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TITLE: MUC16 Juxtamembrane Domain-Binding Peptides for Imaging and Therapy of Ovarian Cancer

PRINCIPAL INVESTIGATOR: Dr. Steven Millward

CONTRACTING ORGANIZATION: M.D. Anderson Cancer Center, University of Texas
Houston, TX

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Ovarian cancer is the most lethal gynecological cancer, usually diagnosed at an advanced stage with poor survival. The lack of effective therapies for advanced ovarian cancer is an important barrier to improving women's health. A key protein that is expressed in the majority of ovarian cancer is called MUC16. A portion of MUC16, called CA-125, is released into the blood stream and is detected by a blood test commonly used to detect and monitor ovarian cancer. Previous attempts to target MUC16 for therapy have failed to produce positive results because they directed the drugs to CA-125. As a result, the CA-125 in the blood would capture the drug before it reaches the tumor. This proposal, in contrast, will instead target the portion of MUC16 that remains with the tumor after CA-125 is released. We will generate targeting agents that will home to the tumor without interacting with components in the blood stream. These agents will be attached to two radioactive substances that will be used to 1) treat the tumor directly and 2) verify that the therapy is working and detect any residual disease by PET imaging.

15. SUBJECT TERMS
None listed.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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1. INTRODUCTION:

The goal of the project was the selection and identification of peptide ligands with high affinity to the MUC16 juxtamembrane domain. During ovarian cancer tumorigenesis, the MUC16 (CA-125) ectodomain is often shed leaving a short juxtamembrane (JMD) peptide protruding into the extracellular space. This epitope represents an attractive target for targeted molecular imaging and radiotherapy of ovarian cancer.

2. KEYWORDS:

MUC16, ovarian cancer, directed evolution, mRNA Display, peptide, affibody, molecular imaging, targeted radiotherapy

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1: Directed evolution and characterization of MUC16 JMD-directed peptide ligands	**Timeline**	**% Complete**
Major Task 1: Directed evolution of MUC16 JMD peptide ligands using mRNA Display	Months	50%
Subtask 1 – Synthesis of MUC16 juxtamembrane domain (JMD) for use as a target for mRNA display.	1-2	100%
Subtask 2 – Affibody mRNA display selections against MUC16 JMD.	3-5	20%
Subtask 3 – Mirror-Image mRNA display selections against MUC16 JMD.	6-8	20%
Subtask 4 – Illumina sequencing and analysis of enriched peptide sequences.	9-10	0%
Milestone(s) Achieved: Generation of JMD-binding peptides for characterization.		0%
Major Task 2: Characterization of candidate peptides	Months	0%
Subtask 1 – Synthesis and fluorescent labeling of peptide candidates.	11	0%
Subtask 2 – Test protease stability of peptide candidates.	12	0%
Subtask 3 – Test JMD binding affinity of peptide candidates with fluorescence anisotropy and surface plasmon resonance.	12-13	0%

Subtask 4 – Determine binding affinity and specificity of peptide candidates for MUC16 expressing cells. Cell lines used: OVCAR-3 (human, sourced from Sigma-Aldrich), OVCAR-5 (human, sourced from Sigma-Aldrich). Experimental groups: OVCAR-3 cells (positive binding); OVCAR-5, MUC16 shRNA in OVCAR-3, anti-JMD antibody blocking (negative binding).	14-15	0%
Milestone(s) Achieved: Identification of lead peptides for in vivo models		0%
Specific Aim 2: Evaluation of MUC16 JMD-directed ligands in OVCAR-3 cell assays and xenograft models	**Timeline**	**% Complete**
Major Task 3: Cytotoxicity and uptake of radionuclide-peptide conjugates.	Months	0%
Subtask 1 – Yttrium-86 and Yttrium-90 radiolabeling of peptide candidates and anti-JMD antibody.	16	0%
Subtask 2 – Evaluate cellular binding of 86Y-peptide and 86Y-antibody. Cell lines used: OVCAR-3 (human, sourced from Sigma-Aldrich), OVCAR-5 (human, sourced from Sigma-Aldrich). Experimental groups: OVCAR-3 cells (high binding); OVCAR-5, excess unlabeled peptide or anti-JMD blocking antibody (low binding)	16-17	0%
Subtask 3 – Evaluate cytotoxicity of 90Y-peptide and 90Y-antibody Cell lines used: OVCAR-3 (human, sourced from Sigma-Aldrich), OVCAR-5 (human, sourced from Sigma-Aldrich). Experimental groups: OVCAR-3 cells (high cytotoxicity); OVCAR-5 cells, untreated OVCAR-3 cells, untreated OVCAR-5 cells, free yttrium-90 (low cytotoxicity)	16-17	0%
Milestone(s) Achieved: Verification of cell binding and cytotoxicity of ligands.		0%
Major Task 4: Treatment and PET imaging of mouse tumor xenografts models.	Months	10%
Subtask 1 – Submit documents for IACUC/ACURO approval.	12-17	100%
Subtask 2 – ACURO Approval	17	100%
Subtask 3 – Evaluate biodistribution of 86Y-peptides and 86Y-antibody in xenograft model. Mice strain: Athymic nude (Envigo/Harlan Labs) Implanted Cells: OVCAR-3 (human, sourced from Sigma-Aldrich), OVCAR-5 (human, sourced from Sigma-Aldrich). 5 mice per group x 4 groups = 20 total mice	17-18	0%
Subtask 4 – Determination of therapeutic efficacy of 90Y-peptides and 90Y-antibodies in xenograft model. Mice strain: Athymic nude (Envigo/Harlan Labs) Implanted Cells: OVCAR-3 (human, sourced from Sigma-Aldrich), OVCAR-5 (human, sourced from Sigma-Aldrich). 10 mice per group x 3 groups = 30 total mice	19-24	0%
Milestone(s) Achieved: Established therapeutic effect and target engagement of radiolabeled peptides and antibodies, publication of 1-2 peer reviewed papers.		0%

What was accomplished under these goals?

Major Task 1: Directed evolution of MUC16 JMD peptide ligands using mRNA Display

The first phase of the project (Specific Aim 1, Major Task 1) was concerned with the synthesis of the MUC16 JMD peptide and the selection of peptides that bind to the JMD using mRNA Display. This was expected to occupy the first 12 months of the project. We have overcome initial challenges in the synthesis and purification of the MUC16 JMD and are now preparing to start the selection experiments.

The MUC16 JMD peptide serves as the target for mRNA Display selections **(Figure 1A)**. As such, we set out to synthesize multi-milligram quantities of peptide which would be subsequently biotinylated for immobilization on streptavidin-agarose resin. Large quantities of peptide are also required for downstream validation studies.

We attempted to synthesize the MUC16 JMD peptide using automated solid-phase peptide synthesis (SPPS, standard FMOC chemistry, HBTU as the coupling agent). Following cleavage, deprotection, and RP-HPLC purification, we were able to obtain minute quantities (< 1mg) of the desired peptide as an impure mixture **(Figure 1B, C)**. We carried out two additional HPLC-based re-purifications yet were unable to achieve compound purities >50% **(Figure 1D)**. We attempted a larger scale automated synthesis which, unfortunately, failed to materially improve yield and purity.

The MUC16 JMD peptide is quite large (65 residues including the thrombin cleavage site, linker, and propargylglycine residue). Large peptides can be challenging to produce using SPPS due to chain aggregation on the solid support which slows coupling and deprotection kinetics. To address this, we modified our synthetic strategy to 1) include the addition of two pseudoproline monomers and 2) the use of a modified reaction/deprotection/wash solvent which has been shown to improve the yield of long peptides during SPPS **(Figure 2A)**. Pseudoproline residues consist of a dipeptide sequence in which one of the residues consists of a serine- or threonine-derived oxazolidine or a cysteine-derived thiazolidine. The oxazolidine/thiazolidine moiety mimics the structure of proline which can disrupt chain aggregation by distorting (or kinking) the peptide backbone during SPPS. The oxazolidine functionality is removed during TFA deprotection at the end of the synthesis. We chose to incorporate two pseudoproline derivatives (shown in red and blue in **Figure 2A)**. We also used a multi-component solvent system (DCM:DMF:NMP 1:1:1 with 1% Triton X-100 and 2M ethylenecarbonate) for coupling reactions and deprotections. This system (also called "Magic Solvent") has previously been shown to inhibit chain aggregation during SPPS. We carried out the modified synthesis as described above and purified the product by RP-HPLC **(Figure 2B,** red arrow**)**. As before, the desired product was accompanied by a contaminant product which could not be removed by subsequent re-purification **(Figure 2C)**. No significant yield was observed using the modified synthetic protocol.

