Agreement No.: HR0011-17-2-0069
PRs: HR0011730376 & HR0011730567
Effective Date: September 26, 2017
DARPA's CFDA Number: 12.910
DODAAC: HR0011

Issued by: Defense Advanced Research Projects Agency (DARPA)
Contracts Management Office (CMO)
675 North Randolph Street
Arlington, VA 22203-2114
Attn: D. Peter Donaghue, Agreements Officer
Desmond.Donaghue@darpa.mil, 703-526-2705

Recipient: Duke University
Office of Research Administration
220 West Main Street, Suite 820
Durham, NC 27705
Attn.: (b)(6) @mc.duke.edu

Recipient Identification Numbers/Codes:

DUNS: 044387793
CAGE: 4B478
TIN: 56-0532129

Cooperative Agreement Amount: $12,833,999.00

Accounting and Appropriation Data:

SUBCLIN 000101 ACRN AA

FUND CITE	AMOUNT
012199 097 0400 000 N 20162017 D 1320 BPPPTT 2016.BT-01.CORE.A DARPA 255	$1,491,338.00

SUBCLIN 000102 ACRN AB

FUND CITE	
012199 097 0400 000 N 20172018 D 1320 BPPPTT 2017.BT-01.CORE.A DARPA 255	$1,500,000.00
Total Funding	$2,991,338.00

Payment Office Information:

Defense Financial Accounting Services IN VP DAI DARPA
8899 East 56th Street
Indianapolis, IN 46249-0002
DODAAC: HQ0685

[AWARD SUBJECT TO ELECTRONIC FUNDS TRANSFER (EFT) REQUIREMENT]

Authority: This Agreement is issued pursuant to the authority of 10 U.S.C. § 2358, as amended.

COOPERATIVE AGREEMENT SCHEDULE

1. Purpose: The purpose of this Cooperative Agreement is to fund research to the Awardee to carry out a public purpose of support or stimulation of the Defense Advanced Research Projects Agency (DARPA) Biological Technologies Office (BTO), Pandemic Prevention Platform (P3) Program. This effort shall be carried out generally as set forth in Exhibit B, Research Description Document (RDD), which has been based on the Awardee's proposal "Duke DARPA Pandemic Prevention Program", dated July 28, 2017, copies of which are in the possession of both parties.

2. Term: The term of this Agreement commences on September 26, 2017 and continues for 30 months, through March 26, 2020.

The Awardee shall make all requests for no-cost period of performance extensions, in writing, to the Agreements Officer (AO) and Administrative Agreements Officer (AAO), no later than 30 days prior to the end of the current period of performance. The AO and AAO are authorized to grant such request, via modification to the Cooperative Agreement, after receiving approval from the Agreement Officer's Representative and DARPA Program Manager.

3. Terms and Conditions: This Agreement is subject to the terms and conditions set forth in the attached Exhibit A, entitled "DARPA Agency Specific Terms And Conditions," dated September 2017 and to any special terms and conditions in this Agreement Schedule.

4. Agreement Officer's Representative: The Agreement Officer's Representative (AOR) representing the Government under this Agreement is:

Space and Naval Warfare Center
ATTN.: Patrick Sims
53560 Hull Street, San Diego, CA 92152
E-mail: pcsims@spawar.navy.mil; Phone: 619-553-0828

5. DARPA Program Manager: The DARPA Program Manager (PM) representing the Government under this Agreement is:

Defense Advanced Research Projects Agency
Biological Technologies Office (BTO)
ATTN.: Colonel Matt Hepburn
675 North Randolph Street, Arlington, VA 22203-2114
Email: Matt.Hepburn@darpa.mil

6. Administrative Agreements Office: The Administrative Agreement Office (AAO) for this Grant is:

Office of Naval Research, Atlanta Office
100 Alabama Street, SW, Suite 4R15
Atlanta, GA 30303-3104
Email: ONR_ATLANTA@NAVY.MIL
Phone: 404-562-1600
DoDAAC: N66020

7. (b)(6) shall be responsible for this effort.

Duke University
Attn.: (b)(6)
909 S. La Salle Street
Durham, NC 27710
Email: (b)(6)@duke.edu, Phone: (b)(6)

The Awardee agrees to notify the AAO before changing the Principal Investigator.

8. Agreement Funding: This Agreement is incrementally funded in the amount of $2,991,338. The Government's obligation to make payments to the Awardee is limited to only those funds obligated by this Agreement or by modification to this Agreement. Subject to availability of funds and continued satisfactory progress on the Agreement as determined by the Government, the Government agrees to provide funding according to the following schedule:

FY16 Commit $1,491,338.00 (HR0011730567 – Base Period)
FY17 Commit: $1,500,000.00 (HR0011730376 – Base Period)
FY18 Planned: $3,173,723.00 (TBD – Base Period)
FY19 Planned: $5,003,544 (TBD - Base Period)
FY20 Planned: $1,665,394.00 (TBD - Base Period)
Total: $12,833,999.00

The Awardee shall notify the AO and AAO in writing promptly whenever the total Agreement amount is expected to exceed the needs of the Awardee for the project period by more than 5%. This notification shall not be required if an application for additional funding is submitted for a continuation award.

9. Payments: Payments will be made to the Awardee on a reimbursement basis and shall be made by electronic funds transfer in accordance with the account information provided by the Contractor and set forth in Exhibit A, provision 3) Payments.

10. Substantial Involvement: Substantial involvement is expected between the U. S. Government and the Awardee when carrying out the activity contemplated in this Agreement. It will include the U. S. Government's (a) sharing responsibility for the management, control, direction, or performance of the project, and (b) retaining the right to intervene in the conduct or performance of the project. The substantial involvement will include the U.S. Government's direction of activities to develop the research protocols necessary to complete the work and the approval of analysis mechanisms.

FOR DUKE UNIVERSITY

(b)(6)

(Signature)

(b)(6)

(Name, Title)

9/25/2017
(Date)

FOR THE UNITED STATES OF AMERICA, DEFENSE ADVANCED RESEARCH PROJECTS AGENCY

DONAGHUE.DESMOND.P. (b)(6) Digitally signed by DONAGHUE.DESMOND.P. (b)(6) Date: 2017.09.26 08:33:09 -04'00'

D. Peter Donaghue
Agreements Officer
Contracts Management Office

09/26/2017
(Date)

EXHIBIT A
SEPTEMBER, 2017
DARPA AGENCY SPECIFIC TERMS AND CONDITIONS

This award is subject to the DoD Research and Development (R&D) general terms and conditions, which can be found at http://www.onr.navy.mil/Contracts-Grants/submit-proposal/grants-proposal/grants-terms-conditions.aspx under the header "DoD Research and Development General Terms and Conditions," dated September, 2017 and are incorporated herein. The DARPA Agency Specific Terms and Conditions supplement the DoD Research and Development general terms and conditions. This document addresses agency-specific concerns in addition to the above referenced regulations. Award recipients (hereafter, recipient) are accountable for all applicable statutory and regulatory requirements that govern these awards, even if not specifically listed in this document or documents referenced herein.

ORDER OF PRECEDENCE

Any inconsistencies in the requirements of this award shall be resolved in the following order:

- Federal statutes
- Federal regulations
- 2 CFR part 200, as modified and supplemented by DoD's interim implementation found in 2 CFR part 1103
- Award-specific terms and conditions (DARPA Agency Specific terms and conditions)
- DoD Research and Development general terms andconditions

In case of disagreement with any requirements of this award, the recipient shall contact the Agreements Officer listed in the award document in order to resolve the issue. The recipient shall not assess any costs to the award or accept any payments until the issue is resolved.

1. Research Responsibility
2. Amendment of Award
3. Payments
4. Prior Approvals
5. Reports and Reports Distribution
6. Public Release or Dissemination ofInformation
7. Acknowledgment of Sponsorship
8. Intellectual Property Matters
9. Activities Abroad
10. Security
11. Research Involving Recombinant DNA Molecules
12. Restrictions on Printing
13. Prohibition on Awarding to Entities that Require Certain Internal Confidentiality Agreements
14. Animal Welfare

1) **Research Responsibility**:

a) The recipient has full responsibility for the conduct of the research activity supported by this award, in accordance with the recipient's proposal, and the terms and conditions specified in this award. Recipients are encouraged to suggest or propose to discontinue or modify unpromising lines of investigation or to explore interesting leads which may appear during the development of the research. However, they must consult the Agreement Officer's Representative (AOR) through the Administrative Agreements Officer (AAO) before significantly deviating from the objectives or overall program of the research originally proposed.

b) The recipient shall immediately notify the Agreements Officer of developments that have a significant impact on the award-supported activities. Also, notification shall be given in the case of problems, delays, or adverse conditions which materially impair the ability to meet the objectives of the award. This notification shall include a statement of the action taken or contemplated, and any assistance needed to resolve the situation.

2) **Amendment of Award:** The only method by which this award can be amended is by a formal, written amendment signed by either the Agreements Officer or the AAO. No other communications, whether oral or in writing, shall modify this award.

3) **Payments**:

a) Submitting Payments Through Wide Area Work Flow (WAWF): All payments shall be made by funds transfers to the bank account registered in the System for Award Management (SAM), http://www.sam.gov. The recipient agrees to maintain its registration in SAM, including information necessary to facilitate payment via Electronic Funds Transfer (EFT). Should a change in registry or other incident necessitate the payment to an account other than that maintained in SAM, it is the recipient's responsibility to notify the AGO/AAO and obtain a modification to this award reflecting the change. The Government shall not be held responsible for any misdirection or loss of payment which occurs as the result of a recipient's failure to maintain correct/current EFT information within its SAM registration.

1) Any request for advance payments must be approved by the AGO/AAO at the Administrative Office designated in the award document.

2) Wide Area Work Flow (WAWF) has been designated as the Department of Defense standard for electronic invoicing and payment. Electronic submission of payment requests requires the recipient to register in WAWF and have the appropriate CAGE code activated. The recipient's SAM Electronic Business Point of Contact (EBPOC) is responsible for activating the CAGE code in WAWF by calling 1-866-618-5988. Once the recipient's CAGE Code is activated, the SAM EBPOC will self-register in WAWF (https://wawf.eb.mil) and follow the instructions for a group administrator. The ONR Regional Offices will assist in this process. The ONR Regional Office is listed as the Administrative Office in the award document. Please call the ONR Regional Office with any questions regarding access to or use of WAWF.

4) Prior Approvals:

In addition to the prior approvals required by the DoD R&D general terms and conditions, prior written approval is required for the following actions:

- The subaward, transfer, or contracting out of any work under this award, unless described in the recipient's proposal and specifically approved and funded in the Award Schedule. The recipient's request for approval shall include the following supporting data:
  (i) Basis for contractor selection;
  (ii) Justification for lack of competition when competitive bids or offers are not obtained;
  (iii) Basis for award cost or price, to include price or cost analysis performed by the recipient; and
  (iv) Approval of the GOR/AOR.

5) **Reports and Reports Distribution**: Reports shall be furnished as specified below:

a) Report Types.

1) *Quarterly R&D Status Report* - This report, due 30 days after the reporting period, shall keep the Government informed of recipient activity and progress toward accomplishment of award objectives and advancement in state-of-the-art on the research and development involved.

2) *Special Technical Report* - This report, due as required, shall document the results of a significant task, test, event or symposium.

3) *Final Technical Report* - This report, due 90 days after expiration or termination of the award, shall document the results of the complete effort. It shall contain brief information on each of the following:

a) A comparison of actual accomplishments with the goals and objectives established for the award, the findings of the investigator, or both.

b) Reasons why established goals were not met, if appropriate.

c) Other pertinent information.

4) *Final Financial Status Report* - This report, due 90 days after completion of the award, shall be submitted on a Standard Form 425 "Federal Financial Report (FFR)". The report shall be on a cash or accrual basis, depending on how the recipient's accounting records are normally kept.

5) *Report of Federal Cash Transactions [applicable only to advance payment awards]* – This report, due 15 days following the end of each quarter, shall be submitted on a Standard Form 425. The recipient shall provide forecasts of Federal cash requirements in the "Remarks" section of the report.

b) Report Distribution:

Addresses	Report Types [7.(a)]	Number of Copies
Agreement Officer's Representative	1, 2, 3, 4, 5	2
DARPA Program Manager	1, 2, 3, 4, 5	1
Administrative Agreements Officer	3, 4, 5	2
DARPA/Research Services	3	1
Defense Technical Information Center ATTN: DTIC-O 8725 John J. Kingman Road Ft. Belvoir, VA 22060-6218	3	2
DARPA Agreements Officer	3, 4, 5	1

6) **Public Release or Dissemination of Information**

a) At this time, DARPA expects the work performed under this award to be fundamental research, and it is, therefore, not subject to publication restrictions. Papers resulting from unclassified contracted fundamental research are exempt from prepublication controls and requirements, pursuant to DoD Instruction 5230.27 dated October 6, 1987.

b) All papers resulting from this award will include the following distribution statement:

"Approved for public release; distribution is unlimited."

c) Should the character of the research change during award performance so that the research is no longer considered fundamental, the award will be modified to impose the restrictions on public release and dissemination of information that apply to those research efforts that are not considered fundamental research.

7) **Acknowledgment of Sponsorship:**

a) The recipient agrees that in the release of information relating to this award, such release shall include a statement to the effect that (1) the project or effort depicted was or is sponsored by the Defense Advanced Research Projects Agency, (2) the content of the information does not necessarily reflect the position or the policy of the Government, and (3) no official endorsement should be inferred.

b) For the purpose of this article, information includes news releases, articles, manuscripts, brochures, advertisements, still and motion pictures, speeches, trade association proceedings, symposia, etc.

c) Nothing in the foregoing shall affect compliance with the requirements of the clause entitled "Security."

8) **Intellectual Property Matters**: Questions regarding intellectual property matters should be referred to Agreements Officer.

All patent reports (interim and final) shall be submitted using the i-Edison.gov reporting website (http://s-edison.info.nih.gov/iEdison). In the event the recipient is unable to submit reports through i-Edison, the recipient may utilize DD Form 882, Report of Inventions and Subcontracts, for submission of interim and final invention reports. The DD Form 882 and all invention disclosures shall be submitted to the AGO/AAO for proper disposition and forwarding to the
Agreements Officer.

9) **Activities Abroad:** The recipient shall assure that project activities carried on outside the United States are coordinated as necessary with appropriate Government authorities and that appropriate licenses, permits, or approvals are obtained prior to undertaking proposed activities. The awarding agency does not assume responsibility for recipient compliance with the laws and regulations of the country in which the activities are to be conducted.

10) **Security:** The recipient may not be granted access to classified information under this award. If security restrictions should happen to apply to certain aspects of the proposed research, the recipient will be so informed. In the event that the scientific work under this award may need classification, or involve access to or storage of any classified data, the Government shall make its decision on the need to classify, or require such access or storage, within 30 days after receipt of written notice from the recipient. If the decision is affirmative, the Government shall invoke the clause in reference to the "Termination" proceedings in the DoD R&D general terms and conditions.

11) **Research Involving Recombinant DNA Molecules**: Any recipient performing research involving recombinant DNA molecules and/or organisms and viruses containing recombinant DNA molecules agrees, by acceptance of this award, to comply with the National Institutes of Health "Guidelines for Research

Involving Recombinant DNA Molecules," July 5, 1994 (59 FR 34496) as amended, or such later revision of those guidelines as may be published in the Federal Register.

12) **Restrictions on Printing**:

Unless otherwise authorized in writing by the AGO/AAO, reports, data, or other written material produced using funds provided by this award and submitted hereunder shall be reproduced only by duplicating processes and shall not exceed 5,000 single page reports or a total of 25,000 pages of a multiple page report. These restrictions do not preclude the writing, editing, and preparation of manuscript or reproducible copy of related illustrative materials if required as a part of this award, or incidental printing such as forms or materials necessary to be used by the recipient to respond to the terms of the award. To satisfy the requirements of the Defense Technical Information Center, at least one copy of each technical report submitted to the Defense Technical Information Center must be black typing or reproduction of black on white paper or suitable for reproduction by photographic techniques. Reprints of published technical articles are not within the scope of this paragraph.

In accordance with Executive Order 12873, dated October 20, 1993, as amended by Executive Order 12995, dated March 25, 1996, the recipient is encouraged to submit paper documents, such as letters or reports, that are printed/copied double-sided on recycled paper that has at least 30 percent postconsumer material.

13) Prohibition on Awarding to Entities that Require Certain Internal Confidentiality Agreements

a) The recipient shall not require employees, contractors, or subrecipients seeking to report fraud, waste, or abuse to sign or comply with internal confidentiality agreements or statements prohibiting or otherwise restricting such employees or contractors from lawfully reporting such waste, fraud, or abuse to a designated investigative or law enforcement representative of a Federal department or agency authorized to receive such information.

b) The recipient must notify its employees, contractors, or subrecipients that the prohibitions and restrictions of any internal confidentiality agreements inconsistent with paragraph (a) of this award provision are no longer in effect.

c) The prohibition in paragraph (a) of this award provision does not contravene requirements applicable to any form issued by a Federal department or agency governing the nondisclosure of classified information.

d) If the Government determines that the recipient is not in compliance with this award provision, it:

1) Will prohibit the recipient's use of any FY 2016 or FY 2015 funds under this award, in accordance with Federal appropriations law; and

2) May pursue other remedies available for the recipient's material failure to comply with award terms and conditions.

