LAWRENCE
LIVERMORE
NATIONAL
LABORATORY

LLNL-TR-745528

Novel Agents for Gram-Negative Biodefense Pathogens Final Report CRADA No. TC02128.0

F. Lightstone, K. W. Turteltaub, J. Finn

January 31, 2018

Disclaimer

This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

Novel Agents for Gram-Negative Biodefense Pathogens

Final Report
CRADA No. TC02128.0
Date Technical Work Ended: December 1, 2012

Date: January 24, 2013 Revision: 2

A. Parties

This project was a relationship between Lawrence Livermore National Laboratory (LLNL) and Trius Therapeutics, Inc.

Lawrence Livermore National Security, LLC
Lawrence Livermore National Laboratory
7000 East Avenue
Livermore, CA 94550
Felice Lightstone
Kenneth W. Turteltaub
Tel: (925) 423-8152
Fax: (925) 422-2282

Truis Therapeutics
6310 Nancy Ridge Drive, Suite 101
San Diego, CA 92121
John Finn
Tel: (858) 452-0370
Fax: (858) 452-677-9975

B. Project Scope

This was a collaborative effort between Lawrence Livermore National Security, LLC as manager and operator of Lawrence Livermore National Laboratory (LLNL) and Trius Therapeutics, Inc. ("Trius") to develop novel agents for gram-negative biodefense pathogens. LLNL and Trius jointly responded to a U.S. Health and Human Services (National Institutes of Health) request for proposals (solicitation # BAA NIH-NIAID-DMID 08-20) to develop new therapeutic countermeasures with a proposal entitled "Development of Therapeutic Agents for Select Biodefense Pathogens" which resulted in this CRADA, with a goal of developing gram-negative, dual-target antibacterial agents that show no cross-resistance to existing drugs. Trius was awarded Contract HHSN272200800042C to accomplish the goals in the proposal, with LLNL as a major subcontractor.

The technical objectives of this CRADA project were to develop a new therapeutic entity with activity against gram-negative category A and B biothreat agents. The therapeutic would be a small molecule that could be administered intravenously (i.v.), preferably once- or twice-a-day and would be available for use both as a prophylactic and as a therapeutic against a wide range of

biodefense pathogens. These included the gram-negative biodefense pathogens: *Escherichia coli, Salmonella (enterica* and Typhi), *Shigella dysenteriae, Yersinia pestis, Burkholderia pseudomallei, Campylobacter jejuni* and *Francisella tularensis,* as well as potentially the Category A gram-positive pathogen *Bacillus anthracis.* This new therapeutic will act through inhibition of bacterial DNA topoisomerase IV and DNA gyrase (ParE and GyrB, respectively).

The project consisted of the following four phases with a total of 15 tasks.

Phase 1 (Year 1-2) - Research of Product. Structural information on ParE and GyraseB will be compared across pathogens, and searches will be conducted to identify small molecule agents that bind in common to these proteins (Trius and LLNL). Pharmacokinetic studies will be conducted with promising small molecule inhibitors will be determined to select compounds with both good inhibition of bacterial growth and good properties for a drug by Trius.

Phase 2 (Year 2-3) - Preclinical Development. Candidates having good inhibition and pharmacokinetic properties will be studied to determine the maximal tolerated doses in animal model (Trius) and to test for efficacy against biodefense-relevant pathogens in vitro (LLNL). Promising compounds will undergo detailed pharmacokinetic analysis using accelerator mass spectrometry (LLNL) and studies will be conducted to assess chronic toxicity in 2 animal models (Trius). Trius will file an exploratory-IND on promising compounds, and accelerator mass spectrometry (LLNL) will be used for detailed pharmacokinetic analysis on the compounds.