Based on the results described above, we next sought to mediate chain aggregation effects by synthesizing fragments of the full-length MUC16 JMD peptide and assembling them into the final product by conventional HBTU-mediated amide coupling **(Figure 2D).** All five fragments were made successfully as fully side chain-protected peptides but could not be assembled into the full-length product. This was likely due to the large size of each fragment, challenges in purifying fully-protected fragments to homogeneity, and the slow kinetics of coupling on solid-phase resin.

Given the absence of an effective synthetic strategy, we elected to express the MUC16 JMD domain as a recombinant peptide in E. coli. The gene for MUC16 was inserted into the pET151/D-TOPO vector resulting in an expression construct with an N-terminal HisTag, tandem protease cleavage sites for tag removal and protease-mediated target elution, and a C-terminal cysteine for biotinylation with biotin-maleimide **(Figure 3A).** Expression in E. coli followed by Ni-NTA purification (imidazole elution) resulted in the expected ~12 kDa product **(Figure 3B)**. While this strategy was sufficient for the production of modest quantities of MUC16 JMD peptide, we observed significant product remaining in the insoluble pellet. To address this, we carried out lysis and purification under denaturing conditions (6M guanidinium or 8 M urea) and used acidic conditions, rather than imidazole, to elute the desired protein. As seen in **Figure 3C**, this resulted in significantly higher peptide yields (~10-20 mg/L) and obviated the need for imidazole removal. We now have more than sufficient MUC16 JMD peptide for mRNA Display selection and subsequent hit validation.

What opportunities for training and professional development has the project provided?

Nothing to Report

How were the results disseminated to communities of interest?

Nothing to report.

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

With the target MUC16 JMD peptide in-hand, we are setting up the mRNA display selections with the affibody library. This library has been validated for selection and has been successfully used to identify high-affinity ligands for the PD-L1 protein (Grindel, B.J. *et. al.* (2022) *ACS Chem. Biol.*). We anticipate that this selection will take up to two months and subsequent hit validation will take an additional 3-5 months. As we have optimized affibody expression and labeling, we expect to rapidly translate the most promising candidates into cell and animal model studies.

We expect to obtain a new CEM microwave-assisted peptide synthesizer as part of our institutional capital cycle in the next 6 months. Microwave-assisted synthesis has been routinely applied to the construction of long/difficult peptide sequences and we believe that this will greatly accelerate the production of synthetic MUC16 JMD as well as the all-D version for mirror-image display.

Known antibodies against the MUC16 JMD have proven challenging to obtain in sufficient quantities for the proposed experiments. As we now have the MUC16 JMD peptide target in large quantities, we have the option to explore the production of monoclonal and polyclonal antibodies against the target. These reagents would provide a positive control in validation experiments and potentially serve as stand-alone lead compounds for imaging and therapeutic applications.

4. IMPACT:

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

Nothing to Report

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

Our initial attempts to chemically synthesize the MUC16 JMD peptide resulted in insufficient compound yield and purity. We subsequently designed an E. coli expression vector for the JMD peptide and expressed it in high yields in E. coli (>10 mg/L). We now have sufficient recombinant peptide in-hand for selection and downstream characterization experiments.
Dr. Grindel was promoted to instructor on June 1, 2023 as a result of his outstanding work on mRNA Display and peptide evolution. Dr. Grindel continues to play a key role in the project, both on the biology side (target validation and evolution) as well as the chemistry side (peptide synthesis)."

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

Our initial approach was to chemically synthesize the MUC16 JMD (~56 amino acids) using solid-phase peptide synthesis (SPPS) methodology. Our initial attempts resulted in successful synthesis of the desired peptide albeit at low yield (<1%) and insufficient purity (<50% pure by analytical HPLC). Subsequent efforts to improve synthetic yield through the use of pseudoproline monomers, fragment ligation, and alternative solvents failed to materially improve upon these results. We suspect that the size of the MUC16 JMD peptide along with a run of arginine residues and beta-branched residues in the N-terminal region is the source of the difficulty. As such, we designed a plasmid containing the MUC16 JMD open reading frame (ORF) and used this to express the peptide in E. coli in high yield (>10 mg/L). We now have sufficient peptide for selection and downstream characterization experiments and are initiating the first round of the directed evolution experiment.

Changes that had a significant impact on expenditures

Ms. Iva Vucic left the institution to pursue her Ph.D. We have been actively recruiting a substitute for her but our efforts have been hindered by the extraordinarily tight labor market and the aftermath of the COVID19 pandemic.

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

Not applicable, nothing to report.

Significant changes in use or care of vertebrate animals

Not applicable, 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.

Nothing to report.

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

Nothing to report.

Other publications, conference papers and presentations.

Nothing to report.

- **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:	Steven Millward
Project Role:	PD/PI
Researcher Identifier (e.g. ORCID ID):	0000-0002-3231-7075
Nearest person month worked:	1.20
Contribution to Project:	Dr. Millward is responsible for the management of the project, experimental design, and data analysis.
Funding Support:	Please see attached Other Support Document.
Name:	Brian Grindel
Project Role:	Instructor, key-personnel
Researcher Identifier (e.g. ORCID ID):	0000-0001-6564-119X
Nearest person month worked:	2.40
Contribution to Project:	Dr. Grindel oversees the setup of expression and selection experiments and provides guidance on peptide synthesis. Dr. Grindel also provides expert assistance on data analysis and experimental troubleshooting.
Funding Support:	Please see attached Other Support Document.

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

Nothing to report

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES:

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Brian John Grindel, Ph.D.

eRA COMMONS USER NAME (credential, e.g., agency login): BRIANGRINDEL

POSITION TITLE: Instructor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Delaware; Newark, Delaware	B.A.	05/2008	Biological Sciences
Rice University; Houston, Texas	Ph.D.	05/2015	Biochemistry & Cell Biology
Rice University / The University of Texas Health Science Center; Houston, Texas	Postdoctoral Fellow	04/2017	Biochemistry / Post-Doctoral Training
The University of Texas MD Anderson Cancer Center; Houston, Texas	Postdoctoral Fellow	04/2022	Biochemistry / Post-Doctoral Training

A. Personal Statement

As an undergraduate Goldwater and Howard Hughes Medical Institute scholar I studied an unusual oncogene mechanism in hepatocellular carcinoma. I dedicated graduate school to studying the bidirectional influence of fibrotic extracellular matrix components on prostate cancer. As well, I helped develop a new diagnostic assay for prostate cancer relapse assessing fragmentation of a heparan sulfate proteoglycan, perlecan, during a collaboration with a biotechnology company. Through this work I learned complex protein synthesis and purification. During my postdoctoral training I expanded my expertise under support of the Translational Cancer Nanotechnology T32 fellowship. During this time, I improved directed evolution by streamlining protein target input systems in mRNA display. I also developed a new scaffold for mRNA display to dual select against mouse and human PD-L1. This scaffold, the affibody, will be used in the current project. These projects expanded my skillset into organic chemistry, radiochemistry, and mouse imaging techniques (PET/CT, MRI, IVIS). During my tenure as a research scientist and now instructor I have expanded to selection against autophagy targets (LC3a, Beclin1) to treat ovarian cancer with cyclic SUPR peptides. As well, I'm helping direct a new study leveraging PTEN collateral lethality to treat ENO1 deleted tumors in glioblastoma with new pro-drugs in mice. I am currently expanding the PD-L1 targeting project by incorporating split-luciferase reporters to track dimerization in live cells. The variety and depth of projects I've worked on over the years give me an encompassing picture of both basic and translational cancer science to advance the goal of targeted cancer treatment and imaging. I'll assist and manage the entire process in regards to protein purification, affibody mRNA display, and post screen validation.

