AWARD NUMBER: W81XWH-18-2-0059

TITLE: A Phase 1 Randomized, Placebo-Controlled, Single Ascending Dose Study to Examine the Safety, Tolerability, and Pharmacokinetics of cP12 in Healthy Adults

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CONTRACTING ORGANIZATION: Neomatrix Therapeutics, Inc.

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Fort Detrick, Maryland 21702-5012

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A Phase I Randomized, Placebo-Controlled Single Ascending Dose Study to Examine the Safety, Tolerability and Pharmacokinetics of cP12 in Healthy Adults

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The overall objective of this proposal is to assess the safety and tolerability of single ascending intravenous doses of cP12 in healthy subjects. This is a randomized double-blind, placebo-controlled single ascending dose study to evaluate the safety, tolerability, and pharmacokinetic profile of cP12 in healthy male and female subjects. Each subject is randomized to receive either a single dose of cP12 or placebo. Initial IRB, FDA and HRPO approval was given to recruit and screen subjects for 4 Cohorts (n=32) plus an option Cohort 5 (n=8) to be given a dose equal to, or less than, the dose given to Cohort 4. Cohorts 1-4 have been completed and no SAE's were noted. We submitted Amendment 4 to give a higher dose in Cohort 5 to the IRB and received approval (Amendments 1-3 were minor and did not need approvals). We then submitted Amendment 4 to the FDA and have received no comments after two months. Amendment 4 was submitted to HRPO December 30, 2019 and received approval on January 7, 2020. Cohort 5 dosing was completed in February 2020. Data for entire Phase1 trial was locked on April 2, 2020

15. SUBJECT TERMS
NONE LISTED

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

The overall objective of this proposal is to assess the safety and tolerability of single ascending intravenous (IV) doses of cP12 in healthy subjects.

This was a randomized double-blind, placebo-controlled single ascending dose (SAD) study to evaluate the safety, tolerability, and PK profile of cP12 in healthy male and female subjects. Each subject was randomized to receive either a single dose of cP12 or placebo.

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

Assess the safety, tolerability and pharmacokinetics

3. **ACCOMPLISHMENTS:** The Principal Investigator (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: Develop and validate bioanalytic assay for cP12 assay in human plasma and urine (months 1-24) UPDATE Status of all tasks below as needed.

Major Task 1: Development and Validation of cP12 Assay in Human Plasma. Months 1-6.

Task 1.1: Set-up of HPLC/MS/MS Human Plasma Assay. Months 1-3. 100%
- STATUS: COMPLETED, MONTH 0. 100%

Task 1.2: Write Validation protocol. Months 1-3. 100%
- STATUS: COMPLETED, MONTH 1. 100%

Task 1.3: Validation of Human Plasma Assay. Months 4-6. 100%
- STATUS: COMPLETED, MONTH 3. 100%

Task 1.4: One-month frozen sample stability at -70° C and -20° C. Months 4-6.
- STATUS: COMPLETED, MONTH 3. 100%

Task 1.5: Audited Validation Report. Months 4-6.
- STATUS: COMPLETED, MONTH 6. 100%

Task 1.6: Extended Human Plasma Stability to 6 months.
- STATUS: COMPLETED, MONTH 10. 100%

Task 1.7: Extended Human Plasma Stability Report.

- STATUS: COMPLETED, MONTH 11. 100%

Milestone Achieved: Development & Validation of cP12 Assay in Human Plasma. Month 6.

- STATUS: COMPLETED, MONTH 6.

Major Task 2: Development and Validation of cP12 Assay in Human Urine. Months 1-6.

Task 2.1: Set-up of HPLC/MS/MS Human Urine Assay. Months 1-3.

- STATUS: COMPLETED, MONTH 0. 100%

Task 2.2: Write Validation protocol. Months 1-3.

- STATUS: COMPLETED, MONTH 1. 100%

Task 2.3: Validation of Human Urine Assay. Months 4-6.

- STATUS: COMPLETED, MONTH 2. 100%

Task 2.4: One-month frozen sample stability at -70° C and -20° C. Months 4-6.

- STATUS: COMPLETED, MONTH 5. 100%

Task 2.5: Audited Validation Report. Months 4-6.

- STATUS: COMPLETED, MONTH 5. 100%

Task 2.6: Extended Human Urine Stability to 6 months.

- STATUS: COMPLETED, MONTH 10. 100%

Task 2.7: Extended Human Urine Stability Report.

