Imaging, Diagnosis, Prognosis

Recombinant Peptides as Biomarkers for Tumor Response to Molecular Targeted Therapy

Ralph J. Passarella,1 Li Zhou,1 John G. Phillips,1 Hongmei Wu,1 Dennis E. Hallahan,1,2 and Roberto Diaz1

Abstract

Purpose: Phage display technology can be used to identify peptide sequences that bind rapidly and specifically to tumors responding to sunitinib therapy. These peptides may help to address problems with current methods of assessing tumor response to therapy that can be slow and have limited usage.

Experimental Design: The peptide of interest was isolated after four rounds of biopanning in MDA-MB-231 and MCF-7 xenografted tumors. The binding location of the peptide was investigated with immunohistochemistry. Its in vivo ability to bind to breast tumors responding to therapy was determined by treating nude mice, xenografted with various tumor cell lines, with sunitinib and using near IR imaging to assess the ability of the peptide conjugated to Alexafluor-750 to bind tumors.

Results: EGEVGLG was the dominant sequence isolated from biopanning. This peptide showed increased binding relative to control groups in two cancer cell lines (MDA-MB-435 and MCF-7 human breast) responding to sunitinib treatment, whereas no elevated binding occurred in vitro when samples were incubated with tumor cells that are unresponsive to sunitinib treatment (B16 melanoma and BxPC3 pancreatic). Mice xenografted with tumors that are responsive to sunitinib therapy showed increased peptide binding when compared with untreated control. Mice bearing tumors unresponsive to sunitinib therapy showed no increased peptide binding between treated and untreated groups.

Conclusion: The use of recombinant peptides to assess the pharmacodynamic response of cancer holds promise in minimizing the duration of ineffective treatment regimens in patients, potentially providing a more rapid and less invasive assessment of cancer response to systemic therapy. (Clin Cancer Res 2009;15(20):6421–9)

Current efforts to detect primary and metastatic tumor response to treatment largely depend on measuring changes in tumor size. Measuring tumor volume is a lengthy process that can take weeks or months before noticeable changes can be observed. Biopsies are not always possible with certain inaccessible metastatic tumor sites such as lung, brain, or liver. Sampling error can factor into this process, which could lead to an inaccurate measurement of tumor response to therapy. Thus, there is a strong need for new methods to rapidly and non-invasively differentiate between tumors that are responding to treatment (1).

The ability to quickly predict the outcome of an individual's treatment would allow physicians to effectively optimize a treatment regimen to target a patient's specific malignancy. Current technologies such as glucose analogue fluorine-18 fluoro-deoxyglucose positron emission tomography/computed tomography scans show the potential of this form of assessment (2, 3). However, many factors limit the effectiveness of this form of fluorodeoxyglucose/positron emission tomography, such as the inability to detect tumors of small size (4), increased fluorodeoxyglucose update during endogenous wound repair (5), and inability to detect slow-growing cancers (6).

Recently, recombinant peptides were identified that are capable of recognizing cancer response to therapy rapidly and non-invasively (7). By implanting tumors in nude mice and treating them with ionizing radiation, biopanning of the T7 phage-displayed peptide library in lung and brain cancers permitted for selection of peptides that bind to receptors activated in response to radiation therapy. Screening of phage-displayed peptide libraries has been established as a way to discover...
peptide ligands that bind to tumor vasculature, cancer cells, or specific molecular targets (8–10). Phage display technology allows for the insertion of random DNA sequences into the bacteriophage genome that encodes the phage capsid proteins. This leads to new peptide expression on the phage surface that binds to cell surface molecules (11, 12).

In the present study, we have discovered a novel set of recombinant peptides selected from phage-displayed peptide libraries that are capable of detecting cancer response to the tyrosine kinase inhibitor sunitinib (SU11248; Sutent). These peptides can be labeled with internal emitters to provide a means to monitor and predict cancer response to sunitinib treatment.