B. Positions, Scientific Appointments, and Honors

Positions

2023 – Present	Instructor, Cancer Systems Imaging, The University of Texas MD Anderson Cancer Center; Houston, Texas
2022 – 2023	Research Scientist, Cancer Systems Imaging, The University of Texas MD Anderson Cancer Center; Houston, Texas

Honors

2021	Best Short Presentation at Departmental Trainee Symposium
2020	Postdoctoral Travel Award Recipient
2018	Best Poster Presentation at NCI Alliance for Nanotechnology in Cancer, 2018 PI Meeting
2017	Translational Cancer Nanotechnology Training Fellowship
2015	George J. Schroepfer, Jr. Award for Outstanding Ph.D. Thesis in Biochemistry & Cell Biology
2014	Best Presentation at Biochemistry and Cell Biology Department Retreat
2013	Most Innovative Research at 40th Anniversary Biochemistry and Cell Biology Department Poster Session
2013	Department of Biochemistry and Cell Biology Travel Award
2010	Nominated for Lindau Award by Rice University Biochemistry and Cell Biology Department
2008	Chemistry / Biology Interface Fellowship
2008	Biological Sciences Academic Achievement Award Recipient; Biological Sciences student with highest GPA
2008	Richard M. Johnson Jr. Memorial Award Recipient; Outstanding Junior in the Biological Sciences Department
2007	Barry Goldwater Scholarship and Excellence in Education Award Recipient
2007	Inducted into Phi Beta Kappa Society; National Honors Society Member
2006	Howard Hughes Medical Institute Scholar Research Stipend Recipient
2005	Howard Hughes Medical Institute Scholar Research Stipend Recipient

C. Contributions to Science

My current lab focuses on directed evolution, a technique designed to select peptides or protein scaffolds for a specific function. We specialize in mRNA display, a powerful *in vitro* directed evolution approach to produce novel peptide/protein-based drugs and imaging agents. mRNA display allows selection from >10^{15} cyclic peptides where unnatural amino acids can be incorporated and pre-selected for serum stability (SUPR peptides: Scanning Unnatural Protease Resistant). I was involved in technical papers to improve the mRNA display process and introduce a new scaffold in the context of mRNA display, the affibody.

Grindel B.J., Engel B.J., Hall C.G., Kelderhouse L.E., Lucci A., Zacharias N.M., Takahashi T.T., Millward S.W. Mammalian Expression and In Situ Biotinylation of Extracellular Protein Targets for Directed Evolution. *ACS Omega*. 2020 Sep 22;5(39):25440-25455.

Engel B.J., **Grindel B.J.**, Gray J.P., Millward S.W. Purification of poly-dA oligonucleotides and mRNA-protein fusions with dT25-OAS resin. Bioorg Med Chem Lett. 2020 Feb 15;30(4):126934.

Kamalinia, Golnaz; **Grindel, B.J.**; Takahashi, Terry; Millward, Steven; Roberts, Richard W. Directing Evolution of Novel Ligands by mRNA Display. (2021) *Chemical Society Reviews*

PD-L1, an immune checkpoint protein, was targeted with mRNA display by myself and collaborators. We developed a linear peptide, SPAM, to bind human PD-L1. This work was continued into an affinity improvement screen producing the 7.7 peptide which binds to PD-L1 with pM affinity. Interestingly, this new reagent only binds to glycosylated PD-L1. I decided to do the first affibody selection with mRNA display targeting both mouse and human PD-L1. The low nM affibody binder M1 was dual specific, blocked PD-1 engagement, and bound to tumors *in vivo*.

Kamalinia G., Engel B.J, Srinivasamani A., **Grindel B.J.**, Ong J.N., Curran M.A., Takahashi T.T., Millward S.W., Roberts R.W. mRNA Display Discovery of a Novel Programmed Death Ligand 1 (PD-L1) Binding Peptide (a Peptide Ligand for PD-L1). ACS Chem Biol. 2020 Jun 19;15(6):1630-1641.

Grindel BJ, Engel BJ, Ong JN, Srinivasamani A, Liang X, Zacharias NM, Bast RC Jr, Curran MA, Takahashi TT, Roberts RW, Millward SW (2022). Directed Evolution of PD-L1-Targeted Affibodies by mRNA Display. *ACS Chem Biol.*
"PD-L1 BINDING PEPTIDES" U.S. Provisional Patent Application No. 63/017,360, filed April 29, 2021

Autophagy is co-opted by cancer to evade chemotherapeutic treatment. Our lab developed an mRNA display driven small cyclic SUPR peptide to inhibit autophagy. The cyclic peptide was able to enter cells and inhibit autophagy in ovarian cancer models to counteract cisplatin treatment evasion. I'm continuing this work to other autophagy proteins like Beclin-1. We are also trying to produce a higher affinity LC3a binder with a 10-mer cyclic peptide library.

Gray, J.P., Uddin, M.N., Chaudhari, R., Sutton, M.N., Yang, H., Rask, P., Locke, H., Engel, B.J., Batistatou, N., Wang, J. and **Grindel, B.J.**, et al. (2021) Directed evolution of cyclic peptides for inhibition of autophagy. *Chemical Science*.

Perlecan is a vital heparan sulfate proteoglycan in the extracellular environment impacting development, angiogenesis, growth, and differentiation. This work focused on determining the functions of perlecan in chondrocyte and osteocyte development and exploiting its properties in 3D culture to regenerate bone. Furthermore, basic biochemical/biophysical characterization of perlecan was carried out to illuminate its functions in bone biology.

Wijeratne, S. S., Martinez, J. R., **Grindel, B. J.**, Frey, E. W., Li, J., Wang, L., Farach-Carson, M.C., et al. (2015). Single molecule force measurements of perlecan/HSPG2: A key component of the osteocyte pericellular matrix. *Matrix Biol*. **50**, 27-38.

Thompson, W. R., Modla, S., **Grindel, B. J.**, Czymmek, K. J., Kirn-Safran, C. B., Wang, L., Duncan, R. L., and Farach-Carson, M. C. (2010) Perlecan/Hspg2 deficiency alters the pericellular space of the lacuno-canalicular system surrounding osteocytic processes in cortical bone. *J Bone Min. Res*.

Chiu, Y-C, Fong, E.L., **Grindel, B.J**., Kasper, F., Harrington, D.A., Farach-Carson, M.C. (2016) Sustained delivery of recombinant human bone morphogenetic protein-2 from perlecan domain I – functionalized electrospun poly(ε-caprolactone) scaffolds for bone regeneration. *Journal of Experimental Orthopaedics*, 3:25.

Martinez, J. R., **Grindel, B. J**., Hubka, K. M., Dodge, G. R. & Farach-Carson, M. C. (2018) Perlecan/HSPG2: Signaling role of domain IV in chondrocyte clustering with implications for Schwartz-Jampel Syndrome. *J. Cell. Biochem*. doi:10.1002/jcb.27521

Perlecan is a large, complex molecule with paradoxical functions in both promoting and restraining prostate cancer. I found that not only is the tissue expression of perlecan in prostate cancer progression important but more informative is its post-translational context. I discovered that matrix metalloproteinase (MMP-7) mediated fragmentation switches perlecan from an indolent actor in cancer to cancer metastasis promoting. Ultimately, I pieced together a new mechanism whereby perlecan binds and interacts with semaphorin/plexin receptors to induce a cancer repulsive phenotype. Even limited cleavage of perlecan by MMP-7 reverses this phenotype to become invasive. As well, we could link perlecan fragments in patient serum to MMP-7 expression in tissue using a novel high throughput assay developed during a collaborative internship with the biotechnology firm Strategic Diagnostics.

Grindel, B.J., Arnold, B., Li, Q., Zayzafoon, M., Muldoon, M., Farach-Carson, M.C. (2016) Perlecan/HSPG2 and Matrilysin/MMP-7 as Indices of Tissue Invasion: Tissue Localization and Circulating Perlecan Fragments in a Cohort of 288 Radical Prostatectomy Patients. *Oncotarget*. 7(9): 10433-10447.

