PI: **Wagner, David H**	Title: Developing a small peptide to control autoimmune inflammation in type 1 diabetes	
Received: 09/02/2016	FOA: PA16-303	Council: 01/2017
Competition ID: FORMS-D	FOA Title: PHS 2016-02 OMNIBUS SOLICITATION OF THE NIH FOR SMALL BUSINESS TECHNOLOGY TRANSFER GRANT APPLICATIONS (PARENT STTR [R41/R42])	
1 R41 AI131784-01	Dual: DK,HD	Accession Number: 3965986
IPF: 10020801	Organization: OP-T-MUNE, INC.	
Former Number:	Department:	
IRG/SRG: ZRG1 EMNR-W (10)B	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: [redacted]	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
David Wagner	Op-T-Mune, Inc.	PD/PI
Martin Yussman	Op-T-Mune, Inc	Other (Specify)-Medical Advisor

OMB Number: 4040-0001
Expiration Date: 06/30/2016

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	**State Application Identifier**

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

2. DATE SUBMITTED | **Application Identifier**

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION

Organizational DUNS*: 016344134

Legal Name*: Op-T-Mune, Inc.
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]

Person to be contacted on matters involving this application

Prefix: Dr. First Name*: David Middle Name: Last Name*: Wagner Suffix:

Position/Title: Chief Scientific Officer
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted] STATES
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email: [redacted]

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** [redacted]

7. TYPE OF APPLICANT* R: Small Business

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).

❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Developing a small peptide to control autoimmune inflammation in type 1 diabetes

12. PROPOSED PROJECT

Start Date* 04/01/2017
Ending Date* 03/31/2018

13. CONGRESSIONAL DISTRICTS OF APPLICANT
CO-001

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. First Name*: David Middle Name: Last Name*: Wagner Suffix:
Position/Title: Chief Scientific Officer
Organization Name*: Op-T-Mune, Inc.
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email*: [redacted]

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$[redacted]
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$[redacted]
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR
○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. 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 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: Dr. First Name*: David Middle Name: Last Name*: Wagner Suffix:
Position/Title*: Chief Scientific Officer
Organization Name*: Op-T-Mune, Inc.
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email*: [redacted]

Signature of Authorized Representative*
David Wagner

Date Signed*
09/02/2016

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:1235-CoverSTTRDiabetes.pdf

424 R&R and PHS-398 Specific Table Of Contents

OMB Number: 4040-0010
Expiration Date: 06/30/2016

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Op-T-Mune,Inc.
Duns Number: [redacted]
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: CO-001

Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: University of Colorado Denver, Anschutz Medical Campus
DUNS Number: [redacted]
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: CO-006

File Name

Additional Location(s)

Funding Opportunity Number: PA-16-303. Received Date: 2016-09-02T13:28:53.000-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ❍ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ❍ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6

If NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ❍ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ● No

IACUC Approval Date: 10-04-2015

Animal Welfare Assurance Number A3269-01

3. Is proprietary/privileged information included in the application?* ● **Yes** ❍ **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	1236-Abstract_Diabetes_STTR.pdf
8. Project Narrative*	1237-Narrative_STTR.pdf
9. Bibliography & References Cited	1238-REFS_STTR_2016.pdf
10.Facilities & Other Resources	1239-Facilities_OpTMune.pdf
11.Equipment	1240-Equipment.pdf
12. Other Attachments	1241-SBC_000405651.pdf

Funding Opportunity Number: PA-16-303. Received Date: 2016-09-02T13:28:53.000-04:00

Abstract: Type 1 Diabetes (T1D) affects an ever growing population. While this disease typically has been associated with juveniles, the disease in adult populations is rapidly increasing. The defining clinical component is insulin loss, which occurs because of sustained inflammation in the islets. At present there is no means to prevent or reverse insulin loss. A major inflammatory pathway in T1D that contributes to insulin loss is the CD40 – CD154 dyad. CD40 is expressed on a wide array of cells and when engaged by CD154 creates localized inflammation. This pathway is decisive in T1D; blocking the interaction prevents diabetes onset and reverses hyperglycemia in new onset diabetic mice. A major impediment to drug development in diabetes has been the failure of therapeutics to translate from mouse to human. Mindful of this, we discovered that CD40 provides a link between mouse and human during T1D. We discovered that NOD mice, the industry standard model for T1D, increase CD40 expression, including on a sub population of T cells during diabetes development. Those cells, termed Th40, not only expand in number as diabetes develops but Th40 cells are singularly capable of transferring T1D to scid recipients. In a translational approach, we discovered that Th40 cells become prominent in human T1D patients, regardless of the age, HLA haplotype, auto-antibody status, or duration of disease. Like in the mouse model, Th40 cells start at low percentages but increase as human subjects progress to T1D and remain at high levels even up to 40 years after diagnosis. New onset as well as long – term diabetic patients have highly expanded numbers of Th40 cells when compared to non-autoimmune, or type 2 diabetic controls. A portion of TrialNet defined Pre-T1D subjects also have expanded Th40 cell numbers, suggesting that these cells become pathogenic over time, depending upon CD40 expression. Controlling CD40 therefore will be therapeutically advantageous. Methods to control CD40 have relied upon monoclonal antibodies or randomly generated, small organic molecules. Both those options have failed clinically. To address this, we developed a series of peptides derived from the CD154 protein sequence that are designed to target CD40 binding sites. These peptides do not function like antibodies and unlike the random generated organic molecule approach, have high specificity for CD40. In preliminary work we determined that some of the peptides prevent diabetes onset in NOD mice and one of the peptides (thus far) reversed hyperglycemia in new onset diabetic mice. The goals of this grant are to establish clinical parameters that will allow further development of a lead candidate for therapeutic development. We propose to determine how candidate peptides impact glucose tolerance testing, serum insulin levels and c-peptide levels.

Methods to control CD40 CD154 interaction have failed because heretofore they have depended upon monoclonal antibody or randomly generated organic molecules. We designed CD40 targeting peptides that prevent diabetes onset and reverse hyperglycemia in new onset diabetic mice.

Facilities and Resources:

Op-T-Mune, Inc., located in Denver, CO is a startup company that originated from the Webb-Waring Center, Denver CO, prior to WWC joining the University of Colorado School of Medicine. WWC operates as an independent center within the University of Colorado Denver Anschutz Medical Campus. WWC has provided guaranteed space to Op-T-Mune for business development purposes. This includes wet-lab space, and office space. Op-T-Mune also has contracted with the Wagner Lab at the University of Colorado School of Medicine, Dr. Wagner serves as Chief Scientific Officer for Op-T-Mune as well as being a senior investigator for WWC and Professor in the University of Colorado School of Medicine. Lab space includes approximately 1500 sq feet with wet bench space, certified sterile hoods, cell culture incubators, dedicated cell culture rooms with sterile hoods and incubators, centrifuges etc. An HPLC for antibody and peptide analysis, two flow cytometers, owned by the Wagner lab, are available. Microscopy core facility including light and fluorescent microscopes, a two photon microscope, and confocal microscopes are available. Histology proceeding facilities, microtomes, both standard and refrigerated are available. Blood collection and procession stations are available with a phlebotomist working in the lab. IRB and IACUC approvals are in place. Use of lab space and equipment is established through contractual agreements with the University of Colorado Denver. The Wagner Lab currently is housed in the School of Pharmacy building and Dr. Wagner has been joined the Pharmaceutical Sciences and Toxicology research group. This adds mass spectrometer and ion source mass spectrometer use capabilities. In addition the expertise of the Pharmaceutical Sciences faculty are available for consult. In addition Op-T-Mune has resources and expertise in Boulder Colorado from Nostix, Inc, a diagnostic test development firm. Mr. Charlie Henry, CEO of OpTMune is currently the CEO of Nostix (which is under sale after IPO on the NYSE).

Equipment :

The equipment available for completion of these studies includes: a functional immunology lab, Wagner group, and a functional protein chemistry/pharmaceutical sciences lab, Carpenter group. Major equipment include 2 flow cytometers, centrifuges, freezers, refrigerators, heating ovens, Fast Protein Liquid Chromotography (FPLC), Mass Spectrometer, electrospray triple quadrupole time of flight mass spectrometer, InfraRed spectrometer, Microplate readers for optical density studies, Agilent HPLC capable of performing reverse phase HPLC and size exclusion HPLC.

