Zoledronic acid</td>
<td>20,000</td>
<td>15,000</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>25</td>
<td>40</td>
</tr>
</tbody>
</table>

Figure 3. Bioluminescence imaging of reporter activity induced by prenylation inhibitors in mammary fat pad xenograft models of breast cancer. A, quantified reporter activity. Tumors were generated by injecting the fourth mammary fat pad on the dorsal side of female nu/nu mice with MDA-MB-231 cells stably transduced with the WT (right) or C→S mutant (left) reporter. Baseline (t = 0) reporter activity was quantified one week after injection as photon flux ratios (WT:C→S tumors) set to a value of 1 for each animal. Reporter activity over time in a given animal following administration of vehicle (open triangles; n = 6), GGTI-298 (closed circles; n = 9), or zoledronic acid (open circles; n = 5) was quantified as the fold change relative to the predrug WT:C→S ratio. Results significantly (P < 0.015) different from vehicle controls are indicated (asterisks). B, representative bioluminescence images of reporter activity in mammary fat pad-localized tumor cells before and after (25 hours) administration of GGTI-298 or vehicle.

Figure 4. Bioluminescence imaging of reporter activity induced by prenylation inhibitors in bone-localized xenograft models of breast cancer. A, quantified reporter activity. Tumors were generated by injecting tibias of female nu/nu mice with MDA-MB-231 cells stably transduced with the WT (right tibia) or C→S mutant (left tibia) reporter. Baseline (t = 0) reporter activity was quantified 2 weeks after injection as photon flux ratios (WT:C→S tumors) set to a value of 1 for each animal. Reporter activity over time in a given animal following administration of vehicle (open triangles; n = 12), GGTI-298 (closed circles; n = 6), zoledronic acid (open circles; n = 5), or clodronate (closed triangles; n = 5) was quantified as the fold change relative to the predrug WT:C→S ratio. Results significantly (P < 0.015) different from vehicle controls are indicated (asterisks). B, representative bioluminescence images of reporter activity in intratibial tumor cells before and after (72 hours) administration of GGTI-298.
To address this question, we used an experimental design identical to that described earlier, except that instead of GGTI-298, we administered zoledronic acid at a dose equivalent to that used clinically in patients with breast cancer. This dose has been shown to inhibit osteoclasts and exert antitumor activity in mouse xenograft models of breast cancer (18). Imaging analysis indicated that reporter induction in tumor cells by zoledronic acid was undetectable in either breast- or bone-localized tumor models (Figs. 3 and 4). However, controls indicated that zoledronic acid administration in these experiments did inhibit a known cell target (osteoclasts, as indicated by markers of bone resorption; Table 2), showing that an effective dose in an established compartment (bone) had been achieved. By showing that zoledronic acid was unable to cause significant prenylation inhibition in breast- or bone-localized tumor cells, these results provide novel evidence supporting hypotheses that zoledronic acid exerts antitumor activity in breast cancer by targeting host rather than tumor cells in vivo.

**Table 2. Effect of mevalonate pathway inhibitors on osteoclast activity in mice (serum CTX level; ng/mL)**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>CTX Level (ng/mL)</th>
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<tbody>
<tr>
<td>Vehicle</td>
<td>30 ± 3.7 (n = 6)</td>
</tr>
<tr>
<td>GGTI-298</td>
<td>28 ± 1.4 (n = 6)</td>
</tr>
<tr>
<td>Zoledronic acid</td>
<td>12 ± 1.1 (n = 4)</td>
</tr>
</tbody>
</table>

Discussion

We have developed a novel bioluminescence–based reporter in which Fluc expression induced by prenylation inhibitors can be imaged quantitatively in living animals. In this system, prenylation inhibition causes a chimeric transcription factor containing the Cdc42 geranylgeranylation site to accumulate in an unprenylated form, redistribute from the nuclear envelope to the nucleoplasm, and augment Fluc expression detectable by bioluminescence-based imaging. Because the reporter uses the geranylgeranylation site of Cdc42, it can indicate the extent that protein geranylgeranylation is inhibited by GGTIs. The reporter also can monitor the extent that protein geranylgeranylation and farnesylation are inhibited by compounds such as anabasphosphonates and statins, which by blocking early steps in the mevalonate pathway deplete pools of isoprenoid precursors used by farnesyl- and geranylgeranyltransferases.

