This is provided as an example proposal.

It is important that you follow the current guidelines.
The mentor letter has been removed.

The John J. Kopchick Molecular and Cellular Biology/Translational Biomedical Sciences Research Fellowship Award

Cover Page

Academic Year 2019-2020

NAME OF APPLICANT: _William Koch_____
NUMBER OF YEARS IN THE MCB OR TBS PROGRAM: _3_____
E-MAIL ADDRESS: _wk922015@ohio.edu_____
DEPARTMENT: _HCOM/TBS_____
EXPECTED GRADUATION DATE (Month and Year): _May 2022_____

TITLE OF PROJECT: Novel therapeutic for type 1 diabetes mellitus: target discovery and mechanism of action for insulin secretion and beta-cell protection_____
FACULTY MENTOR INFORMATION:
NAME: _Craig Nunemaker, PhD_____
E-MAIL ADDRESS: _nunemake@ohio.edu_____
DEPARTMENT: _Biomedical Sciences_____
BUDGET: Total Request _$10,000_____
(May not exceed $10,000 plus an additional $5,000 if participating in an internship)

STATEMENT OF HOW THE RESEARCH IS RELEVANT TO TRANSLATIONAL BIOMEDICAL SCIENCES* (500 character limit)

The objective of this study is to determine MSB-3's mechanism of action for insulin secretion and beta-cell protection. This fellowship will be used as a platform to provide me with the necessary skills to become a physician-scientist who is knowledgeable in the biotechnology industry. Successful completion of this fellowship will lead to the further development of a novel therapeutic that may one day be used in clinical trials to treat patients with type 1 diabetes mellitus (T1DM).

*For the purposes of this program, translational biomedical sciences is defined as the translation of basic research effectively into enhanced healthcare outcomes for the entire population in fields such as biomedical research, bioengineering, drug development, informatics, communications, health policy and planning.

IRB AND IACUC APPROVAL:

To ensure that the University is in compliance with all federal regulations, complete the checklist below.
Note: your proposal can be approved prior to IRB or IACUC approval (put "pending" or "to be submitted" instead of approval number), but funding will be withheld until notification of approval or exemption.

Yes	***No***	***Office of Research Compliance***	***Policy #***
	X	Human Subjects in Research (including surveys, interviews, educational interventions): Institutional Review Board (IRB) Approval #: Expiration Date:	19.052
	X	Animal Species: Institutional Animal Care & Use Committee (IACUC) Approval #: Expiration Date:	19.049

SIGNATURES

Applicant's Signature		**Faculty Mentor's Signature**	
Signature	William Joseph Koch	Signature	Craig S. Nunemaker
Name	William Joseph Koch	Name	Craig S. Nunemaker

Optional: ■ **Yes** ☐ No

If selected for funding, I give permission to the Office of the Vice President for Research and Creative Activity to use my proposal as an example during training and workshop exercises.

Biographical Sketch

William Joseph Koch
Ohio University Heritage College of Osteopathic Medicine, Translational Biomedical Sciences Program

Personal Statement

My long-term interests involve examining the pathogenesis of immune-mediated diabetes in order to develop novel and safe therapeutics. As a DO/PhD student, I will utilize my medical and graduate school training in order to maximize the support of this fellowship to determine the mechanism of action of MSB-3, as well as, gain experience in drug development, intellectual property management, and biotechnology operations.

Education

Ursuline College, Pepper Pike, OH, 8/2009-5/2013, BA, Honors Biology, Chemistry
The Commonwealth Medical College, Scranton, PA, 8/2014-5/2015, MBS, Biomedical Sciences
Ohio University Heritage College of Osteopathic Medicine, Athens, OH, 7/2015-Present, Anticipated Graduation 5/2022, DO/PhD, Translational Biomedical Sciences

Relevant Graduate Courses: Graduate GPA 3.9

MS 502 Biochemistry
MS 504 Human Genetics
MS 503 Epidemiology & Biostatistics
MS 500 Human Physiology
MS 518 Histology Foundations
MS 507/513 Community Health Research I&II
MS 506 Seminars in Biomedical Sciences I&II
MS 510 Introduction to Pharmacology
MS 512 Basic Immunology
MS 520 Histology Organ Systems
MS 509 Cell Biology
MS 511 Foundations of Neuroscience
OUHCOM Year 1&2
TBS 5720 Tools in Translational Research
TBS 5680 Foundations in Bioethics
TBS 6220 Career Development Seminar
TBS 6220 Career Development & Leadership Seminar
PBIO 5150 Statistical Methods in Plant Bio
PBIO 5170 Bio Research & Science Ethics
ELIP 5140 Writing in Graduate Studies
NUTR 5320 Diabetes From Bench to Bedside

