**AWARD NUMBER:** W81XWH-18-1-0160

**TITLE:** Dual PET/Fluorescence Imaging of Glioma with an MMP-14-Activatable Peptide Probe

**PRINCIPAL INVESTIGATOR:** Jason Warram, PhD

**CONTRACTING ORGANIZATION:** University of Alabama at Birmingham
Birmingham, AL 35294

**REPORT DATE:** Sept 2019

**TYPE OF REPORT: Annual**

**PREPARED FOR:** U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



| **1. REPORT DATE** *(DD-MM-YYYY)* | **2. REPORT TYPE** | **3. DATES COVERED** *(From - To)* |
|---|---|---|
| Sept 2019 | Annual | 1 Sept 2018-31 August 2019 |

**4. TITLE AND SUBTITLE**

Dual PET/Fluorescence Imaging of Glioma with an MMP-14-Activatable Peptide Probe

**5a. CONTRACT NUMBER**

**5b. GRANT NUMBER**
W81XWH-18-1-0160

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**

Jason Warram, PhD

**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

University of Alabama at Birmingham
AB 1170
1720 2nd Avenue South
Birmingham AL 35294-0111

Stanford University
1201 Welch Road
Stanford, CA 94305-2004

**8. PERFORMING ORGANIZATION REPORT**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**10. SPONSOR/MONITOR'S ACRONYM(S)**

**11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

**12. DISTRIBUTION / AVAILABILITY STATEMENT**
Approved for public release

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**

The purpose of the project is to develop and test, in preclinical models, a sensitive, specific imaging peptide probe to distinguish GBM tissue from healthy tissue. The novel imaging probe targets a cell-surface marker, matrix metalloproteinase 14 (MMP-14) that is expressed to a much greater degree in GBM cells than in healthy cells. Upon binding to MMP-14, a dual-modality fluorescent tag is activated. This tag is detectable by positron emission tomography (PET) for pre-operative tumor assessment, and by near infrared fluorescence (NIRF) imaging for real-time surgical guidance in distinguishing tumor cells from healthy cells. Results from the project indicate that GBM cells with MMP-14 activity showed activation and retention of NIRF signal from the cleaved peptide probes. Resected mouse brains with patient derived xenograft (PDX) GBM tumors showed tumor-to-background NIRF ratios of 7.6-11.1 at 4 h after *i.v.* injection of the peptides. PET images showed localization of activity in orthotopic PDX tumors after *i.v*. injection of radiolabeled peptide probes; uptake of the radiolabeled probes in tumors was significantly reduced ($p<0.05$) by blocking with the non-labeled peptide. PET and NIRF signals correlated linearly in the orthotopic PDX tumors. Immunohistochemistry showed co-localization of MMP-14 expression and NIRF signal in the resected tumors.

| 15. SUBJECT TERMS<br>Glioma, GBM, molecular imaging, peptide, PET, NIRF, preclinical, MMP-14, dual modality | | | | | |
|---|---|---|---|---|---|
| 16. SECURITY CLASSIFICATION OF: U | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON<br>USAMRMC |
| a. REPORT<br>U | b. ABSTRACT<br>U | c. THIS PAGE<br>U | UU | 74 | 19b. TELEPHONE NUMBER *(include area code)* |

**Standard Form 298 (Rev. 8-98)**
**Prescribed by ANSI Std. Z39.18**

## TABLE OF CONTENTS

## 1. INTRODUCTION:

Malignant glioma (GBM) is the most common brain tumor that occurs in adults, yet the 5-year survival rate is only 5%, with the majority of patients surviving less than 14 months after diagnosis. It is a rapidly growing, aggressive tumor that grows diffusely into neighboring areas of the brain. Exposure to ionizing radiation is the main external risk factor associated with GBM, and individuals exposed to nuclear weapons testing or other types of ionizing radiation are at an increased risk of developing GBM compared to the general population. There are known survival benefits associated with surgical removal of the GBM tumor. However, the diffuse growth of the tumor makes it difficult for a surgeon to clearly distinguish tumor cells from adjacent, healthy cells. As a result, GBM cells remain in the brain post-surgery, leading to tumor recurrence and the death of the patient. Therefore, there is an urgent need for novel tools that improve tumor detection and the prognosis for individuals diagnosed with GBM. The purpose of the project is to develop and test, in preclinical models, an imaging probe that is both sensitive and specific in distinguishing GBM tissue from healthy tissue. The novel imaging probe will target a cell-surface marker, matrix metalloproteinase 14 (MMP-14) that is expressed to a much greater degree in GBM cells than in healthy cells. Upon binding to its target, a dual-modality fluorescent tag will be activated. This tag will be both detectable by positron emission tomography (PET) to allow for pre-operative assessment of tumor burden and localization, and by near infrared fluorescence (NIRF) imaging to allow for real-time surgical guidance in distinguishing tumor cells from healthy cells. Once developed, the use of this probe will be validated in mouse models of GBM to determine *in vivo* pharmacokinetics, biodistribution, signal retention, sensitivity, and specificity for imaging the GBM tumors. The successful development of this innovative tool will provide a crucial foundation to address a critical gap in managing GBM patient care.

## 2. KEYWORDS:

Glioma, GBM, molecular imaging, peptide, PET, NIRF, preclinical, MMP-14, dual modality

## 3. ACCOMPLISHMENTS:

**What were the major goals of the project?**

Specific Aim 1: Design and characterize an MMP-14 activatable dual PET/NIRF peptide probe.

Major goal 1: Develop novel MMP-14 activatable peptide probes.
Milestone achieved: Identification of initial candidate MMP-14 peptide probes at Stanford and shipment to UAB.
Target completion from SOW: February 2019
Actual completion date: Prior to July 2018

Specific Aim 2: Establish the sensitivity and specificity of the MMP-14 activatable dual PET/NIRF peptide probe for in vivo imaging of GBM models.

Major goal 2: Perform in vivo studies (using the peptides in mice with GBM tumors) to identify a lead peptide probe.
Milestone to achieve: Identify a lead substrate-peptide conjugate for further in vivo studies. Criteria for identification will be: candidate with highest tumor uptake with lowest background tissue accumulation.
Target completion from SOW: August 31, 2019
Percentage of completion as of August 1, 2019: 90%

Major goal 3: Determine the optimal dose and imaging time points for *in vivo* imaging with the lead peptide probe, with corresponding sensitivity and specificity metrics.
Milestone to achieve: Identification of optimal dose and imaging time point for the lead peptide probe.
Target completion from SOW: November 31, 2019
Percentage of completion as of August 1, 2019: 50%

Milestone to achieve: Sensitivity, specificity, PPV (positive predictive value), NPV (negative predictive value) of lead peptide probe determined.
Target completion from SOW: December 31, 2019
Percentage of completion as of August 1, 2019: 70%

Major goal 4: Test lead peptide probe in a panel of GBM models *in vivo.*
Milestone to achieve: Global sensitivity, specificity, PPV, NPV of lead peptide probe determined across multiple PDX models.
Target completion from SOW: June 30, 2020
Percentage of completion as of August 1, 2019: 25%

Milestone to achieve: Submit additional extramural research proposals (DOD, NIH) for subsequent stages of investigation (surgical resection in mice with PDX tumors).
Target completion from SOW: August 31, 2020

**What was accomplished under these goals?**

Major activity: Dr. Jianghong Rao at Stanford has been synthesizing and characterizing the novel MMP-14 activatable peptide probes for the project. The objective is to produce MMP-14 activatable peptide probes that could be used for both NIRF and PET imaging of MMP-14 activity and expression (see Figure 1 of the attached manuscript in the appendix). Methods have included a combination of solid phase peptide synthesis and solution phase chemistry to produce the peptides, followed by standard chemical characterization techniques (high performance liquid chromatography and mass spectrometry). Major accomplishments: As shown in Supplementary Figures S2, S3, S4, S5, S6, and S7 (see attached Supplementary Material in the appendix), their work has generated an MMP-14 activatable "substrate peptide" that can be used for NIRF imaging, an MMP-14 "binding peptide" that can be used for PET imaging, and a "substrate-binding peptide" (a covalently joined construct of the substrate and binding peptide moieties) that can be used for both NIRF and PET imaging. These peptides have been sent to Dr. Jason Warram at UAB and used in the following in vitro and in vivo experiments.

Key outcome achieved: an initial series of novel, MMP-14 peptide probes has been successfully produced to fulfill Major Goal 1 above.

Major activity: Characterized MMP-14 expression and activity in several human glioma tumor tissues and cells lines. The objective was to validate the overexpression of MMP-14 in glioma and glioma cell lines that would be suitable in assays that employ peptide probes as enzymatic substrates for MMP-14. Methods have included immunofluorescence staining of a human glioma tissue microarray, GBM cell lines cultured in vitro, and GBM tumor xenografts grown in athymic nude mice in vivo; Western blot analyses of MMP-14 and MMP-2 expression in GBM cell lines cultured in vitro; and gel zymography analyses of soluble MMP-2 activity of GBM cell lines cultured in vitro. Major results: Human glioma tissues, GBM xenografts, and GBM cell lines express MMP-14 on the cell surface, and expression levels are significantly higher in GBM tissues relative to normal brain tissue

($p<0.05$). Different GBM cell lines display different levels of MMP-14 expression and activity (see Figure 2 of the attached manuscript in the appendix, and Supplementary Figure S1 of the attached Supplementary Material in the appendix), and are suitable for assays with novel MMP-14 peptide probes.

Major activity: Determine peptide probe binding, cleavage, and NIRF signal activation in vitro with MMP-14 enzyme, GBM cell lines. The objective is to determine that the novel peptides are substrates for MMP-14 to activate the NIRF signal, and that GBM cells show retention of the NIRF and PET signals from the peptides. Methods have included NIRF activation and microscopy studies using the peptides and recombinant MMP-14 enzyme or GBM cell lines cultured in vitro, and binding assays with the radiolabeled binding peptide or substrate-binding peptide and GBM cell lines cultured as adherent monolayers in vitro. Major results: Both the substrate peptide and substrate-binding peptides showed NIRF signal activation within the first several minutes and up to 2 h after incubation with MMP-14 enzyme or with GBM cells. GBM cells incubated for 4 h with the substrate-binding peptide showed significantly more cell-associated NIRF signal than cells incubated for 4 h with the substrate peptide ($p<0.05$) (see Figure 3 of the attached manuscript in the appendix). These results are consistent with the anticipated mechanism of the substrate-binding peptide, where the MMP-14 binding moiety mediates cellular retention of the residual fluorophore-containing product following cleavage of the substrate sequence by MMP-14. Ongoing studies with the peptides are being performed to determine the enzyme selectivity and kinetics of peptide cleavage in the presence of MMP inhibitors. Cell binding studies showed that the radiolabeled $^{64}$Cu-binding peptide binds to GBM cells cultured in vitro, with increased cell-associated activity present at 2 h than at 30 min (Figure A). As expected, lower activity was associated with the cells when the non-labeled binding peptide was used to block MMP-14 binding sites. Separate studies showed that the radiolabeled $^{64}$Cu-substrate-binding peptide also bound to GBM cells cultured in vitro in a temperature-dependent manner, where more activity was present following incubation at 37 °C relative to 4 °C (Figure B). This result is consistent with internalization of the radiolabeled peptide after binding to MMP-14 on the cell surface. Ongoing studies with the radiolabeled peptides are being performed to determine the affinities and further define the specific binding of the peptides to MMP-14 on GBM cells cultured in vitro.

Key outcomes achieved: MMP-14 and human GBM cells are capable of cleaving the novel peptide substrates in vitro to activate the NIRF signal. The cell-associated NIRF and PET signals from the peptides indicate that the peptides to the GBM cell lines.

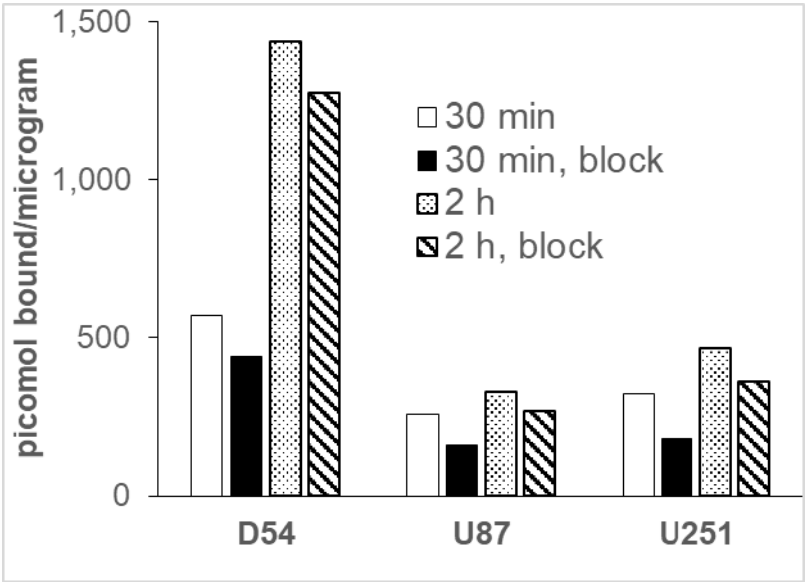



**Figure A**. Radiolabeled binding peptide binds to human GBM cells in vitro. Adherent GBM cells in 24-well plates (0.4 million cells/well) were incubated at 37 °C for 30 min or 2 h with 46 nM $^{64}$Cu-binding peptide. Excess non-labeled binding peptide (1 μM) was added as a block to selected wells. Following incubation, cells were rinsed with PBS and lysed with 1 M NaOH, and the solution was counted on a gamma counter to calculate the cell-associated $^{64}$Cu-binding peptide signal, expressed as picomol bound per microgram of protein present in the lysed cell solution.

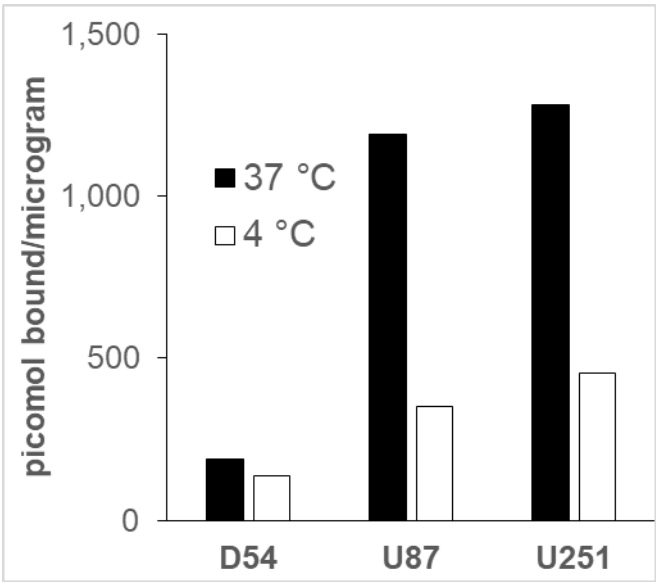



**Figure B**. Radiolabeled substrate-binding peptide binds to human GBM cells in vitro. GBM cells in suspension (0.5 million cells/tube) were incubated at 37 °C or 4 °C for 10 h with 20 nM $^{64}$Cu-substrate-binding peptide. Following incubation, cells were rinsed with PBS, then cell surface-bound activity was collected by rinsing the cells with pH 4 buffer for 10 min. The cells were then lysed with 1 M NaOH, and the collected solutions were counted on a gamma counter to calculate the cell-associated $^{64}$Cu-substrate-binding peptide signal, expressed as picomol bound per microgram of protein present in the collected solutions.

Major activity: Perform NIRF imaging and PET/CT pharmacokinetic and biodistribution studies with the first candidate substrate, binding, and substrate-binding peptide probes in mice with orthotopic PDX GBM tumors (one xenoline). The objective is to characterize the GBM xenograft tumor uptake, specificity and pharmacology of the imaging candidates in mice. Methods have included NIRF imaging studies performed 1-24 h after i.v. injection of the peptides in mice with subcutaneous (s.c.) flank xenografts of GBM cell lines, or in mice with orthotopic (intracranial) patient-derived xenograft (PDX) GBM tumors. PET/CT imaging and biodistribution studies with the peptides have been performed 1-6 h after i.v. injection of the radiolabeled peptides in mice with orthotopic PDX GBM tumors. Major results: At 24 h after *i.v*. injection of the substrate-binding peptide, *in vivo* NIRF imaging showed a low tumor-to-background ratio (TBR, 1.3±0.2 relative to muscle) in s.c. D54 tumors and significantly higher TBR in s.c. U87 tumors (2.2±0.4; $p<0.001$) (see Figure 4A,B of the attached manuscript in the appendix). Low NIRF signal was observed in all normal tissues except the kidneys, indicating predominantly renal clearance of the peptide. Subcutaneous U87 tumors from groups of mice injected *i.v*. with either the substrate peptide or the substrate-binding peptide showed no significant difference in TBR or mean NIRF intensity at the 24 h time point (see Supplementary Figure S8 of the attached Supplementary Material in the appendix). These initial studies demonstrated that glioma tumors show uptake of NIRF signal after administering the quenched peptide substrates.

High contrast of the NIRF signal in orthotopic PDX JX12 tumors relative to adjacent normal brain was apparent in gross slices of resected brains at 1, 4, and 24 h after *i.v*. injection of either the substrate peptide (see Figure 4C of the attached manuscript in the appendix) or the substrate-binding peptide (see Figure 4D of the attached manuscript in the appendix). NIRF analyses of gross brain slices from the 4 and 24 h time points (see Table 1 of the attached manuscript in the appendix) showed significantly higher ($p<0.001$) mean NIRF signal in the tumor relative to adjacent normal brain from mice given the substrate-binding peptide. While the NIRF mean tumor signals and TBRs were higher in mice given the substrate-binding peptide relative to the substrate peptide (see Table 1 of the attached manuscript in the appendix), the TBRs were not significantly different at the time points examined ($p>0.05$, ANOVA). NIRF microscopy showed dispersion of the activated NIRF signal from the peptides throughout the tumors, including near the leading edge of tumor progression (see Figure 4C, D of the attached manuscript in the appendix). Both peptides also yielded positive NIRF signals in regions of diffuse glioma cell growth beyond the bulk tumor. Comparing histological tissue sections with confirmed glioma growth by H+E to NIRF signals measured in tissue sections yielded sensitivities above 83% for the substrate peptide and above 85% for the substrate-binding peptide (see Supplementary Table S1 of the attached Supplementary Material in the appendix) at the 4 h and 24 h time points. These results confirmed that the novel MMP-14 peptides could be used for NIRF imaging of orthotopic models of human glioma.

A preliminary study was performed using the binding peptide labeled with $^{68}$Ga ($^{68}$Ga-binding peptide) in mice with orthotopic PDX JX12 tumors. At 2 h after i.v. dosing, the tumors were visible during PET/CT imaging while normal brain showed low uptake of activity (see Figure 5A of the attached manuscript in the appendix). *Ex vivo* biodistribution analyses indicated significantly more uptake of radioactivity in brains of mice injected with $^{68}$Ga-binding peptide (0.16±0.02 %ID/g) compared to brains of mice injected with $^{68}$Ga-binding peptide and 60-fold excess non-labeled binding peptide as a blocking agent (0.07±0.02 %ID/g, $p<0.01$) (see Figure 5B of the attached manuscript in the appendix), thus supporting the specific retention of the radiolabeled peptide in the PDX tumors.

The amount of $^{68}$Ga in resected brains correlated with qualitative tumor burden determined by H+E tissue analyses (see Supplementary Figure S10 of the attached Supplementary Material in the appendix). PET images and *ex vivo* biodistribution analyses showed high accumulation of activity in the liver, spleen, and kidneys (see Supplementary Figure S11, S12 of the attached Supplementary Material in the appendix), which was likely due to the relative hydrophobicity of the peptide at physiological pH. A separate cohort of mice bearing orthotopic PDX JX12 tumors were used for PET imaging and biodistribution analyses with $^{64}$Cu-labeled substrate-binding peptide ($^{64}$Cu-substrate-binding peptide). At 4 h after i.v. dosing, PET/CT imaging showed significant contrast in the tumor relative to normal brain (see Figure 5C of the attached manuscript in the appendix), yielding a standardized uptake value ratio (SUVR: ratio of tumor $SUV_{mean}$ to normal brain $SUV_{mean}$) of 3.9±0.5. The SUVR was lower in a group of tumor-bearing mice (2.5±1.3) that had been co-injected with 80-fold excess of the non-labeled binding peptide as a blocking agent, although the difference between the two groups of mice was not significant ($p=0.056$). The activity present in whole resected brains from mice in the $^{64}$Cu-substrate-binding peptide group (0.43±0.13 %ID/g) was significantly higher than that in the blocked group (0.23±0.07 %ID/g; $p<0.05$) (see Figure 5D of the attached manuscript in the appendix). This result suggests that the binding peptide is able to partially block binding of the substrate-binding peptide to PDX glioma tumors *in vivo*. Biodistribution analyses of resected tissues was consistent with the PET images, indicating predominantly hepatobiliary accumulation of activity (see Supplementary Figure S14, S15 of the attached Supplementary Material in the appendix). Future studies planned include pharmacology studies of the radiolabeled substrate-binding peptide from 0-6 h after i.v. injection in mice with orthotopic PDX GBM tumors.

Key outcomes achieved: The first candidate peptide probes show specific localization and favorable retention of the NIRF and PET signals in a human PDX GBM tumor model in vivo.

Major activity: Perform histological and zymography analyses of MMP-14 in resected tumors and brain sections from biodistribution studies. The objective is to correlate MMP-14 target expression and probe signal accumulation. Methods include NIRF imaging, histology, MMP-14 immunofluorescence staining, and gel zymography of resected tissues from orthotopic PDX tumor-bearing mice injected with the radiolabeled peptide probes. Correlations between PET, NIRF, and MMP-14 levels in the tumors will be generated and compared to values obtained from normal tissues. Major results: The resected brains from mice injected with the $^{64}$Cu-substrate-binding peptide in the above studies were sectioned and used for NIRF analyses. Gross imaging showed high contrast between the NIRF signal in PDX tumor regions compared to contralateral normal brain, yielding a TBR of 7.2±1.3 ($p<0.001$). The summed NIRF signal from tumor regions in these tissue sections correlated linearly ($R^2=0.84$, $p<0.0001$) with the *in vivo* PET %ID/cm$^3$ signal present in the tumor-bearing brain regions (see Figure 6A of the attached manuscript in the appendix). Microscopic imaging of hematoxylin and eosin (H+E) stained tissue sections confirmed that the NIRF signal co-localized in the PDX glioma tumors, which showed high expression of MMP-14 relative to normal brain (see Figure 6B-G of the attached manuscript in the appendix). These results support the hypothesis that the NIRF signal from the cleavable peptide was specifically retained in the PDX glioma tumors due to the expression of MMP-14 in the tumors. Future studies will compare tissue MMP activity (as determined by gel zymography) and quantified MMP-14 expression with the PET and activated NIRF signals in the tissues.

Key outcomes achieved: The PET and NIRF signals from the first candidate peptide probes show a positive, linear correlation in tumor tissues. Signals from the probes show high spatial congruity with MMP-14 expression in tumor tissues. Based on the cumulative results, the initial candidate peptides meet criteria as “lead” probes for the remaining studies.

Two different postdoctoral scholars (Benjamin Kasten and Hailey Houson) have worked significantly on the project under the mentorship of Dr. Jason Warram and other senior investigators at UAB. Training activities resulting from the project have provided these postdoctoral scholars with greater proficiency in designing and executing multi-modality imaging experiments, NIRF image processing, PET image processing, histological analyses, tumor biology, writing scientific research grants, and manuscript preparation. Thier professional development activities have included the opportunity to attend and present at annual scientific conferences (as specified further below). Four different medical students (Aditi Jani, Denzel Cole, Andrew Prince, Neha Udayakumar) completed a 2-4 month mentored scholarly research activity rotation in Dr. Warram's laboratory. Three different undergraduate students (Savannah Ferch, Himani Modi, Morgan Richardson) also participated in directed research activities in Dr. Warram's laboratory as part of their degree credits. These individuals received training in techniques related to the project, including cell assays, histological tissue processing and analyses, and fluorescence imaging.

**How were the results disseminated to communities of interest?**

Initial results from the project have been disseminated to the scientific research community through presentation at an annual, national meeting (Society of Nuclear Medicine and Molecular Medicine, 2019 Annual Meeting), and through submission of a manuscript to a peer-reviewed journal (*Eur J Nucl Med Mol Imaging*, submitted, under review).

**What do you plan to do during the next reporting period to accomplish the goals?**

Planned activity: Perform blood and tissue pharmacokinetic studies with the initial candidate peptide probes in mice with orthotopic PDX GBM tumors. The objective is to determine the in vivo stability of the peptide and to identify optimal time points to perform in vivo NIRF or PET imaging analyses. Anticipated completion date: December 31, 2019.

Originally planned activity: Identification of additional candidate MMP-14 peptide probes at Stanford and shipment to UAB. This originally planned activity may not be pursued in the next reporting period, because the initial candidate peptides meet criteria as “lead” probes for the remaining studies. If further studies (e.g., pharmacology) do not support further development of the initial candidate peptides developed at the current stage of the project, additional candidate peptides will be generated and tested. Anticipated date to decide if this activity will or will not be further pursued: December 31, 2019.

Originally planned activity: NIRF and PET/CT pharmacokinetic and biodistribution studies with additional candidate substrate, binding, and substrate-binding peptide probes in mice with orthotopic PDX GBM tumors (one xenoline). This task will be performed to characterize the specificity and pharmacology of additional imaging candidates, if they are developed as indicated based on the above criteria. If this activity is pursued, the anticipated target date for completion will be: June 30, 2020.

Milestone Achieved: Lead substrate-peptide conjugate identified for further in vivo studies. The cumulative in vitro and in vivo data from the above studies will be assessed to determine the lead peptide probe for subsequent studies. The probe with the highest specificity for imaging tumor relative to normal brain within 6 h of dosing. Target completion from SOW: August 31, 2019. Anticipated completion date: December 31, 2019.

Planned activity: Explore 1 nmol and 10 nmol doses of the peptides at 3 time points in mice with orthotopic PDX GBM tumors (one xenoline); use peptide block and non-tumor groups as controls. These in vivo PET and NIRF imaging studies will be performed to assess the effects of various doses on signal accumulation and target specificity. Target completion from SOW: November 31, 2019. Anticipated completion date: January 31, 2020.

Planned activity: Histological and zymography analyses of MMP-14 in resected tumors and brain sections from dose optimization studies, according to established methods used in studies to date. This task is performed to correlate target MMP-14 expression and probe signal accumulation. Target completion from SOW: November 31, 2019. Anticipated completion date: March 31, 2020.

Projected Milestone: Identification of optimal dose and imaging time point for the lead peptide probe. Target completion from SOW: November 31, 2019. Anticipated completion date: March 31, 2020.

Planned activity: Fluorescence imaging with 5-ALA, and PET/CT imaging with $^{18}$F[FDG] and $^{18}$F[FET] in mice with orthotopic PDX GBM tumors (one xenoline). This task is performed to compare the lead candidate imaging performance with current gold standards for fluorescence or PET imaging. Target completion from SOW: December 31, 2019. Anticipated completion date: April 30, 2020.

