Integrin αvβ3-Targeted IRDye 800CW Near-Infrared Imaging of Glioblastoma

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Abstract

Purpose: Integrin αvβ3 plays an important role in tumor angiogenesis, growth, and metastasis. We have tested a targeted probe to visualize integrin receptor expression in glioblastomas using near-infrared fluorescent (NIRF) imaging.

Experimental design: A transgenic glioblastoma mouse model (RCAS-PDGF-driven/tv-a glioblastoma, which mimics the infiltrative growth pattern of human glioblastomas) and two human orthotopic glioblastoma models (U-87 MG with high integrin β3 expression and TS543 with low integrin β3 expression) were studied. An integrin-targeting NIRF probe, IRDye 800CW-cyclic-RGD peptide (IRDye 800CW-RGD), was tested by in vivo and ex vivo NIRF imaging.

Results: We show that the IRDye 800CW-RGD peptide: (i) specifically binds to integrin receptors; (ii) is selectively localized to glioblastoma tissue with overexpressed integrin receptors and is retained over prolonged periods of time; (iii) is associated with minimal autofluorescence and photobleaching because of imaging at 800 nm; (iv) provides delineation of tumor tissue with high precision because of a high tumor-to-normal brain fluorescence ratio (79.7 ± 6.9, 31.2 ± 2.8, and 16.3 ± 1.3) in the U-87 MG, RCAS-PDGF, and TS543 models, respectively; (v) P < 0.01; and (v) enables fluorescence-guided glioblastoma resection. Importantly, small foci of residual fluorescence were observed after resection was completed using white light imaging alone, and these fluorescent foci were shown to represent residual tumor tissue by histology. 

Conclusions: NIRF imaging with the IRDye 800CW-RGD probe provides a simple, rapid, low-cost, nonradioactive, and highly translatable approach for improved intraoperative glioblastoma visualization and resection. It also has the potential to serve as an imaging platform for noninvasive cancer detection and drug efficacy evaluation studies. Clin Cancer Res: 18(20); 5731–40. ©2012 AACR.
The integrin receptors are highly expressed on many human solid tumors, including glioblastomas. Treatment of glioblastoma initially involves surgery, and the extent of resection correlates with survival. However, tumor margins are difficult to visualize intraoperatively because of the invasive character of glioblastomas. Here, we show that an IRDye 800CW-conjugated RGD peptide can specifically target the overexpressed integrin receptors in murine RCAS-PDGF-driven/tv-a glioblastomas and human orthotopic glioblastomas (U-87 MG and TS543), and thereby visualize tumor tissue and tumor margins by near-infrared fluorescent (NIRF) imaging. This has significant implications for optical molecular imaging applications, both in research and in potentially clinical settings. We show that IRDye 800CW-RGD NIRF imaging facilitates more sensitive glioblastoma detection and more complete surgical resection. A Drug Master File for the IRDye 800CW dye has been registered with the U.S. Food and Drug Administration and instrumentation capable of intraoperative visualization of IRDye 800CW fluorescence is in existence.

with a peak excitation at 774 nm and emission at 805 nm. It exhibits deeper tissue penetration and a higher signal-to-background ratio than Cy 5.5, due to the higher excitation frequency (8). Using a dual-labeled cyclic RGD motif for NIR fluorescence (NIRF) imaging (IRDye 800CW) and gamma scintigraphy (111In), the NIRF from IRDye 800CW provided greater signal to noise than the radiotracer 111In (9). Furthermore, IRDye 800CW can be functionalized with either an NHS or maleimide reactive group, allowing it to be attached to a number of biomolecules for targeting. Examples include trastuzumab (10) and 2-deoxy-D-glucose (11).

Integrins are heterodimeric transmembrane cell surface receptors coupling the extracellular environment to the cytoskeleton, as well as receptors for transmitting signals important for cell migration, invasion, proliferation, and survival. A subset of integrins, including αVβ3, recognizes a common motif in their ligands, the RGD sequence (arginine–glycine–aspartic acid; ref. 12). The integrin αVβ3 is highly expressed not only on the tumor vasculature and angiogenic endothelial cells, but also on tumor cells, including human gliomas (13). In this report, we focus on showing the use of NIFR-800 imaging for the tumor detection in the well-established RCAS-PDGF-driven/tv-a mouse glioblastoma model (RCAS-PDGF; ref. 14), U-87 MG (15), and TS543 (16) human orthotopic glioblastoma models. The RCAS-PDGF, U-87 MG, and TS543 models exhibit different levels of integrin receptor upregulation. We show that an IRDye 800CW–conjugated RGD peptide can specifically target the overexpressed integrin receptors in these glioblastomas, and visualization of the tumor and tumor margins can be achieved by NIRF imaging. This has significant implications for the use of optical molecular imaging in both the preclinical and potentially clinical setting to facilitate more sensitive cancer detection and more complete surgical resection of glioblastomas.