SBIR.gov SBC Registration Control ID Form

SBC CONTROL ID	SBC_000405651

FIRM INFORMATION					
Company	Op-T-Mune, Inc				
Address	[redacted]				
City	Denver	State	CO	Zip	80206
TIN/EIN	[redacted]	DUNS	[redacted]		
Company URL					
Number of Employees:	5				
Is this SBC majority-owned by multiple venture capital operating companies, hedge funds, or private equity firms?	No				
What percentage (%) of the SBC is majority-owned by multiple venture capital operating companies, hedge funds, or private equity firms?	0%				

SBC CONTROL ID	SBC_000405651

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: David Middle Name Last Name*: Wagner Suffix:

Position/Title*: Chief Scientific Officer
Organization Name*: Op-T-Mune, Inc.
Department:
Division:
Street1*: [redacted]
Street2:
City*: Denver
County:
State*: CO: Colorado
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 80206-3961

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: PD/PI Other Project Role Category:

Degree Type: PhD Degree Year: 1995

Attach Biographical Sketch*: File Name: 1248-Wagner_Bio_2016.pdf

Attach Current & Pending Support: File Name:

Tracking Number: GRANT12240201 Funding Opportunity Number: PA-16-303 . Received Date: 2016-09-02T13:28:53.000-04:00

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Martin Middle Name Last Name*: Yussman Suffix:

Position/Title*: Chief Medical Officer
Organization Name*: Op-T-Mune, Inc
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: Other (Specify) Other Project Role Category: Medical Advisor

Degree Type: MD Degree Year: 1997

Attach Biographical Sketch*: File Name: 1249-Yussman_Biosketch_2016.pdf

Attach Current & Pending Support: File Name:

NAME	POSITION TITLE
David H. Wagner, Jr.	Associate Professor, Univ of Colo School of Med
eRA COMMONS USER NAME (credential, e.g., agency login) [redacted]	Chief Scientific Officer, Op-T-Mune, Inc

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
King College, Bristol, TN	B.S.	06/84	Chemistry/Biology
East Tennessee State Univ. Johnson City, TN	M.S.	12/87	Biochemistry
Quillen College of Medicine, ETSU	PhD	12/94	Biomedical Sciences
National Jewish Medical Center, Denver, CO	Postdoc	12/98	Immunology
Barbara Davis Childhood Diabetes Center	Senior Postdoc	06/01	Diabetes/Immunology

A. Personal Statement: Over the last 15 years I have worked to understand the role of CD40 in autoimmune inflammation. As a PI my lab defined the Th40 cell subset. These are T cells within the CD4+ helper T cell category that express the CD40 co-receptor. We defined these T cells in autoimmune diseases including type 1 diabetes and multiple sclerosis. We demonstrated that Th40 cells achieve full effector function and that CD40 acts as a co-stimulus for these cells, thus describing an importantly overlooked alternative mechanism of T cell activation. We have explored Th40 cells in mouse models of disease and translationally moved these studies to human disease. We have examined over 200 T1D, 70 T2D and 150 control (non-autoimmune) human subjects to date. Now that my lab has developed a unique mechanism to control CD40 induced inflammation through use of a peptide that targets the CD154 binding site on CD40 expressing cells, we will be able to explore this novel therapeutic approach in appropriate atherosclerotic models. In addition this work will define a new means of examining atherosclerosis perhaps defining a new biomarker for this pernicious disease. As Chief Scientific Officer and Co-Founder of Op-T-Mune, Inc., I have worked to develop Intellectual Properties for improving diabetes diagnosis and treating autoimmune inflammation.

B. Positions and Honors:

Employment

2000 - 2002	Instructor	Dept. of Medicine, Univ. of Colorado Denver, School of Medicine
2002 - 2008	Assistant Professor	Dept of Medicine and Webb-Waring Center, UCDSOM
2006 -	Section Head	Immunology Section, Webb-Waring Center
2007-	Co-Founder	Op-T-Mune, Inc., Denver CO
2008 -	**Associate Professor**	Dept of Medicine, Univ. of Colorado Denver, School of Medicine
2009-	Chief Scientific Officer	Op-T-Mune, Inc., Denver, CO
2014 -	Associate Professor	Dept of Neurology, Univ. of Colorado Denver, School of Medicine

Professional Activities and Memberships:

2001 -		Member, American Association of Immunologists
2004 -		Professional Member, American Diabetes Association
Editor,	2011	Type 1 Diabetes: Pathogenesis, Genetics and Immunotherapy\ Publisher: Intech
Editor,	2011	Type 1 Diabetes: Disease Complications\ Intech Open Access Publisher
Editor,	2012	Conference Papers in Immunology/ Online journal
Senior Editor,	2013	American Journal of Clinical and Experimental Immunology
Editor	2014	Medical Genetics and Biomarkers
Session Chair	2005	Aegean Conferences, Santorini, Greece Mechanisms and Treatment of Autoimmunity,
Session Chair	2009	FASEB, San Francisco, CA *T cells and Autoimmunity*
Session Chair	2011	(AAI) Mechanisms in Autoimmunity Block Symposium, San Francisco, CA
Session Chair	2014	(AAI) “Genetic, Environmental and Cellular Risk for Disease”, Pittsburgh, PA

NIH Study Section,	2004-	HAI, Ad Hoc
NIH Study Section,	2013-	ETTN-M,
ADA Study Section	2009-2014	Regular Member

Honors:

2013	AAI Faculty Travel Award
2007	Philip R Lee Outstanding Career Development Award, American Diabetes Association
2002	AAI Outstanding Junior Faculty Award

C. Contributions to Science:

(1) Functional expression of CD40 on CD4$^+$ T cells. CD40 was long considered an Antigen Presenting Cell (APC) molecule. Its function was defined as inducing B cell antibody class switching. On other APCs CD40s' function was described as inducing pro-inflammatory cytokines. In early work I was involved in exploring CD40 on macrophages. My first significant independent discovery was that CD40 is functionally expressed on CD4+ T cells. These cells arise in the thymus and occur in the periphery of all mouse strains tested. Importantly CD40 expressing CD4+ cells occur in the periphery of all human subjects tested. One of the functions of CD40 on T cells is to subvert cell death by promoting Bcl2, Bcl-XL and cFLIP, molecules that promote cell survival. This discovery is related to autoimmune conditions. If fact when CD40 expressing T cells are isolated from autoimmune conditions, CD40 signals override Fas-mediated cell death signals. This discovery described a new biomarker for T cells; therefore we explored this finding further in disease models.

1. Wagner DH, Jr., Vaitaitis G, Sanderson R, Poulin M, Dobbs C, Haskins K: **Expression of CD40 identifies a unique pathogenic T cell population in type 1 diabetes**. *Proc Natl Acad Sci U S A* 2002, **99**(6):3782-3787.
2. Vaitaitis G, Waid DM, Wagner Jr. D: **The Expanding Role of TNF-Receptor Super Family Member CD40 (tnfrsf5) in Autoimmune Disease: Focus on Th40 Cells** *Curr Immunol Rev* 2010, **6**(2):130-137.
3. Vaitaitis GM, Wagner DH, Jr.: **Galectin-9 controls CD40 signaling through a Tim-3 independent mechanism and redirects the cytokine profile of pathogenic T cells in autoimmunity**. *PLoS One* 2012, **7**(6):e38708.
4. Poe JC, Wagner DH, Jr., Miller RW, Stout RD, Suttles J: **IL-4 and IL-10 modulation of CD40-mediated signaling of monocyte IL-1beta synthesis and rescue from apoptosis**. *J Immunol* 1997, **159**(2):846-852.
5. Wagner DH, Jr., Stout RD, Suttles J: **Role of the CD40-CD40 ligand interaction in CD4+ T cell contact-dependent activation of monocyte interleukin-1 synthesis**. *Eur J Immunol* 1994, **24**(12):3148-3154.

(2) CD40 induces RAG1/RAG2 production: In exploring the potential function of CD40, my lab determined that CD40 engagement of peripheral CD4$^+$ T cells induced RAG1 and RAG2 proteins, which constitute the machinery for TCR generation. This was the first demonstration of a cellular mechanism to induce RAG proteins. Secondarily to this we showed that peripheral T cells alter TCR expression in a process called TCR revision. While we not the first to demonstrate TCR revision, we were the first to demonstrate a mechanism to *induce* TCR revision in peripheral T cells. TCR revision is an immunologic paradigm shift; our contribution is the discovery of how revision can be induced. By being able to control TCR revision, this finding could lead to therapeutics to alter auto-aggressive TCR bearing cells, thus serving as an autoimmune treatment; increase vaccine potency by generating antigen specific T cells; and potentially promote the development of tumor specific T cells. Each of these applications have direct therapeutic potential.

1. Vaitaitis GM, Poulin M, Sanderson RJ, Haskins K, Wagner DH, Jr.: **Cutting edge: CD40-induced expression of recombination activating gene (RAG) 1 and RAG2: a mechanism for the generation of autoaggressive T cells in the periphery**. *J Immunol* 2003, **170**(7):3455-3459.
2. Vaitaitis GM, Wagner DH, Jr.: **CD40 interacts directly with RAG1 and RAG2 in autoaggressive T cells and Fas prevents CD40-induced RAG expression**. *Cell Mol Immunol* 2013, **10**(6):483-489.
3. Wagner DH, Jr.: **Re-shaping the T cell repertoire: TCR editing and TCR revision for good and for bad**. *Clin Immunol* 2007, **123**(1):1-6.

Th40 cells and effector functions: We discovered that CD40 expressing CD4$^+$ cells now termed Th40, achieve effector function and that CD40 engagement acts as a T cell co-stimulus. Importantly Th40 cells are capable of producing both Th1 (IFNγ, TNFα, and IL-2) but coincidentally produce IL-17 and IL-23 that are associated with Th17 cells. Th40 cells express both t-bet and RORγt transcription factors. We discovered that Th40 effector cells can express Foxp3, but rather than being classic regulatory cells, Foxp3 functions as an effector cell regulator. This is novel in terms of thinking about Foxp3 as being a T cell regualtory molecule rather than only defining regulatory T cells. In addition CD40 negatively impacts CTLA-4 expression to further ablate tolerance. We created a thymic expressed neo-self antigen to demonstrate that thymic expression of antigen drives T cell CD40 expression. The contribution here is first that self-antigen exposure in the thymus promotes CD40 expressing T cells, that CD40 constitutes an alternative T cell co-stimulatory molecule and that CD40 expression negatively impacts T cell tolerance. This opens entire new approaches to T cell biology. We described that infection models cause significant increases in CD40-bearing T cells. Infection further causes systemic increases in CD154, the natural ligand for CD40. This means that an entire realm of T cell co-stimulation has been overlooked. These findings also are paradigm shifting and require new focus on the biology of T cell responses.

1. Vaitaitis G, Waid DM, Wagner Jr. D: **The Expanding Role of TNF-Receptor Super Family Member CD40 (tnfrsf5) in Autoimmune Disease: Focus on Th40 Cells** *Curr Immunol Rev* 2010, **6**(2):130-137.