Phase 3 (Year 3) - Candidate compounds having good animal efficacy, chronic toxicity and pharmacokinetic profiles will undergo advanced safety testing. Scaled-up synthesis methods (Trius) will be developed for good candidates and these will be tested in non-human primates for pharmacokinetics (LLNL), efficacy (Trius) and toxicity (Trius). Compounds having a therapeutic index of > 3 will be advanced to the investigational new drug status and appropriate Food and Drug Administration filings will occur. Work will begin on developing the protocols to be used in follow-on clinical studies.

Phase 4 (Year 4-5) - Clinical and Phase 1 testing. After IND filings have been completed, work will begin to develop biomarkers of toxicity. Clinical phase 1 studies will be conducted to assess acceptable pharmacokinetics in humans which will be used to define the appropriate dose (Trius) for a once-a-day dosing regimen.

The project consisted the following five (5) major deliverables:

Deliverable 1. Trius will provide crystals and crystal structures of GyrB and ParE and bound candidate structures. LLNL will provide to Trius data from x-ray studies and candidate structures as a result of virtual screening. Trius will provide new chemical entities with inhibitory concentration$_{50}$ (IC_{50})< 50 nM, Minimum inhibitory concentrations under 2 ug/ml, good selectivity for bacterial ParE and GyrB relative to human topoisomerases, and low cytotoxicity. Animal use protocols will be produced and approved. Efficacy will be demonstrated in a septicemic model. (Years 1-2)

Deliverable 2. Compounds resulting from stage 1 will be provided that have a therapeutic index that is greater than 3-times the effective dose (ED_{50}). Safety reports on these entities will be provided for complete toxicity (Trius). Oral bioavailability and pharmacokinetic profiles will be provided (LLNL). Synthesis scale-up will be completed (Trius). Minimum inhibitory concentrations will be established (LLNL) and efficacy in two murine models using *Y. pestis* will be completed and reported on (LLNL).
(Years 2-3)

Deliverable 3. Promising lead candidates will be synthesized (Trius) and formulated (Trius). IND's will be filed (Trius). Institutional Review and approval of human subject's studies will be provided. Microdosing in healthy human volunteers will be conducted and a report provided to Trius (LLNL). Toxicity will be evaluated in animals and a report prepared (Trius). A complete efficacy report will be provided to Trius. (Year 3)

Deliverable 4. Biomarkers will be identified for toxicity (Trius). Phase I clinical study will be completed (Trius) and a clinical outcomes report submitted (Trius). (Years 4-5)

Deliverable 5. Final Report and Abstract due within thirty (30) days of completion or termination of the project, as required under Article XI of the CRADA. (LLNL/Trius)

This CRADA was originally designated as a five (5) year project. At the request of the CRADA Participant, the CRADA was terminated on December 1, 2012. The following major deliverables were successfully completed:

Deliverable 1 was completed by both Trius and LLNL.

Trius provided crystal structures of GyrB and ParE with bound candidate structures. LLNL built compound libraries, ran calculations and simulations on the compounds and protein structures, developed models for virtual screening and provided all the data to Trius. Trius provided new chemical entities. LLNL designed panels of diverse *Bacillus anthracis, Yersinia pestis, Burkholderia pseudomallei, Burkholderia mallei,* and *Francisella tularensis* isolates for minimum inhibitory concentrations (MIC) testing. LLNL performed preliminary MIC testing of initial compounds. Trius completed animal use protocols and demonstrated efficacy in a septicemic model.

Deliverable 2 was completed by both Trius and LLNL.

Trius completed the complete toxicity testing on compounds from Stage 1. LLNL completed dose proportionality, microdose pharmacokinetics, and tissue distribution studies on TR-1491 in Sprague-Dawley rats. LLNL completed MIC testing on panels of diverse *Bacillus anthracis, Yersinia pestis, Burkholderia pseudomallei, Burkholderia mallei,* and *Francisella tularensis* isolates.

Deliverable 3 was modified and completed by both Trius and LLNL.