14) Animal Welfare

(1) The Contractor shall register its research, development, test, and evaluation or training facility with the Secretary of Agriculture in accordance with 7 U.S.C. 2136 and 9 CFR subpart C, and section 2.30, unless otherwise exempt from this requirement by meeting the conditions in 7 U.S.C. 2136 and 9 CFR parts 1 through 4 for the duration of the activity. The Contractor shall have its proposed animal use approved in accordance with Department of Defense Instruction (DoDI) 3216.01, Use of Animals in DoD Programs, by a DoD Component Headquarters Oversight Office. The Contractor shall furnish evidence of such registration and approval to the Contracting Officer before beginning work under this contract.

(2) The Contractor shall make its animals, and all premises, facilities, vehicles, equipment, and records that support animal care available during business hours and at other times mutually agreeable to the Contractor and the United States Department of Agriculture Office of Animal and Plant Health Inspection Service (USDA/APHIS) representative, personnel representing the DoD component oversight offices, as well as the Contracting Officer, to ascertain that the Contractor is compliant with 7 U.S.C. 2131-2159 and 9 CFR parts 1 through 4.

(b) The Contractor shall acquire animals in accordance with DoDI 3216.01, current at time of award

(http://www.dtic.mil/whs/directives/corres/pdf/321601p.pdf).

(c) The Contractor agrees that the care and use of animals will conform with the pertinent laws of the United States, regulations of the Department of Agriculture, and policies and procedures of the Department of Defense (see 7 U.S.C. 2131 et seq., and 9 CFR subchapter A, parts 1 through 4, DoDI 3216.01, Army Regulation 40-33/ SECNAVINST 3900.38C/AFMAN 40-401(I)/DARPAINST 18/USUHSINST 3203). The Contractor shall also comply with DoDI 1322.24, Medical Readiness Training, if this contract includes acquisition of training.

(d) The Contracting Officer may immediately suspend, in whole or in part, work and further payments under this contract for failure to comply with the requirements of paragraphs (a) through (c) of this clause.

(1) The suspension will stay in effect until the Contractor complies with the requirements.

(2) Failure to complete corrective action within the time specified by the Contracting Officer may result in termination of this contract and, if applicable, removal of the Contractor's name from the approved vendor list for live animals used in medical training.

(e) The Contractor may request registration of its facility by contacting USDA/APHIS/AC, 4700 River Road, Unit 84, Riverdale, MD 20737-1234, or via the APHIS Animal Care website at:http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/animalwelfare.

(f) The Contractor shall include the substance of this clause, including this paragraph (f), in all subcontracts involving research, development, test, and evaluation or training that use live vertebrate animals.

Exhibit B

Duke University
Research Description Document
"Duke DARPA Pandemic Prevention Platform (P3)"

The Contractor shall furnish the necessary personnel, materials, facilities, and other services as may be required to manage and complete the contracted effort in accordance with this Research Description Document (RDD) and the schedule set forth herein. The work and services to be performed hereunder shall be subject to the requirements and standards contained in the above titled Contractor's proposal, copies of which are in the possession of both parties. In the event of an inconsistency between the provisions of this Cooperative Agreement and the Contractor's proposal, the inconsistency shall be resolved by giving precedence in the following order: (1) the attachments to the cooperative agreement, and (2) the Contractor's proposal. The contracted effort is unclassified.

(b)(6) Support for the project may not continue without the active direction of the PI. The PI shall keep the DARPA Program Manager, the Agreements Officer Representative (AOR) and the Agreements Officer informed on contract progress through submission of the reports and other deliverables identified in Exhibit A, DARPA Agency Specific Terms and Conditions (July 2016), Paragraph 5.
Reports and Reports Distribution, as described below.

Quarterly R&D Status Report - The contractor shall provide quarterly progress reports as applicable, to the addressees identified in Cooperative Agreement Exhibit A, paragraph 5. b) Report Distribution. The purpose of these reports, 5-10 pages in length, is to present a summary of work completed by RDD tasking and milestones met; the advancement in the state-of-the-art on the research and development involved; discuss any problems encountered; update the program schedule, present the program financial status; and discuss remaining work, using the template below:

(i) A description of progress during the current quarterly reporting period. Please do not include extensive discussions of previous results.
(ii) A table with the following headings: Task (including sub-tasks); milestone/ deliverable; planned start date; planned completion date; percent completion; status (before-, on-, or behind schedule).
(iii) A summary of all problems or areas of concern.
(iv) Planned activities and milestones for the next reporting period.
(v) A description of any major equipment purchased or constructed during the reporting period.
(vi) Notification of any changes in key personnel associated with the contract during the reporting period.
(vii) A summary of substantive information derived from noteworthy trips, meetings, or conferences held in connection with the contract during the reporting period.
(viii) Related accomplishments (publications, patents, media coverage) since the last quarterly report.

Final Technical Report - The Final Technical Report and Final Financial Status Report shall address the topics set forth below and be delivered in accordance with Cooperative Agreement Exhibit A, paragraph 5. b) Report Distribution.

(i) Task Objectives
(ii) Technical Problems
(iii) General Methodology (i.e., literature review, laboratory experiments, surveys, etc.)

(iv) Technical Results
(v) Important Findings and Conclusions
(vi) Significant Hardware Development
(vii) Special Comments
(viii) Implications for Further Research
(vix) Standard Form 298, August 1998

The Contractor shall also submit a Monthly Financial Status to provide financial program progress and inform the DARPA Program Manager of any potential cost issues. The financial status shall be prepared using the Attachment
(1) template and submitted to (b)(6) @darpa.mil.

Reports and papers shall be marked using Distribution Statement A: "Approved for Public Release." Papers and articles published as a result of DARPA sponsored research shall include a statement reflecting the sponsorship. In addition, a bibliography of the titles and authors of all such papers are to be included in the Final Technical Report. The cover or title page of each of the above reports or publications prepared, will have the following citation:

Sponsored by:
Defense Advanced Research Projects Agency
Biological Technologies Office (BTO) Program: Pandemic Prevention Platform (P3) Issued by DARPA/CMO under Contract No. HR0011-17-2-0069

Travel is authorized for attendance at the Kickoff Meeting and PI Meetings.

Animal use is anticipated in this effort. The contractor shall obtain all necessary Institutional Animal Care and Utilization Committee (IACUC) approval and demonstrate this approval to the Government prior to beginning experimentation with animals. If animal use is no longer anticipated, or changes significantly from the approved IACUC then the PI must submit a letter stating the discontinuation of animal use for this effort and/or receive appropriate authorization for IACUC changes of previously specified protocols. Unless prior approval by DARPA is given, IACUC documentation must be provided prior to contract award. Absolutely no funds can be used for animal research and testing (including, but not limited to, animal purchase, housing and care) until DoD veterinary approval is granted.

1.0 SCOPE

For each Task Area below, we describe the scientific team, the task area approach, and proposed innovations. Innovation and platform development is expected to occur within an initial 24-month developmental phase with much of the development activities focused on the first 12 months and waning thereafter. A single capability demonstration is planned 24-30 months (with Duke-selected pathogen influenza).

Justification of Pathogens (Influenza and Chikungunya virus)

The World Health Organization (WHO) estimates that influenza transmission results in up to 500,000 deaths annually [1]. Furthermore, influenza virus has been estimated to be responsible for an annual economic burden in excess of $87 billion [2]. The current standard of care for influenza prevention is seasonal immunization with a multivalent cocktail of inactivated influenza viruses predicted to be antigenically representative of circulating strains (H1N1, H3N2 and B). The majority of influenza virus vaccine preparations available for human vaccination in the United States are generated via propagation in embryonated eggs using a procedure that has remained virtually unchanged since the mid-1900's. The generation/administration of vaccines in this way **1)** is laborious resulting in a minimum five to six-month lag between virus isolation and vaccine availability [1] **2)** may select for egg-adaptation mutations resulting in a loss of immunogenicity with regard to circulating strains [3], and **3)** requires sufficient time post-immunization for the development of virus-specific antibodies (~2 weeks). Thus, there is a critical need for fast-acting antiviral countermeasures, such as therapeutic antibodies, that can be rapidly isolated, evolved, manufactured, and safely delivered to at risk individuals. As such, naturally occurring infection with seasonal or a weaponized, highly pathogenic, influenza strain remains a significant global challenge to both civilians and the US military.

Chikungunya virus (CHIKV) is an *Aedes spp.* mosquito-vectored RNA virus and the etiological agent of chikungunya fever and the associated polyarthralgia disease. In recent decades, CHIKV has been responsible for millions of human infections in Africa, the Indian Ocean Islands, India, South America, the Caribbean, and Southeast Asia [4, 5, 6, 7, 8]. Genetic adaptation of CHIKV has facilitated efficient transmission of the virus by *Aedes albopictus* mosquitoes [9], which have a wide geographic distribution [10]. Epidemic activity of CHIKV has resulted in the emergence of human cases into the United States and Europe with the potential for subsequent localized transmission [11, 12, 13]. As such, CHIKV remains a significant global public health concern and threat to US troops because of the potential for rapid global expansion due to human/mosquito transmission cycles, coupled with the debilitating nature of infection, potential for long-term sequela, and lack of approved vaccines/antivirals. As with influenza virus, there is a critical need for fast-acting antiviral countermeasures against chikungunya virus that can be rapidly isolated, evolved, manufactured, and delivered to at-risk individuals.

Below, we outline the assumptions of expected samples to initiate the viral propagation (TA1), complete antibody evolution (TA2) and produce a countermeasure (TA3) as part of our proposed platform; we have developed a variety of mitigation strategies to ensure we can use a broad array of starting materials. Within the Project Work Description and Requirements section below, we provide clear tasks/subtasks and deliverables for each Task Area, with timelines for each.

Task Area 1 (TA1): Approach/Innovation for On-demand Platform to Grow Virus

We propose a comprehensive P3 virus propagation platform capable of rapid and broad screening of potential culture systems and subsequent down selection for the generation of large-scale virus stocks to support downstream activities in TA2/3. Platform development and optimization will occur at Duke during the Developmental Phase (0-24 months). We will participate in a capability demonstration with influenza (Base).

Virus Propagation – (Task Area 1.1, 1.2)

A major technical hurdle that must be overcome is the design and implementation of a virus propagation

system with sufficient breadth of susceptibility such thatrescue and high titer growth of a diverse range of human viruses (DNA, RNA, enveloped, non-enveloped, etc.) is reasonably assured. Classically, the selection of culture systems for virus rescue/amplification has been predicated on tropism assumptions and knowledge of the virus group of interest. Arboviruses, for example, are inoculated into suckling mice, as well as mammalian and arthropod *in vitro* culture [48]. Clinical/diagnostic virology laboratories often employ commercially available eukaryotic cells for the isolation and identification of virus in clinical samples [49, 50]. The cells used for viral diagnostics, including primary monkey kidney, human foreskin fibroblast, human embryo fibroblast, rabbit kidney, as well as various cancer/immortalized lines (MDCK, A549, HEp-2, etc.) are chosen because of their broad susceptibility to numerous well-characterized.human viruses [49, 50]. Positive cultures are then identified visually (lethality, cytopathic effect (CPE), etc.) or immunocytochemically (ICC; cross-reactive antibodies). These techniques, though effective, are slow and laborious. Furthermore, although cell types used for diagnostics may be susceptible to infection with numerous viruses, they may or may not be competent for the production of sufficient quantities of virus progeny to support antibody discovery, efficacy, and evolution studies. **We therefore propose to develop in TA1 a "Thaw-and-Infect" eukaryotic cell culture array comprised of cell types/lines competent for the isolation and high titer growth of a variety of known and unknown viral isolates**.

Thaw-and-Infect culture array: We will identify appropriate cell types capable of supporting replication and virus stock propagation for all virus families known to induce significant disease in humans. The identified cell types/lines will be curated from appropriate commercial vendors/national repositories (ATCC, BEI Resources, etc.) and amplified (RBL Virology Unit) to generate in-house cryopreserved seed-lots (25 x 1.0 mL aliquots) in accordance with vendor recommendations. As possible, we will develop/adapt cell lines to a minimum number of standard growth conditions (medium composition, temperature, atmospheric conditions, etc.) to optimize downstream efficiency of culture array setup and viral growth competency screens. SOPs will be developed for seed stock generation, recovery, and propagation as part of the TA1 virus propagation platform.

We anticipate the final virus culture platform array format to consist of a single or multiple multi-well culture plates. For all cell lines, we will identify a seeding density that results in an infection-appropriate confluence in this format and use this information to generate working frozen stocks (50 aliquots/line) of appropriate density that can achieve this confluence within 18-24 h of recovery from cryostasis. The "shelf-life" of working lots of all lines, in terms of percent viability, will be evaluated every 3 months and after cryopreservation. Once we have generated seed and working lots of all relevant cell lines, we will evaluate the ability of the culture array to rescue selected viruses. The focus of effort in the Base agreement will be influenza and CHIKV.

In the event of a public/military health crisis, we recognize that the "virus" could be supplied as: **1)** infectious seed stock of known or unknown sequence/identity, **2)** clinical/surveillance sample from an infected individual, or **3)** viral sequence information without infectious material. However, we envision scenarios 1 and 2 being far more probable (i.e., sponsor-supplied infectious material for subsequent propagation). Therefore, we will evaluate the ability of the Thaw-and-Infect culture array to rescue selected viruses in the following contexts: direct inoculation of infectious seed stock (infectious culture supernatant) and virus "spiked" into an appropriate clinical/surveillance milieu, such as Zika virus in mosquito homogenate or human rotavirus in a fecal suspension.

Because we recognize the potential that during a pandemic outbreak only electronic viral sequence information may be available, we will work with Synthetic Genomics Vaccine, Inc. (b)(6) Task 1.2) to optimize their protocols for the synthesis of error-free viral infectious clone genome for direct transfection Synthesis demonstrations will help to identify areas that can be improved to reduce the time required to synthesize varied virus genomes. Once synthesized, the genomes will be sequence- verified and delivered to Duke for virus rescue. Additionally, we will quantify transfection efficiency of all cell lines in the panel using up to five commercially available transfection reagents and DNA/RNA expressing a standard fluorescent protein.

Using our Thaw-and-Infect cell array platform, we will rapidly identify permissive cell lines and use visual estimation of replication metrics (kinetics of spread and median fluorescence intensity; MFI) to select permissive lines for propagation of large-scale cultures. Briefly, 18-24 h prior to inoculation the cell

array will be rescued from cryostasis, re-fed with appropriate growth medium and maintained at standard growth conditions (1 day). We anticipate the sponsor supplying a minimum of 1.0 mL virus source material, which will be divided evenly for identification and propagation. The virus source material will be uniformly suspended in an appropriate volume of infection medium (~5.0 mL representing a maximum 10-fold dilution), sterile filtered if necessary, and used to directly inoculate our culture array. The inoculum will remain in contact with the cell monolayers for 1-2 h and may involve centrifugation-enhanced inoculation as this has been shown to enhance rescue of some viruses [50, 51, 52, 53]. The inoculum will then be removed, and the cells refreshed with appropriate virus growth medium. Viral replication in the cell culture panel will be imaged every 8-12 h, as described below, using virus sequence-specific Fluorescently-tagged Oligonucleotide Probe (FOPs) hybridization to viral nucleic acid and live cell imaging [54, 55, 56]. Virus seed stocks will be harvested at ~72 h from up to n=5 cell lines based on the results of the kinetics/MFI analysis as supernatants/cell lysates (3 days). In addition, culture supernatants will be sent for NGS.

Virus detection and titration using FOP hybridization: Classic methodologies for the visualization of virus infected cells (CPE or ICC) are slow requiring days to weeks [50] for sufficient replication/antigen expression and the availability of appropriate immunological reagents. We propose to significantly expedite this process by instead directly detecting replicating viral nucleic acids via the hybridization of viral sequence-specific (commercially purchased) FOPs in permeabilized live cultures coupled with fluorescent live single cell imaging using a high-throughput multimodal plate reader in the Duke RBL. During the development phase, and in parallel with our cell culture array curating activities, we will develop/optimize conditions for this detection methodology using our selected viruses (influenza, chikungunya) and appropriately matched cell culture systems, which we will subsequently include during our cell culture panel qualification tests.