Projected Milestone: Sensitivity, specificity, PPV, NPV of lead peptide probe determined.
Target completion from SOW: December 31, 2019. Anticipated completion date: May 31, 2020.

Planned activity: Explore *in vivo* imaging of the lead peptide probe at optimal dose/time point in 4 additional PDX models (four xenolines). NIRF and PET imaging studies with the probe will be performed in groups of mice that have orthotopic PDX GBM tumors from xenolines with varying levels of MMP-14 expression and molecular characteristics. This task is performed to assess the broad application of the imaging agent among different GBM tumors. Target completion from SOW: June 30, 2020. Anticipated completion date: June 30, 2020.

Planned activity: Histological and zymography analyses of MMP-14 in resected tumors and brain sections from additional PDX model (one xenoline), according to established methods used in studies to date. This task is performed to correlate target MMP-14 expression and probe signal accumulation. Target completion from SOW: June 30, 2020. Anticipated completion date: July 31, 2020.

Projected Milestone: Global sensitivity, specificity, PPV, NPV of lead peptide probe determined across multiple PDX models. Target completion from SOW: June 30, 2020. Anticipated completion date: July 31, 2010.

Projected Milestone: Submit additional extramural research proposals (DOD, NIH) for subsequent stages of investigation (surgical resection in mice with PDX tumors). Target completion from SOW: August 31, 2020. Anticipated completion date: August 31, 2020.

## *4.* IMPACT:

**What was the impact on the development of the principal discipline(s) of the project?**

The project, if successful in the long-term, has the potential to significantly impact the prognosis for patients with malignant glioma. The proposed strategy is anticipated in the long-term to positively impact both neurosurgeons and patients by mediating rational, individualized, synergistic assessment of GBM for pre-surgical biopsy, marking tumor boundaries, and enhancing successful maximal safe resection in a timely and seamless manner. These factors would overcome significant limitations of currently approved imaging agents to distinguish GBM from normal brain tissue. The proposed project is anticipated to reveal novel cross-correlations between biological parameters (genomic, transcriptomic, kinomic, histologic) of the GBM xenografts and clinically relevant sensitivity, specificity, and predictive metrics that will be defined with the novel MMP-14 activatable PET/NIRF imaging probes. Synergistically employing these novel parameters and integrating them into imaging systems using alternative modalities (e.g., MRI) would foreseeably enable rational, personalized planning for surgical resection or alternative therapeutic interventions in future stages of research. If successful, these advancements are anticipated to improve the prognosis for individuals with GBM.

**What was the impact on other disciplines?**

The project aims to validate a novel molecular imaging approach, where the same enzyme is responsible for both activation of the imaging signal and binding to the cancer cells. Other activatable probes in existing research target separate cellular or physiological pathways. As a result, it can be difficult to distinguish the primary mechanism of action that results in target enhancement. The proposed strategy aims to simplify the straightforward co-registration of NIRF and PET signals as a result of both components detecting a single molecular target. Many different types of cancer (e.g., breast cancer, melanoma) overexpress MMP-14 relative to normal tissues; therefore, the project has the potential to significantly impact management of clinical care across a variety of cancers.

**What was the impact on technology transfer?**

Nothing to Report.

**What was the impact on society beyond science and technology?**

The proposed project has the potential to impact the lives and well-being of individuals diagnosed with brain tumors, as well as their family members and friends. Currently, GBM is understood to be essentially non-curable and has a very short time frame for survival after diagnosis (less than 2 years). People who hear this news understandably face fear and anxiety. As the proposed strategy may significantly impact the prognosis and extend the survival of people with GBM, these negative emotional and psychological impacts could foreseeably be diminished. The result would positively contribute to society, as the individuals could perform normally in their daily lives and work activities.

## 5. CHANGES/PROBLEMS:

As noted above, the results with the initial candidate peptides are consistent with a "lead" candidate for further evaluation. Therefore, it is likely that the originally planned activities to develop "additional candidate" peptides for evaluation will not be necessary. Forthcoming results from planned experiments in the next several months will determine whether or not additional peptide constructs will be developed. This is not a significant change that would alter the course of the project.

**Actual or anticipated problems or delays and actions or plans to resolve them**

Nothing to Report.

**Changes that had a significant impact on expenditures**

Nothing to Report.

**Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

**Significant changes in use or care of human subjects**

Nothing to Report.

**Significant changes in use or care of vertebrate animals**

Nothing to Report

**Significant changes in use of biohazards and/or select agents**

Nothing to Report

## 6. PRODUCTS:

- **Publications, conference papers, and presentations**

**Journal publications.**

Peer-reviewed article: Benjamin B. Kasten, Ke Jiang, Denzel Cole, Aditi Jani, Neha Udayakumar, G. Yancey Gillespie, Guolan Lu, Tingting Dai, Eben L. Rosenthal, James M. Markert, Jianghong Rao, Jason M. Warram; "Targeting MMP-14 for dual PET and fluorescence imaging of glioma in preclinical models;" *European Journal of Nuclear Medicine and Molecular Imaging* (submitted, under review); acknowledgment of federal support: Yes.

Published abstract: Hailey Houson, Benjamin Kasten, Ke Jiang, Jianghong Rao, Jason Warram; "MMP-14 as a noninvasive marker for PET and NIRF imaging of glioblastoma multiforme;" *Journal of Nuclear Medicine*; 60(no. supplement 1):2019;1033; published.

**Books or other non-periodical, one-time publications.**

Nothing to Report.

**Other publications, conference papers and presentations**.

Presentation at international meeting: Hailey Houson, Benjamin Kasten, Ke Jiang, Jianghong Rao, Jason Warram; "MMP-14 as a noninvasive marker for PET and NIRF imaging of glioblastoma multiforme;" poster presentation; SNMMI 2019 Annual Meeting; 2019 Jun 22-25; Anaheim, CA.

- **Website(s) or other Internet site(s)**

Nothing to Report.

- **Technologies or techniques**

Nothing to Report.

- **Inventions, patent applications, and/or licenses**

Nothing to Report.

- **Other Products**

Nothing to Report

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

**What individuals have worked on the project?**

| | |
|---|---|
| Name: | Jason Warram |
| Project Role: | Principal Investigator |
| ORCID ID: | 000-0001-5230-0011 |
| Nearest person month worked: | 1 |
| Contribution to Project: | Dr. Warram has supervised and oversee lab personnel, conducted and analyzed data, reported results. |
| Funding Support: | Partial salary support from the NIH as Co-Investigator on unrelated projects |

| | |
|---|---|
| Name: | Jianghong Rao |
| Project Role: | Site PI |
| ORCID ID: | 0000-0002-5143-9529 |
| Nearest person month worked: | 1 |
| Contribution to Project: | Dr. Jianghong oversees the subtask 1.1 delivered to the UAB team. |
| Funding Support: | Partial salary support from NIH on unrelated projects. |

Name: Catherine Langford "no change from initial submission"
Project Role:
Nearest person month worked:
Contribution to Project
Funding Support:

Name:Yolanda Hartman "no change from initial submission"
Project Role:
Nearest person month worked:
Contribution to Project
Funding Support:

| | |
|---|---|
| Name: | Min Chen |
| Project Role: | Postdoctoral Scholar |
| Nearest person month worked: | 3 |
| Contribution to Project: | Dr. Chen contributed to the subtask 1.1 in developing and testing the imaging probe delivered to the UAB team. |
| Funding Support: | Partial salary support from NIH on unrelated projects |

| | |
|---|---|
| Name: | Mingxi Fang |
| Project Role: | Postdoctoral Scholar |
| Nearest person month worked: | 5 |
| Contribution to Project: | Dr. Fang contributed to the subtask 1.1 in developing and testing the imaging probe delivered to the UAB team. |
| Funding Support: | Partial salary support from NIH on unrelated projects |

**Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

**Warram**

**Dr. Jason Warram**

**New Active Projects:**

**W81XWH1810160** Warram (PI) 09/01/2018 - 08/31/2020 1.2 Cal Mo
DOD PRCRP $206,530
*Dual PET/Fluorescence Imaging of Glioma with an MMP-14 Activatable Peptide Probe*

Specific Aims:

1. To design and characterize an MMP-14 activatable dual PET/NIRF peptide probe.
2. To establish the sensitivity and specificity of the MMP-14 activatable dual PER/NIRF peptide probe for in vivo imaging of GBM models.

The major goal of this project is to validate MMP-14 as a novel, functional target for dual-modality PET/NIRF imaging with peptide probes in preclinical GBM models
Role: Principal Investigator
Grant Administrator: Elayne K. Seiler, Grants Specialist USAMRAA
(301) 619-7358 Elayne.K.Seiler.civ@mail.mil
820 Chandler Street
Fort Detrick MD 21702-5014
Overlap: This is the current project



**Dr. George Y Gillespie**
**New Active Projects:**

**W81XWH1810160** Warram (PI) 09/01/2018 - 08/31/2020 1.2 Cal Mo
DOD PRCRP $206,530
*Dual PET/Fluorescence Imaging of Glioma with an MMP-14 Activatable Peptide Probe*
Specific Aims:
1. To design and characterize an MMP-14 activatable dual PET/NIRF peptide probe.
2. To establish the sensitivity and specificity of the MMP-14 activatable dual PER/NIRF peptide probe for in vivo imaging of GBM models.

The major goal of this project is to validate MMP-14 as a novel, functional target for dual-modality PET/NIRF imaging with peptide probes in preclinical GBM models
Role: Principal Investigator
Grant Administrator: Elayne K. Seiler, Grants Specialist USAMRAA
(301) 619-7358 Elayne.K.Seiler.civ@mail.mil
820 Chandler Street
Fort Detrick MD 21702-5014
Overlap: This is the current project

**R01CA222903** Markert (PI) 09/18/2018 – 08/31/2021 0.48 Cal Mo
NCI $489,620
*A Phase 1 Trial of IRS-1 HSV C134 Administered Intratumorally in Patients with Recurrent Malignant Glioma*
Specific Aims:
1. To conduct a Phase I clinical trial to determine a safe C134 (IND 17296) dose in patients with recurrent malignant glioma.
2. To monitor immune response changes associated with oHSV mediated anti-tumor responses among C134-treated patients.

The goal of this project is to translate the oncolytic herpes virus, C134, to Phase I study and establish how C134-induced immune activity relates with oncolytic HSV anti-tumor response in patients.
Role: Co-Investigator
Grant Administrator: William C. Timmer
(240) 276-6130 timmerw@mail.nih.gov
9609 Medical Center Drive
Room 5W542, MSC #9741
Bethesda, MD 20892-9741
Overlap: There is no budget, scientific, or commitment overlap

**U01CA223976** Wiley, Gillespie et. al. (Co-PI) 09/17/2018 – 08/31/2023 2.4 Cal Mo
NCI $521,691
*Biological Comparisons Among Three Derivative Models of Glioma Patient Cancers Under Microenvironmental Stress*

Specific Aims:

1. To determine whether intrinsic functional and omics of PDMCs predict chemo- and radiosensitivity.
2. To determine whether extrinsic microenvironmental pressures in the perivascular and perinecrotic niche alter PDMC therapeutic resistance.
3. To utilize GeneTerrain to visualize the complex interaction between intrinsic properties and extrinsic pressures to predict therapeutic response.

The goal of this project is to biologically and molecularly compare three derivative patient--derived models of cancer models of glioma under different microenvironmental stresses with accompanying computational modeling to evaluate the robustness of the three models.
Role: Co-Principal Investigator
Grant Administrator: Long Nguyen nguyen1@mail.nih.gov
9609 Medical Center Drive
Bethesda, MD 20892
(240) 276-5360

**Active on-going projects/newly affiliated**

**U01CA224151** Chambers (PI) 9/30/2017 - 8/31/2020 1.8 Cal Mo
NCI $428,817
*Canine Immuno Neurotherapeutics*

Specific Aims:

1. To establish safety and tolerability of M032 alone (Stage I) and in concert with checkpoint inhibitor (1-methyl-D-Trypotophan; Indoximod) (Stage II).
2. To characterize the systemic immune response in dogs treated with an optimal dose of M032 and increasing doses of IDO inhibitor.
3. To conduct genomic (whole exome sequencing) and transcriptomic (RNA-Seq) sequencing and to perform systematic collection, processing, archiving of brain tumor tissue.

The goal of this project is to evaluate the use of a clinical grade oncolytic herpes simplex virus that expresses human interleukin-12 for the treatment of canine malignant glioma with or without an IDO inhibitor.
Role: Co-Investigator
Grants Administrator: Connie L. Sommers
(240) 276-7187 sommersc@mail.nih.gov
9609 Medical Center Drive
BG 9609 RM 2W606
Bethesda, MD 20892
Overlap: There is no budget, scientific, or commitment overlap

**R01NS104339** Hjelmeland (PI) 12/15/2017 – 11/30/2022 1.2 Cal Mo
NINDS $275,675
*A Biosynthetic Metabolic Pathway Regulates Glioma Growth and Initiating Cell Maintenance*

Specific Aims:

1. To determine if GCH1 regulates reactive species balance to increase BTIC maintenance.
2. To determine if elevation of GCH1 and/or the BH4 pathway is a feed forward loop increasing glioma grade and correlating with poor patient prognosis.
3. To determine whether GCH1 and/or the BH4 pathway is a feed forward loop increasing glioma grade and correlating with poor patient prognosis.

The goal of this project is to better understand the importance of guanosine triphosphate cyclohydrolase 1 (GCH1) in Glioblastoma development, brain tumor initiating cell maintenance, and therapeutic resistance.
Role: Co-Investigator
Grant Administrator: Ada O'donnell, Grants Management Specialist, NINDS
(301) 496-3920 odonnella@mail.nih.gov
6001 Executive Boulevard, Suite 3290, MSC 9537
Bethesda, MD 20892-9537
Overlap: There is no budget, scientific, or commitment overlap

**Formerly Active Now Completed**

**N/A** Friedman (PI) 12/31/2016-12/30/2018 Completed
Hyundai Hope on Wheels $250,000
*Enhancement of Immunovirotherapy with IDO Inhibition in Pediatric Medulloblastoma*

Specific Aims:

1. To characterize the expression of IDO in patient medulloblastoma samples and determine how oHSV affects IDO
2. To establish a benefit for combining IDO inhibition with oHSV in medulloblastoma.

The goal of this project was to develop an innovative immunovirotherapy by combining oHSV with IDO inhibition, and then translate this approach to clinical trial in children.
Role: Co-Investigator
Grant Administrator: Zafar Brooks, Executive Program Director
10550 Talbert Avenue
Fountain Valley, CA 92708

**R21NS100054** Griguer (PI) 09/30/2016-08/31/2017 Completed
NINDS $404,250
*Paracrine Signaling in Glioma: Bioenergetics Heterogeneity and Chemoresistance*

Specific Aims:

1. To characterize the paracrine signaling pathway between glycolytic chemosensitive cells and Oxphose-dependent chemoresistant cells.
2. To determine the effect of TMZ treatment on the depletion of glycolytic chemosensitive glioma calls.

The goal of this project was to define a novel tumor growth model where bioenergetics heterogeneity plays a critical role in the growth of the tumor as a whole.
Role: Co-Investigator

**Gillespie**

Grant Administrator: Stephanie Mitchell, Grants Management Specialist
mitchellst@ninds.nih.gov
6001 Executive Boulevard, Suite 3290, MSC 9537
Bethesda, MD 20892-9537
(301) 496-7488

**U01NS093663** Griguer (PI) 09/30/2016-06/30/2018 Completed
NINDS $404,250
*Cytochrome C Oxidase: Biomarker In Newly Diagnosed Glioblastoma Multiforme*
Specific Aims:
1. To determine overall and progression-free survival times according to tumor CcO activity.
2. To compare the prognostic value of tumor CcO activity with the value of other commonly used prognostic markers.

The goal of this project was to determine whether or not high CcO activity correlates with poor outcomes in patients with newly diagnosed, primary GBM.
Role: Co-Investigator
Grant Administrator: Yvonne C. Talley
talleyy@ninds.nih.gov
6001 Executive Boulevard Suite 3309
Bethesda, MD 20892- 9531
(301) 496-7432

**NA** Gillespie (PI) 02/01/2019-07/31/2019 Completed
INCYCUS THERAPEUTICS INC. $150,846
*A Preclinical Study to Evaluate Combination Therapy of Drug Resistant Immunotherapy(DRI) and Checkpoint Inhibitor Therapy in a Mouse Model of Malignant Glioma*
The goal of this project was to test efficiency of gene transfer to murine γδT cells.
Role: Principal Investigator

**Markert**

**Dr. James M. Markert**
**On-going Active Projects**
**U01CA224151 (Chambers, PI)** 9/30/2017 - 8/31/2020 0.12 Cal Mo
NIH $428,817
*Canine Immuno Neurotherapeutics*
Aims 1) To establish safety and tolerability of M032 alone (Stage I) and in concert with a checkpoint inhibitor (1-mehtyl-D-Tryptophan; Indoximod) (Stage II).
2) To characterize the systematic immune response in dogs treated with an optimal dose of M032 and increasing doses of IDO inhibitor.
3) To conduct genomic (whole exome sequencing) and transcriptomic (RNA-Seq) sequencing and to perform systematic collection, processing, archiving of brain tumor tissue.
The goal of this application is to evaluate the use of a clinical grade oncolytic herpes simplex virus that expresses human interleukin-12 for the treatment of canine malignant glioma with or without an IDO inhibitor.
Role: Co-Investigator

Grants Administrator: Connie L Sommers
(240)-276-7187 sommersc@mail.nih.gov
9609 Medical Center Dr.
BG 9609 RM 2W606
Rockville, MD 20850

**N/A** (Friedman, PI) 02/01/2017-01/31/2020 0.01 Cal Mo
Cannonball Kids' Cancer Foundation $NCE
Expansion of G207 Trial
Aims 1) To obtain preliminary information concerning the potential efficacy of and biological response to G207 alone or combined with a single dose of radiation in pediatric patients with recurrent or progressive malignant brain tumors by assessing radiographic response, performing scale, progression-free and overall survival, quality of life, immune response, and presence of G207 in saliva, conjunctiva, and blood
2) To examine NextGen Sequencing data on tumor biopsy specimens for molecular profiling of genes that may predict a treatment response to oHSV
3) To determine expression of HSV entry molecules on tumor biopsy specimens by IHC
The goal of this project is to assess the safety and tolerability of G207 administered intratumorally via stereotactic infusion alone or followed by a single dose of radiation within 24 hours of G207 administration in children with recurrent or progressive malignant infratentorial brain tumors.
Role: Co-Investigator
Grant Administrator: Jennifer Taggart
PO Box 547797
Orlando, FL 32854-7797

**UFRA-154** (Markert, PI) 01/01/2019-12/31/2019 0.12 Cal Mo
University of California San Francisco $15,288
*Wolfe Meningioma Program Project*
Aims 1) To determine the cytotoxic effect of oHSV against patient-derived meningioma cell lines
The goal of this project is to assess viral efficacy in select meningioma cell lines.
Role: Principal Investigator
Grants Administrator: Gina Scott
Work: 415.509.9034 Cell: 949.606.3693
Award Specialist | Research Management Services (RMS)
Office of Sponsored Research (OSR)

**N/A** (Markert, PI) 09/01/2019-08/31/2022 0.96 Cal Mo
Gateway for Cancer Research $239,515
*A Phase I Trial of IRS-1 HSV C134 Administered Intratumorally in Patients with Recurrent Malignant Glioma*
Aims 1) To conduct a C134 Phase I clinical trial in patients with recurrent GBM
2) To demonstrate antiviral and anti-tumor immune response in phase I clinical trial specimens to correlate OV clinical response
3) To assess the quality of life effects in C134-treated patients
The goals of this project are to determine the safety and tolerability of stereotactic intracerebral injections of escalating doses of C134, and to determine the Recommended Phase II dose (RP2D) of this next generation chimeric HSV1 oncolytic HSV and To obtain preliminary information about the potential benefit of IRS1-chimeric HSV1 in the treatment of patients with recurrent malignant gliomas including relevant data on markers of efficacy, including time to tumor progression, patient survival, immune related changes, and quality of life in OV treated patients.

Role: Principal Investigator
Grants Administrator: Jerian Dixon-Evans
(847)-342-7361 Jerian.Dixon-Evans@gatewaycr.org

**N/A** (Boggs, PI) 01/18/2019-01/17/2022 0.01 Cal Mo
Novocure, Inc $12,020
*A Pilot and Feasibility Trail to Determine the Rate of Brain Relapse in Small Cell Lung Cancer Patients with Brain Metastases treated with Stereotactic Radiosurgery followed by Tumor Treating Fields*
This is a pilot and feasibility trail to determine the rate of brain relapse in small cell lung cancer patients with brain metastases treated with stereotactic radiosurgery followed by tumor treating fields.
Role: Co-Investigator
Grants Administrator: Eilon Kirson, M.D., Ph.D. of Novocure Inc.
patientinfo@novocure.com
1500 Broadway, 17th Floor
New York, NY 10036

**N/A** (Markert, PI) 09/30/2016-06/30/2023 0.01 Cal Mo
Massachusetts General Hospital $12,020
*NN106 Cytochrome C Oxidase Activity In Newly Diagnosed Glioblastoma Multiforme (GBM)*
The goal of this project is to determine whether CcO activity represents a novel and valuable prognostic biomarker for patients receiving standard of care therapy. Our hypothesis is that high tumor CcO activity is associated with poor outcomes independently of MGMT promoter methylation status.
Role: Principal Investigator
Grants Administrator: Janice Cordell
301-496-9135 jc53a@nih.gov

**Completed Projects:**

**T32NS48039** (Benveniste, PI) 7/2013 – 6/2018 Complete
NIH/NINDS $174,816
*Training Program in Brain Tumor Biology*
Aims No specific aims are stated for training grants
The goal of this project is to provide superior training opportunities for the next generation of basic and clinical scientists through mentoring.
Role: Co-PI
Grants Administrator: Stephen J Korn, PhD of Training and Workforce Development
(301) 451-5635 korns@ninds.nih.gov
6001 Executive Blvd, Room 2186
Bethesda, MD 20852-4188

**N/A** (Friedman, PI) 12/2016 – 12/2018 Complete
Hyundai Hope on Wheels $125,000
*Enhancement of lmmunovirotherapy with IDO Inhibition in Pediatric Medulloblastoma*
Aims 1) Characterize the expression of IDO in patient medulloblastoma samples
2) Establish the benefit of combining IDO needs of novel, targeting therapies.
The goal of this project is to develop an innovative immunovirotherapy by combining oHSV with IDO inhibition, and then translate this approach to clinical trials in children
Role: Co-Investigator
Grant Administrator: Hyundai Hope on Wheels
(714) 965-3584 grants@hopeonwheels.org
10550 Talbert Ave.
Fountain Valley, CA 92708

**N/A** (Markert, PI) 07/2017 – 06/2019 Complete
UAB School of Medicine $100,000
*Regulation of CD4+ T cell cytotoxic and memory responses to gliomas by oncolytic HSV*
Aims 1) Define the contribution of anti-glioma CD4+ CTL to IL12-oHSV therapy.
2) Define the contribution of CD4+ memory cells to the anti-glioma effects of IL12-oHSV therapy.
3) Define potential CD4+ T cell-related biomarkers of efficacy and demonstrate improvement of IL12-oHSV therapeutic efficacy with adjuvant therapies impacting these biomarkers.
The goal of this project is to measure and observe the T cell populations after IL12 oHSV therapy to determine if they are producing an enhanced anti-glioma response.
Role: Principal Investigator
Grant Administrator: Samone Alexander, of the UAB School of Medicine
(205) 996-1705 Samone@uab.edu
510 20th Street South
Faculty Office Tower 1248
Birmingham, AL 35233

**ImmunoCellular Therapeutics** (Markert, PI) 2/2015 – 2/2018 Complete
ImmunoCellular Therapeutics $46,000
*Immunological targeting of CD-133 in recurrent glioblastoma: A multi-center Phase I translational and clinical study of an autologous CD 133 DC vaccine (ICT-121).*
The goal of this study is to evaluate, in patients with recurrent glioblastoma, the safety, tolerability and clinical response of a CD133 vaccine.
Role: Site PI
Grant Administrator: ImmunoCellular Therapeutics, Ltd.
(818) 264-2300 info@imuc.com
30721 Russell Ranch Road, Suite 140
Westlake Village, CA 91362

**ImmunoCellular Therapeutics** (Markert, PI) 6/2016 – 6/2017 Complete
ImmunoCellular Therapeutics $20,725
*STING (Study of Immunotherapy in Newly Diagnosed Glioblastoma): A Phase III Randomized Double-Blind, Controlled Study of ICT-107 with Maintenance Temozolomide (TMZ) in Newly Diagnosed Glioblastoma following Resection and Concomitant TMZ Chemoradiotherapy*
The goal of this study is to determine the overall survival of subjects treated with ICT-107 and standard of care (RT and TMZ) vs. placebo control and standard of care (RT and TMZ)
Role: Site PI
Grant Administrator: ImmunoCellular Therapeutics, Ltd.
(818) 264-2300 info@imuc.com
30721 Russell Ranch Road, Suite 140
Westlake Village, CA 91362

**Dr. Joshua S. Richman**

**Current:**

01/01/2019-12/31/2021 1.2 Cal

Department of Veterans Affairs $81,750

**Title:** *Genetics of Osteoarthritis and Joint Replacement Recovery: Key to Precision Rehabilitation*

**Time Commitments:** 1.2CM

**Supporting Agency:** Department of Veterans Affairs Million Veterans Program (MVP)

**Address:** MVP Program
810 Vermont Ave NW,
Washington, DC, 20420

**Contracting/Grants Officer:**

**Performance period:** 01/01/2019-12/31/2021

**Level of funding:** $81,750

**Project Goals:** The goal of this project to harness the large sample size of the MVP to identify genetic variants associated with osteoarthritis to improve quality of life for Veterans.

**Role:** Co-Investigator

**Specific Aims:**

1: To identify genetic variants associated with OA.

2: To identify genetic variants prognostic of progression to end-stage OA

Exploratory Aim: To identify genetic variants prognostic of THA/TKA recovery

**Overlap:** None

R35CA220502 Bhatia (PI) 07/01/2018-06/30/2025 0.9 Cal

NCI $585,745

**Title:** *Mitigating Long-term Treatment-related Morbidity in Childhood Cancer*

**Time Commitments:** 0.9 CM

**Supporting Agency:** NCI

**Address:** Department of Health and Human Services
National Institutes of Health
National Cancer Institute

**Contracting/Grants Officer:** Irene Praghudas

**Performance period:** 7/1/18 – 6/30/25

**Level of funding:** $548,745

**Project Goals:** The major goal of this project is to harness and merge novel concepts from the field of molecular biology, pharmacogenomics and cancer survivorship to identify cancer patients by their personal risk of subsequent neoplasms or cardiac dysfunction.