Trius synthesized and formulated promising lead candidates. Truis filed and eIND to test a compound in humans using microdosing. Trius performed toxicity studies and evaluated the data from animals. Trius completed the efficacy report. LLNL did not complete the microdosing study in healthy human volunteers because NIH, the sponsor, deemed it unnecessary to perform

the human microdosing study because the compound seemed to scale from rats to dogs. Because of the change in tasks as requested by the sponsor, LLNL effort ended early and thus this CRADA was terminated on December 1, 2012.

Deliverable 4 was partially completed by Trius.
Trius identified biomarkers for toxicity. Trius filed for a Phase I clinical study and is waiting to perform the study. The current effort is in the 5th year.

Deliverable 5 is hereby completed by LLNL and Truis.

C. Technical Accomplishments

LLNL effort was focused on designing and developing a new antimicrobial that met the necessary metrics to be a lead compound or compound series (Deliverable 1 and 2). As part of our deliverables to Trius, monthly technical and financial reports were provided. Each technical report summarized the monthly effort and provided new findings. Actual data was shared directly with researchers at Trius or provided on an electronically shared storage space, such that large data sets were provided to Trius.

Computational Design and Development

- Numerous virtual libraries were generated and screened for new cores and fragment pieces
- Numerous molecular dynamics simulations were performed to evaluate the interaction between the proposed new ligands and GyrB and ParE.
- Numerous quantum mechanical calculations were performed to understand and evaluate new fragments for R2 and R4 and other R groups.
- Numerous pharmacophore models were generated to maximize screening.
- Numerous QSAR models were generated combining the experimental results with the calculated results.
- Numerous ADMET models were generated to predict the pharmacokinetics of the proposed compounds.
- Specific calculations were done to predict hERG-liability.
- All suggested new compounds were provided to Trius on a weekly basis.

Microbiology
The susceptibility of different bacterial pathogens to the different Trius compounds was tested using panels of diverse virulent *Bacillus anthracis, Yersinia pestis, Burkholderia pseudomallei, Burkholderia mallei,* and *Francisella tularensis* isolates.

Minimum inhibitory concentrations (MICs) were measure at LLNL using inhibitors provided by Trius. Because MIC testing limited the number of compounds that could be tested at one time, inhibitors were provided in batches only after having been tested in non-virulent strains at Trius.

MIC testing occurred in October 2010, December 2010, January 2011, October 2011, November 2011, February 2012. All results were provided to Trius researchers directly.

In the end, eight promising compounds were effective on all tested isolates at concentrations below 0.125 µg/mL.

Pharmacology

For the LLNL effort, three major studies were conducted for one specific Trius inhibitor. The dose proportionality study investigated the pharmacokinetics of one compound after intravenous administrations of inhibitor at three concentrations in Sprague-Dawley rats. Results show that the rats exhibit a multi-compartment profile, which initially shows a rapid decline in plasma concentrations followed by a slower terminal phase with a mean apparent terminal half-life of 4.40 to 7.9 hrs. Other parameters were measured and calculated.

For the microdose pharmacokinetic study, the same inhibitor used in the standard dose study was used for the microdose study in the Sprague-Dawley rat to determne if a microdose can predict the pharmacokinetics parameters of a therapeutic dose. Two IV doses were administered (0.01 mg/kg and 3 mg/kg). The plasma concentrations were quantified by accelerator mass spectroscopy (AMS). Results show that the microdose was indicative of the therapeutic dose and all determined measurements were comparable.

For the tissue distribution study, the same inhibitor used in the PK studies was used again in Sprague-Dawley rats to determine the absorption, disposition and excretion of an IV administered dose, and to determine the tissue residence time of the inhibitor over a 96 hr exposure. Plasma, tissue, and excreta samples were collected following a single IV (10 mg/kg administration of ^{14}C-TR-1491 to the Sprague-Dawley rat. The concentration of TR-1491 in all compartments was determined by AMS analysis.