### Table 1. The peptides discovered after biopanning in the MCF-7 or MDA-MB-231 tumors and the relative abundance of each of these sequences

<table>
<thead>
<tr>
<th>Sequence</th>
<th>From MDA-MB-231</th>
<th>From MCF-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGEVGLG</td>
<td>58%</td>
<td>67%</td>
</tr>
<tr>
<td>MRRS VGS</td>
<td>14%</td>
<td>12%</td>
</tr>
<tr>
<td>SSAVL</td>
<td>8%</td>
<td>18%</td>
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<tr>
<td>VLI</td>
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<td>0%</td>
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<tr>
<td>SAGSVAL</td>
<td>6%</td>
<td>0%</td>
</tr>
<tr>
<td>FGVR</td>
<td>3%</td>
<td>1%</td>
</tr>
<tr>
<td>GFWEGL</td>
<td>3%</td>
<td>0%</td>
</tr>
</tbody>
</table>

### Translational Relevance

We identified recombinant peptides that discern responding from nonresponding tumors after treatment with sunitinib very early in the course of therapy (<5 days). The use of recombinant peptides to assess the pharmacodynamic response of cancer holds promise in minimizing the duration of ineffective treatment regimens in patients, potentially providing a more rapid and less invasive assessment of cancer response to systemic therapy. This is platform technology that shows the principle that recombinant peptide biomarkers are effective at rapidly assessing cancer susceptibility to molecular targeted therapy.

![Fig. 1. EGEVGLG peptide differentiates treated from untreated endothelial cells. A and B, HUVECs were cocultured for 1 d with MDA-MB-231 cells to simulate tumor/tumor vasculature interaction. After treatment with 0.5 μg/mL sunitinib for 1 h and incubation with fluorescently labeled EGEVGLG peptide for another hour, HUVECs were imaged for binding of peptide. The experiment was repeated with HUVEC/MDA cocultures with the peptide incubated on the MDA cells and with HUVEC/HUVEC “cocultures.”](https://www.aacrjournals.org/doi/10.1158/1078-0432.CCR-09-0945)
in tumor-bearing mice. Elevated binding levels of the peptide in the tumor region occur days before the tumor begins displaying a response to therapy. This suggests it may be used as a rapid and noninvasive means of predicting treatment efficacy.

Materials and Methods

Sunitinib synthesis. Sunitinib was synthesized in the Vanderbilt Institute of Chemical Biology using the five-step method previously described (13, 14). Sunitinib was administered intraperitoneally (i.p.) injection at either the subtherapeutic dose of 4 mg/kg or the therapeutic dose of 40 mg/kg (11). All protocols in animal experiments were reviewed and approved by the Vanderbilt University Institutional Animal Care and Use Committee.

Biopanning phage-displayed libraries. In vivo biopanning was done as described (7, 11) with a T7 phage–based random peptide library. The phage-displayed peptide library represents $1 \times 10^8$ independent clones of phages expressing random nonamer peptides that are displayed on T7 phages as fusion proteins with the amino terminus of 10A capsid protein. Mice bearing either MDA-MB-231 or MCF-7 breast tumors were subject to treatments of vehicle control (PBS) or 40 mg/kg sunitinib given i.p. for 3 consecutive days. Treatment began 30 days after tumor implantation. The phage libraries were administered 4 h after the last treatment. Phages were recovered after being in circulation for 16 h by harvesting the tumors in the mice. Certain peptide candidates were eliminated by negative selection, because they would remain bound to other organs in the mice. The first round of biopanning was done with mice implanted with MDA-MB-231 tumors. Phages were recovered from excised tumors and were subjected to three more rounds of selection with either MDA-MB-231 or MCF-7 breast cancer cell lines.

Peptide design and imaging specifications. The isolated peptide, EGEVGLG, was synthesized with two lysine residues for biotin and imaging agent conjugation and three glycine linkers to separate the targeting peptide from the biotin and the imaging agent. Biotinylated-KKKGGGEGEVLG synthetic peptide was purchased from Genemed Synthesis, Inc. with biotin attached to the NH$_2$ terminus lysine residue. This peptide was conjugated with streptavidin (Pierce) for 2 h to create a 1:4 molar ratio of peptide/streptavidin. Conjugated peptide was then incubated with Alexafluor 594 or Alexafluor 750 dye (Invitrogen) for an additional hour, which bound to the remaining lysine residue of the synthesized peptide. Labeled complexes of biotinylated peptide-streptavidin-Alexafluor conjugates were then used in vitro and in vivo for imaging purposes.