Materials and Methods

Reagents

Cyclic Arg-Gly-Asp-D-Phe-Lys peptide (cRGD) and IRDye 800CW–conjugated RGD peptide (IRDye 800CW-RGD) were provided by LI-COR Bioscience. IRDye 800CW–conjugated cyclic Arg-Ala-Asp-D-Phe-Lys peptide (IRDye 800CW-RAD; LI-COR) was used as the nonspecific control.

Quantitative NIR imaging in vivo

All animal experiments were approved by the Institutional Animal Care and Use Committees of Memorial Sloan-Kettering Cancer Center (MSKCC, New York; protocol number: 06-07-11). Mice were anesthetized with 2.5% isoflurane throughout the imaging procedure. Before image acquisition, mouse heads were shaved. The Pearl Impulse Imager (LI-COR) was used for quantitative NIRF imaging in vivo. The excitation/emission settings for the 700 and 800 nm channels were 685/720 and 785/820 nm, respectively.

Animals were imaged before injection to establish a background level of fluorescence. IRDye 800CW-RGD peptide alone (1 nmol), IRDye 800CW-RAD peptide alone (1 nmol), and blocking peptide mixture (1 nmol IRDye 800CW-RGD peptide plus 20 nmol cRGD peptide), respectively, were injected via the tail vein and imaged at 0.5, 24, and 48 hours. The images were analyzed using Pearl Impulse Software (version 2.0). Regions of interest (ROI) for both head and background were selected from equivalent-sized areas containing the same number of pixels. ROIs were quantified for total pixel values.

Quantitative NIR imaging ex vivo

Quantitative analysis of IRDye 800CW-fluorescence in the sliced brain tissues and the frozen sections were measured ex vivo in the 800 nm channel by the Odyssey Infrared Imaging System (LI-COR). Odyssey Application Software (version 3.0) was used to acquire fluorescence (sensitivity = 5, resolution = 21 μm) and to quantify the absolute fluorescence intensities. The background fluorescence was subtracted from the signal intensities. The area-weighted fluorescence signal was used to compare targeting agent specificity.

Statistical analysis

Statistical comparisons were done using the Sigma Plot 8.0 (SPSS Inc.). The presented data are shown as mean ± SEM. Comparisons between mean values were done using the Student paired t test. Statistical significance was set at P < 0.01.

Results

Differential expression of integrins in the RCAS-PDGF, U-87 MG, and TS543 glioblastoma models

Three different glioblastoma models were chosen to test different expression levels of integrin receptor. The RCAS-

Translational Relevance

The integrin receptors are highly expressed on many human solid tumors, including glioblastomas. Treatment of glioblastoma initially involves surgery, and the extent of resection correlates with survival. However, tumor margins are difficult to visualize intraoperatively because of the invasive character of glioblastomas. Here, we show that an IRDye 800CW-conjugated RGD peptide can specifically target the overexpressed integrin receptors in these glioblastomas, and thereby visualize tumor tissue and tumor margins by near-infrared fluorescent (NIRF) imaging. This has significant implications for optical molecular imaging applications, both in research and in potentially clinical settings. We show that IRDye 800CW-RGD NIRF imaging facilitates more sensitive glioblastoma detection and more complete surgical resection. A Drug Master File for the IRDye 800CW dye has been registered with the U.S. Food and Drug Administration and instrumentation capable of intraoperative visualization of IRDye 800CW fluorescence is in existence.
PDGF transgenic mouse glioblastoma model recapitulates many of the histologic and MRI features of human glioblastomas (17, 18). It is a very aggressive and infiltrating tumor, with the formation of oligodendrogliaomas in approximately 60% of mice by 12 weeks of age (19). The U-87 MG human glioblastoma model was selected as a positive control because it has been shown previously to express high levels of integrin αvβ3 (15). The TS543 glioblastoma model was developed from a human patient with the PDGFRA gene amplification (20), and has been used to investigate the function of glioblastoma-associated onco-genes/tumor suppressor genes (21, 22) and miRNA (23, 24), as well as response to receptor tyrosine kinase inhibitors (16, 20). The TS543 orthotopic xenografts are very invasive, with isolated microscopic tumor foci (25). To assess the expression level of integrin β3 in tumor cells and tumor endothelial cells, coimmunofluorescence staining of integrin β3 with tumor cell markers (HA-tag for the RCAS-PDGFR model, EGFP for the U-87 MG model, and human-specific vimentin for the TS543 model) and an endothelial cell marker (CD31) was conducted. In the RCAS-PDGFR model (Fig. 1A), integrin β3 was significantly overexpressed in tumor endothelial cells (right) and in some, but not all glioblastoma cells (left). Overexpression of integrin β3 was shown in the U-87 MG tumor cells and its expression was barely detected in tumor endothelial cells (Fig. 1B). In the TS543 glioblastoma, very low expression of integrin β3 was detected in both tumor cells and tumor endothelial cells (Fig. 1C), and this model was used as an integrin β3 “low model” in further studies. Different expression levels of integrin β3 in the cultured U-87 MG and TS543 tumor cells was confirmed by Western blotting assay (Fig. 1D).