Approximately every 8-12 h following inoculation, the virus growth media will be removed and stored in a matched multi-well plate, followed by reversible permeabilization of the cell monolayers using streptolysin O or comparable reagent. To achieve signal specificity and mitigate background, following permeabilization, we will incubate cultures with tagged oligo-nucleotide pairs capable of fluorescence resonance energy transfer similar as to previously described [54, 55, 56]. We anticipate evaluating n=3-5 probe pairs per virus to determine if there is a preferable target(s) based on virus-specific replication parameters/kinetics. Following completion of this live cell imaging, the monolayers will be washed, virus growth medium returned, and culture incubation continued. Although it may be necessary to incubate propagation cultures for multiple days to obtain peak titers, we anticipate the ability to detect viral nucleic acid using FOPs by 24-48 h post-infection. Once this methodology is fully optimized, we anticipate the ability to rapidly quantify titer (tissue culture infectious dose 50%; TCID50 or focus forming assay; FFA) of in-house generated stocks using readily available commercial reagents (**2 days**).

Culture scale-up: We will aim to generate virus working stocks of 500 mL per lot at a titer of $\geq 10^6$ FFU/mL. In our experience, stocks of this volume/titer are sufficient to support antibody discovery assays **1)** Plaque Reduction Neutralization Test 90% (PRNT90) input range: 2000-6000 FFU/mL and **2)** Whole virus capture ELISA input range ~10^6 FFU/mL. We will compare 72 h titers of the top 5 first round cultures down select three candidate cell lines for the generation of large scale working stocks. To decrease the temporal lag between seed lot generation/titration and working lot amplification, we will rescue larger cultures of all five preliminary cell lines from cryostasis while titration plates are incubating/developing. However, only the three "best" lines (based on kinetics of spread, MFI, and titer) will be inoculated to generate the large working lots.

Eukaryotic cell culture expansion is the major time-limiting step in large-scale virus propagation. It is standard practice (RBL Virology Unit) to cryopreserve eukaryotic cells at a density between $1x10^6$ and $2x10^6$ cells/mL. Starting with this cell density, we estimate it would take 7-8 days of *in vitro* culture expansion to reach a sufficient cell count ($\geq 1x10^8$) to seed a multi-layer flask (~1,720 cm^2) capable of supporting the growth of a ~500 mL culture. Therefore, to reduce this lag time, we propose to identify conditions for the generation of high density frozen cell stocks (~10^8 cells/mL) similar to as previously

described [57]. For all lines included in the Thaw-and-Infect array, cells will be frozen at various densities (up to 10^8 cells/mL) and the effects of freezing density on viability upon recovery following 7-14 days of cryostasis will be evaluated. If necessary, we will optimize the composition of the freezing medium to maximize the viability of high density cell stocks. Working lots (25 x 1.0 mL aliquots) will be generated for all cell lines at a density that can support virus propagation (up to 500 mL in volume) 24-48 h after sample acquisition, or at the maximal density that does not have a negative impact on post-thaw viability. We will also evaluate the "shelf-life" of working lots of all lines in terms of percent viability every 3 months after cryopreservation.

Prior to inoculation, high density cultures will be rescued from cryostasis, re-fed with appropriate growth medium and maintained at standard growth conditions (1-2 days). The respective virus seed stocks will be uniformly suspended in an appropriate volume of infection medium, sterile filtered if necessary, and used to directly inoculate large cultures. Following an appropriate infection time (1-2 h), the inoculum will then be removed and the cells refreshed with appropriate virus growth medium, and the cultures maintained under standard conditions. Virus yield in large cultures will be monitored every 8-12 hours via rapidly developed quantitative real-time PCR. Cultures will be harvested upon reaching a copy number of $\geq 10^{10}$ copies/mL (~10^6 infectious units/mL, assuming a 10,000:1 genome to infectious unit ratio) or when less than a 10-fold increase is observed in three subsequent measurements (3-5 days). Working lots of virus will be harvested as cell culture supernatant/lysate and quantified via TCID50 or FFA with FOP detection. Additionally, the genetic diversity/drift of working virus lots relative to source material (or earliest passage for which sufficient material is available to support this analysis) will be evaluated by NGS.

This platform strategy will result in generation of a working lot of virus sufficient to begin antibody identification and evolution (TA2) and can be used for further amplification/scale-up as necessary in 12-18 days. This will be achieved as follows:

- NGS identification of sponsor provided source material and synthesis of FOPs, 2-4 days
- Primary down-selection of n=5 virus culture systems and generation of n=5 putative virus seed stocks (passage +1 stocks), 3 days
- Infectious unit quantification of virus seed lots and secondary down-selection of n=3 culture systems for working lot amplification, 2 days
- Virus working lot propagation (passage +2 stocks), 500 mL culture volume and titer $\geq 10^6$ infectious units/mL, 3-7 days
- Infectious unit quantification of virus working lots (n=3 culture systems), 2 days

TA1 Analytics: To show progeny viruses are bio-identical to original isolate (or earliest passage for which sufficient material is available to support this analysis), Duke will utilize proteomic analyses to characterize virus isolates and progeny viruses used for subsequent countermeasure development. We have established methods in the DHVI/CGMP Analytics group to evaluate virus protein profiles from samples including SDS-PAGE, western blot and RP-UPLC to resolve and detect individual virus proteins that can be compared between stocks [58]. Furthermore, when paired with mass spectrometry, these methods can verify protein identity, sequence, and detect glycosylation and other protein modifications [59, 60]. The Duke proteomics facility (https://genome.duke.edu/cores-and-services/proteomics-and-metabolomics), led by (b)(6) is equipped to support this analysis, as required. Similarly, virus stocks can be evaluated using LC-MS methods as a reliable and reproducible way to identify and quantify their lipid composition [61]. Data analysis will be supported by the DHVI/DARPA P3 statistics team as needed.

Innovation: Our approaches for virus amplification and quantification are built upon proven classic methodologies. However, we believe the combination of rapid quasi-real-time imaging of replicating viral nucleic acid combined with a culture array comprised of representative cell lines capable of supporting replication of all known human virus families will greatly expedite virus replication/quantification. Specifically, this virus growth platform will **1)** rapidly identify and down-select putative culture systems, **2)** reduce incubation times for virus quantification assays, **3)** reduce the lag time between seed and working stock propagation, **4)** enable quick/accurate multiplicity of infection calculation for optimal

growth in subsequent virus preps, and **5)** facilitate rapid turnaround neutralization assays for the support of TA2/TA3 activities.

Task Area 2 (TA2): Approach/Innovation for System to Isolate/Evolve Antibodies

The Duke DARPA P3 TA2 platform assumes two possible sample input scenarios based on real-world pandemic experiences (convalescent PBMC/plasma from an infected human or viral isolate/clinical specimen). Our current and innovative platform approaches to antibody isolation, evolution, screening and candidate selection, accommodating both pandemic sample types, are detailed below (**Figure 1**).

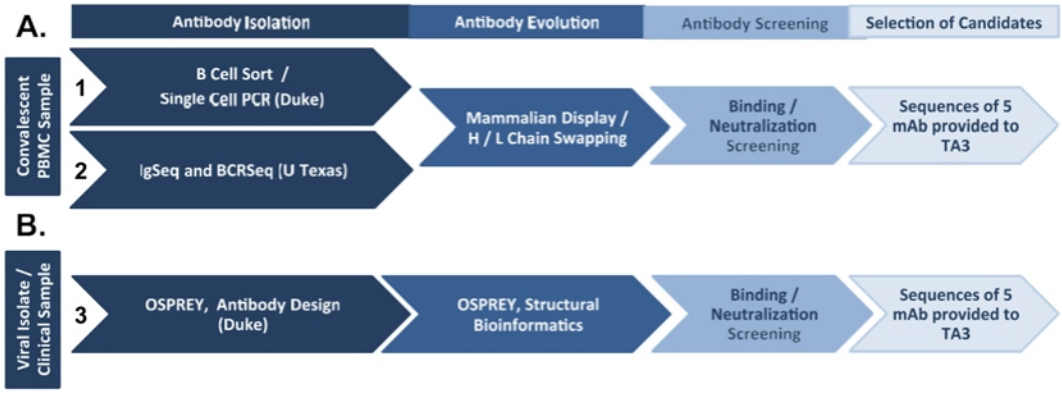

Figure 1. TA2 antibody isolation and evolution platform development approaches. **A**) Tandem approaches if DARPA provides peripheral blood sample. **B)** Optional approach if DARPA only provides viral isolate or virus containing clinical specimen (e.g., sputum, feces).

This schema represents the core existing technologies that the Duke DARPA P3 TA2 team has in place and is currently operational (total 45 days). During the 24-month development phase, this TA2 workflow will be used to develop five candidate neutralizing mAbs against selected viruses (flu and CHKV) described in TA1. With our established and innovative new approaches, we believe we can reduce the time to produce highly potent antibodies/countermeasures to <25 days (**Figure 1**). Methods will be developed in tandem during the 24-month developmental phase and applied as appropriate to meet the TA2 metrics.

Convalescent PBMC sample (Task 2.1-2.2)

If a peripheral blood sample from an infected/convalescent individual is available, then we propose two simultaneous complementary approaches. The (b)(6) [62, 63, 64, 65, 66], (b)(6) [67] and (b)(6) laboratories [68, 69, 70, 71] have developed a suite of technologies that currently enable the identification and affinity optimization/evolution of neutralizing antibodies from memory B cells and plasma cells [62, 63, 64, 65] or from serum/plasma [66, 68, 69]. Together these technology platforms/approaches synergize to provide unprecedented ability to rapidly isolate human antibodies and provide innovation opportunities to shorten the time of antibody isolation and countermeasure delivery.

Approach 1 - Isolation. Pathogen-specific Memory B or Plasma Cells (b)(6) Duke): During the 24-month developmental phase, fluorophore-labeled whole virions (provided by TA1 team), or other recombinant protein/VLP hooks will be used to sort influenza and chikungunya, specific memory B cells for antibody isolation [72, 73, 74]. In addition, plasma cells will be sorted as single cells [63]. The VH and VL genes of both single pathogen-specific memory B cells or single plasma cells will be amplified by PCR, and the full Abs will be produced by transient transfections using overlapping PCR [64]. Bulk plasma cell sorting will also allow us to eliminate the need for use of fluorophore-labeled virions for sorting.

Innovation: Currently, memory B or plasma cell sorting and pathogen-specific Ab isolation, specificity identification and Ab production takes 17 days. We propose to innovate to decrease this time to 12 days. Within the first six months we plan to streamline our existing RT-PCR process by combining PCR

primers used in the nested PCR process. This will reduce the number of PCR plates in our first and second round of PCR. Furthermore, the streamlined process will reduce the number of plates that need to be sequenced. Reductions in PCR and sequencing will speed up isolation of candidate countermeasure antibodies.

Approach 1 - Evolution. Mammalian Display and H/L Chain Swapping (b)(6) *Duke and* (b)(6) *U Texas):* Once isolated, candidate countermeasure antibodies will be evolved in one of two ways that will be probed during the 24 month developmental phase for optimal methods: 1a) mammalian display performed by (b)(6) [67] and 1b) VH and VL swapping (b)(6) [66].

The (b)(6) lab has established an Ab evolution scheme that will be integrated with the Ab isolation approach above for identification of pathogen neutralizing antibodies. If the antibody originates from single B cell cloning, then the VH and VL will be cloned into the single chain variable fragment (scFv) expression vectors (3 days). To evolve antibody fragments to be higher affinity, we will employ random and CDR-targeted mutagenesis of the scFv. This is achieved with error-prone PCR (0.5 day). The mutated gene or gene fragment is then inserted into an expression vector and transformed into bacteria. The mutated DNA is prepared and transfected into multiple liters of 293i cells for cell surface expression [67]. The cells with the highest binding to target virions (TA1) will be FACS sorted under BSL2/3 conditions in the Duke RBL.

To determine improvements in binding/function the scFv of the originally identified Ab will be displayed on the 293i cell surface to determine baseline binding, and then gates will be set to sort cells that have an MFI higher than the original Ab. The sorted cells will be cultured for two days and then treated with HRV-3C to cleave the scFv from the cell surface. Culture supernatant will be removed from the well, cleared of cells, and virus neutralization determined in binding/neutralization screens for candidate countermeasure mAb down-selection (see, *Common Screening/Candidate Selection*).

Innovation: Currently, antibody heavy and light chain sequences are isolated by RT-PCR and then synthesized and ligated into mammalian expression vectors. To increase the throughput and speed of transitioning from the isolation of Ab to evolution of Ab, we will develop a synthesis-free transition process into the cell display system. The synthesis-free transition process will utilized overlapping PCR to link the VH and VL to create a scFv. Once evolved and improved neutralizing antibodies are identified (5 days), the full-length antibody VH/VL chain genes will be synthesized and transferred to the TA3 team for pre-production analysis and ultimately production to support animal/human in vivo studies.

(b)(6) have recently improved potency for a broadly neutralizing HIV antibody by a log using VH and VL swaps [66]. They performed VH/VL chain swaps by transfecting various combinations of heavy and light chain plasmids into 293T cells and then assayed the cell culture supernatant in *in vitro* neutralization assays. This can be performed on the scale of 200-400 antibody transfections in 5 days. The wild-type light and heavy chain pairs will be isolated by RT-PCR from pathogen-specific B cells. Additionally, plasma-circulating antibodies will be affinity purified and protein sequenced as detailed below under Approach 2-isolation.

Innovation: Previous experiments have utilized plasmids for transfections of 293T cells to generate antibodies. Here we will optimize antibody expression from linear expression cassettes so that combinations of immunoglobulin chains can be transfected without gene synthesis or molecular cloning. Additionally, we will optimize antibody production by transfecting Freestyle293 or Expi293 cell lines that have been engineered to express high concentrations of recombinant antibody. The increase in antibody expression will allow a reduction in culture size thereby increasing throughput. We will optimize the overlapping PCR and transfection procedure to obtain high antibody purity from the small cultures since transfection reagents and PCR reagents can be toxic to cells in the downstream assays.

Approach 2 - Isolation. Serum antibody sequencing and recombinant Ab expression (b)(6) *U Texas):* Sera from individuals that have recovered from infectious diseases represent a powerful starting point for the discovery of potent neutralizing antibodies. (b)(6) has invented a suite of technologies (termed IgSeq, BCR-Seq) that now enable the rapid identification and optimization of neutralizing antibodies from plasma of humans or animals. Serum/plasma antibodies are produced at very large amounts and at high rates by long-lived plasma cells. This is important because antibodies in serum have undergone selection

for high expression and folding in mammalian cells and minimal aggregation— all key traits needed for antibodies to be produced in TA3.

Ig-Seq, is a proteomics-based technology for determining the serological repertoire to a specified antigen. Ig-Seq capitalizes on very high-mass resolution LC-MS/MS proteomics of serum antibodies that have been affinity-purified with immobilized virus capsid. MS spectra are interpreted using a patient-specific database of paired VH:VL sequences derived from peripheral B cells, obtained from the BCR-Seq workflow. The BCR-Seq workflow enables the determination of the natively paired VH:VL repertoire from up to $5x10^6$ B cells per one day experiment, with a validated accuracy >97% [68, 75]. The (b)(6) lab has: 1) isolated from serum the broadest HIV-1 neutralizing antibody (with (b)(6) reported to date [66]; 2) identified from serum human Norovirus (NoV) antibodies that block NoV infection (b)(6) et al. in preparation) 3) discovered a new class of antibodies to influenza that confer broad protection [76]; and 4) isolated potent neutralizing antibodies to polio. Currently, the (b)(6) technology collectively takes 30-40 days. We will work to reduce this approach to 15 days during the development phase.

Innovation: To achieve the delivery of validated neutralizing antibody sequences within 15 days as discussed above, it will be necessary to optimize the entire Ig-Seq and BCR-Seq experimental pipelines and implement a number of technical advances which cannot be described in detail here due to space limitations. Very briefly: 1) Activated memory B cells (ABCs) and plasmablasts (PBs) circulating in peripheral blood will be sorted by FACS and native VH:VL pairs will be generated using BCR-Seq using an optimized system that relies on the use of an engineered highly processive RT-DNA polymerase [days 1–4]; 2) Next-Gen sequencing of VH:VL library (for Ig-Seq database) using Ion Torrent or MiSeq platforms will be performed [days 4-6]. In parallel we will perform 3) Ig-Seq bioinformatic identification of virus-specific IgG plasma antibodies using affinity chromatography coupled with mass spectrometry and VH:VL sequence database [days 1–10]. Thus total time for Ab identification will be reduced to 10 days. Subsequently, for antibody validation 4) the VH:VL amplicons from step 2) above will be cloned in a specialized vector for expression of secreted Fabs from *S. cerevisae* [day 11]. 5) *S. cereviase* strains engineered for high level expression of secreted antibody fragments will be transformed and cultured to enable production of 200ug of Fab per culture as needed for testing for neutralization [days 12-15).

Task Area 3 (TA3): Approach/Innovation for Countermeasure Delivery – (Tasks 3.1-3.5)

Messenger RNA (mRNA) is a promising new therapeutic delivery platform. While its application to therapeutic targets including infectious diseases is still in its infancy, work by our team and others has shown that it is potentially transformative [82, 83]. We have established an mRNA platform that has the combined benefits of potent delivery, safety and straightforward, rapid production suitable to deliver medical countermeasures.