2. Vaitaitis GM, Wagner DH, Jr.: **High distribution of CD40 and TRAF2 in Th40 T cell rafts leads to preferential survival of this auto-aggressive population in autoimmunity**. *PLoS One* 2008, **3**(4):e2076.
3. Vaitaitis GM, Carter JR, Waid DM, Olmstead MH, Wagner DH, Jr.: **An alternative role for Foxp3 as an effector T cell regulator controlled through CD40**. *J Immunol* 2013, **191**(2):717-725.
4. Waid DM, Wagner RJ, Putnam A, Vaitaitis GM, Pennock ND, Calverley DC, Gottlieb P, Wagner DH, Jr.: **A unique T cell subset described as CD4loCD40+ T cells (TCD40) in human type 1 diabetes**. *Clin Immunol* 2007, **124**(2):138-148.
5. Carter J, Vaitaitis GM, Waid DM, Wagner DH, Jr.: **CD40 engagement of CD4+ CD40+ T cells in a neo-self antigen disease model ablates CTLA-4 expression and indirectly impacts tolerance**. *Eur J Immunol* 2012, **42**(2):424-435.

(4) Th40 cells and MS and T1D: We discovered that Th40 cells are associated with human disease. Th40 cells occur in all humans tested, over 600 thus far, but are significantly expanded in type 1 diabetes and in multiple sclerosis. The major risk factors associated with either T1D or MS are HLA haplotype. We discovered that regardless of HLA haplotype if autoimmune disease is present Th40 cells are highly expanded in percentage of peripheral lymphocytes as well as in actual number. Importantly, if human subjects carry high risk HLA alleles but do not have autoimmunity, Th40 levels are "normal". As in mice, Th40 cells from human autoimmune patients achieve effector function, with CD40 acting as a crucial co-stimulus. In T1D and MS Th40 cells achieve a unique antigen signature profile. Each patient tested have Th40 cells that display unique responses to associated self-antigens. These studies demonstrate the importance of Th40 cells as pathogenic effector cells in two distinct but related autoimmune diseases. This is especially relevant given the growing number of patients co-presenting with T1D and MS.

1. Wagner, DH. 2016. "**Of the multiple mechanisms leading to type 1 diabetes, T cell receptor revision may play a prominent role (Is type 1 diabetes more than a single disease?)**. Clin Exp. Immunol. In Press.

2. Waid DM, Schreiner T, Vaitaitis G, Carter JR, Corboy JR, Wagner DH, Jr.: **Defining a new biomarker for the autoimmune component of Multiple Sclerosis: Th40 cells**. *J Neuroimmunol* 2014, **270**(1-2):75-85.
3. Siebert JC, Inokuma M, Waid DM, Pennock ND, Vaitaitis GM, Disis ML, Dunne JF, Wagner DH, Jr., Maecker HT: **An analytical workflow for investigating cytokine profiles**. *Cytometry A* 2008, **73**(4):289-298.
4. Waid DM, Wagner RJ, Putnam A, Vaitaitis GM, Pennock ND, Calverley DC, Gottlieb P, Wagner DH, Jr.: **A unique T cell subset described as CD4loCD40+ T cells (TCD40) in human type 1 diabetes**. *Clin Immunol* 2007, **124**(2):138-148.
5. Wagner DH, Jr.: **The specific antigen approach in multiple sclerosis: can it ever be enough?** *Clin Immunol* 2012, **144**(2):139-141.
6. Wagner J, D.H.: **The Role of T cells in type 1 diabetes**. In: *Type 1 Diabetes: Pathogenesis, Genetics and Immuntherapy.* Edited by Wagner J, D.H., vol. 1. Croatia: INTECH; 2011: 660.

(5) A novel means of controlling CD40 mediated inflammation: Controlling CD40 mediated inflammation has long been a research area of hot pursuit. The only available means thus far has been monoclonal antibody, which has created numerous problems. Antibodies against CD40 do not work because they are agonistic. Antibodies against CD154 initially were successful but failed ultimately because human platelets express high levels of CD154 and the treatment antibodies coagulated platelets leading to thrombosis. We created a 15-amino acid peptide that targets the CD154 binding site of CD40. This peptide prevented diabetes onset in NOD mice and reversed hyperglycemia in 50% of new onset mice. The peptide restricted T cell infiltrations in to pancreatic islets. This discovery provides a completely new way of targeting a major inflammatory pathway without the use of antibodies. In fact this discovery may lead to entirely new ways of controlling receptor – ligand interactions by use of small targeting peptides designed to bind directly to receptors.

1. Vaitaitis GM, Olmstead MH, Waid DM, Carter JR, **Wagner DH, Jr**.: **A CD40-targeted peptide controls and reverses type 1 diabetes in NOD mice**. *Diabetologia* 2014, **57**(11):2366-2373.

D. Research Support:

ACTIVE

[redacted]

[redacted]

[redacted]

1R41AI113977-01 (Wagner) 06/15/2014 – 05/31/2015
NIH/NIAID $211,808
Project title: Developing a new treatment for MS: Controlling CD40 in EAE.

The aims of this project are to test a new treatment module, a small peptide designed to interact with CD40, to impact EAE disease development.

Previous Awards:

Granting agency: NIH/NIAID, R21 AI106011, Start and end dates: 04/01/2013 – 03/31/2015
Project title: Mechanism of action of a CD40 control peptide
Total award: $426,250
Role of applicant: PI

Granting agency: NIH/NIAID, R01 DK075013-01, Start and end dates: 04/01/2007 – 03/31/2013
Project title: CD40 induces RAG1 and RAG2: Mechanism for Breach of Tolerance.
Total award: $1,732,500
Role of applicant: PI
The purpose of this grant was to examine how T cell receptor changes induced by CD40 engagement led to alteration in tolerance. The primary focus was in type 1 diabetes. This grant explored CD40 induction of RAG1 and RAG2 proteins that are crucial for T cell receptor changes. The grant provided direct evidence of TCR revision, the ability of T cells to alter TCR expression in the periphery.

Op-T-Mune, Inc.
Granting Agency: NIHCD, R41 (SBIR Phase I), Start/End: 04/01/20013 – 07/01/2014
Project Title: Screening for Type 1 Diabetes: A blood test based on the CD40 biomarker
PI: Peter E. Nelson, Percent Effort: 51%
Consultant: David H. Wagner, Jr., Percent Effort, 5%
The aims of this grant are to determine how Th40 cell levels predict type 1 diabetes onset. Risk levels for high/intermediate/low to develop diabetes are the dominant feature. Pre-diabetic subjects are compared to new onset and long term diabetic subjects. Differences from non autoimmune controls are noted

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

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: Martin Glenn Yussman

eRA COMMONS USER NAME [redacted]

POSITION TITLE: Assistant Professor University of Colorado Medical School

Chief Medical Officer, Op-T-Mune, Inc.

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 Wisconsin, Madison, WI	BA	06/1991	Molecular Biology
University of Wisconsin, Madison, WI	Associate Research Specialist	06/1993	Calcium signaling and Microscopy
Universitatsklinik fur Frauenheilkunde, Innsbruck, Austria	Research Scholars exchange program	8/1994	cytokines and vitamins influence on the expression of EGFr and HER-2
University of Louisville, Louisville, KY	MD	06/1997	Medicine
University of Cincinnati, Cincinnati, OH	Residency	6/2000	Internal Medicine
University of Cincinnati, Cincinnati, OH	Research Fellowship	6/2002	Molecular mechanisms of cardiomyopathy
University of Cincinnati, Cincinnati, OH	Clinical Fellowship	6/2004	Invasive Cardiology

NOTE: The Biographical Sketch may not exceed five pages. Follow the formats and instructions below.

A. Personal Statement

Repaying the cost of medical training detoured my research career. Though I enjoyed success in clinical medicine, my fervor toward research involvement continued. During my career as a clinical cardiologist, I was a site lead investigator for the VIRGO study, IRB member and instructor for residency programs. This experience encouraged ongoing research discussions with Dr. Wagner, co-investigator for this project. Dr. Wagner is an immunologist whose chief interest has been both discovering the etiology of type 1 diabetes as well as developing earlier diagnostics. The collaborative effort with Dr. Wagner as well as previous research experience with groups at the University of Wisconsin and the University of Cincinnati have provided me the expertise necessary to successfully carry out the proposed work. I have become well versed in constructing realistic plans, timelines, and budgets as well as creating collaborative networks instilled from my administrative positions within hospital and office. Inspiring my network of prior community physician partners to collaborate in the research endeavor illustrates these capabilities. With my background in molecular biology and genetics and with advanced microscopy, immunohistochemistry and echo cardiology skills I have successfully researched and published in journals such as Circulation Research and Nature Medicine. The research involved pathologic molecular mechanisms characterized by structural derangements and rearrangements of myocardial tissue, cardiomyocyte death with gain and loss of function design and use of transgenic expression. I have returned to a full-time research career with a more mature outlook, expanding my fellowship goals. I am working toward a successful path with support from the Webb Waring Center and Cardiopulmonary division of the University of Colorado and my prior community cardiology division. I have forfeited my position as senior clinician with that division to return to my research passion which has now matured and expanded by clinical experience. My record is unique with both clinical and basic science expertise and experience preparing me to lead this and future projects. I am become Chief Medical Officer with Op-T-Mune to help this startup company expand its clinical/therapeutic development opportunities.

B. Positions and Honors

List in chronological order previous positions, concluding with the present position. List any honors. Include present membership on any Federal Government public advisory committee.