**Validation of the IRDye 800CW-RGD probe in vitro and in vivo**

To examine the specificity of the IRDye 800CW–labeled RGD probe, tumor sections from the RCAS-PDGFR glioblastomas were stained with the IRDye 800CW-RGD peptide (Fig. 2A). Nonfluorescent cyclic RGD peptide (cRGD) was used as a negative control, as well as a competitive binding reagent. As shown in Fig. 2A, fluorescence emitted at 800 nm (fluorescence-800) was detected in the presence of IRDye 800CW-RGD probe, but not cRGD peptide. Fluorescence-800 decreased significantly when the blocking reagent (cRGD) competed with the IRDye-800CW RGD probe for binding to integrin receptors. Fluorescence-800 from the IRDye 800CW-RAD probe, a nonspecific binding control (26) was detected at very low levels and did not colocalize with the integrin β3 protein. These data showed specific binding of the IRDye 800CW-RGD probe to integrin receptors in vitro.

The IRDye 800CW–labeled RGD probe (1 nmol) was injected intravenously (via tail vein) into normal nude mice to determine its biodistribution, spectrum-specificity, and

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**Figure 1.** Expression of integrin β3 (red) in the RCAS-PDGFR (A), U-87 MG (B), and TS543 (C) glioblastomas by immunofluorescence costaining. CD31 was used as a marker of endothelial cells. HA-tag (A), EGFP (B), and human-specific vimentin (C) were used as tumor cell markers for the RCAS-PDGFR, U-87 MG, and TS543 glioblastomas, respectively. DAPI staining shows nuclei (blue). White scale bars in B and C, 20 μm. Expression of integrin β3 in the cultured U-87 MG and TS543 tumor cells was confirmed by Western blotting (D). β-Actin was used as a loading control.
clearance \textit{in vivo}. At different time points after probe injection (2, 24, 48, and 72 hours), nude mice were monitored by noninvasive dynamic NIRF imaging from both ventral and dorsal views using the Pearl Impulse Imager (Fig. 2B). Fluorescence emitted at 800 nm (from the probe) and 700 nm (fluorescence-700, autofluorescence from the animal) were concomitantly acquired from the same mouse and position. NIRF-800 images from ventral view showed that IRDye 800CW-RGD probe accumulated in the bladder, indicating renal clearance of the agent. NIRF-700 images from both ventral and dorsal views revealed that significant fluorescence-700 was detected from several areas, including the abdomen reflecting gut/food autofluorescence.

The fluorescence signal intensity (fluorescence-800 and fluorescence-700) was quantified using a rectangular ROI, covering the entire body except the tail (indicated in Fig. 2B). Fluorescence-800 from both ventral and dorsal views shared a similar, rapid clearance pattern (Fig. 2B and C). Less than 50% of fluorescence-800 remained 24 hours after injection; at 72 hours, less than 20% of fluorescence-800 was detected. However, fluorescence-700 intensity remained constant from 2 to 72 hours. These results show that fluorescence-800 emissions did not interfere with fluorescence-700 emissions, and that the acquisition of the IRDye 800CW spectrum was channel specific. The IRDye 800CW-RGD peptide NIRF imaging results show low background and high signal-to-background ratio \textit{in vivo}.