We have shown that our nucleoside-modified mRNA is a safe and rapid platform for delivery of therapeutic antibodies [82]. Systemic administration of 1.4 mg/kg of nucleoside-modified mRNA encapsulated in lipid nanoparticles encoding the anti-HIV-1 antibody VRC01 resulted in plasma antibody titers of ~170 µg/mL 24 h post-injection in humanized mice. Protective antibody titers were maintained for >1 week following a single administration and were maintained at ~40 µg/mL with repeat administration for over five weeks. Importantly, treatment with half this dose was sufficient to provide full protection from intravenous HIV-1 challenge, demonstrating it is a viable delivery platform for passive immunotherapy. In total, these results show that the current modified-mRNA platform approach meets pre-determined success criteria (criteria 1, 2, 3 and 4). We expect that appropriate Ab evolution to increase potency (TA2) will significantly reduce dosing requirements. Under the Duke DARPA P3 program, we will improve and apply the mRNA platform for delivery of antibodies as effective medical countermeasures for emerging infectious diseases.

mRNA Platform for Countermeasure Delivery: We are advancing our mRNA platform for a number of clinical applications including therapeutics and vaccines. Scalable GMP processes have been established for mRNA and lipid nanoparticle production in partnership with BioNTech GmbH and Acuitas Therapeutics, respectively. Clinical products using these technology platforms have been safely evaluated in humans (ClinicalTrials.gov identifier: NCT03014089). To facilitate seamless integration into the

overall Duke DARPA P3 program, we will transfer these processes and associated release methods to the DHVI CGMP facility. This facility can produce mRNA countermeasures at the necessary scale (>1,000 doses) and has the required manufacturing, analytical testing, quality, regulatory and clinical expertise to support the program.

The mRNA component of the product is produced *in vitro* from a DNA template (using sequences provided by TA2) through a series of enzymatic reactions utilizing bacterially-expressed enzymes that are commercially available [84]. To initiate the production process, template plasmid DNA produced in *E. coli* is linearized using a restriction enzyme to allow synthesis of runoff transcripts with the desired 3' end.

Next, the mRNA is synthesized with ribonucleotide triphosphates (rNTPs) by T7 DNA-dependent RNA polymerase. Modified nucleoside triphosphates are included in the reaction mixture for incorporation into the mRNA product. The template DNA is then degraded by incubation with DNase. Finally, the mRNA is enzymatically capped by vaccinia capping enzyme, and the cap1 structure is formed by addition of the enzyme 2'-O-methyltransferase. Incorporation of modified nucleosides, such as pseudouridine and 1- methylpseudouridine, and HPLC purification to remove contaminating double-stranded RNA, increases protein translation while rendering the mRNA immunologically silent [85, 86]. CGMP runs up to three-gram scale have been routinely produced and can be scaled-up further, as needed.

Lipid nanoparticles (LNPs) containing an ionizable lipid have proven to be excellent mRNA carriers *in vivo* and are currently in clinical trials for siRNA delivery [87]. Purified mRNA will be formulated into lipid nanoparticles using a self-assembly process in which an aqueous solution of mRNA at pH 4.0 is rapidly mixed with a solution of lipids dissolved in ethanol [88]. This process is conducted using a two pump system to combine the mRNA solution and lipid solution in a controlled reaction using a T-junction connector. The ethanol is then removed from the resulting LNP preparation, and the product is exchanged into final formulation buffer by tangential flow diafiltration. Finally, the LNPs are filtered through a 0.2 μm sterilizing grade filter. This process is readily scalable and has routinely been utilized to produce LNP at the one gram scale.

The production processes for mRNA and LNPs are rapid and straightforward and can be conducted in less than a week, which makes the platform uniquely positioned for rapid response. The rate- limiting steps of the process are the production of sufficient plasmid-DNA template encoding the biologic of interest and safety testing of the product. We will evaluate opportunities to make improvements in both of these areas as the program is developed (see platform improvements below).

Innovation: In order to develop an mRNA-based medical countermeasure with optimal potency, half-life and ease of use in the field; we will evaluate a number of improvements to the platform during the 24-month developmental phase of the project and implement those that support this objective in the capability demonstrations planned throughout the Base. Potential improvements to be evaluated include the following: mRNA modifications such as 5' cap, optimized 5'- and 3'-UTRs and coding sequence, and different lengths of poly(A)-tail, SMART mRNA replicons (Synthetically Modified Alpha Replicon Technology), optimized lipid nanoparticle formulations, improved delivery to utilize less invasive routes of administration, and platform integration improvements. Detailed plans for each improvement area are described below.

Optimize LNP delivery formulation: Our first generation formulation showed that high levels of circulating Ab could be produced with intravenous delivery of mRNA/LNPs [82]. Since a simpler route of administration and more potent formulation is desired, we will evaluate alternative delivery routes including both systemic and inhalation to identify an optimal method that will deliver a simpler and more potent product. Formulations that increase circulating Ab titer (i.e., ≥ 20% of peak titer), and/or increase duration of expression (i.e., titer > 10 ug/mL for 45 days post-administration) though a simpler delivery route, will be advanced for integration into pre-clinical and clinical testing. Our collaborators, Acuitas Therapeutics and Arcturus Therapeutics, continue to optimize LNP formulation to increase both liver translation and for use with alternative routes of delivery [89]. We will select the best formulations that have also passed their toxicity tests by the criteria above.

So far, we have delivered modified mRNA-LNPs by the intradermal (i.d.), intramuscular (i.m.), intravenous (i.v.), intraperitoneal (i.p.) subcutaneous (s.c.), and inhaled (i.n.) routes with very high levels

of protein translation [82, 83, 89]. Based on these data, we intend to move forward with s.c. administration as the primary route of delivery, since it yields high protein expression, is simple, relatively non-invasive with a straightforward regulatory pathway for clinical use. We will examine delivery of modified mRNA-LNP therapeutics using the $FcRn^{-/-}$ hFcRn mouse model and will select the delivery route based on favorable expression profile, ease of administration, and clear path for clinical implementation (see Animal Testing).

Optimize the mRNA ((b)(6) *Duke;* (b)(6) *UPenn;* (b)(6) *SGVI):* The ability of an mRNA to be translated at the highest level (peak and duration) determines the dose of that mRNA required for a therapeutic effect. Increasing inherent translation of mRNA therapeutics reduces the dose required to reach protective Ab concentrations. The optimization of protein translation from mRNA, therefore, becomes an important component. The mRNA encoding each new therapeutic Ab developed needs to be optimized, including the cap structure, 5' and 3' untranslated regions (UTRs), coding sequence conformation, nucleoside modifications, and poly(A) tail length, whose optimums can differ for every coding sequence and the target cell type. Such improvements can increase translation by over 50-fold compared to the wild-type mRNA. As these optimizations are not possible on a 60-day time scale, we propose to perform a formal optimization for the cell types targeted by the delivery route to be used determined above using three existing DHVI human monoclonal antibodies. Our plan is to identify the five best optimizations using these three model mAbs to establish an approach to sequence optimization. Using this platform we can quickly screen/select optimal sequences to deliver countermeasures identified in TA2 for CGMP production.

First, the optimal cap structure (enzymatic, NRcap, "CleanCap (Trilink)", isopropylidene dinucleotide cap analogs, and phosphorothioate caps) and poly(A) tail length will be analyzed for the three model mAb coding sequences using primary cells that translate delivered mRNA for the site of injection followed by *in vivo* delivery. Next, various optimal UTRs and coding sequence optimizations will be analyzed. We have developed a set of plasmids containing a 5' sequence that ensures complete enzymatic capping and 3 different 5' and 2 different 3' UTRs that allows optimization of UTRs for any coding sequence using 6 plasmids. This yields a set of plasmids containing the best UTRs combined with the optimal poly(A) tail length. Two different codon optimizations are cloned into each plasmid and each plasmid is linearized and 3 different mRNAs are made containing Ψ, m1Ψ, or Ψ + m5-cytosine nucleoside modifications. The resulting mRNAs will be used to transfect primary cells that represent the tissues exposed though the selected delivery route (i.e., hepatocytes for IV delivery). A platform including the five best mRNA coding sequences will be defined through testing using model human mAbs. This will allow us to rapidly deploy the best modified mRNA constructs for expression of new human mAbs for delivery as countermeasures once the platform is fully developed.

In addition to improvements in modified mRNA, we will also evaluate whether mRNA replicons can increase peak Ab titer and extend Ab expression *in vivo*. SGVI has developed a self-amplifying RNA vector based on an alphavirus derived from the attenuated TC-83 strain of Venezuelan equine encephalitis virus that can overcome innate immune response shutdown (vector termed SMART: Synthetically Modified Alpha Replicon Technology). Whole body IVIS imaging of mice injected with either SMART or TC-83 replicon RNA expressing luciferase protein revealed that the SMART RNA expressed significantly more luciferase on days 1, 3 and 7 post-injection and remained higher than the TC-83 replicon until day 14. In addition, luciferase was detected at time points out to 28 days post SMART RNA injection demonstrating significant duration of expression.

To select the mRNA improvements to be incorporated into the overall Duke DARPA P3 platform for capability demonstration, we will evaluate mRNA construct designs. Head to head evaluations of the two most potent mRNA designs encoding model mAbs will be first evaluated *in vitro* the relevant human primary cell substrate followed by *in vivo* studies in $FcRn^{-/-}$ hFcRn mice to evaluate pharmacokinetics and in ferrets as a Flu disease challenge model (see Animal Testing, **Table 1**). The selected platform improvements will then be prepared for incorporation into development runs and scaled up for use in capability demonstrations and clinical trial material production.

Platform Integration Improvements ((b)(6) *Duke;* (b)(6) *SGVI):* We will also evaluate platform improvements aimed at seamlessly integrating mRNA production into the overall response and shortening

timelines for delivery. The two areas with the most significant opportunity for improvement are reducing the time from Ab sequence to template DNA and the time for product release. We will work to address these bottlenecks using proven new technologies from SGVI (*in vitro* gene assembly) and others (rapid sterility testing). Details of the approaches are below. Heavy and light chain Ab genes can be engineered into mRNA very rapidly. The process consists of designing overlapping oligos, synthesis of error corrected genes, assembly of the genes into the RNA vector and synthetically amplifying the vector DNA. The amplified DNA can then enter the RNA production process as a critical reagent. Time from Ab gene sequence to template DNA is less than one week; construction of multiple RNA vectors can occur in parallel, so the requirement for more than one construct does not increase time to generate. We will compare performance of synthetically assembled DNA to the traditional plasmid DNA approach in year one to verify resulting mRNAs are of identical quality and potency using the proposed analytical release panel and *in vivo* testing. If selected, the synthetic process would be scaled up to support capability demonstrations and GMP clinical trial lot production.

Animal Testing: Animal studies in support of this program could include a number of animal models including FcRn-/- hFcRn mice [90], IFN-α/βR$^{-/-}$ mice, ferrets, rabbits and non-human primates (NHPs), as well as other appropriate animal models based on the DARPA-provided challenge virus (**Table 1**).

Table 1: Proposed Animal Models for Duke DAPRA P3

Platform Development Phase		
Model	**Purpose**	**Rationale**
FcRn$^{-/-}$ hFcRn Mice	• mRNA comparisons • LNP formulation • Dosing optimization • Route optimization	• Used for pK studies with human IgG • N=5 per group; 35 day study duration • Reduced induction of anti-human IgG responses [91]
Ferret	• Human Influenza challenge studies	• Clinical symptom/disease model of seasonal influenza virus infection [92, 93, 94, 95] • N=10 per group (control, countermeasure + challenge) • 1° Endpoints: weight loss, clinical score,
Capability Demonstrations / Integration Phase		
FcRn$^{-/-}$ hFcRn Mice	• Dosing study for pre-clinical data	• Used for pK studies with human IgG • N=10 per group, 12 groups for dose finding • Reduced induction of anti-human IgG responses [91]
Ferret	• Human Influenza challenge studies (Capability Demonstration #1)	• Clinical symptom/disease model of seasonal influenza virus infection [92, 93, 94, 95] • N=10 per group (control, countermeasure + challenge) • 1° Endpoints: weight loss, clinical score,
Non- Human Primate	• Determine countermeasure peak concentration • Determine countermeasure variability (<10%)	• Large animal suited to replicating recapitulate human physiology • N=5 per group (control, countermeasure + challenge); 60 day study • Endpoint: TBD

Studies during platform development phase are planned to assess *in vivo* pharmacokinetics (pK) and potency. The kinetics of the countermeasure *in vivo* will be determined by ELISA using population pK analysis of multiple animals. We will also conduct challenge studies during development.

Studies during the capability demonstrations will assess countermeasure concentration using multiplex Luminex bead-based assays to determine peak human antibody levels and half-life. We will also determine the inter-NHP antibody concentration variance with the expectation that it will not exceed 10%. Appropriate animal models will be selected for the capability demonstration challenge studies (see **Table 1**) and model-specific (animal model and virus) quantifiable metrics will be defined prior to challenges studies (i.e., death, temperature, weight loss, etc.).

Approach/Innovation for Platform Integration – (Task 4.1)

Across the two-year program we will develop a fully-integrated end-to-end platform that can start with unknown samples from a viral outbreak and be prepared to produce an efficacious and safe CGMP medical countermeasure scalable to 20,000 doses within 60 days (see Figure 2, below). To achieve successful platform integration, all task areas will work together harmoniously to ensure work is proceeding in an efficient manner. Having activities centered at DHVI with contributions from subcontractors will be key to this efficiency.

TA1 will initiate with viral propagation at the Duke RBL. We are confident our TA1 team will have the ability in the end-to-end platform to rapidly identify unknown viral pathogens. Furthermore, the "Thaw and Infect" approach coupled with methods to rapidly quantify virus developed by (b)(6) will enable viral propagation. TA1 and TA2 may be initiated concurrently. For TA2, we have proposed multiple, innovated approaches through the Base (i.e., VH/VL swapping, IgSeq, BCRSeq) that can be used regardless of source samples. Furthermore, by centering isolation/evolution strategies at Duke and UTexas, we can have concurrent activities to yield the most potent antibodies without compromising time. While the antibodies are evolved/isolated, the TA2 analytics teams will be preparing for binding and neutralization testing.

While TA1 and TA2 proceed, TA3 will initiate preparation for CGMP production. Once the top five Ab sequences are selected, the TA3 teams at UPenn/SGVI will verify optimal vector sequence and design in parallel with final potency evaluation. Once potency and vector optimization is complete the Duke team will initiate mRNA production of the best mRNA mAb countermeasure candidate. LNPs will then be produced to encapsulate the mRNA and the mRNA:LNP product will undergo fill/finish and release testing will be done. Concurrent to these activities the regulatory/clinical and/or animal study teams will be preparing for subsequent human or animal studies.

Our approach is unique in that we are combining the best of academia and industry to move pandemic preparedness forward with established platform methodologies and novel innovative approaches to accelerate medical countermeasure development and delivery. Process development and capability demonstration work progressing through the three Task Areas above will be coordinated and synergized by a well-established administrative team of forward leaning project managers, financial/contracts managers, regulatory experts, clinical investigators, etc.

4.1 Background

Biologic countermeasures, such as monoclonal antibodies (mAbs), are a rapid/effective means of controlling and containing outbreaks of emerging pathogens where no licensed therapeutic or vaccine is available [100]. However, standard paradigms for their production and delivery limits their use as a first-line prophylactic or therapeutic, since the process for their identification, optimization and production can take months to years [101]. The Duke DARPA Pandemic Prevention Platform (P3) team seeks to apply its experience, innovations, cutting-edge research portfolio, and in-house CGMP manufacturing capabilities to greatly expedite mAb countermeasures for future pandemics. The fully integrated platform will be a major advancement in rapid pandemic countermeasure development and will address the significant global challenge pandemic outbreaks have on both civilian and military populations.

The Duke University DARPA P3 team will be centrally based at the Duke Human Vaccine Institute (DHVI), a 220+ person organization for bench-to-bedside research, including a 38,000 gross sq.ft. BSL-2/3 NIAID-built regional biocontainment facility, in-house CGMP manufacturing facility, and clinical trials capabilities, including the capacity for first-in-man phase I clinical trials at the Duke-NIAID Vaccine and Treatment Evaluation Unit (VTEU). The DHVI has, for the past decade, carried out innovative basic and translational research through highly effective national and international collaborations in the fight against emerging infectious diseases. For the DARPA P3 initiative, Duke investigators (b)(6) have partnered with outstanding collaborators with, mRNA vaccine and therapeutic platforms and manufacturing expertise (b)(6) (b)(6) UPenn, and (b)(6) Synthetic Genomics Vaccines Inc.), and rapid antibody isolation and evolution expertise (b)(6) University of Texas, Austin). This team is unique because; a) it has been working together for 12+ years, b) members are highly respected leaders in their fields, and c) the team can work quickly and expeditiously in an atmosphere of trust and selfless collaboration.

This multi-disciplinary team has the expertise, novel technology and ability to collaborate to attain the DARPA P3 goals.

Duke's approach to meeting these goals is to focus on developing platform approaches drawing upon experience in growing a variety of different viruses, performing single-cell Ab isolation and evolution, and developing rapid and efficient mRNA CGMP capabilities eliciting active and passive antibody protection.

