Notice of Award

EXPLORATORY/DEVELOPMENT GRANT
Department of Health and Human Services
National Institutes of Health

Federal Award Date: 07/16/2019

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 5R21AI130485-02
FAIN: R21AI130485

Principal Investigator(s):
Abhay R Satoskar (contact), MD
Elyes Zhioua, PHD

Project Title: A live attenuated vaccine for leishmaniasis

Redacted by agreement
Research Foundation
Health Sciences Office
B-034 Graves Hall
Columbus, OH 43210

Award e-mailed to: NIHaward@osu.edu

Period Of Performance:
Budget Period: 08/01/2019 – 07/31/2020
Project Period: 08/01/2018 – 07/31/2020

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $166,973 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to OHIO STATE UNIVERSITY in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R21AI130485. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Redacted by agreement

Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5R21AI130485-02

Award Calculation (U.S. Dollars)

Federal Direct Costs	$129,671
Federal F&A Costs	$37,302
Approved Budget	$166,973
Total Amount of Federal Funds Obligated (Federal Share)	$166,973
TOTAL FEDERAL AWARD AMOUNT	$166,973
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$166,973

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
2	$166,973	$166,973

Fiscal Information:

CFDA Name:	Allergy and Infectious Diseases Research
CFDA Number:	93.855
EIN:	1316025986A1
Document Number:	RAI130485A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2019

IC	CAN	2019
AI	8472350	$166,973

NIH Administrative Data:
PCC: M92 / **OC**: 414E / **Released**: eRA Commons User Name 07/10/2019
Award Processed: 07/16/2019 12:04:24 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R21AI130485-02

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R21AI130485-02

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2**.** As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V,

Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase VI Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots.

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R21AI130485. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

This award represents the final year of the competitive segment for this grant. See the NIH Grants Policy Statement Section 8.6 Closeout for complete closeout requirements at: http://grants.nih.gov/grants/policy/policy.htm#gps.

A final expenditure Federal Financial Report (FFR) (SF 425) must be submitted through the eRA Commons (Commons) within 120 days of the period of performance end date; see the NIH Grants Policy Statement Section 8.6.1 Financial Reports, http://grants.nih.gov/grants/policy/policy.htm#gps, for additional information on this submission requirement. The final FFR must indicate the exact balance of unobligated funds and may not reflect any unliquidated obligations. There must be no discrepancies between the final FFR expenditure data and the Payment Management System's (PMS) quarterly cash transaction data. A final quarterly federal cash transaction report is not required for awards in PMS B subaccounts (i.e., awards to foreign entities and to Federal agencies). NIH will close the awards using the last recorded cash drawdown level in PMS for awards that do not require a final FFR on expenditures or quarterly federal cash transaction reporting. It is important to note that for financial closeout, if a grantee fails to submit a required final expenditure FFR, NIH will close the grant using the last recorded cash drawdown level. If the grantee submits a final expenditure FFR but does not reconcile any discrepancies between expenditures reported on the final expenditure FFR and the last cash report to PMS, NIH will close the award at the lower amount. This could be considered a debt or result in disallowed costs.

A Final Invention Statement and Certification form (HHS 568), (not applicable to training, construction, conference or cancer education grants) must be submitted within 120 days of the expiration date. The HHS 568 form may be downloaded at: http://grants.nih.gov/grants/forms.htm.

This paragraph does not apply to Training grants, Fellowships, and certain other programs—i.e., activity codes C06, D42, D43, D71, DP7, G07, G08, G11, K12, K16, K30, P09, P40, P41, P51, R13, R25, R28, R30, R90, RL5, RL9, S10, S14, S15, U13, U14, U41, U42, U45, UC6, UC7, UR2, X01, X02.

Unless an application for competitive renewal is submitted, a Final Research Performance Progress Report (Final RPPR) must also be submitted within 120 days of the period of performance end date. If a competitive renewal application is submitted prior to that date, then an Interim RPPR must be submitted by that date as well. Instructions for preparing an Interim or Final RPPR are at: https://grants.nih.gov/grants/rppr/rppr_instruction_guide.pdf. Any other specific requirements set forth in the terms and conditions of the award must also be addressed in the Interim or Final RPPR. *Note that data reported within Section I of the Interim and Final RPPR forms will be made public and should be written for a lay person audience.*

NIH strongly encourages electronic submission of the final invention statement through the Closeout feature in the Commons, but will accept an email or hard copy submission as indicated below.

Email: The final invention statement may be e-mailed as PDF attachments to: NIHCloseoutCenter@mail.nih.gov.

Hard copy: Paper submissions of the final invention statement may be faxed to the NIH Division of Central Grants Processing, Grants Closeout Center, at 301-480-2304, or mailed to:

National Institutes of Health
Office of Extramural Research
Division of Central Grants Processing
Grants Closeout Center
6705 Rockledge Drive
Suite 5016, MSC 7986
Bethesda, MD 20892-7986 (for regular or U.S. Postal Service Express mail)
Bethesda, MD 20817 (for other courier/express deliveries only)

NOTE: If this is the final year of a competitive segment due to the transfer of the grant to another institution, then a Final RPPR is not required. However, a final expenditure FFR is required and should be submitted electronically as noted above. If not already submitted, the Final Invention Statement is required and should be sent directly to the assigned Grants Management Specialist.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R21AI130485-02

Clinical Trial Indicator: No
This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

This award may include collaborations with and/or between foreign organizations. Please be advised that short term travel visa expenses are an allowable expense on this grant, if justified as critical and necessary for the conduct of the project.

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This Notice of Award (NoA) includes funds for activity with **Pasteur Institute of Tunis- TUNISIA.**

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This award reflects current Federal policies regarding Facilities & Administrative (F&A) Costs for foreign grantees including foreign sub-awardees, and domestic awards with foreign sub-awardees. Please see: Chapter 16 Grants to Foreign Organizations, International Organizations, and Domestic Grants with Foreign Components, Section 16.6 "Allowable and Unallowable Cost" of the NIH Grants Policy.

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The Research Performance Progress Report (RPPR), Section G.9 (Foreign component), includes reporting requirements for all research performed outside of the United States. Research conducted at the following site(s) must be reported in your RPPR:

Pasteur Institute of Tunis- TUNISIA

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Redacted by agreement
Email: Redacted by agreement **Phone:** Redacted by agreement **Fax:** 301-493-0597

Program Official: Redacted by agreement
Email: Redacted by agreement **Phone:** Redacted by agreement

SPREADSHEET SUMMARY
GRANT NUMBER: 5R21AI130485-02

INSTITUTION: OHIO STATE UNIVERSITY

Facilities and Administrative Costs	Year 2
F&A Cost Rate 1	56%
F&A Cost Base 1	$66,611
F&A Costs 1	$37,302
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A. COVER PAGE

Project Title: A live attenuated vaccine for leishmaniasis	
Grant Number: 5R21AI130485-02	**Project/Grant Period:** 08/01/2018 - 07/31/2020
Reporting Period: 08/01/2018 - 07/31/2019	**Requested Budget Period:** 08/01/2019 - 07/31/2020
Report Term Frequency: Annual	**Date Submitted:** 07/02/2019
Program Director/Principal Investigator Information: ABHAY R SATOSKAR , PHD MD MBBS **Phone number:** Redacted by agreement **Email:** Redacted by agreement	**Recipient Organization:** OHIO STATE UNIVERSITY OFFICE OF SPONSORED PROGRAMS 1960 Kenny Road COLUMBUS, OH 432101016 **DUNS:** 832127323 **EIN:** 1316025986A1 **RECIPIENT ID:**
Change of Contact PD/PI: N/A	
Administrative Official: Redacted by agreement Research Foundation Health Sciences Office B-030 Graves Hall Columbus, OH 43210 **Phone number:** Redacted by agreement **Email:** Redacted by agreement @osu.edu	**Signing Official:** Redacted by agreement Research Foundation Health Sciences Office B-030 Graves Hall Columbus, OH 43210 **Phone number:** Redacted by agreement **Email:** Redacted by agreement @osu.edu
Human Subjects: No	**Vertebrate Animals:** Yes
hESC: No	**Inventions/Patents:** No

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

In this project, we propose to (Aim 1) optimize GLP-LmCen-/- immunogenicity and immunization protocol and determine its safety in dogs and (Aim 2) evaluate efficacy of GLP-LmCen-/- as a vaccine using a novel model of canine VL in which dogs are naturally exposed to bites of L. infantum infected wild Phlebotomus pernicious in VL- hyperendemic regions of Tunisia. The scientific promise of this project, if successful, could provide the foundation for advancing LmCen-/- parasites as a vaccine against leishmaniasis in humans.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: B2.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

We will complete immunological analysis of dogs from the ongoing study. In this study dogs were vaccinated with different doses of LmCen vaccine manufacture by Genova. A group of dogs will also receive a booster immunization. In July, we will immunize a group of 12 dogs with the LmCen dose that is deemed optimal and these dogs will be transported to the field to be housed in VL endemic region during high sand fly season from the months of August to October. The dogs will be transported back to Pasteur Institute Tunis and will be monitored for the development of disease by assessing a variety of clinical, biochemical and hematological parameters. Based on our studies, we believe that the dose of 10^6 LmCen is the optimal dose for the vaccine. Finally, the dogs which have been already vaccinated will be challenged in the laboratory by infecting them intravenously with L. infantum.

Progress report

After this grant was awarded there were some concerns raised by US Department of State about conducting Leishmaniasis research in Tunisia. Drs Satoskar and Zhioua visited US Embassy in Tunis and clarified the objectives of the project alleviating the concerns of the Department of State. Therefore, the start of work on this project was delayed significantly, nonetheless, we have made a significant progress outline below.

Determination of optimal immunogenic LmCen-/- vaccine dose

In this study we used 16 dogs which were immunized as shown below:

G1. 4 dogs : medium with serum (sent from Gennova).

G2. 4 dogs : 10^6 *Lm*Cen-/- per 50 µl (parasites maintained in culture)

G3. 4 dogs : 10^6 *Lm*Cen-/- per 100µl medium with serum

G4. 4 dogs : 10^7 *Lm*Cen-/- per 100µl medium with serum

Immunization was done by intradermal injection of 100µl of vaccine on the ear of dog.

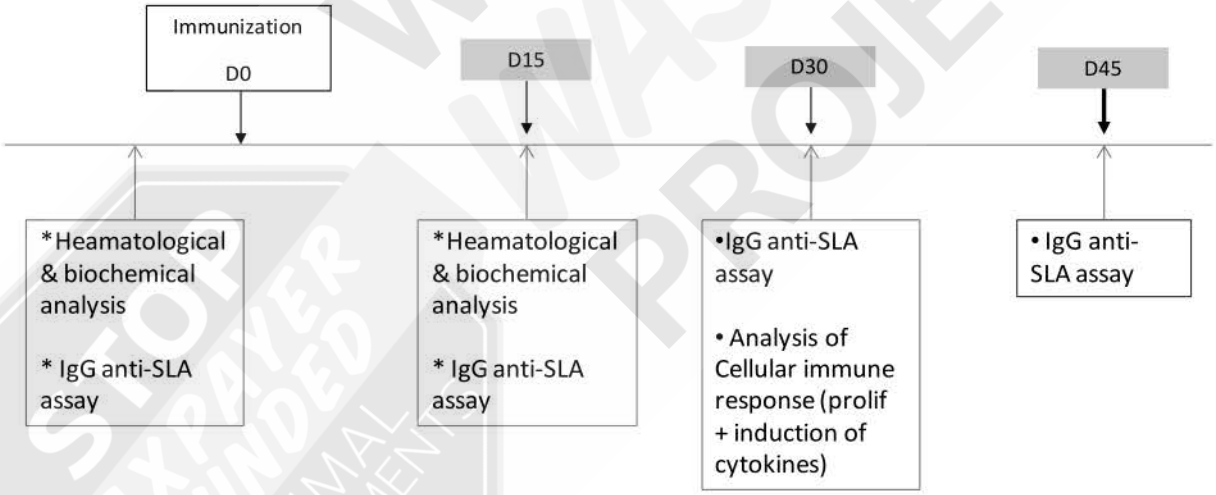

Figure. Experimental protocol of monitoring of clinical, hematological and immunological parameters within immunized dogs

1- **Clinical, hematological & biochemical data on vaccinated dogs**

- ***Results of clinical monitoring of dogs***

	Results of Clinical exam
G1	We did not detect any local reaction or a scratch lesion. Actually, the papule was totally resorbed after 24h of injection.
G2	Dogs showed a local reaction with a persistant papule for one dog (until 28 day

	post-vaccination). A slight adenopathy was noticed in those dogs between the 4th day and one week post-vaccination.
G3	We noticed in two dogs a local reaction and a slow resorption of the papule. A total resorption was perceived after two weeks of injection. One dog has a mild fever in the 3rd day post vaccination
G4	Dogs showed a papule with a local reaction and an adenopathy between the 4th and the 7th day post-vaccination.

Results of hematological & biochemical analysis

Individual results were shown in the attached table. To resume:

Biochemical analysis	Hematological analysis
• ↗ urea level • mild to moderate ↗ liver enzymes (**AST**, **ALT**) For G1, G2, G3 and G4	• Variation in titer of leukocytosis, lymphocytosis • ↗ hemoglobin concentration (might be due to the stress at the time of blood sampling), • mild thrombocytosis.

These variations are not very indicative clinically.

2- Antibody response within vaccinated dogs

Leishmania-specific IgG Total titers and IgG1and IgG2 levels were measured by indirect ELISA. Briefly, 96 well micro titer plates were coated overnight with 2µg/mL of SLA from *L. major*. For IgG Total, IgG1 and IgG 2 analysis, sera were added at a 1:250 dilution. Peroxidase-conjugated rabbit anti-dog IgG, IgG1 and IgG2 antibodies were added at a 1:5000 dilution for 1 h. The reaction was developed using TMB and absorbance was measured at 450 nm.

As shown in the figure below immunization with *Lm*Cen-/- at a dose of 10^6/ 100µl induced the highest levels of *Leishmania* IgG. These levels are higher at 15 days post immunization but decreased in function of time. IgG1/IgG2 ratio is < 1 indicating a predominance of IgG2 suggesting a protective humoral response.

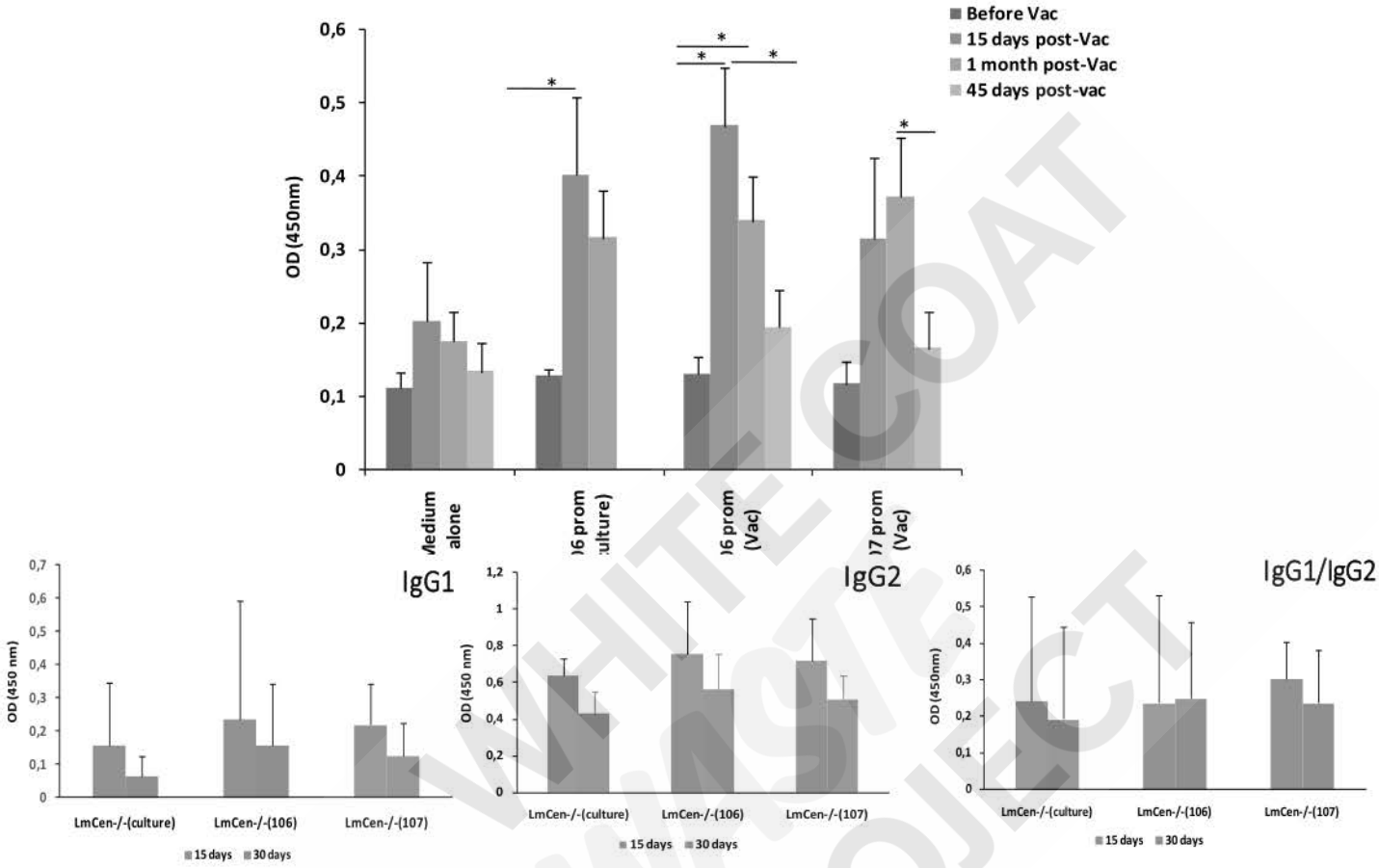

Figure. Specific antibody production at different time after immunization. ELISAs anti-SLA from L. major were performed to detected production of total IgG **(A)**, IgG1 **(B)** and IgG2 **(C)**. **(D)** the ratio IgG1/IgG2. The antibody OD values are shown on the y-axis, and the error bars indicate the standard deviation. Dotted line represents the cut-off value. Significant differences are indicated on the graphs (*p < 0.05)

3. Cellular immune response within vaccinated dogs

One month after immunization, 10 mL of blood taken from dog were used to isolate PBMC by density gradient centrifugation to be used for cell culture experiments.

1- Lymphoproliferative response to *L.major* SLA

Cell culture experiments were performed in triplicate using 1× 10^5 PBMC per well, in a final volume of 100 µL complete RPMI-1640 medium. Cells were stimulated with 10 µg/well of PHA or 10 µg/well of *L. major* SLA. Cell proliferation analysis was performed on PBMC labeled with BrdU.