**NIRF imaging of IRDye 800CW-RGD probe in glioblastomas \textit{in vivo}**

To assess whether we could noninvasively image the binding of the IRDye 800CW-RGD peptide to integrin receptors expressed in the RCAS-PDGF, U-87 MG, and TS543 glioblastomas, tumor-bearing, and nontumor-
bearing control mice were imaged. Both groups of animals were intravenously injected with the IRDye 800CW-RGD probe (1 nmol). Animals were monitored by dynamic NIRF imaging at multiple time points (0, 0.5, 24, and 48 hours). NIRF-800 images from the head area are shown in Fig. 3A. At 0.5 hours postinjection, fluorescence-800 reached a peak in both tumor-bearing and normal animal groups; signal intensity at 0.5 hours was defined as 100% in Fig. 3B. At 24 hours, tumor targeting of IRDye 800CW-RGD peptide was observed, as the fluorescence signal intensity from the tumor areas was clearly greater than the signal intensity from corresponding areas in normal mice. At 48-hour postinjection, the head fluorescence tumor-to-normal brain ratio (T/N) was maximal; 2.98 ± 0.22 in the RCAS-PDGF model (P < 0.01), 3.26 ± 0.18 in the U-87 MG model (P < 0.01), and 1.42 ± 0.11 in the TS543 model.

Binding of the IRDye 800CW-RGD peptide specifically to tumor integrin receptors was established in 2 ways. First, fluorescence-800 signal intensity was compared with that of a control nonspecific IRDye 800CW-RAD peptide. In all 3 glioblastoma models, there was a highly significant difference in signal intensity between the RGD and RAD peptides in tumor-bearing animals (P < 0.01). Furthermore, there was only a small, insignificant difference in signal intensity...
of the IRDye 800CW-RAD control peptide between tumor-bearing and normal animals (Fig. 3B). Second, a blocking assay, involving the coinjection of the IRDye 800CW-RGD probe with 20 times the amount of unlabeled cRGD probe, was conducted. The unlabeled cRGD peptide significantly reduced tumor uptake of the IRDye 800CW-RGD probe in the integrin β3-high glioblastomas (Fig. 3B, left and middle). For example, at 48-hour postinjection in the RCAS-PDGF model, the normalized NIRF-800 intensity from the IRDye 800CW-RGD peptide alone and in the RGD blocking study was 24.8 ± 3.1% and 11.1 ± 3.0%, respectively (P < 0.01).

**NIRF imaging of IRDye 800CW-RGD probe in glioblastomas ex vivo**

To confirm the presence of tumor and further characterize the distribution pattern of IRDye 800CW-RGD fluorescence in the glioblastoma-bearing brains, animals were sacrificed 48 hours after probe injection for ex vivo assays. Whole brains from both glioblastoma-bearing mice and normal mice were harvested and ex vivo NIRF imaging was conducted. In the integrin β3-high glioblastoma-bearing brains (Fig. 4A and B), fluorescence-800 intensity was very high in the tumor area of the IRDye 800CW-RGD–injected animals. Fluorescence-800 from the IRDye 800CW-RAD peptide (nonspecific probe) was barely above background in the fluorescent-800 image showed a high fluorescence signal from the hyperemic tumor area (black arrow heads). A 2-step resection was done to remove the tumor tissue. The resected tissue exhibited very high fluorescence and tumor was confirmed by H&E staining (Fig. 5C). After resection step 1, significant fluorescence in the resection bed was still observed. A second resection step (resection 2) was then done to remove the remaining tissue with strong fluorescence. However, a very small area of residual fluorescence was still observed after resection 2, suggesting residual tumor tissue. To confirm the presence or absence of tumor in the resection bed after resection 2, cryosections (10 μm thick) were prepared for ex vivo NIRF imaging by an Odyssey Imager and histologic examination. The presence of a very small amount of residual tumor tissue in the resection bed after resection 2 was confirmed, showing that the IRDye 800CW-RGD NIRF imaging can aid in the resection of microscopic tumor foci (Fig. 5D).