2014-Present	Affiliate Faculty University of Colorado Denver, Webb Waring Center
2014-Present	Chief Medical Officer, Op-T-Mune, Inc, Denver CO
2011-Oct 2014	General Invasive Cardiologist; SCL Rocky Mountain Cardiovascular Assoc. Cardiology Instructor St. Joes Internal medicine residency and Family practice residency programs. Admin/lead physician EMR transition/coordination
2009-2011	General Invasive Cardiologist; Rocky Mountain Cardiovascular Assoc.

	Cardiology Instructor St. Anthony Family practice residency program. Cardiology Preceptor, University of Colorado Denver school of Medicine Admin/lead physician EMR transition/coordination
2008-2009	Site Principal Investigator VIRGO study; Centura Research Center-Porter Adventist Hospital Heart Failure Quality Director, Porter Adventist Hospital
2004-2009	General Invasive Cardiologist; Cardiovascular Associates IRB Cardiology committee member, St. Anthony Hospital IRB Cardiology committee member, Porter Adventist Hospital Cardiology Instructor, St. Anthony Family practice residency program. Fellow American College of Cardiology
2002	First Prize Award, University of Cincinnati Fellowship Research Program
2002	Accepted to 8th Annual Astra Zeneca Young Investigators Forum
2001	First Prize Award, University of Cincinnati Fellowship Research Program
2001	Accepted to 7th Annual Astra Zeneca Young Investigators Forum

C. Contribution to Science

My publications through the research fellowship addressed the inciting molecular mechanisms regulating subcellular protein trafficking in the heart, and the pathological consequences of aberrant cardiomyocyte protein transport causing dilated cardiomyopathy. Additionally, by use of microarray analysis, a genetic program was identified for myocardial apoptosis in Gq-mediated and pressure –overload cardiac hypertrophy. A critical component of this apoptotic program was Nix/Bnip3L. Nix localized to mitochondria and caused release of cytochrome *c*, activation of caspase-3 and apoptotic cell death, when expressed in HEK293 fibroblasts. Nix overexpression showed the sufficiency of Nix to provoke apoptotic heart failure and identified downstream events mediating this response. This article has been cited over 225 times, and useful as a model for testing potential therapeutic interventions directed at mitochondrial stabilization. I served as the primary or co-investigator in all of these studies.

1. Hahn, H.S., et al., *Protein kinase Calpha negatively regulates systolic and diastolic function in pathological hypertrophy.* Circ Res, 2003. **93**(11): p. 1111-9.
2. Hahn, H.S., et al., *Ischemic protection and myofibrillar cardiomyopathy: dose-dependent effects of in vivo deltaPKC inhibition.* Circ Res, 2002. **91**(8): p. 741-8.
3. Yussman, M.G., et al., *Mitochondrial death protein Nix is induced in cardiac hypertrophy and triggers apoptotic cardiomyopathy.* Nat Med, 2002. **8**(7): p. 725-30.
4. Wu, G., et al., *Increased myocardial Rab GTPase expression: a consequence and cause of cardiomyopathy.* Circ Res, 2001. **89**(12): p. 1130-7.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Op-T-Mune, Inc.

Start Date*: 04-01-2017 **End Date*:** 03-31-2018 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	David		Wagner		PD/PI	[redacted]	3.60			[redacted]	[redacted]	[redacted]
2 .		Dan		Waid		Research Coordinator and Professional Research Assistant	[redacted]	3.60			[redacted]	[redacted]	[redacted]
3 .	Dr.	Martin		Yussman	MD	Chief Medical Officer, Medical Advisor	[redacted]	1.08			[redacted]	[redacted]	[redacted]

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Total Number Other Personnel					**Total Other Personnel**	
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-16-303 . Received Date: 2016-09-02T13:28:53.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: Op-T-Mune, Inc.

Start Date*: 04-01-2017 **End Date*:** 03-31-2018 **Budget Period: 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12240201 Funding Opportunity Number: PA-16-303 . Received Date: 2016-09-02T13:28:53.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: Op-T-Mune, Inc.

Start Date*: 04-01-2017 **End Date*:** 03-31-2018 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	[redacted]
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	[redacted]
8 . Travel to one national meeting	[redacted]
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Direct Costs	40.00	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: 1234-Justification_OpT_Diabetes_2016.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12240201 Funding Opportunity Number: PA-16-303 . Received Date: 2016-09-02T13:28:53.000-04:00

Budget Justification STTR:

Salaries: *Dr. David Wagner PhD*. PI, Chief Scientific Officer. Dr. Wagner is Co-Founder and Chief Scientific Officer of Op-T-Mune. Dr. Wagner will be r responsible for the biomedical research aspect of the grant. The basis for this application has extended from work in the Wagner Laboratory. Dr. Wagner will oversee experiments, assist in analyzing data, oversee maintenance. Dr. Wagner plays a central role in the operations of OpTMune including writing grants, coordinating business relations with the University of Colorado etc. 30% Effort, 3.6 cal months is requested in the program section. Dr. Wagner's effort will be split between Op-T-Mune and the University of Colorado Denver. UCD request is for 10% effort as described in the sub award budget to satisfy the University requirements.

Mr. Dan Waid, BSN, Medical Coordinator, Professional Research Assistant for University of Colorado and Chief Financial Officer for Op-T-Mune. Mr. Waid will be involved with animal experiments including EAE induction, obtaining tissues, flow cytometry including performing flow experiments (the Wagner Lab maintains its own flow cytometer, Mr. Waid oversees all aspects of upkeep for that machine) and flow analysis. Mr. Waid also will perform experiments with human blood (Physician's Assistant United States Navy, 1990 – 2000) and has been instrumental in establishing the CD4CD40/diabetes database. 30% effort, 3.6 Cal Months is requested.

Dr. Martin Yussman, MD. Dr. Yussman is a board certified Internal Medicine physician. He has been recruited as Chief Medical Officer to help Op-T-Mune expand clinical development expertise. For this project Dr. Yussman will be involved in data analysis, and chart reviews. We will be coordinating medical history and clinical observations to our testing module and Dr. Yussman will provide counsel in that respect. We are requesting 9%.

Materials and supplies: Antibodies for CD4, CD3 and CD40 are produced by Op-T-mune. In-house antibodies will be compared to commercial antibodies for titration and standardizations. For QC, fluorescenated antibodies will be purchased from commercial vendors to compare to our antibodies Antibody budget is $12,000. Calibration beads will be purchased and loaded with purchased fluors (Cost, $3100). Supplies including those for cell culture, tissue hood, culture hoods, media etc will be purchased. Peptides are made by the GMP accredited, CMO AmBioPharm, North Augusta, SC, peptides are at 99.5% purity. These peptides will be generated including the 8 different sized peptides for CD40 molecule. Peptides are generated with fluorescent tags. (Cost, $15,364) Buffers for suspending and storing peptides, blood collection supplies, needles, collection tubes, etc., will be purchased ($910). Total $28,740.

Subaward: The nature of the STTR mechanism requires University involvement. The Subaward budget is to the University of Colorado Denver Anschutz Medical Campus. The budget for the subaward is described in that section.

Indirect Costs: After consultation with NIH about small business indirect rates we set indirect at 40%. As reported to us by Mr. Kyle Kroneberger, the NIH Grants policy statement **18.5.4.3.1 cover 40% rule.** We were told that if the grant is awarded, this rate will be further negotiated with NIH. The Indirect costs as allowed by NIH, NIH Grants policy statement, include administrative allowances such as IP protection, business allowance, administrative issues etc.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)
Section A, Senior/Key Person	[redacted]
Section B, Other Personnel	
Total Number Other Personnel	
Total Salary, Wages and Fringe Benefits (A+B)	[redacted]
Section C, Equipment	
Section D, Travel	
1. Domestic	
2. Foreign	
Section E, Participant/Trainee Support Costs	
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
6. Number of Participants/Trainees	
Section F, Other Direct Costs	[redacted]
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	[redacted]
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	[redacted]
8. Other 1	[redacted]
9. Other 2	
10. Other 3	
Section G, Direct Costs (A thru F)	[redacted]
Section H, Indirect Costs	[redacted]
Section I, Total Direct and Indirect Costs (G + H)	[redacted]
Section J, Fee	

OMB Number: 4040-0001
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RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: University of Colorado Anschutz Medical Campus

Start Date*: 04-01-2017 **End Date*:** 03-31-2018 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	David	H	Wagner		PD/PI	[redacted]	1.20			[redacted]	[redacted]	[redacted]
2 .		Gisela		Vaitaitis		Professional Research Assistant	[redacted]	1.20			[redacted]	[redacted]	[redacted]

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Total Number Other Personnel					**Total Other Personnel**	
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-16-303 . Received Date: 2016-09-02T13:28:53.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: University of Colorado Anschutz Medical Campus

Start Date*: 04-01-2017 **End Date*:** 03-31-2018 **Budget Period: 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12240201 Funding Opportunity Number: PA-16-303 . Received Date: 2016-09-02T13:28:53.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: University of Colorado Anschutz Medical Campus

Start Date*: 04-01-2017 **End Date*:** 03-31-2018 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Animal housing	[redacted]
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Indirect Costs	[redacted]	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, Wally Chan, (415) 437-7829

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: 1242-Justification_Subaward_STTR.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12240201 Funding Opportunity Number: PA-16-303 . Received Date: 2016-09-02T13:28:53.000-04:00

Justification: University of Colorado Anschutz Medical Campus

Salaries

Dr. David Wagner PhD. Professor, Dept. of Medicine and Dept. of Neurology. With STTR grants, the University of Colorado requires faculty to include a portion of salary in the University budget. Dr. Wagner is not being paid twice, but his FTE is being divided between Univ. Colorado and Op-T-Mune. Dr. Wagner will be responsible for the biomedical research aspect of the grant. The basis for this application has extended from work in the Wagner Laboratory. Dr. Wagner will oversee experiments, be responsible for analyzing data, oversee maintenance of the diabetes database and coordinate future experiments. FTE will be 10% which is 1.2 calendar months.

