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The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT
Many protein-based drugs have limited efficacy due to a short half-life or require intravenous delivery because of low bioavailability when given subcutaneously. Extend Biosciences is developing proprietary carrier molecules that will allow proteins to access a transport pathway for efficient delivery to the vascular space and then maintain a sustained presence in circulation. This would be of particular importance in the development of longer-lasting versions of bioscavenger proteins that could then be delivered subcutaneously and become bioavailable within minutes of administration. In this project, one of Extend Biosciences' carrier molecules will be conjugated to two

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Report Title

Enhancing the Pharmacokinetic Profile of Protein-Based Drugs

ABSTRACT

Many protein-based drugs have limited efficacy due to a short half-life or require intravenous delivery because of low bioavailability when given subcutaneously. Extend Biosciences is developing proprietary carrier molecules that will allow proteins to access a transport pathway for efficient delivery to the vascular space and then maintain a sustained presence in circulation. This would be of particular importance in the development of longer-lasting versions of bioscavenger proteins that could then be delivered subcutaneously and become bioavailable within minutes of administration. In this project, one of Extend Biosciences' carrier molecules will be conjugated to two bioscavenger proteins of interest to the military as further proof-of-concept for the technology. The modified bioscavengers will be assayed in vitro to ensure that the carrier molecule does not disrupt functional activity. Following success in Phase I, the Phase II studies would test the modified bioscavengers for their improved half-life and bioavailability when delivered subcutaneously in an appropriate animal model, and test whether the modified protein induces toxicity or an immune response. This project will demonstrate the feasibility of improving the half-life and bioavailability of bioscavenger proteins that could be applied to numerous other protein-based drugs including those used in Chemical and Biological Defense treatments.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received Paper

TOTAL:

Number of Manuscripts:

Books

Received Book

TOTAL:

Received	Book Chapter

TOTAL:

Patents Submitted

Patents Awarded

Awards

Graduate Students

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Names of Post Doctorates

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Names of Faculty Supported

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:..... 0.00

Names of Personnel receiving masters degrees

NAME

Total Number:

Names of personnel receiving PHDs

NAME

Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED

FTE Equivalent:

Total Number:

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

Technology Transfer

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MONTHLY PROGRESS REPORT

Contract Number: W911NF-13-C-0033
Phase I option SBIR Proposal Number: C122-101-0073

Title: Enhancing the Pharmacokinetic Profile of Protein-Based Drugs
Performance Period: 02/25/14 – 05/24/14

Contract Amount: $49,999
Total Paid-to-date: $33,332.68
Amount Expended-to-date: $30,460.41

Number of Employees Working on the Project: 3
Number of New Employees Placed on the Contract: 0

Deliverables: Data on the Carrier:Protein ratios (CONFIDENTIAL)

Work Period: 04/25/14 – 05/25/14

Task 1. **Confirm that the carrier molecule(s) are attached to purified BCHE and PON1 by mass spectrometry.**

During the previous work period, BCHE was deglycosylated and purified using ZipTips (Millipore) and submitted for MS analysis to Boston University's Core Facility. We received the results after a long delay due to equipment-related problems. No products within the correct mass range were detected.

An identically prepared sample of BCHE was deglycosylated and purified and sent to the core facility at Yale University as a backup. Again, we experienced a long delay due to instrument calibration and throughput issues. We did receive results and observed a strong signal for deglycosylated BChE with peaks at 66.1, 67.8, and 69.7 kD. This correlates well with the theoretical mass of 65 kD for the completely deglycosylated protein and indicates that glycosylation is not complete. The spectrum for native BChE did not yield any signal above background; this is expected due to the very heterogeneous nature of the glycosylation. Both of the above academic facilities used electrospray ionization (ESI) MS.

As an alternative to ESI and academic core facilities, identical samples were submitted to HT Laboratories (San Diego, CA) for MALDI-TOF MS analysis. A very broad signal for native BChE was obtained with a peak at 78 kD (range at half-peak height = 70-80

kD). A narrower, but still broad peak for deglycosylated BChE was observed at 66 kD (range 63.5-69 kD). This mass range correlates with the expected mass and the ESI MS results described above.

The next goal is to observe an increase in mass upon conjugation of the carrier molecule to BChE. To this end, BChE was reacted with 0, 3, or 10 equivalents of the NHS-activated carrier. The reactions were deglycosylated, desalted by ZipTip, and submitted for MALDI-TOF MS analysis. The 0 equivalent control reaction yielded a peak centered at 65.7 kD, while the 3 and 10 equivalent reactions were 69.4 kD, an increase of 3.7 kD corresponding to 1.5 carriers per BChE.

We have signed the amended Material Transfer Agreement (MTA) from the University of Nebraska to obtain Dr. Oksana Lockridge's CHO-K1 cell line that expresses BCHE for expression and purification for work in Phase II. We expect to obtain the cell line from Dr. Lockridge in the next reporting period.

We are actively interviewing candidates for an open position for cell culture and protein purification related to the project.

During the work period, Extend Biosciences also undertook some independent research and development (IR&D) activities. We would like to obtain additional proof-of-concept data for the use of the technology.

During this work period, no problems were encountered. No changes to the planned schedule have occurred.

Proposed Work for the Following Period: 05/25/14 – 06/25/14

During the next month, we will continue to work on the MS analysis and will move on to testing and characterizing different reaction conditions including higher carrier to BChE ratios. We hope to be able to receive the cell line in-house. We will hope to hire an appropriate candidate that has cell culture expertise.

Extend Biosciences will continue working on a small IR&D project. No bid and proposal work is expected during this period.