1.1 Goals and Impact

The Duke DARPA P3 program will combine world-class expertise in virology, immunology and CGMP manufacturing to create a fully integrated platform capable of responding to a viral pandemic within 60 days. Within each Task Area, we identify the current state of the art of the platform and propose how to improve performance and efficiency of the process. In doing so, we have struck a balance between innovation and taking reasonable risks to achieve workable solutions given the short timeframe. Our approach is unique in that we are combining the best of academia innovation and industry production to move pandemic preparedness forward. Once the platform is developed, we will seek to publish advances to disseminate this important knowledge, as well as commercialize the platform to ensure a global impact to address the significant challenge pandemic outbreaks have on both civilian and military populations.

Figure 2 depicts our overall strategy (Base) for developing a fully integrated platform that will start with a viral isolate and peripheral blood from an outbreak subject and produce an efficacious and safe current good manufacturing practice (CGMP) medical countermeasure scalable to 20,000 doses within 60 days. Shown are our task area-specific objectives and deliverables. Duke DARPA P3 innovations include novel methods for identifying and culturing known/unknown viruses; creating fluorophore-labeled virion reagents to isolate protective antibodies; mass spectroscopy and next generation sequencing approaches to rapidly identify and isolate antibodies from plasma, blood B cells, and/or humanized mouse models; novel computational mAb engineering/evolution platforms; the use of nucleoside-modified mRNA to safely express therapeutic proteins; and rapid mRNA manufacturing and lipid nanoparticle formulation for active and passive antibody delivery. Assembly of existing platform technologies with these low and moderate/high risk innovative approaches will enable the integrated Duke DARPA P3 consortium to ensure product delivery that rises above the current state of the art in the development of pandemic medical countermeasures.

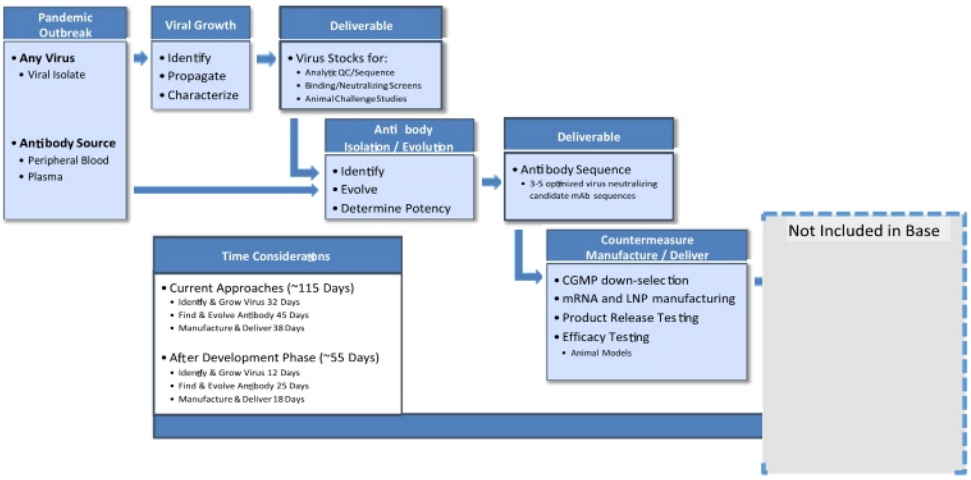

Figure 2. Overall Duke DARPA P3 platform to develop pandemic countermeasures using innovative technologies to accelerate established approaches and transition product to GMP production.

2.0 Project work Description and Requirements

Task Area 1: On-Demand Platform to Grow Virus

The goal of the work in Task Area 1 will to be to develop methods to support viral propagation, so that virus can be used for downstream Tasks (i.e., whole virus neutralization assays). Within Task Area 1, there are two subtasks (Task 1.1 and Task 1.2). The task area approach, deliverables, and timeline are provided in the below.

1.1. Virus Propagation System Approach (Duke, (b)(6)

- "Thaw-and-Infect" eukaryotic cell culture array comprised of cell types/lines competent for the isolation and high titer growth of a variety of known and unknown viral isolates.
    - Identify optimal seeding/freezing density for infection-appropriate confluence between 18-24 hours following recovery from cryostasis.
    - 50 aliquots per cell line.
    - Final format will consist of a single or multiple multi-well culture plates.
- Viral propagation monitored using Fluorescently-tagged oligonucleotide probe (FOPs) hybridization to viral nucleic acid and quantitative real-time PCR.
- Analysis of virus stocks to ensure quality
- High density frozen cell stocks of the "Thaw and Infect" culture array capable of supporting virus culture scale up.
    - Identify optimal conditions to generate high density frozen cell stocks (up to 10^8 cells/mL) that can support virus propagation (up to 500 mL culture volume) 24-48 hours following recovery from cryostasis.
    - 25 aliquots per cell line.
    - Final format will be multi-layer flask.
- The "shelf-life" of working lots of all cell lines, in terms of percent viability, will be evaluated every three months after cryopreservation.
- Evaluate the ability of the Thaw-and-Infect culture array to rescue influenza virus (ss segmented -RNA) and CHIKV (181/25 vaccine strain; ss +RNA).
- Quality control - Progeny viruses will be sequenced (NGS) to monitor drift. We will utilize proteomic and other biochemical analyses to characterize virus isolates (lipid, protein, and carbohydrate) using established methods in the DHVI/CGMP Analytics group, including SDS-PAGE, western blot and RP-UPLC to resolve and detect individual virus proteins that can be compared between stocks [58]. Furthermore, when paired with mass spectrometry, these methods can verify protein identity, sequence, and detect glycosylation and other protein modifications.
-

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Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
1.1.1	• Initial development of "Thaw and Infect" eukaryotic cell culture array reduced panel capable of supporting influenza virus and CHIKV propagation.	0-6
1.1.2	• Continued development of "Thaw and Infect" eukaryotic cell culture array full panel.	6-18
1.1.3	• FOPs specific for influenza virus detection/titration • FOPs specific for CHIKV detection/titration • Optimized methods/data report	6-12
1.1.4	• Quality-controlled seed stocks of influenza virus to support DARPA P3 tasks • Quality-controlled seed stocks of CHIKV to support DARPA P3 tasks	9-15
1.1.5	• High density frozen cell stocks of the "Thaw and Infect" culture array capable of supporting Flu and CHIKV propagation within 24-48 hours after thaw.	9-24
1.1.6	• Quality-controlled working stocks of influenza virus to support DARPA P3 tasks • Quality-controlled working stocks of CHIKV to support DARPA P3	12-18

1.2. Viral Infectious Clone Genomes Approach (SGVI, (b)(6)

- SGVI will demonstrate their ability to rapidly synthesize error-free viral infectious flu genomes should a situation arise where sponsor can only provide viral sequence and not infectious source material.
- Overlapping oligonucleotides composing the Flu Hemagglutinin and Nueraminidase genes will be pooled, ligated and amplified by PCR.
  Amplified PCR products will then be error corrected using enzymatic methods.
- Next promoter ant terminator regions will be added by assembling into linearized plasmid encoding these elements and the final gene expression construct will be amplified.
- Once synthesized, the HA and NA genes will be sequence-verified and delivered to Duke along with plasmids encoding the other 6 Flu genes for virus rescue.
- Quality Control - Progeny viruses will be sequenced (NGS) to monitor drift and ensure that the progeny are bio-identical to traditionally produced flu. We will utilize proteomic and other biochemical analyses to characterize virus isolates using established methods in the DHVI/CGMP Analytics group, including SDS-PAGE, western blot and RP-UPLC to resolve and detect individual virus proteins that can be compared between stocks [58].

Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
1.2.1	• One Influenza virus infectious clone genome	0-12

Task Area 2: System to Identify and Evolve Antibodies

The goal of the work in Task Area 2 will to be to isolate neutralizing antibody to Flu and CHKV from convalescent PBMC and plasma, improve the antibody 100-fold in function by *in vitro* antibody evolution and transfer antibody sequence to Task Area 3 for production and delivery optimization/testing. We propose overlapping approaches to isolation that will be optimized and down-selected during the

performance period. Within Task Area 2, there are two subtasks (**Tasks 2.1 and 2.2**). The task area approaches, deliverables and timelines are provided below.

2.1. Optimize Antibody Isolation from Peripheral Blood Cells and Plasma

- ***Isolation Approach 1.*** *Pathogen-specific Memory B or Plasma Cells (Duke,* (b)(6) Fluorophore-labeled whole virions (provided by TA1 team) or recombinant labeled protein and/or VLPs will be used to sort influenza and chikungunya specific memory B cells for antibody isolation. In addition, plasma cells will be sorted as single cells. The VH and VL genes of both single pathogen-specific memory B cells or single plasma cells will be amplified by PCR, and the full Abs will be produced by transient transfections using overlapping PCR.
  - mAb candidates will be produced in small batches and progress through high-throughput binding assays (whole virus ELISA) and be down-selected for pathogen neutralization in collaboration with TA1 to establish baseline potency prior to handing off to evolution team.
  - Neutralization titers will be measured as concentration of antibody inhibiting ≥80% of target virus replication
- ***Isolation Approach 2.*** *Plasma antibody sequencing* (U Texas, (b)(6) has invented a suite of technologies (termed IgSeq, BCR-Seq) that will enable the rapid identification and optimization of neutralizing antibodies from plasma of humans or animals. Ig-Seq, is a proteomics-based technology for determining the serological repertoire to a specified antigen. Ig-Seq capitalizes on very high-mass resolution LC-MS/MS proteomics of serum antibodies that have been affinity-purified with immobilized virus capsid. MS spectra are interpreted using a patient-specific database of paired VH:VL sequences derived from peripheral B cells, obtained from the BCR-Seq workflow. The BCR-Seq workflow enables the determination of the natively paired VH:VL repertoire from up to $5x10^6$ B cells per one day experiment, with a validated accuracy >97%.
  - mAb candidates will be produced in small batches (at Duke from UTexas sequences) and progress through high-throughput binding assays (whole virus ELISA) and be down-selected for pathogen neutralization in collaboration with TA1 to establish baseline potency prior to handing off to evolution team.
  - Neutralization titers will be measured as concentration of antibody inhibiting ≥80% of target virus replication

Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
2.1.1	• Pathogen-specific antibody sequences from PBMCs from Flu-infected subjects using single memory B cell sorts or bulk plasma cells	0-6
2.1.2	• Pathogen-specific antibody sequences from PBMCs from CHKV-infected subjects using single memory B cell sorts or bulk plasma cells	6-12
2.1.3	• Method for sorting antigen-specific B cells using FOP-tagged viruses (TA1)	6-12
2.1.4	• Pathogen-specific antibody sequences from PBMCs from Flu and CHKV-infected subjects using single B cell sorts with using FOP-tagged viruses	6-12
2.1.5	• Pathogen-specific antibody sequences from Plasma from Flu-infected subjects (IgSeq phase)	0-6
2.1.6	• Pathogen-specific antibody sequences from Plasma from Flu-infected subjects (BCR Seq phase)	6-12

2.2. Optimize In Vitro Antibody Evolution

- ***Evolution Approach 1.*** *Mammalian Display and H/L Chain Swapping (Duke, (b)(6) U Texas, (b)(6)* – Once isolated, candidate countermeasure antibodies will be evolved by mammalian display and VH and VL swapping. We have established an Ab evolution scheme that will be integrated with the down-selected Ab isolation approach for identification of pathogen neutralizing antibodies.
- The VH and VL will be cloned into the single chain variable fragment (scFv) expression vectors. To evolve antibody fragments to be higher affinity, we will employ random and CDR-targeted mutagenesis of the scFv. This is achieved with error-prone PCR. The mutated gene or gene fragment is then inserted into an expression vector and transformed into bacteria. The mutated DNA is prepared and transfected into multiple liters of 293i cells for cell surface expression. The cells with the highest binding to target virions (TA1) will be FACS sorted.
- To determine improvements in binding/function the scFv of the originally identified Ab will be displayed on the 293i cell surface to determine baseline binding, and then gates will be set to sort cells that have an MFI higher than the original Ab. The sorted cells will be cultured for two days and then treated with HRV-3C to cleave the scFv from the cell surface. Culture supernatant will be removed from the well, cleared of cells, and virus neutralization determined in binding/neutralization screens for candidate countermeasure mAb down-selection.

Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
2.2.1	• Whole virus binding ELISA and neutralization assays for Flu and CHKV	0-6
2.2.2	• Optimized methods/protocols for heavy/light chain swapping and mammalian display	0-12
2.2.3	• Evolved antibody sequences from Flu-infected subjects	12-18
2.2.4	• Evolved antibody sequences from CHKV-infected subjects	18-24

Task Area 3: Deliver Medical Countermeasures

To develop an mRNA-based medical countermeasure with optimal potency, half-life and ease of use in the field; we will evaluate a number of improvements to the platform during the 24-month performance period and implement those that support this objective in a pre-clinical capability demonstration using ferrets and NHPs. Potential improvements to be evaluated include mRNA modifications SMART mRNA replicons (Synthetically Modified Alpha Replicon Technology), optimized lipid nanoparticle formulations. Within Task Area 3, there are five subtasks (**Subtasks 3.1 - 3.5**). The task area approaches, deliverables and timelines are provided below.

3.1. Optimize RNA Platform Approach (Duke, UPenn, SGVI)

- We will develop modified RNA using model antibodies to deliver optimal expression. Parameters to be evaluated include the following: mRNA modifications such as 5' cap, optimized 5'- and 3'-UTRs and coding sequence, and different lengths of poly(A)-tail. We will develop SMART mRNA replicons (Synthetically Modified Alpha Replicon Technology), evaluating 5' cap, UTR improvements as well as replicon improvements. Included in this effort we will compare template DNA derived from standard plasmid mini preps with synthetic DNA produced by SGVI's gene assembly method.
- Using DHVI model influenza monoclonal antibody sequences U Penn will optimize mRNA modifications; SGVI will develop mRNA replicon platform; Duke will perform head-to-

head-comparison studies in FcRn-/- hFcRn line 32 Tg mice (T32).

Animal Model	Purpose	Rationale
FcRn$^{-/-}$ hFcRn Mice	• mRNA comparisons • LNP formulation • Dosing optimization	• Used for pK studies with human IgG
		• N=5 per group; 35 day study duration • Reduced induction of anti- human IgG

Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
3.1.1	• Optimize mRNA for in vivo production of model antibodies.	0-9
3.1.2	• Evaluation of modified mRNA and mRNA replicon platforms in cell based in vitro models	0-9
3.1.3	• Head-to-head comparison of modified mRNA and mRNA replicon platforms in FcRn-/- hFcRn line 32 Tg mice (T32)	9-12

3.2. Optimize LNP formulation Approach

- Our first generation formulation showed that high levels of circulating Ab could be produced with intravenous delivery of mRNA/LNPs. We have evaluated delivery of mRNA-LNPs by the intradermal (i.d.), intramuscular (i.m.), intravenous (i.v.), intraperitoneal (i.p.) subcutaneous (s.c.), and inhaled (i.n.) routes with very high levels of protein translation.
- Based on these data, we intend to move forward with s.c. administration as the primary route of delivery, since it yields high protein expression, is simple, relatively non-invasive with a straightforward regulatory pathway for clinical use.
- Formulations that increase circulating Ab titer (i.e., ≥ 20% of peak titer), and/or increase duration of expression (i.e., titer > 10 ug/mL for 45 days post-administration) in T32 mice experiments described in the table below will be advanced for integration into the final capability demonstrations in Ferrets and NHPs.

Animal Model	Purpose	Rationale
FcRn$^{-/-}$ hFcRn Mice	• mRNA comparisons • LNP formulation • Dosing optimization	• Used for pK studies with human IgG • N=5 per group; 35 day study duration • Reduced induction of anti-human IgG responses

Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
3.2.1	• Evaluate potential mRNA formulations with Acuitas and Arcturus for packaging and stabilizing mRNAs	0-9
3.2.2	• Test formulations in small animal models (Mice) including FcRn-/- hFcRn Mice	9-12

3.3. Knowledge/Platform Transfer to DHVI-GMP

- At the end of this effort described in Task 3.1 and 3.2, we will deliver an optimized process for production of mRNA encoded antibodies and an effective system for s.c. delivery. For this task, the mRNA and LNP production platforms will be implemented at DHVI allowing future options for development and clinical trial material production to be conducted in DHVI's GMP facility.

Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
3.3.1	• Platform transition to DHVI (Duke) - Method / Transfer Package	6-15
3.3.2	• Produce countermeasure material for flu ferret and NHP in vivo studies	12-21*

* The time to produce the countermeasure is not expected to take this duration. However, the timeline must align with 2.2.3 and 2.2.4, which is extending the timeperiod.

