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TITLE: Targeting the Nociceptin/Orphanin FQ Receptor for Scleroderma Therapy

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RECIPIENT: Palo Alto Veterans Institute for Research
Palo Alto, CA 94304

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Fort Detrick, Maryland 21702-5012

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Approved for public release; distribution unlimited

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none

14. ABSTRACT
Scleroderma is a debilitating disease of the connective tissue that affects about 75,000–100,000 individuals in the United States and accounts for an estimated $400 million in total annual medical costs. There is no treatment that stops or cures scleroderma: novel therapies are therefore urgently needed. The overall objective of this proposal is to demonstrate that small molecule compounds that target receptor-ligand pair NOP receptor–Nociceptin/Orphanin FQ can limit the damage and malfunctions that occur in the immune system and in blood vessels during scleroderma. We anticipate that our approach will demonstrate that a single drug can limit inflammation, heal blood vessel damage, and increase blood flow.

15. SUBJECT TERMS
Nociceptin/orphanin FQ (N/OFQ), N/OFQ peptide receptor (NOPR), vascular endothelial cells, macrophages, partial agonist, scleroderma, fibrosis, vasculopathy, autoimmunity.

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TABLE OF CONTENTS

1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

This study addresses new approaches to treat scleroderma. The overall objective is to demonstrate that small molecules that target nociception/orphanin N/OFQ peptide receptor NOPR can limit the damage and malfunctions that occur in the immune system and in blood vessels during scleroderma. Our approach uses a combination of targeted in vitro cell-based assays and a preclinical rodent model of scleroderma to evaluate NOPR partial agonists for efficacy.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Nociceptin/orphanin FQ (N/OFQ), N/OFQ peptide receptor (NOPR), vascular endothelial cells, macrophages, partial agonist, scleroderma, fibrosis, vasculopathy, autoimmunity.

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Specific Aim 1 **To test the hypothesis that distinct partial agonists for NOPR limit macrophage migration and vasculopathy.**	**Target Date**	**Actual Completion Date** *or →*	**Percentage Completion at Time of 1 Year Update**
Major Task 1: Administrative tasks and experimental preparation	Months 1-6		80%
Subtask 1. Obtain local IACUC and ACURO approval for the animal studies	1-2	-1	
Subtask 2. Obtain and expand SVEC4-10 mouse cell line (ATCC)	1-2	2	
Subtask 3. Obtain NOPR-/- animals and establish breeding colony	1-6		50%
Milestone(s): Local IACUC and ACURO Approval	-1	-1	
Major Task 2: To test NOPR partial agonists for activity in three in vitro assays: endothelial cell wound healing, macrophage chemotaxis, and blood vessel tension (aortic ring assay)	3-12		50%
Subtask 1. Determine optimal NOPR agonist (N/OFQ) and antagonist (JTC-801) concentrations for the three proposed in vitro assays: scratch wound assay, macrophage chemotaxis using transwell inserts, and blood vessel tension using an aortic ring assay. In the scratch wound assay, a pipet tip is used to scratch or wound an endothelial monolayer, and the 'time-to-wound closure' is monitored			66% (Defined experimental parameters for the scratch wound assay and macrophage chemotaxis assay, but not the aortic ring