Coculture assay. Four coverslips, each containing $1 \times 10^4$ human umbilical vein endothelial cells (HUVEC), in the sixth passage (Lonza), were placed on the bottom layers of coculture plates (Fisher). Cells were grown for 1 day in the plate before $3 \times 10^5$ of either MDA-MB-231, B16, or BxPC3 cells were added to the superior layer of the plate. HUVECs were allowed to interact for an additional day before 0.5 μg/mL of sunitinib was added into the bottom dish. The cells were incubated for 1 h before they were harvested. Coverslips were blocked for 30 min with 5% bovine serum albumin and 1% Streptavidin and incubated for 1 h with a Streptavidin-Peptide-Alexafluor 594 complex (Invitrogen). The HUVEC nuclei were stained with 4′,6-diamidino-2-phenylindole and images of nuclei and peptide binding were taken using a Zeiss Axiophot fluorescent microscope at ×40 magnification.

In a second assay, $3 \times 10^5$ MDA-MB-231 cells were plated on coverslips and cocultured with HUVECs. HUVECs were also layered in coculture plates. Both culture types were either treated with 0.5 μg/mL sunitinib.
sunitinib or left untreated, incubated with the EGEVGLG peptide and imaged as before. Positive and negative controls of sunitinib-treated and untreated MDA/HUVEC cocultures with the peptide incubated on HUVECs were used.

Background fluorescence of sunitinib was determined by coculturing cells as before, treating with sunitinib and imaging for fluorescence. These values were subtracted from all treated groups to normalize them against background fluorescence. Quantification of peptide and cell co-localization was done using Metamorph Offline software.

Tumor models. B16 murine melanoma, BxPC3 human pancreatic adenocarcinoma, MDA-MB-231 human breast cancer, and MCF-7 human breast cancer cell lines were purchased from American Type Culture Collection. MDA-MB-435 cells transfected with green fluorescent protein (GFP; MDA-MB-435-GFP) was a gift from G. Mundy (Vanderbilt University, Nashville, TN). Heterotopic tumor models were developed by s.c. inoculating cell suspensions (6 × 10⁶ cells or adjusted for different cell types) into nude mice. Estradiol pellets (Innovative Research of America) were implanted s.c. in nude mice 2 d before they were xenografted with the estrogen receptor/progesterone receptor–positive MCF-7 tumor cell line. Nude mice had tumors implanted into their right hind limbs and were used for experiments when the tumor size reached ~300 mm³ in volume (30 d after implantation).

Immunohistochemistry. Paraffin-embedded tumor samples were taken from mice that had received 4 mg/kg sunitinib, 40 mg/kg sunitinib, or vehicle control. Samples were stained using an antibody for the von Willebrand Factor (DakoCytomation) at a 1:100 dilution from the original stock solution of 3.1 g/L and incubated overnight. A final three washes with PBS for 5 min per wash was done before placing a coverslip over the sample. Images were taken using a fluorescent microscope at ×20 magnification.

Tumor growth study. Mice were implanted with MDA-MB-435-GFP tumors into their right hind limbs. Treatment was started when tumor sizes reached ~300 mm³ in volume. Treatment conditions included sunitinib at 4 or 40 mg/kg, and vehicle control. Treatments were given through i.p. injection daily for 5 d. Tumor size was measured every other day using calipers. Fold increase in tumor volume (compared with tumor size on the first day of treatment) was calculated to show tumor responsiveness to treatment. Intensity of GFP was also measured on the days when tumor volume was measured with calipers.

Near IR imaging. Labeled complexes of biotinylated peptide-streptavidin-Alexafluor conjugates were injected into circulation using tail vein injection in tumor-bearing mice being treated with vehicle control or sunitinib 4 h after the final sunitinib treatment. Near IR images were taken using the IVIS imaging system with an ICG filter setting.
at various time points after the injection. Radiance (photons/s/cm²) was measured in the region of interest by using the LivingImage software.

**Statistical analyses.** Student’s t test was used to perform group comparisons. Linear correlations of peptide binding and tumor response to treatment were developed by use of the correlation coefficient of tumor growth and radiance data sets (SigmaPlot). The same linear correlation was done with GFP radiance and tumor growth.

**Results**

**Selection of phage-displayed peptides.** The primary goal of this study was to identify novel recombinant peptides capable of differentiating responsive mouse models of cancer from those that are unresponsive to sunitinib therapy. Due to the spatial separation of organs, in vivo selection allows for the differentiation between peptides that bind specifically to responsive tumors versus those binding to unresponsive tumors and other normal tissues (7). The phages that bound to responsive tumors were enriched through a total of four serial rounds of biopanning. The first round of biopanning was done in MDA-MB-231 tumors, with three subsequent rounds of selection done in either MDA-MB-231 or MCF-7 tumors. This helped select for peptides that bound multiple types of tumors.