3.4. Approach to Challenge/pK Animal Studies in Ferret

- After development and down selection of the best RNA and LNP candidates in small animal experiments using model antibodies, we will develop and deliver a Flu countermeasure to Ferrets as a relevant disease model.
- Studies are planned to assess in vivo pharmacokinetics (pK) and potency.
- The kinetics of the countermeasure in vivo will be determined by ELISA using population pK analysis of multiple animals. We will also conduct virus challenge study.
- Studies during this animal capability demonstration will assess countermeasure concentration using multiplex Luminex bead-based assays to determine peak human antibody levels and half-life.

Animal Model	Purpose	Rationale
Ferret	• Human Influenza challenge studies	• Clinical symptom/disease model of seasonal influenza virus infection • N=10 per group (control, countermeasure + challenge) • 1° Challenge Endpoints: weight loss, clinical score, viral load

Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
3.4.1	• In vivo pK mRNA/LNP data for Flu counter-measure • Reproducibility data on mRNA/LNP for Flu counter-measure	18-24
3.4.2	• In vivo protection capability of Flu counter-measure in ferret challenge model	18-24

3.5. Approach to pK studies conducted in NHP with developed countermeasure

- After testing the selected Flu countermeasure in Ferrets as a relevant disease model, studies will be done to assess in vivo pharmacokinetics (pK) in NHPs as a suitable animal model for human countermeasure expression.
- The kinetics of the countermeasure in vivo will be determined by ELISA using population pK analysis of multiple animals.
- Studies during this capability demonstration will assess countermeasure concentration using multiplex Luminex bead-based assays to determine peak human antibody levels and half-life.
- We will also determine the inter-NHP antibody concentration variance with the expectation that it will not exceed 10%.

Animal Model	Purpose	Rationale
Non-Human Primate	• Determine countermeasure peak concentration • Determine countermeasure variability (<10%)	• Large animal suited to replicating recapitulate human physiology • N=5 per group (control, countermeasure); 60 day study

Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
3.5.1	• In vivo pK mRNA/LNP data for Flu counter-measure • Reproducibility data on mRNA/LNP for Flu counter-measure	18-24

Platform Integration

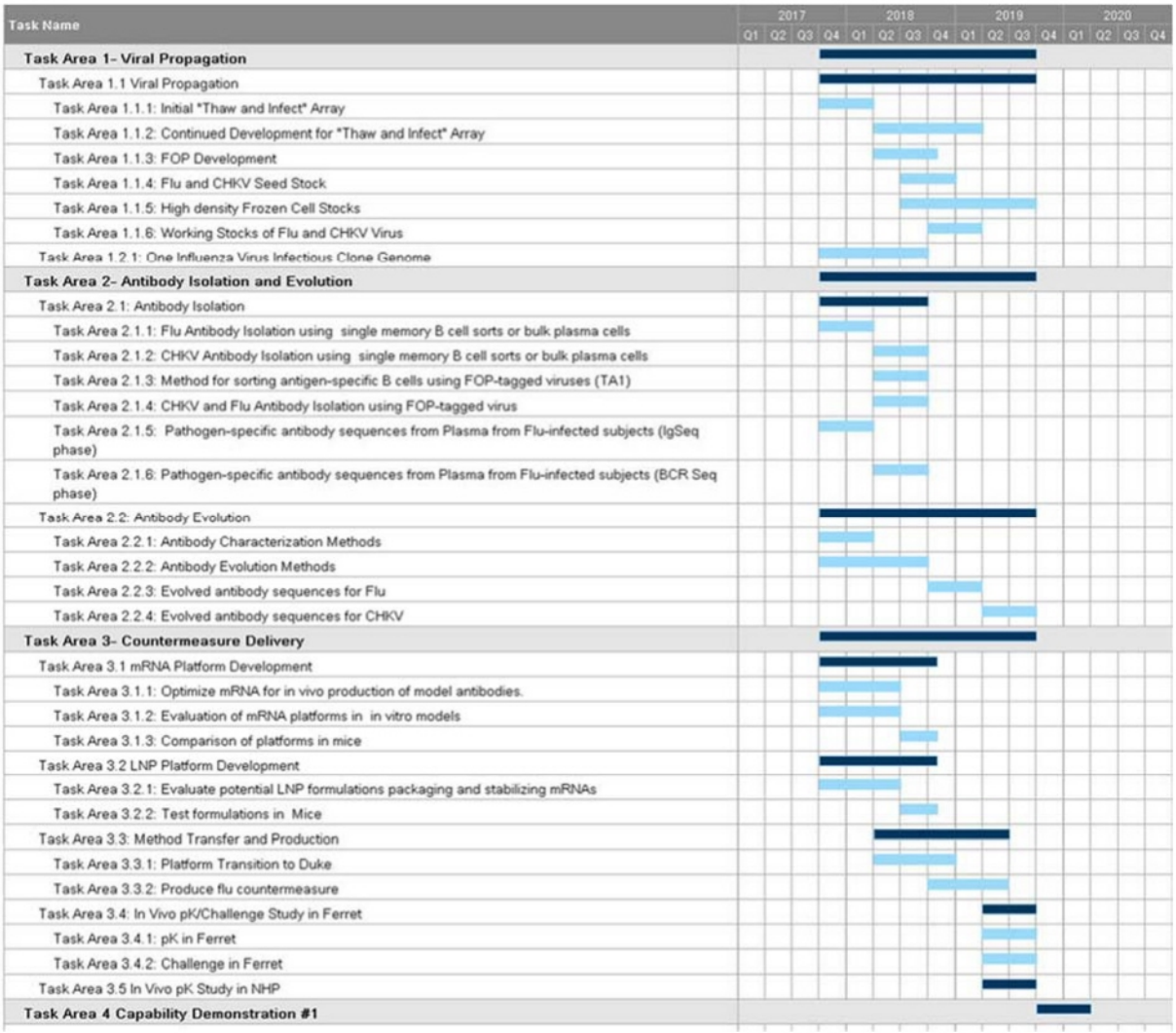
4.1. Capability Demonstration #1 Approach

- Capability demonstration performed with influenza virus at Duke.
- Virus received by Task Area 1 team at Duke. Initiate propagation of virus in thaw and infect arrays to develop virus for isolating antigen-specific B cells and for testing antibody neutralization/binding. Virus will be provided to Task Area 2 team.
- PBMCs received by Task Area 2 team at Duke. Duke will work on antibody isolation from B cells.
- Antibody sequences will be provided to UPenn or SGVI for mRNA modification.
- Duke will make final mRNA sequence selected and enclose the mRNA in an LNP (produced at Duke).
- Resulting countermeasure will be tested in vivo in the Ferret small Flu challenge animal model.

Deliverables and Timeline

Subtask	Deliverable	Timeline (months)
4.1	Data Summary and Report, and Countermeasure	24-30

Overall Project Timeline



DEFENSE ADVANCED RESEARCH PROJECTS AGENCY
675 NORTH RANDOLPH STREET
ARLINGTON, VA 22203-2114

FROM: DARPA Contracts Management Office (CMO)

TO: Patrick Sims.
Space and Naval Warfare Center
53560 Hull Street
San Diego, CA 92152

FOR: DARPA/BTO

SUBJECT: Appointment as Agreement Officer's Representative (AOR)

1. You are hereby appointed as the Agreement Officer's Representative for:

Agreement Number:	HR0011-17-2-0069
Recipient:	Duke University
Program Description:	Pandemic Prevention Platform (P3)
Proposal:	"Duke DARPA Pandemic Prevention Platform"

2. This appointment authorizes and designates you to perform the following duties and/or responsibilities as specified herein:

   a. Furnish plans, schedules, specifications, descriptions, and other documents to the recipient as required by the agreement.

   b. Assist the recipient in interpreting technical aspects of the agreement specifications/statement of work. Differences of opinion and interpretations which could affect the terms and conditions of the agreement will be referred to the Agreements Officer for resolution.

   c. Provide Government recommendations/approvals to the recipient promptly in all cases where the agreement calls for technical approval.

   d. Observe, monitor, and assess the recipient's performance under the terms of the agreement. This includes reporting promptly to the Agreements Officer any failures, delays, or significant deviations of performance, quality, costs, or other actions which might jeopardize agreement performance.

3. In the performance of the duties delegated to you in this letter, you are cautioned that you could be held personally liable for actions taken or directions given by you to the recipient that are beyond the authorities given to you in this letter. The duties or authorities in this letter are not delegable;

therefore, you must advise the Agreements Officer or the Administrative Agreements Officer immediately when you are unable to perform these duties.

4. In your dealings with the recipient you must not give technical direction as though the recipient's employees are Government employees. You must maintain a formal, arms-length relationship with the recipient in order to avoid even an appearance that the agreement is one for personal services. If the recipient's performance takes place in a Government facility, then to the maximum extent practicable, the recipient's work area should be physically separated from areas in which Government employees work, and communication with the recipient's employees on agreement matters should be only through that recipient employee(s) designated by the recipient to supervise them. You must not give any direction to the recipient that is not authorized by the statement of work because it is not the intent of the Government that a recipient be required to do anything that is not included in the agreement.

5. You are responsible for providing prompt notification to the Agreements Officer any significant deficiencies with respect to recipient performance or other actions which might jeopardize agreement performance.

6. You are not authorized by this letter to take any action, either directly or indirectly, that could result in a change in the pricing, quantity, quality, place of performance, delivery schedule or any other terms and conditions of the basic agreement, or to direct the accomplishment of effort which would exceed the scope of the basic agreement. You must be especially cautious in providing interpretation of the specifications/ statement of work. The agreement reached or technical direction given must be formalized in writing with copies to the Agreements Officer. You shall also notify the recipient that if he believes that the AOR's interpretation is erroneous, the recipient must notify the Agreements Officer in writing concerning the details of his position.

7. Specific duties in addition to those above are as follows:

    a. Control all government technical interfaces with the recipient.

    b. Ensure that copies of government technical correspondence are forwarded to the Agreements Officer for placement in the agreement file.

    c. Promptly furnish documentation on any requests for change, deviation, or waiver (whether generated by the Government or the recipient) to the Agreements Officer for appropriate action.

    d. Review and, if required, accept invoices submitted through WAWF as stipulated in Article V of the Agreement. In order to approve the Recipient's invoices, you must obtain access to Wide Area Work Flow (WAWF) through your own agency. DARPA will not assign WAWF extensions to AORs who are not assigned to DARPA. Your Group Administrator (GAM) will be able to provide you access to WAWF. If you are not sure who your GAM is, you can email the WAWF Help Desk at cscassig@csd.disa.mil to request this information.

e. Provide required review of Patent/Invention Disclosures made in I-Edison (http://www.iedison.gov).

f. The AOR must maintain a separate file for each agreement for which he/she acts as AOR. The file should serve as a repository and record of all documents and communications between the recipient and the AOR. At a minimum, the file must include a copy of the agreement and all modifications as well as the AOR appointment letter. Examples of other file documentation may include:

- Reports required per the agreement, e.g. interim and final technical or patent reports
- Memoranda for Record documenting important agreement discussions
- Records of formal meetings, e.g. post award conference, program reviews, etc.

f. Assist Administrative Agreements Officer and/or Agreements Officer, as requested, with closeout activities at completion of agreement period of performance.

8. Prior to appointment, you must have completed CLC 222/COR 222 or DAU-recognized equivalent course and Combatting Trafficking in Persons (CTIP) General Awareness training or equivalent (See website ctip.defense.gov for equivalents).

9. Your appointment as AOR for this effort expires upon final disposition of the agreement.

10. Your good judgment in performing your duties under the agreement will have an important effect on the value of the performance obtained by the Government.

DONAGHUE.DESMOND.P.(b)(6)
(b)(6)
Digitally signed by DONAGHUE.DESMOND.P.(b)(6)
Date: 2017.09.18 13:41:14 -04'00'

D. Peter Donaghue
Agreements Officer
DARPA/Contracts Management Office (CMO)

(Date)

SIMS.PATRICK.C.(b)(6)
Digitally signed by SIMS.PATRICK.C.(b)(6)
Date: 2017.09.14 08:26:51 -07'00'

Patrick Sims
Agreement Officer Representative

(Date)

LINK TO TEMPLATE (click here)

Please use this template to provide monthly financial updates to the Program Management team. As you input your data, the graph will automatically update. ***Please keep past reports in this file and create a new tab each month. We want to see all reports in the same file. Title tabs "Phase-Month-Year, " e.g., "Base - January - 2015"***

LINK TO EXAMPLE (click here)

An example of a completed template is also provided. The example graph illustrates a scenario where the performer is under spending. It is designed to show how this template will make it easy for Biological Control performers to clearly communicate the status of their effort to DARPA so that both can plan for and initiate contractual actions quickly and effectively.

Spend Plan Data	
Period of Performance	The financial report will only cover the current phase (e.g., Base, Option 1, etc.). Use a format similar to "Sep-2013," not "Month 6." In order to plan for continuing resolution requests, DARPA may reach out to you separately to request your projected spend rate for future phases.
Phase Total	Total for current phase *(Example Graph - total is $1,000,000)* .
Funds Received	Funds awarded to date; most efforts are funded incrementally *(Example Graph - this effort received an increment for $500,000 in Oct-2012 to exercise the base, and received the remainder of their base period funding in Mar-2013 (remaining $500,000))* .
Spend Plan	Projected Expenditures must cover the entire phase.
Actual Expenditures (est.)	Actual Expenditures should not be solely based off of invoices you have submitted or received to date. Instead, it should be an accurate (to the extent that is possible) account of the expenses you have actually incurred to date. For example, if a subcontractor has incurred but hasn't invoiced $100,000 worth of work, include the $100,000 in your actual expenditures. Or a large amount of equipment valued at $50,000 that hasn't yet been invoiced should also be factored in to the actual expenditures.
Invoiced to Date	Report the invoices you have submitted to date *(Example Graph - the scenario used in the example graph submits invoices quarterly)* .
Issues/Updates Summary (if applicable)	
Use this section as an opportunity to bring issues, concerns, or updates to the attention of DARPA. For example, you can summarize reasons for over/under-spending, potential no-cost extension requests, invoicing problems, etc.	

[Name of Prime Institution]
Quarterly Progress Report

Period Covered by the Report: [Date] through [Date]

Date of Report:

Project Title:
Contract Number:
Total Dollar Value:
Subcontractors:
Program Manager: Dr. Matt Hepburn, Defense Advanced Research Projects Agency, Biological Technologies Office

Submitted by:

[Name]
[Address]

Telephone:
Email:

TABLE OF CONTENTS

Global Information:

- **Please do NOT wait until submitting a report to let us know if you are having a financial OR technical issue!**
- Clearly indicate if funding from another federal agency (e.g., NIH) has been used to support anything presented
- Clearly indicate if any content on it is pre-publication sensitive. Material that is not sensitive could be presented or shared by the Program Manger with the public
- Delete all instructional text from this document before submitting your report
- Support your claims with data, images, or videos in this document
- Please always update the header of this document with the period the report covers
- Name your files the following way: QR - Team Name – Month/Year Due (e.g., QR – Univ XYZ – Sep 2017)
- Due Months: June, September, December, March
- Quarterly teleconference calls will be scheduled with the Program Manager and his team

Definitions (as DARPA defines them):

- Functional block diagram: describes the functions and interrelationships of a system in a flock-block diagram style so that one can easily and thoroughly understand the system and the relationship of each of the parts to the whole. If the hardware evolves throughout your project, please provide a block diagram for each evolution (example included).
- Work-breakdown Structure (WBS): a hierarchical and incremental decomposition of the project into phases, deliverables and work packages. It is a tree structure, which shows a subdivision of effort required to achieve an objective (example included).
- Deliverable: a deliverable is a measurable and verifiable outcome or object that a project team must create and deliver according to the terms of an agreement. An intangible deliverable is a particular outcome that the team achieves. A tangible deliverable is a concrete or material object created by the team.
- Milestone: a milestone describes the status of the project as represented by an event or moment at which one or more project activities are complete. Milestones can represent the completion of key project tasks, conclusions reached, or questions answered that affect the project schedule in a major way.
- Big-win: a significant accomplishment
- Go/no-go criteria: a simple “pass-fail” way of viewing accomplishments; accomplishments that are essential for the continuation of your effort.
- SETA: Science, Engineering, and Technical Assistant (internal DARPA term for technical support staff)

1 High-Level Project Progress

1.1 Big Wins

Briefly describe the most significant and salient accomplishment(s) that you have achieved during this ***most recent quarter***. How has this compared to your original project plan?

