First Year Report: COE Pancreatic Cancer Detection and Treatment

Dear Dr. Chapes,

Below you'll find a concise report of our COE's activities and successes during the first year. We have decided to present this report investigator-centered to allow you to discern the individual contributions. In year 1 of the COE, a total of $50,000 was distributed among Drs. Culbertson, Hua, Li, and Prakash (Om). Please do not hesitate to contact me if you and/or the Advisory Board require more information.

University Distinguished Professor Dr. Duy Hua (Chemistry)

My group works on the electronic biosensors project (Thrust 1) with Dr. Li's group and on the synthesis of pancreatic cancer overexpressed protease inhibitors and modification of anti-pancreatic molecules (Thrust 2).

1) Thrust 1: Early detection of pancreatic cancer using electronic biosensors: We have designed and synthesized a library of nine hexapeptide substrates for cathepsin B and selected three most active peptides, peptide 3, peptide 15, and peptide 16, for the profiling of overexpressed proteases in pancreatic cancer. We have also designed and synthesized six and one hexapeptide substrates for ADAM 10 and ADAM17, respectively. Results indicate the hexapeptide substrate for ADAM17 is good, but the ADAM10 hexapeptides require some optimization due to the weak reactivity of ADAM10. The work is being carried out by a graduate student and a postdoctoral fellow in my laboratory.
   Thrust 2: Synthesis of pancreatic cancer protease inhibitors: We have re-synthesized cathepsin B inhibitors, CB1 and CB2 (both are tripeptidyl molecules), with IC50 values of 50 and 40 nm, respectively. These molecules are being studied for their anti-pancreatic cancer activities. We will study the modification of tegafur using our C-H oxidation protocol as described in Specific Aim 2 in the grant application in Year 2. A graduate student is working on this project.
2) New funded grant: the following NIH R01 grant was funded for 4 years: NIH General Medical Science, R01 GM128659-01 (Hua, single investigator). Title: "Catalytic asymmetric oxidation of alkenes and alkanes", 4/1/2019 – 2/28/2023; awarded amounts: $1,199,260.
3) New grants that are within the funding range, but not yet approved: None.

Prof. Dr. Jun Li (Chemistry)

Besides the work described by Dr. Hua under Thrust 1, I can add the following:
One postdoc, one graduate student and one undergraduate student in my group are working on developing an electrochemical method to detect the activity of cancer proteases, which is mainly supported by an active NIH R01 grant (PI: Jun Li and Co-PI: Duy Hua). This is also partially supported by Johnson Cancer Center with the COE funding, a graduate summer stipend and undergraduate cancer research award. The postdoc just received a fellowship award from NASA empowered Translational Research Institute for Space Health (**TRISH**) and will continue working with the team as a collaborator from NASA from Sept. 1, 2019.

The collaborative work between the Li lab and the Hua lab has generated the following products:
Publication:

1. Song Y, Fan H, Anderson MJ, Wright JG, Hua DH, Koehne J, Meyyappan M, Li J. Electrochemical Activity Assay for Protease Analysis Using Carbon Nanofiber

Nanoelectrode Arrays. Anal Chem. 2019;91(6):3971-9. doi: 10.1021/acs.analchem.8b05189.

Conference Presentations:

1. Quantitative Electrochemical Analysis of Cathepsin B Activity Using Carbon Nanofiber Nanoelectrode Arrays with Optimized Peptide Substrate Length and Temperature, Yang Song, Huafang Fan, Morgan Anderson, Jestin G. Wright, Duy Hua, Jessica Koehne, M. Meyyappan and J. Li*, ACS Midwest Regional Meeting, Oct. 18, 2019, Wichita, KS.
2. Quantitative Electrochemical Analysis of Protease Activity Using Carbon Nanofiber Nanoelectrode Arrays toward Cancer Diagnosis, Yang Song, Huafang Fan, Morgan J. Anderson, Jestin Gage Wright, Duy H. Hua, Jessica Koehne, Meyya Meyyappan, and Jun Li, 2019 Designing Molecules Workshop and Conference, Manhattan, KS, Aug. 16th, 2019.
3. Quantitative Electrochemical Analysis of Cathepsin B Activity Using Carbon Nanofiber Nanoelectrode Arrays with Optimized Peptide Substrate Length and Temperature, Yang Song, Huafang Fan, Morgan Anderson, Jestin G. Wright, Duy Hua, Jessica Koehne, M. Meyyappan and J. Li*, 235th ECS Meeting, May 29th, 2019, Dallas, TX.