Grindel, B. J., Martinez, J. R., Pennington, C. L., Muldoon, M., Stave, J., Chung, L. W., and Farach-Carson, M. C. (2014) Matrilysin/matrix metalloproteinase-7(MMP7) cleavage of perlecan/HSPG2 creates a molecular switch to alter prostate cancer cell behavior. *Matrix Biol.* **36**, 64–76.

Warren, C. R., **Grindel, B. J.**, Francis, L., Carson, D. D., and Farach-Carson, M. C. (2014) Transcriptional Activation by NFκB Increases Perlecan/HSPG2 Expression in the Desmoplastic Prostate Tumor Microenvironment. *J. Cell. Biochem.* **115**, 1322–33.

Farach-Carson MC, **Grindel, B.J.** (2009). "HSPG2 (heparan sulfate proteoglycan 2)." *Atlas Genet. Cytogenet. Oncol. Haematol*.; 13 (9): 1039-1049.

Grindel, B. J., Martinez, J.R., Tellman, T.V., Harrington, D.A., Zafar, H., Nakhleh, L., Chung, L.W., Farach-Carson, M.C. (2018) Matrilysin/MMP-7 Cleavage of Perlecan/HSPG2 Complexed with Semaphorin 3A Supports FAK-Mediated Stromal Invasion by Prostate Cancer Cells. *Sci. Rep*. 8.

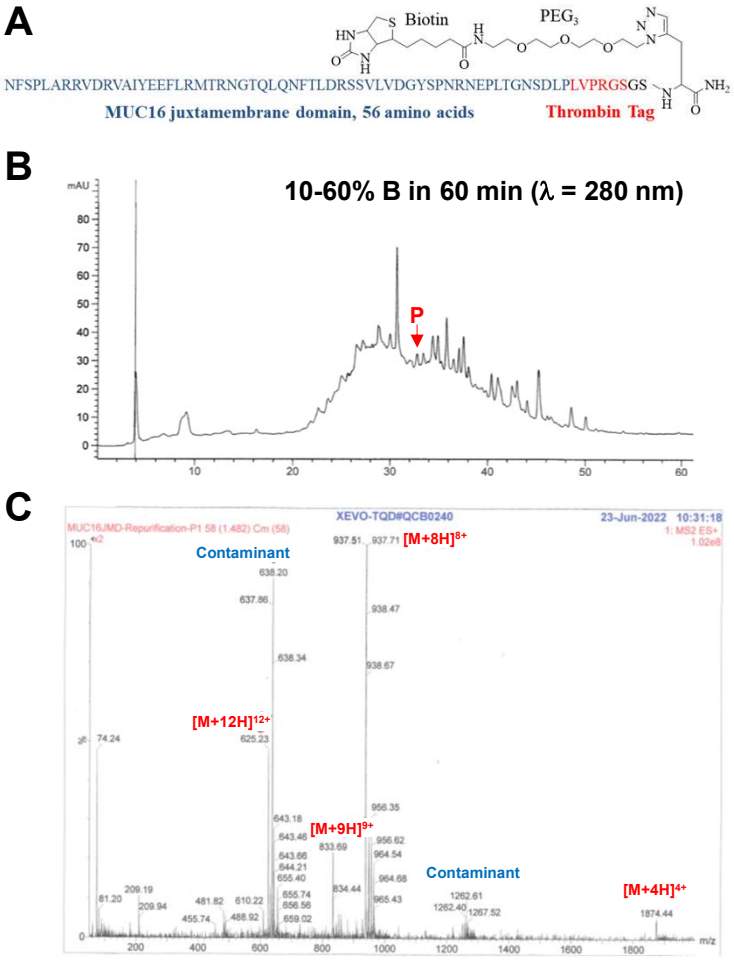
Tellman T.V., Cruz L.A., **Grindel B.J**., Farach-Carson M.C. Cleavage of the Perlecan-Semaphorin 3A-Plexin A1-Neuropilin-1 (PSPN) Complex by Matrix Metalloproteinase 7/Matrilysin Triggers Prostate Cancer Cell Dyscohesion and Migration. Int J Mol Sci. 2021 Mar 22;22(6):3218

My undergraduate work explored a novel function of a normally endoplasmic reticulum resident (ER) protein, ERp57, both in chondrocyte/bone responses to vitamin D_3 and TNF-α in hepatic carcinogenesis. I found ERp57 escaped ER retention due to a weak signal sequence and translocated into the nucleus using its putative bipartite nuclear localization sequence.

Chen, J., Lobachev, K. S., **Grindel, B. J.**, Farach-Carson, M. C., Hyzy, S. L., El-Baradie, K. B., Olivares-Navarrete, R., Doroudi, M., Boyan, B. D., and Schwartz, Z. (2013) Chaperone properties of pdia3 participate in rapid membrane actions of 1α,25-dihydroxyvitamin d3. *Mol. Endocrinol.* **27**, 1065–77.

Grindel, B. J., Rohe, B., Safford, S. E., Bennett, J. J., and Farach-Carson, M. C. (2011) Tumor necrosis factor-α treatment of HepG2 cells mobilizes a cytoplasmic pool of ERp57/1,25D_3-MARRS to the nucleus. *J. Cell. Biochem.* **112**, 2606–15.

Undergraduate Senior Thesis: Regulation and expression of ERp57 in hepatocellular carcinoma



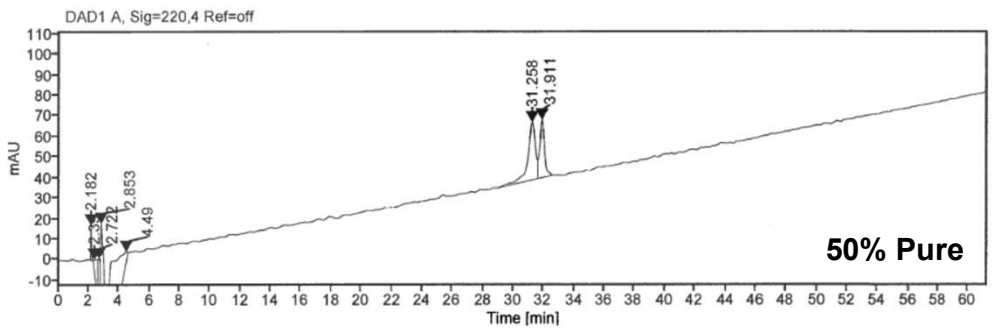


Figure 1. Initial Synthesis and Purification of MUC16 JMD. A) Sequence of the MUC16 JMD peptide. The C-terminal thrombin site is shown in red. Biotinylation is effected by conjugation of Biotin-PEG--azide to the C-terminal propargylglcine side chain. **B)** The MUC16 JMD peptide was assembled by solid-phase synthesis (automated synthesizer), deprotected in TFA, and purified by RP-HPLC. The desired product (P) was collected and re-purified using a gradient of 20-50% B in 60 min. **C)** LC/MS of the re-purified MUC16 JMD showed adduct peaks corresponding to the desired product (red) as well as a set of adduct peaks corresponding to a contaminant product (blue). Subsequent analysis of the re-purified product by analytical RP-HPLC confirmed the presence of two partially resolved products. A second re-purification using a gradient of 30-40% B in 60 minutes yielded no fundamental improvement in purity. The final yield after three purifications was < 1mg of 50% pure material. This was judged to be insufficient for selection.