**Specific Aims:**

1: Develop risk prediction models for SN & CD in <u>*childhood cancer survivors*</u> (***COG-ALTE03N1) (Training set)***

2: Replicate optimized prediction model (from Phase I) in an independent cohort of <u>*childhood cancer survivors*</u>, *using St. Jude Lifetime Cohort (**SJLIFE**).* ***(Test set)***

3: Apply risk-prediction models to <u>*newly-diagnosed children*</u> with cancer and determine association of models with incident SN/CD, primary cancer relapse, and mortality (all-cause and cause-specific) *using COG Biospecimen and Pathology Core (**COG-BPC**) with access to germline DNA at diagnosis.* ***(Application)***

*4: Determine the functional relevance of genomic variants (**Burridge lab, Nakamura lab**)* ***(Pathogenesis)***

**Overlap:** *None*

**1K08CA234225** Willilams (PI) 09/01/2018-08/31/2023 0.24 Cal
NCI $191,593

**Title:** *Myopenia and Mechanism of Chemotherapy Toxicity in Older Adults with Colorectal Cancer: The M&M Study*

**Time Commitments:** 0.24 calendar

**Supporting Agency:** NCI

**Address:** Clinical Investigator Award
Department of Health and Human Services
National Institutes of Health
National Cancer Institute

**Contracting/Grants Officer:** Susan E. Lim

**Performance period:** 9/1/2018 – 8/31/2023

**Level of funding:** $191,593

**Project Goals:** The major goal of this study is to examine the trajectories of myopenia, its association with chemotoxicity and survival, and the role of altered pharmacokinetics and genetic variants as underlying mechanisms of increased chemotoxicities in older adults with metastatic CRC in order to better understand the variability in treatment outcomes and ultimately target interventions to improve them.

**Specific Aims:**

1: Characterize muscle mass at baseline (before initiation of chemotherapy), and 3 and 6 months after initiation of chemotherapy in patients with metastatic CRC

1.1 Characterize muscle mass at baseline and over time, and identify distinct trajectories of myopenia.

1.2 Evaluate the impact of age, sex, race/ethnicity, chemotherapy, nutrition, and inflammation on myopnia trajectories.

Hol: There will be two or more distinct trajectories of myopenia, and trajectories with greater decline in muscle mass will be associated with older age, undernutrition and markers of systemic inflammation.

2: Examine the association between myopenia and cumulative grades 3-5 chemotoxicity and 1y OS

2.1 Assess the association between myopenia <u>at CRC diagnosis</u> and cumulative grades 3-5 chemotoxicity, and ly OS.

2.2 Assess the association between myopenia trajectories and cumulative grades 3-5 chemotoxicity and ly OS.

Ho2 Individuals with myopenia at CRC diagnosis and those with trajectories of worsening myopenia will be at highest risk for grades 3-5 chemotoxicity and worse OS at ly.

Exploratory Aim: Explore the underlying mechanisms of the myopenia-chemotoxicity association.

E.1 Explore the role of germline genetic variants in mediating the association between myopenia and cumulative grades 3-5 chemotoxicity (in all patients).

E.2 Explore the role of altered oxaliplatin PKs in mediatiating the association between myopenia and cumulative grades 3-5 chemotoxicity (in a subset of 60 patiens at UAB only).

HoE Genetic variants in telomere homeostasis, DDR, inflammation, myostatin and/or altered chemotherapy PKs (oxaliplatin as example) will mediate the association between myopenia & cumulative grades 3-5 chemotoxicity.

**Overlap:** None

No Number (Johnston) 01/15/2019 – 01/14/2020 0.60 Cal
UAB Comprehensive Cancer Center $40,000
**Title:** *End-of-Life Care of Young Adults with Cancer Alabama: Disparities and Parent Priorities*
**Time Commitments:** 0.60 calendar
**Supporting Agency:** The University of Alabama at Birmingham O'Neal Comprehensive Cancer Center
**Address:** UAB Wallace Tumor Institute
1824 6th Avenue South
Birmingham, AL 35233
**Contracting/Grants Officer:** Susan Ruppert, PhD
**Performance Period:** 01/15/2019 – 01/14/2020
**Level of funding:** $40,000
**Project goals:** The major goal of this study is to determine disparities in the medical intensity (intubation, CPR, etc) in Alabama.
**Specific Aims:**

1: To understand childhood cancer families' perspective on markers of quality end-of-life care Methods: Interviews with a diverse set of bereaved family members will address what aspects of end-of-life care are most important. Hypothesis: Pain and symptoms management and the presence of supportive services will be most important.
2: To determine rates of high-intensity end-of-life care in pediatric cancer patients in Alabama. Methods: We will use Alabama State Medicaid data (2000 to 2015) to determine rates of and clinical and sociodemographic characteristics associated with high-intensity end-of-life care and other markers deemed important by families in Aim 1. Hypothesis: Over 60% of children will die in the hospital with the highest rates of medically-intense end-of-life care in rural, low income, and African American children and in children with leukemia.

**Overlap:** None
No Number Johnston (PI) 02/01/2019-01/31/2021 0.24 Cal
Kaul Pediatric Research Institute $35,000
**Title:** *End-of-Life Care of Children with Cancer in Alabama: Disparities and Parent Priorities*
**Time Commitments:** 0.24 calendar
**Supporting Agency:** Kaul Pediatric Research Institute
**Address:** 1600 7th Avenue South
Birmingham, AL 35233
**Contracting/Grants Officer:** Coke Matthews
**Performance Period:** 02/01/2019 – 01/31/2021
**Level of funding:** $35,000
**Project goals:** The major goal of this study is to determine disparities in the medical intensity (intubation, CPR, etc) in Alabama.
**Specific Aims:**

1: To understand childhood cancer families' perspective on markers of quality end-of-life care Methods: Interviews with a diverse set of bereaved family members will address what aspects of end-of-life care are most important. Hypothesis: Pain, effective symptom management and availability of supportive care services will be most important.
2**:** To determine rates of high-intensity end-of-life care in pediatric cancer patients in Alabama. Methods: We will use Alabama State Medicaid data (2000 to 2015) to determine rates of and clinical and sociodemographic characteristics associated with high-intensity end-of-life care and

other markers deemed important by families in Aim 1. Hypotheses: Over 60% of children will die in the hospital with the highest rates of medically-intense end-of-life care in rural, and African American children and in children with leukemia.

**Overlap:** None

No Number Johnston (PI) 07/01/2019-06/30/2022 0.18 Cal
Conquer Cancer Foundation of ASCO $33,000
**Title:** *Hospice Use at End-of-Life in Children with Cancer*
**Time Commitments:** 0.18 calendar
**Supporting Agency:** Conquer Cancer; The ASCO Foundation
**Address:** 2318 Mill Road, Suite 800
Alexandria, VA 22314
**Contracting/Grants Officer:** Thomas G. Roberts, Jr.
**Performance Period:** 7/1/19 – 6/30/22
**Level of funding:** $33,000
**Project goals:** The major goal of this study is to better understand drivers of variability in pediatric hospice and what models may help agencies provide high quality pediatric hospice services.

**Specific Aims:**

1**:** Determine rates of hospice use in pediatric oncology and identify clinical and sociodemographic factors associated with hospice use
Methods: We will use Truven (national insurance claims data) to examine clinical and sociodemographic disparities in hospice use
Hypothesis: Less than 30% of pediatric oncology patients will enroll in hospice and hospice enrollment will be associated with white race, solid tumor diagnosis, and private insurance
2**:** Obtain diverse perspectives on home death and hospice use in pediatric oncology
**Sub Aim 1:** Determine racially/ethnically diverse bereaved families' perspective on home death and hospice use
**Sub Aim 2:** Determine providers' perspectives on home death and hospice, including barriers and facilitators of hospice enrollment
Methods: Semi-structured interviews with 1) racially/ethnically diverse bereaved families and 2) pediatric oncology and hospice healthcare provider teams
Hypothesis: Hypothesis generating
Measurable Objective: Qualitative themes around pediatric oncology hospice enrollment
Learning Objective: Deepen qualitative skills and coordination of multisite studies

**Overlap:** None

No Number Wadhwa (PI) 02/01/2019-01/31/2021 0.60 Cal
Kaul Pediatric Research Institute $35,000
**Title:** *Body Composition and Adverse Outcomes in Childhood Cancer*
**Time Commitments:** 0.60 calendar
**Supporting Agency:** Kaul Pediatric Research Institute
**Address:** 1600 7th Avenue South
Birmingham, LA 35233
**Contracting/Grants Officer:** Coke Matthews
**Performance Period:** 02/01/2019 – 01/31/2021
**Level of funding:** $35,000
**Project goals:** The major goal of this project is to identify a method to personalize the chemotherapy dose for each child and minimize serious side-effects but at the same time, maximize cure rates.

Specific Aims:

Primary 1: Characterize skeletal muscle mass (using SMI) and height-adjusted total adipose tissue (hTAT) in children with HL, NHL, RMS, NBL from diagnostic CT scans.

**Aim 1.1:** Model the relation of SMI with BMI%ile and BSA at cancer diagnosis, overall and by specific cancer type; **Aim 1.2:** Model the relation of hTAT with BMI%ile and BSA at cancer diagnosis, overall and by specific cancer type. Hypothesis 1: SMI and hTAT will show modest correlation with BMI%ile and BSA with a r2< 0.5.

Primary 2: Examine the association between SMI, hTAT and toxicities experienced in the first 6 months after cancer diagnosis in children with HL, NHL, RMS and NBL. **Aim2.1:** Model the association between SMI and the following outcomes within the first 6 months after cancer diagnosis: occurrence of any grade 3-5 toxicity, >1 grade 3-5 toxicity, toxicity-related treatment delays, and any toxicity-related dose reduction or dose omission. **Aim 2.2**: Model the association between hTAT and same outcomes as in Aim 2.1 within the first 6 months after cancer diagnosis. Hypothesis 2: We hypothesize that patients with a lower SMI (Ho2.1) or higher hTAT (Ho2.2) will experience greater toxicities, treatment delays and dose reductions or omissions.

**Exploratory Aim 1:** Estimate the association of SMI and hTAT at cancer diagnosis with progression-free and overall survival in patients with HL, NHL, RMS and NBL.

**Overlap:** None

No Number Wolfson (PI) 07/01/2018-06/30/2020 0.3 Cal
The Rally Foundation $100,000

**Title:** *Understanding Causes of Outcome Disparities in AYA with ALL*
**Time Commitments:** 0.3 calendar
**Supporting Agency:** The Rally Foundation
**Address:** 5775 Glenridge Drive
Building B, Suite 370
Atlanta, GA 30328
**Contracting/Grants Officer:** Dean Crowe
**Performance Period:** 7/1/18 – 6/30/20
**Level of funding:** $100,000
**Project goals:** The major goal of this project is to develop an AYA consortium to investigate a broad range of factors on AYA disparities in AYAs with ALL

**Specific Aims:**

**COHORT A: Children (10-14y) and AYA (15-39y) enrolled at diagnosis of ALL:**

1: Evaluate the difference in leukemia biology and germline genetic variants between AYA and children. Ho1: There will be an over-representation of high-risk somatic mutations and germline alleles in AYA.

2: Evaluate difference in therapeutic approach (pediatric vs. adult-inspired) and enrollment on clinical trials between AYA and children. Aim 2.1: Describe the role of sociodemographics (race/ethnicity, income, education, material hardship, insurance), in therapeutic approach and enrollment on clinical trials. Ho2: A smaller proportion of AYA will be treated with pediatric-inspired therapy/enrolled on clinical trials, after adjustment for sociodemographics.

**COHORT B: Children (10-14y) and AYA (15-39y) with ALL enrolled during maintenance:**

3**:** Evaluate the difference in 6MP adherence between AYA and children using electronic medication monitoring (MEMS). Aim 3.1: Describe the role of sociodemographics (race/ethnicity, income, education, material hardship, and HoE: Genetic variants in telomere homeostasis, DDR, inflammation, myostantin and/or altered chemotherapy PKs (oxaliplatin

as example) will mediate the association between myopenia & cumulative grades 3-5 chemotoxicity.

**Overlap:** None

MRSG-18-020-01-CPPB Kenzik (PI) 07/01/2018-06/30/2023 0.3 Cal
American Cancer Society $145,800

**Title:** *Health Care Utilization and Associated Costs Among Older Cancer Survivors*
**Time Commitments:** 0.3 calendar
**Supporting Agency:** American Cancer Society
**Address:** 250 Williams Street
Atlanta, GA 30303
**Contracting/Grants Officer:** Elvan Daniels
**Performance Period:** 7/1/18 – 6/30/23
**Level of funding:** $145,800
**Project goals:** The major goal of this project is to utilize the evidence generated to inform survivorship care delivery and to develop strategies and infrastructure for older cancer survivors requiring long-term follow-up care.
**Specific Aims:**

1: Examine patterns of high-intensity healthcare use associated with cancer survivorship in the elderly

**Aim 1.1** Determine trajectories of high-intensity healthcare use during survivorship and compare to age-, sex-, and race-matched non-cancer population to establish excess use. H1.1: Survivors will demonstrate excess healthcare use compared with age-, sex-, and race-matched non-cancer population

**Aim 1.2** Among survivors, examine trajectories of high-intensity healthcare utilization by adherence to standardized healthcare recommendations. H1.2: Non-adherent survivors will demonstrate greater utilization of high-intensity healthcare when compared with adherers, accounting for new-onset morbidities

2: Determine the costs of healthcare utilization among older cancer survivors

**Aim 2.1** Estimate the healthcare utilization costs (high-intensity; PCP/specialty care) to Medicare for older cancer survivors when compared with age-, sex-, and race-matched non-cancer population and establish excess cost. H2.1 The healthcare utilization costs will be significantly higher for cancer survivors when compared matched non-cancer population

**Aim 2.2** Among survivors, compare healthcare utilization costs by adherence to standardized healthcare guidelines. H2.2: Healthcare costs will be lower among adherers, accounting for sociodemographic predictors; the lower healthcare costs in
adherers will be due to a lower incidence of new-onset morbidities and less high-intensity healthcare use.

**Aim 2.3** Among survivors, determine healthcare utilization costs attributable to key new-onset morbidity. H2.3: Healthcare costs will vary by type of specific new-onset morbidity; healthcare costs will be higher for non-adherers

3: Examine self-reported financial hardship among older cancer survivors

H3.1: Financial hardship will be greater among those with morbidities when compared with those without, accounting for sociodemographics. H3.2: Financial hardship will be greater among non-adherers, explained by risk of new morbidity.

**Overlap:** None

**Richman**

**Completed:**

**Title:** *Can Electronic Data and Natural Language Processing Accurately Reproduce a Surgical Quality Measure?*
**Time Commitments:** 3.6 calendar (Completed)
**Supporting Agency:** VA HSR&D
**Address: HSR&D** Central Office
1100 1st Street NE,
Suite 6. Washington,
DC, 20002
E-mail: miho.tanaka@va.gov
**Contracting/Grants Officer:** Miho Tanaka, Program Officer
**Performance Period:** 02/01/2017-01/31/2018
**Level of funding:** $95,800
**Project Goals:** The primary objective was to provide pilot data for whether combining data from the VA's electronic medical record with Natural Language Processing methods applied to unstructured clinic notes can successfully reproduce the SCIP-Card-2 surgical quality measure.
**Specific Aims:**.

1: Use clinical data to divide cases into those that meet the measure using pharmacy data vs. those that require NLP
2: Use NLP data in combination with clinical data to recreate the SCIP-Card-2 measure
3: Assess the reliability and accuracy of the replicated measure

**Overlap:** No scientific or budgetary overlap with the current project

**Title:** *Improving Surgical Quality: Risks and Impact of Readmission*
**Time Commitments:** 1.0 calendar (Completed)
**Supporting Agency:** VA HSR&D
**Address: HSR&D** Central Office
1100 1st Street NE,
Suite 6. Washington,
DC, 20002
**Contracting/Grants Officer:** George Fitzelle
**Performance period:** 10/01/14 - 09/30/17
**Level of funding:** $876,964
**Project Goals:** Hospital readmissions are associated with increased costs, resource utilization, and poor patient outcomes. To understand determinants of surgical readmission, we will perform a retrospective cohort study of patients undergoing inpatient surgery within VA from 2008 to 2013
**Specific Aims:**

1: Evaluate the contribution of patient, procedure, post-operative complication and system factors on readmission within 30 days of hospital discharge following surgery, and use these data to (1) develop and validate a readmission risk prediction model that can be used real-time, (2) *develop a classification of readmission reasons, and (3) explore processes of care linked with readmission.*
2: Assess potential patient factors not currently collected by VASQIP at discharge and determine their association with readmission.

3: Rank reasons for readmission categories developed from Aims 1 and 2 for (a) potential preventability and (b) appropriateness as a measure of surgical quality**.**

**Overlap:** No scientific or budgetary overlap with the current project

No Number (Landier, PI) 01/15/2017 – 01/14/2019 0.30 calendar
Alex's Lemonade Stand Foundation $100,000

**Title:** *A Nurse-Led Structured Discharge Teaching Intervention for Patents of Newly Diagnosed Pediatric Oncology Patients*
**Time Commitments:** 0.3 (Completed)
**Supporting Agency:** Alex's Lemonade Stand Foundation
**Address:** 111 Presidential Blvd #203, Bala Cynwyd, PA 19004
**Contracting/Grants Officer:**
**Performance period:** 01/15/2017 – 01/14/2019
**Level of funding:** $100,000
**Project Goals:** The major goal was to test a nurse-led, structured discharge teaching intervention that incorporates these expert recommendations (including standardized and streamlined content and individualized approaches tailored to parental learning preferences) into discharge teaching for parents of newly diagnosed children.
**Specific Aims:**

1: Estimate differences in parent/caregiver ratings of readiness for discharge, perceived quality of education and post-discharge coping difficulty without and with implementation of the SDTI
2: Estimate differences in unscheduled healthcare utilization (hospital readmissions, unscheduled clinic and/or emergency room visits) for 30 days following the initial hospital discharge without and with implementation of the SDTI.
3: Measure differences in nurse and parent satisfaction with the discharge education process, and identify barriers to and facilitators of the provision of discharge education without and with the SDTI.

**Overlap:** No scientific or budgetary overlap with the current project

**Title:** Improving Medication Adherence in the Alabama Black Belt
**Time Commitments:** 0.6CM (Completed)
**Supporting Agency:** PCORI
**Address:** 1828 L St NW, Washington, DC 20036
**Contracting/Grants Officer:**
**Performance period:** 05/01/14-6/30/17
**Level of funding:** $498,467
**Project Goals:** To test an intervention to improve medication adherence in residents Alabama's Black Belt with type 2 diabetes, a historically disadvantaged and underserved region.
**Specific Aims:**

1: Build on already developed culturally-tailored education material to develop the medication adherence intervention. The intervention will consist of educational DVDs with integrated storytelling about how community members accepted their disease and overcame barriers to medication adherence, plus one-on-one telephonic peer coaching. Activities include conducting focus groups with patients; creating the DVDs and the coaching intervention protocol; training peer coaches; and pilot testing.
2: Conduct a randomized controlled trial with 500 individuals with type 2 diabetes and medication nonadherence. The trial will compare the effect of usual care and the intervention on medication adherence and physiologic risk factors including A1c, blood pressure, and low density lipoprotein cholesterol (primary outcomes), and quality of life

and self-efficacy (secondary outcomes). This innovative approach would be a major shift in how we help patients in under resourced areas living with chronic diseases commit to taking medications, improving health, and eventually reducing health disparities.

17MCPRP33350072 Levitan (PI) 01/01/2017-12/31/2018 0.3 Cal (Completed)
American Heart Association $150,217
**Title:** *Improving Participation and Reducing Acute Cardiovascular Hospitalizations with Weight Management (ReACH)*
**Time Commitments:** 0.3 Cal (Completed)
**Supporting Agency:** AHA
**Address:** 7272 Greenville Avenue Dallas, TX 75231
**Contracting/Grants Officer:**
**Performance period:** 01/01/2017-12/31/2018
**Level of funding:** $150,217
**Project Goals:** This study utilizes data from the Veterans Affairs (VA) Healthcare MOVE! program and no-VA community-level resources to assess factors affecting recruitment and engagement in lifestyle interventions targeting improved cardiovascular health. The study also strives to examine factors affecting long-term cardiovascular among individuals enrolled in lifestyle interventions.
**Specific Aims:**
1: Describe variation in hospital and program characteristics of MOVE! across hospitals.
2: Identify hospital and MOVE! components associated with higher patient recruitment and engagement in MOVE!.
3: Examine secular ecologic trends and changes in monthly rates of long-term CVD outcomes before and after implementation of MOVE!
**Overlap:** No scientific or budgetary overlap with the current project

**Title:** *Predicting Surgical Outcomes with NSQIP and Clinical Monitoring Data*
**Time Commitments:** 12 Cal (VA) (Completed)
**Supporting Agency:** VA HSR&D
**Address:** HSR&D Central Office
1100 1st Street NE,
Suite 6. Washington,
DC, 20002
**Contracting/Grants Officer:**
**Performance period:** 01/01/2011-01/01/2016
**Level of funding:** $904,692
**Project Goals:** This VA-funded career-development award is to provide the training and support to help Dr. Richman develop an independently funded research program in health-services research. Specific scientific aims include integrating new data sources and novel analytical methods to better-predict adverse surgical outcomes in the VA.
**Specific Aims:**
1: Identify and calculate summary measures and key elements of the AIMS data to include in risk classification and modeling along with the VASQIP variables.
2: To conduct standard analyses of the relative predictive value of summary measures of the AIMS data and the VASQIP preoperative risk factors in predicting the VASQIP 30-day mortality and morbidity postoperative outcomes.
3: To conduct similar analyses to predict the VASQIP 30-day mortality and morbidity postoperative outcomes using tree-based approaches.

4: To compare the prediction capabilities of the standard hierarchical logistic regression analyses vs. the tree-based methods. In addition to global comparisons, particular attention will be paid to identifying subgroups where either method demonstrates superior performance.
5: Construct a hybrid tree-logistic model and compare its performance to tree-based methods and logistic regression.
6: To develop the surgical APGAR score from the AIMS and VASQIP data and compare the predictive ability of the APGAR score vs. the results of standard hierarchical logistic regression and tree-based methods in predicting the VASQIP 30-day mortality and morbidity postoperative outcomes.

**Overlap:** No scientific or budgetary overlap with the current project

**Title:** *REasons for Geographic And Racial Differences in Stroke-Myocardial Infarction-2 (REGARDS-MI-2) Study*
**Time Commitments:** 3.6 calendar (Completed)
**Supporting Agency:** NIH
**Address:** 6130 Executive Blvd # 2123, Rockville, MD 20852
**Contracting/Grants Officer:**
**Performance period:** 09/30/2011-08/31/2016
**Level of funding:** $350,000
**Project Goals:** This extension of the REGARDS-MI study will extend follow-up of adjudicated acute coronary heart disease events from 3.5 to 5 years, permitting a host of analyses designed to better understand root causes for racial and geographic differences in acute coronary heart disease outcomes in US Blacks and Whites.
**Specific Aims:**

1: To estimate region and race-specific rates of definite or probable MI, acute CHD mortality, including in- and out-of-hospital deaths, and sudden death.
2. To identify potential explanatory factors for racial difference in nonfatal and fatal CHD.

**Title:** *Quality of Epilepsy Treatment and Costs in Older Americans by Race (QUIET CARE)*
**Time Commitments:** 1.8 Cal (Completed)
**Supporting Agency:** NIH
**Address:** 6130 Executive Blvd # 2123, Rockville, MD 20852
**Contracting/Grants Officer:**
**Performance period:** 12/01/2012-11/30/2015
**Level of funding:** $362,927
**Project Goals:** In this project we will examine the quality of epilepsy care and adherence to antiepileptic drug treatment across racial groups of Medicare beneficiaries with Part D drug coverage, and explore opportunities for better care, reduced disparities, and reduced health care costs.
**Specific Aims:**

1: Determine the quality of AED treatment for different racial groups of Medicare beneficiaries with epilepsy.
2: Determine the use of appropriate interventions after seizure recurrence for different racial groups of Medicare beneficiaries with epilepsy.
3. Determine if lower health care cost are associated with improving the quality of epilepsy care across racial groups.

**Overlap:** No scientific or budgetary overlap with the current project

**Richman**

**Title:** *Phase I, Open-Label Study Evaluating the Safety and Pharmacokinetics of Escalating Doses of cetuximab-IRDye800 as an Optical Imaging Agent to Detect Cancer During surgical procedures*
**Time Commitments:** 0.6 Cal (Completed)
**Supporting Agency:** NCI
**Address:** 6130 Executive Blvd # 2123, Rockville, MD 20852
**Contracting/Grants Officer:**
**Performance period:** 04/01/2014 – 03/31/2016
**Level of funding:** $250,000
**Project Goals:** The study is a clinical trial to assess the use of fluorescently labeled cetuximab with an FDA approved imaging device to detect subclinical cancer intraoperatively to guide surgical resection of head and neck cancer.
**Specific Aims:**

1: Administer escalating doses of cetuximab-IRDye800 to determine safety and imaging efficacy in humans

2**:** Perform histopathological review of resected tumors to determine sensitivity and specificity of cetuximab-IRDye800.

**Overlap:** No scientific or budgetary overlap with the current project

**Title:** ***Using Cers to Optimize Quality of Life for Persons with Diabetes and Chronic Pain***
**Time Commitments:** 2.16 Cal (Completed)
**Supporting Agency:** AHRQ
**Address:** 5600 Fishers Ln, Rockville, MD 20857
**Contracting/Grants Officer:**
**Performance period:** 09/29/10 – 09/28/13
**Level of funding:** $346,274
**Project Goals:** Our study is designed to not only provide robust evidence on the implementation of CER content in clinical decision-making, a highly relevant and important outcome for any health-related education material, but also to determine the incremental effectiveness of a CHW-delivered, CBT-based patient activation intervention with integrated CER content, beyond simple dissemination of CME to providers, directly informing policy. AL's Medicare QIO is currently implementing a CHW model targeting older rural patients with multiple morbidities; other QIOs and health plans are examining where this model can be implemented to greatest effect. Further, the study will advance the science on the role of CHW in the delivery of care to complex patients because we study 2 conditions simultaneously, and address a common clinical syndrome (chronic pain) in need of better quality of care. We also target a highly vulnerable, hard-to-reach population: rural, poor AA are at extraordinary risk of chronic disease and face enormous barriers to receiving high quality care, yet they have been studied far less than inner city minorities.
**Specific Aims:**

**1: ADAPT Five CERs on OA and DM** to enhance use and value in decision-making, using formative evaluation to assess needs. We will work iteratively with (1) primary care providers, to incorporate adapted materials into a state-of-the-art interactive, multimodal CME program; and (2) with Black Belt residents, to develop culturally relevant patient education materials, and incorporate them into a CHW-delivered DM-pain intervention designed to empower and activate patients, based on CBT principles.

**2: TEST a 16-week intervention developed in Aim 1 in a group RCT**, randomizing at the practice level, and including 400 patients with both DM and OA. The trial will have 80% power

to detect differences in functional status scores as low as 0.7, A1c ≥0.4%, systolic BP ≥4 mmHg, and LDL ≥6 mg/dl (primary outcomes). Secondary outcomes include CER utilization in clinical decision-making, pain ratings and behaviors, and self-care behaviors. Providers in both arms will receive CME on the CERs. Intervention arm patients will receive the CHW empowerment intervention on pain and DM self-care with integrated CER content, and "attention" control arm patients will receive a didactic health education program.