**Discussion**

NIRF imaging in the 650 to 900 nm range has specific advantages: efficient NIRF photon penetration of tissue over several millimeters, minimal intratissue photon scattering, and low absorption by water and biomolecules, as well as minimal autofluorescence in this spectral range. Different integrin-targeted NIRF optical probes have been developed. The NIR fluorophores include cyanine analogs [such as Cy5 (ref. 27), Cy5.5 (ref. 15), and Cy7 (ref. 28)] and fluorescent nanoparticles (such as semiconductor nanocrystals, i.e. quantum dots; ref. 29). Studies have shown that NIRF dyes conjugated to cyclic RGD peptides were able to visualize subcutaneously inoculated integrin-positive tumors. However, both unconjugated Cy5.5 and Cy7 showed relatively high tumor-nonspecific targeting, which may be because of the high lipophilicity of the cyanine dyes (30). Furthermore, these NIRF probes were unable to visualize U-87 MG orthotopic gliomas noninvasively, although the dissected tissue showed tumor-specific uptake of the NIRF probes. Quantum dots represent an attractive NIRF contrast agent because of their composition-tunable fluorescence emission, good quantum yield, increased brightness, and photostability (31). However, their uptake may be restricted in gliomas (particularly low-grade gliomas) because of their...
size and the blood–tumor barrier. In addition, the uptake and retention of quantum dots in other organs (e.g., spleen, liver, and lung), and the potential toxicity may provide obstacles to further clinical applications (29).

Here, we have evaluated an alternative NIRF dye with excitation and emission characteristics that provide enhanced tissue penetration and thus better sensitivity and ultimately more accurate quantification of integrin expression in vivo. IRDye 800CW ($\lambda_{ex}/\lambda_{em} = 774/805$ nm) is a good candidate because the RGD peptides coupled with IRDye 800CW showed deeper tissue penetration, less scattering, and lower background fluorescence compared with Cy5.5.

Figure 4. NIRF imaging of the RCAS-PDGF (A and D), U-87 MG (B and E), and TS543 (C and F) glioblastomas ex vivo. Mice brains were dissected 48 hours after injection of IRDye 800CW-RGD alone, IRDye 800CW-RAD alone (nonspecific peptide control), and IRDye 800CW-RGD plus cRGD peptide (blocking assay). Whole brains (A–C) and sliced brains (D–F) were imaged by a Pearl Impulse Imager and an Odyssey Imager, respectively. Tumor areas are indicated by the red arrowheads in the photograph. The black background in the NIRF-800 columns (A–C) and rows (D–F) was converted to a white-background to facilitate visualization of the fluorescence emissions. Fluorescence at 800 nm from the tumor area and the corresponding normal brain area was quantified (right panel in D–F); error bars, SEM. H&E staining was used to confirm the presence of a glioma. Blue scale bars in the insets, 50 μm.
Ex/Eₘ = 675/694 nm) and Cy7 (Ex/Eₘ = 743/767 nm) in a U-87 MG subcutaneous tumor model (30), as well as a M21 melanoma model (31). However, integrin-targeting NIRF probes have not yet been tested in the more clinically relevant spontaneous or induced glioblastoma mouse models. Such models show a unique intracranial microenvironment and infiltrating character, which cannot be replicated by subcutaneous or intracranial xenografts. In this study, a commercially available IRDye 800CW-conjugated cyclic RGD peptide was investigated in a preclinical mouse model of glioblastoma tumorigenesis, namely, the RCAS-PDGF glioblastoma model, as well as in 2 human glioblastoma intracranial xenografts models, U-87 MG and TS543.

We first showed specific binding of the IRDye 800CW-RGD peptide to integrin receptors. Conjugation of IRDye 800CW to cRGD peptide did not have significant effect on the optical properties of IRDye 800CW nor on the receptor binding affinity and specificity of the cRGD peptide. Comparing the NIRF images in the 800 and 700 nm channels, we observed that the IRDye 800CW-RGD peptide resulted in very low background fluorescence in vivo in the 800 nm channel, whereas significant background and autofluorescence were observed in the 700 nm channel. The NIRF-800 images were not “contaminated” by fluorescence from the 700 nm channel, showing the spectrum-specificity of IRDye 800CW-RGD peptide. This would also allow for fluorescence multiplexing, by combining the IRDye 800CW with other NIR fluorophores with different emission wavelengths. Ex vivo binding assays also showed that the IRDye 800CW-RGD peptide had significantly higher affinity to integrin receptors than the nonspecific peptide (IRDye 800CW-RAD), and ex vivo blocking assays confirmed that the receptor binding of IRDye 800CW-RGD peptide was saturable.