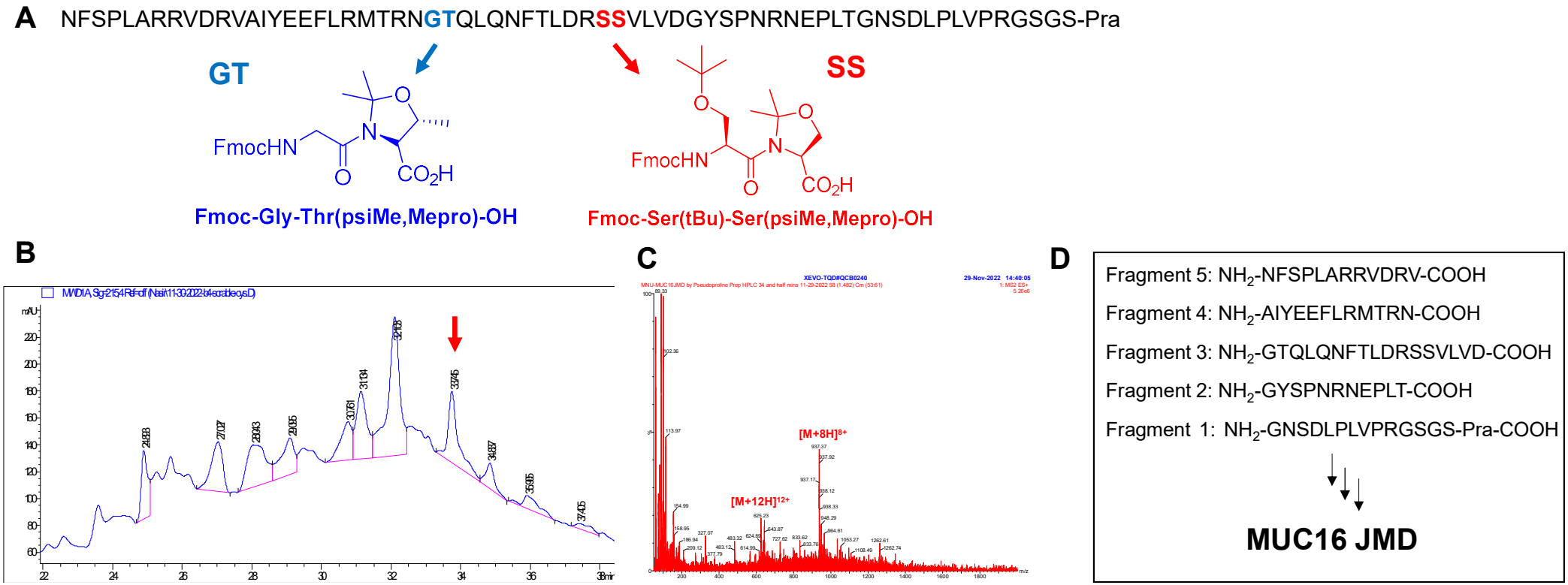


Figure 2. Alternate Synthetic Strategies for MUC16 JMD. A) Sequence of the MUC16 JMD peptide showing the sites of pseudoproline incorporation. **B)** Purification of cleaved MUC16 JMD by RP-HPLC. The product is shown with the red arrow. **C)** ESI-MS (positive ion mode) of purified product. Although we observe the expected m/z peaks for the full-length MUC16 JMD peptide, there is significant evidence of a contaminating peptide side-product. **D)** Schematic of the fragment ligation strategy.

A

HisTag V5 TEV Thrombin

MHHHHHHGKPIPNPLLGLDSTENLYFQGIDPFTGSLVPRGSN

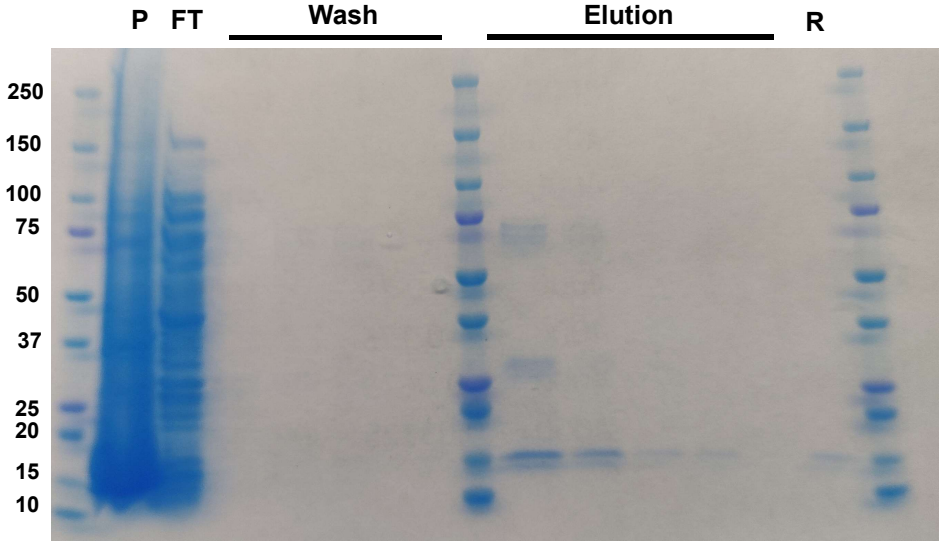
MUC16 JMD

FSPLARRVDRVAIYEEFLRMTRNGTQLQNFTLDRSSVLVDGY

SPNRNEPLTGNSDLPSGSGSGC

11.7 kDa

B



P = Pellet

FT = Flow through

R = Ni-NTA resin

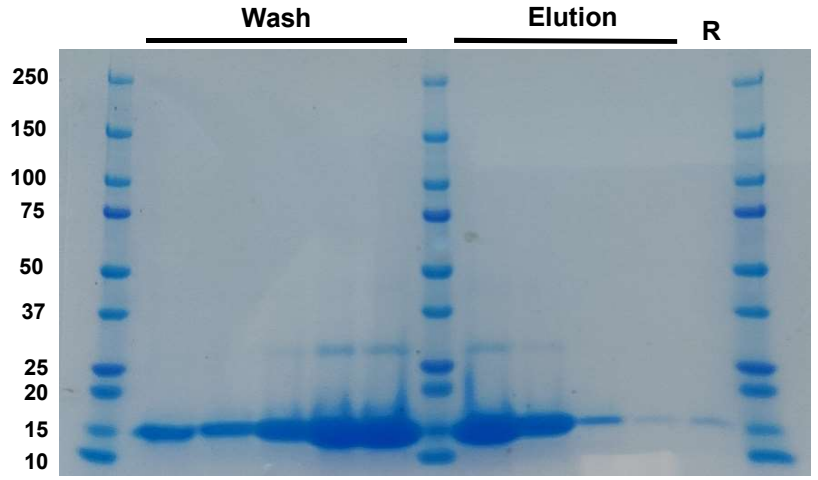


Figure 3. Expression and Purification of MUC16 JMD. A) Sequence of the MUC16 JMD peptide expression construct. The C-terminal cysteine used for maleimide-based conjugation is underlined. **B)** Coomassie-stained SDS-PAGE gel showing the purification of the MUC16 JMD peptide under non-denaturing conditions. Elution was effected by increasing concentrations of imidazole. Note the significant quantity of peptide remaining in the insoluble pellet (P). **C)** Coomassie-stained SDS-PAGE gel showing the purification of the MUC16 JMD peptide under denaturing conditions. Elution was effected by decreasing the pH from 8.0 to 4.0 in the presence of 8M urea.

Previous/Current/Pending Support for DOD

Millward, Steven

PREVIOUS:

Title:	DIRAS3 Induces Autophagy and Enhances Sensitivity to Anti-autophagic Therapy by Inhibiting KRAS Signaling In Pancreatic Ductal Adenocarcinoma (Bast)
Effort:	0.60 calendar months, 5% effort
Supporting Agency:	Emerson Collective Cancer Research Fund Grant
Grants Officer:	Emerson Collective Cancer Research Fund Grant 2200 Geng Road, Ste 100 healthgrants@emersoncollective.com
Performance Period:	07/15/2020 – 07/14/2022
Level of Funding:	
Project Goals	Delineate the molecular mechanisms whereby DIRAS3 prevents KRAS-mediated signaling and activates autophagy in PDAC cells.
Specific Aims	Specific Aim 1: To determine the mechanism(s) by which DIRAS3 induces autophagic flux in PDAC Specific Aim 2: To determine whether DIRAS3 or its peptide fragment enhances PDAC cancer cell sensitivity to autophagy inhibition.
Overlap:	None

Title:	PET Imaging of Apoptosis and Inflammation for the Prevention of Hepatocellular Carcinoma (HCC) (Millward)
Effort:	1.20 calendar months, 10% effort
Supporting Agency:	The University of Texas MD Anderson Cancer Center/CABI Pilot Project Program
Grants Officer:	Lori Armstrong The University of Texas MD Anderson Cancer Center Mid Campus Building 1 1515 Holcombe Blvd Houston, TX 77030 laarmstrong@mdanderson.org
Performance Period:	07/01/2020 – 03/31/2022 (NCE)
Level of Funding:	
Project Goals	To change the clinical practice of HCC diagnosis, and prevention through non-invasive imaging technologies to identify NASH and monitor its treatment.
Specific Aims	Specific Aim 1: Utilize [18F]-TBD PET to distinguish NASH from NAFLD and measure its value as a predictive imaging biomarker for dietary and pharmacological intervention with Emricasan.