All results and data for all three studies were provided to Trius directly.

D. Expected Economic Impact

The economic impact for this project is difficult to predict. For the drug industry, the failure rate of new drugs in clinical trial 1 is approximately 90%. If the outcome of this CRADA is a successfully licensed new broad spectrum antibiotic, then the economic impact is enormous for the following reasons: 1) a new class of antibiotics will be on the market for general public use against drug resistant strains of bacteria, hopefully saving lives and decreasing health care expenses; 2) a medical countermeasure against will have been developed to combat biological weapons pathogens; and 3) new technologies, such as high performance computing and accelerator mass spectroscopy, will have successfully been used to create this new drug and may stimulate the drug industry to use these technologies in other drug development efforts, ultimately speeding up drug development and saving 100s of millions to billions of dollars for the companies while stimulating the US economy.

D.1 Specific Benefits

Benefits to DOE

At present, no broad spectrum antimicrobial exists that are proven for use against such biological weapon agents. This CRADA will result in new antimicrobials that will protect the public

against Bacterial Category A and B agents that could be used by terrorists. Thus, the development of the new antibiotic is a national security need. Benefits to DOE include the use of DOE technologies to speed up drug development. This effort shows that DOE is at the forefront of advancing new technologies for use in therapeutic design and development to better human health. This activity is funded by the U.S. Govnment through the National Institutes of Health, and the resulting product is slated to be incorporated into the National Strategic Stockpile funded under BARDA.

Benefits to Industry

In the process of developing new antibiotics, new advanced technologies were used. This CRADA demonstrated the successful use of high performance computing and accelerator mass spectroscopy in a drug design and development effort. These new technologies will benefit industry by showing a reduction in time to clinical trial 1 and, hopefully, a successful new drug entity. With the reduction in time to successful clinical trial, a new paradigm to drug development will have been demonstrated. This new paradigm should have beneficial impact on the drug industry.

E. Partner Contribution

This effort for the NIH was a collaborative effort between Trius and LLNL. Trius contributed all as described in the Deliverables section, including but not limited to synthesis, crystallography, enzymatic assays, sample preparation, MIC testing of non-virulent bacterial strains, pharmacology studies, safety testing, eIND filing, and IND filing.

F. Documents/Reference List

Reports

Monthly technical and financial reports from LLNL were provided to Trius for FY2009, 2010, 2011, and 2012.

Two publications are in press:

Leslie W. Tari, Michael Trzoss, Daniel C. Bensen, Xiaoming Li, Zhiyong Chen, Thanh Lam, Junhu Zhang, Christopher J. Creighton, Mark L. Cunningham, Bryan Kwan, Mark Stidham, Karen J. Shaw, Felice C. Lightstone, Sergio E. Wong, Toan B. Nguyen, Jay Nix and John Finn (2012) Pyrrolopyrimidine inhibitors of DNA gyrase B and topoisomerase IV, part I: structure guided discovery and optimization of dual targeting agents with potent, broad spectrum enzymatic activity. *Bioorg Med Chem Lett.* In press.

Michael Trzoss, Daniel C. Bensen, Xiaoming Li, Zhiyong Chen, Thanh Lam, Junhu Zhang, Christopher J. Creighton, Mark L. Cunningham, Bryan Kwan, Mark Stidham, Kirk Nelson, Vickie Driver, Amanda Castellano, Karen J. Shaw, Felice C. Lightstone, Sergio E. Wong, Toan B. Nguyen John Finn and Leslie W. Tari (2012) Pyrrolopyrimidine inhibitors of DNA gyrase B (GyrB) and topoisomerase IV (ParE), part II: development of inhibitors with broad spectrum, Gram-negative antibacterial activity. *Bioorg Med Chem Lett.* In press.

Copyright Activity

None

Subject Inventions

The following joint subject invention was disclosed by LLNL on 3/25/11:

IL12370, Patent pending, U.S. and PCT patent application filed by Trius Therapeutics

Truis Therapeutics, Inc. has expressed an interest in licensing the above subject invention.