We have also showed the selective retention and long-lasting tumor accumulation of the IRDye 800CW-RGD peptide in the integrin β₃-high glioblastomas, both in vivo and ex vivo. Noninvasive dynamic NIRF imaging showed rapid clearance of the IRDye 800CW-RGD peptide in the brains of normal mice and high tumor-targeting of the IRDye 800CW-RGD peptide in the glioblastoma-bearing mice. The maximum tumor-to-normal brain ratio of IRDye 800CW-RGD fluorescence in living animals (T/N = 3.3 in U-87 MG model, T/N = 3.0 in RCAS-PDGF model, and T/N = 1.4 in the TS543 model) was observed at 48-hour postinjection.

NIRF imaging of tumor-bearing brains ex vivo (comparable to a neurosurgical setting) showed high and specific...
tumor uptake of the IRDye 800CW-RGD peptide in all 3 glioblastoma models. In the integrin $\beta_3$-“low” TS543 glioblastomas, tumor-targeting of the IRDye 800CW-RGD peptide was lowest with a $T/N = \text{ratio of 16}$. In contrast, the $T/N$ = ratios in the other integrin $\beta_3$-high glioblastomas were considerably higher ($T/N = 80$ in U-87 MG model and $T/N = 31$ in RCAS-PDGF model), reflecting the different expression levels of integrin $\beta_3$ in these glioblastomas. Overexpression of other integrin family members in the TS543 glioblastomas is also possible, because it has been reported that all 5 $\alpha_v$ integrins, 2 $\beta_3$ integrins, and $\alpha_v\beta_3$ share the ability to recognize ligands containing an RGD tripeptide active site (32).

The potential for translation to clinical applications exists. For example, intraoperative fluorescence-guided resection emerged as a promising technique to facilitate tumor resection of malignant gliomas in the late 1940s. More recently, 5-ALA/PpIX has been used as a fluorescent probe for visualization of malignant glioma tissues intraoperatively. 5-ALA is a nonfluorescing molecule that is metabolized to fluorescent protoporphyrin IX (PpIX). PpIX accumulates preferentially in glioma cells and emits a red-violet light (635–704 nm) when excited with blue light (400–410 nm). Stummer and colleagues showed in a phase III trial that 5-ALA/PpIX fluorescence-assisted neurosurgical resection of gliomas led to a significantly higher frequency of complete resections (MRI contrast–enhancing tumor area) as compared with the conventionally operated white light resection group (65% vs. 36%, respectively; ref. 33). However, there remain several inherent limitations in using 5-ALA during surgical resection, including specificity (34, 35) and photobleaching (36).

An advantage of the IRDye 800CW is that both the excitation and emission wavelengths (774 and 805 nm) are centered at a wavelength amenable to intraoperative imaging, which allows deeper tissue penetration of both excitation and emission photons. For example, we were able to detect IRDye 800CW-RGD fluorescence from intracranial glioblastomas in intact animals, with photons having to penetrate the skull and skin. We also did not observe problematic photobleaching effects of IRDye 800CW-RGD peptide under ambient room light and NIRF laser excitation.

Another advantage of the IRDye 800CW-RGD peptide, in comparison to fluorescein and 5-ALA, is its tumor-targeting properties (binds specifically to integrin receptors). As the integrin $\alpha_v\beta_3$ is overexpressed in both malignant and low-grade gliomas (although at lower levels; ref. 37), it should be possible to visualize low-grade gliomas using the IRDye 800CW-RGD probe. The toxicity of IRDye 800CW dye has been tested in a preclinical model with good results (7). IRDye 800CW dye is currently on record with European regulatory authorities and a drug master file has been registered with the U.S. Food and Drug Administration in the anticipation of similar clinical trials in the United States. A cyclic RGD peptide-based drug (cilengitide) for the treatment of glioblastoma is also in phase III clinical trials (38).

Instrumentation capable of visualizing the IRDye 800CW fluorescence is in existence. The Frangioni group developed the FLARE intraoperative NIRF imaging system, which has been used in clinical trials to map sentinel lymph nodes in breast cancer (39) or image the thoracic duct (40). In addition, the Zeiss Penero (Carl Zeiss; ref. 41) and the Leica FL800 (Leica Microsystems; ref. 42) are adaptable for intraoperative surgical resection of tumor tissue using IRDye 800CW-targeting agents. Thus, the IRDye 800CW-RGD peptide is a potential alternative to 5-ALA for fluorescence-guided glioblastoma resection.

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

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Conception and design: R. Huang, J. Vider, M. F. Kircher, R. G. Blasberg
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