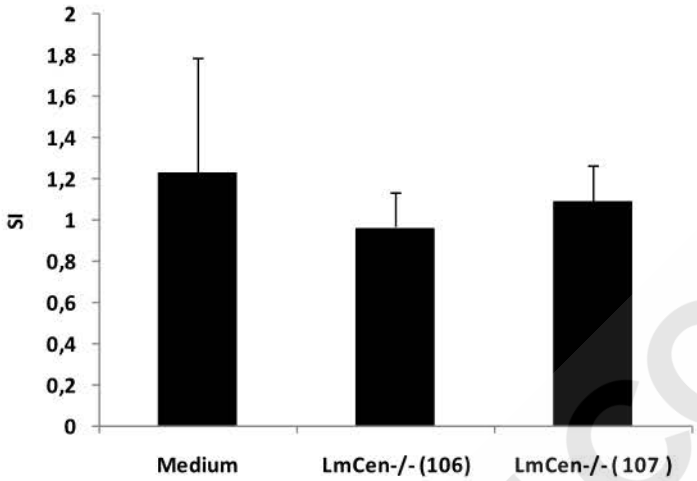

Figure. Cell proliferative responses in animals immunized with different doses of *Lm*Cen-/-. PBMC from dogs were incubated with *L. major* SLA or PHA for 5 days. Proliferation responses were expressed in terms of stimulation ratio that was calculated as: mean proliferation response of cultures stimulated SLA *L. major*/mean proliferation response of unstimulated cultures.

No significant differences were detected between proliferation responses within dogs immunized with LmCen-/- (at different doses) and those injected with medium.

2. Cytokines production in response to *L.major* SLA

Cell culture experiments were performed in triplicate using 5× 10^5 PBMC per well, in a final volume of 500 µL complete RPMI-1640 medium. Cells were stimulated with 10 µg/well of PHA or 10 µg/well of *L. major* SLA. Supernatants collected after 24, 48 and 72 h incubation, supernatants were used for detection of IL-12 (24h), IL-4 and IL-10 (48h) and IFN-γ and TNF-α (72h).

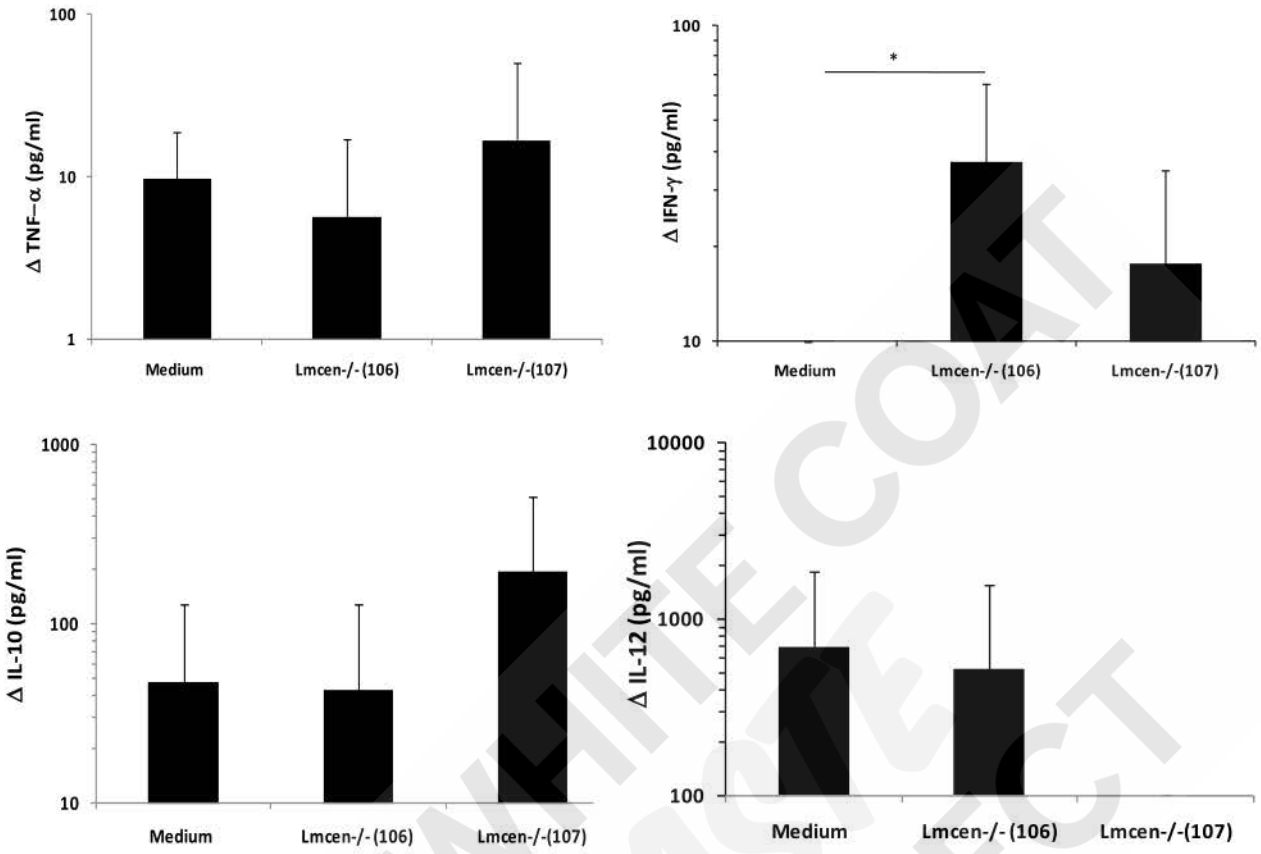

Figure. Production of cytokines in dogs immunized with different doses of *Lm*Cen-/-. Detection of TNFα, IFN-γ, IL-10 and IL-12/IL-23p40 in cell cultures after stimulation with *L. major* SLA. Results are expressed as delta (production of cytokines in cultures stimulated with SLA *L. major* minus production of cytokines in unstimulated cultures

We detected significant levels of IFN-γ in culture supernatants from dogs immunized with 106 *Lm*Cen-/- compared to the other groups. Nonetheless, we pointed out high levels of TNFα and IL-10 within dogs immunized with 10^7 *Lm*Cen-/-.

Conclusions and prospects:

We showed that the *Lm*Cen$^{-/-}$ at dose of 10^6/ 100µl seems to be better. It induces a humoral response (high levels of IgG specific of *Leishmania*) but a weak cellular immune response (induction of IFN-γ).

	Biochemicalparameters						Hematoligicalparameters											
							Red blood cells						White blood cells					
	Urea	Creatinine	Total proteins	AST	ALT	GGT	Red blood cells	Hemoglobin	Hematocrit	MCV	MCH	MCHC	White blood cells	PNE	Lymphocytes	Neutrophiles	Monocytes	Thrombocytes
Usuel Values	3,3 - 6,6 mmol/l	60-130 µmol/L	54 -71 g/l	6-13 IU/l	5-25 IU/l	2-10 IU/l	5,3-8,3 10^12/l	12-18 g/dl	37- 55 %	60- 77 fl	20-25 pg	32 – 36g/dl	6 – 18 10^9/l	2-10 %	12 à 30%	60-75%	3 à 10%	200-500 10^9/l
4452	8,83	65	66,6	52	42	5	7,93	19,1	52,2	65,8	24,1	36,6	13,620	0,2	26	73,8	0	400
4454	10,33	85	70,7	61	35	5	7,19	17,9	48,5	67,5	24,9	36,9	12,520	0,3	37,3	62,4	0	435
4458	10,83	94	66,8	67	30	3	6,73	15,6	44,6	66,3	23,2	35	14,010	0,3	34,7	64,8	0	471
4471	6,83	65	66,6	47	36	4	7,18	16,8	47,1	65,6	23,4	35,7	10,870	0,5	28	71,4	0	401
4457	8	71	69,2	44	26	3	7,20	17,7	48,8	67,8	24,6	36,3	9,660µl	0,7	28,2	71,1	0	486
4459	12,50	77	67,2	53	39	4	7,61	18,4	52,1	68,5	24,2	35,3	14,930µl	0,2	44,6	55,1	0	526
4460	8,50	67	-	45	48	3	6,99	18	47,5	68	25,8	37,9	19,220µl	0	27,7	72,3	0	514
4493	9,83	86	65,3	44	37	4	7,45	19	52,3	70,2	25,5	36,3	12,220µl	0,1	26,7	73,1	0	376
4455*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4485	11	79	71,5	61	42	3	7,70	18,4	48,9	63,5	23,9	37,6	14,090µl	0	28	71,8	0,1	383
4486	10,33	70	68,3	53	49	2	6,59	16,4	45,1	68,4	24,9	36,4	18,720µl	0	28	72	0	274
4488	6,17	70	65,3	63	52	1	7,44	17,8	49	65,9	23,9	36,3	18,240µl	0	25,3	74,6	0	446
4469	7,83	74	62,2	61	32	5	7,37	15	42,1	57,1	20,4	35,6	14,600µl	0	53,6	45,9	0,2	265
4473	6	66	65,8	33	34	5	7,58	18,5	49,8	65,7	24,4	37,1	13,460µl	0,6	26	73,2	0,1	448
4475	8,33	70	61,2	48	36	0	7,57	18	50,6	66,8	23,8	35,6	13,190µl	0,1	43,5	56,4	0	493
4494	8,17	70	67,9	49	48	8	7,74	19,8	53,9	69,6	25,6	36,7	12,280	0,1	30,5	69,4	0	413

*- : For the dog 4455, the blood sample was hemolyzed, which was the cause of the rejection of the sample

: Group of Medium
: Group of 10^6(Lm Cen -/- fromLab Culture)
: Group of 10^6(Lm Cen -/- from GENNOVA)
:Group of 10^7(Lm Cen -/- from GENNOVA)

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

No

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Nothing to report

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? No

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization?

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
eRA Commons User Name	Y	Satoskar, Abhay R	PHD,MBBS,MD	PD/PI	EFFORT					NA
	Y	Zhioua, Elyes	PHD	PD/PI				Institut Pasteur de Tunis	TUNISIA	NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

Yes

Dr. Zhioua's effort was reduced because of delays and will be adjusted accordingly in the next project period.

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

Yes

File uploaded: Active OS 6.2019.pdf

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

NA

OTHER SUPPORT

Abhay R. Satoskar, MBBS, MD, PhD

Active

R21AI138555 (PI: Satoskar) 07/01/2018 – 06/30/2020
NIH $150,000
EFFORT
Characterization of bystander immunosuppression during visceral leishmaniasis
The data generated from this study will provide foundation for future studies on understanding the mechanisms of immunosuppression during *L. donovani* infection and will enable the development of novel strategies to treat VL as well as prevent secondary bacterial infections and sepsis in VL patients.

R21AI131227 (PI: McGwire) 02/06/2017-01/31/2020
NIH $125,000
EFFORT
Trypanosoma cruzi cyclophilin 19 induced host inflammation
At the conclusion of this project we will understand whether Cyp19 contributes to *T. cruzi* infection and tissue pathogenesis and the results will provide the groundwork for further mechanistic studies on this pathologic process and the development of small molecule inhibitors for the potential treatment Chagas heart disease.
Role: Co-Investigator

W81XWH-16-1-0036 (PI: Satoskar) 02/01/2016-01/31/2020
Department of Defense $554,484
EFFORT
Prevention and treatment of breast cancer and its metastasis by targeting macrophage migration inhibitory factor (MIF)
The goal of this project is to determine how the pro-inflammatory cytokine macrophage migration inhibitory factor (MIF) promotes breast cancer development and progression, and determine whether MIF is a viable drug target for prevention and/or treatment of breast cancer.

Private Source ?I: Satokar) 05/21/2018-05/20/2020
$324,714
EFFORT
A live attenuated vaccine for leishmaniasis
The goal of this grant is to develop a vaccine by undertaking live attenuation of *Leishmania* by deleting *centrin* gene

R01AI107250 (PI: Seveau) 02/01/2014 – 01/31/2020
NIH/NIAID $250,000
EFFORT
Multifaceted activity of listeriolysin O during host cell invasion by Listeria
This project will elucidate fundamental mechanisms used by the pore-forming toxin listeriolysin O to mediate host cell invasion by the foodborne pathogen Listeria monocytogenes.
Role: Co-Investigator

W81XWH1810805 (PI: Cebulla) 09/30/2018 - 09/29/2021
Department of Defense $407,193
EFFORT
Macrophage migration inhibitor (MIF) therapeutics for neuroprotection and prevention of scar in traumatic retinal detachment
Role: Co-Investigator

R21AI130485 (PI: Satoskar) 08/01/2018 - 07/31/2020
NIH $156,529
EFFORT
A live attenuated vaccine for leishmaniasis
In this project, we propose to (Aim 1) optimize LmCen-/- immunogenicity and immunization

protocol and determine its safety in dogs and (Aim 2) evaluate efficacy of LmCen-/- as a vaccine using a novel model of canine VL in which dogs are naturally exposed to bites of L. infantum infected wild Phlebotomus pernicious in VL- hyperendemic regions of Tunisia.

Changes

Private Source ended 05/05/2019
Private Source nded 12/31/2018
R21AI127582 ended 11/30/2018
R21AI123692 ended 03/31/2019
W81XWH-14-2-0168 no effort as of 04/2019

Overlap

None

OTHER SUPPORT

Elyes Zhioua

R21AI130485 (PI: Satoskar) 08/01/2018 - 07/31/2020
NIH $156,529
EFFORT
A live attenuated vaccine for leishmaniasis
In this project, we propose to (Aim 1) optimize LmCen-/- immunogenicity and immunization protocol and determine its safety in dogs and (Aim 2) evaluate efficacy of LmCen-/- as a vaccine using a novel model of canine VL in which dogs are naturally exposed to bites of L. infantum infected wild Phlebotomus pernicious in VL- hyperendemic regions of Tunisia.

Private Source (PI: Satokar) 05/21/2018-05/20/2020
$324,714
EFFORT
A live attenuated vaccine for leishmaniasis
The goal of this grant is to develop a vaccine by undertaking live attenuation of *Leishmania* by deleting *centrin* gene
Role: Co-I

Changes

None

Overlap

None

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Dollar Amount	Country
$60,000	TUNISIA

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

After this grant was awarded there were some concerns raised by US Department of State about conducting Leishmaniasis research in Tunisia. Drs Satoskar and Zhioua visited US Embassy in Tunis and clarified the objectives of the project alleviating the concerns of the Department of State. Therefore, the start of work on this project was delayed significantly, nonetheless, we have made a significant progress outline below.

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Sub-Project ID:	Study ID	Study Title:

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address
Primary: The Ohio State University	832127323	OH-003	1960 Kenny Road Columbus OH 432101016
Institut Pasteur de Tunis	499250553		13 Place Pasteur BP74 Tunis

G.9 FOREIGN COMPONENT

Organization Name: Institut Pasteur de Tunis
Country: TUNISIA
Description of Foreign Component:
Dr. Zhioua will oversee the field and laboratory work.

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

Yes

Estimated unobligated balance: 187040

G.10.b Provide an explanation for unobligated balance:
As mentioned before the start of the work on this project was delayed significantly due to concerns raised by US State Department of doing Leishmanaisis research in Tunisia after this grant was awarded.

G.10.c If authorized to carryover the balance, provide a general description of how it is anticipated that the funds will be spent
Dr. Satoskar and Zhioua had to have several meetings with officials including meetings at US Embassy in Tunis to provide additional clarifications requested by them and alleviating the concerns which was accomplished successfully. In addition, there were delays in recruiting trained personnel in Satoskar lab due to visa issues. However, these have been resolved.

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No

Notice of Award

EXPLORATORY/DEVELOPMENT GRANT
Department of Health and Human Services
National Institutes of Health

Federal Award Date: 07/31/2018

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 1R21AI130485-01A1
FAIN: R21AI130485

Principal Investigator(s):
Abhay R Satoskar (contact), MD
Redacted by agreement

Project Title: A live attenuated vaccine for leishmaniasis

Redacted by agreement
The Ohio State University
B-034 Graves Hall
333 West 10th Avenue
Columbus, OH 432101239

Award e-mailed to: NIHaward@osu.edu

Period Of Performance:
Budget Period: 08/01/2018 – 07/31/2019
Project Period: 08/01/2018 – 07/31/2020

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $208,827 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to OHIO STATE UNIVERSITY in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R21AI130485. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Redacted by agreement
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 1R21AI130485-01A1

Award Calculation (U.S. Dollars)

Federal Direct Costs	$156,529
Federal F&A Costs	$52,298
Approved Budget	$208,827
Total Amount of Federal Funds Obligated (Federal Share)	$208,827
TOTAL FEDERAL AWARD AMOUNT	$208,827
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$208,827

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
1	$208,827	$208,827
2	$166,973	$166,973

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name:	Allergy and Infectious Diseases Research
CFDA Number:	93.855
EIN:	1316025986A1
Document Number:	RAI130485A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2018

IC	CAN	2018	2019
AI	8472350		$166,973
AI	8480847	$208,827	

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M92 / **OC**: 414A / **Released**: eRA Commons User Name 07/25/2018
Award Processed: 07/31/2018 12:08:01 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 1R21AI130485-01A1

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 1R21AI130485-01A1

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain

references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2**.** As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase VI Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots.

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R21AI130485. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:

Additional Costs

Clinical Trial Indicator: No
This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

This is a Modular Award without direct cost categorical breakdowns in accordance with the guidelines published in the NIH Grants Policy Statement, October 2017, see https://grants.nih.gov/grants/policy/nihgps/HTML5/section_13/13.5_post-award_administration.htm. Recipients are required to allocate and account for costs related to this award by category within their institutional accounting system in accordance with applicable cost principles.

****

This Notice of Award (NoA) includes funds for activity with **Pasteur Institute of Tunis- TUNISIA**.

****

This award may include collaborations with and/or between foreign organizations. Please be advised that short term travel visa expenses are an allowable expense on this grant, if justified as critical and necessary for the conduct of the project.

****

This award reflects current Federal policies regarding Facilities & Administrative (F&A) Costs for foreign grantees including foreign sub-awardees, and domestic awards with foreign sub-awardees. Please see: Chapter 16 Grants to Foreign Organizations, International Organizations, and Domestic Grants with Foreign Components, Section 16.6 "Allowable and Unallowable Cost" of the NIH Grants Policy Statement effective October 1, 2017.