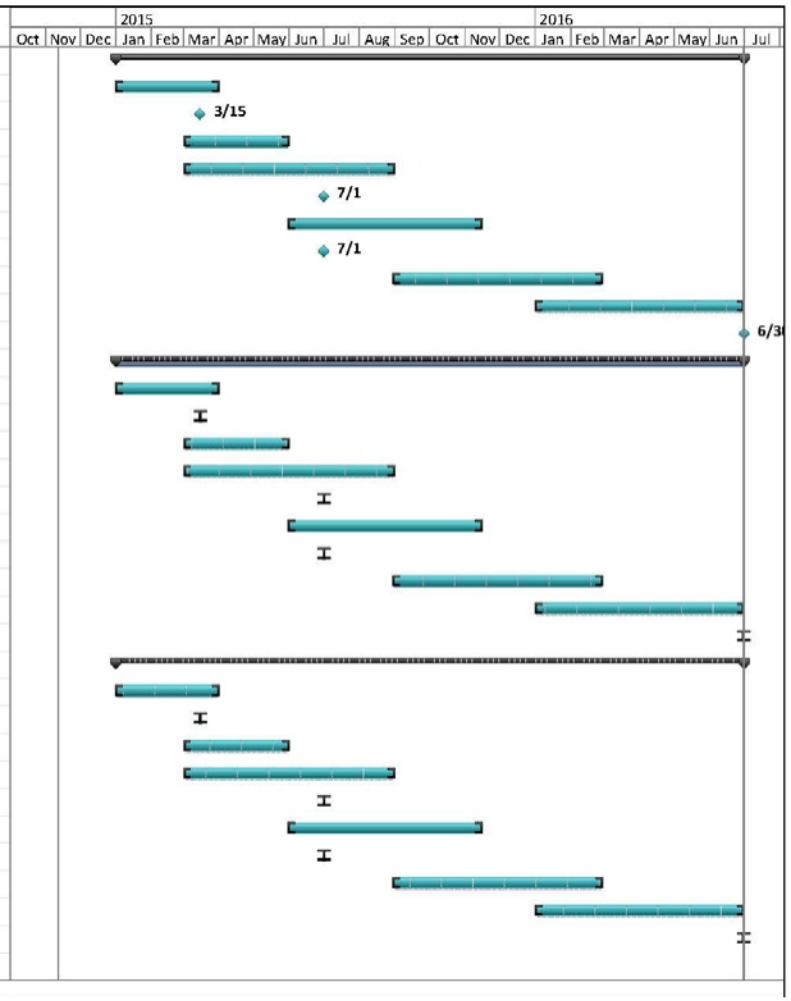
1.2 Go/no-go Progress

Accomplishment *Why is this significant? ID TA.*	**Month** *From Kick-off Presentation*	**Update** *What is the current status? Explain any discrepancies (behind/ahead of schedule). What is the next step?*

2 Schedule – Milestones and Deliverables

Include a readable high-level Gantt chart for Phase I that lists the major milestones and deliverables for each task (example included below). Use the month and year to label your chart (e.g., Mar-15), not the quarter or month (e.g., Quarter 2, Month 1). Below is an example of an acceptable chart.

ID	Task Mode	Task Name	Duration	Start	Finish	Predecessors
1		**Task**	**391 days**	**Thu 1/1/15**	**Thu 6/30/16**	
2		Subtask	64 days	Thu 1/1/15	Tue 3/31/15	
3		MS	0 days	Sun 3/15/15	Sun 3/15/15	
4		Subtask	67 days	Sun 3/1/15	Sun 5/31/15	
5		Subtask	132 days	Sun 3/1/15	Mon 8/31/15	
6		MS	0 days	Wed 7/1/15	Wed 7/1/15	
7		Subtask	122 days	Sun 5/31/15	Sun 11/15/15	
8		MS	0 days	Wed 7/1/15	Wed 7/1/15	
9		Subtask	132 days	Sun 8/30/15	Sun 2/28/16	
10		Subtask	130 days	Fri 1/1/16	Thu 6/30/16	
11		MS	0 days	Thu 6/30/16	Thu 6/30/16	
12		**Task**	**391 days**	**Thu 1/1/15**	**Thu 6/30/16**	
13		Subtask	64 days	Thu 1/1/15	Tue 3/31/15	
14		MS	1 day	Sun 3/15/15	Sun 3/15/15	
15		Subtask	67 days	Sun 3/1/15	Sun 5/31/15	
16		Subtask	132 days	Sun 3/1/15	Mon 8/31/15	
17		MS	1 day	Wed 7/1/15	Wed 7/1/15	
18		Subtask	122 days	Sun 5/31/15	Sun 11/15/15	
19		MS	1 day	Wed 7/1/15	Wed 7/1/15	
20		Subtask	132 days	Sun 8/30/15	Sun 2/28/16	
21		Subtask	130 days	Fri 1/1/16	Thu 6/30/16	
22		MS	1 day	Thu 6/30/16	Thu 6/30/16	
23		**Task**	**391 days?**	**Thu 1/1/15**	**Thu 6/30/16**	
24		Subtask	64 days	Thu 1/1/15	Tue 3/31/15	
25		MS	1 day	Sun 3/15/15	Sun 3/15/15	
26		Subtask	67 days	Sun 3/1/15	Sun 5/31/15	
27		Subtask	132 days	Sun 3/1/15	Mon 8/31/15	
28		MS	1 day	Wed 7/1/15	Wed 7/1/15	
29		Subtask	122 days	Sun 5/31/15	Sun 11/15/15	
30		MS	1 day	Wed 7/1/15	Wed 7/1/15	
31		Subtask	132 days	Sun 8/30/15	Sun 2/28/16	
32		Subtask	130 days	Fri 1/1/16	Thu 6/30/16	
33		MS	1 day	Thu 6/30/16	Thu 6/30/16	
34						



Include a corresponding table that lists the following:

- Short text-identifier for each milestone and deliverable
- Status (on schedule, behind schedule, ahead of schedule, complete)
- If behind or ahead of schedule, explain why

Milestone/ Deliverable Description	**Responsible team members**	**Start Date**	**Due Date**	**Actual State Date**	**Actual End Date**	**Status**	**Dependencies** *Across tasks and teams(if applicable)*

3 Task Progress, Accomplishments, and Plans

*The Program Manager wants to receive this information in a **well-organized** and **systematic** manner. He only wants updates from your **most recent quarter**, not a cumulative discussion of your project's progress to date. Back up all your claims with actual data. Highlight accomplishments to date that:*

- *The Program Manager can show his leadership to protect your program from being cut when cut directives are passed down from above*
- *Motivate funding extension and expansion of existing or follow-on programs*
- *Understand deviations from negotiated schedule and spending plan*

These reports are an opportunity to garner support from DARPA.

Identify the following for each major task in your SOW (i.e., Task 1, Task 2, Task 3, etc.); this section will form the bulk of your report:

- Task ID number mapped with SOW and TA that it supports (in example table below)
- Basic high-level description of the task (in example table below)
- Funding associated with the task (spent to date vs. remaining to spend); explain any deviations from your original spend plan (in example table below)

Task #/Title	Brief Description	% Complete	Total for Task	Total Spent	Remaining to Spend	Explain Deviations between Planned vs. Actual Expenditures

- **Describe your expected vs. actual progress towards the goals, milestones, and deliverables of the task; discuss why you have met expectations, why you didn't meet expectations, or why you have exceeded expectations; highlight significant accomplishments**
- Next steps (projected vs. actual)
- Support your claims with data, images, or videos
- Identify and describe all significant challenges and risks encountered during work towards the goals of this task
    - Identify critical dependencies across tasks and teams

- Describe your mitigation plan
- Characterize the level of risk (high, medium, or low)
- Explain if the risk has changed since your proposal or last report
- When will you resolve the risk?

4 Project Coordination, Dissemination, and Translation Efforts

4.1 Project Coordination

- Describe the steps you have taken to coordinate your team during this most recent quarter.
- Summarize key meetings for project planning and coordination, provide the following:
  - Meeting name, location, dates
  - Who attended from your team?
  - What was the general purpose?
  - What were the meaningful outputs?

4.2 Dissemination and Translation (if applicable)

- Include mention of any new partnerships, collaborators, users, etc. that directly resulted due to outcomes from this DARPA-supported effort
- Describe any possible consumers and commercialization pathways/partners for the results from your research

5 Publications and Presentations

Please update the table below with any current or upcoming publications. This section will be cumulative for your effort, keep this information for all future reports.

Title, Authors	Description/Type	Status
	Presentation to Conference Name	Published
	Paper, Name of Journal	Under Review by Journal
	Letter to the Editor, Scientific Organization	In preparation

6 Patents, Invention Disclosures, IDEs, etc…

Please update the table below with any current or upcoming patents, inventions, Investigational Device Exemption (IDE), etc... This information will be held strictly confidential. It will be cumulative for your effort, keep this information for all future reports.

Title, Authors	Description/Type	Status
	E.g. Patent; Name of Patent	Accepted
	E.g. FDA IDE	Filed/submitted
	Invention Disclosure	Preparing now

Appendix I – Project Context

For future reports, only update this section if any information changes, indicate any changes with blue text.

Teaming and Personnel

Organizational Chart

Insert an organizational chart for your entire team

Contact Information

For each member of your team (i.e., prime and subcontractors) fill out the table of the following page to provide information about their key personnel. The short descriptive "topic" under the Role column identified for each role should correspond to the general area of expertise they will be providing. The longer descriptive "major role" column should describe the work they will be doing on the tasks and subtasks they are assigned to.

Prime Team Members and Contact Information: [Name]

Role	Full Name	Contact Information (phone and email)	Major Role(s)
PI	Name	XXX@univ.edu (888) 888-8888	
Co-I (topic)	Name	XXX@univ.edu (888) 888-8888	
Postdoc (topic)	Name	XXX@univ.edu (888) 888-8888	

Subcontract Team Members and Contact Information: [Name]

Role	Full Name	Contact Information (phone and email)	Major Role(s)
PI	Name	XXX@univ.edu (888) 888-8888	
Co-I (topic)	Name	XXX@univ.edu (888) 888-8888	
Postdoc (topic)	Name	XXX@univ.edu (888) 888-8888	

Work Breakdown Structure

Provide a WBS-style breakdown of your tasking and assign team members to each (example included below).

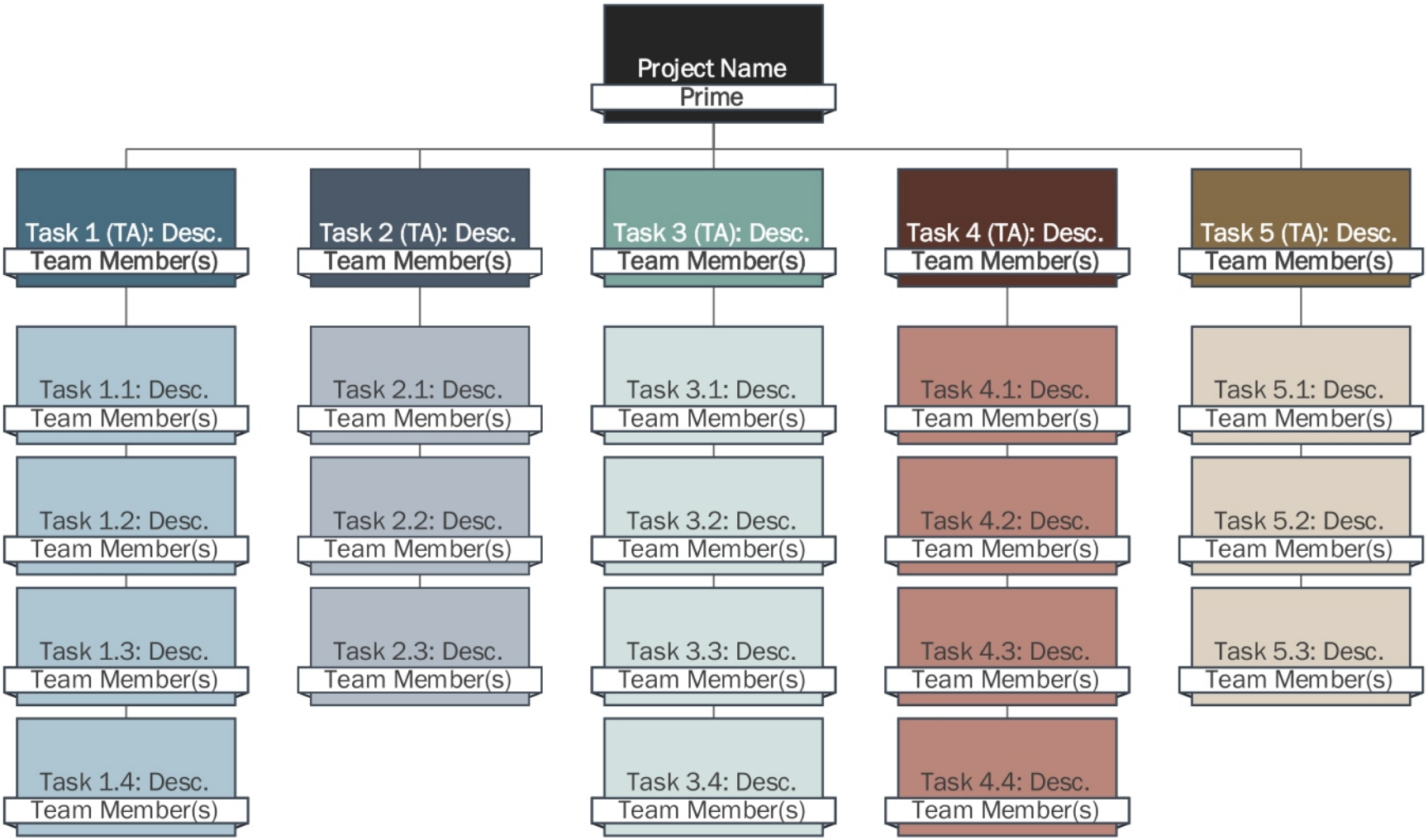


Exhibit F – Intellectual Property Assertions

IDENTIFICATION AND ASSERTION OF USE, RELEASE, OR DISCLOSURE RESTRICTIONS (JAN 2011)

(a) The terms used in this provision are defined in following clause or clauses contained in this solicitation—

(1) If a successful offeror will be required to deliver technical data, the Rights in Technical Data--Noncommercial Items clause, or, if this solicitation contemplates a contract under the Small Business Innovation Research Program, the Rights in Noncommercial Technical Data and Computer Software--Small Business Innovation Research (SBIR) Program clause.
(2) If a successful offeror will not be required to deliver technical data, the Rights in Noncommercial Computer Software and Noncommercial Computer Software Documentation clause, or, if this solicitation contemplates a contract under the Small Business Innovation Research Program, the Rights in Noncommercial Technical Data and Computer Software – Small Business Innovation Research (SBIR) Program clause.

(b) The identification and assertion requirements in this provision apply only to technical data, including computer software documentation, or computer software to be delivered with other than unlimited rights. For contracts to be awarded under the Small Business Innovation Research Program, the notification and identification requirements do not apply to technical data or computer software that will be generated under the resulting contract. Notification and identification is not required for restrictions based solely on copyright.

(c) Offers submitted in response to this solicitation shall identify, to the extent known at the time an offer is submitted to the Government, the technical data or computer software that the Offeror, its subcontractors or suppliers, or potential subcontractors or suppliers, assert should be furnished to the Government with restrictions on use, release, or disclosure.

(d) The Offeror's assertions, including the assertions of its subcontractors or suppliers or potential subcontractors or suppliers, shall be submitted as an attachment to its offer in the following format, dated and signed by an official authorized to contractually obligate the Offeror:

Identification and Assertion of Restrictions on the Government's Use, Release, or Disclosure of Technical Data or Computer Software.

The Offeror asserts for itself, or the persons identified below, that the Government's rights to use, release, or disclose the following technical data or computer software should be restricted:

Technical Data or Computer Software to be Furnished With Restrictions*	Basis for Assertion**	Asserted Rights Category***	Name of Person Asserting Restrictions****
Noncommercial Items			
NONE – Duke University, University of Pennsylvania, University of Texas at Austin	NONE – Duke University, University of Pennsylvania, University of Texas at Austin	NONE – Duke University, University of Pennsylvania, University of Texas at Austin	NONE – Duke University, University of Pennsylvania, University of Texas at Austin

Commercial Items			
NONE – Duke University, University of Pennsylvania, University of Texas at Austin	NONE – Duke University, University of Pennsylvania, University of Texas at Austin	NONE – Duke University, University of Pennsylvania, University of Texas at Austin	NONE – Duke University, University of Pennsylvania, University of Texas at Austin

*For technical data (other than computer software documentation) pertaining to items, components, or processes developed at private expense, identify both the deliverable technical data and each such item, component, or process. For computer software or computer software documentation identify the software or documentation.
**Generally, development at private expense, either exclusively or partially, is the only basis for asserting restrictions. For technical data, other than computer software documentation, development refers to development of the item, component, or process to which the data pertain. The Government's rights in computer software documentation generally may not be restricted. For computer software, development refers to the software. Indicate whether development was accomplished exclusively or partially at private expense. If development was not accomplished at private expense, or for computer software documentation, enter the specific basis for asserting restrictions.
***Enter asserted rights category (e.g., government purpose license rights from a prior contract, rights in SBIR data generated under another contract, limited, restricted, or government purpose rights under this or a prior contract, or specially negotiated licenses).
****Corporation, individual, or other person, as appropriate.
*****Enter "none" when all data or software will be submitted without restrictions.

Date 9/5/2017

Printed Name and Title (b)(6)

Duke University

Signature

(End of identification and assertion)

(c) An offeror's failure to submit, complete, or sign the notification and identification required by paragraph (d) of this provision with its offer may render the offer ineligible for award.