The peptides discovered after biopanning in the MCF-7 or MDA-MB-231 tumors are displayed on Table 1. Thirty-six phage plaques were amplified by use of PCR and were sequenced. The relative abundance of each of these sequences is listed in Table 1. The peptide EGEVGLG was found to be the predominant phage-encoded peptide isolated from both the MDA-MB-231 and MCF-7 tumor screens and was selected for subsequent experiments.

**EGEVGLG peptide recognizes sunitinib-treated endothelium.** We have previously shown that screened phage peptides bind to tumor vasculature (7). As a preliminary assessment of the differentiating capability of the EGEVGLG peptide, an in vitro assay was done. Using coculture plates, MDA-MB-231 cells were cocultured with HUVECs and treated with 0.5 μg/mL sunitinib or vehicle control for 1 h. HUVECs were then incubated for another hour with the fluorescent-labeled EGEVGLG peptide. The HUVECs were then imaged for peptide binding. We found (Fig. 1A and B) a significant increase in the amount of EGEVGLG peptide binding in the sunitinib-treated HUVECs when compared with the untreated control group (P < 0.05).

Investigation of the mechanism of EGEVGLG binding was done with a second coculture assay for differences in binding of EGEVGLG between sunitinib-treated and untreated groups (Fig. 1A and B). The groups were (using the nomenclature upper level/lower level of coculture plate) MDA/HUVEC, HUVEC/MDA, and HUVEC/HUVEC. This showed a statistically significant increase in binding of EGEVGLG in only the coculture of MDA/HUVEC with the peptide incubated on the HUVEC (P < 0.05). There was no significant increase in binding in treated versus untreated in both the HUVEC/HUVEC and HUVEC/MDA with the peptide incubated with MDA (P > 0.05). There was also a baseline of fluorescence of sunitinib that was accounted for by incubating cells with the drug and imaging for fluorescence. This baseline level was subtracted from all sunitinib-treated groups (Fig. 1A and B).

To further investigate the location of peptide binding, tumor samples were resected from mice that were treated with either 40 mg/kg sunitinib or vehicle control once daily for 5 d. MDA-MB-231 and MCF-7 tumor samples were taken at 96 and 48 hours, respectively, after the final treatment. Immunohistochemistry was done and samples were stained for tumor vasculature and incubated with peptide for 1 hour (Fig. 1C and D). MDA-MB-435 and MCF-7 samples treated with sunitinib (Fig. 1C and D, bottom) showed EGEVGLG peptide binding that colocalized with tumor vasculature stained with von Willebrand Factor, whereas vehicle-treated groups showed minimal peptide binding (Fig. 1C and D, top). These results indicate that the EGEVGLG peptide binds to tumor vasculature after sunitinib treatment in an ex vivo model.

**Sunitinib treatment elicits tumor growth delay in MDA-MB-435-GFP tumors.** To develop a model that could address metastatic disease changes, a stable cell line of MDA-MB-435 cancer cells transfected with GFP was implanted into the right hind limbs of nude mice. The mice were then given daily treatments of sunitinib at 40 mg/kg while their tumor volumes were measured by caliper. At the same time, images were taken that captured GFP radiance from the tumors. It was observed that the treated mice had smaller average tumor volume than the control group (Fig. 2A). A significant difference in volume was observed when compared with the untreated group (P < 0.05). There was no baseline of fluorescence of sunitinib that was accounted for by incubating cells with the drug and imaging for fluorescence. This baseline level was subtracted from all sunitinib-treated groups (Fig. 1A and B).

Fig. 3. Nude mice implanted with MDA-MB-435-GFP tumor cells show increased peptide binding relative to control. Nude mice (n = 5 per group) were injected with MDA-MB-435-GFP tumor cells and were given vehicle control, 4 mg/kg, or 40 mg/kg of sunitinib once daily for 5 consecutive days. Peptide-dye complexes were injected 4 h after the 5th day of treatment. A, mice were imaged 48 h after initial peptide injection. B, radiance of groups treated with control or 40 mg/kg were quantified and significant difference was observed on day 5 posttreatment (*, P < 0.05).
the 5th day after the end of treatment ($P < 0.05$). Imaging reveals an increase in GFP radiance for both control and treated groups over time (Fig. 2B). We found a significant correlation ($P < 0.05$) between fold-change in tumor volume and fold change in GFP signal in the sunitinib-treated group over time (Fig. 2C).