****

The Research Performance Progress Report (RPPR), Section G.9 (Foreign component), includes reporting requirements for all research performed outside of the United States. Research conducted at the following site(s) must be reported in your RPPR: **Pasteur Institute of Tunis-TUNISIA**.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Redacted by agreement
Email: Redacted by agreement **Fax:** Redacted by agreement

Program Official: Redacted by agreement
Email: Redacted by agreement **Phone:** Redacted by agreement

SPREADSHEET SUMMARY
GRANT NUMBER: 1R21AI130485-01A1

INSTITUTION: OHIO STATE UNIVERSITY

Budget	Year 1	Year 2
TOTAL FEDERAL DC	$156,529	$129,671
TOTAL FEDERAL F&A	$52,298	$37,302
TOTAL COST	$208,827	$166,973

Facilities and Administrative Costs	Year 1	Year 2

F&A Cost Rate 1	56%	56%
F&A Cost Base 1	$93,389	$66,611
F&A Costs 1	$52,298	$37,302

PI: **Satoskar, Abhay R**	Title: A live attenuated vaccine for leishmaniasis	
Received: 06/30/2017	FOA: PA16-161	Council: 01/2018
Competition ID: FORMS-D	FOA Title: NIH EXPLORATORY/DEVELOPMENTAL RESEARCH GRANT PROGRAM (PARENT R21)	
1 R21 AI130485-01A1	Dual:	Accession Number: 4070489
IPF: 6218701	Organization: OHIO STATE UNIVERSITY	
Former Number:	Department: Pathology	
IRG/SRG: VMD	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 150,000 Year 2: 125,000	Animals: Y Humans: N Clinical Trial: N Current HS Code: Evaluative Info HESC: N	New Investigator: Early Stage Investigator:
Senior/Key Personnel:	*Organization:*	*Role Category:*
Elyes Zhioua	Pasteur Intitute	MPI
Redacted by agreement		Other (Specify)-Collaborator
		Other (Specify)-Other Significant Contributor
Abhay Satoskar	The Ohio State University	PD/PI

OMB Number: 4040-0001
Expiration Date: 10/31/2019

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
AI130485

b. Agency Routing Number

2. DATE SUBMITTED
2017-06-30

Application Identifier

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION

Organizational DUNS*: 832127323

Legal Name*: The Ohio State University
Department:
Division:
Street1*: 1960 Kenny Road
Street2:
City*: Columbus
County: Franklin
State*: OH: Ohio
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 43210-1016

Person to be contacted on matters involving this application
Prefix: First Name*: Redacted by agreement Suffix:
Position/Title: Sr. Sponsored Program Officer
Street1*: B-034 Graves Hall
Street2: 333 West 10th Avenue
City*: Columbus
County: Franklin
State*: OH: Ohio
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 43210-1239
Phone Number*: Redacted by agreement Fax Number: 614-292-9779 Email: Redacted by agreement @osu.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 1-316025986-A1

7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education

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*
A live attenuated vaccine for leishmaniasis

12. PROPOSED PROJECT
Start Date*: 04/01/2018
Ending Date*: 03/31/2020

13. CONGRESSIONAL DISTRICTS OF APPLICANT
OH-003

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Abhay Middle Name: R Last Name*: Satoskar Suffix:
Position/Title: Professor
Organization Name*: The Ohio State University
Department: Pathology
Division: College of Medicine
Street1*: 1645 Neil Avenue
Street2:
City*: Columbus
County: Franklin
State*: OH: Ohio
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 43210-1292
Phone Number*: Redacted by agreement Fax Number: Email*: Redacted by agreement @osu.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$375,567.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$375,567.00
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: First Name*: Redacted by agreement Suffix:
Position/Title*: Sr. Sponsored Program Officer
Organization Name*: The Ohio State University
Department: Office of Sponsored Programs
Division: Health Sciences Office
Street1*: B-034 Graves Hall
Street2: 333 West 10th Avenue
City*: Columbus
County: Franklin
State*: OH: Ohio
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 43210-1239
Phone Number*: Redacted by agreement Fax Number: 614-292-9779 Email*: Redacted by agreement @osu.edu

Signature of Authorized Representative*
Redacted by agreement

Date Signed*
06/30/2017

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:

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

OMB Number: 4040-0010
Expiration Date: 10/31/2019

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:	The Ohio State University
Duns Number:	832127323
Street1*:	1960 Kenny Road
Street2:	
City*:	Columbus
County:	Franklin
State*:	OH: Ohio
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	43210-1016
Project/Performance Site Congressional District*:	OH-003

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:	Institut Pasteur de Tunis
DUNS Number:	499250553
Street1*:	13 Place Pasteur BP74
Street2:	
City*:	Tunis
County:	
State*:	
Province:	
Country*:	TUN: TUNISIA
Zip / Postal Code*:	
Project/Performance Site Congressional District*:	

Additional Location(s) File Name:

Funding Opportunity Number: PA-16-161. Received Date: 2017-06-30T09:27:25.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

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:

Animal Welfare Assurance Number A3261-01

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: Tunisia, India

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Abstract6_301034676970.pdf
8. Project Narrative*	Project_Narrative1034676980.pdf
9. Bibliography & References Cited	Refernces6_101034676975.pdf
10.Facilities & Other Resources	Facilities_Resources_FINAL_EZ_June_71034676977.pdf
11.Equipment	Equipment_EZ_June71034676979.pdf

Funding Opportunity Number: PA-16-161. Received Date: 2017-06-30T09:27:25.000-04:00

Abstract

Infections caused by the protozoan parasite *Leishmania* include cutaneous (CL), mucosal (ML), and visceral leishmaniasis (VL). Over 12 million people currently suffer from leishmaniasis, and approximately 2 million new cases occur annually. Currently no vaccine is available for this disease for humans. However, patients who recover from leishmaniasis develop immunity against reinfection indicating that a vaccine is feasible. In the past, leishmanization, a process in which deliberate infection with a low dose of *Leishmania major*, etiologic agent of zoonotic cutaneous leishmaniasis (ZCL) causes a controlled skin lesion and provides > 90% protection against reinfection, was a common practice. Although such practice may not be acceptable under the current regulatory environment due to possibility of complications, these observations suggest that live-attenuated parasites that provide a complete array of antigens without causing disease could be an effective vaccine for leishmaniasis. Genetically attenuated *L. infantum* and *L. donovani* have shown promise as a vaccine in animal models. However, using these parasites in humans could raise safety concerns due to their visceralizing potential. Attenuated dermatotrophic *Leishmania* that cross-protects against VL could be a safer vaccine because potential adverse events (e.g. development of a lesion at vaccination site) can be easily monitored and effectively treated using approved topical interventions. Several clinical as well as animal studies have shown that an infection with dermatotrophic *Leishmania* such as *L. major* or immunization with antigens from these parasites confers significant cross-protection against VL. However, it is not known whether immunization with attenuated cutaneous disease causing species such *centrin* gene deficient *L. major* will protect against VL. Using CRISPR-Cas technology, we have generated antibiotic selection marker free *centrin* gene deficient *L. major* (*LmCen*$^{-/-}$). In this project, we propose to use a novel canine model of VL to test the safety and efficacy of GLP-grade *LmCen*$^{-/-}$. Whole genome sequencing of *LmCen*$^{-/-}$ has confirmed stable deletion of *centrin* gene without other mutations in the parasite genome. Our preliminary findings show that *LmCen*$^{-/-}$ are highly attenuated and fail to cause disease in immunocompromised mice. We have also found that immunization with *LmCen*$^{-/-}$ parasites induces a disease protective Th1 response in hamster as well as mice and completely protects against homologous challenge with virulent *L. major*. Our industry partner Gennova Biopharma has already established *LmCen*$^{-/-}$ production under GLP conditions at their US-FDA approved facility. In this project, we propose to **(Aim 1)** optimize GLP-*LmCen*$^{-/-}$ immunogenicity and immunization protocol and determine its safety in dogs and **(Aim 2)** evaluate efficacy of GLP-*LmCen*$^{-/-}$ as a vaccine using a novel model of canine VL in which dogs are naturally exposed to bites of *L. infantum* infected wild *Phlebotomus pernicious* in VL- hyperendemic regions of Tunisia. The scientific promise of this project, if successful, could provide the foundation for advancing *LmCen*$^{-/-}$ parasites as a vaccine against leishmaniasis in humans.

Project Narrative

Visceral leishmaniasis (VL) is caused by *Leishmania donovani* (*L. donovani*) or *L. infantum* and is a life-threatening neglected tropical disease of global health concern. Half a million people are infected each year, and over 60,000 succumb to disease annually, although these numbers are likely underestimated. No vaccine is currently available for VL. The goal of this project is to develop a vaccine for VL and test its efficacy in protecting dogs against naturally acquired VL.

RESOURCES AND FACILITIES

Laboratory

Dr. Satoskar has a laboratory space in [Redacted by agreement] circa [Specific Animal Location] with bench space for 6 people and M405 as an office on the [Redacted by agreement]. The laboratory has three BL2 biosafety hoods, C02 water jacketed incubators-Napco 5430 double, refrigerated centrifuge-Fisher Marathon 3000R, two microfuges-Taylor TE1624, electrophoretic equipment, microscope- Nikon Labophot-2, an inverted phase microscope, refrigerator-True 3-Door, -20 Freezer-GE, PCR machine- MJ research PTC-100, MJ-mini cycler, UV-Vis spectrophotometer (Beckmann DU550) and microplate reader (VersaMax, Molecular Devices). Dr. Satoskar's laboratory is also equipped with cytospin, BD FACSCalibur 4 color flowcytometer and cell sorter, FlowSight Imaging Flowcytometer (EMD Millipore), MJ Research Opticon 2 and CFX96 Biorad real time PCR machines, Zeiss Immunofluorescence microscope with digital imaging system and Leica cryostat It is equipped to carry out the proposed project.

Dr. Zhioua main lab is approximately [Specific Animal Location] including two insectaries with [Specific Animal] each, three laboratory spaces with [Specific Animal] Main laboratory area and sand fly insectaries are rated BSL-2 and studies involving infection of sand flies via artificial feeding is routinely performed.

OSU's Shared Resources include a central flow cytomtery and cell sorting facility, electron microscopy and protein sequencing/proteomic facility. Within the university campus, there are four gene array facilities with robotic gene array spotting and high resolution scanners. The Microarray Shared Resource offers comprehensive services including mRNA transcriptional profiling, microRNA/non-coding small RNA transcriptional profiling, Single Nucleotide Polymorphism genotyping, genomic DNA gain/loss detection on BAC CGH Array, microRNA genomic gain/loss on oligo CGH array, RNA characterization and microarray processing and data analysis. Access to a gene array facility is available at the Nationwide Children's Hospital, Columbus, OH.

Computer

Dell computers are available for graphics, data entry, and analysis in the Department of Pathology. Direct access to the World Wide Web, modem links with gene analysis, electronic communication, and library resource packages are also present.

Two desktop PC's, one Dell laptop are available in Dr. Zhioua's laboratory. Additional office support is also provided by the Institut Pasteur de Tunis

Office

Dr. Satoskar's office is located in [Redacted by agreement] in [Redacted by agreement] The Department of Pathology office is located in the Hamilton Hall which [Redacted by agreement] In support of the University's strategic goals, allocation of space is based on the overall needs of the research, education and clinical missions. Faculty office space includes standard office resources such as personal computers, fax machines and copiers. The offices of the faculty are within convenient walking distance to classrooms, laboratories, administrative offices and each other.

Office infrastructure for the Zhioua laboratory: 230 sq ft. of office space (PI) and 280 sq ft office space for students.

Animal facility

The Dog kennel is located at [Redacted by agreement] from Dr. Zhioua's laboratory. The dog kennel is devide to 3 sections with [Redacted by agreement] each. Each sections contains 5 compartiments. Eight dogs are housed per compartiment. Dogs in each compartiemnt have access to the pation where they can exersice twice a day. A total of [Redacted] is available for each dog. All windows are screen against flies. The facility includes an electrically grounded, fully equipped surgical operating room. The facility is completely equipped for histological sectioning and immunohistochemical staining.

Biostatistics Core

The Biostatistics Core Shared Resource (BCSR) offers cancer investigators a centralized resource for expertise in the biostatistical analysis and design of clinical, basic and population-based research, allowing for collaboration on all facets of study design, data management and statistical analysis of clinical, epidemiological, public health and laboratory research data. Researchers, investigators, and clients are provided statistical services when the BCSR is supported through grants as FTE collaborators, written into

departmental contracts (MOUs), written into external contracts, or billed directly for hourly projects. The BCSR offers the following statistical services: Complex Model Development, Data Management, Data Retrieval, Design of Clinical Trials, Development and Application of New Statistical Methodologies, Development of Randomization Strategies, Experimental Design, Grant Proposal Preparation, Manuscript Preparation, Measurement Selection, Microarray Analysis, Sample Size Determination, Statistical Analysis, Statistics Seminars/Courses, and Survey Sampling.

Clinical and Translational Resources

PIs are member of OSU's Center for Clinical and Translational Science (CCTS) and the goal of this center is to speed new translational discoveries to enhance patient outcomes. The CCTS provides biomedical informatics, biostatistics, clinical research services, community engagement, novel clinical and translational methodologies and technologies. The CCTS also has a laser capture microscope and provides services for laser capture of multicellular structures and of single cell populations from histological sections.

Other

The PIs have access to more than 20 core research laboratories at the Ohio State University for shared use by health-sciences investigators. Clinical research faculty, basic scientists and students all benefit from the shared cost of these resources, and the research environment at Ohio State benefits from the economies of scale that enable timely acquisition of new instrumentation and technologies. The core facilities include the following: **A.** Scanning and transmission electron microscopy **B.** Microarray facilities that include genome-wide analysis of multiple genes using Affymetrix GeneChips. Services include mRNA transcriptional profiling, microRNA/non-coding small RNA transcriptional profiling, single nucleotide polymorphism genotyping, genomic DNA gain/loss detection on BAC CGH Array, microRNA genomic gain/loss on oligo CGH array, consultation, RNA characterization, and microarray processing and data analysis **C.** Fluorescent microscopes fully equipped for optimized magnification, time-lapse video microscopy and multi-channel visualization, the lab offers other instrumentation, including multiphoton confocal microscopy that allows investigators to probe delicate living cells or tissues longer and deeper without damaging samples. **D.** DNA sequencing, genotyping, real time polymerase chain reaction (PCR), RNA/DNA extraction, imaging and DNA synthesis support. **E.** Proteomics **F.** X-ray Crystallography **G.** Viral Vector Core: Facility producing research-grade retroviral and lentiviral vectors, generates stable producer lines, and offers non-GMP quality control testing, including vector titer by functional assay or PCR, mycoplasma, sterility, RCR and RCL testing.

MAJOR EQUIPMENT

Dr. Satoskar's lab is equipped with centrifuge, inverted microscope, carbon-dioxide incubator, light microscope, refrigerator, -80 and -20^0 C freezer, BioRad Gel Doc XR/ChemiDoc XRS gel documentation system, spectrophotometer, microplate ELISA reader, water baths, PCR thermal cyclers, fluorescence microscope, DNA and protein gel electrophoresis apparatus, blot transfer apparatus and a liquid nitrogen tank and BioTek Synergy II Multi-Mode Microplate Reader. Dr. Satoskar's laboratory is also equipped with cytospin, BD FACSCalibur 4 color flowcytometer and cell sorter, FlowSight Imaging Flowcytometer (EMD Millipore), MJ Research Opticon 2 and CFX96 Biorad real time PCR machines, Zeiss Immunofluorescence microscope with digital imaging system and Leica cryostat. Cell sorting, ultracentrifuges and scintillation counters are available in the Department of Pathology. Equipment such as high speed centrifuges, a walk-in incubator and a cold room, a huddle room, and a lecture room are available on the shared basis on the 8th floor the OSU-Biomedical Research Tower (BRT). The OSU in general and Department of Pathology have central computing and imaging facilities consisting of confocal microscopes, phosphoimager and required software for image analyses. The university also has a central electron microscopy and protein sequencing/ proteomic facilities. Within the University campus, there are four gene arrays facilities with robotic gene array spotting and high resolution scanners. The PIs also have access to a gene array facility at the Nationwide Children's Hospital, Columbus. OH.

Major equipment available in the Zhioua laboratory include, one biosafety cabinet (Techgen), Three refrigerator, -80 and -20^0 C freezer, tabletop centrifuge (Sigma), on standard thermocycler (Applied biosystems), two growth chambers for rearing sand flies (Sanyo), two refrigerators, two gel electrophoresis apparatus (BioRad), two dissecting microscopes, (Zeiss), one inverted microscope, and two stereo microscopes (Zeiss), one incubator (Thermo), on multiplex ELISA reader (Tecan) and other small equipment necessary for molecular biology. The lab also boosts various small equipments such as one vortex, balance, pH meter, and microwave. A 4WD pick-up with four doors is at Zhioua's laboratory disposal for field work. Flow cytometry Core facility equipped with BD FACS Calibur flowcytomter is available at the Pasteur Institute, Tunis.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: Abhay Middle Name R Last Name*: Satoskar Suffix:

Position/Title*: Professor
Organization Name*: The Ohio State University
Department: Pathology
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E-Mail*: satoskar.2@osu.edu

Credential, e.g., agency login: eRA Commons User Name

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

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: Satoskar_Biosketch_R21_06_20171034676983.pdf

Attach Current & Pending Support: File Name:

Funding Opportunity Number: PA-16-161 . Received Date: 2017-06-30T09:27:25.000-04:00

PROFILE - Senior/Key Person

Prefix: First Name*: Elyes Middle Name Last Name*: Zhioua Suffix:

Position/Title*: Professor
Organization Name*: Pasteur Intitute
Department: Vector Ecology
Division:
Street1*: 13 Place Pasteur BP74
Street2:
City*: Tunis
County:
State*:
Province:
Country*: TUN: TUNISIA
Zip / Postal Code*:

Phone Number*: Personal Info Fax Number:

E-Mail*: Personal Info @gmail.com

Credential, e.g., agency login: eRA Commons User Name

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

Degree Type: PhD Degree Year:

Attach Biographical Sketch*: File Name: Biosketch_Zhioua_Elyes1034676984.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Redacted by agreement

PROFILE - Senior/Key Person

Redacted by agreement

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: Abhay R. Satoskar

POSITION TITLE: Professor and Vice Chair for Research, Department of Pathology

eRA COMMONS USER NAME (credential, e.g., agency login): eRA Commons User Name

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 Bombay	MBBS	1/1989	Medicine
University of Bombay	MD	2/1992	Pathology
University of Strathclyde, Glasgow	PhD	5/1996	Immunology

A. Personal Statement

The research in my laboratory focuses on studying the mechanisms of immunity and immune evasion in parasitic diseases, particularly infections caused by kinetoplastids. I am a pathologist with broad background in Immunology. As a graduate student at University of Strathclyde, I got interested in studying the regulation of immune responses in parasitic infections which is a major global health issue. As a postdoctoral fellow at Harvard school of Public Health in the lab of Redacted by agreement my research continued to focus in the field of immunoparasitology, particularly understanding the role of different cytokines in controlling outcome of leishmaniasis. At the Ohio State University, I have continued my research in this field and have also extended it to discovery of novel antiparasitic and/or immunomodulatory drugs from the natural products. We are also involved in the development and implementation of non-pharmacological therapies for cutaneous leishmaniasis in endemic regions in Africa, Middle East, Asia and Latin America. My laboratory has developed/acquired several tools and techniques including fluorescent parasites, conditionally targetable reporter mice and imaging flow cytometry to study the mechanisms of immunity and immune evasion *in vivo.* Additionally, we have partnered with industry to develop vaccines and test novel immune modulators for host-targeted therapies for parasitic diseases. I am currently leading a team of investigators from Japan, Canada and USA who are focused on developing a liver attenuated vaccine for leishmaniasis. I have been NIH funded continually for the last 14 years and has also received funding from Department of Defense, WHO and private foundations. I have graduated 8 Ph.D. students and have mentored 10 postdoctoral fellows, and several undergraduate, high school and medical students. For the last 7 years, we have been collaborating with Redacted by Lab at US – FDA studies on anti-*Leishmania* vaccine. These studies have shown that attenuated *LdCen-/-* parasites confer cross protection against challenge with virulent demotropic *Leishmania* (Dey et. al., Immunol. 2014 Oct 1;193(7):3513-27; Gannavaram et al., Methods Mol Biol. 2016;1403:623-38). I have successfully completed and continue to administer several research projects and have been collaborating with Dr. Zhioua since 2012. I have a consistent record of successful and productive research for the last 18 years, and my experience and expertise have prepared me to participate in this project on development of a vaccine for leishmaniasis.