Gisela Vaitaitis, MS, Professional Research Associate, 10% FTE year 1 and 8% for the last 6 months in year 2. Mrs. Vaitaitis will perform molecular biology and protein chemistry experiments. She oversees the Wagner Lab animal facility. She induces EAE in mice.

Animals: Because animals will be housed at the University of Colorado Anschutz Medical Campus, we are requesting a portion of animal housing on the University sub-award. Based on the number of animals requested in the vertebrate animal form, and housing for 1 year, we are requesting $12,000.

Supplies: Supply budget includes basic lab supplies, buffers, protein chemistry supplies, supplies for Mass spectrometer, mass spec rental time paid to school of pharmacy, MOG protein, pertussis toxin, CFA etc.

Indirect Costs: The University of Colorado Anschutz Medical Campus has a negotiated rate of 55.5%. We are including that as a separate Modified Direct Cost.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)
Section A, Senior/Key Person	[redacted]
Section B, Other Personnel	
Total Number Other Personnel	
Total Salary, Wages and Fringe Benefits (A+B)	[redacted]
Section C, Equipment	
Section D, Travel	
1. Domestic	
2. Foreign	
Section E, Participant/Trainee Support Costs	
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
6. Number of Participants/Trainees	
Section F, Other Direct Costs	[redacted]
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other 1	[redacted]
9. Other 2	
10. Other 3	
Section G, Direct Costs (A thru F)	[redacted]
Section H, Indirect Costs	[redacted]
Section I, Total Direct and Indirect Costs (G + H)	[redacted]
Section J, Fee	

Total Direct Costs less Consortium F&A

NIH policy (NOT-OD-05-004) allows applicants to exclude consortium/contractual F&A costs when determining if an application falls at or beneath any applicable direct cost limit. When a direct cost limit is specified in an FOA, the following table can be used to determine if your application falls within that limit.

Category	Budget Period 1	Budget Period 2	Budget Period 3	Budget Period 4	Budget Period 5	TOTALS
Total Direct Costs less Consortium F&A	[redacted]	0	0	0	0	[redacted]

OMB Number: 4040-0001
Expiration date: 06/30/2016

SBIR/STTR Information

Program Type (select only one)*

❍ SBIR ● STTR ❍ Both (See agency-specific instructions to determine whether a particular agency allows a single submission for both SBIR and STTR)

SBIR/STTR Type (select only one)*

● Phase I ❍ Phase II ❍ Fast-Track (See agency-specific instructions to determine whether a particular agency participates in Fast-Track)

Questions 1-7 must be completed by all SBIR and STTR Applicants:

1a. Do you certify that at the time of award your organization will meet the eligibility criteria for a small business as defined in the funding opportunity announcement?* ● Yes ❍ No

1b. Anticipated Number of personnel to be employed at your organization at the time of award.* 5

2. Does this application include subcontracts with Federal laboratories or any other Federal Government agencies?* ❍ Yes ● No

If yes, insert the names of the Federal laboratories/agencies:*

3. Are you located in a HUBZone? To find out if your business is in a HUBZone, use the mapping utility provided by the Small Business Administration at its web site: http://www.sba.gov * ● Yes ❍ No

4. Will all research and development on the project be performed in its entirety in the United States?* ● Yes ❍ No

If no, provide an explanation in an attached file. Explanation:*

5. Has the applicant and/or Program Director/Principal Investigator submitted proposals for essentially equivalent work under other Federal program solicitations or received other Federal awards for essentially equivalent work?* ❍ Yes ● No

If yes, insert the names of the other Federal agencies:*

6. Disclosure Permission Statement: If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?* ● Yes ❍ No

7. Commercialization Plan: If you are submitting a Phase II or Phase I/Phase II Fast-Track Application, include a Commercialization Plan in accordance with the agency announcement and/or agency-specific instructions.*

Attach File:*

OMB Number: 4040-0001
Expiration date: 06/30/2016

SBIR/STTR Information

SBIR-Specific Questions:

Questions 8 and 9 apply only to SBIR applications. If you are submitting ONLY an STTR application, leave questions 8 and 9 blank and proceed to question 10.

8. Have you received SBIR Phase II awards from the Federal Government? If yes, provide a company commercialization history in accordance with agency-specific instructions using this attachment.* ❍ Yes ❍ No

Attach File:*

9. Will the Project Director/Principal Investigator have his/her primary employment with the small business at the time of award?* ❍ Yes ❍ No

STTR-Specific Questions:

Questions 10 and 11 apply only to STTR applications. If you are submitting ONLY an SBIR application, leave questions 10 and 11 blank.

10. Please indicate whether the answer to BOTH of the following questions is TRUE:* ● Yes ❍ No

(1) Does the Project Director/Principal Investigator have a formal appointment or commitment either with the small business directly (as an employee or a contractor) OR as an employee of the Research Institution, which in turn has made a commitment to the small business through the STTR application process; AND

(2) Will the Project Director/Principal Investigator devote at least 10% effort to the proposed project?

11. In the joint research and development proposed in this project, does the small business perform at least 40% of the work and the research institution named in the application perform at least 30% of the work?* ● Yes ❍ No

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OMB Number: 0925-0001

Expiration Date: 10/31/2018

1. Human Subjects Section

Clinical Trial? ○ Yes ● No

*Agency-Defined Phase III Clinical Trial? ○ Yes ○ No

2. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ○ No

If "No" to AVMA guidelines, describe method and proved scientific justification

3. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ○ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

☐ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

5. Inventions and Patents Section (RENEWAL)

*Inventions and Patents: ○ Yes ○ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ○ Yes ○ No

6. Change of Investigator / Change of Institution Section

☐ Change of Project Director / Principal Investigator

Name of former Project Director / Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

☐ Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 10/31/2018

Introduction	
1. Introduction to Application (Resubmission and Revision)	
Research Plan Section	
2. Specific Aims	1243-Specific_Aims_Diabetes.pdf
3. Research Strategy*	1244-STTR_Diabetes_Peptide_Body.pdf
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Significance: In 2012, 9.3% of the population corresponding to over 29.1 million Americans were diagnosed with diabetes; this from the American Diabetes Association Statistics Fact Sheet in 2016. There are estimated to be an additional 7 million undiagnosed and 86 million “pre-diabetic” people in the US , up from 79 million in 2010. It is estimated from the most recent statistics than in 2012 1.25 million people had type 1 diabetes with possibly 350,000 undiagnosed [American Diabetes Association 2016 Fact Sheet]. Both the American Diabetes Association and the NIH estimate the yearly rate of new T1D diagnoses in the US to be approximately 95,000 people with that number inexplicably continuing to increase. T1D certainly affects children, but the fastest expansion in disease is occurring in individuals over age 20. Treatment for the disease is very limited; at present there are no medications that either prevent insulin loss, or restore insulin production.

Impact: Op-T-Mune, Inc. is a startup company created by Mr. Charlie Henry, entrepreneur, former CEO of Nostix, Inc., and Dr. David Wagner, Associate Professor at the University of Colorado School of Medicine. Our goals include the prevention and reversal of T1D by controlling the pathogenic cells that target islet beta cells and lead to loss of insulin production [1-10]. The Wagner Lab identified T cells demarcated by CD40 expression and reference them as Th40, in the NOD mouse model of T1D [3, 4, 6, 8-16]. An important translational link followed in that Th40 cells are significantly ($p < 10^{-4}$) increased in number in human T1D subjects, including long term and new onset diabetic subjects, compared to non-autoimmune controls [10]. Importantly, Th40 cells are expanded in number in a cohort of diagnosed pre-diabetic, type 1 human subjects (preliminary data), suggesting that induction of CD40 expression on T cells is involved early in disease development. Multiple studies show that controlling CD40 induced inflammation is both disease preventative and therapeutic [2, 9, 17-22]. Because CD40-expressing cells are highly pro-inflammatory and expand in number as T1D progresses [1, 5, 6, 9, 23, 24], they are a prime target for therapeutic control. Until now the only way to control CD40/CD154 interaction has been by monoclonal antibody or randomly generated, synthetic small molecules. Neither of these options has been successful and each creates unique complications [25-28]. *A therapeutic alternative that is effective and can be tolerated for long periods would therefore prove paradigm altering.*

We created a unique means to modulate CD40 through peptides derived from the CD154 protein sequence that encompass known hot spots for receptor – ligand interaction. These peptides avoid the complications associated with antibodies, and are physiologically precise, unlike random generated CD40-like small organic molecules. We have identified potential candidates one of which has proven effective at preventing diabetes onset in 96% of NOD mice, total of 200 tested, and reversing hyperglycemia in 57% of treated new onset diabetic mice, totaling 24 mice thus far (preliminary data).

Goals for Phase I: Establish a lead candidate peptide through:

1. Determining the impact on glucose tolerance testing (GTT)
2. Determining the duration of protection KGYY15 versus KGYY6
3. Determining the impact on insulin and c-peptide production

Goals for Phase II:

Determine the cells types that are impacted by the CD40 targeting peptide(s)
Determine potential immunogenicity of treatment peptide in short-term and long-term studies
Determine how the treatment impacts natural immunity
Perform extended pharmaco-kinetic studies
Perform toxicity studies

The end deliverable of the phase II funding will be to submit the IND application with the FDA.