Subtask 1. Determine optimal NOPR agonist (N/OFQ) and antagonist (JTC-801) concentrations for the three proposed in vitro assays: scratch wound assay, macrophage chemotaxis using transwell inserts, and blood vessel tension using an aortic ring assay. In the scratch wound assay, a pipet tip is used to scratch or wound an endothelial monolayer, and the 'time-to-wound closure' is monitored over the next 24 hr by microscopy. In the macrophage chemotaxis assay, cells are loaded into the top well of a transwell insert and exposed to chemoattractants in the bottom well; the number of migrating cells is quantified by flow cytometry. In the aortic ring assay, freshly isolated thoracic aorta rings will be harvested and mounted in a small-vessel myograph. Vasodilation will be determined by micrometer measurements derived from small steel wires inserted into the aortic ring lumen.	3-4		66% (Defined experimental parameters for the scratch wound assay and macrophage chemotaxis assay, but not the aortic ring assay).
Subtask 2. Test 7 candidate partial agonist compounds in wound healing assays using the SVEC4-10 mouse endothelial cell line. These compounds are commercially available (no MTA required).	3-6		50% (Tested 3 candidate partial agonists in the scratch assay)
Subtask 3. Test 7 candidate partial agonist compounds in chemotaxis assays with macrophages from WT C57BL/6 mice and NOPR-deficient mice, the latter to confirm specificity	5-12		20% (successfully revived NOPR$^{+/-}$ mice from cryopreservation)
Subtask 4. Test 7 candidate partial agonist compounds in aortic ring vasodilation assays using tissues from WT C57BL/6 and NOPR-deficient mice, the latter to confirm specificity	5-12		20% (successfully revived NOPR$^{+/-}$ mice from cryopreservation)
Milestone(s): Select one partial agonist compound based on its wound healing, chemotaxis, and vasodilation profile for in vivo testing. The criteria for compound selection will be based on the comparative performance of compounds in the in vitro assays. The compound we seek will have strong inhibitory effects on macrophage migration, strong activating effects in wound healing and vasodilation, and its effects will be NOPR-specific. Of the 7 compounds we will screen in the in vitro assays, we will select the partial agonist that best embodies these properties.			20% (Compound 12 improved in vitro wound healing slightly better than compounds 7 or 8)
Specific Aim 2 **To test the hypothesis that NOPR partial**			

Milestone(s): Select one partial agonist compound based on its wound healing, chemotaxis, and vasodilation profile for in vivo testing. The criteria for compound selection will be based on the comparative performance of compounds in the in vitro assays. The compound we seek will have strong inhibitory effects on macrophage migration, strong activating effects in wound healing and vasodilation, and its effects will be NOPR-specific. Of the 7 compounds we will screen in the in vitro assays, we will select the partial agonist that best embodies these properties.			20% (Compounds 12 and 8 improved in vitro wound healing slightly better than compound 7 when used at 1 uM)
Specific Aim 2 **To test the hypothesis that NOPR partial agonists prevent and reverse scleroderma pathogenesis.**			
Major Task 3: Test the hypothesis that NOPR partial agonists ameliorate scleroderma pathogenesis in vivo	13-18		10%
Subtask 1. Perform 1-2 in-life bleomycin-induced scleroderma studies using C57BLl/6 and NOPR-/- mice treated prophylactically (treatment start on day 1) or therapeutically (treatment start on Day 15) with NOPR agonist (N/OFQ), antagonist (JTC-801), or partial agonist to be determined from in vitro studies in Major Task 2.	13-16		10% (performed one pilot study to establish model; successfully revived NOPR$^{+/-}$ mice from cryopreservation)
Subtask 2. Perform histological evaluation of tincture-stained skin study samples (H&E for general skin architecture and leukocyte infiltrates, trichrome for fibrosis evaluation).	15-18		10% (performed one pilot study to establish model)
Subtask 3. Perform immunoassay evaluation of scleroderma pathology biomarkers in skin study samples.	15-18		0%
Milestone(s): Submit manuscript for publication			0%

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the

project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Major Activity 1:
Administrative tasks and experimental preparation
Specific Objectives:
Subtask 1. Obtain local IACUC and ACURO approval for the animal studies.
Subtask 2. Obtain and expand SVEC4-10 mouse cell line (ATCC).
Subtask 3. Obtain NOPR-/- animals and establish breeding colony
Results and Conclusions:
1) We obtained the necessary local IACUC and ACURO approval for the proposed animal studies.
2) We obtained and expanded the endothelial cell line SVEC4-10 from the ATCC.
3) We obtained 2 NOPR$^{+/-}$ animals and confirmed the genotype by in-house PCR (**Fig. 1**). We have established a breeding colony to eventually generate the NOPR$^{-/-}$ needed for the experiments proposed in the SOW.