	Specific Aim 2: Utilize [18F]4FN PET to distinguish NASH from NAFLD and measure its value as a predictive imaging biomarker for dietary and pharmacological intervention with Atorvastatin.
Overlap:	None

Title:	Targeted Molecular Imaging of Cell Death in Ovarian Cancer (R21CA181994 – Millward, Lu)
Effort:	2.40 calendar months, 20% effort
Supporting Agency:	National Institutes of Health / National Cancer Institute
Grants Officer:	Anne Menkens National Institutes of Health 9900 Rockville Pike Bethesda, MD 20892 menkensa@mail.nih.gov
Performance Period:	02/03/2016 – 01/31/2019 (NCE)
Level of Funding:	
Project Goals	Develop targeted molecular imaging agents to visualize apoptosis and autophagy in vivo by PET and MRI.
Specific Aims	Specific Aim 1: Employ targeted molecular imaging to visualize apoptosis in mouse models of ovarian cancer by PET/CT. Investigate the apoptotic levels immediately following administration of platinum-based therapies and measure the sensitivity and selectivity of imaging procedure. Specific Aim 2: Develop fluorescent, PET, and MRI proves for autophagy-specific protease activity to image autophagy in vitro and in vivo. Determine the sensitivity and selectivity of the lead substrate-based PET radiotracer and measure the pharmacodynamics of autophagy-targeted drugs in ovarian cancer mouse models.
Overlap:	None

Title:	Development of SUPR Peptides as PET Imaging Agents for Her2-positive Breast Cancer (R44CA206771 – Millward, Fiacco)
Effort:	3.00 calendar months, 25% effort
Supporting Agency:	National Institutes of Health / National Cancer Institute
Grants Officer:	Ming Zhao National Institutes of Health 9900 Rockville Pike Bethesda, MD 20892 Zhaoming3@mail.nih.gov
Performance Period:	05/06/2016 – 04/30/2019 (NCE)
Level of Funding:	

Project Goals	Carry out preclinical evaluation of a SUPR peptide-based PET radiotracer for visualization of Her2 expression in breast cancer.
Specific Aims	Specific Aim 1: We will optimize the lead EVO-004 scaffold for chemical yield, stability, circulation time, and target affinity. Specific Aim 2: We will adapt our optimized milligram-scale SUPR peptide synthetic protocols to gram-scale GMP synthesis using a GMP synthesis facility (Bachem). Specific Aim 3: We will test our GMP-certified EVO-004 radiotracer in rodent and primate models using dynamic PET/CT.
Overlap:	None

Title:	Directed Evolution of Selective Autophagy Inhibitors for the Treatment of Ovarian Cancer (Millward) (P50CA217685 – SPORE in Brain Cancer – Bast)
Effort:	1.20 calendar months, 10% effort
Supporting Agency:	The University of Texas MD Anderson Cancer Center/NCI – SPORE in Ovarian Cancer – Developmental Research Project
Grants Officer:	Lori Armstrong The University of Texas MD Anderson Cancer Center Mid Campus Building 1 1515 Holcombe Blvd Houston, TX 77030 laarmstrong@mdanderson.org
Performance Period:	09/01/2018 – 02/28/2020 (NCE)
Level of Funding:	
Project Goals	To develop cell-permeable, macrocyclic peptides that selectively target LC3 to inhibit autophagosome maturation through disruption of LC3-mediated PPIs.
Specific Aims	Specific Aim 1: Develop high affinity SUPR peptides against LC3 to inhibit autophagosome maturation and autophagic flux
Overlap:	None

Title:	Interdisciplinary Translational Pre/Postdoctoral Program in Cancer Nanotechnology (1T32CA196561-01 - Sokolov)
Effort:	0.12 calendar months, 1% effort
Supporting Agency:	National Institutes of Health / National Cancer Institute
Grants Officer:	Susan E. Lim 9609 Medical Center Dr. Bethesda, MD 20892-9707 lims@mail.nih.gov
Performance Period:	09/01/2015 – 08/31/2020 (NCE)
Level of Funding:	

Project Goals	To enhance the education of the next generation of young scientists in biomedical imaging research using nanotechnologies by combining training in fundamental aspects of nanoscience and nanotechnology with clinical questions faced in cancer imaging and therapy.
Specific Aims	Specific Aim 1: All trainees will work with at least two program faculty mentors (one from Rice and one from MD Anderson) to define and carry out an independent research problem. Specific Aim 2: Incoming trainees will participate in a unique 2-week-long boot camp in "Cancer Management and Nanotechnology" that provides an overview of current opportunities and barriers in the field. Specific Aim 3: Trainees will develop foundational background in the field by taking four courses related to translational cancer or nanotechnology topics. Specific Aim 4: Trainees will gain important lab management skills by participating in a short hands-on course providing an introduction to laboratory and project management.
Overlap:	None

Title:	SUPR Peptides for Tumor Glycosylated MUC1 Imaging (F32EB024379 - Engel)
Effort:	0.00 calendar months, 0% effort
Supporting Agency:	National Institutes of Health / National Cancer Institute
Grants Officer:	Tatjana Atanasijevic 6707 Democracy Blvd. Bethesda, MD 20817 atanasijevict@mail.nih.gov
Performance Period:	12/01/2018 – 11/30/2020
Level of Funding:	
Project Goals	To develop novel Scanning Unnatural Protease Resistant (SUPR) peptide-based radiotracers to detect the expression of tumor Mucin 1 (MUC1) by PET/CT.
Specific Aims	Specific Aim 1: Synthesize chemically-defined core-1 glycosylated MUC1 tandem repeat. Specific Aim 2: Generate and characterize SUPR peptides against T-TR peptide. Specific Aim 3: *In vivo* imaging of MUC1 with Cy5- and 18F-labeled SUPR peptides.
Overlap:	None

Title:	SUPR Peptides for Immune Checkpoint Blockade Imaging (Millward)
Effort:	0.12 calendar months, 1% effort
Supporting Agency:	The University of Texas MD Anderson Cancer Center and CABI/G.E. In-Kind Research Award
Grants Officer:	Lori Armstrong The University of Texas MD Anderson Cancer Center Mid Campus Building 1 1515 Holcombe Blvd Houston, TX 77030 laarmstrong@mdanderson.org
Performance Period:	07/01/2016 – 06/30/2021
Level of Funding:	
Project Goals	Develop a SUPR peptide-based PET tracer for PD-L1 imaging and prediction of response to PD-1/PD-L1 therapies.
Specific Aims	Specific Aim 1: Generate and Characterize SUPR Peptides Against PD-L1. Specific Aim 2: *In vivo* Optical Imaging of PD-L1 with Cy5-labeled SUPR Peptides. Specific Aim 3: PET/CT Imaging of PD-L1 using 18F-SUPR Peptides.
Overlap:	None

Title:	Passenger Deletion of ENO1 as a Targetable Vulnerability in Cancer (128307-RSG-15-145-01-CDD – Muller)
Effort:	1.20 calendar months, 10% effort
Supporting Agency:	American Cancer Society
Grants Officer:	Leigh Hoffner Program Coordinator – American Cancer Society 250 Williams Street NW Atlanta, GA 30303 Leigh.hoffner@cancer.org
Performance Period:	01/01/2016 – 06/30/2021 (NCE)
Level of Funding:	
Project Goals	Probe the biochemical consequences, adaptations and possible bypasses of Enolase inhibition in vitro as well as directly validate ENO1 deletion as a therapeutic target in intracranial gliomas in vivo, and finally, to identify a clinical candidate Enolase inhibitor.
Specific Aims	Specific Aim 1: To determine optimal pro-drugging strategy for the intracellular deliver of Enolase inhibitors. Specific Aim 2: To determine pharmacology and anti-neoplastic activity of lead compounds in subsutaneous and intracranial orthotopic xenografts.