Innovation: While blocking CD40/CD154 interaction to control autoimmune inflammation is not innovative, the method we have developed is. Current immuno-modulatory therapies predominantly rely upon monoclonal antibody treatments, which are less than ideal. Antibodies cross-react with unintended targets and cause nephritic complications. We propose using small peptides that bind to the CD40 receptor interactive site to prevent CD154 interaction. The other approach has been randomly generated, small organic molecules to theoretically target CD40. The approach, thus far, has proven completely imprecise and does not show clinical positive outcome. Our approach constitutes an entirely new way to precisely target known receptor/ligand pairs and avoid the complications and shortcomings of other methods.

Background of the Invention: T1D pathogenesis occurs when auto-aggressive cells infiltrate pancreatic islets, creating localized inflammation that leads to loss of insulin production. We determined that $CD4^+$ effector T cells become capable of expressing the CD40 receptor [3, 4, 6, 8-10, 12-15, 29], which then correlates with auto-inflammation. In the NOD model of T1D, CD40 is expressed on diabetogenic T cell clones [6] and as NOD mice progressively develop insulitis and eventually diabetes, CD40 expressing T cells (referred by us as Th40 for simplicity) drastically increase as a percentage of the $CD4^+$ compartment and in total number [3, 6, 8, 9, 11]. Th40 cells alone from pre-diabetic or diabetic NOD mice are necessary and sufficient to transfer diabetes to NOD.scid recipients, thus confirming cell type pathogenicity [6, 9]. Mice that receive $CD40^-$ T cells, even if those T cells were pre-activated, do not develop diabetes [8, 9]. Depletion of CD40 followed by depletion of Tregs also does not transfer diabetes [8]. These data demonstrate that controlling CD40 expression or function is therapeutic in T1D.

Important translational studies showed that Th40 cells are significantly increased in number in human T1D subjects compared to non-autoimmune or type 2 diabetes controls [10] and Figure 1]. This includes new onset as well as long – term (diagnosed up to 40 years in some cases) T1D subjects. In addition, we examined blood samples from recipients of islet transplants. That protocol requires severe immunosuppressive therapy for one year that is withdrawn over a 6-month period. Samples were taken 6 months after immunosuppressive therapy had ceased and Th40 cells already had rebounded to pathogenic levels (Fig. 1). All those subjects eventually experienced islet transplant failure suggesting a pathogenic link between Th40 cell levels and islet loss in human diabetes.

We examined samples from subjects meeting the American Diabetes Association (ADA) and NIDDK criteria for "pre-diabetes" determining that a cohort of those subjects has elevated Th40 cells (Fig. 2). Pre-diabetic subjects, normal glycemic index but many with abnormal glucose tolerance test (GTT) results, with Th40 cell levels at "high risk' responded to human islets while pre-diabetic subjects with Th40 cell levels at "low risk" did not respond to human islets (not shown). These data demonstrate that elevated Th40 levels pre-date hyperglycemia onset but more importantly may be causal in diabetes pathogenesis. Th40 cells are significantly increased in subjects carrying the T1D risk factors, HLA-DR4, DR3, DQ2 or DQ8 [10]. Importantly in subjects who do not carry the predictive haplotypes but nonetheless are diabetic, Th40 cells occur at highly expanded numbers [10]. Furthermore, in non-autoimmune subjects who carry diabetes associated haplotypes but do not develop T1D, Th40 percentages remain at normal levels [10]. These findings suggest that expanded percentages of Th40 cells are a more reliable risk factor/biomarker than HLA haplotypes and that disease occurs irrespective of HLA haplotype. I*n a double blind study measuring the levels of Th40 cells in individual subject (268 subjects thus far), we were successfully able to identify 97% of T1D subjects, differentiating T1D from controls or T2D subjects.*

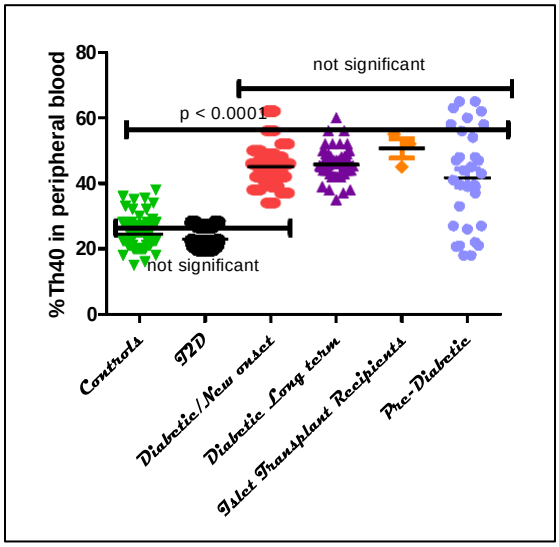


Figure 1: Th40 cells in human T1D subjects compared to controls. Th40 cell levels were determined from peripheral blood of T1D (n=268); controls (n=172); T2D (n=82); pT1D (n=32) subjects recruited from the Barbara Davis Childhood Diabetes Clinic. pT1D are NIH TrialNet subjects, meeting NIH and ADA criteria for pre-diabetes. Subjects were all healthy. T1D diagnosis met the ADA and NIH criteria. Average age of T1D is 26.2, control is 33.7. T1D subjects were further categorized as new onset (diagnosis < 6 months) and long term (diagnosis > 2 years, in some cases 40 years). Islet Transplant recipients (4 total) were > 2 years' post-transplant.

Th40 cells from T1D but not from control subjects proliferate and achieve effector function when exposed to insulin peptides, GAD peptides, and human islets [10, 13]. Th40 cells from T1D subjects but not from non-autoimmune controls produce significantly elevated levels of IL-6, IFNγ and TNFα, pro-inflammatory cytokines [10]. CD40 on T cells serves as a functional co-stimulus even independently of CD28, the classic T cell costimulus, inducing T cell activation, this makes CD40 an ideal candidate for therapeutic control.

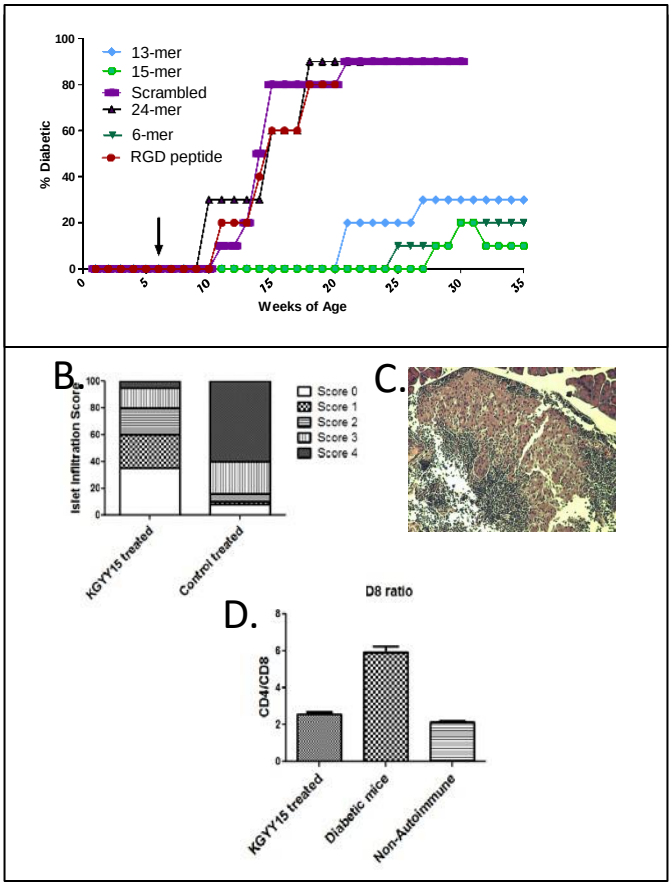
Controlling CD40 in Autoimmunity: Injecting an antibody to block CD40 – CD154 interaction (anti-CD154) prevents diabetes onset in NOD mice [9, 17]. We determined that the mechanism of action was to control the number of Th40 cells [9]. Importantly, Th40 cells were not eliminated but were contained at normal levels [9]. Based on these observations, CD40 proves to be not only a reliable biomarker in T1D but serves as a target molecule for controlling destructive T cell mediated inflammation.

Modulating CD40 – CD154 interactions thus far has relied

upon monoclonal antibodies, specifically anti-CD154, or randomly generated small synthetic molecules. Using anti-CD154 proved unsuccessful in clinical trials because platelets express CD154 and the treatment resulted in platelet aggregation and life-threatening thrombotic events [30] halting the Phase II trials. The block of CD40/CD154 was efficacious towards controlling the autoimmune diseases; the problems were that the anti-CD154 antibodies coagulated platelets [31-33]. Attempting to control CD40 using anti-CD40 has likewise proven non-fruitful, because the anit-CD40 antibodies that have been developed thus far are all agonistic. While this approach works well in cancer treatment (there currently is a Phase 2 trial anti-CD40 antibody) it is ineffective in autoimmunity that results from CD40 induced inflammation. Treating with anti-CD40 antibody will only exacerbate the autoimmune condition. The organically synthesized, randomly generated small molecule option also proved ineffective, lacking any specificity and demonstrating general performance failure as well as resulting in relatively high toxicity [34]. In view of these studies, we devised an innovative approach to blocking CD40 – CD154 interactions avoiding use of antibodies or synthetic organic chemical approaches. We designed *peptides* derived from the CD154 protein sequence that are capable of binding directly to the CD40 receptor to interrupt the inflammatory signal pathways. This approach is highly specific while avoiding toxicity problems.