- STATUS: COMPLETED, MONTH 11. 100%

Milestone Achieved: Development & Validation of cP12 Assay in Human Urine. Month 5

- STATUS: COMPLETED, MONTH 5.

~~**Major Task 3: Development and Validation of cP12 Assay in Dosing Solutions.**~~

- STATUS: Deleted as not required.

Major Task 4: Pharmacokinetic cP12 assays of Human Plasma samples and Human Urine samples. Months 13-31

Task 4.1: Analysis of human plasma and urine samples. Months 13-24.

- STATUS: Complete: Plasma and urine analysis for Cohorts 1-5 have been analyzed.

Task 4.2: If appropriate reanalysis will be performed. Months 14-24.

- STATUS: Complete.

Task 4.3: Incurred Sample Reproducibility. Months 13-24.

- STATUS: Complete.

Task 4.4: Sample storage. Months 13-30.

- STATUS: Complete.

Task 4.5: Sample disposal. Months 31.

- STATUS: No sample disposal.

Task 4.6: If appropriate Expedited analysis will be performed. Months 13-24.

- STATUS: Complete.

Milestone Achieved: Analyses of all samples completed and results relayed to Celerion.

Month 24

- STATUS: Complete. Data were locked by Celerion on April 2, 2020

Specific Aim 2: IRB and HRPO submission and approval (months 1-9)

Major Task 5: IRB Approval.

Task 5.1: Submit Protocol, Informed consent form and Investigator Brochure to IRB. Month 1.

- STATUS: COMPLETED, MONTH 0.

Task 5.2: Respond to IRB comments. Month 2.

- STATUS: COMPLETED, MONTH 0.

Milestone Achieved: IRB approval. Month 3.

- STATUS: COMPLETED, MONTH 0.
- Approval of Protocol Amendment 1 (minor) - 10 July 2018
- Approval of Protocol Amendment 2 (minor) - 25 February 2019
- Approval for Continuing Review - 9 July 2019
- Approval of Protocol Amendment 3 (minor) - 12 July 2019
- Approval of Protocol Amendment 4 (major) - 29 October 2019

Major Task 6: HRPO Approval.

Task 6.1: Submit Protocol, Informed consent form and Investigator Brochure to HRPO. Month 3.

- STATUS: COMPLETED, MONTH 1.

Task 6.2: Respond to HRPO comments. Months 3-6.

- STATUS: COMPLETED, MONTH 4.

Milestone Achieved: HRPO approval. Month 6

- STATUS: COMPLETED, MONTH 5.
- HRPO notified of IRB approval of Continuing Review July 9, 2019- Renewal approval, Expiration July 5, 2020
- STATUS: USAMRMC HRPO renewal approval: Not needed as study is complete.
- Protocol Amendment 4 submitted and approval received January 7, 2020.

Major Task 7: Obtain FDA approval of any required changes.

Task 7.1: Submit Amended Protocol, Informed Consent Form and Investigator Brochure to FDA. Month 6-7.

- STATUS: FDA submission, April 3, 2019. Month 7.
- FDA submission of Protocol Amendment 4 - Nov 13 - Dec 13 2019.

Task 7.2: Respond to FDA comments. Month 8.

- STATUS: No comments

Milestone Achieved: *FDA approval. Month 9*

- STATUS: Complete.

Specific Aim 3: Study design for first-in-human study to assess safety in humans of IV delivered cP12 (months 10-18)

Major Task 8: Recruit and Screen Subjects.

Task 8.1: Subject Recruitment (N=40). Months 10-18

- STATUS: Complete.

Task 8.2: Subject Screening. Months 10-18

- STATUS: Complete.

Milestone Achieved: *All subjects needed for study recruited and screened. Month 16*

- STATUS: Completed.

Major Task 9: Conduct Clinical Trial of lowest (0.003mg/kg) cP12 dose.

Task 9.1: Test lowest cP12 dose (0.003mg/kg) in sentinel pair (one drug, one placebo). Month 10.

- STATUS: Dosing Complete.

Task 9.2: If appropriate. test 0.003mg/kg cP12 dose in six additional subjects (5 receiving cP12 dose, 1 receiving placebo). Month 10.

- STATUS: Infusion Complete, Safety data reviewed by Safety Review Committee. No concerns.

Milestone Achieved*: 0.003mg/kg cP12 dose safe in 6 test subjects compared to 2 placebo controls. Month 10*

- STATUS: Dosing completed on July 9, 2019.
- Safety Review Committee Meeting occurred on 19 July 2019. Dose escalation approved.