B. Positions and Honors

Positions and Employment:

1992-1992 Research Fellow, Max-Planck Institute for Immunobiology, Freiburg, Germany
1996-1998 Research Fellow, Immunology and Infectious Diseases, Harvard School of Public Health
1998-1999 Instructor, Immunology and Infectious Diseases, Harvard School of Public Health, Boston,
1999-2001 Research Scientist, Immunology and Infectious Diseases, Harvard School of Public Health
2001-2006 Assistant Professor, tenure track, Microbiology, The Ohio State University
2001-2006 Assistant Professor, Adjunct t, Department of MVIMG, The Ohio State University
2003-2008 Assistant Professor (Adjunct), Department of IID, Harvard School of Public Health, Boston, MA
2006-2010 Associate Professor, with tenure, Microbiology, The Ohio State University
2010-present Professor, with tenure, Pathology and Microbiology, The Ohio State University
2013-present Vice Chair for Research, Department of Pathology, The Ohio State University

2014-present University Pathology Services Endowed Anatomic Pathology Professorship, OSU
2014-present Expert Educator, LSI Curriculum Foundation Block, College of Medicine, OSU

Service:

1996-present Reviewer for 70 journals including Nat. Immunol., J. Exp. Med., PNAS, J. Clin. Invest., FASEB J., Mol. Cancer; Infect. Immun. Immunology, Europ. J. Immunol., Am. J. Pathol.
2003 Sheikh Hamdan Medical Research Grant Foundation, Dubai, UAE, Grant Reviewer
2003-04 Ad hoc Member, NIH, TMP Study Section
2005-2007 Reviewer, Wellcome Trust, United Kingdom, Program Projects
2006-2007 Member, Leishmania panel, US Army Medical Research and Materiel Command's (USAMRMC) Military Infectious Disease Research Program (MIDRP)
2008-2012 Associate Editor, The Journal of Immunology
2009 Member, NIH, ICIDR, Parasitology SEP
2010 Member, AIBS/MIDRP Leishmaniasis Panel
2011 Grant Reviewer, Health Research Board, Ireland
2011-15 Ad hoc Member, NIH, PTHE Study section
2012 Grant Reviewer, WELBIO, Belgium; Czech Science Foundation
2012-present Associate Editor, PLoS *Neglected Tropical Diseases, Frontiers in Microbial Immunology*
2012-present Editorial Board Member, F1000 Research
2013-present Academic Editor, PLoS ONE
2013-present International Expert, Research Equipment Program Panel, NRF South Africa
2014 Chair, NIH, Topics in Antimicrobial Drug resistance and Drug Discovery Study Panel
2014 Expert Advisor in Immunology, DNDi, Geneva, Switzerland
2015-present Member, NIH, PTHE Study section
2016-present Reviewer, Swiss National Foundation
2016-pressent Member, NSERC Scholarships and Fellowships Committee, Ottawa, Canada
2017 Grant reviewer, Medical research Council, UK, Grant Reviewer

Memberships:

1997-present American Association of Immunologists
1998-present American Society of Microbiology
1998-present American Society for Tropical Medicine and Hygiene
2006-present American Society for Investigative Pathology
2006-pressent International Society for Interferon and Cytokine Research
2007-present Society for Leukocyte Biology

Awards/Honors:

1990 University Merit Scholarship, University of Bombay
1992 Dr. C.K. Deshpande Gold Medal, University of Bombay
1992 David Livingston Bursary from the University of Strathclyde for postgraduate research in Immunology
1992 Overseas Research Student Award from the Committee of Vice Chancellors and Principals, London
2000 Visiting Scientist Award, University of Cape Town, South Africa
2003 AAI Junior Faculty Travel Award
2005 Dean's Award for Undergraduate Research Mentoring
2012 Visiting Professor University of Cape Town, South Africa

C. Contribution to Science

1. As a graduate student in the laboratory of Redacted by agreement at the University of Strathclyde, my project focused on understanding the immunological basis for gender-associated susceptibility to the New world cutaneous leishmaniasis (CL) as well as examining the role of IL-4 and Th2 response in regulating outcome of different forms of *Leishmania* infections using mouse models. Gender differences in susceptibility to CL have been reported in humans and females are more resistant to infection than males. Our studies discovered that gender-associated resistance of females to CL is due to their ability to mount a preferential Th1 response and suggested that T cells that develop in the presence of estrogen have tendency to differentiate towards a Th1 phenotype. Our studies on IL-4 and *Leishmania* were the first ones to show that IL-4 plays a disease protective role in visceral leishmaniasis. These findings challenged the well-established dogma because role of IL-4 as a susceptibility factor in CL was well-established in 1990s. Subsequent studies by us and other investigators validated our results and showed that although IL-4 and

IL-4 signaling pathway mediate susceptibility to CL, it is critical for immunity as well as for effectiveness of antileishmanial therapy against VL.

a. **Satoskar, A***, and Alexander, J. Sex determined susceptibility and differential IFN-gamma and TNF-alpha mRNA expression in DBA/2 mice infected with *Leishmania mexicana*. Immunology. **1995**; 84:1. PMCID: PMC1415181
b. **Satoskar, A***, Bluethmann, H, and Alexander, J. Disruption of murine Interleukin-4 gene inhibits disease progression during *L. mexicana* infection but does not increase the control of *L. donovani* infection. Infect. Immun. **1995**; 63:4894.
c. **Satoskar, A**, Brombacher, F, Dai, W, McInness, I, Liew, FY, Alexander, J, and Walker, W. SCID mice reconstituted with IL-4-deficient lymphocytes, but not immunocompetent lymphocytes, are resistant to cutaneous leishmaniasis. J. Immunol. **1997**; 159:5005.
d. **Satoskar, A***, Al-Quassi, HH, and Alexander, J. Sex-determined resistance against *Leishmania mexicana* is associated with the preferential induction of a Th1-like response and IFN-gamma production by female but not male DBA/2 mice. Immunol. Cell Biol. **1998**; 76: 159.

2. Cytokines play a critical role in regulating immunity against *Leishmania*. On the other hand, it is well documented that *Leishmania* can subvert immunity by manipulating cytokine signaling pathways in the host cell. At Harvard School of Public Health I continued to work in the field of leishmaniasis with the focus on understanding the role of cytokines and cytokine signaling pathways in regulating outcomes of cutaneous and visceral leishmaniasis. These studies demonstrated the protective roles of IL-12, STAT4 and IL-1R1 signaling in resistance against leishmaniasis, established the role of STAT6-signaling in mediating susceptibility and showed that IL-18 and NK cells play a role in innate immunity but not required for resolution of leishmaniasis. Furthermore, in studies conducted with the labs of Redacted by agreement Redacted by agreement I used the experimental model of leishmaniasis to discover the critical roles of T-bet and SAP transcription factors in induction of IFN-γ and IL-4 from helper T cells respectively.
   a. Stamm, LM, Raisanen-Solokowski, A, Okano, M, Russell, M, David, JR, and **Satoskar, AR***. Mice with STAT6-targeted disruption develop a Th1 response and control cutaneous leishmaniasis. J. Immunol. **1998**; 161: 6180.
   b. Stamm, LM, Satoskar, AA, Ghosh, SK, David, JR and **Satoskar, AR***. STAT4-mediated IL-12 signaling pathway is critical for the development of protective immunity in cutaneous leishmaniasis. Eur. J. Immunol. **1999**; 29:2524.
   c. Monteforte, GM, Takeda, K, Rodriguez-Sosa, M, Akira, S, David, JR and **Satoskar, AR*.** Genetically resistant mice lacking IL-18 gene develop Th1 response and control cutaneous *Leishmania major* infection. J. Immunol. **2000**; 164: 5890.
   d. Wu, C, Nguyeng, KB, Pien, GC, Wang, N, Gullo, C, Howie, D, Rodriguez Sosa, M, Edwards, MJ, Borrow, P, **Satoskar, AR**, Sharpe, AH, Biron, CA, and Terhorst, C. SAP controls T cell responses to virus and terminal differentiation of Th2 cells. Nature Immunol. **2001**; 2: 410.

3. Inflammation is a hall mark of infection and is required for clearance of pathogens but it also contributes the pathology. One of the major focus in my laboratory is studying mechanisms of inflammation during parasitic infections. Early studies from laboratory had shown that chemokine receptors CXCR3 is critical for the clearance of cutaneous leishmaniasis. Another study from our lab discovered the role of IL-27 in limiting inflammation and suppressing immunity in VL. In subsequent studies, we discovered the mechanisms by which expression of CXCR3 on immune cells. To further investigate the role of CXCR3 and study its regulation we have generated a CXCR3-EGFP reporter mice as well as T cell specific CXCR3 transgenic mice. Characterization of these mice led to discovery of two distinct subsets of innate CD8+ T cells.
   a. Rosas, LE, Barbi, J, Lu, B, Fujiwara, N, Gerard, C, Sanders, VM, and **Satoskar AR*.** CXCR3$^{-/-}$ mice mount an efficient Th1 response but fail to control *L. major* infection. Eur. J. Immunol. **2005**; 35:515.
   b. Rosas, LE, Satoskar, AA, Roth, K, Keiser, T, Barbi, J, Hunter, CA, de Sauvage, F, and **Satoskar, AR*.** IL-27R (WSX-1/TCCR) gene deficient mice display enhanced resistance to *Leishmania donovani* infection but develop severe liver immunopathology. Am. J. Pathol. **2006**; 168:158.
   c. Oghumu S, Terrazas C, Varikuti S., Kimble J., Vadia, S., Yu, L., Seveau, S., and **Satoskar AR*** . CXCR3 expression defines a novel subset of innate CD8^{+} T cells that enhance immunity against bacterial infection and cancer upon stimulation with IL-15. FASEB J. **2014** Dec. 2. Pii: fj. 14-264507. PMCID: PMC4422364
   d. Terrazas, CA, Varikuti, S, Kimble, J., Moretti, E., Boyaka, P., and **Satoskar AR*.** Interleukin 17A promotes susceptibility to experimental visceral leishmaniasis caused by *Leishmania donovani.* FASEB J **2016;** 30:1135-1143.

4. The pentavalent antimonials (Sb) introduced 100 years ago still remain the first line drugs for leishmaniasis world-wide, despite the fact that these drugs have low-dose cardiac, renal and hepatic toxicity, and require the daily parenteral administration for at least 20 days. In addition, emergence of drug-resistant parasites, particularly visceral strains (VL), is also rapidly increasing worldwide. Since 2009, our team is also involved discovering novel therapies as well as vaccines to prevent and/or treat leishmaniasis. We have discovered that PI3Kγ is critical for parasite entry into the host cell and as such is a therapeutic target for host-directed therapy in leishmaniasis. Work from our recently completed NIH-funded project has also led to the discovery of antileishmanial molecules from several plants including *Pentalinon andrieuxii*, which has been used by Mayan traditional healers for cutaneous leishmaniasis (CL) for many years. Furthermore, our collaborative studies conducted with Redacted by [redacted] Lab showed that vaccination centrin gene deficient attenuated *L. donovani* parasites confers cross-protection against CL.
   a. Cummings, HE, Barbi, J, Zorko, N., Sarkar, A., Keiser, T., Lu, B., Gerard, C., Ruckle, T., Wewers, M., Whitracre, CC., Lezama-Davila, CM, Rommel, C., Seveau, S., and **Satoskar, AR*.** Critical role for phosphoinositide 3-kinase gamma in parasite invasion and disease progression of cutaneous leishmaniasis. PNAS, **2012;** 109:1251-6. PMCID: PMC3268302
   b. Pan L, Lezama-Davila CM, Isaac-Marquez AP, Calomeni EP, Fuchs JR, **Satoskar AR**, Kinghorn AD. Sterols with antileishmanial activity isolated from the roots of Pentalinon andrieuxii. Phytochemistry **2012.** 82:128-35. PMCID: PMC3572536
   c. Dey, R., Natarajan, G., Cummings, HE, Sevapandiyan, A., Duncan, R., **Satoskar, AR*,** and Nakhasi, H. Characterization of cross-protection by genetically modified liver attenuated *Leishmania donovani* parasites against *L. mexicana.* J. Immunol. **2014;** 1:193(7):3513-27.
   d. Gupta, G., Peine, KJ, Abdelhamid D., Snider H, Shelton A., Rao L., Kotha S., Huntsman A., Varikuti S., Oghumu S., Naman B., Pan L, Parinandi NL, Pappenfuss TL, Kinghorn AD, Bachelder E., Ainslie KM, Fuchs JR and **Satoskar AR*.** A novel sterol isolated from a plant used by Mayan traditional healers is effective in treatment of visceral leishmaniasis caused by *Leishmania donovani*. ACS Infect. Dis. **2015;** 1 (10), pp 497–506.
5. Systemic pentavalent antimonials such as sodium stibogluconate (SSG) are widely used for treatment of CL world-wide, but they are toxic, and compliance with treatment is poor because daily injections for 3 weeks or longer are required. In addition, antimonial treatment in immunocompromised individuals. Therefore, there is a strong need for new CL treatments that are safe, affordable, easy to administer and that possess broad-spectrum activity against different species of *Leishmania* that causes CL. Clinical studies from our group conducted in India have discovered that topical Radio frequency-induced heat therapy (RFHT) is safe and highly effective for treating CL. More importantly, we have discovered that this treatment is effective in immunocompromised HIV patients infected with CL who are difficult to manage with conventional treatment. Our recent field studies in Turkey have focused on evaluating the efficacies of chemotherapies that are currently used for treatment of CL in this region.
   a. Prasad, N., Ghiya, BC, Bumb, RA, Kaushal, H., Satoskar AA., Lezama-Davila, CM, Salotra, P. and **Satoskar AR*** Heat, Oriental sore and HIV. The Lancet. **2011;** 377:610**.**
   b. Ahuja, AA, Bumb, RA, Mehta, RD, Prasad, N., Tanwar, RK and **Satoskar, AR*.** Successful treatment of canine cutaneous leishmaniasis using radiofrequency induced heat (RFH) therapy. Am. J. Trop Med. Hyg. **2012.** Aug;87(2):261-3.
   c. Bumb, RA, Prasad, N, Aara, N, Khandelwal, K, Mehta, RD, Ghiya, BC, Salotra, P, Lai, W., Peters, S., and **Satoskar AR***. Long-term efficacy of a single-dose Radio Frequency Heat therapy versus intralesional antimonials for cutaneous leishmaniasis in India. Br. J. Dermatol. **2013.** Jan 8. doi: 10.1111/bjd.12205.
   d. Yesilova Y, Surucu HA, Ardic N, Aksoy M, Yesilova A, Oghumu S, and **Satoskar AR.** Meglumine antimoniate is more effective than sodium stibogluconate in the treatment of cutaneous leishmaniasis. J Dermatolog Treat. **2015** Jun 24:1-5. [Epub ahead of print]

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/pubmed/?term=satoskar+ar

D. Research Support
Ongoing Research Support
W81XWH-14-2-0168 (PI: Satoskar) 09/30/2014-09/29/2017
Army Medical Res Acquisition Activity
Development of novel therapeutics for neglected tropical disease leishmaniasis

The goal is to development therapeutics for leishmaniasis.

Private Source 12/01/2015-11/30/2017

Development of a vaccine for leishmaniasis
The goal is to attenuate dermotropic strains of *Leishmania* and evaluate their immunogenicity

R21AI127582 (PI: Satoskar/Ibba) 12/14/2016-11-30/2018
NIH
A Systems Biology Approach for Targeted Drug Discovery for Leishmaniasis
The goal of this project is to use a systems biological approach to search for aminoacyl-tRNAsynthetase inhibitors with anti-leishmanial activity in a unique marine natural products library.

Private Source 04/15/2015-10/31/2017

Response determination of PBMC collected from PKDL patients when stimulated with CpG-D35
The overarching goal of this project is to evaluate the efficacy of type D CpG as immunotherapeutic agents for treatment of PKDL

WX81XWH-16-1-0036 (PI: Satoskar) 02/01/2016 – 01/31/2019
Army Medical Res Acquisition Activity
Prevention and treatment of breast cancer and its metastasis by targeting macrophage inhibitory factor (MIF)
The goal of this grant is to determine whether MIF is a prevention and/or therapeutic target in breast cancer

R01AI107250 (PI: Seveau) 02/01/2014-01/31/2019
NIH/NIAID
Multifaceted activity of listeriolysin O during host cell invasion by Listeria
The goal is to identify host pathways that can be targeted for therapeutic interventions against a wide range of diseases caused by intracellular pathogens.
Role: Co-Investigator

R21AI131227 (PI: McGwire) 02/06/2017-01/31/2019
NIH
Trypanosoma cruzi cyclophilin 19 induced host inflammation
At the conclusion of this project we will understand whether Cyp19 contributes to *T. cruzi* infection and tissue pathogenesis and the results will provide the groundwork for further mechanistic studies on this pathologic process and the development of small molecule inhibitors for the potential treatment Chagas heart disease.
Role: Co-Investigator

R21CA198024 (PI: Oberyszyn) 04/01/2016-03/31/2018
National Cancer Institute
Post-transplant Cutaneous Squamous Cell Carcinoma and Macrophage Migration Inhibitory Factor
The goal of this project is to use the pre-clinical model to test the hypothesis that increases in UVB-induced SCC observed in OTR are mediated at least in part by elevated cutaneous MIF levels.
Role: Co-Investigator

R21AI123692 (PI: Ainslie) 04/15/2016-03/31/2018
Univ. of North Carolina (Prime: NIH)
Microparticle resiquimod for the treatment of visceral leishmaniasis
We propose the co- treatment of both resiquimod, a TLR 7/8 agonist and Amphotericin B for visceral leishmania which will allow for the synergistic clearance of the infection.
Role: Co-Investigator

Completed Research Support
RC4 AI092624 (PI: Satoskar) 10/01/2010-09/30/2013
NIH/NIAID
Novel therapeutics of neglected tropical disease leishmaniasis
Goal was to isolate, synthesize and test efficacy of anti-parasitic molecules from *P. andrieuxii* leaves and stems as well as endophytic fungi from the plant in treatment of visceral and cutaneous leishmaniasis.