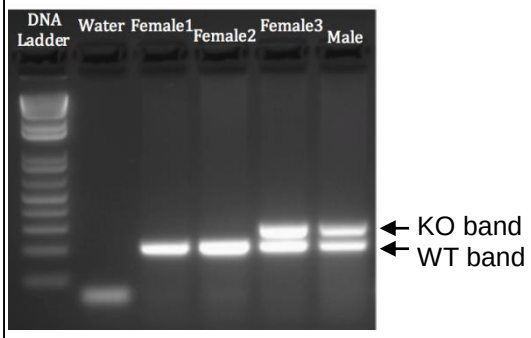


Figure 1. Successful resuscitation of NOPR$^{-/+}$ mice from cryopreserved sperm by the Mutant Mouse Regional Resource Center (MMRRC). The MMRRC was successful on its second attempt at resuscitating NOPR$^{-/+}$ mice from cryopreserved sperm. We received four mice, two of which (one male and one female) were confirmed by our in-house PCR genotyping analysis to be NOPR$^{-/+}$. We have since mated the heterozygous founders with WT C57bl/6 mice to generate additional NOPR$^{-/+}$ mice. We will then intercross these NOPR$^{-/+}$ to generate NOPR$^{-/-}$ mice (we require 8 KO mice for the proposed experiments in the SOW).

Stated Goals Not Met:
By the month 6 target date we did not have in hand the NOPR$^{-/-}$ mice needed for the experiments proposed in the SOW. The NOPR-targeted animals were not commercially available as "live mice", but need to be resuscitated from frozen sperm. The first attempt by the MMRRC was unsuccessful, but the second attempt resulted in 2 live heterozygous founder mice. These mice are currently being used as breeders in our Veterinary Medical Unit to generate additional heterozygous and eventually homozygous NOPR KO mice.

Major Activity 2:
To test NOPR partial agonists for activity in three in vitro assays: endothelial cell wound healing, macrophage chemotaxis, and blood vessel tension (aortic ring assay)
Specific Objectives:
Subtask 1. Determine optimal NOPR agonist (N/OFQ) and antagonist (JTC-801) concentrations for the three proposed in vitro assays: scratch wound assay, macrophage chemotaxis using transwell inserts, and blood vessel tension using an aortic ring assay. In the scratch wound assay, a pipet tip is used to scratch or wound an endothelial monolayer, and the 'time-to-wound closure' is monitored over the next 24 hr by microscopy. In the macrophage chemotaxis assay, cells are loaded into the top well of a transwell insert and exposed to chemoattractants in the bottom well; the number of migrating cells is quantified by flow cytometry. In the aortic ring assay, freshly isolated thoracic aorta rings will be harvested and mounted in a small-vessel myograph.

Vasodilation will be determined by micrometer measurements derived from small steel wires inserted into the aortic ring lumen.
Subtask 2. Test 7 candidate partial agonist compounds in wound healing assays using the SVEC4-10 mouse endothelial cell line. These compounds are commercially available (no MTA required).
Subtask 3. Test 7 candidate partial agonist compounds in chemotaxis assays with macrophages from WT C57BL/6 mice and NOPR-deficient mice, the latter to confirm specificity
Subtask 4. Test 7 candidate partial agonist compounds in aortic ring vasodilation assays using tissues from WT C57BL/6 and NOPR-deficient mice, the latter to confirm specificity

Results and Conclusions

1) We successfully procured NOPR agonist Ro-64-6198, antagonist SB612111, and three partial agonists (compounds 7, 8, and 12) (**Revised Table 1**). For our initial studies, we elected to focus on evaluating a single NOPR agonist (Ro-64-6198), a single antagonist (SB612111), and three partial agonists, rather than N/OFQ agonist, J113397 and JTC-801 antagonists, and the four other proposed partial agonists to conserve resources. We selected Ro-64-6198 over NOPR peptide ligand N/OFQ because Ro-64-6198 uses the same formulation vehicle (DMSO) as the test articles, thus reducing the number of vehicle controls needed for each experiment and making comparisons among compounds more accurate and independent of possible vehicle effects. We selected antagonist SB612111 based on its favorable Ki compared with the other two antagonists. We selected partial agonists 7, 8, and 12 based on their Ki and GTPγS profiles, with the goal of spanning the range of binding affinities and GTPγS stimulation. Compound 7 has high binding affinity and good GTPγS stimulation; compound 8 has lower binding affinity and weaker GTPγS stimulation capacity; compound 12 has middle-of-the-road properties (**Revised Table 1**).