Overlap:	None

Title:	The Lee Project – Using Evolution to Target Glioblastoma
Effort:	0.12 calendar months, 1% effort
Supporting Agency:	Glioblastoma Research Foundation
Grants Officer:	Amber Barbach 154 W 14 St New York, NY 10011 amber@gbmfntn.org
Performance Period:	09/01/2020 – 08/31/2021
Level of Funding:	
Project Goals	Employ directed evolution strategies to improve the diagnosis and treatment of glioblastoma.
Specific Aims	Specific Aim 1: Employ directed evolution strategies to improve the diagnosis and treatment of glioblastoma.
Overlap:	None

Title:	Evaluating the Predictive Power of Novel PET Imaging Agents in the Transition from NAFLD through NASH to HCC (Millward/Gammon)
Effort:	1.20 calendar months, 10% effort
Supporting Agency:	National Institutes of Health / The University of Texas MD Anderson Cancer Center – HCC SPORE DRP
Grants Officer:	Lori Armstrong The University of Texas MD Anderson Cancer Center Mid Campus Building 1 1515 Holcombe Blvd Houston, TX 77030 laarmstrong@mdanderson.org
Performance Period:	09/01/2020 – 08/31/2021
Level of Funding:	
Project Goals	To test the hypothesis that PET imaging of inflammation and apoptosis, supported by a panel of serum biomarkers, can be used to construct an integrated, robust diagnostic model that discriminates HCC progression from the following confounders: normal liver, NAFLD, and NASH livers with an AUROC of $\geq$0.85
Specific Aims	Specific Aim 1: Utilize 18F-TBD in combination with serum biomarkers to discriminate progression to HCC from NASH, NAFLD, and normal murine livers.
Overlap:	None

Title:	Inhibition of Autophagy-mediated Tumor Dormancy and Chemoresistance in Ovarian Cancer (Millward)
Effort:	0.24 calendar months, 2% effort
Supporting Agency:	The University of Texas MD Anderson Cancer Center/Institutional Research Grant
Grants Officer:	Lori Armstrong The University of Texas MD Anderson Cancer Center Mid Campus Building 1 1515 Holcombe Blvd Houston, TX 77030 laarmstrong@mdanderson.org
Performance Period:	02/01/2020 – 07/31/2021 (NCE)
Level of Funding:	
Project Goals	To develop a set of macrocyclic peptides that selectively target key components of the autophagic machinery to inhibit autophagosome maturation.
Specific Aims	Specific Aim 1: Identify SUPR peptides with nanomolar affinity to LC3 that inhibit autophagosome maturation in cell culture at sub-micromolar concentrations. Specific Aim 2: Determine the efficacy, toxicity, and pharmacokinetics of LC3-binding SUPR peptides in mouse models of autophagy-mediated chemoresistance and dormancy.
Overlap:	None

Title:	Start-Up Funding (Millward)
Effort:	1.20 calendar months, 10% effort
Supporting Agency:	The University of Texas MD Anderson Cancer Center
Grants Officer:	Lori Armstrong The University of Texas MD Anderson Cancer Center Mid Campus Building 1 1515 Holcombe Blvd Houston, TX 77030 laarmstrong@mdanderson.org
Performance Period:	04/01/2015 – 08/31/2023 (NCE)
Level of Funding:	
Project Goals	Initial funding to develop a research program focusing on the directed evolution of molecular imaging agents and therapeutics for the diagnosis and treatment of cancer.
Specific Aims	Specific Aim 1: Develop molecular imaging agents for tumor apoptosis. Specific Aim 2: Develop macrocyclic peptides that block key protein-protein interactions in autophagy. Specific Aim 3: Develop SUPR peptides for molecular imaging.

Overlap:	None

CURRENT:

Title:	DIRAS3 Disrupts K-RAS Clustering and Signaling, Enhancing Autophagy and Response to Autophagy Inhibition (R01CA266187-01A1 – Bast/Lu)
Effort:	0.60 calendar months, 5% effort
Supporting Agency:	National Institutes of Health / National Cancer Institute
Grants Officer:	TBD
Performance Period:	09/01/2022 – 08/31/2027
Level of Funding:	
Project Goals	The overall goal of this proposal is to study effect of DIRAS3 on KRAS-dependent cell growth, and investigate the mechanisms by which DIRAS3 induces autophagy in KRAS-driven PDAC and LGSOC to enhance apoptosis and growth inhibition in PDAC and LGSOC.
Specific Aims	Specific Aim 1: To study the effect of DIRAS3 on KRAS-dependent cell growth, migration and effector signaling pathways in MEF cells with mutant KRAS, as well as in KRAS-driven pancreatic ductal adenocarcinoma (PDAC) and low-grade serous ovarian cancer (LGSOC), Specific Aim 2: To investigate the mechanisms by which DIRAS3 induces autophagy in KRAS-driven PDAC and LGSOC. Specific Aim 3: To test the ability of DIRAS3 or a stapled peptide derived from the DIRAS3 α5 domain in combination with autophagy inhibitors (CQ or DC661).
Overlap:	None

Title:	Membrane Permeant Peptides for Imaging Cell Function (R01EY019587 – Piwnica-Worms)
Effort:	1.20 calendar months, 10% effort
Supporting Agency:	National Institutes of Health / National Eye Institute
Grants Officer:	Hemin R Chin National Institutes of Health 31 Center Drive MSC 2510 Bethesda, MD 20892-2510
Performance Period:	09/30/2018 – 08/31/2024 NCE
Level of Funding:	
Project Goals	To conduct quantitative pre-clinical testing in advanced glaucoma models to monitor disease progression and quantify dietary (niacin) and gene therapy (Nmnat1) interventions, as well as toxicology analysis, and metabolite profiling. We will advance a lead peptide toward the clinic through a statistically-robust non-human primate model of

	glaucoma.
Specific Aims	Specific Aim 1: Test the hypothesis that our caspase-3-activated cell-penetrating peptides can quantify apoptosis in pre-clinical rodent models of RGC degeneration and treatment interventions. Specific Aim 2: Rigorously test the hypothesis that our lead caspase-3-activated cell-penetrating peptide can quantify apoptosis in an advanced non-human primate model of glaucoma. Specific Aim 3: Perform advanced pre-clinical characterization of a lead activatable cell-penetrating peptide in preparation for eIND filing.
Overlap:	None

Title:	Targeted Inhibition of Autophagy for Treatment of Dormant Ovarian Cancer (RP200166 – Millward)
Effort:	3.60 calendar months, 30% effort
Supporting Agency:	Cancer Prevention and Research Institute of Texas – Individual Investigator Research Award
Grants Officer:	Patty Moore Cancer Prevention and Research Institute of Texas 1701 North Congress Avenue, Suite 6-127 Austin, TX 78701 pmoore@cprit.texas.gov
Performance Period:	08/31/2020 – 02/29/2024 NCE
Level of Funding:	
Project Goals	To develop macrocyclic peptides that selectively inhibit the PPIs involved in autophagosome initiation and maturation.
Specific Aims	Specific Aim 1: Develop high affinity macrocyclic peptides against LC3 to inhibit autophagosome maturation. Specific Aim 2: Develop high affinity macrocyclic peptides against Beclin1 to inhibit autophagosome initiation. Specific Aim 3: Identify Beclin1 and LC3 SUPR peptides that inhibit DIRAS3- and chemotherapy-induced autophagy in ovarian cancer xenograft models.
Overlap:	None

Title:	Pro-Drug Enolase Inhibitors in Precision Oncology (R01CA231509 – Millward)
Effort:	1.80 calendar months, 15% effort
Supporting Agency:	National Institutes of Health / National Cancer Institute
Grants Officer:	Weiwei Chen