**EGEVGLG peptide detects treatment response in tumor-bearing mice.** Having shown the potential of EGEVGLG to bind selectively to treated tumor sections ex vivo, the next step was to test the peptide’s binding specificity in vivo. Nude mice were implanted with MDA-MB-435-GFP tumors. Tumor-bearing mice were treated with once daily doses of sunitinib for 5 days. Four hours after the final sunitinib treatment, the mice were injected with EGEVGLG peptide-dye complex. Images of the mice 48 hours after peptide injection (Fig. 3A) showed a graded increase in tumor radiance as dose of drug increased from vehicle control (left) to 4 mg/kg sunitinib (middle) to 40 mg/kg sunitinib (right). Radiance of images of the mice was quantified and the peptide radiance from the tumor’s region of interest was quantified relative to the control (Fig. 3B). The average radiance of the treated group was noticeably higher than the control group beginning 24 hours after peptide injection, and this difference became statistically significant ($P < 0.05$) on the 5th day after peptide injection.

MCF-7 was the second in vivo tumor model used to assess the differential binding ability of the EGEVGLG peptide. Such as the aforementioned experiment, MCF-7 was implanted into the hind limbs of nude mice treated daily with sunitinib. Groups treated with 4 or 40 mg/kg of sunitinib had average tumor volumes that were smaller than the control (Fig. 4A). These differences became significant on days 9 and 11 after treatment. Images of the mice taken at 48 hours after initial injection of the peptide-dye complex (Fig. 4B) showed a marked increase in peptide binding in the 4 and 40 mg/kg treated groups (middle and right, respectively) when compared with the control group (left). The fold change in tumor radiance was higher in...
both treated groups and significantly higher ($P < 0.05$) for the 40 mg/kg treatment group starting at 24 hours and was maintained even at 96 hours after peptide injection (Fig. 4C). Figure 4D shows a significant correlation between the average peptide radiance in the three experimental groups at 24 hours and their average tumor volumes on day 11 ($P < 0.05$). These imaging studies with the EGEVGLG peptide showed that this peptide preferentially binds to sunitinib-treated responsive tumors when compared with untreated controls, as the intensity of peptide binding correlates with a decrease in tumor size and decrease in GFP activity.

**EGEVGLG differentiates between responsive and unresponsive tumors.** The previously described coculture experiments were done using B16 melanoma and BxPC3 pancreatic tumor cell lines that have been shown in previous studies (7) to be unresponsive to sunitinib therapy. HUVECs were cocultured with MDA-MB-231, B16, or BxPC3 cells to simulate tumor vasculature. After treatment with 0.5 μg/mL sunitinib for 1 hour and incubation with fluorescently labeled EGEVGLG peptide for another hour, HUVECs were imaged for peptide binding. MDA-MB-231 showed a significant differential binding (over nine times; $P < 0.05$) in the sunitinib-treated group relative to the untreated control (Fig. 5A). No differential binding was observed in either the B16 or BxPC3 cells lines between the sunitinib-treated and untreated control groups (Fig. 5A).

In nude mice, these B16 or BxPC3 tumors were implanted and treated with either vehicle control or 40 mg/kg of sunitinib once daily for 5 consecutive days. The tumor growth rates in both tumor models showed no significant difference in tumor volumes between treated and untreated groups (Fig. 5B). The EGEVGLG peptide-dye complex was injected 4 hours after the 3rd day of treatment. No significant difference was observed between the radiance of the sunitinib-treated and untreated groups (Fig. 5C and D). Neither of these two tumor models responded to treatment with sunitinib, and this correlated with no preferential binding of the EGEVGLG peptide to these treated tumors.
**Discussion**