Revised Table 1. Receptor binding affinities K_i and GTPγS binding (EC_{50} and % stimulation) to NOPR of procured compounds.

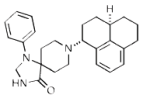
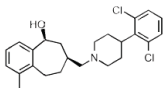
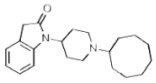
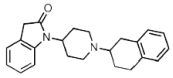
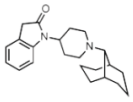
Compound	Structure	K_i (nM)	EC_{50} (nM) [% stim.]	Quantity (mg)	Cost ($)	Note
2 Ro 64-6198		0.39	38 [100]	750	6,500	NOPR agonist
4 SB612111		1.42±0.12	NA	200	2,770	NOPR antagonist
7 (WuXi 1c)		1.39±0.42	19.9±3.4 [59.1±7.1]	750	1,600	test compound
8 (WuXi 1d)		29.3±11.4	92.5±16 [30.9±3.1]	616	1,600	test compound
12 (WuXi 1m)		7.49±0.78	28.7±0.6 [45±5]	668	1,600	test compound

Table shows K_i and EC_{50} from different publications[6,7]. EC_{50} values are not available for antagonists.

2) We successfully established the in vitro endothelial cell scratch assay (**Fig. 2A**), and evaluated Ro-64-6198, SB612111, 7, 8, and 12 for activity. In preliminary studies, the two partial agonists 8 and 12, as well as antagonist SB612111 improved wound closure compared with vehicle alone (0.1% DMSO) or NOPR agonist Ro-64-6198 when tested at 1 µM (**Fig. 2B**). At 100 uM, all three partial agonists and SB612111 improved wound closure compared with vehicle or Ro-64-6198 (**Fig. 2C**). Compounds 7 and 12 showed a dose-dependent response in improving wound closure (**Fig. 2B,C**). Thus, partial agonist 12 has the most promising wound closing properties in the SVEC4-10 endothelial cell scratch assay.