Major Task 10: Conduct Clinical Trial of presumptive optimal cP12 dose

Task 10.1: Test presumptive optimal cP12 dose (0.01mg/kg) in sentinel pair (one drug, one placebo). Month 11

- STATUS: Dosing Complete, Month 10.

Task 10.2: If appropriate. test 0.01 mg/kg cP12 dose in six additional subjects (5 receiving cP12 dose, 1 receiving placebo). Months 11-12

- STATUS: Infusion Complete, Safety data reviewed by Safety Review Committee. No concerns.

***Milestone Achieved**: 0.01mg/kg cP12 dose safe in 6 test subjects compared to 2 placebo controls. Month 13*

- STATUS: Dosing completed on July 26, 2019
- Safety Review Committee Meeting occurred on August 7, 2019. Dose escalation approved.

Major Task 11: Conduct Clinical Trial of 0.02mg/kg cP12 dose

Task 11.1: Test next cP12 dose (0.02mg/kg) in sentinel pair (one drug, one placebo). Month 17

- STATUS: Dosing Complete, Month 11.

Task 11.2: If appropriate test 0.02 mg/kg cP12 dose in six additional subjects (5 receiving cP12 dose, 1 receiving placebo). Month 19

- STATUS: Dosing Completed August 13, 2019, Month 11.

***Milestone Achieved:** 0.02mg/kg cP12 dose safe in 6 test subjects compared to 2 placebo controls. Month 19.*

- STATUS: Safety Review Committee Meeting occurred on August 21, 2019. Dose escalation approved.

Major Task 12: Conduct Clinical Trial of 0.04mg/kg cP12 dose

Task 12.1: Test highest dose cP12 dose (0.04mg/kg) in sentinel pair (one drug, one placebo). Month 20

- STATUS: Dosing Complete, Month 11.

Task 12.2: If appropriate. test 0.04 mg/kg cP12 dose in six additional subjects (5 receiving cP12 dose, 1 receiving placebo). Month 22

- STATUS: Dosing Completed August 30, 2019, Month 11.

***Milestone Achieved**: 0.04mg/kg cP12 dose safe in 6 test subjects compared to 2 placebo controls. Month 22*

- STATUS: Safety Review Committee Meeting occurred on September 11, 2019. Dose escalation approved for Cohort 5.

Major Task 13: Conduct Clinical Trial of Optional Cohort

Task 13.1: Test additional/repeat cP12 dose in sentinel pair (one drug, one placebo). Month 23

- STATUS: Dosing complete.

Task 13.2: If appropriate, test additional/repeat cP12 dose in six additional subjects. (5 receiving cP12 dose, 1 receiving placebo). Month 25
Note: Protocol Amendment 4; Cohort 5 to be tested with 0.08mg/kg cP12 in 6 test subjects compared to 2 placebo Controls. IRB approval received, FDA submission (Nov 13- Dec 13, 2019) no comments received, HRPO submission December 30, 2019, HRPO approval January 7, 2020.

- STATUS: Dosing completed February 2020, Month 14.

Milestone Achieved: Additional/repeat cP12 dose safe in 6 test subjects compared to 2 placebo controls. Month 25

STATUS: Dosing completed on February 21, 2020. Month 14.

Major Task 14: Biometrics of all data collected from Clinical Trial

Task 14.1: PK Analysis. Month 13-26.

- STATUS: Clinical Study Report completed July 2020.

Task 14.2: Statistical Data Analysis and Summarization. Month 27-30.

- STATUS: Completed

Task 14.3: Celerion formatted Clinical Study Report. Month 31-32

- STATUS: Completed

Milestone to Achieve: Biometrics completed of all collected data from Clinical Trial. Month 32.

- STATUS: Completed July 2020

<u>Specific Aim 4:</u> Submit clinical study report to FDA (months 10-30)

Major Task 15: Data capture, verification and disposition

Task 15.1: Data capture. Months 10-25.

STATUS: Complete: Database lock on April 2, 2020

Task 15.2: Data verification. Month 10-26.

STATUS: Complete: Database lock on April 2, 2020

Task 15.3: Data disposition. Month 27-30.

Complete: Database lock on April 2, 2020

<u>Milestone Achieved:</u> Data capture, verification and disposition completed. Month 30.