BIOGRAPHICAL SKETCH

NAME: Zhioua, Elyes

eRA COMMONS USER NAME (credential, e.g., agency login): eRA Commons User Name

POSITION TITLE: Full Professor

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 Bizerte, Tunisia	B.S.	9/1985	Biology
University of Neuchatel, Switzerland	M. Sc.	03/1987	Parasitology
Institut Pasteur de Paris, France	Certificate	06/1991	Medical Entomology
University of Neuchatel, Switzerland	D. Sc.	02/1993	Parasitology
University of Rhode Island, USA	Postdoctoral	03/1993-06/1998	Medical Entomology

A. Personal Statement

I have over 20 years of experience on vector ecology. From 1992 to 2002, my main field of research focus was the ecology of ticks in Europe and in the US. I developed a biological approach to control Lyme disease ticks based on the use of entomopathogenic fungi. For the last ten years, my research focus is the eco-epidemiology of leishmaniasis. Pasteur Institute of Tunis has a long history of research on Leishmaniasis. For the first time in Tunisia, I set up colonies of sand flies including *Phlebotomus papatasi* and *Phlebotomus perniciosus*, vector of zoonotic cutaneous leishmaniasis (ZCL) and zoonotic visceral leishmaniasis (ZVL), respectively. I developed an environmentally-friendly method to control populations of *P. papatasi* based on zooprophylaxis. I investigated the effects of pre-immunization with salivary gland proteins of colonized and wild-caught *P. papatasi* on the development of ZCL in BALB/c mice. Thus, for the development of a vaccine against leishmaniasis, the wild populations of sand flies should be taken into consideration. I tested the efficacy of rodent bait treated with systemic and feed through insecticide for controlling populations of *P. papatasi* associated with rodent reservoir hosts of *Leishmania major*, etiologic agent of ZCL under laboratory and field conditions. In addition to leishmaniasis, I showed that sand flies are main vector of phleboviruses in North Africa including the neurotropic arbovirus Toscana virus. I studied the impact of environmental changes mainly the development of irrigation in arid areas of North Africa on the emergence of ZVL. I investigated the vertical transmission of *Leishmania infantum*, etiologic agent of ZVL in dogs. I studied the eco-epidemiology of ZVL in endemic areas of Tunisia and concluded that controlling ZVL in dogs is a cornerstone is reducing the incidence of the disease in humans. I developed a canine model to evaluate the safety and efficacy of vaccine candidate against ZVL in which dogs are naturally exposed to sand flies vectors of *L. infantum* in a hyperendemic foci of Tunisia. Dr Satoskar, visited Pasteur Institute of Tunis in the past and he is familiarized with leishmaniais in Tunisia and we shared common interest in the development of new approaches to control ZVL. My expertise in the ecology of sand flies is well suited to oversee all aspects of this project in Tunisia.

B. Positions and Honors

Positions and Employment

2008-Present: Professor, Pasteur Institute of Tunis, Tunisia
2003-2007: Associate Professor, Pasteur Institute of Tunis, Tunisia
2000-2002. Director of the Tick Research Laboratory, University of Rhode Island, Department of Veterinary Sciences, Kingston, RI 02881, USA

1998-2002: Research Assistant Professor, University of Rhode Island, Department of Veterinary Sciences, Kingston, RI 02881, USA
1993-1998: Postdoctoral Fellow, University of Rhode Island, Department of Plant Sciences, Kingston, RI 02881, USA
1991-1992: Research Associate, Pasteur Institute of Paris, France

Reviewer
Manuscripts for numerous scientific journals (Acta Tropica, Plos One, Plos NTD, Parasites & Vectors, Journal of Medical Entomology, Parasitology Research, Journal of Clinical Microbiology)

C. Contributions to Science

1. **Eco-epidemiology of zoonotic cutaneous leishmaniasis in North Africa.** Zoonotic cutaneous leishmaniasis (ZCL) is endemic in North Africa. A basic understanding of the eco-epidemiology is of major importance in the development of new approaches to reduce the incidence of this disease.

   a. Chelbi I., and **E. Zhioua**. 2007. Phenology of *Phlebotomus papatasi* Scopoli (Diptera: Psychodidae) relative to seasonal variation of the prevalence of zoonotic cutaneous leishmaniasis in Central Tunisia. Journal of Medical Entomology, 44: 385-388.
   b. Chelbi I., and **E. Zhioua.** 2007. Biology of *Phlebotomus papatasi* Scopoli (Diptera: Psychodidae) in the laboratory. Journal of Medical Entomology, 44: 597-600.
   c. Chelbi, I., B. Kaabi, M. Béjaoui, and **E. Zhioua**. 2009. Spatial correlation between *Phlebotomus papatasi* Scopoli (Diptera: Psychodidae) and Zoonotic Cutaneous Leishmaniasis in Tunisia. Journal of Medical Entomology: 46: 400-402.

2. **Development of sand fly-based saliva as vaccine against *Leishmania major*.** Several studies showed that pre-immunization with salivary proteins of *Phlebotomus papatasi* protect against ZCL in mice model. In addition, pre-exposure of mice to bites of uninfected *P. papatasi* confer protection against ZCL. However, in natural setting, people at risk are more exposed to uninfected sand fly bites than infected ones but still succumb to the disease. Laboratory colonies of insects are often accepted as being representative of field populations from which they have been derived, but I showed that this assumption is not valid, because colonies frequently incorporate only a fraction of the original populations' genetic variability. I showed that for the development of a vaccine based on salivary gland proteins we need to take in consideration the natural populations of *P. papatasi*.

   a. Ben Hadj Ahmed S, Chelbi I, Kaabi B, Cherni S, Derbali M, and **Zhioua E**. 2010. Differences in the salivary effects of wild-caught vs. colonized *Phlebotomus papatasi* Scopoli (Diptera: Psychodidae) on the development of zoonotic cutaneous leishmaniasis in BALB/c Mice. Journal of Medical Entomology, 47: 74-79.
   b. Ben Hadj Ahmed S, Kaabi B, Chelbi I, Derbali M, Cherni S, Laouini D, and **Zhioua E.** 2010. Pre-immunization with saliva of long-term colonized *Phlebotomus papatasi* is not protective following experimental challenge with *Leishmania major* and saliva of wild-caught *Phlebotomus papatasi*. American Journal of Tropical Medicine and Hygiene. 83: 512-514.
   c. Ben Hadj Ahmed S, Kaabi B, Chelbi I, Derbali M, Cherni S, Laouini D, and **Zhioua E.** 2011. Colonization of *Phlebotomus papatasi* changes the effect of pre-immunization with saliva from lack of protection towards protection against experimental challenge with *Leishmania major* and saliva. Parasites and Vectors 4: 126. PMCID: PMC3143093
   d. Abdeladhim M., R. Jochim, M. Ben Ahmed**, E. Zhioua**, I. Chelbi, S. Cherni, , H. Louzir, J.M.C. Ribeiro and J.G. Valenzuela. 2012. Updating the salivary gland transcriptome of *Phlebotomus papatasi* (Tunisian strain): the search for sand fly-secreted immunogenic proteins for humans. PLoS ONE 7(11): e47347. DOI:10.1371/journal.pone.0047347. PMCID: PMC3491003

3. **Development of new approaches to control populations of *Phlebotomus papatasi*.** Control of populations of *P. papatasi* is based on insecticide residual spraying and impregnated bed nets. These methods are costly and require several applications. I developed an environmentally-friendly method to reduce the indoor abundance of *P. papatasi* based on zooprophylaxis. In addition, reducing the infection

rates of *P. papatasi* with *L. major* is based on controlling rodent reservoir by zinc phosphide. In Collaboration with Redacted by agreement from Genesis Laboratory Inc., we showed that rodent bait treated with fipronil for feed through and systemic is effective in controlling populations of *P. papatasi*. In collaboration with Redac Redacted by we showed that attractive toxic sugar baits (ATSB) are also effective in controlling populations of *P. papatasi*.

a. Chelbi, I. B. Kaabi, M. Derbali, S. Ben Hadj Ahmed, K. Dellagi, and **E. Zhioua**. 2008. Zooprophylaxis: Impact of breeding rabbits in man-made underground holes around houses on reducing the indoor density of *Phlebotomus papatasi*, principal vector of *Leishmania major*, etiologic agent of zoonotic cutaneous leishmaniasis in the Old World. Vector-Borne and Zoonotic Diseases, 8: 741-747.

b. Derbali, M., L. Polyakova, A. Boujaâma, S. Cherni, D. Burruss, W. Barhoumi, I. Chelbi, R. Poché, **E. Zhioua.** 2014. Laboratory and field evaluation of rodent bait treated with fipronil for feed through and systemic control of *Phlebotomus papatasi*. Acta Tropica, 135: 27-32.

c. Qualls, W.A., G.C. Muller, K. Khallaayoune, E.E. Revay, E. **Zhioua**, V.D. Kravchenko, K.L. Arheart, R.D. Xue, Y. Schlein, A. Hausmann, and J.C. Beier. 2015. Control of sand flies with attractive toxic sugar baits (ATSB) and potential impact on non-target organisms in Morocco. Parasites & Vectors, 8: 87. PMCID: PMC4333173

4. **Eco-epidemiology of phleboviruses transmitted by sand flies in North Africa.** Several studies performed in Southern Europe showed that sand flies of the subgenus *Larroussius* are vectors of phlebovirus particularly Toscana virus, etiologic agent of meningitis. However, few data concerning phlebovirus in North Africa are available. I showed that *P. pernicious* is the main vector of Toscana virus and Toscana like virus. People living in rural areas are at high risk for Toscana infection. I showed that dogs are also good sentinels for phleboviruses.

a. **Zhioua E**, Moureau G, Chelbi I, Ninove L, Bichaud L, Derbali M, Champs M, Cherni S, Salez N, Cook S, de Lamballerie X, and Charrel R. 2010. Punique virus, a novel phlebovirus, related to Sandfly fever Naples virus isolated from sandflies collected in Tunisia. Journal of General Virology, 91: 1275- PMCID: PMC3496376

b. Bichaud, L., K. Dachraoui, G. Piorkowski, I. Chelbi, G. Moureau,S. Cherni, X. De Lamballerie, S. Sakhria, R. N Charrel, and **E. Zhioua**. 2013. Isolation of Toscana virus from sand flies, Tunisia. Emerging and Infectious Diseases, 19: 322-324. PMCID: PMC3559066

c. Sakhria, S., L. Bichaud, M. Mensi, N. Salez, K. Dachraoui, L. Thirion, S. Cherni, I. Chelbi, X. De Lamballerie, **E. Zhioua**, and R. Charrel. 2013. Co-Circulation of Toscana virus and Punique virus in Northern Tunisia using a microneutralization-based serological study. Plos Neglected Tropical Diseases 7(9) :é2429.doi :10.1371/journal.pntd.0002429. PMCID: PMC3772032

d. Fares, W., R.N. Charrel, K. Dachraoui, L. Bichaud, W. Barhoumi, M. Derbali, S. Cherni, I. Chelbi, X. de Lamballerie, and **E. Zhioua**. 2015. Infection of sand flies collected from different bio-geographical areas of Tunisia with phleboviruses. Acta Tropica, 141: 1-6.

5. **Eco-epidemiology of zoonotic visceral leishmaniasis in North Africa.** ZVL is endemic in North Africa. The disease is fatal if left untreated. A basic understanding of the eco-epidemiology is of major importance in the development of new approach to reduce the incidence of this disease. I showed that environmental changes due to the development of irrigation systems in the arid areas of North Africa are at the origin of the emergence of ZVL in Central Tunisia. I showed that canine visceral leishmaniasis is the main factor for transmission to humans and subsequently, it is an important parameter for controlling transmission to humans. In addition, I developed a model to naturally challeng vaccinated dogs against *Leishmania infantum*.

a. **Zhioua, E**., B. Kaabi and I. Chelbi. 2007. Entomological investigations following the spread of visceral leishmaniasis in Tunisia. 2007. Journal of Vector Ecology, 32: 371-374.

b. Barhoumi W, Qualls WA, Archer RS, Fuller DO, Chelbi I, Cherni S, Derbali M, Arheart KL, **Zhioua E**, Beier JC. 2014. Irrigation in the arid regions of Tunisia impacts the abundance and apparent density of sand fly vectors of *Leishmania infantum*. Acta Tropica, 141: 73-78. PMCID: PMC4312219

c. Zoghlami, Z., E. Chouihi, W. Barhoumi, K. Dachraoui, Nabil Massoudi, K. Ben Helel, Z. Habboul, M. H. Hadhri, , S. Limam, M. Mhadhbi, M. Gharbi, **E. Zhioua.** 2014. Interaction between canine and human visceral leishmaniases in a holoendemic focus of Central Tunisia. Acta Tropica, 139: 32-38.

d. Ben Slimane, T., E. Chouihi, S. Ben Hadj Ahmed, I. Chelbi, W. Barhoumi, S. Cherni, Z. Zoghlami, M. Gharbi, **E. Zhioua**, 2014. An investigation on vertical transmission of *Leishmania infantum* in experimentally infected dogs and assessment of offspring's infectiouness potential by xenodiagnosis. Veterinary Parasitology, 206: 282-286.

Complete list of published work available at http://www.ncbi.nlm.nih.gov/pubmed/?term=Zhioua+E

D. Research Support

Completed Research Support

Private Source **Zhioua (PI)** 06/01/2012-05/31/2016

Natural challenge of dogs with *Phlebotomus perniciosus* in endemic foci of zoonotic visceral leishmaniasis
The goal of this project is to test the efficacy of a candidate vaccine against canine leishmaniasis.
Role: PI

Private Source **Zhioua (PI)** 08/01/2015-07/31/2016

Evaluation of rodent bait treated with fipronil for feed through and systemic control as preventive measures against zoonotic cutaneous leishmaniasis.
The goal of this project is to assess the efficacy of rodent bait treated with fipronil for controlling populations of *Phlebotomus papatasi*, vector of zoonotic cutaneous leishmaniasis
Role: PI

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PHS 398 Cover Page Supplement

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 Modular Budget

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

Budget Period: 1

Start Date: 04/01/2018 End Date: 03/31/2019

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium Indirect (F&A)*	150,000.00
Consortium Indirect (F&A)	6,529.00
Total Direct Costs*	156,529.00

B. Indirect (F&A) Costs

	Indirect (F&A) Type	Indirect (F&A) Rate (%)	Indirect (F&A) Base ($)	Funds Requested ($)
1.	On Campus Organized Research	55.75	93,389.00	52,065.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, 214-767-3261

Indirect (F&A) Rate Agreement Date: 04/25/2015

Total Indirect (F&A) Costs: 52,065.00

C. Total Direct and Indirect (F&A) Costs (A + B)

Funds Requested ($): 208,594.00

Funding Opportunity Number: PA-16-161. Received Date: 2017-06-30T09:27:25.000-04:00

PHS 398 Modular Budget

Budget Period: 2

Start Date: 04/01/2019 End Date: 03/31/2020

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium Indirect (F&A)*	125,000.00
Consortium Indirect (F&A)	4,671.00
Total Direct Costs*	129,671.00

B. Indirect (F&A) Costs

	Indirect (F&A) Type	Indirect (F&A) Rate (%)	Indirect (F&A) Base ($)	Funds Requested ($)
1.	On Campus Organized Research	56.00	66,611.00	37,302.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, 214-767-3261

Indirect (F&A) Rate Agreement Date: 04/25/2015

Total Indirect (F&A) Costs: 37,302.00

C. Total Direct and Indirect (F&A) Costs (A + B)

Funds Requested ($): 166,973.00

Funding Opportunity Number: PA-16-161. Received Date: 2017-06-30T09:27:25.000-04:00

PHS 398 Modular Budget

Cumulative Budget Information

1. Total Costs, Entire Project Period

Section A, Total Direct Cost less Consortium Indirect (F&A) for Entire Project Period ($)	275,000.00
Section A, Total Consortium Indirect (F&A) for Entire Project Period ($)	11,200.00
Section A, Total Direct Costs for Entire Project Period ($)	286,200.00
Section B, Total Indirect (F&A) Costs for Entire Project Period ($)	89,367.00
Section C, Total Direct and Indirect (F&A) Costs (A+B) for Entire Project Period ($)	375,567.00

2. Budget Justifications

Personnel Justification	Budget_Justification1034676976.pdf
Consortium Justification	Consortium_Justification1034676967.pdf
Additional Narrative Justification	

BUDGET JUSTIFICATION

Senior / Key Personnel

Abhay R. Satoskar, M.D., Ph.D., Principal Investigator EFFORT **month/year)** will be responsible for administration and supervision of the project and sub-contracts. His laboratory will be involved in training in immunological techniques. He will also visit Institut de Pasteur, Tunis 4 times during the project to provide training to personnel in Zhioua lab in immunological techniques and assist if necessary. He will participate in experimental design for all aims and the preparation of progress reports and manuscripts describing the findings from the proposed work.

Redacted by agreement **Collaborator** EFFORT **month/year)** is a biostatistician at Redacted by agreement Redacted by agreement She will be involved in sample size calculation and statistical analysis of the data.

Consortium Justification – Institut Pasteur de Tunis (Foreign)

Senior / Key Personnel

Elyes Zhioua, PhD, Principal Investigator (no effort requested). Dr. Zhioua will oversee the field and laboratory work.

Other Personnel

Redacted by agreement Other Significant Contributor. Redacted by agreement will oversee all experiences related to xenodiagnosis. Redacted by agreement is a medical entomologist with an outstanding experience in sand fly ecology.

Redacted by agreement Other Significant Contributor, and Redacted by agreement Other Significant Contributor, are immunologists with an outstanding experience in the immunology of leishmaniasis. Redacted by agreement will oversee all experience related to the analysis of the immune responses in the vaccinated and control dogs.

Redacted by agreement Veterinarian (EFFORT . Redacted by agreement will be responsible for all experiments related to dogs. Redacted by agreement is in Good Clinical Practice.

TBN, Care Taker (12 calendar months). He will be in charge of managing the dog kennel during the exposition and between transmission seasons.