Prof. Dr. Om Prakash (Department of Biochemistry and Molecular Biophysics)

My group is ready to characterize the composition and biophysical properties of the therapeutic peptides coming out of the Hua and Bossmann groups.

New funded grants:
Console upgrade and Cryogenic Probe for a 500 MHz NMR System for Biomedical Research'. Om Prakash-PI, NIH, Period of Support, 08/10/2019 – 08/09/2020, Total amount of the project $815,000.00.

Associate Prof. Dr. Punit Prakash (Electrical and Computer Engineering)

My work focuses on Thrust 3 (Ultra-high Field Magnetic Resonance Imaging and Hyperthermia)

Research progress: We have completed the development of an experimental platform for delivering mild hyperthermia to pancreatic tumor targets experimental small animals, integrated with the 14.1 T ultra-high field MRI housed in the KSU Dept of Chemistry. This recently published work allows considerably improved control over the shape of heating patterns, limiting heating of non-targeted tissue, and is suitable for heating both sub-cutaneously implanted tumors, as well as tumors within the mouse pancreas. In September 2019, we will work closely with Prof. Bossmann's group to use this system for delivering hyperthermia to pancreatic tumor targets in mice as part of preliminary experimental studies to inform an R01 application currently under development.

The collaborative work between the Prakash lab and the Bossmann lab has generated the following publication:

1) P. Faridi, S. H. Bossmann, P. Prakash, "Simulation-based design and characterization of a microwave applicator for MR-guided hyperthermia experimental studies in small animals," *Biomedical Physics and Engineering Express*, Accepted for publication, 2019. doi: 10.1088/2057-1976/ab36dd

New funded grants: Below I've listed all my active grants. I anticipate renewal of numbers 2 and 3 for another 12 months, starting ~November 2019.

1. NIH/NCI, R01 CA218357, “Bronchoscope-guided microwave ablation of early-stage lung tumors.” (2018 – 2022).
   PI: Prakash

2. Neurent Medical, “Phase III: Computational modeling and in vivo assessment of RF devices and energy delivery strategies for rhinitis” (2018-2019)
   PI: Prakash

3. Hologic, Inc., “Microwave ablation for treatment of uterine fibroids: technical feasibility assessment” (2018-19).
   PI: Prakash

4. NSF IIP 1819177, “Directional minimally invasive microwave antenna for precise spatial control of thermal ablation,” (2018 – 2019).
   PI: Pfannenstiel; Prakash Role: co-PI

New Grants that are within the funding range, but not yet approved
NIH/NIBIB, R01 “Treating primary aldosteronism-induced hypertension via microwave thermal therapy.”
PI: Prakash, co-PI Bossmann
Percentile: 13; Council meets: October 2019
The most recently published R01 payline at NIBIB is 19 percentile, suggesting we are well within the funding range.

Research Assistant Professor Dr. Thomas Müller (Biology) (Thrust 3)

Research Progress: The Johnson Cancer Center funding helped us to secure additional funding to promote imaging-based cancer research using zebrafish as a model as well as generate results for comparative brain research. Based on these results, we have submitted one article about the zebrafish amygdaloid complex to Frontiers in Neuroscience. Moreover, I am writing two manuscripts that will be submitted before the end of the year. One article describes the topology of the zebrafish forebrain and the origin of the amygdaloid nucleus of the lateral olfactory tract (nLOT). A second article will focus on the expression of parvalbumin-expressing neuron types critical for fear learning and emotional well-being. In addition, we have established an international collaboration on the organization of neural systems that mediate cognition and spatial navigation in (teleost) fishes. Here, we are writing a theory-based review article about the zebrafish pendant of the mammalian hippocampal formation which holds the most critical neural networks for spatial orientation.