**3: DISSEMINATE the CER products of the study** through AHRQ and the UAB CME department to physicians nationally, and through the UAB School of Nursing's on-line curriculum to nurses worldwide. Dissemination products include: (1) Robust evidence on whether CHWs improve CER implementation in a hard-to-reach vulnerable group; (2) On-line CME for primary care physicians and nurses on five CER topics; (3) On-line CHW training curriculum to improve health outcomes for individuals with chronic pain, OA and DM; (4) Consumer information materials tailored for southern rural AA communities on five CER topics.

**Overlap:** No scientific or budgetary overlap with the current project

**Hackney**

**Dr. James R. Hackney**

**New Active Projects:**

No newly active projects.

**Formerly Active Now Completed:**

**R21CA216227** Nabors (PI) 5/11/17 – 4/30/19 0.24 Mo
NIH/H. Lee Moffitt Cancer Center $11,612
*Sequence Variation in MtDNA and Extreme Survival in Glioblastoma*

Specific Aims:

1. To identify germline variants in mtDNA associated with ES in GBM
2. To examine somatic variants in mtDNA as determinants of ES in GBM

The primary aim of this study is to identify germline variants in mtDNA associated with extreme survival in GBM.

Role: Co-Investigator

Grand Administrator: Joy Kearse
Phn: 301-631-3002 kearsej@mail.nih.gov
Fax: 301-451-5391

Overlap: There is no budget, scientific or commitment overlap with any agency.

**U01NS093663** Markert (PI) 09/01/16 – 08/31/20 1.2 Mo
NIH/NINDS $591,993
*Prospective Study of Cytochrome C. Oxidase Activity as a Novel Biomarker in Subjects with Newly Diagnosed Primary Glioblastoma Multiforme (GBM)*

Specific Aims:

1. To determine the correlation between CcO activity and Progression Free Survival
2. To compare the prognostic value of tumor CcO activity with the value of other commonly used prognostic markers.

This biomarker trial is designed to prospectively evaluate the hypothesis that the overall survival (OS) time of a subject with newly diagnosed primary GBM tumors, treated by standard of car (SOC), is a function of the CcO enzymatic activity in the tumor (OS; time interval from date of first diagnosis to death from any cause, irrespective of post-SOC therapies, assessed up to 24 months from accrual). In particular, tumors with high CcO

activity, defined as CcO/citrate synthase (CcO/CS) ratio >4, are associated with shorter OS time as compared to tumors with low CcO activity (CcO/CS ≤ 4). SOC consists of post-surgical radiation therapy with concurrent Temozolomide followed by up to 12 cycles of adjuvant Temozolomide.

Role: Collaborator ended 09/2018

Grant Administrator: Janice Cordell of the National Institute of Neurological Disorders and Stroke
(301) 451-4299 cordellj@ninds.mih.gov
6001 Executive Boulevard
Suite 3309
Bethesda, MD 20892-9531

**What other organizations were involved as partners?**

| | |
|---|---|
| Organization Name: | Stanford University |
| Location of Organization: | 450 Serra Mall<br>Stanford, CA 94305-2004 |
| Site Principal Investigator: | Dr. Jianghong Rao at Stanford has been synthesizing and characterizing the novel MMP-14 activatable peptide probes for the project. Please see details in Section 3, Accomplishments. |

## 8. SPECIAL REPORTING REQUIREMENTS

**COLLABORATIVE AWARDS:** N/A

**QUAD CHARTS: N/A**

## 9. APPENDICES:

- Award Chart
- Abstract "MMP-14 as a noninvasive marker for PET and NIRF imaging of glioblastoma multiforme" presented at the Society of Nuclear Medicine and Molecular Medicine, 2019 Meeting
- Submitted Publication "Targeting MMP-14 for dual PET and fluorescence imaging of glioma in preclinical models" under review at Eur J Nucl Med Mol Imagaing

## CA170769: Dual PET/Fluorescence Imaging of Glioma with an MMP-14-Activatable Peptide Probe

**PI:** Jason Warram, University of Alabama at Birmingham, Alabama **Budget:** $295,600
**Topic Area:** Brain Cancer **Mechanism:** W81XWH-17-PRCRP-IA

**Research Area(s):** Brain Cancer **Award Status:** September 1, 2018-August 1, 2019

**Study Goals:** Develop a novel, MMP-14 activatable peptide imaging probe and determine pharmacokinetic, dosage, and imaging parameters for dual-modality PET/NIRF imaging in preclinical orthotopic models of human GBM.

**Specific Aims:**

***Aim 1: Design and characterize an MMP-14 activatable dual PET/NIRF peptide probe.***
***Aim 2: Establish the sensitivity and specificity of the MMP-14 activatable dual PET/NIRF peptide probe for in vivo imaging of GBM models.***

**Key Accomplishments and Outcomes:**

**Publications:** Benjamin B. Kasten, Ke Jiang, Denzel Cole, Aditi Jani, Neha Udayakumar, G. Yancey Gillespie, Guolan Lu, Tingting Dai, Eben L. Rosenthal, James M. Markert, Jianghong Rao, Jason M. Warram. "Targeting MMP-14 for dual PET and fluorescence imaging of glioma in preclinical models." *European Journal of Nuclear Medicine and Molecular Imaging* (submitted, under review).

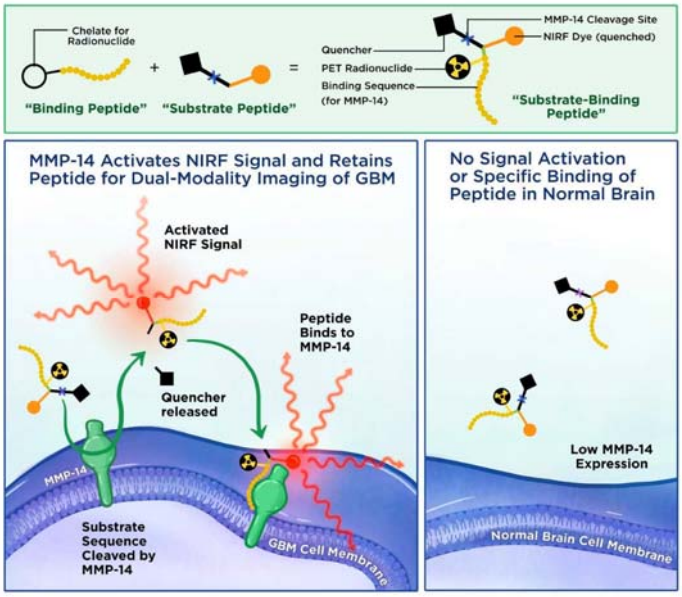
**Patients: none to date**

**Funding Obtained: none to date**

**MMP-14 as a noninvasive marker for PET and NIRF imaging of glioblastoma multiforme**

Hailey Houson, Benjamin Kasten, Ke Jiang, Jianghong Rao, Jason Warram

**Background:** Glioblastoma multiforme (GBM) is a rapidly proliferating and invasive cancer originating from the glial cells of the brain. GBMs are diffuse, with indistinct tumor margins leading to incomplete surgical resection and subsequent recurrence. Matrix metalloproteinase 14 (MMP-14) is an enzyme that degrades the extracellular matrix. MMP-14 is highly expressed in GBM, and is involved in the invasion of the cancerous cells into the surrounding tissue. The primary treatment for GBM is surgery, with an increasing percentage of the tumor removed correlating to improved survival of the patient. However, current imaging of GBM pre-surgery is difficult to translate during surgery due to shift in the tissue. Combinatorial imaging of MMP-14 pre-surgery with PET and intra-operatively with near infrared fluorescence (NIRF) would allow for improved surgical resection of the tumor.
We have developed peptides to bind MMP-14, which are suitable for both PET and NIRF imaging.
**Methods**: Several peptide constructs were developed and used for imaging in mice bearing intracranial implants of patient derived xenograft GBMs. Construct 1 exhibited binding affinity to MMP-14, construct 2 was a substrate for MMP-14, and construct 3 was a combination of 1 and 2. Constructs 1 and 3 were labeled with $^{68}$Ga or $^{64}$Cu and used for PET imaging. Constructs 2 and 3 were fluorescent and were used for NIRF imaging.
**Results:** Immunohistochemistry showed the presence of MMP-14 in the tumor areas, which was co-localized with fluorescence signal from probes 2 and 3. Radiolabeled probes 1 and 3 showed accumulation in the tumor, which could be significantly reduced with the addition of non-labeled blocking peptides ($p<0.01$ and $p<0.05$ respectively). Additionally, in vivo PET and ex vivo NIRF was well correlated as shown using construct 3 ($R^2 = 0.80$).
**Conclusions**: All 3 constructs showed accumulation in the tumor area. Results warrant further investigation of probes in additional preclinical GBM models. Development of probes to image MMP-14 could improve noninvasive detection of the tumor area before surgery, guided tumor resection, and surveillance for recurrence.



**Targeting MMP-14 for dual PET and fluorescence imaging of glioma in preclinical models**

Benjamin B. Kasten[1∞], Ke Jiang[2∞], Denzel Cole[3], Aditi Jani[4], Neha Udayakumar[4], G. Yancey Gillespie[1], Guolan Lu[5], Tingting Dai[2], Eben L. Rosenthal[5], James M. Markert[1], Jianghong Rao[2*], Jason M. Warram[3*]

[1]Department of Neurosurgery, University of Alabama at Birmingham, Birmingham, AL 35294, USA
[2]Departments of Radiology and Chemistry, Molecular Imaging Program at Stanford, Stanford University School of Medicine, Stanford, CA 94305, USA
[3]Department of Otolaryngology, University of Alabama at Birmingham, Birmingham, AL 35294, USA
[4]School of Medicine, University of Alabama at Birmingham, Birmingham, AL 35294, USA
[5]Department of Otolaryngology – Head and Neck Surgery, Stanford University, Stanford, CA 94305, USA
[∞]These authors contributed equally.

*Corresponding authors:
Jason M. Warram, PhD
Department of Otolaryngology
University of Alabama at Birmingham
Volker Hall G082
1670 University Boulevard
Birmingham, AL 35294, USA
Telephone: 1-205-996-5009
Fax: 1-205-975-6522
Email: mojack@uab.edu

Jianghong Rao, PhD
Departments of Radiology and Chemistry
Stanford University
1201 Welch Road, Lucas Center P093
Mail Code 5484
Stanford, CA 94305-5484, USA
Telephone: 1-650-736-8563
Fax: 1-650-736-7925
Email: jrao@stanford.edu

**ACKNOWLEDGMENTS**

Yolanda Hartman, Catherine Langford, Savannah Ferch, Kurt Zinn, Andrew Prince, Marilyn Shackelford, Sally Jordan, Lauren Radford, Charlotte Jeffers, Jennifer Burkemper, Tolulope Aweda, Adriana Massicano, Kiranya Tipirneni, Jonathan McConathy, Suzanne Lapi, Jinda Fan, Jennifer Coleman, Norio Yasui, Dattatray Devalankar, Sharon Samuel, Sheila Bright, Erika McMillian, Himani Modi, Hailey Houson and Morgan Richardson are gratefully acknowledged for their contributions. LI-COR Biosciences is gratefully acknowledged for supplying

IRDye800CW-maleimide and QC-1-NHS ester to prepare the peptide probes. Funding was provided by the NIH/NINDS T32 UAB Training Program in Brain Tumor Biology (T32 NS048039), the UAB Brain Tumor Core Facility (USPHS NCI P20CA151129), the National Center for Advancing Translational Research of the National Institutes of Health (UL1TR001417), the Department of Defense Congressionally Directed Medical Research Program (CA170769), and the Comprehensive Cancer Center at UAB (NIH P30CA013148).

## ABSTRACT

**Purpose**: There is a clinical need for agents that target glioma cells for non-invasive and intraoperative imaging to guide therapeutic intervention and improve the prognosis of glioma. Matrix metalloproteinase (MMP)-14 is overexpressed in glioma with negligible expression in normal brain, presenting MMP-14 as an attractive biomarker for imaging glioma. In this study, we designed a peptide probe containing a near infrared fluorescence (NIRF) dye/quencher pair, a positron emission tomography (PET) radionuclide, and a moiety with high affinity to MMP-14. This novel substrate-binding peptide allows dual modality imaging of glioma only after cleavage by MMP-14 to activate the quenched NIRF signal, enhancing probe specificity and imaging contrast.

**Methods**: MMP-14 expression and activity in human glioma tissues and cells were measured *in vitro* by immunofluorescence and gel zymography. Cleavage of the novel substrate and substrate-binding peptides by glioma cells *in vitro* and glioma xenograft tumors *in vivo* was determined by NIRF imaging. Biodistribution of the radiolabeled MMP-14 binding peptide or substrate-binding peptide was determined in mice bearing orthotopic patient-derived xenograft (PDX) glioma tumors by PET imaging.

**Results:** Glioma cells with MMP-14 activity showed activation and retention of NIRF signal from the cleaved peptides. Resected mouse brains with PDX glioma tumors showed tumor-to-background NIRF ratios of 7.6-11.1 at 4 h after *i.v.* injection of the peptides. PET/CT images showed localization of activity in orthotopic PDX tumors after *i.v*. injection of $^{68}$Ga-binding peptide or $^{64}$Cu-substrate-binding peptide; uptake of the radiolabeled peptides in tumors was significantly reduced ($p<0.05$) by blocking with the non-labeled binding peptide. PET and NIRF signals correlated linearly in the orthotopic PDX tumors. Immunohistochemistry showed co-localization of MMP-14 expression and NIRF signal in the resected tumors.

**Conclusions**: The novel MMP-14 substrate-binding peptide enabled PET/NIRF imaging of glioma models in mice, warranting future image-guided resection studies with the probe in preclinical glioma models.

## KEY WORDS

MMP-14, glioma, NIRF, PET, molecular imaging, dual modality

## INTRODUCTION

Malignant glioma is the most common and deadly primary brain malignancy in adults. The current standard-of-care, comprised of maximal safe surgical resection followed by radio-chemotherapy, is associated with a median survival of less than 18 months [1]. Clinical trials are testing novel therapeutic strategies in attempts to improve the current prognosis of patients with glioma. Studies have shown that the extent of surgical resection correlates with patient outcomes [2-3]. Unfortunately, malignant tissues are frequently difficult to differentiate from normal brain

parenchyma, making complete surgical resection of all glioma while sparing vital healthy brain a significant clinical challenge. Developing agents that specifically target aggressive glioma cells for both non-invasive and intraoperative imaging is an attractive strategy to guide effective therapeutic intervention. Glioma is known for its invasive and diffuse growth pattern, which is indicative of matrix metalloproteinase (MMP) activity [4-7]. Activated MMP-14, also called MT1-MMP, is overexpressed in glioma while it is not expressed at significant levels in normal glial tissue. MMP-14 expression is known to increase with the grade of glioma and correlates with poor patient outcome [8-11]. Preclinical studies have demonstrated that MMP-14 can be exploited as a biomarker for molecular imaging of glioma with various non-invasive or intra-operative reporting modalities [12-14].

Non-invasive imaging of glioma patients through positron emission tomography (PET) with radiolabeled amino acid analogs has enabled pre-operative planning for biopsy or surgery, mapping for radiotherapy, and assessing therapeutic response [15-22]. However, clinical PET suffers from low spatial resolution (>3 mm) and does not permit real-time surgical guidance. Problems with brain shift further hamper co-registration of PET and anatomical images. While clinical PET complements the information from MRI [23], these modalities remain insufficient to guide real-time surgical resection of occult glioma. The United States Food and Drug Association (FDA) recently approved 5-aminolevulinic acid (5-ALA), which is converted into the fluorescent metabolite protoporphyrin IX (PpIX) in rapidly proliferating tumor cells (e.g., glioma), for fluorescence guided surgical resection of glioma. Phase III trials have shown 5-ALA fluorescence guided resection in glioma patients was well tolerated and mediated greater rates of complete resection compared to MRI alone [24-25]. Nonetheless, the optical properties of PpIX are poorly suited for *in vivo* imaging. Significant tissue autofluorescence and photon attenuation at the wavelengths of excitation (~405 nm) and emission (635 nm) reduce spatial resolution and tumor-to-background ratios (TBRs) associated with 5-ALA imaging of glioma [26-28]. Molecular imaging strategies that use near infrared fluorescence (NIRF) reporters are attractive for *in vivo* imaging and fluorescence guided resection due to the minimal tissue autofluorescence and significantly less photon attenuation in the NIR window (700-900 nm) [29-31]. Several molecular targeted compounds with NIRF reporters are currently being evaluated for resecting glioma in preclinical and clinical studies [14, 29-30, 32-34].

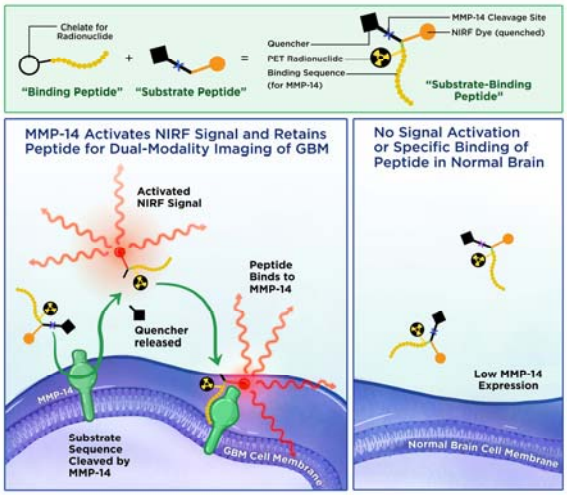



**Figure 1.** Diagram showing general scheme for dual-modality PET/NIRF imaging of glioma (GBM) with an MMP-14 activatable peptide.

The purpose of the present work was to design a novel, MMP-14-activatable dual PET/NIRF peptide probe for imaging and guiding resection of glioma (Figure 1). The peptide probe combined 1) a NIRF reporter and quencher pair separated by a peptide sequence (MMP-14 "substrate peptide") that is cleaved specifically by activated MMP-14 to release the quencher and allow visualization of the NIRF dye, and 2) a chelate for radionuclides attached to a peptide sequence that binds to MMP-14 (MMP-14 "binding peptide") and enables PET imaging. The following *in vitro* and *in vivo* studies tested the ability of these MMP-14-targeted imaging probes to detect preclinical models of human glioma. Glioma cells *in vitro* and orthotopic xenograft tumors in mice *in vivo* cleaved the substrate and the substrate-binding peptides to activate the NIRF signal of the initially quenched peptide probes, yielding favorable imaging contrast in tumors relative to normal brain. *In vivo* PET/CT imaging showed notable activity in orthotopic glioma tumors relative to normal brain after *i.v*. injection of the radiolabeled binding or substrate-binding peptides. PET and NIRF signals from the substrate-binding peptide correlated linearly in the orthotopic PDX tumors and co-localized with MMP-14 expression in the resected tumors. The results from these initial studies indicate the success of the proposed dual-modality imaging strategy to detect MMP-14 in glioma models with the first-generation substrate-binding peptide probe.

## RESULTS

### Human glioma tumor tissues and cell lines express varying levels of MMP-14.

Immunofluorescence staining of a human glioma tissue microarray was performed to determine the relative protein expression of MMP-14 in glioma and normal brain specimens. Consistent with previous reports [8-11], the immunofluorescence results showed significant overexpression of MMP-14 in all grades of glioma relative to normal cerebral tissue (1.6±0.6, 2.2±0.7, 2.5±0.8, 3.0±1.2 glioma/normal brain ratios for grade 1, grade 2, grade 3, and grade 4, respectively; $p<0.05$), with highest expression in grade 4 glioma (Figure 2A). Western blot, immunofluorescence, and gel zymography studies were then performed to characterize the *in vitro* expression and activity of MMP-14 and MMP-2 in immortalized human glioma cell lines. Absolute expression of MMP-14 does not necessarily correlate to MMP-14 activity, as the latter is regulated partly through tissue inhibitors of metalloproteinases (TIMPs). For instance, TIMP-2 binds to the catalytic domain of MMP-14, thus blocking enzymatic activity. TIMP-2 is also required in a ternary complex with MMP-14 and proMMP-2 prior to enzymatic cleavage by a second MMP-14 enzyme to activate MMP-2 [7]. U251 cells in our studies displayed the highest expression of MMP-14 by immunofluorescence (Figure 2B) and Western blot analyses (Figure S1A,B), while U87 cells displayed the highest level of MMP-14 activity as indirectly determined through gel zymography analyses of MMP-2 activity (Figure 2C), which is catalyzed by MMP-14 as described [5, 35]. U87 cells showed the highest MMP-2 expression by Western blot (Figure S1C,D). Although TIMP expression was not determined in our studies, the zymography assay confirmed MMP-14 activity in the glioma cells. These results indicate the cell lines would be suitable in assays that employ peptide probes as enzymatic substrates for MMP-14.

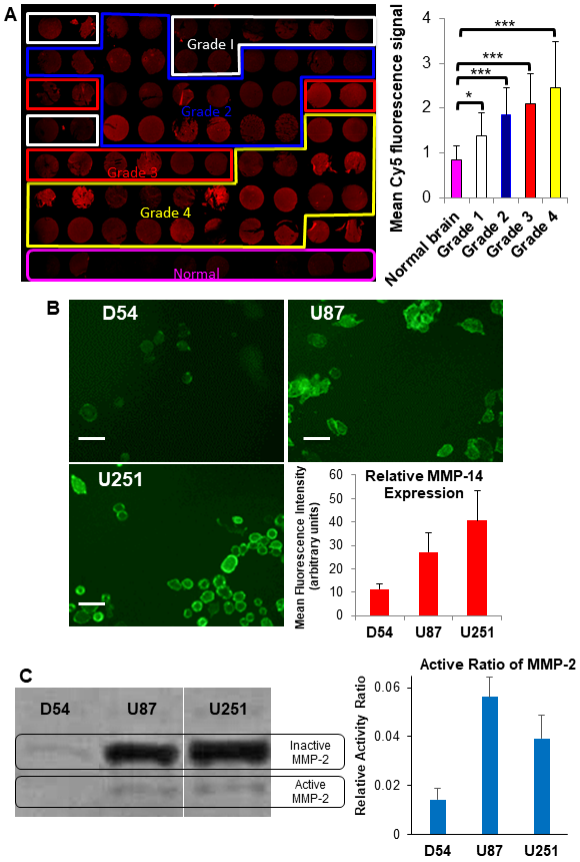



**Figure 2.** Human glioma patient specimens and cell lines grown *in vitro* express different levels of MMP-14. A, MMP-14 immunofluorescence (left) and quantification of fluorescence signal (right) in a human tissue microarray (mean±SD, n=10-24 tissue sections/group); B, MMP-14 immunofluorescence of adherent GBM cells grown *in vitro* and quantification of fluorescence signal (bottom right; mean±SD, n=25-30/group); C, MMP-2 gel zymography (left) and quantification of relative active/latent MMP-2 band intensity (right) from supernatants of adherent GBM cells grown *in vitro* (mean±SD, n=3/group); scale bar in B, 20 μm; *p<0.05; ***p<0.001

## Description of the novel MMP-14 activatable peptide probes.

We and others have used an MMP-14 substrate peptide sequence (RSCitG-HPhe-YLY) to generate peptide probes for imaging and therapy studies in human xenograft tumors that over-express MMP-14 [36-38]. For the present studies, this sequence was used between the NIRF IRDye800 and quencher IR QC-1 pair [39-40] to generate the MMP-14 activatable NIRF substrate peptide (Supplementary Figure S2). This strategy allows the initially quenched signal from IRDye800, which is suitable for NIRF imaging *in vivo*, to become activated upon cleavage of the peptide by MMP-14. For the second peptide component, an MMP-14 binding peptide sequence (HWKHLHNTKTFL) was selected that has previously been described with an apparent $k_d$ of 47.4 nM for MMP-14 [41]. This binding peptide sequence has been joined to various reporting moieties and used in a rat orthotopic glioma model [12] as well as human xenograft tumors that over-express MMP-14 [41-43]. For the present studies, a derivative of the NOTA (1,4,7-triazacyclononane-1,4,7-triacetic acid) chelate for coordination with $^{64}$Cu(II) or $^{68}$Ga(III) was attached to the peptide sequence to generate the MMP-14 binding peptide (Supplementary Figure S3) for *in vivo* PET imaging of MMP-14 expression. The two peptide precursors were synthesized separately by standard solid phase synthesis, NHS and maleimide coupling reactions, and then joined via a cycloaddition reaction between the azide and alkyne

moieties present on the corresponding precursor peptides to generate the combined MMP-14 substrate-binding peptide (Supplementary Figure S4). Mass spectroscopic analyses were consistent with the anticipated peptide structures (Supplementary Figures S5, S6, S7).

**MMP-14 and human glioma cells activate the NIRF signals of the MMP-14 peptide probes *in vitro.***

With the novel MMP-14-targeted peptides in hand, *in vitro* NIRF activation studies with the catalytic domain of MMP-14 were performed. Previous studies have shown that appending fluorescence dyes or other moieties to the N- and C-termini of the core substrate peptide sequence does not abrogate cleavage of the peptide by MMP-14 [37, 44]. However, studies to date have not determined if incorporation of an MMP-14 binding ligand into the same scaffold as an MMP-14 substrate sequence affects cleavage of the substrate peptide by the enzyme.

As presented in Figure 3A, both the substrate and the substrate-binding peptides showed NIRF activation over time relative to the quenched starting moieties during incubation with MMP-14. The relative NIRF activation of the substrate peptide appeared to stabilize within the first 10 minutes, while NIRF activation of the substrate-binding peptide continued to increase beyond 1 h. The fold of increase in the fluorescence with the substrate-binding peptide was much higher than that with the substrate peptide. Based on these initial results, it is possible that the binding sequence may influence the kinetics of the substrate-binding peptide cleavage reaction by MMP-14.

NIRF signal activation of the substrate and substrate-binding peptides was apparent in supernatant solutions (Figure 3B) and associated with the cells (Figure 3C) during *in vitro* incubation with the glioma cell lines. Similar increases in NIRF intensities released into the supernatant were observed during incubation of the peptides with all three glioma cell lines (Figure 3B) regardless of their relative expression of active MMP-14 (Figure 2C). NIRF microscopy showed that U87 and U251 cells had significantly higher cell-associated mean NIRF signal at both 1 and 4 h after incubation with the substrate-binding peptide relative to the substrate peptide ($p<0.001$) at the respective time points (Figure 3D). In D54 cells, which had lower MMP-14 expression and activity relative to the other cells (Figure 2B,C), the cell-associated NIRF signal was significantly higher for the substrate-binding peptide compared to the substrate peptide at 4 h ($p<0.001$) but not at 1 h ($p>0.05$; Figure 3D). These results are consistent with the anticipated mechanism of the substrate-binding peptide, where the MMP-14 binding moiety mediates cellular retention of the residual fluorophore-containing product following cleavage of the substrate sequence by MMP-14. The observed NIRF signal associated with cells incubated with the substrate peptide, which lacks the MMP-14 binding component, likely indicates non-specific cell uptake of the substrate peptide or the fluorophore-containing product following cleavage by MMP-14. Collectively, these results indicate that glioma cells, including those with relatively low MMP-14 activity, are capable of cleaving the novel peptide substrates *in vitro*.