	YEAR 1	YEAR 2	TOTAL
DIRECT COSTS	82,000	58,000	**140,000**
INDIRECT COSTS	6,000	5,000	**11,000**
TOTAL COSTS	**88,000**	**63,000**	**151,000**

PHS 398 Research Plan

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

Introduction	
1. Introduction to Application (Resubmission and Revision)	Introduction_to_revised_application6_281034676969.pdf
Research Plan Section	
2. Specific Aims	SpecificAims6_301034676973.pdf
3. Research Strategy*	ResearchPlan6_301034676971.pdf
4. Progress Report Publication List	
Human Subjects Section	
5. Protection of Human Subjects	
6. Data Safety Monitoring Plan	
7. Inclusion of Women and Minorities	
8. Inclusion of Children	
Other Research Plan Section	
9. Vertebrate Animals	Vertebrate_Animals6_81034676972.pdf
10. Select Agent Research	
11. Multiple PD/PI Leadership Plan	MPIPlan6_101034676974.pdf
12. Consortium/Contractual Arrangements	Consortium_Letter1034676968.pdf
13. Letters of Support	LOS6_281034676981.pdf
14. Resource Sharing Plan(s)	Resource_Sharing1034676978.pdf
15. Authentication of Key Biological and/or Chemical Resources	authentication1034676982.pdf
Appendix	
16. Appendix	

Funding Opportunity Number: PA-16-161. Received Date: 2017-06-30T09:27:25.000-04:00

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reviewers' comments

of the Freedom of Information and Privacy Act

SPECIFIC AIMS

Leishmania are obligate intracellular parasites that are transmitted by a sand fly vector and cause a wide range of diseases, such as cutaneous (CL), diffuse cutaneous (DCL), mucosal (MCL) and visceral leishmaniasis (VL)[1]. VL is caused by *Leishmania donovani* (*L. donovani*) or *L. infantum* and is a potentially life-threatening neglected tropical disease of global health concern. Half a million people are infected each year, and over 60,000 succumb to disease annually, although these numbers are likely underestimated (www.who.int).

Our team is focused in developing a prophylactic vaccine against leishmaniasis because none is currently available. It is well documented that patients who recover from leishmaniasis develop protective immunity against reinfection[1], which altogether indicates that a vaccine is feasible. In the past, leishmanization, a process in which deliberate infections with a low dose of *Leishmania major,* etiologic agent of zoonotic cutaneous leishmaniasis (ZCL) causes a controlled skin lesion and provides greater than 90% protection against reinfection, was a common practice in *Leishmania*-endemic regions[2-5]. However, under the current regulatory environment such practice may not be acceptable due to the complications that can occur. Nonetheless, these studies suggest that for an effective vaccine against leishmaniasis, a controlled infection with attenuated *Leishmania* that provides a complete array of antigens of the parasite may be necessary for developing protective immunity. Therefore, live-attenuated parasites that are nonpathogenic should induce the same protective immunity as leishmanization and thus would be effective vaccines. Past experience with other pathogens including viruses such as small pox and bacteria such as *S. typhi* have demonstrated that live-attenuated pathogens can be highly successful vaccines.

Genetically attenuated *L. infantum* and *L. donovani* including *LdCen*$^{-/-}$ have shown promise as a vaccine candidate in animal models[6-10]. However, using live-attenuated *L. donovani* as a vaccine in humans could raise safety concerns due to visceralizing potential of this species of *Leishmania.* Several studies have shown that an infection with dermatotrophic *Leishmania* such as *L. major* and *L. tropica* or immunization with antigens from these parasites or non-pathogenic *Leishmania* confers significant cross-protection against VL caused by *L. donovani* or *L. infantum* in experimental animals[11-17] and humans[18-20]. Therefore, attenuated dermatotrophic *Leishmania* such as *L. major* that also cross-protects against VL could be a safer vaccine against leishmaniasis because adverse events (e.g. development of a lesion as vaccination site) will be easy to monitor and can be treated easily with approved topical interventions.

Using CRISPR-Cas technology, our team has successfully generated for the first time antibiotic selection marker free *centrin* gene deficient *L. major* (*LmCen*$^{-/-}$). This was an essential step since it is not possible to conduct clinical trials with parasites containing antibiotic resistance genes. We propose to use a novel canine model of VL to test the safety and efficacy of *centrin* gene deficient *L. major* (*LmCen*$^{-/-}$). Whole genome sequencing of *LmCen*$^{-/-}$ passed through mice multiple times has confirmed stable deletion of *centrin* gene without other mutations in the parasite genome. Our preliminary findings show that *LmCen*$^{-/-}$ are highly attenuated and fail to cause disease in immunocompromised mice. We have also confirmed that immunization with *LmCen*$^{-/-}$ parasites induces disease protective Th1 response in hamster as well as mice and completely protects mice against homologous challenge with virulent *L. major.* Our industry partner Gennova Biopharma has already established *LmCen*$^{-/-}$ production under GLP conditions (GLP-*LmCen*$^{-/-}$) at their US-FDA approved facility. The goals of this project are to: (1) optimize *LmCen*$^{-/-}$ immunization protocol and immunogenicity in dogs using GLP-*LmCen*$^{-/-}$ and determine its safety and (2) evaluate efficacy of GLP-*LmCen*$^{-/-}$ as a vaccine using a model of canine VL in which dogs are naturally exposed to bites of *L. infantum* infected wild *Phlebotomus pernicious* in VL-hyperendemic regions of Tunisia. The proposed studies in this natural infection model of dogs will represent a significant advance towards testing this vaccine in humans. The specific aims of this project are:

Aim1: To optimize *LmCen*$^{-/-}$ immunogenicity and immunization protocol in dogs and determine its safety

- Determine dose, route of vaccination and immunization regimen that elicits optimal protective immune response in *LmCen*$^{-/-}$ -immunized dogs by monitoring CD4+ and CD8+ T cell responses, parasite-specific antibody responses, Th1/Th2 cytokine production
- Analyze safety of *LmCen*$^{-/-}$ vaccine by monitoring lesion development at the inoculation site, lesion histopathology and analyzing parasites loads at the site and peripheral blood temporally

Aim 2: Evaluate efficacy of *LmCen*$^{-/-}$ vaccine against canine VL caused by *L. infantum* infection transmitted by wild sand flies in the Western Mediterranean

- Monitor the course of VL in *LmCen*$^{-/-}$ vaccinated dogs following naturally acquired *L. infantum* infection transmitted by wild *Phlebotomus pernicious.*
- Analyze immune responses in the infected dogs by phenotypic characterization of immune cells and by measuring antibodies and cytokine levels in the peripheral blood temporally.

RESEARCH STRATEGY

BACKGROUND AND SIGNIFICANCE

- Over 12 million people currently suffer from leishmaniasis, and approximately 2 million new cases occur annually, making it a major global health problem and a WHO classified neglected tropical disease (NTD) (www.who.int). Cutaneous leishmaniasis (CL) is the most common form of *Leishmania* infection which manifests as localized skin lesions that can become chronic, leading to significant tissue destruction and disfigurement[1]. Other forms of infections are mucosal leishmaniasis (ML), or life-threatening visceral leishmaniasis (VL) caused by *L. donovani* and *L. infantum*[1]. VL is the second most common fatal parasitic infection after malaria and is characterized by dissemination of the parasites to liver, spleen and bone marrow[21].
- The zoonotic potential of human leishmaniasis has also been escalating with increased incidence of canine infections, which is the main reservoir host for *L. infantum* worldwide including the United States[22-23].
- No licensed human vaccine is currently available against any parasitic disease including leishmaniasis.
- Leishmanization, a process in which deliberate infection with a low dose of virulent *Leishmania major* causes a controlled skin lesion and provides > 90% protection against re-infection, was a common practice in *Leishmania*-endemic regions in the Middle East and is currently used in Uzbekistan[2-5]. However, under the current regulatory environment, such practice is not acceptable globally because of the complications that often occur including vaccine induced lesions that do not cure.
- It is also well documented that the majority of the patients with leishmaniasis develop a long-term protective immunity after curing primary infection[24-28], which suggests that an effective vaccine could be developed against leishmaniasis.
- A live attenuated strain of *Leishmania* will provide a complete array of antigens without causing disease and therefore could be an effective vaccine for leishmaniasis.
- A study supported by Private Source has found that even a modestly efficacious vaccine (50% efficacy) could provide substantial value and will be highly cost-effective in VL endemic regions[29].
- Live attenuated vaccines are currently being used in humans and have been found to be safe and effective against viruses (small pox, yellow fever, polio, MMR) and bacteria (typhoid, BCG).
- *Leishmania* Centrin is a calcium-binding cytoskeletal protein located in the centrosome/basal body and is involved in duplication or segregation in *Leishmania*[30-31].
- *Centrin* gene deficient *Leishmania donovani* (*LdCen-/-*) generated are avirulent and do not cause disease even in immunocompromised T cell[10] and IFN-γ deficient host[10], are completely eliminated from the host and are therefore safe[10]. Previous studies have shown that vaccination with *LdCen-/-* parasites induces a Th1 response and confers long-term protection against experimental VL in mice[10], hamster[10] and dogs[6-7] as well as cross-protects against CL in mice[8, 10].
- Several genetically attenuated *L. infantum* and *L. donovani* including *LdCen*$^{-/-}$ have shown promise as a vaccine candidate in animal models[9]. However, using live-attenuated *L. donovani* as a vaccine in humans could raise safety concerns due to visceralizing potential of this species of *Leishmania*.
- Attenuated dermatotrophic *Leishmania* that cross-protects against visceral disease-causing *L. donovani* could be a safer vaccine against VL and CL because adverse events (e.g. development of a lesion as vaccination site) will be easy to monitor and can be treated using approved non-pharmacological interventions such as topical thermotherapy.
- A clinical study by Redacted by agreement (1943) had reported that CL patients infected with *L. tropica* were protected against VL after intravenous challenge with 1 x 10^7 *L. donovani*[18]. Furthermore, two subsequent studies found that infection with *L. major* induced protective immunity against VL in human volunteers[19-20].
- Studies using a variety of animal models have also shown that infection with virulent dermatotrophic *Leishmania* such as *L. major* which causes self-resolving infection[11-12] or immunization with antigens from these parasites confers significant cross-protection against VL caused by *L. donovani* or *L. infantum*[13-14, 16-17].
- Despite these previous studies, it is not known whether immunization with attenuated cutaneous disease-causing species such as *centrin* gene deficient *L. major* (*LmCen*$^{-/-}$) will protect against the visceral disease-causing species *L. infantum*.
- A canine model of VL developed at Pasteur Institute, Tunisia will be used for testing the efficacy of *LmCen*$^{-/-}$ as a vaccine against VL. In this preclinical model dogs acquire infection in natural habitat in the Western Mediterranean through exposure to wild sand flies infected with *L. infantum*. *Leishmania* are delivered into skin through a sand fly bite together with molecules that modulate the bite site, which include salivary proteins and parasite-derived promastigote secretory gel. These important aspects of parasite transmission are often overlooked when testing vaccines using preclinical animal models of VL.
- The data generated from these studies will provide rationale for advancing *LmCen*$^{-/-}$ as a vaccine against leishmaniasis and critical information needed for future clinical trials in humans.

Scientific Premise:

- There are no licensed vaccines for human leishmaniasis, and chemotherapy is the mainstay to combat this disease, which relies on antileishmanial drugs which are toxic and have poor patient compliance.

- As described above, data from preclinical animal models of VL and CL show that various attenuated *L. infantum* and *L. donovani* including *LdCen*$^{-/-}$ could be promising vaccine candidates but visceralizing potential of these species of *Leishmania* preclude their further advancement as vaccines for leishmaniasis.
- Several clinical and preclinical animal studies show that infection/immunization with dermatotrophic *Leishmania* such as *L. major* confers significant protection against visceralizing *Leishmania.* Therefore, attenuated *L. major* parasites (*LmCen*$^{-/-}$) could be safe and viable vaccine candidate for leishmaniasis.
- The scientific promise of this project, if successful, could result in the first highly effective regulatory approved vaccine for a human parasitic disease, Leishmaniasis.

INNOVATION

- This is the first study to use a novel attenuated cutaneous disease-causing species, *centrin* gene deficient *L. major* (*LmCen*$^{-/-}$) developed using CRISPR-Cas technology to protect against VL caused by *L. infantum* in a large animal model. CRISPR-Cas enables to the development of attenuated strains without antibiotic resistance genes.
- A novel model of canine VL in which dogs acquire *L. infantum* infection in their natural habitat following exposure to infected wild sand flies will be used to test the safety and efficacy of *LmCen*$^{-/-}$ parasites in protecting against VL. These studies will also revaluate immune determinants *LmCen*$^{-/-}$ parasites.
- The proposed studies will provide a rationale for advancing *LmCen*$^{-/-}$ parasites as a vaccine against leishmaniasis in humans and dogs as a proof of principal and a large animal model for humans. Currently no vaccine is available for this disease for humans.

APPROACH

Our overall goal is to evaluate the safety and efficacy of GLP-*LmCen*$^{-/-}$ as a vaccine against naturally acquired *L. infantum* infection in the wild acquired thorough bites of infected sand flies. The following preliminary data supports our study.

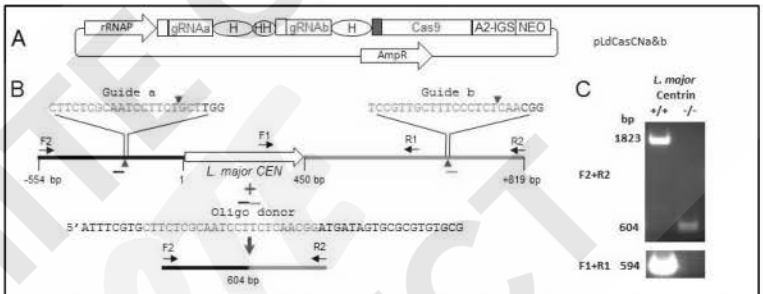

Figure 1: Generation of an antibiotic selection marker free *Centrin* gene deficient *L. major* (LmCen$^{-/-}$)using CRISPR-Cas Plasmid pLdCasCNa&b expresses the Cas 9 gene and two *Centrin* gene targeting gRNAs from the Leishmania ribosomal promoter (rRNAP) which are processed by the Hepatitis virus ribozymes (H) and hammerhead ribozyme (HH). **B.** Targeting strategy used, where an oligo donor was used to replace the Centrin gene. As detailed in ref 35, addition of the oligo donor increases efficiency of gene targeting by homologous recombination. **C.** PCR showing that the *Centrin* gene has been removed (also confirmed by sequence analysis). ***Important note:*** *Following Centrin gene removal, the pLdCasCNa&b plasmid was removed by growing the Centrin deleted mutant in media without G418 and then verified the absence of the targeting plasmid by PCR and sensitivity to G418. This resulted in the L. major centrin mutant with no antibiotic resistance gene.*

1. Generation of antibiotic selection marker free *centrin* gene deficient *L. major* (*LmCen*$^{-/-}$) using CRIPR-Cas technology: Our team has deleted *centrin* gene from *L. major* (Friedlin) using CRISPR-Cas to successfully generate antibiotic selection marker free *centrin* gene deficient *L. major* (*LmCen*$^{-/-}$) **(Figure 1).** Whole genome sequencing of *LmCen*$^{-/-}$ passed multiple times through mice has confirmed stable deletion of *centrin* gene without other mutations in the parasite genome.

2. *LmCen*$^{-/-}$ are highly attenuated and fail to cause disease in immunocompetent as well as immunocompromised host: To determine safety and long-term persistence of *LmCen*$^{-/-}$ parasites *in vivo*, we infected WT BALB/c and immunodeficient STAT1$^{-/-}$ BALB/c mice with different doses of WT *L. major* or *LmCen*$^{-/-}$ and monitored the course of infection. As anticipated, at week 7 post-infection WT as well as STAT1$^{-/-}$ mice infected with virulent *L. major* (Friedlin) developed large non-healing lesions full of parasites **(Figure 2)**. In contrast, none of the mice infected with *LmCen*$^{-/-}$ developed any visible lesions. Similarly, hamsters infected with *LmCen*$^{-/-}$ also failed to develop lesions and no viable parasites were recovered from their draining lymph node (dLN) **(Figure 3C)**, infected ears **(Figure 3D)**, liver, spleen or bone marrow (data not shown)

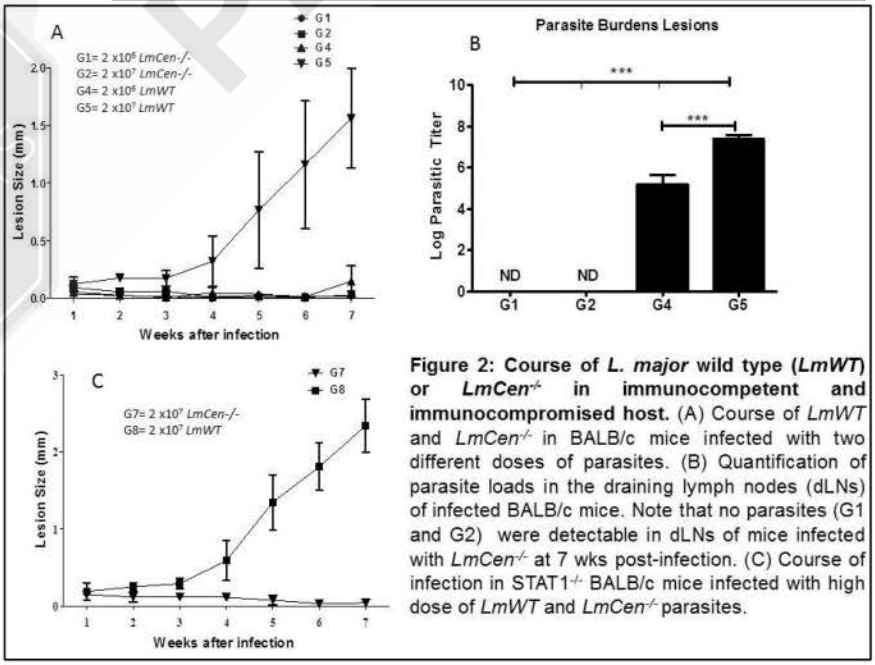

Figure 2: Course of *L. major* wild type (*LmWT*) or *LmCen*$^{-/-}$ in immunocompetent and immunocompromised host. (A) Course of *LmWT* and *LmCen*$^{-/-}$ in BALB/c mice infected with two different doses of parasites. (B) Quantification of parasite loads in the draining lymph nodes (dLNs) of infected BALB/c mice. Note that no parasites (G1 and G2) were detectable in dLNs of mice infected with *LmCen*$^{-/-}$ at 7 wks post-infection. (C) Course of infection in STAT1$^{-/-}$ BALB/c mice infected with high dose of *LmWT* and *LmCen*$^{-/-}$ parasites.

indicating that *LmCen*$^{-/-}$ parasites were cleared from the hamsters **(Figure 3)**. These findings show that the vaccine dose (10^6-10^7) is safe and does not cause disease.