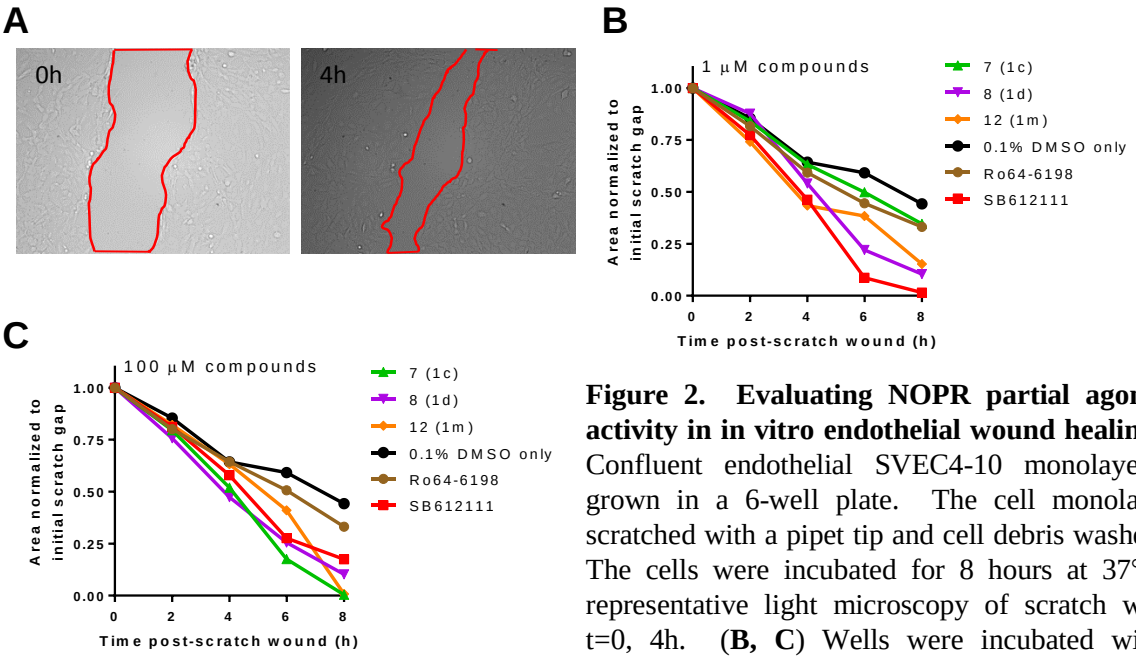


Figure 2. Evaluating NOPR partial agonists for activity in in vitro endothelial wound healing assay. Confluent endothelial SVEC4-10 monolayers were grown in a 6-well plate. The cell monolayer was scratched with a pipet tip and cell debris washed away. The cells were incubated for 8 hours at 37°C. (**A)** representative light microscopy of scratch wound at t=0, 4h. (**B, C**) Wells were incubated with three different NOPR partial agonists (1 µM, **B**; or 100 µM, **C**) or 0.1% DMSO (vehicle control), and the plate was photographed every 2 hours. ImageJ was used to quantify the scratch area over time. Data presented normalized to the initial area. N=1 experiment, one well per compound.

3) We successfully established in vitro transwell chemotaxis of thioglycollate-elicited mouse peritoneal monocyte/macrophages to CXCL12 (~4-fold assay window comparing background to migration to 100 nM CXCL12) (**Fig. 3A**). Migration to NOPR agonist Ro-64-6198 (1 nM) was only slightly above background (**Fig. 3A**), even though Ro-64-6198 was reported by others to induce mouse macrophage migration. We also evaluated migration of human CD14+ blood monocytes to Ro-64-6198, which showed a similar response, slightly above background migration (**Fig. 3B**). We are attempting to optimize assay conditions to establish at least a 2-fold assay window for Ro-64-6198-stimulated mouse monocyte/macrophage migration as follows: i) testing a wide range of Ro-64-6198 for activity, ii) increasing the migration time from 2 to 4 h, iii) using resident macrophages and 8 µm pore filters, and iv) using the more motile bone-marrow-derived monocyte macrophages (culturing mouse bone marrow with M-CSF for 7 days) for migration experiments.

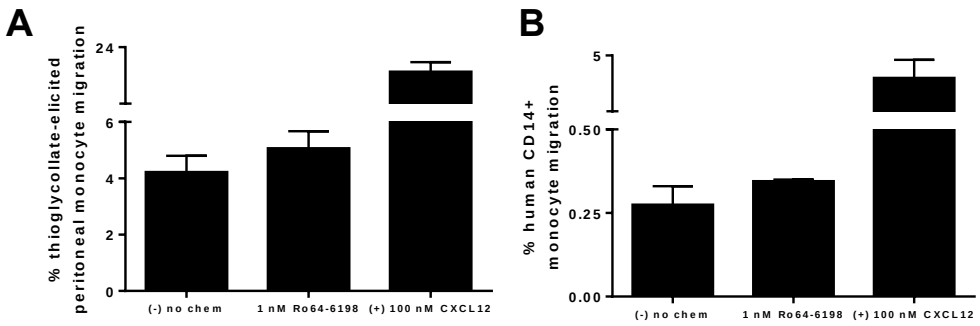


Figure 3. Testing NOPR agonist Ro-64-6198 for chemotactic activity using mouse and human monocytes. One million thioglycollate-elicited mouse peritoneal monocytes (**A**) or human peripheral blood mononuclear cells (**B**) were added to the top well of a 5 um pore transwell filter. 1 nM Ro-64-6198 was added to the bottom chamber, and after 2 h incubation at 37°C the cells that migrated to the bottom well were quantified by flow cytometry. For human PBMC, the cells were stained for monocyte marker CD14 prior to quantification. Mean ± range, duplicate wells. Negative control: (-) no chemoattractant. Positive control: (+) 100 nM CXCL12 (CXCR4 agonist).