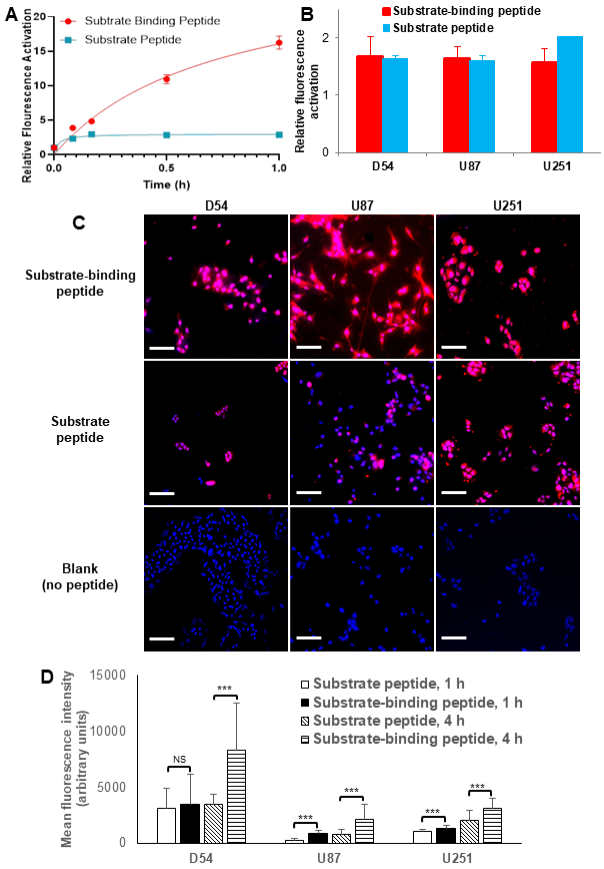



**Figure 3.** Human MMP-14 enzyme and glioma cells activate the NIRF signals of the MMP-14 peptides *in vitro.* A, relative increase in NIRF activation at various time points (0-1 h) after incubating the substrate-binding peptide or the substrate peptide (0.5 µM) with the recombinant catalytic domain of MMP-14 at 37 °C. B, relative increase in NIRF activation released into the supernatant after incubating the substrate-binding peptide or the substrate peptide (0.5 µM) with human glioma cell lines (D54, U87, U251) at 37 °C for 2 h (mean±SD, n=2-4/group). C, NIRF microscopy showing the cell-associated NIRF signal (red) in glioma cells (D54, U87, U251) 1 h after incubation with the substrate-binding peptide (top), substrate peptide (middle), or buffer control without peptide (bottom); cell nuclei were counter-stained with DAPI (blue); scale bar: 50 µm. D, quantification of cell-associated NIRF signal at 1 or 4 h after incubating glioma cells *in vitro* with the substrate or substrate-binding peptide (mean±SD, n=30/group). ***p<0.001; NS, not significant.

## NIRF signals of the MMP-14 activatable peptide probes localize in human glioma xenografts in nude mice *in vivo*

Having confirmed that glioma cells activate the NIRF signal of the peptides *in vitro*, NIRF imaging studies were performed to determine if the peptide probes showed uptake in glioma xenograft tumors *in vivo*. At 24 h after *i.v.* injection of the substrate-binding peptide, *in vivo* NIRF imaging showed a low TBR (1.3±0.2 relative to muscle) in subcutaneous D54 tumors and significantly higher TBR in subcutaneous U87 tumors (2.2±0.4; p<0.001) (Figure 4A, B). Low NIRF signal was observed in all normal tissues except the kidneys (Figure 4A), indicating predominantly renal clearance of the peptide. Subcutaneous U87 tumors from groups of mice injected *i.v.* with either the substrate peptide or the substrate-binding peptide showed no significant difference in TBR or mean NIRF intensity at the 24 h time point (Supplementary Figure S8). These initial studies demonstrated that glioma tumors show uptake of NIRF signal after administering the quenched peptide substrates. The moderate NIRF TBRs observed for the flank tumors could be due to endogenous expression of MMP-14 in the tissues surrounding the

tumors (e.g., muscle, skin) [45].

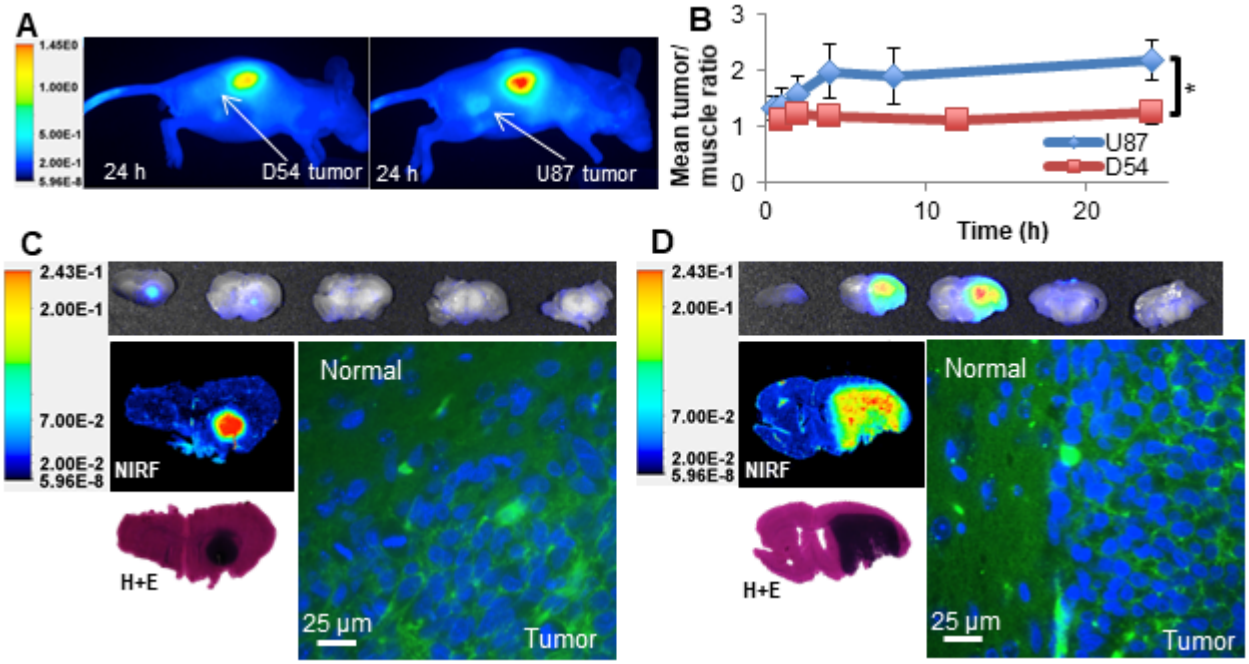



**Figure 4.** NIRF signals from the MMP-14 peptide probes localize in human glioma xenograft tumors in mice *in vivo.* A, *in vivo* NIRF images of live mice bearing flank D54 or U87 glioma tumors at 24 h after *i.v.* injection of the substrate-binding peptide. B, mean *in vivo* tumor/muscle NIRF ratios at various time points (0.25-24 h) after injection of the substrate-binding peptide in mice bearing flank D54 or U87 glioma tumors (mean±SD, n=5 mice/group). C,D, mice bearing orthotopic PDX JX12 glioma tumors were injected *i.v.* either with the substrate peptide (C) or with the substrate-binding peptide (D). Mice were euthanized 1 h later, when the brains were resected, fixed in formalin overnight, and serially sectioned (2 mm). Gross tissue NIRF imaging (Pearl system) showed localization of NIRF signals in the serial tumor-bearing sections (2 mm sections, top panels), as confirmed by higher resolution NIRF imaging (Odyssey system) of 5 µm tissue sections and H+E stained 5 µm tissue sections. NIRF microscopy of DAPI-stained tissue sections (5 µm, right panels, 630x magnification) showed NIRF signal accumulation (yellow channel) in the leading tumor edge but not adjacent normal brain (blue channel, DAPI). *$p<0.05$

NIRF imaging studies with the substrate or substrate-binding peptides were subsequently performed in mice bearing orthotopic patient-derived xenograft (PDX) glioma tumors. Relative to flank xenografts of immortalized cell lines, these orthotopic PDX tumors more accurately retain characteristics of clinical glioma [46-47]. Furthermore, orthotopic tumors were anticipated to better demonstrate the signal contrast from the peptide probes in glioma tumors relative to the endogenous surrounding tissue (e.g., normal brain). High contrast of the NIRF signal in PDX JX12 tumors relative to adjacent normal brain was apparent in gross slices of resected brains at 1, 4, and 24 h after *i.v.* injection of either the substrate peptide (Figure 4C) or the substrate-binding peptide (Figure 4D). NIRF analyses of gross brain slices from the 4 and 24 h time points (Table 1) showed significantly higher ($p<0.001$) mean NIRF signal in the tumor relative to adjacent normal brain from mice given the substrate-binding peptide. This result is consistent with retention of the cleaved peptide moiety containing the fluorophore within the tumor and low accumulation of the cleaved peptide in normal brain, which has negligible expression of MMP-14. The NIRF signal in the tumor was significantly higher than in normal brain of mice at 4 h ($p<0.001$), but not at 24 h ($p=0.074$), after injection of the substrate peptide. The group of mice analyzed at 24 h after injecting the substrate peptide had fewer evaluable tumors compared to the

other groups, which could have impacted the statistical outcome of the NIRF signal comparison in these mice. While the NIRF mean tumor signals and TBRs were higher in mice given the substrate-binding peptide relative to the substrate peptide (Table 1), the TBRs were not significantly different at the time points examined ($p>0.05$, ANOVA). NIRF microscopy showed dispersion of the activated NIRF signal from the peptides throughout the tumors, including near the leading edge of tumor progression (Figure 4C, D). Both peptides also yielded positive NIRF signals in regions of diffuse glioma cell growth beyond the bulk tumor (data not shown). Comparing histological tissue sections with confirmed glioma growth by H+E to NIRF signals measured in tissue sections yielded sensitivities above 83% for the substrate peptide and above 85% for the substrate-binding peptide (Supplementary Table S1) at the 4 h and 24 h time points. These results confirmed that the novel MMP-14 peptides could be used for NIRF imaging of orthotopic models of human glioma. These findings support future studies that utilize the peptides for intra-operative resection of preclinical glioma tumors *in vivo*.

**Table 1.** *Ex vivo* NIRF signal intensities and TBRs from the substrate peptide or the substrate-binding peptide measured in 2 mm gross brain slices from mice bearing orthotopic PDX glioma tumors.

| | Mean NIRF signal in tumor | Mean NIRF signal in normal brain | p value (tumor vs. normal brain NIRF signal) | Mean±SD TBR (range) |
|---|---|---|---|---|
| Substrate peptide, 4 h | 0.012±0.007 | 0.002±0.001 | <0.001 | 7.6±2.9 (2.7-10.9) |
| Substrate peptide, 24 h | 0.011±0.010 | 0.001±0.00 | 0.074 | 8.7±6.3 (2.9-17.1) |
| Substrate-binding peptide, 4 h | 0.024±0.011 | 0.002±0.001 | <0.001 | 11.1±4.5 (7.8-18.9) |
| Substrate-binding peptide, 24 h | 0.021±0.010 | 0.002±0.001 | <0.001 | 13.3±3.7 (6.0-17.6) |

**PET signals of the radiolabeled MMP-14 peptide probes localize in human glioma orthotopic xenografts in nude mice *in vivo***

The second goal of this work was to determine if the peptides could be used for *in vivo* PET imaging of orthotopic models of human glioma tumors in mice. A preliminary study was performed using the binding peptide labeled with $^{68}$Ga ($^{68}$Ga-binding peptide) in mice with orthotopic PDX JX12 tumors. The radiolabeled peptide was obtained in 87-91% radiochemical conversion after heating with $^{68}$Ga at 90 °C for 20 min; further heating did not improve the yield of the product $^{68}$Ga-binding peptide (Supplementary Figure S9). Tumor-bearing mice were dosed *i.v.* with 0.4-0.8 nmol $^{68}$Ga-binding peptide from the diluted reaction solution (molar activity approximately 6.1 GBq/μmol at time of dosing). At 2 h after dosing, the tumors were visible during PET/CT imaging while normal brain showed low uptake of activity (Figure 5A). *Ex vivo* biodistribution analyses indicated significantly more uptake of radioactivity in brains of mice injected with $^{68}$Ga-binding peptide (0.16±0.02 %ID/g) compared to brains of mice injected with $^{68}$Ga-binding peptide and 60-fold excess non-labeled binding peptide as a blocking agent (0.07±0.02 %ID/g, $p<0.01$) (Figure 5B), thus supporting the specific retention of the radiolabeled peptide in the PDX tumors. The amount of $^{68}$Ga in resected brains correlated with qualitative tumor burden determined by H+E tissue analyses (Supplementary Figure S10). PET images and *ex vivo* biodistribution analyses showed high accumulation of activity in the liver, spleen, and

kidneys (Supplementary Figure S11, S12), which was likely due to the relative hydrophobicity of the peptide at physiological pH.

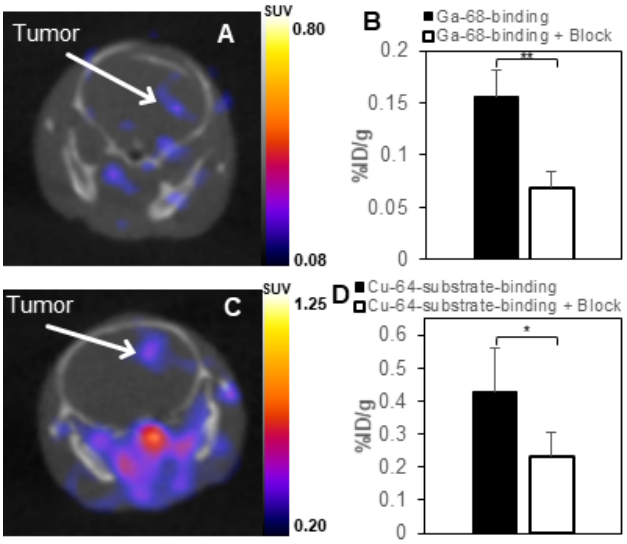



**Figure 5.** PET imaging and biodistribution show specific localization of radiolabeled peptide probes in orthotopic PDX glioma tumors *in vivo*. A, representative transverse PET/CT image at 2 h after *i.v.* injection of $^{68}$Ga-binding peptide in athymic nude mice bearing orthotopic PDX JX12 glioma tumors, showing localization of activity in the tumor. B, *ex vivo* biodistribution showing whole-brain activity at 3.5 h after *i.v.* injection of $^{68}$Ga-binding peptide or of $^{68}$Ga-binding peptide + block (non-labeled binding peptide) in athymic nude mice bearing orthotopic PDX JX12 glioma tumors. Full biodistribution analyses are available in the Supplementary Data. C, representative transverse PET/CT image at 4 h after *i.v.* injection of $^{64}$Cu-substrate-binding peptide in athymic nude mice bearing orthotopic PDX JX12 glioma tumors, showing localization of activity in the tumor. Activity in soft tissues of the neck (e.g., blood vessels, muscle, lymph nodes) was apparent at the scaling intensity shown in the PET image. D, *ex vivo* biodistribution showing whole-brain activity at 5.5 h after *i.v.* injection of $^{64}$Cu-substrate-binding peptide or $^{64}$Cu-substrate-binding peptide + block (non-labeled binding peptide) in athymic nude mice bearing orthotopic PDX JX12 glioma tumors. Full biodistribution analyses are available in the Supplementary Data. *p<0.05; **p<0.01; SUV, standardized uptake value.

A separate cohort of mice bearing orthotopic PDX JX12 tumors were used for PET imaging and biodistribution analyses with $^{64}$Cu-labeled substrate-binding peptide ($^{64}$Cu-substrate-binding peptide). $^{64}$Cu (12.7 h half-life) was used as the radionuclide with the substrate-binding peptide to allow PET imaging analyses at later time points (e.g., 4 h) that were used in the NIRF imaging studies. The relatively short half-life of $^{68}$Ga (67.7 min) precludes PET imaging analyses beyond 3 h. The $^{64}$Cu-substrate-binding peptide was generated quantitatively after labeling with $^{64}$Cu at room temperature for 20 min (Supplementary Figure S13). Tumor-bearing mice were dosed *i.v.* with 0.13 nmol $^{64}$Cu-substrate-binding peptide from the diluted reaction solution (molar activity approximately 46.6 GBq/µmol at time of dosing). At 4 h after dosing, PET/CT imaging showed significant contrast in the tumor relative to normal brain (Figure 5C), yielding a standardized uptake value ratio (SUVR: ratio of tumor $SUV_{mean}$ to normal brain $SUV_{mean}$) of 3.9±0.5. The SUVR was lower in a group of tumor-bearing mice (2.5±1.3) that had been co-injected with 80-fold excess of the non-labeled binding peptide as a blocking agent, although the difference between the two groups of mice was not significant (p=0.056). The activity present in whole resected brains from mice in the $^{64}$Cu-substrate-binding peptide group (0.43±0.13 %ID/g) was significantly higher than that in the blocked group (0.23±0.07 %ID/g; p<0.05) (Figure 5D). This result suggests that the binding peptide is able to partially block binding of the substrate-binding peptide to PDX glioma tumors *in vivo*.

Biodistribution analyses of resected tissues was consistent with the PET images, indicating predominantly hepatobiliary accumulation of activity (Supplementary Figure S14, S15).

**NIRF signals from the $^{64}$Cu-substrate-binding peptide correlate with PET signals and co-localize with MMP-14 expression in human glioma orthotopic xenografts**

The resected brains from mice injected with the $^{64}$Cu-substrate-binding peptide in the above studies were sectioned and used for NIRF analyses. Gross imaging showed high contrast between the NIRF signal in PDX tumor regions compared to contralateral normal brain, yielding a TBR of 7.2±1.3 ($p<0.001$). The summed NIRF signal from tumor regions in these tissue sections correlated linearly ($R^2=0.84$, $p<0.0001$) with the *in vivo* PET %ID/cm$^3$ signal present in the tumor-bearing brain regions (Figure 6A). Microscopic imaging of hematoxylin and eosin (H+E) stained tissue sections confirmed that the NIRF signal co-localized in the PDX glioma tumors, which showed high expression of MMP-14 relative to normal brain (Figure 6B-G). These results support the hypothesis that the NIRF signal from the cleavable peptide was specifically retained in the PDX glioma tumors due to the expression of MMP-14 in the tumors. The absolute NIRF signal from brains of mice injected with the $^{64}$Cu-substrate-binding peptide was lower than that from mice in the study with the non-labeled substrate-binding peptide. This result was anticipated due to the different mass dose of the peptides used for the two studies. These studies support the feasibility of dual-modality PET and NIRF imaging with the radiolabeled peptide probe in a PDX glioma model. A goal of future work will be to determine the effect of the labeled and non-labeled peptide doses on the TBR for the PET and NIRF signals separately.

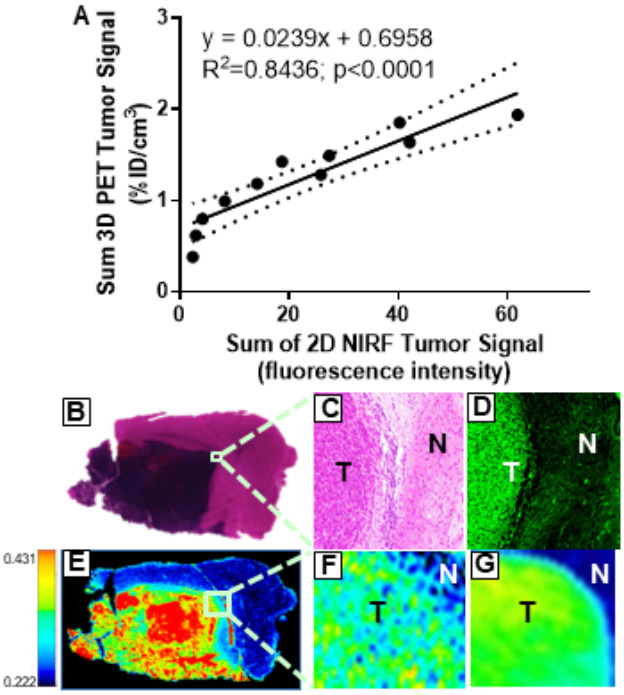



**Figure 6**. NIRF signals from the radiolabeled substrate-binding peptide show high concordance with PET signal, orthotopic PDX glioma localization, and MMP-14 expression. A, linear correlation between *in vivo* PET (%ID/cm$^3$) and *ex vivo* NIRF signals from brains from mice bearing orthotopic PDX JX12 glioma tumors after *i.v.* injection of the $^{64}$Cu-substrate-binding peptide (0.13 nmol); dotted lines show the 95% confidence interval of the correlation. Co-localization of H+E stained tumor (B,C), IRDye800 fluorescence on Odyssey scanner (E,F), and MMP-14 expression (D,G) in brain sections from mice bearing orthotopic PDX JX12 glioma tumors after *i.v.* injection of the $^{64}$Cu-substrate-binding peptide. Separate sections were probed using an anti-MMP-14 antibody conjugated to either

dye AlexaFluor 488 for microscopy (D) or dye Cy5 for Odyssey scanner (G, 21 μm resolution). Microscopic images (C,D) are 100x. T, tumor; N, normal brain.

## DISCUSSION

The membrane-bound collagenase MMP-14 is a key enzyme in initiating and propagating the invasive phenotype associated with malignant glioma progression. MMP-14 processes other MMPs (e.g., proMMP-2) to their active state and cleaves adhesion proteins (e.g., CD44, integrins, etc.) in addition to extracellular collagen [5, 35, 48-50]. MMP-14 is also expressed on tumor-associated glial cells and macrophages, which can comprise up to 30% of the tumor bulk and have been implicated in promoting glioma cell invasion, expansion, and pathogenesis [51-53]. These combined factors formed the rationale for exploiting MMP-14 as a biomarker for imaging glioma. The peptide probes developed here may also be relevant for imaging other malignancies (e.g., breast cancer, melanoma) that overexpress MMP-14 [54-57].

Our imaging approach is unique in that the substrate-binding peptide probe exploits MMP-14 for both enzymatic NIRF signal activation and specific localization of the PET and activated NIRF signals. Most existing PET and activatable fluorescence dual-imaging probes utilize different molecular targets for fluorescence signal activation and binding to the tumor cells. The proposed approach exploits enzymatic amplification of the NIRF signal to enable high-contrast intra-operative imaging of tumors that express activated MMP-14. Exploiting a single biological target for both PET and NIRF reporters can potentially enable straightforward co-registration of the imaging signals for intra-operative fluorescence imaging with high spatial resolution [58-62], which is otherwise challenging when employing separate probes that have differing pharmacokinetic and localization patterns [63]. It remains to be determined if the substrate-binding peptide first binds to MMP-14, thus preventing the substrate from being activated by the same MMP-14. Mechanistic studies to determine factors affecting peptide cleavage were beyond the scope of our proof-of-concept experiments discussed above. Future experiments will more fully characterize peptide cleavage kinetics and potential mechanisms of inhibition caused by the peptides on MMP-14 enzymatic activity. Since MMP-14 is located on the cell surface, the kinetics *in vivo* may not be the same as that determined in solution. As endogenous substrates of MMP-14 are large tertiary protein complexes (e.g., proMMP-2, TIMP-2, MMP-14), it is likely a second MMP-14 on the cell surface could process the substrate sequence in the peptide-MMP-14 complex to produce fluorescence activation. Other MMP-14 activatable fluorescence imaging probes do not include a targeted binding moiety, resulting in diffusion of the activated probe away from the target cells [64-65]. Incorporating the binding peptide into the substrate-binding probe in our studies was anticipated to cause specific retention of the PET and activated NIRF signals on glioma cells, which would enhance signal contrast of the tumor cells relative to adjacent healthy brain. Glioma cell lines showed up to 2.8-fold higher cell-associated NIRF signal at 4 h after *in vitro* incubation with the substrate-binding peptide relative to the substrate peptide (Figure 3D; $p<0.001$), thus supporting the hypothesis that the binding peptide component enhances cell retention of the activated NIRF signal. In addition to ligand-protein interactions that result in signal retention on target cells, factors such as probe extravasation and clearance from tumors also influence TBRs *in vivo*. The above NIRF imaging results of PDX glioma tumors in resected brain tissues showed comparable TBRs for the substrate-binding peptide and substrate peptide at 4 and 24 h p.i. (Table 1). At the 24 h time point, however, the substrate peptide resulted in the lowest NIRF signal among the groups examined, which is consistent with lower specific retention of the cleaved substrate peptide due to its lack of a binding component.

The results from this work are in agreement with prior studies that have targeted MMP-14 as a biomarker for imaging glioma in preclinical animal models. Favorable PET SUVR (3.9) and NIRF TBR (13.3) signals from the substrate-binding peptide probe were observed in the present studies with an orthotopic PDX glioma model. Previous studies in glioma models or in humans with glioma have used dual-modality imaging probes targeted to other proteins that are overexpressed in glioma relative to normal brain tissue. For instance, preclinical studies using a dual PET/NIRF peptide that binds to EphB4 (Cy5.5-TNYL-RAWK-$^{64}$Cu-DOTA) in rodents with orthotopic human glioma xenografts indicated TBRs of 9 for PET imaging and 6-7 for fluorescence imaging [58]. A clinical trial using a dual PET/NIRF probe that targets the gastrin releasing peptide receptor ($^{68}$Ga-BBN-IRDye800) in glioma patients showed a PET SUVR of 13.4 and a NIRF TBR of 4.9 [66].

A primary goal of future studies is to assess if the activatable NIRF probes are suitable for optical-guided resection of glioma models *in vivo*. The TBRs and specific localization of the NIRF signals in the resected PDX glioma tumors from the present studies suggest the probes would be worthwhile candidates for NIRF-guided resection of glioma. Several preclinical studies in rodents with orthotopic brain tumors have shown TBRs ranging from 3 to 16 for various established (e.g., 5-ALA) or experimental fluorescence imaging agents [14, 32-33, 63, 67]. Clinical therapy trials in patients with glioma are in progress to evaluate fluorescence-guided resection using imaging agents that include 5-ALA (NCT02119338, NCT01502280, NCT02632370, NCT00752323, NCT02191488, NCT01811121, NCT02379572), ABY-029 (NCT02901925), BLZ-100 (recently completed NCT02234297), panitumumab-IRDye800 (NCT03510208), $^{68}$Ga-BBN-IRDye800 (NCT03407781) and fluorescein (NCT03291977, NCT02691923, [68]). These studies highlight the continued thrust to incorporate fluorescence-guided resection to improve outcomes for patients with glioma.