Awards and Scholarships

2016 Research and Scholarly Advancement Fellowship (RSAF), Ohio University Heritage College of Osteopathic Medicine
2018 Student Research and Creative Activity Expo, Ohio University, First Place Poster, TBS Program
2018 John J. Kopchick Molecular and Cellular Biology/Translational Biomedical Sciences Research Fellowship Award
2019 Student Research and Creative Activity Expo, Ohio University, First Place Poster, TBS Program
2019 Student Research and Creative Activity Expo, Ohio University, First Place Poster, DI

Abstracts, Presentations and Publications

- Eswarappa, S, Potdar, A, **Koch, WJ**, Fan, Y, Vasu, K, Lindner, D, Willard, B, Graham, L, DiCorleto, P, Fox, P (2014). Programmed Translational Readthrough Generates Anti-Angiogenic VEGF-Ax. *Cell*, *157*(7), 1605–1618.
- **Student Research & Creativity Activity Expo**, Ohio University, Athens, OH (2018). Abstract and Poster Presentation.
- **11th Annual Midwest Islet Club**, Washington University, St. Louis, MO (2018). Abstract and Poster Presentation.
- **33rd National MD/PhD Student Conference**, University of Colorado School of Medicine, Keystone, CO (2018). Abstract and Poster Presentation.
- **Islet Biology Workshop,** Vanderbilt University, Nashville, TN (2018). Abstract and Poster Presentation.
- **Student Research & Creativity Activity Expo**, Ohio University, Athens, OH (2019). Abstract and Poster Presentation.
- **15th Annual Association of Physicians / American Society for Clinical Investigation / American Physician Scientist Association Joint Meeting**, Chicago, IL (2019). Abstract and Poster Presentation.
- **12th Annual Midwest Islet Club**, Washington University, St. Louis, MO (2018). Abstract and Poster Presentation.

Project Narrative

Objective & Scope: At diagnosis of type 1 diabetes mellitus (T1DM), the immune system has already destroyed enough beta cells to reduce insulin-secreting capacity by 70-90% [1]. For this reason, current therapeutics for the treatment of T1DM focuses primarily on enhancing exogenous insulin replacement in light of decreased beta-cell mass. However, this approach neglects the direct cytokine-mediated immune destruction of pancreatic beta cells. Our laboratory has discovered a novel group of small molecules with the ability to treat T1DM by protecting pancreatic beta cells from immune-mediated destruction and restoring insulin secretion in a glucose-dependent manner. In isolated pancreatic islets from CD-1 mice (a common outbred strain), MSB-3 has the ability to stimulate a 4-fold increase in insulin secretion. In addition, MSB-3 robustly protects pancreatic islets against cytokines such as IL-1β, TNF-α and IFN-γ. The favorable effects of MSB-3 are also appreciated in vitro in both a type 1 (NOD) mice model, as well as, deceased donor human pancreatic islets. The focus of this proposal is to define the mechanism(s) of this dual-acting compound.

Materials & Methods: Studies show that a kinase-independent JAK/STAT inhibitor has the ability to protect beta cells from cytokine-induced cell death [2]. Interestingly, we have identified one of our compounds (EPB-1) as structurally identical to a commercially available STAT5 inhibitor that was developed for the treatment of cancer [3]. This commercially available compound is a potent and specific STAT5 inhibitor that produces effects consistent with inhibition of JAK/STAT signaling [4,5]. Based on the possibility that more of our compounds may interact with STAT5, our lead compound MSB-3 was docked to the SH2

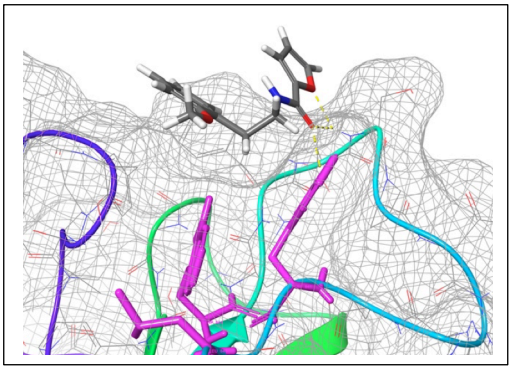

Figure 1. Glide docking of MSB-3 to the SH2 domain of STAT5.

domain of STAT5. Glide docking of MSB-3 shows hydrogen bonds to Ser635 and Trp641 in the SH2 domain of STAT5 proximal to a hydrophobic pocket involved in STAT5 dimer interface contacts (Trp631, Trp641, and L643 shown in magenta in **Figure 1**). This computational docking suggests that MSB-3 may interact with STAT5, thus indicating a rationale to investigate the potential role of MSB-3 on the JAK/STAT signaling pathway.