Background Intellectual Property

LLNL disclosed the following Background Intellectual Property for this project:

IL-11554A, Patent not pursued

U.S. Patent No. 5,209,919 - *Method of Measurement in Biological Systems;* Inventors: Kenneth W. Turteltaub, John S. Vogel, James S. Felton, Barton Levan Gledhill, Jay C. Davis, Larry H. Stanker (IL-8567B) (expired 5/11/10)

U.S. Patent No. 5,376,355 - *Method of Measurement in Biological Systems;* Inventors: Kenneth W. Turteltaub, John S. Vogel, James S. Felton, Barton Levan Gledhill, Jay C. Davis (IL-8567C) (expired 5/11/10)

U.S. Patent No. 5,366,721 - *Method for Detection of Long-Lived Radioisotopes in Small Biochemical Samples*; Inventors: Kenneth W. Turteltaub, John S. Vogel, James S. Felton, Barton Levan Gledhill, Jay C. Davis (IL-8567D) (expired 5/11/10)

Truis Therapeutics, Inc. has not expressed an interest in licensing the above LLNL Background Intellectual Property.

Truis Therapeutics, Inc. did not disclose any Background Intellectual Property for this project.

This page intentionally left blank

G. Acknowledgement

Industrial Participant's signature of the final report indicates the following:

1) The Participant has reviewed the final report and concurs with the statements made therein.
2) The Participant agrees that any modifications or changes from the initial proposal were discussed and agreed to during the term of the project.
3) The Participant certifies that all reports either completed or in process are listed and all subject inventions and the associated intellectual property protection measures generated by his/her respective company and attributable to the project have been disclosed and included in Section E or are included on a list attached to this report.
4) The Participant certifies that if tangible personal property was exchanged during the agreement, all has either been returned to the initial custodian or transferred permanently.
5) The Participant certifies that proprietary information has been returned or destroyed by LLNL.

Feb 25, 2013

John Finn, Chief Scientific Officer — Date
Truis Therapeutics, Inc.

May 15, 2013

Felice C. Lightstone, LLNL Principal Investigator — Date
Lawrence Livermore National Laboratory

May 23, 2013

Veronica Lanier, Acting Technology Commercialization Manager — Date
Lawrence Livermore National Laboratory

Attachment I – Final Abstract

Novel Agents for Gram-Negative Biodefense Pathogens

Final Abstract (Attachment I)
CRADA No. TC02128.0
Date Technical Work Ended: December 1, 2012

Date: January 24, 2013 Revision: 2

A. Parties

This project was a relationship between Lawrence Livermore National Laboratory (LLNL) and Trius Therapeutics, Inc.

Lawrence Livermore National Security, LLC
Lawrence Livermore National Laboratory
7000 East Avenue
Livermore, CA 94550
Felice Lightstone
Kenneth W. Turteltaub
Tel: (925) 423-8152
Fax: (925) 422-2282

Truis Therapeutics
6310 Nancy Ridge Drive, Suite 101
San Diego, CA 92121
John Finn
Tel: (858) 452-0370
Fax: (858) 452-677-9975

B. Purpose and Description

This was a collaborative effort between Lawrence Livermore National Security, LLC as manager and operator of Lawrence Livermore National Laboratory (LLNL) and Trius Therapeutics, Inc. ("Trius") to develop novel agents for gram-negative biodefense pathogens. LLNL and Trius jointly responded to a U.S. Health and Human Services (National Institutes of Health) request for proposals (solicitation # BAA NIH-NIAID-DMID 08-20) to develop new therapeutic countermeasures with a proposal entitled "Development of Therapeutic Agents for Select Biodefense Pathogens" which resulted in this CRADA, with a goal of developing gram-negative, dual-target antibacterial agents that show no cross-resistance to existing drugs. Trius was awarded Contract HHSN272200800042C to accomplish the goals in the proposal, with LLNL as a major subcontractor.