The collaborative work within the COE has generated the following products:

We have presented or will present soon some of these results on six posters on national and international meetings:

2019 Newell J, Waner L, Stonebreaker J, Kenney JW, Dallman J, Bossmann S, Prakash P and Mueller T: Assessing inhibitory GABAergic and excitatory glutamatergic signaling during fear conditioning in a shank3ab zebrafish mutant model of autism. Zebrafish

Neural Circuits and Behavior Meeting, Cold Spring Harbor Laboratory (CSHL), Cold Spring Harbor, NY, November 20-23, 2019.

2019 Kenney JW, Steadman PE, Mueller T, She MT, Young O, Josselyn SA, Frankland PW: AZBA: A Digital Adult Zebrafish Brain Atlas. Zebrafish Neural Circuits and Behavior Meeting, Cold Spring Harbor Laboratory (CSHL), Cold Spring Harbor, NY, November 20-23, 2019.

2019 Tudor C, Waner L, Stonebreaker J, Newell J, and Mueller T: "A Custom Movement Tracking Software for Behavioral Analyses of an Adult Zebrafish *Shank3* Mutant Model of Autism." Society for Neuroscience (SfN) Conference, Chicago, IL, October 19-23, 2019.

2019 Kenney JW, Steadman PE, Mueller T, Shi MT, Josselyn SA, and Frankland PW: "AZBA: An Adult Zebrafish Brain Atlas for the Digital Age." ZDM12 –Zebrafish Disease Models Society Meeting, Harvard Medical Center, Boston, MA, July 15-18.

2019 Kenney JW, Steadman PE, Mueller T, Shi MT, Josselyn SA, and Frankland PW: "AZBA: An Adult Zebrafish Brain Atlas." 2019 Midwest Zebrafish Meeting, University of Kentucky, Lexington KY, June 7-9, 2019.

2019 Tudor C, Waner L, and Mueller T: "Analysis of *lhx5*-driven GFP Expression in Zebrafish Suggests Early Evolution of Accessory and Main Olfactory-Like Telencephalic Pathways in Fish". AChemS XLI meeting, Bonita Springs, FL, April 12-15, 2019.

I have secured three extramurally funded grants:

2019 Human Frontier Science Program (HFSP): Navigating the Waters – A Neural Systems Approach to Spatial Cognition in Fish.
Funding amount (total): ca. $1,350,000.00/3 years/4PIs.
Awarded March 28, 2019.

Jacob Engelmann (Lead-PI, University of Bielefeld, Germany), Ronen Segev (Ben-Gurion-University, Israel), Theresa Burt de Perera (University of Oxford, UK), and Thomas Mueller (Kansas State University, USA).

Grant No. RGP0016/2019. Period: 12/01/2019 – 11/31/2022.

2019 Pilot Grant "Neural Circuits of Associative Emotional Learning in a Zebrafish Model of Autism," of the COBRE Center of Cognitive and Neurobiological Approaches to Brain Plasticity (CNAP) as part of the COBRE

Grant No. 5P20GM113109-03, FAIN: P20GM113109. Period: 6/1/2019 to 5/31/2020.

2018 Pilot Grant "Amygdala Circuits of Associative Learning and Reward in Zebrafish: An MRI Approach," of the COBRE Center of Cognitive and Neurobiological Approaches to Brain Plasticity (CNAP) as part of the COBRE

Grant No. 1P20GM113109-02A1, FAIN: P20GM113109. Period: 6/01/2018 - 5/31/2019.

PS: Although the COBRE grants are centered on imaging the zebrafish brain, the imaging development (especially MRI and light sheet microscopy methods) are of value for this Center of Excellence.

Prof. Dr. Chris Culbertson, Associate Dean for Research, College of Arts & Sciences

My group works on the electronic biosensors project (Thrust 1) with Dr. Bossmann's group. We are designing an Isoelectric Focusing Device (IEF) for the detection of ductal pancreatic cancer.

Earliest detection of pancreatic cancer is mandatory to open the longest possible window for cancer treatment.