In our pilot studies above we observed relatively low PET signal in the orthotopic glioma tumors. This result may be due to moderate expression of MMP-14 relative to alternative biomarkers, restricted extravasation of the peptide into tumor parenchyma, moderate specific activity of the radiolabeled probes, sub-optimal mass doses of the probes, or other pharmacokinetic factors. Many small molecule PET probes show moderate accumulation and contrast (TBR 2-5 [69-73]) in glioma, partly due to rapid blood clearance kinetics and short PET radionuclide half-life, while macromolecule-based PET probes often show high accumulation due to significantly longer circulation times that allow the tracer to access tumors through disrupted BBB regions [13, 74]. The results from the *in vivo* blocking experiment support the conclusion that the radiolabeled peptide probes showed specific binding in the tumor. It was beyond the scope of these proof-of-concept studies to quantify non-specific pooling of the probes in glioma tumors due to a disrupted BBB. In human patients, glioma often contains significant portions of tumor cell infiltration in non-contrast-enhancing regions of the brain. Therefore, it would be worthwhile in future experiments to investigate the accumulation of the novel probes in spontaneous glioma models with tumor invasion in intact BBB regions. While MMP-14 is expressed in systemic tissues, the presence of tissue inhibitor of MMP-14 (TIMPs) may help minimize its activity in the circulation. Future studies to determine the specificity of glioma-mediated NIRF activation would benefit from comparing NIRF imaging with the probes above to control peptides that either lack the quencher molecule or that have a non-cleavable sequence separating the dye and quencher pair. It would also be useful to determine if different tumor infiltrating cells (e.g., tumor associated glial cells, macrophages) besides glioma cells contribute

to the NIRF signal localization from the MMP-14 peptides within the tumor microenvironment [49, 51, 53].

## CONCLUSION

The novel MMP-14 targeted and activatable peptide probes enabled dual PET and NIRF imaging of glioma in preclinical studies. High NIRF signal TBRs were observed in the resected brain sections of mice bearing PDX glioma tumors. Correlations between *in vivo* PET and *ex vivo* NIRF signals support the concept for dual-modality imaging of glioma with a single, MMP-14-targeted probe scaffold. The co-localization of NIRF signals and MMP-14 expression in the tumors observed by tissue staining confirmed the specific localization of the peptide probes. These results support future preclinical studies designed to test the efficacy of surgical resection of glioma with the MMP-14-targeted probes.

## MATERIALS AND METHODS

### General reagents

All general reagents were from commercial suppliers (Thermo Fisher, Waltham, MA, USA; Sigma, St. Louis, MO, USA) unless specified otherwise. Primary antibodies for MMP-14 (rabbit anti-MMP-14 monoclonal antibody (mAb), clone EP1264Y), MMP-2 (rabbit anti-MMP-2 polyclonal, ab37150), or isotype control (rabbit mAb, clone EPR25A) were from Abcam (Cambridge, MA). Fluorophore-conjugated goat anti-rabbit polyclonal secondary antibodies were from Invitrogen (Thermo Fisher). HRP-conjugated secondary antibodies were from Santa Cruz Biotechnology, Inc. (Dallas, TX). The recombinant catalytic enzyme domain of MMP-14 was from Invitrogen. $^{64}$Cu was obtained in 0.1 M HCl from the *Mallickrodt Institute of Radiology PET Nuclear Pharmacy & Cyclotron Facility of the Washington University Medical Center* or from the University of Alabama at Birmingham (UAB) Cyclotron Facility. $^{68}$Ga, eluted in 0.1 M HCl from a $^{68}$Ge/$^{68}$Ga generator, was obtained from the UAB Cyclotron Facility.

### Gel zymography

Gel zymography was performed as previously described [75]. Briefly, adherent monolayers of glioma cells were rinsed with PBS and incubated in serum-free medium for 24 h. Supernatants were collected, analyzed for protein content, and loaded on zymogram gels (10% gelatin, Novex® Zymogram Gels, Thermo Fisher) according to the manufacturer's recommendations. Relative band intensities were quantified by ImageJ.

### Immunofluorescence

*Immunocytochemistry*: Glioma cells were seeded at 100,000 cells/well in a 12-well plate and allowed to attach overnight. Adherent cells were rinsed in PBS, blocked with 1% BSA in PBS at room temperature for 45 min, incubated with the anti-MMP-14 mAb (Abcam ab51074) or isotype control mAb (Abcam ab172730) at 1 μg/mL in 0.1% BSA in PBS at room temperature for 30 min, rinsed 4 times with 0.1% BSA in PBS, incubated with Ready Probes AlexaFluor 488-goat anti-rabbit antibody solution (R37116, Molecular Probes, Thermo Fisher) at room temperature for 30 min, rinsed 3 times with 0.1% BSA in PBS, and imaged on an inverted fluorescence microscope at 200x magnification. Mean fluorescence intensity per cell was quantified using ImageJ by randomly selecting 20-25 cells over four fields of view and measuring the mean fluorescence intensity.

*Immunohistochemistry*: A slide with a formalin fixed paraffin embedded (FFPE) human glioma tissue microarray (BS17016b, US Biomax, Inc, Derwood, MD) was deparaffinized and antigen was retrieved by heating for 10 min at 90 °C in citrate buffer pH 6 with 1 mM EDTA. Tissues were blocked in 5% BSA/TBST at room temperature, incubated overnight at 4 °C with anti-MMP-14 mAb at 1 μg/mL in 5% BSA/TBST, washed in TBST, incubated for 2 h at room temperature with Cy5-secondary antibody (A10523, Invitrogen, Thermo Fisher) at 1/1000 dilution in 5% BSA/TBST, rinsed in TBST, and mounted in DAPI-Fluoromount-G (Southern Biotech, Birmingham, AL). Microarray tissues were imaged on an Odyssey scanner (LI-COR Biosciences, Lincoln, NE) using the 700 nm channel. Integrated instrument software (ImageStudio, LI-COR) was used to determine mean fluorescence intensity as total counts/region of interest (ROI) pixel area. 5-6 μm FFPE sections from resected brains of mice bearing orthotopic PDX JX12 glioma tumors were processed using the same protocol, except AlexaFluor 488-conjugated secondary antibody (A11008, Invitrogen, Thermo Fisher) was used (1/2000 dilution).

**Production of peptide probes**

The MMP-14 binding, substrate, and substrate-binding peptide probes were synthesized by solid phase techniques using protected amino acids and commercially available IRDye800CW-maleimide and quencher QC-1-NHS ester (LI-COR). The synthesized peptides were purified by semi-preparative reverse-phase high performance liquid chromatography (RP-HPLC) and characterized by mass spectrometry. Additional details are provided in the Supplementary Materials.

***In vitro* NIRF activation and NIRF microscopy studies**

The catalytic enzyme domain of MMP-14 (5 nM, 5 ng/well) or adherent glioma cells in 96-well plates (Corning Costar, Corning, NY) were incubated with 0.5 μM solutions of the substrate or substrate-binding peptide in 50 μL MMP-14 assay buffer (PBS with 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 10 μM $ZnCl_2$) at 37 °C, 5% $CO_2$ for 0-24 h. At designated time points, aliquots (1.5 μL) were removed and spotted on Whatman 1 chromatography paper. After drying at room temperature, NIRF signal of the blots was quantified by the Pearl imaging system (LI-COR). Alternatively, for quantification of the NIRF signal from the substrate peptide, the NIRF signal in the 96-well plates containing glioma cells was directly imaged on the Pearl system. As controls to determine relative fluorescence signal activation at each time point, solutions were used that contained the respective peptide solution without MMP-14 or glioma cells. Cell studies were performed in duplicate wells. For NIRF microscopy studies, glioma cell lines were seeded in 8-chamber slides ($10^4$ cells/chamber) two days before the experiment. On the day of the experiment, cells were rinsed with PBS and incubated at 37 °C, 5% $CO_2$ with the substrate or substrate-binding peptides (1 μM) in cell medium containing 1% FBS. Chambers were placed on ice at designated time points and medium was aspirated; chambers on ice were washed with ice-cold buffer (PBS, 1% BSA), fixed in formaldehyde at room temperature for 15 min, washed to remove formaldehyde, and mounted in Fluoromount-G prior to NIRF microscopy imaging at 100x magnification. Mean fluorescence intensity per cell was quantified using ImageJ by randomly selecting 30 cells over three fields of view and measuring the mean fluorescence intensity.

***In vivo* NIRF imaging studies**

Mice bearing flank D54 or U87 xenografts (n=5/group) were injected *i.v.* with 10 nmol substrate peptide or substrate-binding peptide (0.2 mL in PBS). Live, anesthetized mice were imaged on the Pearl system (LI-COR) from 0-24 h *p.i*. Mice bearing orthotopic PDX JX12 xenografts were injected *i.v.* with 0.8-0.9 nmol substrate peptide or substrate-binding peptide (0.2 mL in PBS) and euthanized at 1 h (n=1 mouse/group), 4 h (n=4-6 mice/group), or 24 h (n=3-5 mice/group) *p.i*. Immediately after euthanizing anesthetized mice by cervical dislocation, the skin over the skull was removed and NIRF signal was determined by the Pearl system. The whole brains were resected, fixed in 10% formalin overnight, and imaged by the Pearl system. Brains were then serially sectioned (1 or 2 mm coronal slices), and all slices were imaged by the Pearl system. Three or four slices containing tumor and adjacent normal brain without tumor were then dehydrated in 70% EtOH and processed for embedding in paraffin and further sectioning (5-6 μm). Un-stained sections were imaged on an Odyssey system (LI-COR) or stained for H+E or immunofluorescence (anti-MMP-14 mAb or isotype control) as described above. NIRF signals from the Odyssey scans were defined as positive if they were above the following threshold: three-times the standard deviation above the pooled mean NIRF signal in negative regions (no tumor cells present by H+E histology). Odyssey images and H+E images at the same resolution were compared by ImageJ to define true positive, true negative, false positive, or false negative NIRF signal regions.

***In vivo* PET/CT imaging studies and biodistribution studies.**

Mice bearing orthotopic PDX JX12 xenografts were used for PET/CT imaging and subsequent biodistribution studies with the radiolabeled peptide probes. *Mice injected i.v. with $^{68}$Ga-binding peptide (0.7-1.0 nmol, 2.2-4.5 MBq) with or without unlabeled binding peptide (50 nmol) (n=3-4 mice/group) were imaged at 1 and 2 h time points p.i. Mice injected i.v. with $^{64}$Cu-substrate-binding peptide (0.3 nmol, 6.3-6.5 MBq) with or without unlabeled binding peptide (22 nmol) (n=5-6 mice/group) were imaged at 1 and 4 h time points p.i. PET (energy window 350-650 KeV; 15 min acquisition for $^{64}$Cu studies, 20 min acquisition for $^{68}$Ga studies) and CT (voltage 80 kVp, current 150 μA, 720 projections) images were acquired on a GNEXT PET/CT small animal scanner (Sofie Biosciences, Culver City, CA). The PET images were reconstructed using a 3D-Ordered Subset Expectation Maximization algorithm (24 subsets and 3 iterations), with random, attenuation, and decay correction.* The CT images were reconstructed using a Modified Feldkamp Algorithm. Reconstructed images were analyzed using VivoQuant software (version 3.5patch 2, Invicro, LLC, Boston, MA). Following the last imaging time point, anesthetized mice were euthanized by cervical dislocation. Whole brains and other selected tissues were resected, weighed, and counted on a gamma counter (1480 Wizard$^2$, Perkin Elmer, Shelton, CT). Percent uptake of the injected dose per gram (% ID/g) was calculated by comparing the tissue activity to solutions with known activity of the radionuclide of interest. Resected brains were then fixed in 10% formalin overnight, serially sectioned (1 or 2 mm coronal slices), and processed for further NIRF imaging, paraffin embedding, and tissue sectioning for H+E or immunofluorescence analyses as described above.

**Statistical analyses**

Data were analyzed using Microsoft Excel or GraphPad Prism (Version 6.1, GraphPad Software, La Jolla, CA, USA). Student's *t*-test was used when comparing two groups. When comparing multiple groups, one-way ANOVA tests, followed by Bonferroni corrections for

multiple comparisons, were performed. All $p$-values correspond to two-tailed tests; significance was considered to be at $p < 0.05$.


## ACKNOWLEDGMENTS

Yolanda Hartman, Catherine Langford, Savannah Ferch, Kurt Zinn, Andrew Prince, Marilyn Shackelford, Sally Jordan, Lauren Radford, Charlotte Jeffers, Jennifer Burkemper, Tolulope Aweda, Adriana Massicano, Kiranya Tipirneni, Jonathan McConathy, Suzanne Lapi, Jinda Fan, Jennifer Coleman, Norio Yasui, Dattatray Devalankar, Sharon Samuel, Sheila Bright, Erika McMillian, Himani Modi, Hailey Houson and Morgan Richardson are gratefully acknowledged for their contributions. LI-COR Biosciences is gratefully acknowledged for supplying IRDye800CW-maleimide and QC-1-NHS ester to prepare the peptide probes. Funding was provided by the NIH/NINDS T32 UAB Training Program in Brain Tumor Biology (T32 NS048039), the UAB Brain Tumor Core Facility (USPHS NCI P20CA151129), the National Center for Advancing Translational Research of the National Institutes of Health (UL1TR001417), the Department of Defense Congressionally Directed Medical Research Program (CA170769), and the Comprehensive Cancer Center at UAB (NIH P30CA013148).


## DECLARATIONS

The authors declare no financial conflicts of interest.

## ABBREVIATIONS

5-ALA, 5-aminolevulinic acid; ANOVA, analysis of variance; BBB, blood brain barrier; BSA, bovine serum albumin; CT, computed tomography; FDA, Food and Drug Administration; FFPE, formalin fixed paraffin embedded; GBM, glioblastoma multiforme; %ID/g, percent injected dose per g; *i.v.*, intravenous; mAb, monoclonal antibody; MMP, matrix metalloproteinase; NIR, near infrared; NIRF, near infrared fluorescence; NOTA, 1,4,7-triazacyclononane-1,4,7-triacetic acid; PDX, patient derived xenograft; PET, positron emission tomography; *p.i.*, post injection; PpIX, protoporphyrin IX; RIPA, radioimmunoprecipitation assay; *s.c.*, subcutaneous; SUVR, standardized uptake value ratio; TBR, tumor-to-background ratio; UAB, University of Alabama at Birmingham

**REFERENCES**


1. Delgado-López, P. D.; Corrales-García, E. M., Survival in glioblastoma: a review on the impact of treatment modalities. *Clin. Transl. Oncol.* **2016,** *18* (11), 1062-1071.
2. Daniel Orringer; Darryl Lau; Sameer Khatri; Grettel J. Zamora-Berridi; Kathy Zhang; Chris Wu; Neeraj Chaudhary; Oren Sagher, Extent of resection in patients with glioblastoma: limiting factors, perception of resectability, and effect on survival. *J. Neurosurg.* **2012,** *117* (5), 851-859.
3. Li, Y. M.; Suki, D.; Hess, K.; Sawaya, R., The influence of maximum safe resection of glioblastoma on survival in 1229 patients: Can we do better than gross-total resection? *J. Neurosurg.* **2016,** *124* (4), 977-988.
4. Shiomi, T.; Okada, Y., MT1-MMP and MMP-7 in invasion and metastasis of human cancers. *Cancer and Metastasis Reviews* **2003,** *22* (2), 145-152.
5. Kessenbrock, K.; Plaks, V.; Werb, Z., Matrix Metalloproteinases: Regulators of the Tumor Microenvironment. *Cell* **2010,** *141* (1), 52-67.
6. Beliën, A. T. J.; Paganetti, P. A.; Schwab, M. E., Membrane-type 1 Matrix Metalloprotease (MT1-MMP) Enables Invasive Migration of Glioma Cells in Central Nervous System White Matter. *The Journal of Cell Biology* **1999,** *144* (2), 373-384.
7. Fillmore, H. L.; VanMeter, T. E.; Broaddus, W. C., Membrane-type Matrix Metalloproteinases (MT-MMP)s: Expression and Function During Glioma Invasion. *J. Neurooncol.* **2001,** *53* (2), 187-202.
8. Nakada, M.; Nakamura, H.; Ikeda, E.; Fujimoto, N.; Yamashita, J.; Sato, H.; Seiki, M.; Okada, Y., Expression and Tissue Localization of Membrane-Type 1, 2, and 3 Matrix Metalloproteinases in Human Astrocytic Tumors. *The American Journal of Pathology* **1999,** *154* (2), 417-428.
9. Wang, L.; Yuan, J.; Tu, Y.; Mao, X.; He, S.; Fu, G.; Zong, J.; Zhang, Y., Co-expression of MMP-14 and MMP-19 predicts poor survival in human glioma. *Clin. Transl. Oncol.* **2013,** *15* (2), 139-145.
10. Forsyth, P. A.; Wong, H.; Laing, T. D.; Rewcastle, N. B.; Morris, D. G.; Muzik, H.; Leco, K. J.; Johnston, R. N.; Brasher, P. M. A.; Sutherland, G.; Edwards, D. R., Gelatinase-A (MMP-2), gelatinase-B (MMP-9) and membrane type matrix metalloproteinase-1 (MT1-MMP) are involved in different aspects of the pathophysiology of malignant gliomas. *Br J Cancer* **1999,** *79* (11-12), 1828-1835.
11. Yamamoto, M.; Mohanam, S.; Sawaya, R.; Fuller, G. N.; Seiki, M.; Sato, H.; Gokaslan, Z. L.; Liotta, L. A.; Nicolson, G. L.; Rao, J. S., Differential Expression of Membrane-Type Matrix Metalloproteinase and Its Correlation with Gelatinase A Activation in Human Malignant Brain Tumors *in Vivo* and *in Vitro*. *Cancer Res.* **1996,** *56* (2), 384-392.
12. Gu, G.; Gao, X.; Hu, Q.; Kang, T.; Liu, Z.; Jiang, M.; Miao, D.; Song, Q.; Yao, L.; Tu, Y.; Pang, Z.; Chen, H.; Jiang, X.; Chen, J., The influence of the penetrating peptide iRGD on the effect of paclitaxel-loaded MT1-AF7p-conjugated nanoparticles on glioma cells. *Biomaterials* **2013,** *34* (21), 5138-5148.
13. de Lucas, A. G.; Schuhmacher, A. J.; Oteo, M.; Romero, E.; Cámara, J. A.; de Martino, A.; Arroyo, A. G.; Morcillo, M. Á.; Squatrito, M.; Martinez-Torrecuadrada, J. L.; Mulero, F., Targeting MT1-MMP as an ImmunoPET-Based Strategy for Imaging Gliomas. *PLoS ONE* **2016,** *11* (7), e0158634.

14. Shimizu, Y.; Temma, T.; Hara, I.; Makino, A.; Kondo, N.; Ozeki, E.; Ono, M.; Saji, H., In vivo imaging of membrane type-1 matrix metalloproteinase with a novel activatable near-infrared fluorescence probe. *Cancer Sci* **2014,** *105* (8), 1056-62.
15. Albert, N. L.; Weller, M.; Suchorska, B.; Galldiks, N.; Soffietti, R.; Kim, M. M.; la Fougere, C.; Pope, W.; Law, I.; Arbizu, J.; Chamberlain, M. C.; Vogelbaum, M.; Ellingson, B. M.; Tonn, J. C., Response Assessment in Neuro-Oncology working group and European Association for Neuro-Oncology recommendations for the clinical use of PET imaging in gliomas. *Neuro Oncology* **2016,** *18* (9), 1199-1208.
16. Kondo, A.; Ishii, H.; Aoki, S.; Suzuki, M.; Nagasawa, H.; Kubota, K.; Minamimoto, R.; Arakawa, A.; Tominaga, M.; Arai, H., Phase IIa clinical study of [$^{18}$F]fluciclovine: efficacy and safety of a new PET tracer for brain tumors. *Annals of Nuclear Medicine* **2016,** *30* (9), 608-618.
17. Filss, C. P.; Galldiks, N.; Stoffels, G.; Sabel, M.; Wittsack, H. J.; Turowski, B.; Antoch, G.; Zhang, K.; Fink, G. R.; Coenen, H. H.; Shah, N. J.; Herzog, H.; Langen, K.-J., Comparison of $^{18}$F-FET PET and Perfusion-Weighted MR Imaging: A PET/MR Imaging Hybrid Study in Patients with Brain Tumors. *J. Nucl. Med.* **2014,** *55* (4), 540-545.
18. Piroth, M. D.; Holy, R.; Pinkawa, M.; Stoffels, G.; Kaiser, H. J.; Galldiks, N.; Herzog, H.; Coenen, H. H.; Eble, M. J.; Langen, K. J., Prognostic impact of postoperative, pre-irradiation $^{18}$F-fluoroethyl-l-tyrosine uptake in glioblastoma patients treated with radiochemotherapy. *Radiother. Oncol.* **2011,** *99* (2), 218-224.
19. la Fougère, C.; Suchorska, B.; Bartenstein, P.; Kreth, F.-W.; Tonn, J.-C., Molecular imaging of gliomas with PET: Opportunities and limitations. *Neuro Oncol* **2011,** *13* (8), 806-819.
20. Tsuyuguchi, N.; Takami, T.; Sunada, I.; Iwai, Y.; Yamanaka, K.; Tanaka, K.; Nishikawa, M.; Ohata, K.; Torii, K.; Morino, M.; Nishio, A.; Hara, M., Methionine positron emission tomography for differentiation of recurrent brain tumor and radiation necrosis after stereotactic radiosurgery —In malignant glioma—. *Annals of Nuclear Medicine* **2004,** *18* (4), 291-296.
21. Lodge, M. A.; Holdhoff, M.; Leal, J. P.; Bag, A. K.; Nabors, L. B.; Mintz, A.; Lesser, G. J.; Mankoff, D. A.; Desai, A. S.; Mountz, J. M.; Lieberman, F. S.; Fisher, J. D.; Desideri, S.; Ye, X.; Grossman, S. A.; Schiff, D.; Wahl, R. L., Repeatability of $^{18}$F-FLT PET in a multicenter study of patients with high-grade glioma. *J. Nucl. Med.* **2017,** *58* (3), 393-398.
22. Pafundi, D. H.; Laack, N. N.; Youland, R. S.; Parney, I. F.; Lowe, V. J.; Giannini, C.; Kemp, B. J.; Grams, M. P.; Morris, J. M.; Hoover, J. M.; Hu, L. S.; Sarkaria, J. N.; Brinkmann, D. H., Biopsy validation of $^{18}$F-DOPA PET and biodistribution in gliomas for neurosurgical planning and radiotherapy target delineation: Results of a prospective pilot study. *Neuro Oncol* **2013,** *15* (8), 1058-67.
23. Bangiyev, L.; Rossi Espagnet, M. C.; Young, R.; Shepherd, T.; Knopp, E.; Friedman, K.; Boada, F.; Fatterpekar, G. M., Adult Brain Tumor Imaging: State of the Art. *Semin. Roentgenol.* **2014,** *49* (1), 39-52.
24. Díez Valle, R.; Tejada Solis, S.; Idoate Gastearena, M. A.; García de Eulate, R.; Domínguez Echávarri, P.; Aristu Mendiroz, J., Surgery guided by 5-aminolevulinic fluorescence in glioblastoma: volumetric analysis of extent of resection in single-center experience. *J. Neurooncol.* **2011,** *102* (1), 105-113.
25. Schucht, P.; Beck, J.; Abu-Isa, J.; Andereggen, L.; Murek, M.; Seidel, K.; Stieglitz, L.; Raabe, A., Gross Total Resection Rates in Contemporary Glioblastoma Surgery: Results of an Institutional Protocol Combining 5-Aminolevulinic Acid Intraoperative Fluorescence Imaging and Brain Mapping. *Neurosurgery* **2012,** *71* (5), 927-936.

26. Stummer, W.; Stocker, S.; Wagner, S.; Stepp, H.; Fritsch, C.; Goetz, C.; Goetz, A. E.; Kiefmann, R.; Reulen, H. J., Intraoperative detection of malignant gliomas by 5-aminolevulinic acid-induced porphyrin fluorescence. *Neurosurgery* **1998,** *42* (3), 518-25; discussion 525-6.
27. Tonn, J. C.; Stummer, W., Fluorescence-guided resection of malignant gliomas using 5-aminolevulinic acid: practical use, risks, and pitfalls. *Clin Neurosurg* **2008,** *55*, 20-6.
28. FDA Briefing Information for the May 10, 2017 Meeting of the Medical Imaging Drugs Advisory Committee: NDA 208630 5-ALA (5-aminolevulinic acid HCl). https://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/MedicalImagingDrugsAdvisoryCommittee/ucm557135.htm (accessed May 24, 2017).
29. Liu, J. T.; Meza, D.; Sanai, N., Trends in fluorescence image-guided surgery for gliomas. *Neurosurgery* **2014,** *75* (1), 61-71.
30. Belykh, E.; Martirosyan, N. L.; Yagmurlu, K.; Miller, E. J.; Eschbacher, J. M.; Izadyyazdanabadi, M.; Bardonova, L. A.; Byvaltsev, V. A.; Nakaji, P.; Preul, M. C., Intraoperative Fluorescence Imaging for Personalized Brain Tumor Resection: Current State and Future Directions. *Frontiers in Surgery* **2016,** *3* (55).
31. Zhang, R. R.; Schroeder, A. B.; Grudzinski, J. J.; Rosenthal, E. L.; Warram, J. M.; Pinchuk, A. N.; Eliceiri, K. W.; Kuo, J. S.; Weichert, J. P., Beyond the margins: real-time detection of cancer using targeted fluorophores. *Nat Rev Clin Oncol* **2017,** *14* (6), 347-364.
32. Huang, R.; Vider, J.; Kovar, J. L.; Olive, D. M.; Mellinghoff, I. K.; Mayer-Kuckuk, P.; Kircher, M. F.; Blasberg, R. G., Integrin $\alpha_v\beta_3$-Targeted IRDye 800CW Near-Infrared Imaging of Glioblastoma. *Clin. Cancer Res.* **2012,** *18* (20), 5731-5740.
33. de Souza, A. L. R.; Marra, K.; Gunn, J.; Samkoe, K. S.; Hoopes, P. J.; Feldwisch, J.; Paulsen, K. D.; Pogue, B. W., Fluorescent Affibody Molecule Administered In Vivo at a Microdose Level Labels EGFR Expressing Glioma Tumor Regions. *Mol. Imaging Biol.* **2017,** *19* (1), 41-48.
34. Miller, S. E.; Tummers, W. S.; Teraphongphom, N.; van den Berg, N. S.; Hasan, A.; Ertsey, R. D.; Nagpal, S.; Recht, L. D.; Plowey, E. D.; Vogel, H.; Harsh, G. R.; Grant, G. A.; Li, G. H.; Rosenthal, E. L., First-in-human intraoperative near-infrared fluorescence imaging of glioblastoma using cetuximab-IRDye800. *J. Neurooncol.* **2018,** *139* (1), 135-143.
35. Cepeda, M. A.; Evered, C. L.; Pelling, J. J. H.; Damjanovski, S., Inhibition of MT1-MMP proteolytic function and ERK1/2 signalling influences cell migration and invasion through changes in MMP-2 and MMP-9 levels. *Journal of Cell Communication and Signaling* **2017,** *11* (2), 167-179.
36. Atkinson, J. M.; Falconer, R. A.; Edwards, D. R.; Pennington, C. J.; Siller, C. S.; Shnyder, S. D.; Bibby, M. C.; Patterson, L. H.; Loadman, P. M.; Gill, J. H., Development of a novel tumor-targeted vascular disrupting agent activated by membrane-type matrix metalloproteinases. *Cancer Res.* **2010,** *70* (17), 6902-6912.
37. Ansari, C.; Tikhomirov, G. A.; Hong, S. H.; Falconer, R. A.; Loadman, P. M.; Gill, J. H.; Castaneda, R.; Hazard, F. K.; Tong, L.; Lenkov, O. D.; Felsher, D. W.; Rao, J.; Daldrup-Link, H. E., Development of Novel Tumor-Targeted Theranostic Nanoparticles Activated by Membrane-Type Matrix Metalloproteinases for Combined Cancer Magnetic Resonance Imaging and Therapy. *Small* **2014,** *10* (3), 566-575.
38. Mohanty, S.; Chen, Z.; Li, K.; Morais, G. R.; Klockow, J.; Yerneni, K.; Pisani, L.; Chin, F. T.; Mitra, S.; Cheshier, S.; Chang, E.; Gambhir, S. S.; Rao, J.; Loadman, P. M.; Falconer, R. A.; Daldrup-Link, H. E., A novel theranostic strategy for MMP-14-expressing glioblastomas impacts survival. *Mol Cancer Ther* **2017,** *16* (9), 1909-1921.