The technical objectives of this CRADA project were to develop a new therapeutic entity with activity against gram-negative category A and B biothreat agents. The therapeutic would be a small molecule that could be administered intravenously (i.v.), preferably once- or twice-a-day

and would be available for use both as a prophylactic and as a therapeutic against a wide range of biodefense pathogens. These included the gram-negative biodefense pathogens: *Escherichia coli, Salmonella (enterica* and Typhi), *Shigella dysenteriae, Yersinia pestis, Burkholderia pseudomallei, Campylobacter jejuni* and *Francisella tularensis,* as well as potentially the Category A gram-positive pathogen *Bacillus anthracis.* This new therapeutic will act through inhibition of bacterial DNA topoisomerase IV and DNA gyrase (ParE and GyrB, respectively).

Deliverable 1 was completed by both Trius and LLNL.
Trius provided crystal structures of GyrB and ParE with bound candidate structures. LLNL built compound libraries, ran calculations and simulations on the compounds and protein structures, developed models for virtual screening and provided all the data to Trius. Trius provided new chemical entities. LLNL designed panels of diverse *Bacillus anthracis, Yersinia pestis, Burkholderia pseudomallei, Burkholderia mallei,* and *Francisella tularensis* isolates for minimum inhibitory concentrations (MIC) testing. LLNL performed preliminary MIC testing of initial compounds. Trius completed animal use protocols and demonstrated efficacy in a septicemic model.

Deliverable 2 was completed by both Trius and LLNL.
Trius completed the complete toxicity testing on compounds from Stage 1. LLNL completed dose proportionality, microdose pharmacokinetics, and tissue distribution studies on TR-1491 in Sprague-Dawley rats. LLNL completed MIC testing on panels of diverse *Bacillus anthracis, Yersinia pestis, Burkholderia pseudomallei, Burkholderia mallei,* and *Francisella tularensis* isolates.

Deliverable 3 was modified and completed by both Trius and LLNL.
Trius synthesized and formulated promising lead candidates. Truis filed and eIND to test a compound in humans using microdosing. Trius performed toxicity studies and evaluated the data from animals. Trius completed the efficacy report. LLNL did not complete the microdosing study in healthy human volunteers because NIH, the sponsor, deemed it unnecessary to perform the human microdosing study because the compound seemed to scale from rats to dogs. Because of the change is tasks as requested by the sponsor, LLNL effort ended early and thus this CRADA was terminated on December 1, 2012.

Deliverable 4 was partially completed by Trius.
Trius identified biomarkers for toxicity. Trius filed for a Phase I clinical study and is waiting to perform the study.

The overall NIH project is on time.

C. Benefit to Industry

In the process of developing new antibiotics, new advanced technologies were used. This CRADA demonstrated the successful use of high performance computing and accelerator mass spectroscopy in a drug design and development effort. These new technologies will benefit industry by showing a reduction in time to clinical trial 1 and, hopefully, a successful new drug entity. With the reduction in time to successful clinical trial, a new paradigm to drug

development will have been demonstrated. This new paradigm should have beneficial impact on the drug industry.

D. Benefit to DOE/LLNL

At present, no broad spectrum antimicrobial exists that are proven for use against such biological weapon agents. This CRADA will result in new antimicrobials that will protect the public against Bacterial Category A and B agents that could be used by terrorists. Thus, the development of the new antibiotic is a national security need. Benefits to DOE include the use of DOE technologies to speed up drug development. This effort shows that DOE is at the forefront of advancing new technologies for use in therapeutic design and development to better human health. This activity is funded by the U.S. Government through the National Institutes of Health, and the resulting product is slated to be incorporated into the National Strategic Stockpile funded under BARDA.

E. Project Dates

March 17, 2009 to December 1, 2012