- STATUS: Complete: Database lock on April 2, 2020

Major Task 16: Report formatted for FDA submission

Task 16.1: Report formatted for FDA submission. Months 30-32.

- STATUS: Clinical Study Report published July 2020

Task 16.2: Clinical Study Report submitted to FDA. Month 32.

- STATUS: Complete September 19, 2020 (Month 24)

Milestone Achieved: Clinical Study Report submitted to FDA Month 32.

- STATUS: Clinical Study Report submitted to FDA Month 24

Specific Aim 5: Request Fast Track and Develop Phase 2a clinical trial protocol (months 1-12)

Major Task 17: Fast Tract Designation

Task 17.1: Request and prepare Briefing Package for FDA meeting regarding Phase 2a Clinical Trial Design. Month 1.

- STATUS: Month 6, FTD sent to FDA 5 March 2019

Task 17.2: Respond to FDA comments. Month 2.

- STATUS: Month 5, complete

Milestone Achieved: Fast Track Designation received. Month 3.

- STATUS: Month 6, complete

Major Task 18: Develop Phase 2a Clinical Trial Protocol

Task 18.1: Prepare Briefing Package for FDA meeting regarding Phase 2a Clinical Trial Design. ~~Month 3~~. This subtask should have been Month 30-32 as it could not have been done until the Clinical Study Report was complete, i.e. Major Task 16 Month 32. Hence for Task 18.1 completion target should have been Month 33.

STATUS: Phase 2a protocol and Briefing Package compiled and submitted to FDA. Completed.

Task 18.2: Respond to FDA comments. This Task should have been "Request a Phase 2a Guidance Meeting with the FDA" ~~Month 3=~~6 Month 33.

- STATUS: NMT had their End of Phase 1 (EOP1) meeting with the FDA on June 28th 2021. Completed.

Task 18.3: Type C Phase 2a Guidance Meeting schedule and attended. ~~Month 6-9.~~ This subtask completion target should have been Month 35.

- STATUS: Completed on June 28th 2021.

Task 18.4: Respond to FDA comments from Phase 2a Guidance Meeting. ~~Month 9-12.~~ This subtask completion target was Month 36.

STATUS: FDA meeting minutes were received on August 12, 2021. The FDA advised that a small Phase 2a safety study in outpatients with 5% or less Total Body Surface Area (TBSA) burns be completed and approved prior to a Phase 2 study in hospitalized patients with 5-20% TBSA burns. A protocol for the small safety study in burn patients is being drafted. Quotes are still needed for the new outpatient study design. NMT had requested RRPs for the inpatient Phase 2 CT from two CROs and received budget quotes from both. Completion expected Month 36.

Milestone to Achieve: Phase 2a Clinical Trial Design Amended and writing of full Phase 2a protocol to proceed. ~~Month 12.Month 36.~~ Month 42-48

-STATUS: Phase 2a clinical protocol was developed and included in the Briefing Package which was submitted prior to EOP1 meeting with FDA. The FDA advised a Phase 2a Safety Study with 5-15 (possibly more) subjects with 5% Total Body Surface Area burns of which some percentage is Partial Thickness Burns. NMT is working on revising our plans to include a Phase 2a Safety Study with a follow-on full Phase 2 Study for Safety and Efficacy as previously planned. This work was not completed by Month 36. Therefore, a No Cost Extension was requested, approved and the latter document forwarded to NMT on September 7th, 2021. New completion target date: Month 42-48.

Major Task 19. This is a new task. It was noted that prior to a Phase 2a Clinical Trial a rat micronucleus study is required by I.C.H. and FDA.

Task 19.1: Draft protocol and submit to ACURO for review and approval.

- STATUS: ACURO approval received 11 June 2021, Month 33

Task 19.2: Complete Study and analysis (1 month) and obtain draft report (12-15 weeks). Month 35-36.

STATUS: Study was completed (7/28/21). A draft report was received 9/20/21 for NMT review. The final report will not be completed by Month 36.

Milestone to Achieve: Rat micronucleus study, data analysis and final report completed. ~~Month 35-36.~~ Month 38-40.

STATUS: Completion expected to be October - December 2021. Therefore, a No Cost Extension was requested and the approval forwarded to NMT on September 7th 2021.

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.

Key Findings or Accomplishments:

Task 18.4: Respond to FDA comments from Phase 2a Guidance Meeting. This subtask completion target was Month 36.