Design of the peptides: The interaction motif between CD154 and CD40 was identified [35, 36]. Mutational analysis in the CD154 protein sequence suggested that a lysine (K) and two tyrosine (Y) residues at positions 145, 147 and 148, respectively, are necessary for CD40 interaction [36]. Considering this, we designed a series of small peptides that encompass those three amino acids (Fig. 2). Each peptide

Figure 2: Design of the KGYY15 peptide. Based on the amino acid sequence of the murine CD154 sequence (the human sequence is >90% homologous) a series of peptides was generated. The known CD40/CD154 interaction amino acids are highlighted. The 15-mer peptide is in green. The 15-mer sequence at the bottom shows the amino acids that are essential for functionality (orange) of the KGYY15 peptide in diabetes prevention assays. The relative peptide stability assessed by ExPaSY analysis is shown to the far right.



contains the K-G-Y-Y motif and the additional amino acids were taken from the actual CD154 protein sequence, thus all the peptides represent naturally occurring products. The stability of each peptide was initially assessed by ExPaSY, a software program that evaluates chemical stability based on numerous parameters; those values are indicated (Fig. 2). Stability was further measured using mass spec. The most stable peptide determined by both measures was the 15-mer (green), even though that peptide contains methionine in position 12 (Fig. 3). Structure to Activity Relationship (SAR) analysis revealed that the methionine residue surprisingly was critical to diabetes prevention functionality (Figure 5). The SAR was done under the

Figure 3, KGYY15 prevents diabetes onset in NOD mice. (**A**) NOD mice at 6 weeks of age were injected weekly iv with 25 ug (1 mg/kg) of each peptide through 45 weeks. Each cohort included 10 animals. Blood glucoses were measured and levels at 250 mg/dl or higher for 3 consecutive measures were considered diabetic. The RGD peptide is a 15 amino acid sequence from the CD154 sequence that does not include the CD40 binding motif. (**B**) Pancreata were excised and examined by histology for cellular infiltrates: 0 = no infiltrate; 1 = one pole infiltrate; 2 = peri-insulitis, bi-polar infiltrates; 3 = 75% infiltrate and 4 = full infiltration. (**C**) Representative level 2 islet. (**D**) CD4 to CD8 ratio in treated versus diabetic mice. Mice were gender and age matched. Data represent several experiments over a 5-year period.

guidance of Dr. John Carpenter, PhD, Co-Director of the University of Colorado Center for Pharmaceutical Biotechnology. Dr. Carpenter has extensive expertise in peptide to drug development and drug formulation and his expertise is sought out by many Pharmaceutical companies.

Preliminary Data: We established that a monoclonal antibody recognizing CD154 prevents diabetes onset in NOD mice and that prevention coincides with lower levels of Th40 cells in treated mice only [9]. Numerous studies show that blocking CD40 prevents inflammation and prevents or improves a variety of autoimmune diseases [9, 17, 18, 37, 38]. To address how our CD40 - targeting peptides perform, we administered each peptide to NOD mice and monitored diabetes onset (Fig. 3). Cohorts of 6 – week old mice, 10 in each group, were treated with the different peptides (Fig. 3). The data from six different experiments were consolidated. Based on the size, i.e. number of amino acids in the peptide, diabetes was increasingly controlled in treated animals (Fig. 3). The peptides at 8 and 10 amino acids were ineffective at diabetes prevention (not shown); while the 13-mer and 15-mer, were quite effective at diabetes prevention. The 15-mer in fact was highly effective, preventing diabetes in 90% of recipients. A larger 24-mer peptide was completely ineffective at controlling diabetes (Fig. 3). Interestingly, the 6 amino acid peptide was reasonably effective at prevention. A control peptide, RGD, composed of a 15 amino acid region upstream from the KGYY section that does not contain the CD40 binding motif, did not impact diabetes prevention (Fig. 3). In addition, a scrambled version of the KGYY15 peptide did not impact diabetes prevention. Mice were monitored for onset and severity of diabetes as defined by hyperglycemia and glucosuria. *We determined that KGYY15 treated mice had significantly ($p < 0.01$) sustained islet insulin granulation (not shown).* Histology of pancreata from KGYY15 treated mice demonstrated some islet infiltration (Fig. 3 B and C) but significantly ($p < 0.001$) less than untreated mice (Fig. 3 B). These data demonstrate certain of the peptide promotes islet cyto-protection. The numbers and proportionality of CD4 and CD8 cells were normal in long term (15 weeks) peptide treated mice (Fig. 3 D). B cell numbers were also at normal levels (not shown). While not conclusive, these data suggest that the peptide has limited negative effect on the overall immune system, but impacts the Th40 pathogenic compartment of that system.

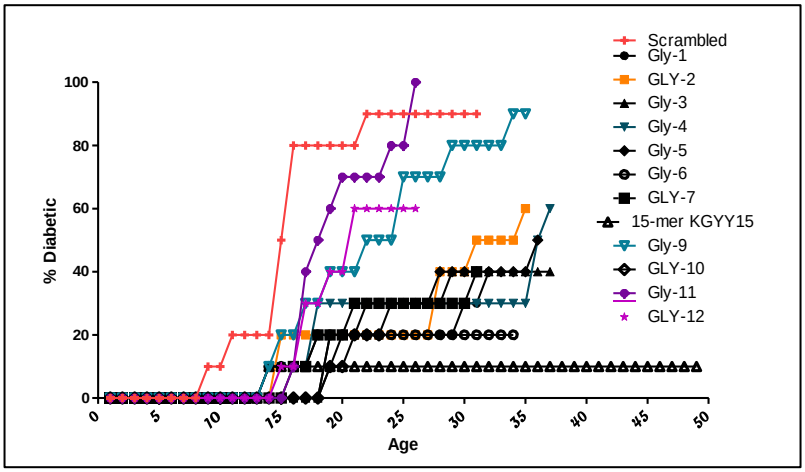


Figure 4: Structure to Activity Relationship in the KGYY15 peptide: Each amino acid of the 15-mer peptide was sequentially replaced by Glycine. Solubility and stability of each was assessed by mass spec, as described above. 25 ug (1 mg/kg) doses were administered weekly for 45 weeks. Each treatment group included 10 female NOD mice. Diabetes was considered blood glucose levels of > 250 mg/dl for 3 separate readings in a one-week period. Controls included the scrambled peptide (control) and the 15-mer KGYY15 unaltered (control) peptide. Positions 2, 9, 11 and 12 were determined to be critical. Interestingly position 12 methionine was critical for diabetes prevention functionality.

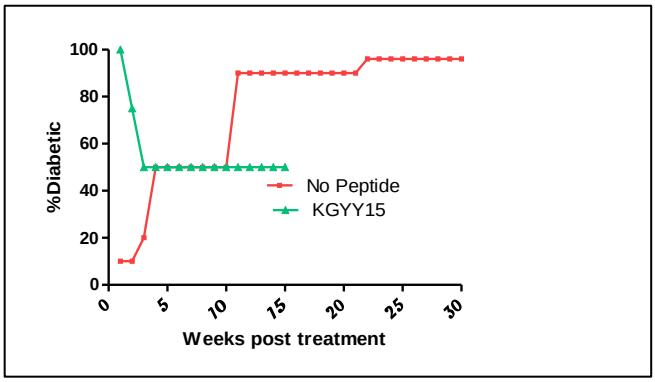


Figure 5: KGYY15 reverses hyperglycemia in new onset diabetic NOD mice. NOD mice were monitored for blood glucose levels > 250 mg/dl and were then treated with 4 mg/kg KGYY15 followed by an additional dose 2 days later and a half dose weekly after that. Blood glucose levels were monitored with 'normal' determined to be 120 mg/dl or less. Untreated NOD mice demonstrate normal diabetes kinetics (red line). Treated diabetic mice demonstrate reversal of hyperglycemia (green line). Data represent 24 mice, with 12 reversals.

Structure to Activity Relationship (SAR). We addressed the amino acids in the 15-mer peptide that are necessary for diabetes prevention activity. A glycine walk, sequential substitution of Gly for each amino acid in the peptide, was conducted. Substitutions at the Tyr position number 9, Thr 11 and Met 12 proved critical for diabetes prevention activity (Fig. 4). Methionine reportedly undergoes oxidation more easily than other amino acids and is considered to restrict peptide stability. However, from this SAR data the methionine in KYGG15 is

critical for function. We determined that the KGYY15 peptide containing methionine at position 12 is stable, demonstrating < 1% breakdown (determined by mass spec analysis) over 6 weeks in solution. The 24-mer peptide that did not prevent hyperglycemia experienced rapid breakdown, within 60 minutes, as measured by mass spec and as predicted by ExPaSY analysis. This suggests that placement of methionine in the peptide dictates overall stability as methionine is more centrally located in the 24-mer peptide and occurs near the terminus of the 15-mer peptide.

Reversing Diabetes: We examined how KGYY15 treatment affects established hyperglycemia in new onset diabetic NOD mice. Mice with blood glucose greater than 250 mg/dl, were treated with KGYY15 at hyperglycemia onset. KGYY15 at a concentration of 4 mg/kg was administered intravenously (i.v.) in a single dose and boosted daily for 7 days. Within 2 days 25% of treated mice had normal (< 120 mg/dl) blood glucose levels and at 3 weeks 57% were euglycemic (Fig. 5).

We determined that KGYY15 directly binds to CD40 cells using in vitro assays. PBL from mice were purified and stained with an anti-CD40 antibody and co-stained with a fluorescenated peptide. Human cells were stained with anti-CD4 and the peptide. The peptide remained stably bound to T cells for 5 days in cell culture. Importantly we determined that KGYY15 does not induce clotting (determined with the Department of Hematology at the University of Colorado). Human blood treated with KGYY15 had the same clot time as untreated (data not shown). Blood treated with anti-CD154 clots much more rapidly as the antibody binds and coagulates.