Furthermore, through the use of STAT5a-b-deficient hypomorph (SKO) mice, which have a > 90% inhibition of STAT5 signaling, we were able to isolate pancreatic islets to examine the importance of STAT5 signaling. In **Figure 2A**, MSB-3 reduces cytokine-induced apoptosis in wild type (blue) islets, but not in islets from SKO mice (dark red). The addition of MSB-3 has no additional protective effects. MSB-3 even increases necrosis associated cell death in SKO mice as measured by propidium iodide (PI) in **Figure 2B**. Of interest, the commercially available STAT5 inhibitor had similar effects on apoptosis (**Figure 2C**) and cell death (**Figure 2D**), although it should be noted that the commercially available STAT5 inhibitor failed to protect wild type islets as measured by PI. Overall, the data suggest JAK/STAT

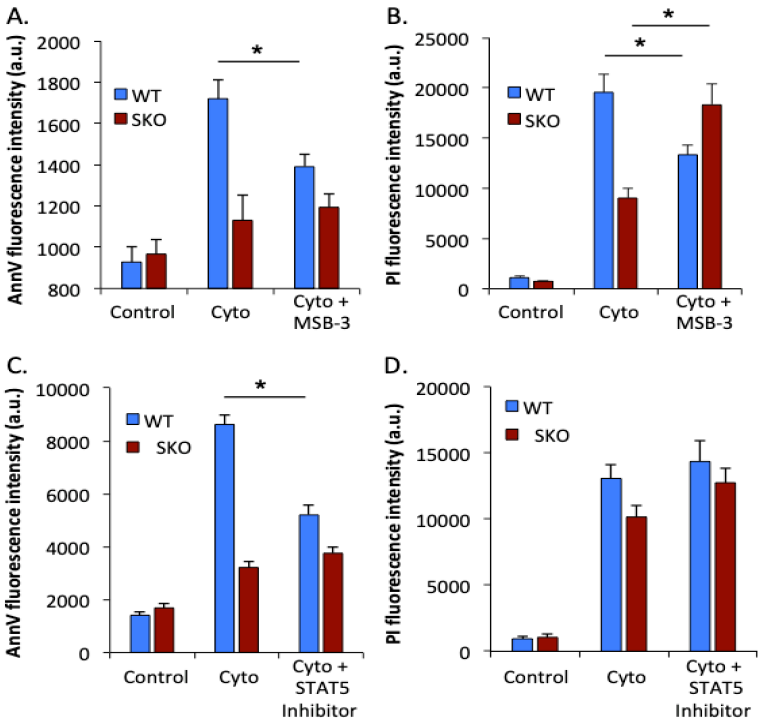

Figure 2. Cell protection may involve STAT5. (A) MSB-3 (200μM) reduces cytokine-induced apoptosis (AnnV) in wild type (wt) islets, but not in islets from SKO mice. (B) MSB-3 protects wt islets from cell death/necrosis (PI), but augments death in SKO islets. (C-D) STAT5 inhibitor (200uM) also fails to protect SKO islets. Cyto = 10ng/mL IL-1β + 20ng/mL TNF-α. Identical conditions were used for data collected within each panel. * $P < 0.05$.

involvement in the mechanism of action. *Thus, we hypothesize* the positive therapeutic effects of MSB-3 on pancreatic islets are due to its undefined role in the JAK/STAT pathway. **My specific aim is** to determine if reducing STAT5 signaling is key to protection of pancreatic beta cells and insulin secretion.