The goal of this study was to discover a means to rapidly and noninvasively assess cancer response to the tyrosine kinase inhibitor sunitinib. Sequence analysis of phage recovered from in vivo biopanning of MDA-MB-231 cells showed the following distribution: EGEVGLG, SSAVL, MRRSVGS, FGVR, VLI, SAGSVAL, and GFWEGLL. The first four peptides were also isolated from in vivo biopanning of MCF-7 cells (Table 1). Further investigation on the most active peptide, EGEVGLG, using immunohistochemical and in vitro coculture analyses show that although the peptide seems to bind to the vascular endothelium, a necessary interaction between vascular endothelium and tumor must occur for EGEVGLG to bind to its putative receptor (Fig. 1). Imaging studies using the EGEVGLG peptide-dye complex show that the peptide preferentially binds to sunitinib-treated estrogen receptor/progesterone-positive (MCF-7) and estrogen receptor/progesterone-negative (MDA-MB-435) breast tumors (Figs. 3 and 4). These two different cancer tumor models showed differential binding between the sunitinib-treated groups and untreated control groups in a dose-dependent fashion. In 5 days or less following peptide injection into treated, tumor-bearing mice, a significant difference in peptide binding as indicated by increased tumor radiance was observable between the treated and the untreated groups. Also, we found that the peptide is capable of differentiating between responding and nonresponding tumor types. The EGEVGLG peptide binds to a treated, responding cancer such as MDA-MB-435 but not to the treated, nonresponding cancer cell lines B16 and BxPC3 (Fig. 5).

By correlating this difference in radiance with tumor growth delay in these experiments, the EGEVGLG peptide can predict response to sunitinib therapy early in the course of therapy in tumor-bearing mice. An increase in peptide binding and tumor radiance detected within 72 hours of beginning sunitinib treatment correlates with a significant delay in tumor growth observed several days later (Figs. 3 and 4). This peptide's specificity could potentially reduce the time necessary to assess cancer response to therapy to sunitinib and allow a physician to personalize a patient's treatment regimen more rapidly. Furthermore, it could also eliminate the need for invasive procedures such as biopsies and less accurate methods such as tumor volume measurements to determine response.

Previously studies have shown that various receptors and adhesion molecules are induced in tumor microvasculature in response to therapy, making them potential targets for imaging tumor response and targeted drug delivery (11, 15–25). Thus, there is a strong incentive to identify the putative receptor to which the EGEVGLG peptide binds. Because the peptide binds to the HUVECs in the coculture experiments as well as in the immunohistochemistry samples (Fig. 1), it suggests the target is a membrane receptor with an extracellular domain. Experiments designed to identify the putative cellular receptor for EGEVGLG and the other screened recombinant peptides are currently under way. By determining the properties of these lipid or protein receptors, peptides, and/or antibodies that bind to them with an even higher affinity can be synthesized for use as more accurate biomarkers for tumor response to sunitinib.

In addition, the apparent specificity of EGEVGLG to tumors that are responsive to sunitinib could open up opportunities to explore novel forms of drug delivery (26, 27). Our finding that the EGEVGLG peptide seems to preferentially bind only to tumors responding to sunitinib suggests that conjugating it to a nanoparticle drug delivery system could be an excellent form of adjuvant therapy. This could allow for the delivery of chemotherapeutic agents to metastatic tumors by using these recombinant peptides to target tumor vasculature responding to systemic therapy. The peptide could concentrate the chemotherapeutic drug in responding (cancerous) areas, potentially reducing systemic toxicity and allowing for an increased drug load to be administered to the subject. In the future, efforts to convert this technology from mice to humans will require implementation of many new studies to determine how the peptide interacts with many different systems, particularly the immune system.

Because the peptide can bind to responding cancer cells that have MDA-MB-435 cells stably transfected with GFP, we have developed a model to detect treatment response in metastatic tumors. In our experiments, the observed correlation between GFP radiance and tumor volume changes in the transfected tumor line (Figs. 2 and 3) suggests the potential of these experimental models as a viable means to assess tumor volume, and ultimately tumor response, in a metastatic setting. In vivo use of GFP-expressing cancer cells for imaging offers a robust method of visualizing tumors in situ (26, 27). This technique could potentially allow for the evaluation of the screened recombinant peptides’ ability to assess a tumor’s response to tyrosine kinase inhibitor therapy in metastatic tumor models.

In conclusion, we identified recombinant peptides that discern responding from nonresponding tumors after treatment with sunitinib very early in the course of therapy (<5 days). This is platform technology that shows the principle that recombinant peptide biomarkers are effective at rapidly assessing cancer susceptibility to molecular targeted therapy.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Acknowledgments**

We thank G. Mundy (Vanderbilt University) for the gift of the MDA-MB-435-GFP cell line; E. Ruoslahti (Burnham Institute) for the gift of T7 phage–based random peptide library; J. Huamani and A. Fu for technical support; and Z. Han for multiple suggestions and helpful discussions.

**References**

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