STATUS: FDA meeting minutes were received on August 12, 2021. The FDA advised that a small Phase 2a safety study in outpatients with 5% or less TBSA burns be completed and approved prior to a Phase 2 study in hospitalized patients with 5-20% TBSA burns. A protocol for the small safety study in burn patients is being drafted. Quotes are still needed for the new outpatient study design. NMT had requested RFPs for the inpatient Phase 2 CT from two CROs and received budget quotes from both.

Completion expected Month 42-48.

Milestone to Achieve: Phase 2a Clinical Trial Design Amended and writing of full Phase 2a protocol to proceed. ~~Month 12. Month 36~~ Month 42-48.

STATUS: Phase 2a clinical protocol was developed and included in the briefing Package which was submitted prior to the EOP1 meeting with FDA. The FDA advised a Phase 2a Safety Study with 5-15 subjects with 5% or less TBSA burns of which some percentage is Partial Thickness Burns. NMT is working on revising our plans to include a Phase 2a Safety Study with a follow-on full Phase 2 Study for Safety and Efficacy as previously planned. This work was not completed by Month 36. Therefore, a No Cost Extension was requested and the approval document was received on September 7th 2021. New completion Target date: Month 42-48.

Major Task 19. This is a new task. It was noted that prior to a Phase 2a Clinical Trial a rat micronucleus study is required by I.C.H. and FDA.

Task 19.2: Complete Study and analysis (1 month) and obtain draft report (12-15 weeks). Month 35-36.

STATUS: Study was completed 7/28/21). A draft report was received 9/20/21 for NMT review. The Final report will not be completed by Month 36.

Milestone to Achieve: Rat micronucleus study, date analysis and final report completed. ~~Month 35-36~~. Month 38-40.

STATUS: Completion expected to be October - December 2021. Therefore, a No Cost Extension was requested and the approval forwarded to NMT on September 7th 2021.

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.

A Briefing was provided to our DoD Scientific Officer on 19 March 2020.

Abstracts were submitted to the Military Health System Research Symposium (MHSRS) for the August 2020 and 2021 meetings (both meetings were cancelled due to COVID-19). The 2020 was selected for a podium presentation and the 2021 abstract was selected for a poster presentation.

2020: A novel bioactive peptide, cP12, for intravenous (IV) treatment of burns, produces pharmacokinetic (PK) concentrations in a Phase 1 Clinical Trial that correlate with cP12 concentrations causing microvascular vasodilation.

2021: Fibronectin-derived peptide P12 mitigates burn conversion, speeds healing and reduces scarring through its ability to dilate the microvasculature, decrease microvascular occlusion, and increase angiogenesis.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

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

The Rat Micronucleus Study was completed (August 15th 2021). NMT awaits the Draft report for our review. The Final report will most likely not be delivered until October 2021 or later.

The FDA advised a Phase 2a Safety Study with 5-15 subjects with 5% or less TBSA burns of which some percentage is Partial Thickness Burns. NMT is working on revising our plans to include a Phase 2a Safety Study with a follow-on full Phase 2 Study for Safety and Efficacy as previously planned.

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).

The PK analysis at the dose that gave optimal efficacy in the preclinical porcine burn model, gave us blood levels in humans that were similar to the blood levels in swine at that dose. Now we know that the blood concentrations presumably needed for optimal efficacy in humans is the same as in swine, being 100pM to 10nM.

AEs of itching or transient hives occurred in several subjects of the 5th Cohort receiving 0.08mg/kg cP12 during or shortly after the 30 min intravenous infusing while subjects in the 4th Cohort receiving 0.04mg/kg had little reaction (no hives, but transient warmth of the skin or transient itching). No Adverse Events (AEs) were observed in the 2nd or 3rd Cohorts receiving 0.01 and 0.02mg/kg. At present we are planning to use doses no higher than 0.03mg/kg in our Phase 2a Clinical Trial.

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.
we

Addition of Major Task 19 to the SOW. As previously reported, this is a new task. It was noted that prior to a Phase 2a Clinical Trial a rat micronucleus study is required by I.C.H. and FDA.

This task is costing approximately $35K which is a cost over-run. We received approval from ACURO on June 11, 2021 and the study began July 2021. The study is expected to be completed in about 1 month. Data analysis and a follow-on draft report will begin. We expect that the draft report and final report will be delivered after Month 36. Therefore, we contacted the DoD Contracting Officer and Science Officer requesting a No Cost Extension. NMT requested a No Cost Extension and provided justification. We received approval of our request on September 7th 2021 for one year extension.