Research Progress: The microfluidic device has been integrated into a question-in answer-out style workstation. The workstation consists of a mini computer, a linear scanning moving stage, a stationary laser induced fluorescence detector, a motorized magnet for on chip nanoparticle manipulation and the microfluidic chip. Under a pH gradient 3-10, the peptide standards were focused according to the PI (point of zero electric charge) in the pH gradient. This demonstrated that peptides can be designed with an expected isoelectric point. This opens up possibilities to use multiple MMP substrates simultaneously with each substrate designed to be focused at different isoelectric points.

The collaborative work within the COE has generated the following products:

1. Culbertson, C. T. In *Enhancing the information content of single cell analysis on microfluidic devices using optical fiber bridges for the analysis of reactive nitrogen species and kinases in immune system cells*, American Chemical Society: 257th ACS National Meeting & Exposition, Orlando, FL, United States, Mar. 31-Apr. 4, 2019; pp I+EC-0183.
2. Sibbitts, J.; Sellens, K. A.; Jia, S.; Klasner, S. A.; Culbertson, C. T., Cellular Analysis Using Microfluidics. *Anal. Chem. (Washington, DC, U. S.)* **2018,** *90* (1), 65-85.

University Distinguished Professor Dr. Stefan H. Bossmann (Chemistry)

My group works on the electronic biosensors project (Thrust 1) with Dr. Culbertson's group and Dr. Kasi (The University of Kansas Cancer Center (KUCC)). We are designing an Isoelectric Focusing Device (IEF) for the detection of ductal pancreatic cancer. Earliest detection of pancreatic cancer is mandatory to open the longest possible window for cancer treatment. In Thrust 2, we work on novel deep-tissue targeting sequences for pancreatic tumors and on copper-activated drugs against pancreatic cancer. In Thrust 3, we work on optimizing mouse models and MRI imaging conditions for pancreatic cancer.

Research progress:

1) Thrust 1: The progress working on microfluidic IEF-devices was already reported by Dr. Culbertson. My group is synthesizing the required superparamagnetic nanoparticles and peptide sequences. Recently, I was able to secure an American Cancer Society Institutional Research Grant that enables us to receive 120 liquid biopsies (serum samples) from KUCC to optimize our protease-based method for early pancreatic cancer detection. Dr. Kasi is responsible for the selection of serum samples and the statistical analysis of the results.
2) Thrust 2: My group has developed a new deep-tissue penetration peptide sequence named WTAS. First results indicate that it may be capable of delivering drugs and immune stimuli to pancreatic tumors, which are often not well connected to the blood supply because of a physical condition named desmoplasia. Furthermore, we have discovered a new class of antitumor agents, which require copper(I) for activation. These drugs are virtually non-toxic in the absence and quite cytotoxic in the presence of copper(I). Since the human body is practically free of non-protein bound copper(I), with the exception of tumors and infected tissue, we have discovered a very effective targeting mechanism. This concept is currently being patented by Kansas State University.
3) Thrust 3: We have secured the KPC cell line from KUCC after months of negotiations between our institutions. This enables us to optimize mouse models for pancreatic cancer,

which are based on syngeneic mice with intact immune system. In collaboration with Dr. Tej B. Shrestha and Clinical Assistant Prof. Dr. Matthew Basel (Anatomy & Physiology), we succeeded in optimizing ultra-high-field magnetic resonance imaging of pancreatic tumors in live mice. We are now able to image tumors, metastases and micrometastases, which will enable to monitor “population dynamics” of metastases and micrometastases during pancreatic cancer therapy. Furthermore, we have succeeded in Diffusion Tensor Imaging in live mice bearing pancreatic tumors. With this technology, we were able to quantify desmoplasia in live mice. This will enable us to test the ability of novel peptides, small molecules, and nanoscopic drugs to cross the barrier around pancreatic cancer and to effectively act against tumors and (micro)metastases.