Approach: Our goal is to prevent and reverse diabetes onset by controlling the inflammatory CD40 pathway. We are developing targeted peptides that block the CD40 receptor. We tested 8 different peptides, each based on the CD40 interaction site of CD154 protein (Fig. 2). Two peptides show high efficacy at prevention and one, thus far, has shown high potential for hyperglycemia reversal (Fig. 4). In order to advance this therapeutic, we require reliable clinical readouts that eventually can be tested in human clinical trials. The goal of this phase 1 study is to determine a lead candidate peptide based on impact on glucose tolerance testing, sustained insulin production, measured in serum and observed in pancreatic islets, and impact on c-peptide levels as a measure of native insulin production.

Research Approach:

Goal # 1: Impact of the lead candidates on glucose tolerance testing. A final diagnosis of diabetes is relatively straight forward, high serum glucose and a failed GTT. Determining risk for developing diabetes is much less obvious. In the Diabetes Prevention Trial – Type 1 (DPT-1), a 12-hr fasting blood glucose of >126 mg/dl or a sustained oral GTT > 200 mg/dl, constitutes a diagnosis of T1D [39]. As subjects progress towards diabetes, glucose tolerance becomes less stable. For example, the clinical designation of pre-T1D includes an abnormal or deviated GTT. NOD mice, like human subjects who eventually develop T1D, undergo a relatively extensive pre-diabetes phase [40]. Over time, beta cell mass reduces substantially, finally insulin secretion is halted, thus establishing a diabetic state. As mice progress towards diabetes and beta cell mass is lost, the mice experience abnormal glucose tolerance [40]. To develop a clinical measure for our therapeutic, we will determine how each peptide impacts GTT over time. We will perform GTT on NOD mice at different stages of diabetes development; 2 - 4 weeks (no insulitis), 6 – 9 weeks (moderate insulitis, early “pre-Diabetes”, 12 – 15 weeks (extensive insulitis, late ‘pre-diabetes”) and after diabetes onset. As controls we will perform GTT on diabetes resistant, BALB/c mice. We will inject KGYY15 or KGYY6 into all mice using the prevention (1 mg/kg weekly for 12 weeks) strategy. Routes of administration will include IV, intra-peritoneal (IP), and subcutaneous (SC). Tests will be done on 5 of each test animal (NOD mice) compared to 2 controls (BALB/c); this powers the study to 95% confidence as determined by GraphPad Prism statistical analysis program. After 1 week, and every week following for up to 12 weeks, a GTT will be performed. Recovery rates, how quickly each animal reduces injected glucose will be determined. Changes over time will be recorded.

Outcomes and concerns: The technical issues of this type of analysis are straight forward, the GTT is straight forward. We predict that the therapeutics will improve GTT scores over the proposed time periods. Our strategy will determine if the 15-mer or 6-mer peptide provide optimal protection, thus establishing a single lead candidate. If no differences are detected, we will alter the dosing strategy and re-examine. If successful, this analysis will provide important documentation that GTT after therapeutic treatment is an appropriate monitor. We established both protective and reversal strategies in preliminary data. This aim clarifies a more clinically appropriate monitor that can be developed for eventual human trials. *Future considerations*: In Phase 2 studies we will address more long-term issues. Can either peptide be used after diabetes onset to 1) improve GTT; 2)

improve natural insulin production and 3) reverse hyperglycemia. ***Time frame***. Because we will be following mice for long periods, up to 45 weeks of age, 12 months will be required to complete the proposed studies. *Note:* In phase 2 studies, PK will be done on both the 15-mer and the 6-mer. The 15-mer peptide is more stable than other peptides and therefore may prove to have more long-lasting effect. The 6-mer has a much shorter predicted stability profile and may therefore prove to be fast – acting. Thus both options could prove highly valuable for drug development.

Goal #2: Determine the duration of protection using KGYY peptides? KGYY15 [2] and KGYY6 (Fig. 3) efficaciously control hyperglycemia when administered at 1 mg/kg on a weekly basis. It is important to determine how long each peptide is effective at sustaining euglycemia. This will be necessary for developing dosing standards. *Experiment #1:* Female NOD mice, 11 from each age group compared to 22 controls (This study is powered at 90% confidence determined by ClinCal.com sample size calculator) at the ages described above will be treated with 1, 2, 4, 8, 16 and 32 mg/kg of KGYY15 and KGYY6 in a single dose. Routes of administration will include: IV, IP, SC and IM. Mice then will be monitored until hyperglycemia occurs.
Experiment #2: Female NOD mice will be treated with 1, 2, 4 and 8 mg/kg of each peptide at 2 and 3 week intervals for 18 weeks. Mice will be continually monitored for hyperglycemia. *Outcomes and concerns*: The proposed experiments are straight forward. We have extensive experience with this experimental approach [2]. By performing dose escalation studies, we will effectively determine the most efficacious dose and route of administration. While the primary concern is lack of success, we showed that a 1mg/kg dose of KGYY15 administered weekly, prevents diabetes onset (Fig 3 and [2]); therefore, relative success is assured.

Goal #3: We will determine how each of the peptides maintain insulin and c-peptide. Mice will be treated as described in Goal 2, i.e. untreated, versus the various concentrations of KGYY15 and KGYY6. NOD and control BALB/c mice will be treated. At weekly intervals we will examine serum levels of insulin and c- peptide, using commercially available kits. At each time point, some mice will be removed for pancreatic analysis, including islet mass determination, and histology to determine islet infiltration and insulin production. Histologic sections will be stained by H&E to measure cell infiltrates and with anti-insulin. *Outcomes and concerns*: These studies will correlate insulin production in the pancreas to corresponding levels that can be detected in serum. This type of analysis cannot be performed in human. However, given that the NOD T1D model is a reasonable model for human disease; and more importantly, because CD40 now has been clearly demonstrated to have strong pathogenic roles in NOD mice [1, 2, 4, 5, 8, 11, 23, 24, 41] and human disease [7, 10, 13], this approach is valid. The outcome will be to determine that serum insulin levels and c-peptide levels will be sustained by the peptide treatments. That is, while the peptides are preventing hyperglycemia, insulin production and c-peptide can be detected in serum. This will translate as another clinical measure for eventual human clinical trials.

Milestones and Rationale: Preliminary data establish proof of principle i.e., the 15-mer peptide prevents and reverses diabetes onset. An interesting aspect is that the 15-mer peptide is extremely stable, even though it contains methionine at position 12 and the 6-mer, while relatively effective at diabetes prevention has a short half-life. Either option binds extracellularly as opposed to requiring cellular uptake for functionality. Thus our peptides are less subjected to cellular breakdown. When the receptor with bound peptide is internalized, the drug can be re-administered; this may explain the functionality of the 6-mer, which will be addressed specifically in phase 2. We speculate that the cell surface binding alters cellular responses. It is possible that the peptide binds to dendritic cells, B cells, or monocytes and alters the response of those cells to generate pathogenic effector cells. In either case, the administration of the peptide prevents T1D onset.
Time course*:* Goal 1 will require 12 months. Goals 2 and 3 can be done concurrently. These projects should be completed in 12 months.

Phase II studies will focus on PK and toxicology studies and will require up to 4 years. An expert in peptide drug development, Dr. John Carpenter will be recruited as a collaborator for the phase 2 application. Dr. Carpenter and his team will greatly facilitate development of KGYY15 from a bulk drug substance to a viable drug option. In addition, Op-T-mune has hired a consulting firm, CliniPace, LLC, for advancing IND filing. **Patent coverage** for the peptides is in place PCT Application No. PCT/US2015/022033. *Office action:* We received notification that the patent will be awarded in the US and Europe.

1. **Animals to be used in this study.** Animals used in this study will include NOD mice that spontaneously develop type 1 diabetes, NOD.scid for adoptive transfers and BALB/c as controls. Mice will be injected with each of the test peptides, scrambled control, non-relevant peptide control and vehicle controls. Based on previous observations where significant differences in disease onset were achieved sample size powered to 90% require 10 mice per treatment group. There will be 5 treatment groups and 5 different treatment concentrations requiring 250 mice. Glucose tolerance testing will be done on each set of mice. NOD.scid recipient mice will be used for adoptive transfers requiring 80 mice based on previous cell transfer studies.

2. **Justification of animal numbers.** The number of mice required for statistical significance was determined by t-test sample size calculations based on an alpha of 0.05, confidence of 0.95 and known differences of the mean between age of onset and delayed onset with treatments.

3. **Veterinary care for animals**. All animals will be housed at the University of Colorado Denver, vivarium. Four full time veterinarians and a large Vet Tech staff are available. The UCD animal facility is full accredited by PHS and AAALAC. All procedures are performed under IACUC approved protocols.

4. **Discomfort, distress, pain and injury are always alleviated**. Most procedures require only simple injections. There is limited discomfort, distress pain or injury with injections. Discomfort and distress are associated with diabetes development, but animals are never allowed to proceed to severe categories. At hyperglycemia animals typically are euthanized immediately. If animals are maintained for longer than 1 week when diabetic, animals are administered insulin and blood glucose levels monitored. Analgesics will be provided when they don't alter experimental results. Insulin will be provided when animals become hyperglycemic. Glucose tolerance testing will involve injecting mice with high dose glucose and monitoring by tail vein clip to obtain blood. Blood glucose is analyzed every 30 mins, but additional tail clip is not required, just removing the scab.

5. **Euthanasia of Mice:** Euthanasia of mice will be done by controlled CO2 exposure followed by cervical dislocation. This method is the more common and least painful method available. This method is approved by the Panel on Euthanasia of the American Veterinary Medical Association, and by the UCD IACUC.

No select agents will be used.

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Resource Sharing: The resources in this grant include proprietary peptides. Patent coverage for the peptides is in place. Otherwise, standard mice that are commercially available will be used.