Development of a new Phase 2a Safety Study is ongoing in response to the FDA recommendations received during the EOP1 meeting on June 28, 2021.

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.

The rat micronucleus study was completed (August 15th 2021) and NMT is awaiting the Draft report for our review. The Final report new target for completion is December, 2021 (Month 39).

Development of a new Phase 2a Safety Study protocol is ongoing in response to the FDA recommendations received during the EOPh1 meeting on June 28, 2021.

The new Target date for completion and submission of the new protocol to the FDA is Month 42 and the Target date for FDA approval is Month 48.

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.

To Date NeoMatrix Therapeutics incurred the following costs that were not budgeted for in original budget submitted to DoD:

Cost Over Runs:

--Drafting a new Phase 2a Safety Study per FDA's comments at our EOP1 meeting is an unanticipated effort and will have costs that were unanticipated. Follow up with FDA as needed will also incur future costs.

--Rat micronucleus study required by I.C.H. & FDA $34,619.00

--Microconstants sample kits and yearly sample storage $19,635.00

--Propharma: DoD Required Independent Medical Monitor, $72,300.00

--Shipping: CSN $3,200.00, World Courier $987.00, FedEx $12,436 (cP12 stability studies).

--Licenses: MedDRA and WHODrug, $2,584.00

--Other: Celerion Change Order for Amendment 3 & 4 IRB submissions and inclusion of Injection Site Exam (to monitor for hypersensitivity) $3,418.00

--Stratum: IV infusion set compatibility with low dose cP12 under GMP conditions, $7,500

--University of Iowa Pharmaceuticals: stability testing and storage fees, $15,300

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

Not applicable.

Significant changes in use or care of vertebrate animals.

Significant changes in use of biohazards and/or select agents

Not applicable.

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).*

None

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.*

Webinar presented to all NMT DoD contract Program Managers at the American Burn Association virtual meeting on March 19, 2020 entitled, "**NeoMatrix Therapeutics Portfolio of intravenous and topical treatments for burns and other battlefield wounds**".

An abstract submitted to the 2020 MHSRS for presentation of NMT findings during Phase 1 Clinical Trial entitled, "**A novel, bioactive peptide, cP12, for intravenous (IV) treatment of burns, produces pharmacokinetic (PK) in a Phase 1concentrations in a Phase1 Clinical Trial that correlate with cP12 concentrations causing microvascular vasodilation**". The abstract was accepted for a podium talk but the meeting was canceled and NOT replaced by a virtual meeting. However, the abstract was published in a special online synopsis of what was intended to be presented at the meeting.

A presentation entitled, "**Burn conversion: clinical problem, large animal model**," was presented at the American Burn Association virtual meeting, April 15, 2021.

Since the 2020 MHSRS meeting was cancelled, a revised abstract with additional information on PK in the cP12 Phase 1 Clinical Trial was submitted for the 2021 MHSRS. Its title is "**cP12 plasma levels in Phase 1 Clinical Trial correlates with cP12 concentrations that induce microvascular vasodilation and mitigate burn conversion in animal models."** This was selected for a poster presentation. (See below under Abstracts)

Furthermore, an additional abstract was submitted for the 2021 MSHSRS, entitled "**Fibronectin-derived peptide P12 mitigates burn conversion, speeds healing and reduces scarring through its ability to dilate the microvasculature, decrease microvascular occlusion, and increase angiogenesis."** This was selected for a poster presentation. (See below under Abstracts). MHSRS was cancelled due to COVID 19.

- **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.

- **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.

None.

- **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.

- **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;* An abstract submitted to the 2020 MHSRS for presentation of NMT findings during Phase 1 Clinical Trial entitled, "**A novel, bioactive peptide, cP12, for Intravenous (IV) treatment of burns, produces pharmacokinetic (PK) in a Phase1 concentrations in a Phase1 Clinical Trial that correlate with cP12 concentrations causing microvascular vasodilation**". The abstract was accepted for a podium talk but the meeting was canceled and NOT replaced by a virtual meeting. However, the abstract was published in a special online synopsis of what was intended to be presented at the meeting.
  - *audio or video products;*
  - *software;*
  - *models;* An oral presentation entitled, "**Burn conversion: clinical problem, large animal model**," was presented at the American Burn Association virtual meeting, April 15, 2021.
  - *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."

Nothing to report.

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.