Stated Goals Not Met:

We have not yet tested the NOPR compounds in the aortic ring assay (target date was month 12), but we have identified a local lab (Phil Tsao) with expertise in the assay that will be assisting us with the experiment. We tested 3 representative partial agonists (of the 7 proposed) in the wound healing assay by month 8 (instead of month 6). We were delayed due to the failure of our initial commercial chemistry company (Proactive Molecular Research) to deliver compounds 7, 8, and 12 (they were unable to complete the synthesis with the desired purity). The second company we selected (WuXi) was able to provide adequate quantities of the compounds with the necessary purity. We are in the process of establishing an adequate chemotactic window (migration to Ro-64-6198); once we have a window, we can test our partial agonist compounds for inhibitory activity as proposed (the target for this was month 12).

Major Activity 3:

Test the hypothesis that NOPR partial agonists ameliorate scleroderma pathogenesis in vivo

Specific Objectives:

Subtask 1. Perform 1-2 in-life bleomycin-induced scleroderma studies using C57BLl/6 and NOPR-/- mice treated prophylactically (treatment start on day 1) or therapeutically (treatment start on Day 15) with NOPR agonist (N/OFQ), antagonist (JTC-801), or partial agonist to be determined from in vitro studies in Major Task 2.

Subtask 2. Perform histological evaluation of tincture-stained skin study samples (H&E for general skin architecture and leukocyte infiltrates, trichrome for fibrosis evaluation).

Subtask 3. Perform immunoassay evaluation of scleroderma pathology biomarkers in skin study samples.

Results and Conclusions:

1) We performed a pilot study using bleomycin to induce experimental scleroderma in C57bl/6 mice. Twenty-eight days after the initial s.c. bleomycin injections we euthanized the mice and evaluated the skin by histology. Bleomycin induced significant dermal skin thickening in a dose-dependent manner, with homogeneous deposits visible by H&E staining that stained light blue

upon Masson trichrome stain, indicating collagen deposition/fibrosis (**Fig. 4**). Thus, we have successfully established the in vivo scleroderma in our lab and are prepared to evaluate NOPR compounds for efficacy and effects on disease pathogenesis.