3. Immunization with *LmCen*$^{-/-}$ induces protective Th1 response in mice and hamsters: To verify if immunization of hamsters and mice with *LmCen*$^{-/-}$ parasites results in an immune response consistent with protection, Syrian golden hamsters and BALB/c mice were infected intradermally with 2 x 10^6 *LmCen*$^{-/-}$ parasites and immune responses in spleens (Hamster and mice) and draining lymph nodes (mice) were analyzed **(Figures 4 and 5)**. Results in hamster showed that *LmCen*$^{-/-}$ immunization significantly induces the expression of IFN-γ **(Figure 4A)** with a concomitant down regulation of IL-10 **(Figure 4B)** suggesting the induction of a Th1 type of immunity. A ratio of IFN-γ and IL-10 further indicated induction of Th1 response by *LmCen*$^{-/-}$ parasites **(Figure 4C).** In addition, compared with *LmWT* control group, *LmCen*$^{-/-}$ immunized hamsters showed a protective Th1-associated IgG2 dominant response as observed by a higher IgG2/IgG1 ratio **(Figure 4D).** These results demonstrate that immunization with *LmCen*$^{-/-}$ parasites induces a host protective immune response. Studies in mice showed that immunization results in a significantly strong induction of a protective Th1 type response indicated by IFN-γ, IL-2 and TNF-α dominated response with concomitant suppression of IL-10 production compared to non-immunized control (**Figure 5**). This Th1 dominated protective response was evident in all the tissues sampled indicating that immunized mice developed a protective immunity.

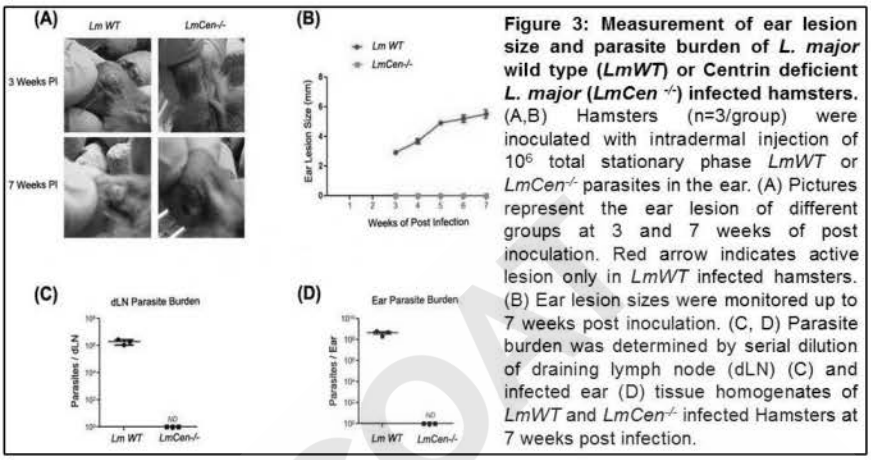

Figure 3: Measurement of ear lesion size and parasite burden of *L. major* wild type (*LmWT*) or Centrin deficient *L. major* (*LmCen* $^{-/-}$) infected hamsters. (A,B) Hamsters (n=3/group) were inoculated with intradermal injection of 10^6 total stationary phase *LmWT* or *LmCen*$^{-/-}$ parasites in the ear. (A) Pictures represent the ear lesion of different groups at 3 and 7 weeks of post inoculation. Red arrow indicates active lesion only in *LmWT* infected hamsters. (B) Ear lesion sizes were monitored up to 7 weeks post inoculation. (C, D) Parasite burden was determined by serial dilution of draining lymph node (dLN) (C) and infected ear (D) tissue homogenates of *LmWT* and *LmCen*$^{-/-}$ infected Hamsters at 7 weeks post infection.

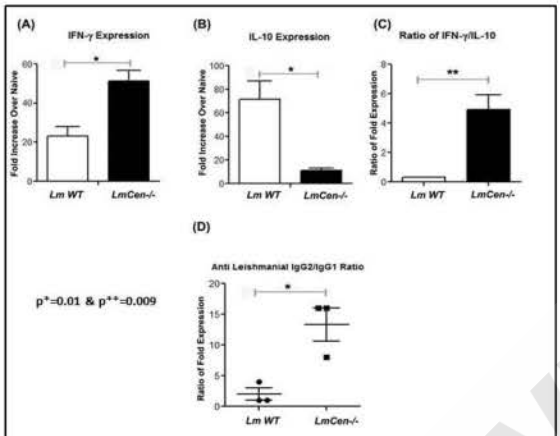

Figure 4: Analysis of immune responses in hamsters infected with *L. major* wild type (*LmWT*) or *LmCen*$^{-/-}$. Levels of (A) IFN-γ and (B) IL-10 gene transcripts in the spleens of infected hamsters were measured by real time PCR and (C) IFN-γ/IL-10 ratio was calculated. (D) Blood was collected at the time of euthanasia and levels of parasite specific Th1 associated IgG2a and Th2 associated IgG1 were determined by ELISA.

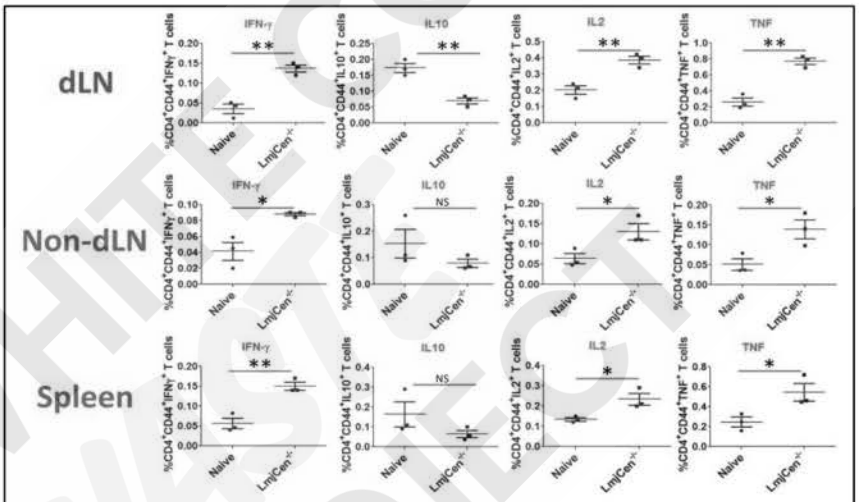

Figure 5: Analysis of immune responses in BALB/c mice infected with *LmCen*$^{-/-}$ parasites. Mice were sacrificed 7 weeks after immunization and multifunctional T cell response from draining, non-draining lymph nodes and spleen was measured independently using flow cytometry. * p<0.05

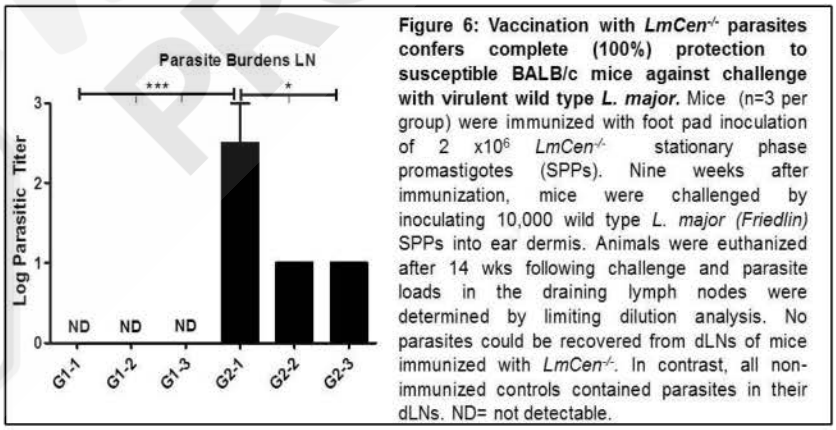

Figure 6: Vaccination with *LmCen*$^{-/-}$ parasites confers complete (100%) protection to susceptible BALB/c mice against challenge with virulent wild type *L. major*. Mice (n=3 per group) were immunized with foot pad inoculation of 2 x10^6 *LmCen*$^{-/-}$ stationary phase promastigotes (SPPs). Nine weeks after immunization, mice were challenged by inoculating 10,000 wild type *L. major (Friedlin)* SPPs into ear dermis. Animals were euthanized after 14 wks following challenge and parasite loads in the draining lymph nodes were determined by limiting dilution analysis. No parasites could be recovered from dLNs of mice immunized with *LmCen*$^{-/-}$. In contrast, all non-immunized controls contained parasites in their dLNs. ND= not detectable.

4. Vaccination with *LmCen*$^{-/-}$ confers complete protection against homologous challenge with virulent *L. major:* To determine whether immunization with *LmCen*$^{-/-}$ induces immunity against leishmaniasis, we investigated whether susceptible BALB/c mice vaccinated with *LmCen*$^{-/-}$ are protected against disease following challenge with virulent wild type *L. major* (Friedelin) **(Figure 6).** We found that *LmCen*$^{-/-}$ vaccination conferred

complete protection against *L. major* challenge. While significant number of parasites could be cultured from dLNs of unvaccinated control mice, no parasites were detectable in dLNs of vaccinated mice **(Figure 6).**

Aim1: To optimize *LmCen*$^{-/-}$ immunogenicity and immunization protocol in dogs and determine its safety

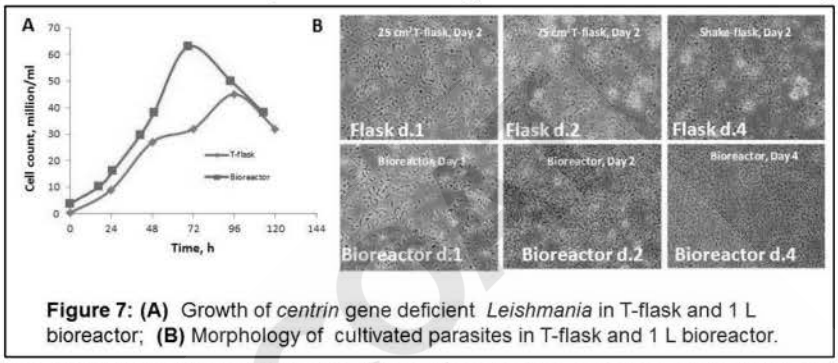

Figure 7: (A) Growth of *centrin* gene deficient *Leishmania* in T-flask and 1 L bioreactor; **(B)** Morphology of cultivated parasites in T-flask and 1 L bioreactor.

Rationale: Previously published clinical as well as experimental studies have reported that an infection with dermatotrophic *Leishmania* such as *L. tropica* or *L. major* confers protection against visceralizing *L. donovani* and *L. infantum*. Our preliminary studies show that *LmCen*$^{-/-}$ parasites are safe and induce a protective Th1 response in hamster and mice. We also found that *LmCen*$^{-/-}$-immunized BALB/c mice were completely protected against homologous challenge with wild type *L. major*. Collectively, these findings indicate that *LmCen*$^{-/-}$ could be a safe and promising vaccine against leishmaniasis. The overall goal of our project is to evaluate the efficacy of GLP-*LmCen*$^{-/-}$ parasite as a vaccine against VL using a novel canine model of zoonotic visceral leishmaniasis (ZVL) where dogs acquire *L. infantum* in their wild habitat. In this aim, we will establish the protocol for *LmCen*$^{-/-}$ vaccination in dogs by optimizing parasite dose, number of injections and route of inoculation as well as monitor its safety. Our industry partner Gennova Biopharma has already established production of GLP-*LmCen*$^{-/-}$ parasites **(Figure 7)**.

The Experiments: ***A) What is the dose and route of inoculation of LmCen*$^{-/-}$ *that elicits an optimal protective Th1 response in dogs?*** In this sub-aim, we will optimize the dose and route of injection for *LmCen*$^{-/-}$ vaccination in dogs. Six months old 18 purposed bred healthy beagle dogs will be immunized with different doses of *LmCen*$^{-/-}$ via intradermal as well as subcutaneous routes **(Table 1).** These doses were chosen on the basis of a previous study which reported that dogs immunized by subcutaneous injection of 10^7 *centrin* gene deficient *L. donovani* are protected against VL caused by *L. infatum*[6]. A group of dogs will be infected with wild type *L. major* as controls. Course of infection will be monitored by assessing lesion development and measuring lesion sizes every week. Peripheral blood will be collected from immunized dogs by venepuncture under general anaesthesia once every 15 days up to 2 months. Plasma levels of protective (IFN-γ, TNF-α), disease exacerbating cytokines (IL-10) as well as *Leishmania*-specific IgG_{Total}, IgG_1 and IgG_2 will be measured by ELISA[6]. Activation status of CD4+ and CD8+ T cells in the peripheral blood will also be assessed by analysing surface levels if MHC class II and CD11/18 by flow cytometry as described previously[6]. For measuring T cell activation, PBMCs will be isolated from the heparinized blood by density gradient centrifugation and *in vitro* proliferation assay will be performed[6]. Briefly, CFSE-labelled 5 x 10^5 PBMCs per well in 1 ml complete RPMI-1640 medium in triplicate will be plated in 48 well flat-bottom tissue culture plate. Cells will be stimulated with PHA (25µg/ml) or soluble *L. infantum* antigen (10µg/ml) for 72 hours. Cells will be labelled with $CD4^+$ and $CD8^+$ T cell specific antibodies and T proliferation will be assessed by flow cytometry. Levels of IL-2, IFN-γ, TNF-α and IL-10 in culture supernatants will be measured by ELISA using commercially available kits (Abcam Inc., USA; Sigma Aldrich Inc., USA). At the last time point (i.e. 4 months post-immunization), delayed-type hypersensitivity (DTH) responses will be measured by injecting 200 µg freeze-thawed *L. infantum* antigen into the ear dermis, and measuring the increase in skin thickness 48 h later with a dial-gauge micrometer. The lowest dose of *LmCen*$^{-/-}$ that induces the highest DTH response and IFN-γ production will be used for vaccination studies proposed in Aim 2. ***B) Does administration of LmCen*$^{-/-}$ *booster injection to LmCen*$^{-/-}$*-immunized dogs induce significantly higher and/or long-lasting Th1 response?*** We will compare immune responses in dogs immunized with *LmCen*$^{-/-}$ with and without booster injection. The dose and route of injection for these studies will be determined in experiments proposed in sub-aim A. Six months old sex matched 8 purposed bred healthy beagle dogs will be infected with *LmCen*$^{-/-}$. Two months following immunization, a group of 4 dogs will be administered second injection of *LmCen*$^{-/-}$ as a booster. Lesion development will be monitored weekly up to 6 months following first injection. Immune responses will be

Table 1

Dose of *LmCen*$^{-/-}$	Route of injection	Analysis of immune response and safety
10^7 (n=3)	Intradermal	1. Monitor lesion growth 2. Measure cytokine levels in the blood as well as analyze production (e.g. IL-10 and IFN-γ) by PBMCs once a month up to 6 months 3. Analyze *Leishmania*-specific $CD4^+$ and $CD8^+$ T cell responses 4. Detection of parasites in the blood and bone marrow by PCR 5. Analysis of hematological parameters and biochemical parameters for liver and kidney function 6. Biopsy of inoculation site at the end of study for histopathology and detection of parasites.
10^8 (n=3)	Intradermal	
10^9 (n=3)	Intradermal	
10^7 (n=3)	Subcutaneous	
10^8 (n=3)	Subcutaneous	
10^9 (n=3)	Subcutaneous	

analyzed in both groups once a month by evaluating activation status of immune cells as well as T cell responses and cytokine production as described above. ***C) Analysis of the safety of LmCen$^{-/-}$* vaccination:** Vaccinated dogs will be followed up to 6 months for development of lesion at the site of inoculation or any other site. Quantitative real-time PCR (qPCR) will be used to determine the parasite loads in peripheral blood collected twice a month for first two months and once a month thereafter up to 6 months. Biopsy of inoculation site will be performed at 4 months post-challenge for histopathology and parasite detection by qPCR.

Expected outcomes, pitfalls and alternatives: Based on published studies and our preliminary findings, we anticipate that none of the dogs infected with *LmCen$^{-/-}$* will develop lesion or contain parasites in their blood 2 months after immunization. We expect that dogs immunized with *LmCen$^{-/-}$* will mount a strong Th1 response which could vaccine dose dependent. It is likely that *LmCen$^{-/-}$* -immunized dogs receiving a booster may develop a more sustained Th1 response. It is possible that cytokine responses in the peripheral blood may be inconclusive. In this case, we will perform splenic biopsies under anesthesia and analyze cytokine responses in the spleen by measuring gene transcript levels of Th1 and Th2 associated cytokines by qPCR.

We do not expect difficulties to make sufficient quantities of GLP grade *LmCen$^{-/-}$* demonstrated by Gennova Biopharma **(Figure 7)**. We also do not expect any issues in shipping *LmCen$^{-/-}$* to Tunisia for canine studies. Gennova has also demonstrated stability of cryopreserved GLP- *LmCen$^{-/-}$* with ~90% recovery and viability after 60 days in frozen condition. Furthermore, recovered GLP-*LmCen$^{-/-}$* could infect mouse macrophages similar to non-cryopreserved parasites and transform into amastigotes.

Aim 2: Evaluate efficacy of *LmCen$^{-/-}$* vaccine against canine VL caused by *L. infantum* infection transmitted by wild sand flies in the Western Mediterranean

Rationale: Preclinical animal models of VL that are frequently used for vaccine and/or drug testing require inoculation of large doses of parasites for the induction of the disease. Furthermore, disease in some laboratory animals such as mice does not resemble human VL. For example, unlike humans, mice self-resolve *L. donovani* infection in their liver and do not succumb to infection. In contrast, dogs are the major reservoir for *L. infantum* and the wild sand fly transmission model in dogs developed at Pasteur Institute closely mimics the human VL disease. Similar to VL patients *L. infantum*-infected dogs develop weight loss, anemia, cachexia and hepatosplenomegaly and succumb to infection if left untreated **(Figure 8).** *Leishmania* parasites are delivered into skin together with molecules that modulate the bite site, which include salivary proteins and parasite-derived promastigote secretory gel. Additionally, an infected wild sand fly displays modified feeding behavior with prolonged and persistent probing. These important aspects of parasite transmission by wild sand flies are often overlooked in needle inoculation or sand fly transmission models of VL in the laboratory. Indeed, previous studies performed by Dr. Zhioua's group showed that mice immunized with salivary gland homogenate (SGH) of long-term colonized (F29) female *Phlebotomus papatasi* Scopoli induced protection against *L. major* co-inoculated with the same type of SGH. In contrast, pre-immunization of mice with SGH of wild-caught sand flies did not confer protection against *L. major* co-inoculated with the same type of SGH[32]. These results suggest that vaccine against leishmaniasis should be tested under natural condition. In this aim we will use a natural transmission model *of L. infantum* infection in dogs to test the efficacy of *LmCen$^{-/-}$* parasites as a vaccine against VL. The natural transmission model detailed below is more physiological, relevant to natural transmission and will provide information critical for human studies in future.