NMT recently received an email and attached letter with a "Funding Status Notification", indicating funding was available for our 2020 full proposal submitted to DoD CDMRP Joint Warfighter Medical Research Program (JWMRP) Military Medical Research and Development Award. Title "cNP8 Delivered Topically in a Tyrosine-Derived Fiber Mat to Reduce Burn Injury Progression, Speed Wound Closure, and Mitigate Scarring". We are waiting to hear back from JWMRP on two full proposals we submitted upon request for 2021.

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.

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: In addition to embedding an updated Quad Chart within this annual / final technical report, also submit a standalone copy as an attachment in PowerPoint file only (.ppt or

.pptx) to CDMRP Reporting at usarmy.detrick.medcom-cdmrp.mbx.cdmrp-reporting@mail.mil and copy the assigned CDMRP Science Officer.

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.

cP12 plasma levels in Phase 1 Clinical Trial correlates with cP12 concentrations that induce microvascular vasodilation and mitigate burn conversion in animal models.

Fubao Lin, PhD and Richard A. Clark, MD; NeoMatrix Therapeutics, Inc., Stony Brook, NY

Background: P12, a bioactive, 14-residue, fibronectin-derived peptide, binds PDGF-BB with a K_D = 200nM. P12 enhances growth and survival activity in adult human dermal fibroblasts (AHDF) cultured under stress conditions, e.g. nutrient deprivation, reactive oxygen stress, and endoplasmic reticulum stress (*J Invest Dermatol* 134:1119-1127, 2014). For *in vivo* studies, P12 was cyclized (cP12) to resist digestion by exopeptidases found in normal blood and tissue. When cP12 is administered intravenously (IV) at 0.01mg/kg, 1-4h post-burn, it limits burn injury progression, speeds healing, and reduces scarring in our porcine vertical injury progression burn model (*Wd Repair Regen* 24:501-513, 2016). In the same study, we found that aggregated red blood cells (RBCs) occlude the peri-burn microvasculature as early as 1h and increasingly for at least 48h post-burn. Furthermore, RBC occlusion decreases when cP12 is administered at the same dose that optimally speeds healing and reduces scarring. Concurrently, we discovered that P12 (100pM to 10nM) dilates the microvasculature of the hamster cheek pouch through a β-adrenergic-dependent pathway (*Microcirculation*, 24:e12369, 2017). Therefore, P12/cP12 has two distinct mechanisms of action that work at entirely different concentrations, i.e. 1) P12 induces PDGF-BB receptor signal biasing toward cell survival and growth *in vitro* at 10 to 30μM (*J Invest Dermatol*, 134:921-929, 2014) and 2) P12 induces microvascular vasodilation in the hamster cheek pouch when applied *ex vivo* at 100pM to 10nM (*Ibid*).

Methods:
1. AHDF cultures under nutrient deprivation (no serum) with 1nM PDGF-BB ± P12 (*Ibid*).
2. Hamster cheek pouch intravital microscopy model for microvascular measurements (*Ibid*).
3. Yorkshire pig vertical injury progression burn model (*J Burn Care Res*, 6:638-646, 2011).
4. A Phase 1 Randomized, Placebo-Controlled, Single Ascending Dose Study to Examine Safety, Tolerability, and Pharmacokinetics of cP12 in Healthy Adults (FY17/18 MBRP Burn Program)

Results: From our porcine burn model results (*Wd Repair Regen* 24:501-513, 2016), it appeared that IV administered cP12 might be working through a vasodilation mechanism. Unfortunately, porcine plasma levels of cP12 could not be detected (assay sensitivity limit 100nM) when the optimal dose (0.01mg/kg) for mitigation of burn conversion was administered. With a more sensitive assay, cP12 could be detected in human plasma at picomolar concentrations. In fact, Phase 1 Clinical Trial volunteers given 0.01mg/kg cP12 infusions attained plasma levels between 180pM to 11.2nM from 15 to 90min post-infusion. This human plasma concentration range is similar to the P12 levels that stimulate microvascular dilation in the hamster cheek pouch (100pM to 10nM). Following optimal dosing of IV cP12 (0.01mg/kg) in pigs that reduces peri-burn microvascular occlusion and burn conversion, cP12 plasma levels were <100nM, a low nM or pM concentration consistent with plasma levels detected in humans given the same dose.