The use of this imaging reporter is illustrated by comparing the efficacy of various classes of prenylation inhibitors toward breast cancer cells in vitro versus in vivo. All inhibitors (GGTI-298, simvastatin, and zoledronic acid) targeting 3 different enzymes of the mevalonate pathway effectively induced reporter activity in breast cancer cells in vitro. Furthermore, in breast- or bone-localized breast cancer models, GGTI-298 induced reporter activity in tumor cells in vivo, supporting former evidence and indicating that this compound acts systemically (28). In contrast, zoledronic acid administered at a therapeutically relevant dose that effectively inhibits osteoclast activity did not cause detectable induction of reporter activity in breast-localized tumor cells, as might be expected because peak circulating concentrations of this drug (1–3 μmol/L; ref. 29) are approximately 10-fold lower than the apparent EC50 for inducing reporter expression or inhibiting prenylation of Rap1A in cultured breast cancer cells (Fig. 2). Crucially, however, zoledronic acid also failed to induce reporter expression even in bone-localized tumor cells, despite evidence showing that this compound and related anabasphosphonates accumulate at a high level (up to 800 μmol/L) in osteoclast-resorbed bone (30). Accordingly, because our imaging studies showed no indication that zoledronic acid significantly inhibits protein prenylation in breast cancer cells in living animals, these findings indicate that future work should pivot away from tumor cells and toward testing the hypothesis that zoledronic acid exerts antitumor activity indirectly by targeting host cells (6, 7, 10, 18, 19).

Further evidence supports this concept. Depending on menopausal status, zoledronic acid administration in patients with localized breast cancer in the ABCSG and AZURE trials is associated with positive effects on disease-free survival and breast response or recurrence that are distinct from its effects on bone (11, 31, 32). Indeed, osteoclast function apparently is dispensable for zoledronic acid to exert antitumor activity, as indicated by several studies using xenograft models of breast cancer in various mouse mutants with defective osteoclasts (6, 7, 10, 18, 19, 33). Accordingly, identifying therapeutically relevant nonosteoclast host cell targets of zoledronic acid is likely to become a critical goal. Candidates include cells of the myeloid lineage (34–36), which mediate tumor surveillance by the immune system, transit the bone microenvironment, and are highly endocytic, potentially enabling them to access and accumulate zoledronic acid from bone at levels sufficient to inhibit FPP synthase activity and blunt protein prenylation. It will be intriguing to test these and other hypotheses directly in living animals by determining whether zoledronic acid induces the bioluminescence reporter that has been introduced transgenically or retrovirally into distinct host cell populations. Identification of host cell types targeted by zoledronic acid in turn could advance understanding of how this compound affects processes such as tumor angiogenesis or immune surveillance. Such information might lead to improved clinical outcomes for patients with breast cancer receiving zoledronic acid or other FPP synthase–targeted drugs as adjuvant chemotherapeutics.

Our bioluminescence reporter also could be used to study the action of other important classes of prenylation inhibitors, including FTIs, GGTIs, and statins, in cancer. Because certain tumors are resistant to FTIs, whereas others are sensitive (14), mechanisms governing tumor susceptibility to FTIs could be investigated in animal models using modified reporters in which the Gal4-VP16-GFP transactivator bears a farnesylation site from H-Ras or other relevant proteins. Moreover, because FTI/GTI combination therapy is being investigated in tumors driven by oncopogenic
K-Ras [14], which can be prenylated alternatively by farnesyltransferase or geranylgeranyltransferase activity [25, 37], reporters using a K-Ras prenylation site could be used to detect the combined pharmacodynamic action of these 2 drug classes in tumors in vivo.

In conclusion, our imaging reporter removes a principal hurdle that heretofore has impeded progress toward developing prenylation inhibitors as therapeutics for treating cancer and other diseases. It does so by providing a quantitative tool for precise detection of cell targeting by various classes of prenylation inhibitors at therapeutically relevant dosing regimens in animal models of cancer or potentially other diseases. Such investigations hold promise for improving clinical outcomes of cancer therapy using amidobisphosphonates or other prenylation inhibitors and suggesting new clinical uses for these drugs.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K.M. Kalenbronn, A. Penly, D. Piwnica-Worms, K.J. Blumer

Study supervision: D. Piwnica-Worms, K.J. Blumer

Conceived the reporter system: K.J. Blumer

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