First, we will examine the mechanism of MSB-3 in vitro using a hamster beta-cell line (HIT-T15 beta cells) to knockdown STAT5. HIT-T15 beta cells will be transfected with siRNA against STAT5a/b (Thermo Fisher Scientific, MA) using Lipofectamine 2000 (Invitrogen, CA) in a confluent 6-well plate. Expression of STAT5 protein will be tested 72h after transfection with Western blot using anti-STAT5 antibody (Cell Signaling Technology, MA). Anti-actin antibody (Cell Signaling Technology, MA) will be used as a control. We will use STAT5 siRNA knockdown in HIT-T15 beta cells to test for cell death rates using Annexin V (AnnV) and propidium iodide (PI), as well as, TUNEL staining. siRNA STAT5 reduced HIT-T15 beta cells will then be treated with MSB-3 +/- cytokines (10ng/mL IL-1β + 20ng/mL TNF-α) in order to assess protection. The advantage of this siRNA approach is that STAT5 will be knocked down relatively acutely so that developmental compensation does not interfere.

Next, we will overexpress STAT5 in HIT-T15 cells to test for cell death as described above. HIT-T15 beta cells will be infected with adenovirus specific for inducing STAT5 overexpression (Vector Biolabs, PA) in a confluent 6-well plate. Expression of STAT5 protein will be tested 24h after infection as described above. The purpose of overexpressing STAT5 is to confirm the role of STAT5 in beta-cell protection, as well as, determine the effect of our lead compound in altering this signaling pathway. In addition, we will also measure phosphorylated STAT5a/b related to total STAT5 protein to determine the effects of our compounds on STAT5 activity. Lastly, insulin secretion will be measured from overnight supernatant following STAT5

knockdown and overexpression +/- MSB-3 to address whether STAT5a/b signaling is involved in enhancing insulin secretion.

Significance: According to the Centers for Disease Control and Prevention (CDC), 30 million Americans have diabetes, of which about 5% have T1DM. The pathogenesis of T1DM is the result of a surge in cell signaling molecules, known as cytokines, which induce immune-mediated cell death of beta cells within pancreatic islets. Our in vitro results indicate that MSB-3 has the potential to protect islets from cytokine-mediated cell death and enhance insulin secretion. Collectively, the outcome of this research will provide insight into pancreatic beta-cell immune defense and glucose-dependent augmentation of insulin secretion, two vastly important qualities in the design of T1DM therapeutic treatments.

Intellectual Property: With the collaboration of my co-mentor, Dr. Stephen Bergmeier, we hope to identify more compounds with the same benefits as MSB-3, but with greater efficacy, solubility, and potency. Our long-term goal is to design a novel compound that qualifies for a *composition of matter* patent based on this novel dual-acting therapeutic to treat T1DM, as well as, potentially T2DM.

Bibliography:

1. Cernea S, Dobreanu M. Diabetes and beta cell function: from mechanisms to evaluation and clinical implications. Biochemia Medica. 2013 May 24;23(3):266-280.
2. Chou DH-C, Vetere A, Choudhary A, Scully SS, Schenone M, Tang A, *et al.* Kinase-Independent Small-Molecule Inhibition of JAK-STAT Signaling. J Am Chem Soc. 2015 Jun 24;137(24):7929–34.
3. Muller J, Sperl B, Reindl W, Kiessling A, Berg T. Discovery of chromone-based inhibitors of the transcription factor STAT5. Chembiochem Eur J Chem Biol. 2008 Mar 25;9(5):723–7.
4. Bhavsar SK, Hosseinzadeh Z, Brenner D, Honisch S, Jilani K, Liu G, *et al.* Energy-sensitive regulation of Na+/K+-ATPase by Janus kinase 2. Am J Physiol Cell Physiol. 2014 Feb 15;306(4):C374–84.
5. Betts BC, Veerapathran A, Pidala J, Yu X-Z, Anasetti C. STAT5 polarization promotes iTregs and suppresses human T-cell alloresponses while preserving CTL capacity. J Leukoc Biol. 2014 Feb;95(2):205–13.

Budget and Justification

Budget to Support Academic Advancement and Achievement	
Conference/Workshop Travel	**Total**
ENDO 2020 – Endocrine Society Annual Meeting	
o Registration o Membership o Airfare o Lodging (3 nights @ $280 per night) o Per diem (3 nights @ $50 per day) o Cab fair	$469 $15 $400 $840 $150 $40
Student Stipend	$586
Total Budget to Support Translational Biomedical Sciences Research	**$2500**
Total Budget Requested	**$2500**

The purpose of the ENDO 2020 – Endocrine Society Annual Meeting will be to focus on scientific and medical advances in endocrinology. This conference brings together scientists, physicians, physician-scientists, and students interested in new technologies available for endocrinology and metabolic diseases, including technological advancements from both the biologic and device technology fields. ENDO 2020 will be held on March 28th – 31st, 2020 in San Francisco, CA. This conference will provide an opportunity to gain insight on novel advancements in diabetes treatment, as well as, network with national endocrinologists and specialists in the field.