Conclusion: We posit that a main mechanism of IV cP12 in burns is peri-burn microvascular dilation. At 0.01mg/kg IV dose of cP12, no central cardiovascular dilation, as manifested by increased heart rate or decreased blood pressure, occurred in either humans or animals. Thus, IV cP12 could be extremely useful in the setting of Prolonged Field Care and may be useful for other microvascular occlusive diseases while avoiding adverse effects on the central circulation.

What do you expect the attendee to be able to do at the end of the session?
1. Understand how pharmacokinetics in a Phase 1 Clinical Trial can be important.
2. Describe how clinical pharmacokinetics can give important information on a drug product's mechanisms of action.
3. Describe how clinical pharmacokinetics can be related to preclinical animal studies.

Fibronectin-derived peptide P12 mitigates burn conversion, speeds healing and reduces scarring through its ability to dilate the microvasculature, decrease microvascular occlusion, and increase angiogenesis.

Monica McTique, PhD[1], Marcia G. Tonnesen, MD,[1,2] and Richard A. Clark, MD[1]*, [1]Department of Biomedical Engineering and Dermatology, Stony Brook, NY, and [2]Dermatology Section, VAMC, Northport, NY

Background: When cyclized P12 (cP12) is administered intravenously (IV) at 0.01mg/kg, 1-4h post-burn, it limits burn injury progression, speeds healing, and reduces scarring in our porcine vertical injury progression burn model (*Wd Repair Regen* 24:501-513, 2016). In the same study, we demonstrated that aggregated red blood cells (RBCs) occlude the peri-burn microvasculature as early as 1h and increasingly for at least 48h post-burn, and that RBC occlusion is decreased when 0.01mg/kg cP12 is administered IV 1-4h post-burn. Concurrently, we found that P12 at 100pM to 10nM dilates the mucocutaneous microvasculature of the hamster cheek pouch through a β-adrenergic-dependent pathway (*Microcirculation*, 24:e12369, 2017). This is the same concentration range found in human plasma after 0.01mg/ml cP12 IV infusion during a Phase 1 clinical trial (180pM to 11.2nM), and in pig plasma (<100nM) after the IV 0.01mg/kg cP12 that optimally reduces peri-burn microvascular RBC occlusion.

Methods: For studies on angiogenesis we used two previously established methods:
1. An *in vitro* angiogenesis assay using human dermal microvascular endothelial cells (EC) cultured on collagen-coated microbeads that were dispersed in a human fibrin gel (XD Feng, MG Tonnesen, S Mousa, RAF Clark *Int J Cell Biol*, 13:231-279, 2013).
2. An *in vivo* angiogenesis assay using nude mice and a commercially available assay kit containing angioreactors and matrix for implanting into the flanks of the mice. Although the kit also contained Matrigel™ as a hydrogel delivery system for potential angiogenesis factors, we found that human fibrin for delivery of peptides was far superior.

Results: P12 increased angiogenesis both *in vitro* and *in vivo*.
1. The *in vitro* angiogenesis assay demonstrated that 3μM P12 with vascular endothelial growth factor (VEGF) and Fibroblast Growth Factor-2 (FGF-2) at suboptimal doses (15μg/ml and 12.5 μg/ml, respectively) doubled sprout angiogenesis from EC-covered beads compared to no P12 under the same conditions. Higher P12 concentrations showed a lesser effect.
2. The *in vivo* angiogenesis assay demonstrated that the addition of 10 or 30μM cP12 to a low dose of FGF-2 (50ng/ml) in fibrin gel increased angiogenesis 7-fold and 15-fold compared to no cP12

added to the same conditions. Similarly, 10 or 30μM cP12 addition to a low dose of VEGF (125ng/ml) in fibrin gel increased angiogenesis 80% and 130% compared to control.

Conclusions: We posit that IV cP12 abates burns by complimentary mechanisms, including dilation of peri-burn microvasculature, reduction of BV occlusion, and enhancement of angiogenesis. At an optimal dose of IV cP12 (0.01mg/kg), no central cardiovascular dilation, as manifested by increased heart rate or decreased blood pressure, occurs in either humans or animals. Thus, IV cP12 could be extremely beneficial in the setting of Prolonged Field Care, and may be useful for other microvascular occlusive diseases, while avoiding adverse effects on the central circulation.

Learning Objectives

1. Understand how peptides can have a multitude of activities that work together to effect important biologic outcomes.
2. Learn how *in vitro* angiogenesis assays can give important clues about *in vivo* angiogenesis.
3. Acquire knowledge about a simple *in vivo* angiogenesis assay in small animals.