39. Peng, X.; Chen, H.; Draney, D. R.; Volcheck, W.; Schutz-Geschwender, A.; Olive, D. M., A nonfluorescent, broad-range quencher dye for Förster resonance energy transfer assays. *Anal. Biochem.* **2009,** *388* (2), 220-228.
40. Simard, B.; Tomanek, B.; van Veggel, F. C.; Abulrob, A., Optimal dye-quencher pairs for the design of an "activatable" nanoprobe for optical imaging. *Photochem Photobiol Sci* **2013,** *12* (10), 1824-9.
41. Zhu, L.; Wang, H.; Wang, L.; Wang, Y.; Jiang, K.; Li, C.; Ma, Q.; Gao, S.; Wang, L.; Li, W.; Cai, M.; Wang, H.; Niu, G.; Lee, S.; Yang, W.; Fang, X.; Chen, X., High-affinity peptide against MT1-MMP for in vivo tumor imaging. *J. Controlled Release* **2011,** *150* (3), 248-255.
42. Kondo, N.; Temma, T.; Shimizu, Y.; Ono, M.; Saji, H., Radioiodinated Peptidic Imaging Probes for *in Vivo* Detection of Membrane Type-1 Matrix Metalloproteinase in Cancers. *Biological and Pharmaceutical Bulletin* **2015,** *38* (9), 1375-1382.
43. Min, K.; Ji, B.; Zhao, M.; Ji, T.; Chen, B.; Fang, X.; Ma, Q., Development of a Radiolabeled Peptide-Based Probe Targeting MT1-MMP for Breast Cancer Detection. *PLOS ONE* **2015,** *10* (10), e0139471.
44. Gill, J. H.; Loadman, P. M.; Shnyder, S. D.; Cooper, P.; Atkinson, J. M.; Ribeiro Morais, G.; Patterson, L. H.; Falconer, R. A., Tumor-targeted prodrug ICT2588 demonstrates therapeutic activity against solid tumors and reduced potential for cardiovascular toxicity. *Mol. Pharmaceutics* **2014,** *11* (4), 1294-1300.
45. Snyman, C.; Niesler, C. U., MMP-14 in skeletal muscle repair. *J Muscle Res Cell Motil* **2015,** *36* (3), 215-225.
46. Giannini, C.; Sarkaria, J. N.; Saito, A.; Uhm, J. H.; Galanis, E.; Carlson, B. L.; Schroeder, M. A.; James, C. D., Patient tumor EGFR and PDGFRA gene amplifications retained in an invasive intracranial xenograft model of glioblastoma multiforme. *Neuro Oncol* **2005,** *7* (2), 164-176.
47. Oliva, C. R.; Nozell, S. E.; Diers, A.; McClugage, S. G., 3rd; Sarkaria, J. N.; Markert, J. M.; Darley-Usmar, V. M.; Bailey, S. M.; Gillespie, G. Y.; Landar, A.; Griguer, C. E., Acquisition of temozolomide chemoresistance in gliomas leads to remodeling of mitochondrial electron transport chain. *J. Biol. Chem.* **2010,** *285* (51), 39759-39767.
48. Bourboulia, D.; Stetler-Stevenson, W. G., Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs): Positive and negative regulators in tumor cell adhesion. *Seminars in Cancer Biology* **2010,** *20* (3), 161-168.
49. Ulasov, I.; Yi, R.; Guo, D.; Sarvaiya, P.; Cobbs, C., The emerging role of MMP14 in brain tumorigenesis and future therapeutics. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer* **2014,** *1846* (1), 113-120.
50. Devy, L.; Huang, L.; Naa, L.; Yanamandra, N.; Pieters, H.; Frans, N.; Chang, E.; Tao, Q.; Vanhove, M.; Lejeune, A.; van Gool, R.; Sexton, D. J.; Kuang, G.; Rank, D.; Hogan, S.; Pazmany, C.; Ma, Y. L.; Schoonbroodt, S.; Nixon, A. E.; Ladner, R. C.; Hoet, R.; Henderikx, P.; TenHoor, C.; Rabbani, S. A.; Valentino, M. L.; Wood, C. R.; Dransfield, D. T., Selective inhibition of matrix metalloproteinase-14 blocks tumor growth, invasion, and angiogenesis. *Cancer Res.* **2009,** *69* (4), 1517-1526.
51. Markovic, D. S.; Vinnakota, K.; Chirasani, S.; Synowitz, M.; Raguet, H.; Stock, K.; Sliwa, M.; Lehmann, S.; Kälin, R.; van Rooijen, N.; Holmbeck, K.; Heppner, F. L.; Kiwit, J.; Matyash, V.; Lehnardt, S.; Kaminska, B.; Glass, R.; Kettenmann, H., Gliomas induce and exploit microglial MT1-MMP expression for tumor expansion. *Proc. Natl. Acad. Sci. U. S. A.* **2009,** *106* (30), 12530-12535.

52. Markovic, D. S.; Vinnakota, K.; van Rooijen, N.; Kiwit, J.; Synowitz, M.; Glass, R.; Kettenmann, H., Minocycline reduces glioma expansion and invasion by attenuating microglial MT1-MMP expression. *Brain, Behavior, and Immunity* **2011,** *25* (4), 624-628.
53. Charles, N. A.; Holland, E. C.; Gilbertson, R.; Glass, R.; Kettenmann, H., The brain tumor microenvironment. *Glia* **2011,** *59* (8), 1169-1180.
54. McGowan, P. M.; Duffy, M. J., Matrix metalloproteinase expression and outcome in patients with breast cancer: analysis of a published database. *Annals of Oncology* **2008,** *19* (9), 1566-1572.
55. Määttä, M.; Soini, Y.; Liakka, A.; Autio-Harmainen, H., Differential Expression of Matrix Metalloproteinase (MMP)-2, MMP-9, and Membrane Type 1-MMP in Hepatocellular and Pancreatic Adenocarcinoma: Implications for Tumor Progression and Clinical Prognosis. *Clin. Cancer Res.* **2000,** *6* (7), 2726-2734.
56. Hofmann, U. B.; Westphal, J. R.; Zendman, A. J.; Becker, J. C.; Ruiter, D. J.; van Muijen, G. N., Expression and activation of matrix metalloproteinase-2 (MMP-2) and its co-localization with membrane-type 1 matrix metalloproteinase (MT1-MMP) correlate with melanoma progression. *J Pathol* **2000,** *191* (3), 245-56.
57. Imanishi, Y.; Fujii, M.; Tokumaru, Y.; Tomita, T.; Kanke, M.; Kanzaki, J.; Kameyama, K.; Otani, Y.; Sato, H., Clinical significance of expression of membrane type 1 matrix metalloproteinase and matrix metalloproteinase-2 in human head and neck squamous cell carcinoma. *Human Pathology* **2000,** *31* (8), 895-904.
58. Huang, M.; Xiong, C.; Lu, W.; Zhang, R.; Zhou, M.; Huang, Q.; Weinberg, J.; Li, C., Dual-modality micro-positron emission tomography/computed tomography and near-infrared fluorescence imaging of EphB4 in orthotopic glioblastoma xenograft models. *Mol Imaging Biol* **2014,** *16* (1), 74-84.
59. Li, C.; Wang, W.; Wu, Q.; Ke, S.; Houston, J.; Sevick-Muraca, E.; Dong, L.; Chow, D.; Charnsangavej, C.; Gelovani, J. G., Dual optical and nuclear imaging in human melanoma xenografts using a single targeted imaging probe. *Nucl. Med. Biol.* **2006,** *33* (3), 349-358.
60. Sampath, L.; Kwon, S.; Ke, S.; Wang, W.; Schiff, R.; Mawad, M. E.; Sevick-Muraca, E. M., Dual-labeled trastuzumab-based imaging agent for the detection of human epidermal growth factor receptor 2 overexpression in breast cancer. *J. Nucl. Med.* **2007,** *48* (9), 1501-1510.
61. Kimura, R. H.; Miao, Z.; Cheng, Z.; Gambhir, S. S.; Cochran, J. R., A dual-labeled knottin peptide for PET and near-infrared fluorescence imaging of integrin expression in living subjects. *Bioconjugate Chem.* **2010,** *21* (3), 436-444.
62. Olson, E. S.; Jiang, T.; Aguilera, T. A.; Nguyen, Q. T.; Ellies, L. G.; Scadeng, M.; Tsien, R. Y., Activatable cell penetrating peptides linked to nanoparticles as dual probes for in vivo fluorescence and MR imaging of proteases. *Proc. Natl. Acad. Sci. U. S. A.* **2010,** *107* (9), 4311-4316.
63. Elliott, J. T.; Marra, K.; Evans, L. T.; Davis, S. C.; Samkoe, K. S.; Feldwisch, J.; Paulsen, K. D.; Roberts, D. W.; Pogue, B. W., Simultaneous *in vivo* fluorescent markers for perfusion, protoporphyrin metabolism, and EGFR expression for optically guided identification of orthotopic glioma. *Clin. Cancer Res.* **2017,** *23* (9), 2203-2212.
64. Zhu, L.; Zhang, F.; Ma, Y.; Liu, G.; Kim, K.; Fang, X.; Lee, S.; Chen, X., In vivo optical imaging of membrane-type matrix metalloproteinase (MT-MMP) activity. *Mol. Pharmaceutics* **2011,** *8* (6), 2331-2338.

65. Gao, S.; Zhang, L.; Wang, G.; Yang, K.; Chen, M.; Tian, R.; Ma, Q.; Zhu, L., Hybrid graphene/Au activatable theranostic agent for multimodalities imaging guided enhanced photothermal therapy. *Biomaterials* **2016,** *79*, 36-45.
66. Li, D.; Zhang, J.; Chi, C.; Xiao, X.; Wang, J.; Lang, L.; Ali, I.; Niu, G.; Zhang, L.; Tian, J.; Ji, N.; Zhu, Z.; Chen, X., First-in-human study of PET and optical dual-modality image-guided surgery in glioblastoma using $^{68}$Ga-IRDye800CW-BBN. *Theranostics* **2018,** *8* (9), 2508-2520.
67. Ribeiro de Souza, A. L.; Marra, K.; Gunn, J.; Samkoe, K. S.; Hull, S.; Paulsen, K. D.; Pogue, B. W., Optimizing glioma detection using an EGFR-targeted fluorescent affibody. *Photochem. Photobiol.* **2018,** *94* (6), 1167-1171.
68. Acerbi, F.; Broggi, M.; Schebesch, K.-M.; Höhne, J.; Cavallo, C.; De Laurentis, C.; Eoli, M.; Anghileri, E.; Servida, M.; Boffano, C.; Pollo, B.; Schiariti, M.; Visintini, S.; Montomoli, C.; Bosio, L.; La Corte, E.; Broggi, G.; Brawanski, A.; Ferroli, P., Fluorescein-guided surgery for resection of high-grade gliomas: A multicentric prospective phase II study (FLUOGLIO). *Clin. Cancer Res.* **2018,** *24* (1), 52-61.
69. Suchorska, B.; Jansen, N. L.; Linn, J.; Kretzschmar, H.; Janssen, H.; Eigenbrod, S.; Simon, M.; Pöpperl, G.; Kreth, F. W.; La Fougere, C.; Weller, M.; Tonn, J. C., Biological tumor volume in $^{18}$FET-PET before radiochemotherapy correlates with survival in GBM. *Neurology* **2015,** *84* (7), 710-719.
70. Verger, A.; Filss, C. P.; Lohmann, P.; Stoffels, G.; Sabel, M.; Wittsack, H. J.; Kops, E. R.; Galldiks, N.; Fink, G. R.; Shah, N. J.; Langen, K.-J., Comparison of $^{18}$F-FET PET and perfusion-weighted MRI for glioma grading: a hybrid PET/MR study. *Eur. J. Nucl. Med. Mol. Imaging* **2017,** *44* (13), 2257-2265.
71. Dunet, V.; Pomoni, A.; Hottinger, A.; Nicod-Lalonde, M.; Prior, J. O., Performance of $^{18}$F-FET versus $^{18}$F-FDG-PET for the diagnosis and grading of brain tumors: systematic review and meta-analysis. *Neuro Oncol* **2016,** *18* (3), 426-434.
72. Yoo, M. Y.; Paeng, J. C.; Cheon, G. J.; Lee, D. S.; Chung, J. K.; Kim, E. E.; Kang, K. W., Prognostic value of metabolic tumor volume on $^{11}$C-methionine PET in predicting progression-free survival in high-grade glioma. *Nuclear Medicine and Molecular Imaging* **2015,** *49* (4), 291-297.
73. Doi, Y.; Kanagawa, M.; Maya, Y.; Tanaka, A.; Oka, S.; Nakata, N.; Toyama, M.; Matsumoto, H.; Shirakami, Y., Evaluation of trans-1-amino-3-$^{18}$F-fluorocyclobutanecarboxylic acid accumulation in low-grade glioma in chemically induced rat models: PET and autoradiography compared with morphological images and histopathological findings. *Nucl. Med. Biol.* **2015,** *42* (8), 664-672.
74. Yang, Y.; Hernandez, R.; Rao, J.; Yin, L.; Qu, Y.; Wu, J.; England, C. G.; Graves, S. A.; Lewis, C. M.; Wang, P.; Meyerand, M. E.; Nickles, R. J.; Bian, X.-w.; Cai, W., Targeting CD146 with a $^{64}$Cu-labeled antibody enables in vivo immunoPET imaging of high-grade gliomas. *Proc. Natl. Acad. Sci. U. S. A.* **2015,** *112* (47), E6525-E6534.
75. Merrell, M. A.; Ilvesaro, J. M.; Lehtonen, N.; Sorsa, T.; Gehrs, B.; Rosenthal, E.; Chen, D.; Shackley, B.; Harris, K. W.; Selander, K. S., Toll-like receptor 9 agonists promote cellular invasion by increasing matrix metalloproteinase activity. *Mol. Cancer Res.* **2006,** *4* (7), 437-447.

# SUPPLEMENTARY MATERIAL

## Supplementary Materials and Methods

### Cell lines and cell culture

Human GBM cell lines and animal models were maintained and generated by the UAB Brain Tumor Animal Models Core Facility. D-54MG (D54 cells) and U-251MG (U251 cells) were gifts from Darell D. Bigner (Duke University, Durham NC), and U-87MG (U87 cells) was obtained from the American Tissue Type Collection (Manassas, VA). Cell lines were genetically modified with a luciferase-expressing construct (Addgene, Cambridge, MA). Cells were cultured as adherent monolayers in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum and prophylactic plasmocin. Experiments with cell lines were performed within 10-12 months of thawing frozen stocks. Cell line authentication analyses were not performed during the time of these studies. For *in vitro* NIRF activation studies, cells were seeded in 96-well plates at 5,000-40,000 cells/well 1-2 days before the experiment. For immunocytochemistry, Western blot, and gel zymography analyses, cells were seeded near confluency in 12-well or 6-well plates the day before the experiment.

### Western blot

Adherent monolayers of glioma cells were rinsed 2x with ice-cold PBS and lysed with RIPA buffer containing protease inhibitors on ice for 5 min. The supernatants were clarified by centrifugation at 4 °C, analyzed for protein concentration, aliquoted, and frozen at -20 °C until use. Cell lysates (20-25 μg) were separated by SDS-PAGE and transferred to a PVDF membrane. Membranes were blocked with 3% BSA in TBST, probed with primary antibodies overnight at 4 °C (1/2000 dilution for anti-MMP-14 mAb, Abcam ab51074; 1/1000 dilution for anti-MMP-2 antibody, Abcam ab37150; 1/2000 dilution for anti-β-actin antibody, Santa Cruz Biotechnology sc-47778), rinsed, probed with HRP-conjugated secondary antibody (Santa Cruz Biotechnology sc-2004, 1/1000 dilution), rinsed, and visualized by enzymatic chemical luminescence substrate (ECL) through exposure to X-ray film. Relative band intensities on the developed film were quantified by ImageJ. The experiment was performed in triplicate.

### Animal subjects and husbandry, and *in vivo* tumor xenografts

Animal studies were approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee (20366) and performed in compliance with guidelines from the Public Health Service Policy and Animal Welfare Act of the United States. 5-8 week old female athymic nude mice from Envigo (Indianapolis, IN) or from Charles River (Wilmington, MA) were used for all *in vivo* studies. Standard show and water was available *ad libitum.* All *i.v.* injections were performed *via* the lateral tail vein. For NIRF imaging of flank GBM xenografts, anesthetized mice were implanted *s.c.* in the right hind flank with 2 million D54 or U87 cells (n=5 mice per tumor type). NIRF imaging experiments with the MMP-14 peptide probes were performed 3-4 weeks later, when tumors were 5-8 mm in diameter. Orthotopic PDX GBM xenografts were generated as previously described [1]. Briefly, PDX JX12 tumors passaged in the flanks of athymic nude mice were harvested and dissociated into single cell suspensions. Anesthetized mice were implanted intracranially with 500,000 dissociated PDX tumor cells (5 μL methylcellulose suspension) 2 mm anterior and 1 mm lateral to the bregma at a depth of 2 mm over 2 min. Mice were monitored until fully recovered from anesthesia. Imaging and biodistribution studies were performed 13-17 days after PDX tumor cell implantation. Mice were

anesthetized with isoflurane during all live imaging procedures.

**General chemistry methods**

All chemicals were purchased from commercial sources (such as Aldrich, Synpeptide). Commercially available reagents were used without further purification, unless noted otherwise. All chemicals were reagent grade or better. The rink amide MBHA resin (100-200 mesh) was purchased from Millipore Sigma. The peptides were synthesized according to standard solid-phase peptide synthesis (SPPS); The NHS ester or maleimide coupling reactions were according to thermos fisher scientific coupling protocols. MALDI-MS spectrometric analyses were performed at the Mass Spectrometry Facility of Stanford University. HPLC was performed on a Dionex HPLC System (Dionex Corporation) equipped with a GP50 gradient pump and an in-line diode array UV-Vis detector. A reversed-phase (RP) C18 (Phenomenax, 5 μm, 4.6x250 mm, 5 μm, 10x250 mm or 21.2x250 mm) column was used for analysis and semi-preparative purification. UV absorbance of the probe was recorded on an Agilent 8453 UV spectrophotometer. Fluorescence was recorded on a Fluoromax-3 spectrafluorometer (Jobin Yvon). Unless noted, the compounds were analyzed by RP-HPLC (1 mL/min flow rate) or purified by semi-preparative RP-HPLC (3 mL/min flow rate), using water containing 0.1% trifluoroacetic acid (TFA) (solvent A) and acetonitrile containing 0.1% TFA (solvent B) as the eluates, or using 50 mM pH 5.5 triethylammonium acetate buffer (solvent C) and acetonitrile (solvent D) as the eluates, according to the following gradient: 0-21 min linear gradient from 0% B (or D) to 50% B (or D), 21-25 min linear gradient to 100% B (or D), 25-27 min hold 100% B (or D), 27-31 min linear gradient to 0% B (or D) and equilibrate in 0% B (or D).

*Synthesis of substrate peptide*: The peptide 1 (Figure S2) was synthesized using rink amide MBHA resin (100-200 mesh) according to standard SPPS methods. Peptide X (1.27 mg, 1 μmol, 1 eq), IRDye800CW-maleimide (1.19 mg, 1 μmol, 1 eq) and 0.5 mL of pH 7.4 PBS buffer were combined in a 1 mL vial and stirred at room temperature for 2 h. Then the quencher QC-1-NHS ester (1.24 mg, 1 μmol, 1 eq) was added to solution and stirred overnight. The reaction was purified directly by semi-preparative RP-HPLC using solvents C and D as eluates to afford light blue substrate-peptide 2 (1.1 mg, 32%). >99% purity was achieved after purification. MS: calcd. for $C_{160}H_{205}ClN_{23}O_{43}S_9$ [$(M+H)^+$]: 3459.17; found Maldi-Tof MS: m/z 3460.32 (Figure S5).

*Synthesis of binding peptide*: The peptide 2 (Figure S3) was synthesized using rink amide MBHA resin (100-200 mesh) according to standard SPPS methods. Peptide Y (1.9 mg, 1 μmol, 1 eq), NOTA-maleimide (0.85 mg, 2 μmol, 2 eq) and 0.5 mL of pH 7.4 PBS buffer were combined in a 1 mL vial and stirred at room temperature for 2 h. The reaction was purified directly by semi-preparative RP-HPLC using solvents A and B as eluates to afford white binding peptide 1 (1.65 mg, 71%). >99% purity was achieved after purification. MS: calcd. for $C_{105}H_{157}N_{34}O_{26}S$ [$(M+H)^+$]: 2342.16; found Maldi-Tof MS: m/z 2342.30 (Figure S6).

*Synthesis of substrate-binding peptide*: To a 1 mL glass vial was added 0.34 mg of substrate peptide (~0.1 μmol), 150 μL of pH 7.4 PBS buffer, 100 μL of *N,N*-dimethylformamide, 0.46 mg of binding peptide (~0.2 μmol), a premixed solution of 25 μL of 20 mM $CuSO_4$ and 50 μL of 50 mM THPTA (tris(3-hydroxypropyltriazolylmethyl)amine), 100 μL of 500 mM sodium ascorbate, and 100 μL of 500 mM aminoguanidine hydrochloride. The vial was capped tightly and stirred at room temperature for 1 h. Excess EDTA was added to the stirred solution and then the mixture

was purified directly by preparative RP-HPLC using solvents C and D as eluates to afford the substrate-binding peptide (0.31 mg, 54%). >99% purity was achieved after purification. MS: calcd. for $C_{265}H_{361}ClN_{57}O_{69}S_{10}$ [$(M+H)^+$]: 5800.33; found Maldi-Tof MS: m/z 5803.03 (Figure S7).

**Radiolabeling and radio HPLC analyses**

Radioactive RP-HPLC analyses were performed on an Agilent 200 liquid chromatography system outfitted with an Agilent Zorbax SB-C18 RP-HPLC column (3x250 mm, 5 µm particle size) and a matching guard cartridge (3x7 mm). Gradient elutions at a flow rate of 0.5 mL/min were performed with solvent A and solvent D according to the following method: 0-15 min linear gradient from 90% A/10% B to 60% A/40% B, 15-17 min linear gradient to 5% A/95% B, 17-20 min hold at 5% A/95% B, 20 min return to 90% A/10% B and equilibrate. UV-Vis was monitored at 254 and 775 nm. An attached radiodetector (model 105-S, Carroll & Ramsey Associates) was used to monitor elution of radioactive compounds.

*$^{68}$Ga-binding peptide*: 135 MBq $^{68}$Ga eluate (0.18 mL) in a screw-cap vial was adjusted to pH ~4.5 with 2 M NaOH and 1 M sodium acetate. To this solution (0.2 mL) was added 7.5 nmol binding peptide and the solution was heated at 90 °C for 20 min, after which it was cooled on ice and an aliquot was analyzed by radio RP-HPLC (Supplementary Figure S5). Aliquots of the crude radiolabeling reaction were diluted with PBS, or with PBS containing 50 nmol unlabeled binding peptide (block dose), to give 0.2 mL (2.2-4.5 MBq) per mouse dose. Molar activity at the time of dosing was approximately 6.1 GBq/µmol (non-blocked group).

*$^{64}$Cu-substrate-binding peptide*: 198 MBq $^{64}$Cu (7 µL), 0.5 M pH 6.0 sodium acetate buffer containing 1% gentisic acid (50 µL), acetonitrile (25 µL), and 5 nmol substrate-binding peptide were combined in a plastic microcentrifuge tube. After sitting at room temperature for 20 min, and aliquot was analyzed by radio RP-HPLC (Supplementary Figure S9). Nitrogen gas was blown into the reaction tube for 20 min to evaporate residual acetonitrile, and aliquots of the residual crude solution were diluted with PBS containing 4.3% EtOH, or with PBS containing 4.3% EtOH and 22 nmol unlabeled binding peptide (block dose), to give 0.2 mL (6.3-6.5 MBq) per mouse dose. Molar activity at time of dosing was approximately 46.6 GBq/µmol (non-blocked group).

## Supplementary Figures

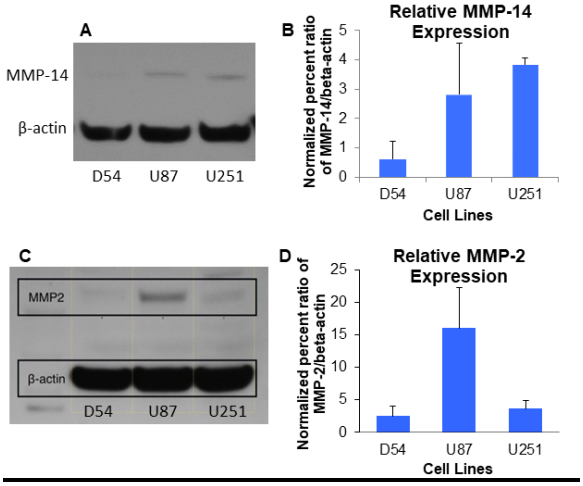



**Figure S1**. Western blots of MMP-14 (A) or MMP-2 (C) with quantification of respective blot intensities (B,D) from lysates of adherent GBM cells grown *in vitro* (average±SD, n=3/group).