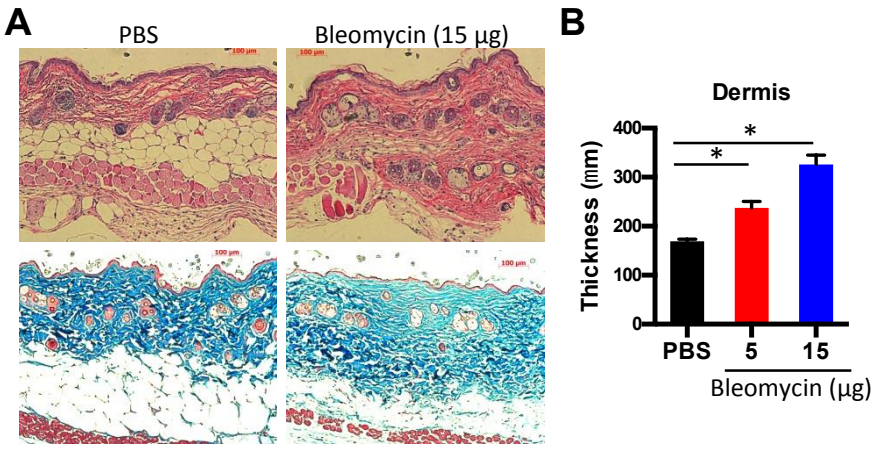


Figure 4. Local bleomycin injections induce clinical and histological scleroderma-like disease including dermal thickening and collagen deposition. C57B6 mice were injected with 100ul PBS or bleomycin (dissolved in PBS at two different concentrations; 50ug/ml or 150ug/ml) subcutaneously into the shaved back 5 times/week for 4 weeks. One day following the final injection, lesional skin was removed, fixed in 10% formalin, embedded in paraffin and sections were stained with either hematolxylin and eosin (H&E) (**A**, *top panels*) or Masson trichrome stain. (**A,** *bottom panels***).** Representative images of the skin stained for H&E or Masson trichrome, 10X magnification. Note the dense collagen network (light blue trichrome stain) and dermal thickening in the bleomycin-treated skin compared with controls. *(***B)** Dermal thickness defined as the distance between the epidermal-dermal junction and the dermal-adipose layer junction was determined in the H&E-stained sections. Mean ± SEM, 3 mice/group (8 measurements/section, 2 sections/mouse were averaged), *$p<0.05$ by t-test.

Stated Goals Not Met:

None (these tasks are targeted for completion by month 16-18).

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to Report

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

During the next reporting period we will focus on completing all outstanding tasks in the SOW as follows:
1) We will intercross NOPR$^{+/-}$ mice to generate the required number of NOPR$^{-/-}$ animals.
2) We will finalize our in vitro wound healing experiments and nominate the best NOPR compound for in vivo evaluation in experimental scleroderma.

3) We will optimize our in vitro chemotaxis assay and test the NOPR partial agonists for inhibitory activity against NOPR agonist Ro-64-6198. We will nominate the most effective chemotactic inhibitor for in vivo evaluation in experimental scleroderma.
4) We will test our partial agonists for activity in the aortic ring assay, and select the best vasodilator for in vivo evaluation in experimental scleroderma.
5) We will evaluate Ro-64-6198, SB612111, 7, 8, 12, and vehicle control in vivo for efficacy against in bleomycin-induced scleroderma. We will analyze the skin for histological, immunological, and cytokine signs of aberrant wound healing/fibrosis.
6) We will prepare and submit manuscripts, meeting materials (poster/oral presentations), and patent applications as appropriate to disclose these results.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to Report (still in progress)

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report

What was the impact on technology transfer?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Obtaining the NOPR-targeted mice and NOPR small molecules was delayed due to issues with the commercial entities supplying these materials. This was discussed in more detail in section 3 above. We are intercrossing NOPR+/- mice to generate the required NOPR-/- animals, and we identified a second chemistry company able to provide the NOPR partial agonists.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals.

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted,*

awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to Report (yet)

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to Report (yet)

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

Nothing to Report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report (yet)

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*

- *biospecimen collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Name:	Brian Zabel
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	ZABEL.BRIAN (eRA Commons ID)
Nearest person month worked:	3
Contribution to Project:	Dr. Zabel has overseen all aspects of the project. He successfully sourced the NOPR small molecules (**Revised Table I**), managed the resuscitation and delivery of NOPR KO mice, designed in vivo and in vitro experiments (**Figs. 1-4**), and analyzed results.
Name:	Nicole Salazar
Project Role:	Research Assistant
Researcher Identifier (e.g. ORCID ID):	nsala001 (eRA Commons ID)
Nearest person month worked:	2
Contribution to Project:	Dr. Salazar assisted with the in vitro wound closing assay (**Fig. 2**) and genotyping the NOPR KO mice (**Fig. 1**).

Name:	Melissa LaJevic
Project Role:	Research Assistant
Researcher Identifier (e.g. ORCID ID):	LAJEVIC.MELISSA (eRA Commons ID)
Nearest person month worked:	1
Contribution to Project:	Dr. LaJevic performed initial in vivo experiments to successfully induce scleroderma-like disease in mice and prepared and evaluated the affected skin by histology (**Fig. 4**). She also performed in vitro chemotaxis experiments (**Fig. 3**).

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

ZAB0003APR
Pulmonary Fibrosis Foundation **7/1/14-6/30/16**
Role of Chemerin and Its Receptors in TGF-beta-induced Experimental Pulmonary Fibrosis
This is a new award.

ZAB0004ARG **8/20/14-7/31/16**
LakePharma, Inc.
Hybridoma Monoclonal Antibody Generation
This is a new award.