	National Institutes of Health 31 Center Drive MSC 2510 Bethesda, MD 20892-2510 chenw6@mail.nih.gov
Performance Period:	02/08/2021 – 01/31/2026
Level of Funding:	
Project Goals	To broaden the therapeutic applicability of Enolase inhibition to treat ENO1-heterozygous deleted cancers, which constitute ~20% of all human cancers – a significant portion of which is currently considered untreatable.
Specific Aims	Specific Aim 1: Synthesis and biological evaluation of liver-specific Enolase inhibitor pro-drugs. Specific Aim 2: Synthesis and biological evaluation of CNS-targeted Enolase inhibitor pro-drugs.
Overlap:	None

Title:	MUC16 Juxtamembrane Domain-Binding Peptides for Imaging and Therapy of Ovarian Cancer (OC210142 – Millward)
Effort:	1.20 calendar months, 10% effort
Supporting Agency:	Department of Defense (DoD) – CDMRP Ovarian Cancer Pilot Award
Grants Officer:	Abigail L. Strock Department of the Army 820 Chandler Street Fort Detrick, MD 21702
Performance Period:	09/01/2022 – 08/31/2024
Level of Funding:	
Project Goals	To generate MUC16 JMD-binding peptide ligands for use in molecular imaging and targeted radiotherapy of ovarian cancer.
Specific Aims	Specific Aim 1: Directed evolution and characterization of MUC16 JMD-directed peptide ligands. Specific Aim 2: Evaluation of MUC16 JMD-directed ligands in OVCAR-3 cell assays and xenograft model
Overlap:	None

Title:	Evolution of Hypoxia Inducible Factor Inhibitors for Renal Cell Carcinoma (KC210213 – N. Millward)
Effort:	0.84 calendar months, 7% effort
Supporting Agency:	Department of Defense (DoD) – CDMRP Kidney Cancer Concept Award
Grants Officer:	TBD
Performance Period:	06/01/2022 – 05/31/2024 NCE
Level of Funding:	
Project Goals	That cyclic peptides selected for HIF1α binding will have enhanced affinity and selectivity relative to previously

	identified small molecules and will effectively block the HIF1α/ARNT interaction.
Specific Aims	Specific Aim 1: We will test our SUPR peptide libraries on cancer cell lines derived from resected ccRCC and RMC tumor tissue. Specific Aim 2: We will validate the in vivo activity of the most promising HIF1α binding SUPR peptides in patient derived xenograft (PDX) models of RCC. For ccRCC, our HIF1α SUPR peptides will be tested on M80 and M62 cell lines and PDX models.
Overlap:	None

Title:	Interdisciplinary Translational Pre/Postdoctoral Program in Cancer Nanotechnology
Effort:	0.00 calendar months, 0% effort
Supporting Agency:	National Institutes of Health / National Cancer Institute
Grants Officer:	TBA
Performance Period:	04/01/2022 – 03/31/2027
Level of Funding:	
Project Goals	The overall goal of this project is to develop a novel training program to prepare Ph.D. scientists who will transfer the many promises of cancer nanotechnology into clinical reality of the future.
Specific Aims	N/A
Overlap:	None

PENDING

Title:	NanoScan SPECT 4H / CT1512 / PET122S in-vivo imager (1S10OD036241-01- Hazle, J.)
Effort:	0.00 calendar months, 0% effort
Supporting Agency:	National Institutes of Health
Grants Officer:	TBA
Performance Period:	02/01/2024 – 01/31/2025
Level of Funding:	
Project Goals	To provide The University of Texas MD Anderson Small Animal Imaging Facility (SAIF) users with a PET/SPECT/CT preclinical imaging system to replace an aging, first generation PET/SPECT/CT system that will no longer be supported by the manufacturer as of next year. The SAIF facility offers the only PET/SPECT/CT instrument in the Texas Medical Center, and our researchers need access to this critical technology to further our mission to eliminate cancer.

Specific Aims	N/A
Overlap:	None

CERTIFICATION STATEMENT

I, PD/PI, Partnering PI, or other senior/key personnel, certify that the statements herein are current, accurate, and complete to the best of my knowledge; I agree to update such disclosure at the request of the agency prior to the award of support and at any subsequent time the agency determines appropriate during the term of the award; and I have been made aware of the requirements under Section 223(a)(1) of the William M. (Mac) Thornberry National Defense Authorization Act for Fiscal Year 2021. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties (U.S. Code, Title 218, Section 1001).

*Signature: DocuSigned by: 403EBE83981F4C8...

Date: 9/18/2023 | 3:29 PM CDT

Previous/Current/Pending Support for DOD

Grindel, Brian

PREVIOUS:
None.

CURRENT:

Title:	Targeted Inhibition of Autophagy for Treatment of Dormant Ovarian Cancer (RP200166 – Millward)
Effort:	7.80 calendar months, 65% effort
Supporting Agency:	Cancer Prevention and Research Institute of Texas – Individual Investigator Research Award
Grants Officer:	Patty Moore Cancer Prevention and Research Institute of Texas 1701 North Congress Avenue, Suite 6-127 Austin, TX 78701 pmoore@cprit.texas.gov
Performance Period:	08/31/2020 – 02/29/2024 NCE
Level of Funding:	
Project Goals	To develop macrocyclic peptides that selectively inhibit the PPIs involved in autophagosome initiation and maturation.
Specific Aims	Specific Aim 1: Develop high affinity macrocyclic peptides against LC3 to inhibit autophagosome maturation. Specific Aim 2: Develop high affinity macrocyclic peptides against Beclin1 to inhibit autophagosome initiation. Specific Aim 3: Identify Beclin1 and LC3 SUPR peptides that inhibit DIRAS3- and chemotherapy-induced autophagy in ovarian cancer xenograft models.
Overlap:	None

Title:	Pro-Drug Enolase Inhibitors in Precision Oncology (R01CA231509 – Millward)
Effort:	2.40 calendar months, 20% effort
Supporting Agency:	National Institutes of Health / National Cancer Institute
Grants Officer:	Weiwei Chen National Institutes of Health 31 Center Drive MSC 2510 Bethesda, MD 20892-2510 chenw6@mail.nih.gov
Performance Period:	02/08/2021 – 01/31/2026
Level of Funding:	
Project Goals	To broaden the therapeutic applicability of Enolase inhibition to treat ENO1-heterozygous deleted cancers, which constitute ~20% of all human cancers – a significant

	portion of which is currently considered untreatable.
Specific Aims	Specific Aim 1: Synthesis and biological evaluation of liver-specific Enolase inhibitor pro-drugs. Specific Aim 2: Synthesis and biological evaluation of CNS-targeted Enolase inhibitor pro-drugs.
Overlap:	None

Title:	MUC16 Juxtamembrane Domain-Binding Peptides for Imaging and Therapy of Ovarian Cancer (OC210142 – Millward)
Effort:	1.80 calendar months, 15% effort
Supporting Agency:	Department of Defense (DoD) – CDMRP Ovarian Cancer Pilot Award
Grants Officer:	Abigail L. Strock Department of the Army 820 Chandler Street Fort Detrick, MD 21702
Performance Period:	09/01/2022 – 08/31/2024
Level of Funding:	
Project Goals	To generate MUC16 JMD-binding peptide ligands for use in molecular imaging and targeted radiotherapy of ovarian cancer.
Specific Aims	Specific Aim 1: Directed evolution and characterization of MUC16 JMD-directed peptide ligands. Specific Aim 2: Evaluation of MUC16 JMD-directed ligands in OVCAR-3 cell assays and xenograft model
Overlap:	None

PENDING

None.

CERTIFICATION STATEMENT

I, PD/PI, Partnering PI, or other senior/key personnel, certify that the statements herein are current, accurate, and complete to the best of my knowledge; I agree to update such disclosure at the request of the agency prior to the award of support and at any subsequent time the agency determines appropriate during the term of the award; and I have been made aware of the requirements under Section 223(a)(1) of the William M. (Mac) Thornberry National Defense Authorization Act for Fiscal Year 2021. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties (U.S. Code, Title 218, Section 1001).

*Signature: DocuSigned by: Brian J Grindel B0B30A1C30574CD...

Date: 9/19/2023 | 9:30 AM CDT