The stipend will go towards scholarly supplies in support of my education in the DO/PhD Translational Biomedical Sciences Program.

Budget to Support Translational Biomedical Sciences Research		
Research Materials	**Quantity**	**Total**
STAT5 Expression		
o STAT5 Rabbit-monoclonal primary antibody (Cell Signaling, 4205T)	1 @ $260 per 100uL	$260
o pSTAT5 Rabbit-monoclonal primary antibody (Cell Signaling, 4322T)	1 @ $134 per 20uL	$134
o Beta-actin Mouse primary antibody (Cell Signaling, 12262S)	1 @ $305 per 100uL	$305
o Anti-mouse IgG, HRP-linked secondary antibody (Cell Signaling, 7076S)	1 @ $142 per 1mL	$142
o siRNA STAT5 (Thermo Fisher Scientific, AM16708)	1 @ $231 per 5nmol	$231
o Ham's F-12K Medium (Thermo Fisher Scientific, 21127030)	1 @ $249 per 500mL pack of 10	$249
o Lipofectomine 200 Transfection Reagent (Invetrogen, 11668-019)	1 @ $652 per 1.5mL	$652
o Adenovirus-STAT5 over-expression (Vector Biolabs, 1416)	1 @ $690 per 200uL	$690
o 10% Mini-PROTEAN Precast Protein Gels (BioRad, 4561035)	2 @ $116 per pack of 10	$232
o Pierce ECL (Thermo Fisher Scientific, 32209)	1 @ $215 per 500mL	$215
o Pierce ECL Plus (Thermo Fisher Scientific, 32132)	1 @ $302 per 100mL	$302
Cell Death		
o TUNEL Assay Kit – BrdU-Red (abcam, ab66110)	1 @ $749 per kit	$749
o Annexin V FITC conjugate (Thermo Fisher Scientific, A13199)	1 @ $299 per 500uL	$299
Insulin Secretion		
o Mouse Insulin ELISA (ALPCO, 80-INSMS-E01)	4 @ $399 per plate	$1596
Total Research Supplies		**$6,056**
Conference Travel		**Total**
Islet Biology: from Gene to Cell to Micro-Organ / Diabetes: Glucose Control and Beyond Joint Meeting		
o Registration		$620
o Lodging (4 nights @ $142 per night)		$568
o Airfare		$400
o Per diem (4 nights @ $50 per day)		$200
o Cab fair		$60

Total Conference Travel	**$1848**
Total Budget to Support Translational Biomedical Sciences Research	**$7904**
Total Budget Requested	**$7500**

Justification for Research Materials

The mentor will provide all basic laboratory supplies. All necessary equipment for the proposed in vitro studies found within the Project Narrative will be available and provided by the mentor or Ohio University. Funds for the remaining cost of research supplies ($404) after disbursement of the fellowship will be provided by the mentor's research funds.

The proposed research will include assays to determine mechanism of action of MSB-3. These in vitro studies will involve using HIT-T15 beta cells to knockdown and overexpress STAT5. We will run a Western blot and use select antibodies listed above to quantify STAT5 levels. Next, these manipulated HIT-T15 cells will be challenged with a cytokine cocktail (IL-1β + TNF-α) in order to determine whether reducing STAT5 signaling is key to protection of pancreatic beta cells. Cell death will be measured using Annexin V, propidium iodide, and TUNEL staining. An insulin ELISA kit will be used to assess insulin secretion levels post manipulation of STAT5 expression in HIT-T15 cells.

Justification for Conference Travel

The purpose of the Islet Biology: from Gene to Cell to Micro-Organ / Diabetes: Glucose Control and Beyond Joint Meeting is to bring together physician-scientists, research scientists, clinicians, and students to focus on scientific advances in bet-cell biology and diabetology. This conference will be held on January 27th – 31st, 2020 in Santa Fe, New Mexico. I will present my research findings at the poster session. At this conference, I hope to establish collaborations with researchers who share a common interest in diagnosing and developing treatments for immune-mediated diabetes.