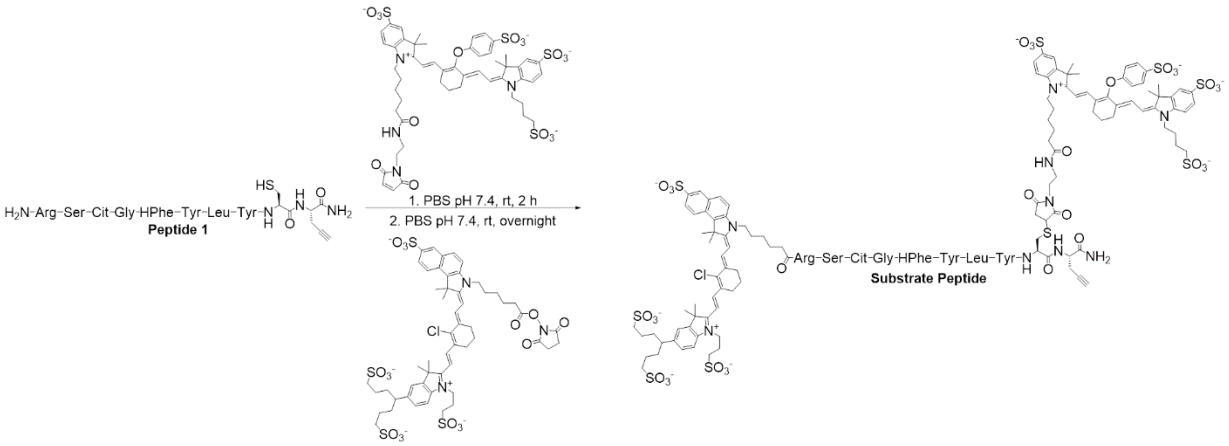



**Figure S2**. Synthetic scheme and structure of MMP-14 substrate peptide.

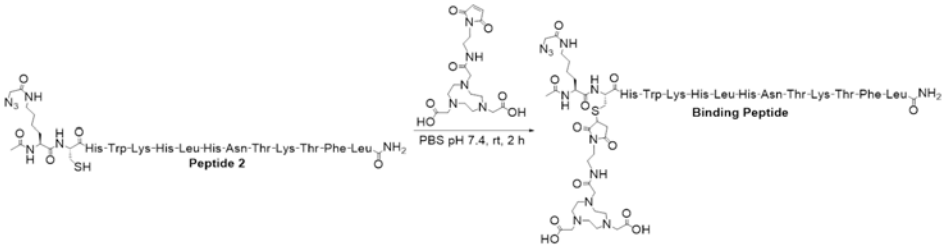



**Figure S3**. Synthetic scheme and structure of MMP-14 binding peptide.

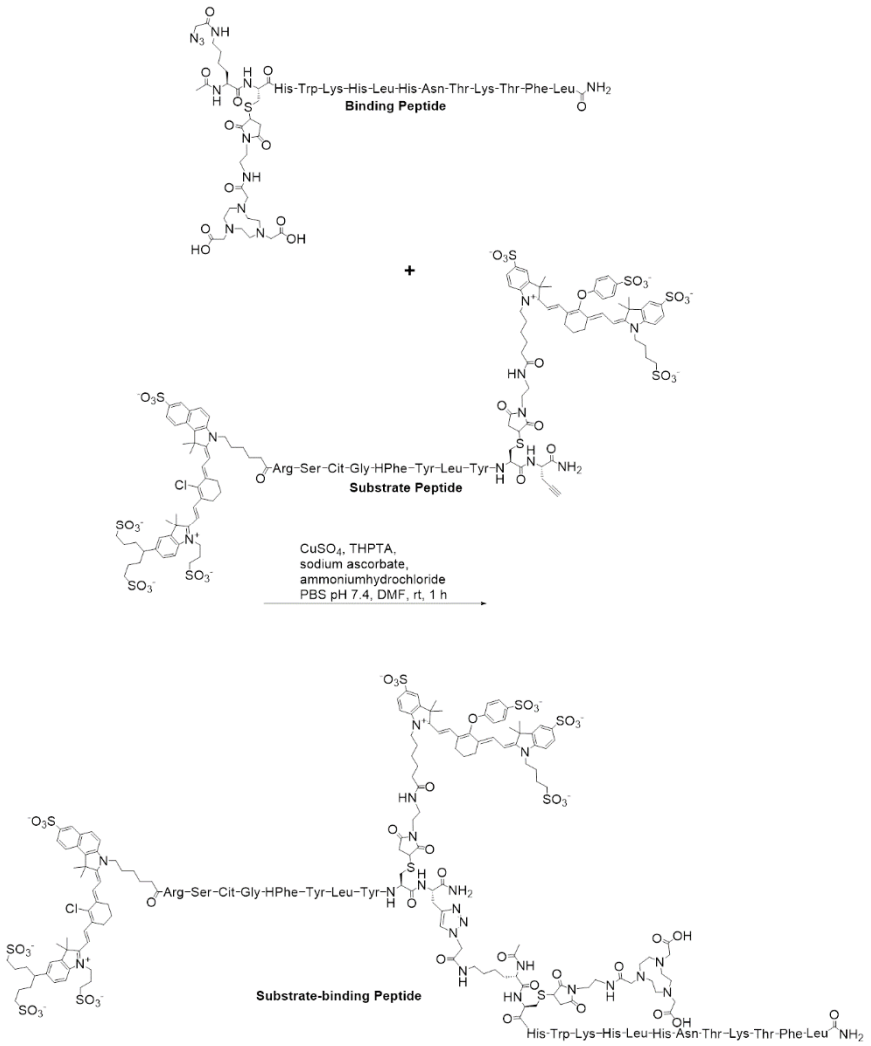
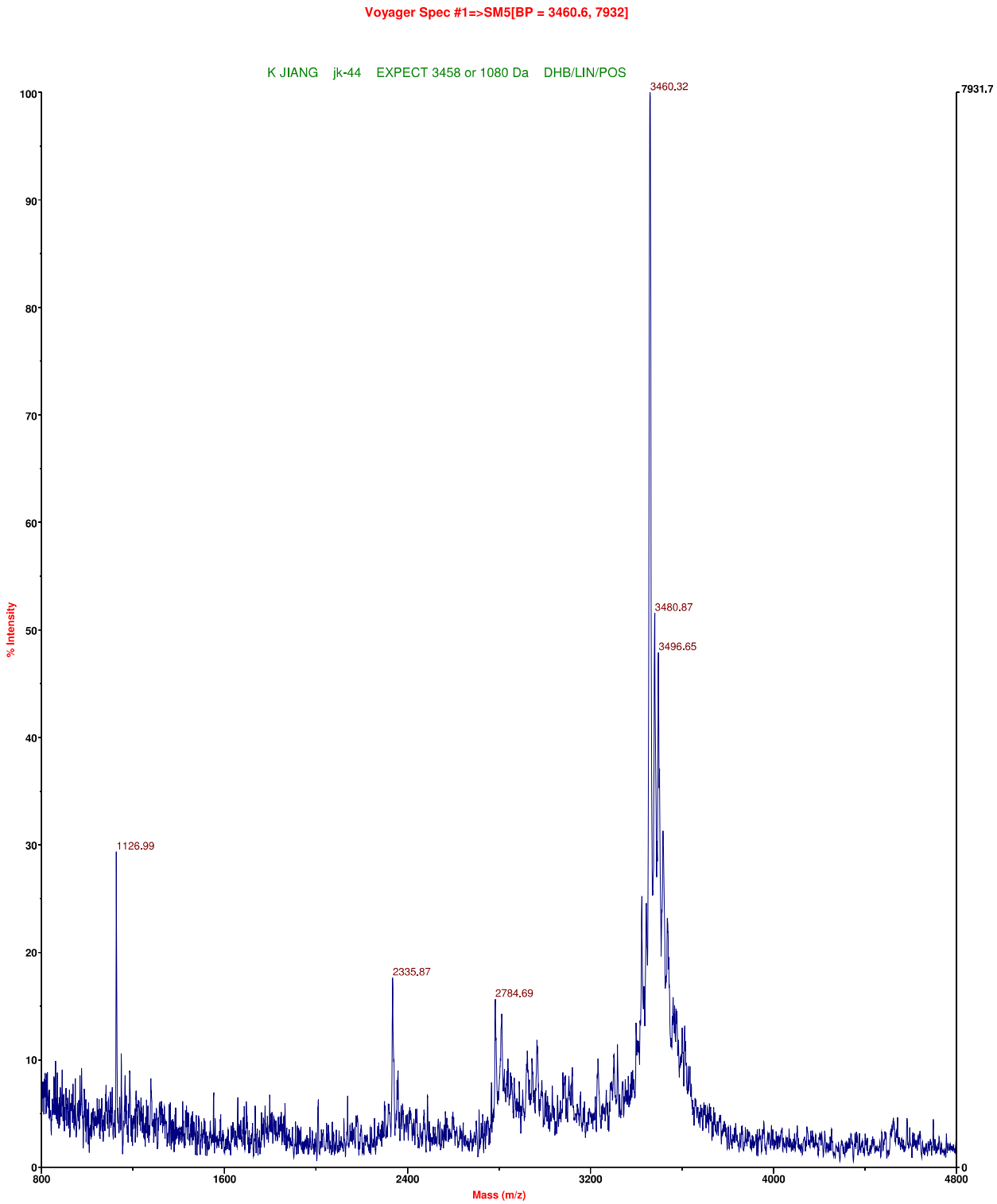
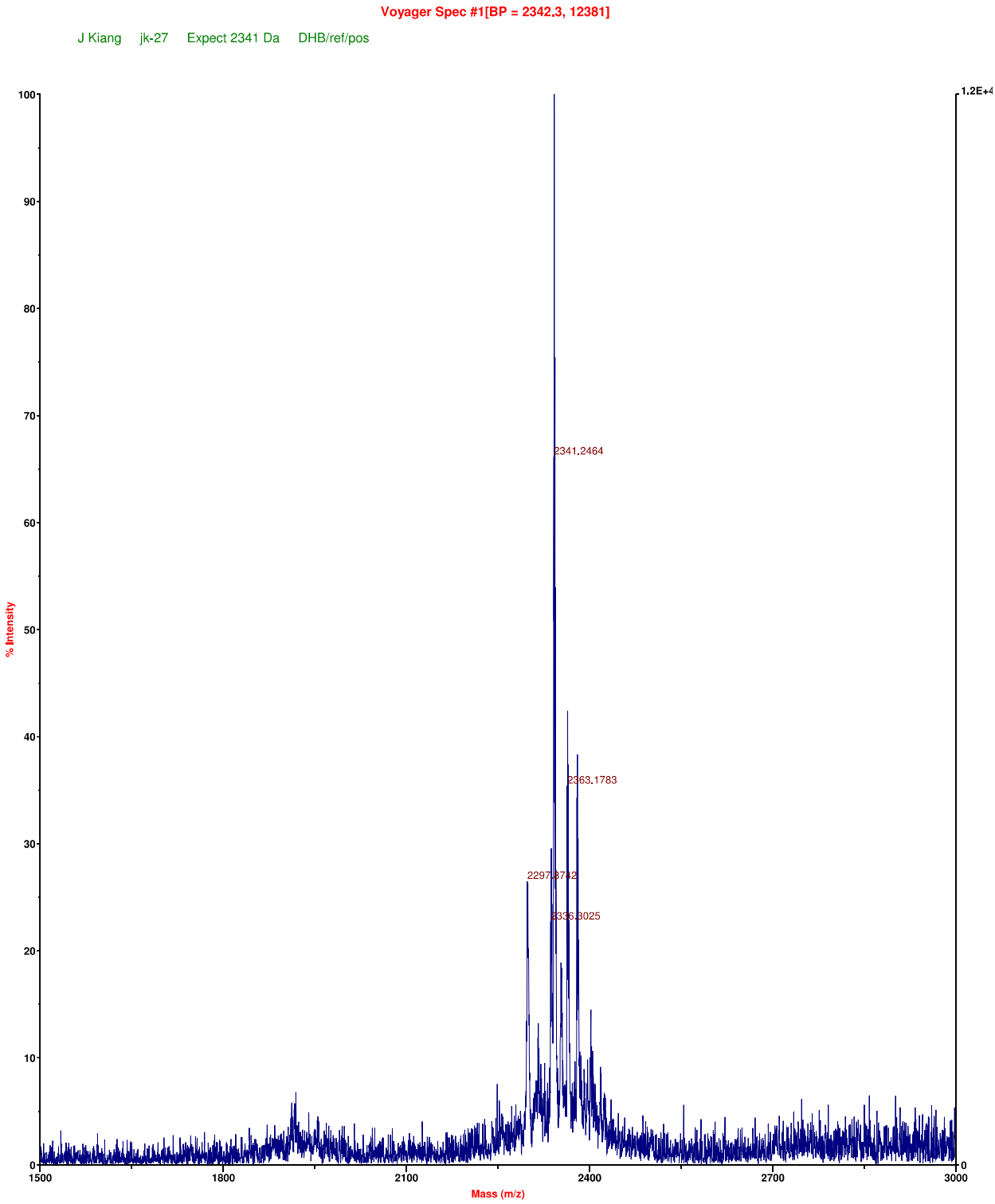



**Figure S4**. Synthetic scheme and structure of MMP-14 substrate-binding peptide.



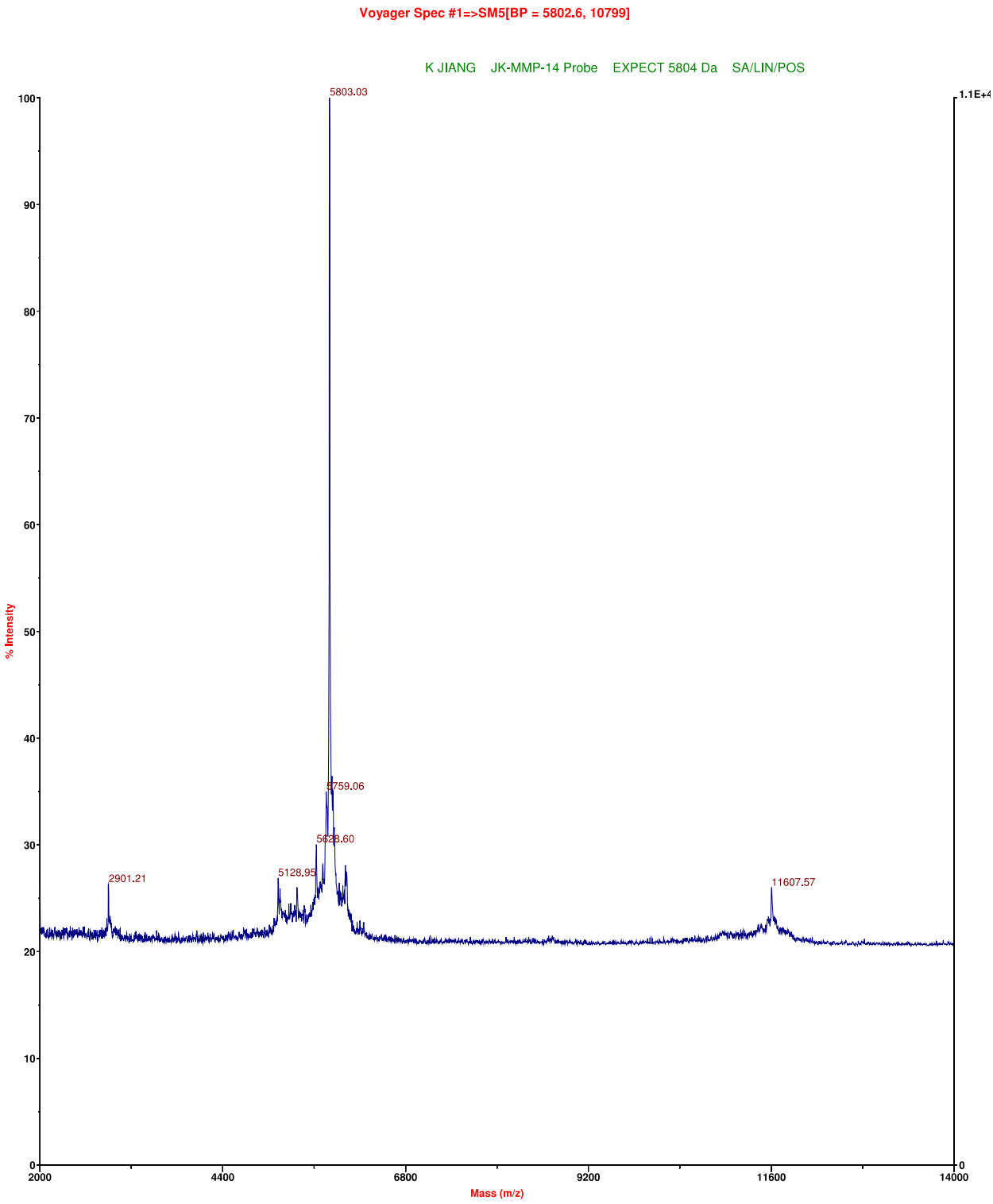

K JIANG jk-44 EXPECT 3458 or 1080 Da DHB/LIN/POS
C:\...\jk-44_0002.dat
Acquired: 14:57:00, February 13, 2018

**Figure S5**. Maldi-Tof MS spectrum of substrate peptide.




J Kiang jk-27 Expect 2341 Da DHB/ref/pos
C:\...\jk-27_0002.dat
Acquired: 12:57:00, July 14, 2016

**Figure S6**. Maldi-Tof MS spectrum of binding peptide.




K JIANG JK-MMP-14 Probe EXPECT 5804 Da SA/LIN/POS
C:\...\JK-MMP-14 Probe_0002.dat
Acquired: 13:54:00, March 06, 2018

**Figure S7**. Maldi-Tof MS spectrum of substrate-binding peptide.

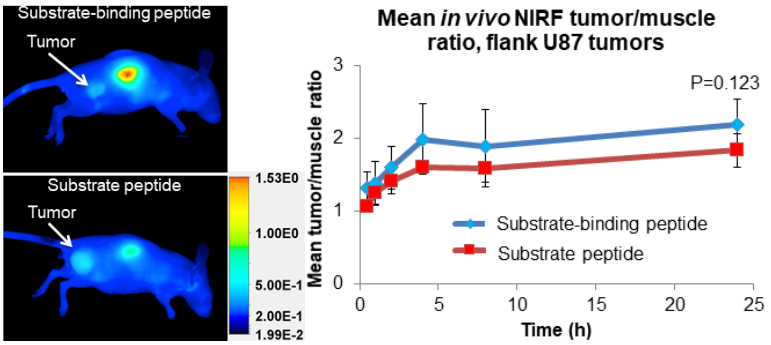



**Figure S8**. Mice bearing *s.c.*U87 xenografts were injected *i.v.* with 10 nmol of the substrate peptide or the substrate-binding peptide (n=5 mice/group) and imaged from 0-24 h on a Pearl imaging system (LI-COR). Left, representative *in vivo* NIRF images of mice at the 24 h time point after injection of the substrate-binding peptide (top) or the substrate peptide (bottom). Tumors are indicated by arrows. Right, mean *in vivo* NIRF tumor/muscle ratios at various time points (0.5-24 h) after injection of the substrate-binding peptide. No significant difference in NIRF tumor/muscle ratios between groups of mice was observed (p=0.123 at 24 h p.i.).

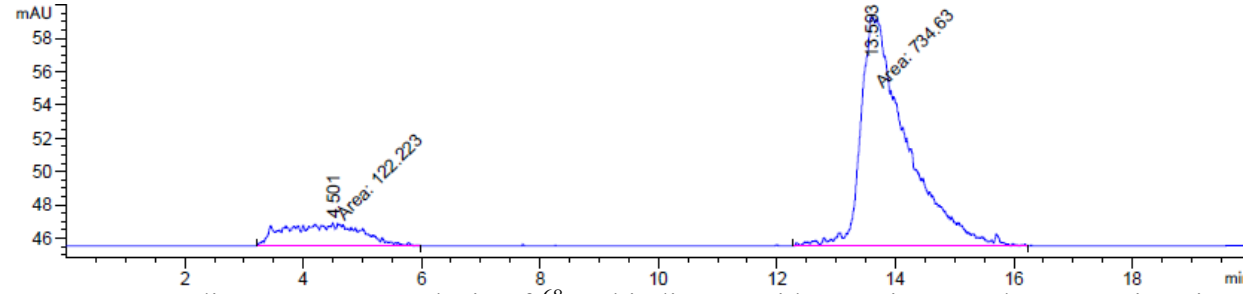



**Figure S9**. Radio RP-HPLC analysis of $^{68}$Ga-binding peptide reaction. Product retention time: 13.6 min. Retention time of the non-labeled binding peptide: 13.4 min.

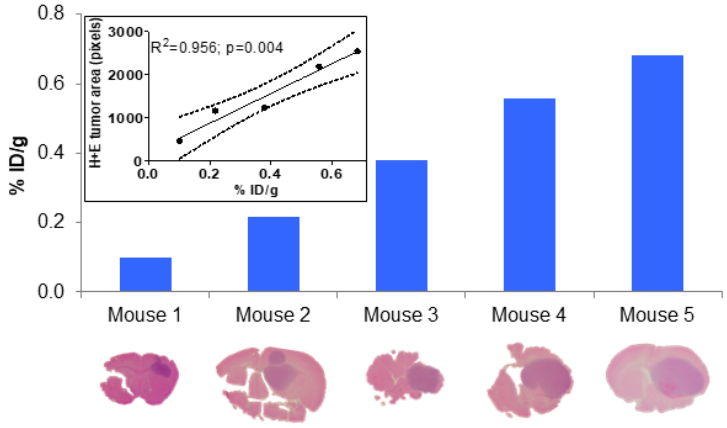



**Figure S10**. Correlation between the activity of $^{68}$Ga-binding peptide in brains of mice bearing orthotopic PDX JX12 GBM tumors and tumor size. Athymic nude mice (n=5) with orthotopic PDX JX12 GBM tumors were injected *i.v.* with the $^{68}$Ga-labeled binding peptide (4.4-9.6 MBq, 5.8 nmol peptide), imaged by PET/CT at 1 h, and euthanized for biodistribution analyses at 1.5 h *p.i.* The graph shows the % injected dose per gram (%ID/g) of radioactivity in individual mouse

brains after resection. Inset, correlation between %ID/g in resected brains and quantification of tumor area in H+E stained sections (5 µm) containing the widest tumor diameter (as shown in lower panels). Tumor area was quantified in ImageJ by calculating the pixel area from pictures of H+E stained sections.

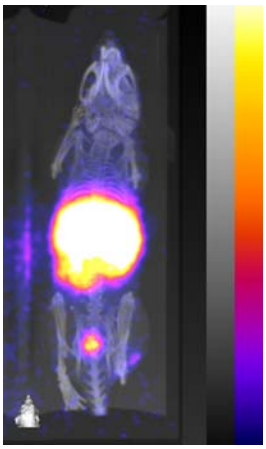


**Figure S11**. Representative maximum intensity projection PET/CT image of a mouse bearing an orthotopic PDX JX12 GBM tumor at 2 h after *i.v.* injection of $^{68}$Ga-binding peptide. (PET SUV scale 0.1 to 3.0)

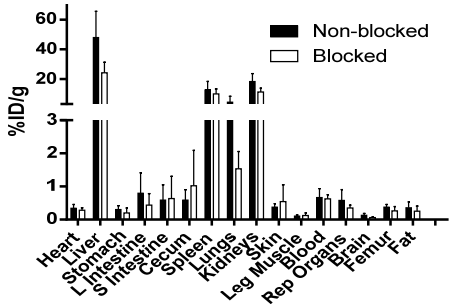



**Figure S12**. Biodistribution results (average±SD) of $^{68}$Ga-binding peptide from resected tissues of mice bearing orthotopic PDX JX12 GBM tumors. Mice (n=5 mice for non-blocked group, n=4 mice for blocked group) were euthanized at 3.5 h *p.i.* of $^{64}$Cu-substrate-binding peptide.

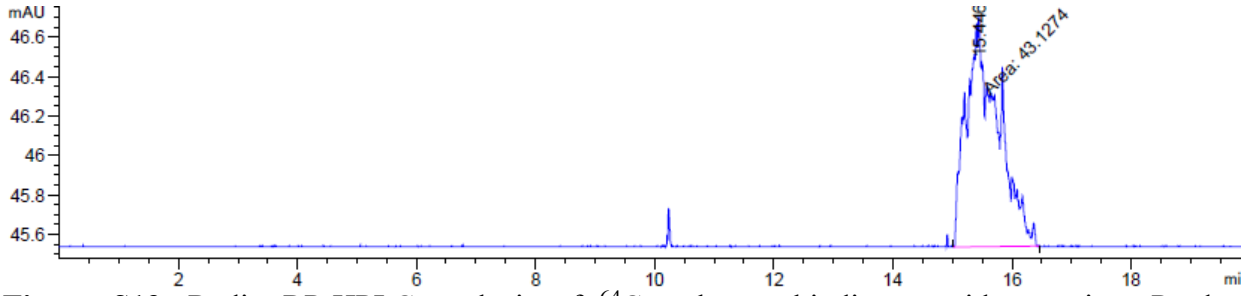



**Figure S13**. Radio RP-HPLC analysis of $^{64}$Cu-substrate-binding peptide reaction. Product retention time: 15.4 min. Retention time of the non-labeled substrate-binding peptide: 15.3 min.

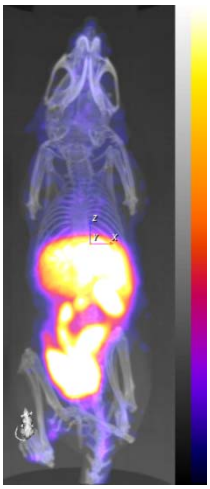


**Figure S14**. Representative maximum intensity projection PET/CT image of a mouse bearing an orthotopic PDX JX12 GBM tumor at 2 h after *i.v.* injection of $^{64}$Cu-substrate-binding peptide. (PET SUV scale 0.25 to 3.15)

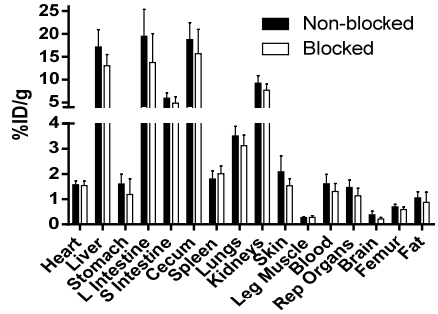



**Figure S15**. Biodistribution results (average±SD) of $^{64}$Cu-substrate-binding peptide from resected tissues of mice bearing orthotopic PDX JX12 GBM tumors. Mice (n=8 mice for non-blocked group, n=7 mice for blocked group) were euthanized at 5.5 h *p.i.* of $^{64}$Cu-substrate-binding peptide.

**Table S1.** *Ex vivo* NIRF signal (measured by the Odyssey scanner) metrics of positive predictive value, negative predictive value, sensitivity, and specificity from the substrate peptide or the substrate-binding peptide, relative to histologic verification of tumor (by H+E), measured in 5 µm sections of paraffin-embedded brain slices from mice bearing orthotopic PDX glioma tumors.

| | **Positive predictive value** | **Negative predictive value** | **Sensitivity** | **Specificity** |
|---|---|---|---|---|
| Substrate peptide, 4 h | 67.9% | 100.0% | 100.0% | 58.3% |
| Substrate peptide, 24 h | 50.0% | 91.7% | 83.3% | 73.3% |
| Substrate-binding peptide, 4 h | 85.7% | 50.0% | 85.7% | 100.0% |
| Substrate-binding peptide, 24 h | 83.3% | 100.0% | 100.0% | 60.0% |

**Supplementary References**

1. Warram, J. M.; de Boer, E.; Korb, M. L.; Hartman, Y. E.; Kovar, J.; Markert, J. M.; Gillespie, G. Y.; Rosenthal, E. L., Fluorescence-guided resection of experimental malignant glioma using cetuximab-IRDye 800CW. *British Journal of Neurosurgery* **2015,** *29* (6), 850-858.