ZAB0006AOM **2/10/15-1/31/19**
National Science Centre, Poland
The Role of Antimicrobial Chemerin in Skin Biology
This is a new award.

R01 CA169354 (Butcher) **4/1/13-3/31/18**
National Cancer Institute
Chemerin in Tumor Immunity and Surveillance
This is a new award.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.
Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. **APPENDICES:** Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Targeting the Nociceptin/Orphanin FQ Receptor for Scleroderma Therapy

PR131037
W81XWH-15-1-0001

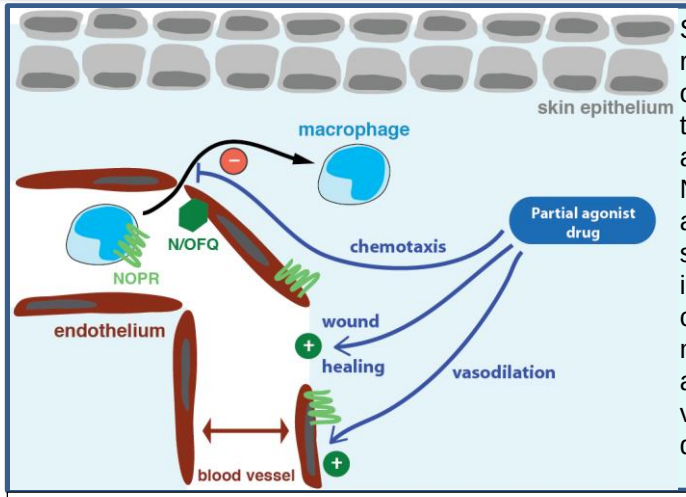
PI: Dr. Brian Zabel **Org:** Palo Alto Veterans Institute for Research **Award Amount:** $175K

Study/Product Aim(s)

- Test the hypothesis that distinct partial agonists for the Nociceptin/Orphanin FQ receptor (NOPR) limit macrophage migration and vasculopathy
  - Test the hypothesis that N/OFQ increases macrophage recruitment into inflamed sites via expression of NOPR
  - Test the hypothesis that NOPR partial agonists counter scleroderma vasculopathy
- Test the hypothesis that NOPR partial agonists ameliorate scleroderma pathogenesis

Approach

The effect of small molecule drug candidates will be tested in macrophage chemotaxis, endothelial wound healing and vasodilation assays *in vitro*. Drug candidate(s) will be selected and tested in a bleomycin-induced scleroderma model *in vivo*.



Schematic representation of our hypothesis that partial agonist drugs for NOPR ameliorate scleroderma by inhibiting chemotaxis of macrophages and limiting vascular dysfunction

Activities: ACURO approval has been obtained for mouse studies. Preliminary in vitro wound healing and macrophage chemotaxis have been performed. Preliminary in vivo bleomycin-induced scleroderma studies have been performed.

Timeline and Cost

Activities CY	14	15	16
NOPR *in vitro* assays Milestone: Select 1-2 compounds			
NOPR scleroderma *in vivo* study Milestone: Submit manuscript			
Estimated Budget ($K)	**$28**	**$115**	**$32**

Updated: 10/30/2015

Goals/Milestones (Example)

FY15 Goal – NOPR *in vitro* assays

☑Obtain ACURO approval for animal studies
☑ Obtain NOPR$^{-/+}$ animals, start breeding colony
☑ Obtain and expand SVEC4-10 mouse cell line
☑ Perform chemotaxis assays
☑ Perform wound healing assays
☐Perform vasodilation assays
☐Select 1–2 compounds based on their *in vitro* profile

FY16 Goals – NOPR scleroderma *in vivo* studies

☑Establish preclinical bleomycin-induced scleroderma model
☐Test compounds for efficacy in experimental scleroderma
☐Submit manuscript for publication

Comments/Challenges/Issues/Concerns

- Unanticipated delays in obtaining NOPR$^{-/-}$ mice and NOPR compounds

Budget Expenditure to Date

Projected Expenditure: $110,870
Actual Expenditure: $94,967