The collaborative work within the COE has generated the following products:

1. Yapa, A. S.; Shrestha, T. B.; Wendel, S. O.; Kalubowilage, M.; Yu, J.; Wang, H.; Pyle, M.; Basel, M. T.; Toledo, Y.; Ortega, R.; Malalasekera, A. P.; Thapa, P. S.; Troyer, D. L.; Bossmann, S. H., Peptide Nanosponges Designed for the Delivery of Perillyl Alcohol to Glioma Cells. *ACS Appl. Bio Mater.* **2019,** *2* (1), 49-60.
2. Kasi, A.; Bajwa, S.; Williamson, S. K.; Sun, W.; Baranda, J. C.; Zambrano, O. C.; Kalubowilage, M.; Bossmann, S. H., Novel prognostic biomarkers and their association with survival in pancreatic cancers. *Journal of Clinical Oncology* **2019,** *47* (4), 296.
3. Kalubowilage, M., Janik, K., Bossmann, S. H., Magnetic Nanomaterials for Magnetically-Aided Drug Delivery and Hyperthermia, *Applied Sciences*, **2019**, *9* (14), 2927-2941.
4. Basel, M. T.; Bossmann, S. H. *Methods in Molecular Biology*, Springer: New York City, 2019, in print.

Dr. Culbertson I have secured two extramurally funded grants:

1. NSF, Emerging Frontiers in Research and Innovation (EFRI) (1933321), *EFRI CEE: Opening the Gates of Apoptosis in Cancer,* Co-PI’s: Dr. Christopher T. Culbertson, Dr. Bala Natarajan, Dr. Massoud Motamedi (UTMB), Dr. Michael Sheetz (UTMB), Dr. Larry Sowers (UTMB), Dr. Gracie Vargas (UTMB), 2019-2023, $2,000,000 Role: PI
2. NSF, Division of Chemical, Bioengineering, Environmental, and Transport Systems (1940790) *EAGER: A Microfluidic Device for Studying Environment-Triggered Migration of Glioblastoma Cells,* 2019-2021, $300,000 Role: PI

Dr. Kasi and I have secured an

3. American Cancer Society Institutional Research Grant to The University of Kansas Medical Center (IRG-16-194-07). Pilot project: *Novel Biomarkers for Early Detection and Prognosis of Pancreatic Cancers in Liquid Biopsies by Ultrasensitive Fluorescence Nanobiosensors* PI: Anup Kasi, MD, 2019-2020, $35,000

Conclusion: The COE Pancreatic Cancer Detection and Treatment had a very successful first year. In all three thrusts, we are either on-track or beyond. We have also attracted a very significant amount of funding from NIH, NSF, NASA, and the American Cancer Society.

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Relevance of the new grants to pancreatic cancer

1. NIH General Medical Science, R01 GM128659-01 (Hua, single investigator). Title: “Catalytic asymmetric oxidation of alkenes and alkanes”, 4/1/2019 – 2/28/2023; awarded amounts: $1,199,260.

Dr. Duy Hua is developing novel catalytic asymmetric oxidation technology that will enable the synthesis of new drugs with (hopefully) unprecedented action against (pancreatic) cancer. The new catalysts will be able to synthesize chiral drugs in unprecedented yields.

2. Console upgrade and Cryogenic Probe for a 500 MHz NMR System for Biomedical Research’. Om Prakash-PI, NIH, Period of Support, 08/10/2019 – 08/09/2020, Total amount of the project $815,000.00.

This grant from NIH will enable Dr. Om Prakash to characterize the peptide-based drugs against pancreatic cancer that are being synthesized by the Hua and Bossmann groups with exceptional resolution. Especially the 3D geometries of the therapeutic peptides are of great interest, because they permit the prediction of drug action.

3. Neurent Medical, “Phase III: Computational modeling and in vivo assessment of RF devices and energy delivery strategies for rhinitis” (2018-2019)
   PI: Prakash

Radiofrequency-hyperthermia will be an important method for treating pancreatic cancer and for reversing desmoplasia in mouse models in year 2 of the COE. This grant permits the optimization of hyperthermia treatment (RF-frequency and dose) for (pancreatic) tumor treatment.

4. NSF IIP 1819177, “Directional minimally invasive microwave antenna for precise spatial control of thermal ablation,” (2018 – 2019).
   PI: Pfannenstiel; Prakash Role: co-PI

This antenna will be used for treating pancreatic tumors in mice. We will observe both, the spatial and temporal heat distribution and the physiological effects of the heat by ultra-high field MRI.