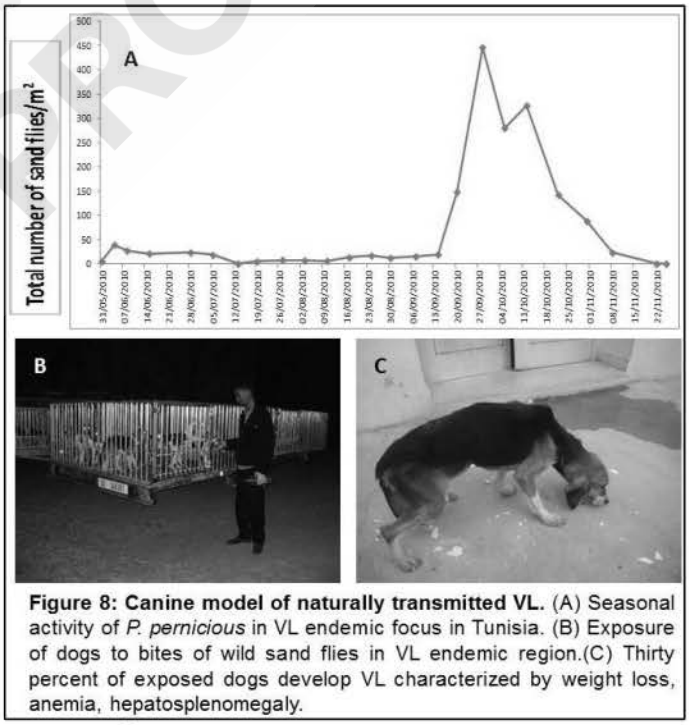

Figure 8: Canine model of naturally transmitted VL. (A) Seasonal activity of *P. pernicious* in VL endemic focus in Tunisia. (B) Exposure of dogs to bites of wild sand flies in VL endemic region.(C) Thirty percent of exposed dogs develop VL characterized by weight loss, anemia, hepatosplenomegaly.

The Experiments: *A) Evaluate efficacy of LmCen$^{-/-}$ as a vaccine against VL*: The activity period of *Phlebotomus perniciosus*, the main vector of *L. infantum* in the Western Mediterranean spreads between May and November[33] **(Figure 8A).** *L. infantum* infection rates in sand flies were found to range from 3.8 to 9.4%[33]. In a cohort of exposed dogs, the incidence of *L. infantum* infection and canine leishmaniasis were 50% and 30%, respectively[33]. Human incidence of VL was found to be 39.2/100,000 inhabitants[33]. To take advantage of this natural transmission, 6 months old 36 purposed bred healthy beagle dogs will be divided into two groups (18 males and 18 females per group). All dogs will be

confirmed PCR negative and seronegative for *L. infantum* immediately before the vaccination phase will commence. The *LmCen*$^{-/-}$ group will be vaccinated with *LmCen*$^{-/-}$ parasites (dose/regimen will be determined in Aim 1) and the control group will be inoculated with PBS only. One month after vaccination, all animals will be exposed to wild *L. infantum* infected *P. perniciosus* at the study site. Dogs will spend overnight and brought back the following day early morning to the dog kennel **(Figure 8B)**. Dogs will be exposed to sand fly bites each night throughout the sand fly season to ensure transmission. Following exposure, dogs will be housed at the kennel and monitored for disease development by measuring a variety of clinical and laboratory parameters (e.g. weight loss, cachexia, hepatosplenomegaly and anemia, **Figure 8C**). At 3, 6 and 12 months post-challenge, bone marrow as well as peripheral blood will be collected from dogs under general anaesthesia. Parasite loads in the bone marrow will be determined by quantitative real-time PCR[6] and/or by culture using NNN medium. Blood levels of albumin, AST, ALT, total bilirubin, calcium, total cholesterol, creatinine, urea, alkaline phosphatase, gamma GT, glucose, and total proteins will be determined. Serum levels of anti-*L. infantum* antibodies will be determined by IFAT to identify dogs that are infected with *L. infantum*[33]. Haematological parameters will be assessed by performing whole blood counts. Xenodiagnosis will be performed on each dog at 3, 6, 12, 15, 20 months post-immunization. *P perniciosus* used for xenodiagnoses will be from a colony maintained at the Vector Ecology laboratory at Redacted by agreement as described by Maroli et al[34] with some modifications[35]. Dogs will be anaesthetized by subcutaneous injection of 200 µl of ketamine (10 mg/ml) (Merial, Lyon, France) and for 2 hours will be placed in a cage containing between 15 to 30 females *P. perniciosus*. Engorged sand flies will be placed in new cages containing sugar for blood digestion. Seven days post-blood meal, sand flies will be dissected under dissecting microscope and examined for the presence of promastigotes in their midguts. ***B) Determination of immunological parameters of protection in dogs:*** Cell mediated immune response mediated by IFN-γ producing CD4+ T helper 1 (Th1) cells is indispensable for protection against VL whereas the IL-10 increases susceptibility to infection. Some studies also suggest a role for CD8+ T cells in mediating immunity against VL[7, 36-39]. Therefore, analysis of immunological parameters of protection in dogs will focus on evaluation of both CD4+ and CD8+ T cell immunity by analyzing cytokine production, proliferation and cytotoxicity as described previously[6-7]. Blood will be collected by venepuncture at 3, 6 and 12 months post-immunization. Plasma levels of protective (IFN-γ, TNF-α) and disease exacerbating cytokines (IL-10) will be measured by ELISA. Immune cells in the peripheral blood, T cell activation status and proliferation and levels of cytokines (IL-2, IFN-γ, TNF-α and IL-10) in culture supernatants will be analyzed as described in Aim 1.

Pitfalls and alternatives: It is possible that immunization with *LmCen*$^{-/-}$ may not confer complete protection against *L. infantum* infection transmitted by wild sand flies. However, as reported previously even modest protection (50% efficacy) could provide substantial value and will be highly cost-effective in VL endemic regions[29] We do not anticipate any technical issues in using wild sand fly transmission model of canine VL for the proposed studies. However, if necessary we will use a laboratory model canine VL in which dogs are infected by intravenous inoculation of *L. infantum*. Zhioua Lab has published experience with this model[40].

Statistical Considerations: For experiment A) in Aim 1, n=3 dogs will be used in each vaccinated group (6 treatment groups in total with 3 dose levels (10^7, 10^8, 10^9), 2 routes (intradermal and subcutaneous) and a control group (unvaccinated). DTH will be measured and summarized within each group. The dose and regimen that induces highest DTH response will be used for the following vaccination studies. For experiment B) in Aim 1, n=10 infected dogs per group (vaccinated once with or without a booster, 8 dogs in total) will be used to provide 80% power to detect a 2-fold change in Th1 responses at the study point end with CV=50% and two-sided alpha of 0.05. Lesion size, immune responses, T cell responses and cytokine production will be summarized at each time point and compared between the groups using linear mixed effect model. For Aim 1 C), linear mixed effect model will also be used to model the parasite loads over time as the outcome measurements are correlated. For experiments in Aim 2, n=6 disease dogs with VL (3 males and 3 females) will be used in each group (vaccinated and unvaccinated control group, 12 diseased dogs in total) to provide at least 80% power to detect a 2.5-fold change in parasite load at the study point end (12 months) with CV=50% and two-sided alpha of 0.05. Laboratory parameters (e.g. weight loss, cachexia, hepatosplenomegaly and anemia), parasite loads, blood levels of albumin, AST, ALT, total bilirubin, calcium, total cholesterol, creatinine, urea, alkaline phosphatase, gamma GT, glucose, and total proteins will be also determined and summarized using descriptive statistics at each time point for each group. Linear mixed effect model will be used to model the parasite burdens over time (3, 6, and 12 months) as the outcome measurements are correlated. Level of cytokines such as IFN-γ, TNF-α, IL-2, and IL-10 will be summarized and compared among the two groups. T-test or non-parametric Wilcoxon test will be used for group comparisons. Holm's procedure will be used to adjust for multiplicities. Linear mixed effect model will also be used to model these measurements over the time.

Vertebrate Animals

The research will involve use of mice and dogs. These will be housed in Institute approved animal facility at the Pasteur Institute, Tunisia. The institute has an approved Animal Welfare Assurance from the NIH Office of Laboratory Animal Welfare.

1. For studies in Aim 1 to determine the optimal vaccine dose and regimen we will use 21 (6 treatment groups and one control group; n=3 per group) purposed bred 6 months old beagle dogs. This is necessary for analyzing vaccine induced immune responses and determining whether differences between the groups are statistically significant.

For experiments in Aim 2, we will require to use 36 dogs to ensure that we have sufficient number of dogs that develop VL. A previous study by Zhioua Lab has found that in a cohort of exposed dogs, the incidence of *L. infantum* infection and canine leishmaniasis were 50% and 30%, respectively. Both male and female mice will be used for animal studies as gender differences have been reported in susceptibility to VL in humans. Thirty six purposed bred healthy beagle dogs will be divided into two groups (18 males and 18 females per group) for the studies outlines in Aim 2. One groups will be vaccinated with *LmCen-/-* and other group will be control. Dogs will be vaccinated subcutaneously with 1 x 10^7 attenuated CRISP-*LmCen*$^{-/-}$ parasites. One month after vaccination, all animals will be exposed to wild *L. infantum* infected *Phlebotomus perniciosus*, the main vector of VL in the Western Mediterranean basin. Dogs will spend overnight and brought back the following day early morning to the dog kennel. Dogs will be exposed to sand fly bites each night throughout the sand fly season It is expected that 30% of exposed dogs (total n=12; 6 dogs per group) will develop the disease. This will provide at least 80% power to detect a 2.5-fold change in parasite load at the study point end with CV=40% and two sided alpha of 0.05.

2. We are testing efficacy of a live attenuated *L. major* as a vaccine against VL and therefore require an animal model that closely mimics human disease. Similar to humans, dogs infected with *L. infantum* develop VL characterized by weight loss, anemia and hepatosplenomegaly. These experiments require the use of dogs.

3. Board certified veterinarians are present full time in the animal facilities at Pasteur Institute, Tunis. Each room in the kennel at Pasteur Institute is assigned to an animal technician who is responsible for the daily care. Veterinarians are involved in daily monitoring of the dogs

4. We do everything to minimize any pain or discomfort to dogs. Dogs infected with *L. infantum* are watched closely and euthanized if they develop sever disease as evident by a significant weight loss, development of ascites and refusal to feed. All procedure such as peripheral blood draw and bone marrow aspiration is performed under general anesthesia with aseptic precautions
.
5. Euthanasia is carried out using methods recommended by the Panel on Euthanasia of the American Veterinary Medical Association.

Multiple Principal Investigator Leadership Plan

Roles and responsibilities of the PIs:

Drs. Satoskar and Zhioua will provide oversight of the entire project. They will be responsible for the implementation of the project aims and scientific agenda.

Data Sharing Plan

Both PIs will jointly implement the proposed scientific tasks, execute the specific aims, and ensure that systems are in place to guarantee institutional compliance with U.S. laws, DHHS and NIH policies on biosafety, animal research, data and facilities. Both PIs will also be responsible for all animal research approvals. Dr. Satoskar will serve as contact PI. Both PIs will share fiscal and administrative management, and both will be responsible for communication with the NIH including submission of annual reports. Both of the PIs will meet at least once a month via Skype. In addition, regular joint lab meetings will also be held to discuss the progress of the research at least twice a month through Skype or video conferencing. Dr. Satoskar will travel to Institut de Pasteur at least 4 times during 2 year project period to train personnel in immunological techniques and monitor the progress in the field.

Conflict Resolution

In case of conflicts, the PIs will try to resolve any issues amicably. However, if they are unable to reach an agreement, the conflict will be referred to an arbitration committee consisting of two impartial senior Faculty members , one each from The Ohio State University Medical Center and Institut de Pasteur. No members of the arbitration committee would be directly involved in the research, grant or disagreement.

Publication and Intellectual Property

Publication authorship will be based on the related scientific contributions of the PIs and the key personnel. The Technology Transfer Offices at the OSU Medical Center and Institut de Pasteur will be responsible for preparing and negotiating an agreement for the conductive research, including any intellectual property generated from these studies.

Change in PI Location

In case one of the PI moves to a new institution, attempts will be made to transfer the relevant portion of the grant to the new institution. In the event that the one PI can no longer carry out his duties, a new PI from the institution will be recruited as a replacement subject to the approval of the institution's authorities.

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MINISTERE DE LA SANTE PUBLIQUE

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Tunis, Juin 12, 2017

RE: Letter of Support

Institut Pasteur de Tunis (IPT), Tunisia is participating in the proposal entitled "A live attenuated vaccine for leishmaniasis" " in accordance to the program announcement to the US Department of Health and Human Services, National Institute of Health program on NIH Exploratory/Developmental Research Grant Program (Parent R21) (PAR-13-303).

This proposal represents a multi-institutional collaboration between Dr. Abhay Satoskar from the Ohio State University and Dr. Elyes Zhioua (Principal Investigator) from Institut Pasteur de Tunis.

As General Director of the Institut Pasteur de Tunis which is the foreign organization, I confirm that Institut Pasteur de Tunis is willing to comply with all pertinent U.S. Federal regulations and policies. Institut Pasteur de Tunis is willing to support Dr. Elyes Zhioua as Co-Principal Investigator with time and resources on this project. Institut Pasteur de Tunis agrees to provide necessary documentation to the Office of Human Research Protections (OHRP) for human subject involvement and to the Office of Laboratory Animal Welfare (OALW) for animal subjects. Institut Pasteur de Tunis agrees to comply with required NIH policies on awards involving foreign institutions.

The appropriate administrative officials at the Institut Pasteur de Tunis have reviewed the proposal information and the total budget in the direct cost amount of US$140,000.00. The indirect cost requested by the Institut Pasteur de Tunis requests at the rate of 8% is US$11,200.00. Requested information for the research activity at Institut Pasteur de Tunis is enclosed.

Redacted by agreement
General Director
Redacted by agreement
Institut Pasteur de Tunis

Gennova

Date: June 1, 2017

Abhay R Satoskar MBBS, MD, PhD
Professor & Vice Chair for Research
University Pathology Services Endowed Anatomic Pathology Professor
Departments of Pathology and Microbiology
Wexner Medical Center
The Ohio State University
129 Hamilton Hall
1645 Neil Avenue
Columbus, Ohio 43210
Tel:614-366-3417

Dear Abhay

I am happy to collaborate with you to strengthen the efforts on developing anti-Leishmania vaccine. Gennova as a collaborator will contribute providing GLP grade centrin deficient *L. major* that we are currently growing for the studies proposed in your revised R21AI-130485 grant titled "A live attenuated vaccine for leishmaniasis". As you are aware our facility is US-FDA approved for manufacturing biological products including vaccines.

Wishing you great success.

Yours Sincerely

Personal Signature

Redacted by agreement

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Phone: Redacted by agreement

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Division of Emerging and transfusion Transmitted Diseases

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June 12, 2017

Abhay R Satoskar MD, PhD
Professor and Vice Chair
Department of Pathology
Wexner Medical Center
The Ohio State University
Columbus, Ohio 43210

Dear Abhay,

I am happy to continue our ongoing collaboration and serve as consultant on your R21 project to evaluate efficacy of centrin gene deficient *L. major* as a vaccine using a canine model of visceral leishmaniasis (VL) transmitted by wild sand flies. Based on preliminary data I think selection marker free *LmCen-/-* parasites could be a promising vaccine candidate for *anti-Leishmania* vaccine for humans. I also think that canine model of VL developed at the Pasteur Institute will an ideal model to test such a vaccine. My lab will be happy to provide antibodies agaist centrin for performing western blots to confirm lack of centtrin protein in *LmCen-/-* parasites. I am also happy to share my knowledge and expertise as needed for this project.

Looking forward to collaborate in this exciting project

Sincerely,

Personal Signature

Redacted by agreement

Division of Emerging and Transfusion Transmitted Diseases

McGill

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June 14, 2017

Abhay R Satoskar MD, PhD
Professor
Department of Pathology
Wexner Medical Center
The Ohio State University
Columbus, Ohio

Dear Abhay

I am delighted to continue our ongoing collaboration on developing an anti-*Leishmania* vaccine. As you know, selection marker GMP- *LmCen-/-* parasites that will be used in your project were developed in my lab as a part of our GHIT project. My laboratory will be able to assist in authentication of GMP- *LmCen-/-* manufactured by Gennova. In addition, I am also happy to share my expertise in undertaking field studies in VL endemic regions.

I am looking forward to working with you on this exciting project

Best regards

Personal Signature

Redacted by agreement
Professor
Department of Microbiology and Immunology
McGill University

Resource Sharing

This project is an early product development project and does not involve clinical data sets. The data generated from this project will be shared with the research community through presentations at the scientific meetings as well as publications in appropriate peer-reviewed journals. In addition, all manuscripts at the accepted stage will be deposited in PubMed Central, either by the journal to which the paper is submitted or by the corresponding author in each case. Accordingly, the papers will be available for open access one year after publication in the literature. Recorded factual data which is necessary for documentation of research findings will be provided in a timely manner upon request to the individual PIs for a period of 3 years following publication of the study. We also propose to create a web site for the project which will include copies of all materials presented at scientific meetings and publications.

Plan for Sharing Model Organisms:

The Ohio State University is committed to advancing research by making research resources, reagents and model organisms available to the scientific community. In accordance with its patent policy, the Ohio State University in conjunction with Institut de Pasteur will execute its intellectual property rights

L.mCen-/- used in this project will be shared with interested parties through OSU. Transfer of materials to non-commercial organizations for teaching and research will be governed by the OSU's standard Material Transfer Agreement. This agreement is in accordance with the NIH Grants Policy Statements' (2001) guidelines on the sharing of biomedical research resources.

Patentable research resources may be licensed to for-profit third parties for commercial development and dissemination, which enables OSU to develop technologies and make them available for the public benefit. If the patented research is licensed to for-profit third parties, then OSU will retain the right to utilize that resource for educational and research purposes, and also to distribute it to the non-profit educational and research enterprises.

Authentication of Key Biological and/or Chemical Resources

Deletion of *Centrin 1* gene in the parasites *LmCen-/-* will be authenticated by 1) sequencing as well as 2) Southern blot analysis (see attached letter from Redacted by agreement. In addition, Western blot analysis will be used to confirm the absence of centrin protein in the parasites. Anti—Centrin antibodies have been generated and validated in Redacted Lab (see attached letter). Specificity of commercially purchased kits for canine cytokine ELISA will be tested using recombinant cytokines from other species (e.g. mice).