5. New grant that is within the funding range, but not yet approved
   NIH/NIBIB, R01 “Treating primary aldosteronism-induced hypertension via microwave thermal therapy.” PI: Prakash, co-PI Bossmann
   Percentile: 13; Council meets: October 2019
   The most recently published R01 payline at NIBIB is 19 percentile, suggesting we are well within the funding range.

This grant will enable us to test the antenna for hyperthermia treatment on large animals (pigs). Furthermore, we will be able to develop peptide sequences to target selective cell populations. This technology will secure us an advantage when applying for R01s/U01s in February and May.

6. Human Frontier Science Program (HFSP): Navigating the Waters – A Neural Systems Approach to Spatial Cognition in Fish.
   Funding amount (total): ca. $1,350,000.00/3 years/4PIs.
   Awarded March 28, 2019.

   Jacob Engelmann (Lead-PI, University of Bielefeld, Germany), Ronen Segev (Ben-Gurion-University, Israel), Theresa Burt de Perera (University of Oxford, UK), and Thomas Mueller (Kansas State University, USA).

   Grant No. RGP0016/2019. Period: 12/01/2019 – 11/31/2022.

7. Pilot Grant "Neural Circuits of Associative Emotional Learning in a Zebrafish Model of Autism," of the COBRE Center of Cognitive and Neurobiological Approaches to Brain Plasticity (CNAP) as part of the COBRE, Thomas Mueller (PI)

   Grant No. 5P20GM113109-03, FAIN: P20GM113109. Period: 6/1/2019 to 5/31/2020.

8. Pilot Grant "Amygdala Circuits of Associative Learning and Reward in Zebrafish: An MRI Approach," of the COBRE Center of Cognitive and Neurobiological Approaches to Brain Plasticity (CNAP) as part of the COBRE, Thomas Mueller (PI)

   Grant No. 1P20GM113109-02A1, FAIN: P20GM113109. Period: 6/01/2018 - 5/31/2019.

Grants 6, 7, and 8 are funding / will fund the development of high-resolution MRI technology at Kansas State. This concerns hardware, as well as software development. By lowering the resolution of ultra-high field MRI, we will be able to image micrometastases in tumors without the use of a contrast agents. This novel technology will enable us to observe the population dynamics of micrometastases in pancreatic cancer in response to treatment attempts (chemotherapy, hyperthermia, immunotherapy).

9. NSF, Emerging Frontiers in Research and Innovation (EFRI) (1933321), *EFRI CEE: Opening the Gates of Apoptosis in Cancer,* Co-PI's: Dr. Christopher T. Culbertson, Dr. Bala Natarajan, Dr. Massoud Motamedi (UTMB), Dr. Michael Sheetz (UTMB), Dr. Larry Sowers (UTMB), Dr. Gracie Vargas (UTMB), 2019-2023, $2,000,000 Role: PI

This prestigious collaborative grant will allow us to investigate the biochemical and biophysical connections between mechanobiology and cancer metabolism. The ultimate goal is to trigger the onset of apoptosis by using mechanical cues.

10. NSF, Division of Chemical, Bioengineering, Environmental, and Transport Systems (1940790) *EAGER: A Microfluidic Device for Studying Environment-Triggered Migration of Glioblastoma Cells,* 2019-2021, $300,000 Role: PI

This grant is concerned with the interaction of CD44 with hyaluronic acid. Although it is mainly concerned with glioblastoma, it should be noted that CD44 is highly overexpressed in pancreatic cancer we well. Therefore, we anticipate significant "technology transfer".

11. American Cancer Society Institutional Research Grant to The University of Kansas Medical Center (IRG-16-194-07). Pilot project: *Novel Biomarkers for Early Detection and Prognosis of Pancreatic Cancers in Liquid Biopsies by Ultrasensitive Fluorescence Nanobiosensors* PI: Anup Kasi, MD, 2019-2020, $35,000

This grant studies the early detection and disease prognosis of pancreatic cancer vs. pancreatitis.