(f) If the Offeror is awarded a contract, the assertions identified in paragraph (d) of this provision shall be listed in an attachment to that contract. Upon request by the Contracting Officer, the Offeror shall provide sufficient information to enable the Contracting Officer to evaluate any listed assertion.

(End of provision)

TECHNICAL DATA OR COMPUTER SOFTWARE PREVIOUSLY DELIVERED TO THE GOVERNMENT (JUN 1995)

The Offeror shall attach to its offer an identification of all documents or other media incorporating technical data or computer software it intends to deliver under this contract with other than unlimited rights that are identical or substantially similar to documents or other media that the Offeror has produced for, delivered to, or is obligated to deliver to the Government under any contract or subcontract. The attachment shall identify--

(a) The contract number under which the data or software were produced;
(b) The contract number under which, and the name and address of the organization to whom, the data or software were most recently delivered or will be delivered; and
(c) Any limitations on the Government's rights to use or disclose the data or software, including, when applicable, identification of the earliest date the limitations expire.
(End of clause)

[ ] Attachment submitted

[ ] No Attachment required

[Team Name] Monthly Technical Report
[P3 Program]

Project PoP: [Start and End Dates for the project]
Reporting Period: [dates report covers]

USRTK v DARPA / 22cv7377(DoD 21-L-0004) / 0053

Summary Slide

- *Brief bulleted or pictorial summary of the data that will be presented as part of this monthly technical update*

USRTK v DARPA / 22cv7377(DoD 21-L-0004) / 0054

Detailed Technical Update Slides

- *As many slides as are necessary to detail the monthly technical progress*
- *This should include data from subcontractors/vendors*
- *The data slides should be technical in nature and show data and results not just text/bullets describing the data*
- *Whenever possible please send the monthly reports as a powerpoint file, if the file is too large, PDF is acceptable*

USRTK v DARPA / 22cv7377(DoD 21-L-0004) / 0055

Additional Items for Discussion

- *Administrative Issues*
- *Questions or Concerns not addressed in previous slides*
- *Major challenges*

USRTK v DARPA / 22cv7377(DoD 21-L-0004) / 0056

Detailed spend plan

- *Include a detailed spend plan indicating how much funds you have received to date, expenditures and remaining funds*

USRTK v DARPA / 22cv7377(DoD 21-L-0004) / 0057

Agreement No.: HR0011-17-2-0069
PRs: HR0011832648
Modification: P00001
Effective Date: January 25, 2018
DARPA's CFDA Number: 12.910
DODAAC: HR0011

Issued by: Defense Advanced Research Projects Agency (DARPA)
Contracts Management Office (CMO)
675 North Randolph Street
Arlington, VA 22203-2114
Attn: Susan K. Shean, Agreements Officer
susan.shean@darpa.mil, 703-526-4128

Recipient: Duke University
Office of Research Administration
2200 West Main Street, Suite 710
Durham, NC 27705
Attn.: (b)(6) @mc.duke.edu

Recipient Identification Numbers/Codes:

DUNS: 044387793
CAGE: 4B478
TIN: 56-0532129

Cooperative Agreement Amount: $12,833,999.00

Accounting and Appropriation Data:

SUBCLIN 000101 ACRN AA	
FUND CITE	AMOUNT
012199 097 0400 000 N 20162017 D 1320 BPPPTT 2016.BT-01.CORE.A DARPA 255	$1,491,338.00
SUBCLIN 000102 ACRN AB	
FUND CITE	
012199 097 0400 000 N 20172018 D 1320 BPPPTT 2017.BT-01.CORE.A DARPA 255	$1,500,000.00
SUBCLIN 000102 ACRN AB	
FUND CITE	
012199 097 0400 000 N 20172018 D 1320 BPPPTT 2017.BT-01.CORE.A DARPA 255	**$1,000,000.00**
Total Funding	**$3,991,338.00**

Payment Office Information:

Defense Financial Accounting Services IN VP DAI DARPA
8899 East 56th Street
Indianapolis, IN 46249-0002
DODAAC: HQ0685

[AWARD SUBJECT TO ELECTRONIC FUNDS TRANSFER (EFT) REQUIREMENT]

Authority: This Agreement is issued pursuant to the authority of 10 U.S.C. § 2358, as amended.

COOPERATIVE AGREEMENT SCHEDULE

The purpose of this Modification is to provide incremental funding as authorized by PR#: HR0011832648, as shown below.

1. Paragraph 8 Agreement Funding is revised as shown below, to incorporate incremental funding in the amount of $1,000,000, and to revise future planned funding.

FROM:

8. Agreement Funding: This Agreement is incrementally funded in the amount of $2,991,338. The Government's obligation to make payments to the Awardee is limited to only those funds obligated by this Agreement or by modification to this Agreement. Subject to availability of funds and continued satisfactory progress on the Agreement as determined by the Government, the Government agrees to provide funding according to the following schedule:

FY16 Commit $1,491,338.00 (HR0011730567 – Base Period)
FY17 Commit: $1,500,000.00 (HR0011730376 – Base Period)
FY18 Planned: $3,173,723.00 (TBD – Base Period)
FY19 Planned: $5,003,544 (TBD - Base Period)
FY20 Planned: $1,665,394.00 (TBD - Base Period)
Total: $12,833,999.00

The Awardee shall notify the AO and AAO in writing promptly whenever the total Agreement amount is expected to exceed the needs of the Awardee for the project period by more than 5%. This notification shall not be required if an application for additional funding is submitted for a continuation award

TO:

8. Agreement Funding: This Agreement is incrementally funded in the amount of **$3,991,338**. The Government's obligation to make payments to the Awardee is limited to only those funds obligated by this Agreement or by modification to this Agreement. Subject to availability of funds and continued satisfactory progress on the Agreement as determined by the Government, the Government agrees to provide funding according to the following schedule:

FY18 Planned: **$2,173,723.00** (TBD – Base Period)
FY19 Planned: $5,003,544 (TBD - Base Period)
FY20 Planned: $1,665,394.00 (TBD - Base Period)
Total: $12,833,999.00

The Awardee shall notify the AO and AAO in writing promptly whenever the total Agreement amount is expected to exceed the needs of the Awardee for the project period by more than 5%. This notification shall not be required if an application for additional funding is submitted for a continuation award.

2. The recipient address on the cover page has been updated to match the System for Award Management information. All other terms and conditions of the Cooperative Agreement remains unchanged.

FOR THE UNITED STATES OF AMERICA,
DEFENSE ADVANCED RESEARCH PROJECTS AGENCY

(b)(6)

Susan K. Shean
Agreements Officer, Contracts Management Office
1/25/18
(Date)

Agreement No.: HR0011-17-2-0069
PR: HR0011832875
Modification: P00002
Effective Date: January 31, 2018
DARPA's CFDA Number: 12.910
DODAAC: HR0011

Issued by: Defense Advanced Research Projects Agency (DARPA)
Contracts Management Office (CMO)
675 North Randolph Street
Arlington, VA 22203-2114
Attn: Susan K. Shean, Agreements Officer
susan.shean@darpa.mil, 703-526-4128

Recipient: Duke University
Office of Research Administration
2200 West Main Street, Suite 710
Durham, NC 27705
Attn.: (b)(6) @mc.duke.edu

Recipient Identification Numbers/Codes:

DUNS: 044387793
CAGE: 4B478
TIN: 56-0532129

Cooperative Agreement Amount: $12,833,999.00

Accounting and Appropriation Data:

SUBCLIN 000101 ACRN AA
FUND CITE — AMOUNT
012199 097 0400 000 N 20162017 D 1320 BPPPTT 2016.BT-01.CORE.A DARPA 255 — $1,491,338.00

SUBCLIN 000102 ACRN AB
FUND CITE
012199 097 0400 000 N 20172018 D 1320 BPPPTT 2017.BT-01.CORE.A DARPA 255 — $1,500,000.00

SUBCLIN **000103** ACRN AB
FUND CITE
012199 097 0400 000 N 20172018 D 1320 BPPPTT 2017.BT-01.CORE.A DARPA 255 — $1,000,000.00

SUBCLIN 000104 ACRN AC
FUND CITE
012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255 — **$1,500,000.00**
Total Funding — **$5,491,338.00**

Payment Office Information:

Defense Financial Accounting Services IN VP DAI DARPA
8899 East 56th Street
Indianapolis, IN 46249-0002
DODAAC: HQ0685

[AWARD SUBJECT TO ELECTRONIC FUNDS TRANSFER (EFT) REQUIREMENT]

Authority: This Agreement is issued pursuant to the authority of 10 U.S.C. § 2358, as amended.

COOPERATIVE AGREEMENT SCHEDULE

The purpose of this Modification is to provide incremental funding as authorized by PR# HR0011832875, as shown below and to correct the SubCLIN added under Contract Modification P00002.

1. The SubCLIN added under Contract Modification P00002 is corrected from 000102 to 000103 as shown in bold on the cover page.

2. Paragraph 8 Agreement Funding is revised as shown below, to incorporate incremental funding in the amount of $1,500,000, and to revise future planned funding.

FROM:

8. Agreement Funding: This Agreement is incrementally funded in the amount of **$3,991,338**. The Government's obligation to make payments to the Awardee is limited to only those funds obligated by this Agreement or by modification to this Agreement. Subject to availability of funds and continued satisfactory progress on the Agreement as determined by the Government, the Government agrees to provide funding according to the following schedule:

FY18 Planned: $ 2,173,723.00 (Base Period)
FY19 Planned: $ 5,003,544.00 (Base Period)
FY20 Planned: $ 1,665,394.00 (Base Period)
Total: $8,842,661.00

The Awardee shall notify the AO and AAO in writing promptly whenever the total Agreement amount is expected to exceed the needs of the Awardee for the project period by more than 5%. This notification shall not be required if an application for additional funding is submitted for a continuation award

TO:

8. Agreement Funding: This Agreement is incrementally funded in the amount of **$5,491,338**. The Government's obligation to make payments to the Awardee is limited to only those funds obligated by this Agreement or by modification to this Agreement. Subject to availability of funds and continued satisfactory progress on the Agreement as determined by the Government, the Government agrees to provide funding according to the following schedule:

FY18 Planned: **$ 673,723.00** (Base Period)
FY19 Planned: $5,003,544.00 (Base Period)
FY20 Planned: $1,665,394.00 (Base Period)
Total: $7,342,661.00

The Awardee shall notify the AO and AAO in writing promptly whenever the total Agreement amount is expected to exceed the needs of the Awardee for the project period by more than 5%. This notification shall not be required if an application for additional funding is submitted for a continuation award.

3. All other terms and conditions of the Cooperative Agreement remain unchanged.

FOR THE UNITED STATES OF AMERICA,
DEFENSE ADVANCED RESEARCH PROJECTS AGENCY

(b)(6)

Susan K. Shean
Agreements Officer, Contracts Management Office

1/31/18
(Date)

Agreement No.: HR0011-17-2-0069
PR: HR0011833965
Modification: P00003
Effective Date: April 10, 2018
DARPA's CFDA Number: 12.910
DODAAC: HR0011

Issued by: Defense Advanced Research Projects Agency (DARPA)
Contracts Management Office (CMO)
675 North Randolph Street, Arlington, VA 22203-2114
Attn: Susan K. Shean, Agreements Officer
susan.shean@darpa.mil, 703-526-4128

Recipient: Duke University
Office of Research Administration
2200 West Main Street, Suite 710, Durham, NC 27705
Attn.: (b)(6) @mc.duke.edu

Recipient Identification Numbers/Codes:

DUNS: 044387793
CAGE: 4B478
TIN: 56-0532129

Cooperative Agreement Amount: $12,833,999.00

Accounting and Appropriation Data:

SUBCLIN 000101 ACRN AA
FUND CITE — AMOUNT
012199 097 0400 000 N 20162017 D 1320 BPPPTT 2016.BT-01.CORE.A DARPA 255 — $1,491,338.00

SUBCLIN 000102 ACRN AB
FUND CITE
012199 097 0400 000 N 20172018 D 1320 BPPPTT 2017.BT-01.CORE.A DARPA 255 — $1,500,000.00

SUBCLIN 000103 ACRN AB
FUND CITE
012199 097 0400 000 N 20172018 D 1320 BPPPTT 2017.BT-01.CORE.A DARPA 255 — $1,000,000.00

SUBCLIN 000104 ACRN AC
FUND CITE
012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255 — $1,500,000.00

SUBCLIN 000104 ACRN AC
FUND CITE
012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255 — **$1,673,723.00**
Total Funding — **$7,165,061.00**

Payment Office Information:
Defense Financial Accounting Services IN VP DAI DARPA
8899 East 56th Street, Indianapolis, IN 46249-0002
DODAAC: HQ0685

[AWARD SUBJECT TO ELECTRONIC FUNDS TRANSFER (EFT) REQUIREMENT]
Authority: This Agreement is issued pursuant to the authority of 10 U.S.C. § 2358, as amended.

COOPERATIVE AGREEMENT SCHEDULE

The purpose of this Modification is to provide incremental funding as authorized by PR# HR0011833965, as shown below.

1. Paragraph 8 Agreement Funding is revised as shown below, to incorporate incremental funding in the amount of $1,673,723, and to revise future planned funding.

FROM:

8. Agreement Funding: This Agreement is incrementally funded in the amount of **$5,491,338**. The Government's obligation to make payments to the Awardee is limited to only those funds obligated by this Agreement or by modification to this Agreement. Subject to availability of funds and continued satisfactory progress on the Agreement as determined by the Government, the Government agrees to provide funding according to the following schedule:

FY18 Planned: $ 673,723.00 (Base Period)
FY19 Planned: $5,003,544.00 (Base Period)
FY20 Planned: $1,665,394.00 (Base Period)
Total: $7,342,661.00

The Awardee shall notify the AO and AAO in writing promptly whenever the total Agreement amount is expected to exceed the needs of the Awardee for the project period by more than 5%. This notification shall not be required if an application for additional funding is submitted for a continuation award

TO:

8. Agreement Funding: This Agreement is incrementally funded in the amount of **$7,165,061**. The Government's obligation to make payments to the Awardee is limited to only those funds obligated by this Agreement or by modification to this Agreement. Subject to availability of funds and continued satisfactory progress on the Agreement as determined by the Government, the Government agrees to provide funding according to the following schedule:

FY18 Planned: $ 673,723.00 (Base Period)
FY19 Planned: $5,003,544.00 (Base Period)
FY20 Planned: $1,665,394.00 (Base Period)
Total: **$5,668,938.00**

The Awardee shall notify the AO and AAO in writing promptly whenever the total Agreement amount is expected to exceed the needs of the Awardee for the project period by more than 5%. This notification shall not be required if an application for additional funding is submitted for a continuation award.

2. This modification incorporates an ACURO approval letter, protocol DARPA-0376.02 dated March 5, 2018.

3. All other terms and conditions of Cooperative Agreement HR0011-17-2-0069 remain unchanged and in full force and effect.

FOR THE UNITED STATES OF AMERICA,
DEFENSE ADVANCED RESEARCH PROJECTS AGENCY

(b)(6)

Susan K. Shean
Agreements Officer, Contracts Management Office

4/10/18
(Date)

Agreement No.: HR0011-17-2-0069
PR: N/A
Modification: **P00004**
Effective Date: April 25, 2018
DARPA's CFDA Number: 12.910
DODAAC: HR0011

Issued by: Defense Advanced Research Projects Agency (DARPA)
Contracts Management Office (CMO)
675 North Randolph Street, Arlington, VA 22203-2114
Attn: Susan K. Shean, Agreements Officer,
susan.shean@darpa.mil, 703-526-4128

Recipient: Duke University
Office of Research Administration 2200 West Main Street, Suite 710, Durham, NC 27705
Attn.: (b)(6) @mc.duke.edu

Recipient Identification Numbers/Codes:

DUNS: 044387793
CAGE: 4B478
TIN: 56-0532129
Cooperative Agreement Amount: $12,833,999.00

Accounting and Appropriation Data:

SUBCLIN 000101 ACRN AA	
FUND CITE	AMOUNT
012199 097 0400 000 N 20162017 D 1320 BPPPTT 2016.BT-01.CORE.A DARPA 255	$1,491,338.00
SUBCLIN 000102 ACRN AB	
FUND CITE	
012199 097 0400 000 N 20172018 D 1320 BPPPTT 2017.BT-01.CORE.A DARPA 255	$1,500,000.00
SUBCLIN 000103 ACRN AB	
FUND CITE	
012199 097 0400 000 N 20172018 D 1320 BPPPTT 2017.BT-01.CORE.A DARPA 255	$1,000,000.00
SUBCLIN 000104 ACRN AC	
FUND CITE	
012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255	$1,500,000.00
SUBCLIN 000104 ACRN AC	
FUND CITE	
012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255	$1,673,723.00
Total Funding	$7,165,061.00

Payment Office Information:

Defense Financial Accounting Services IN VP DAI DARPA
8899 East 56th Street, Indianapolis, IN 46249-0002
DODAAC: HQ0685

[AWARD SUBJECT TO ELECTRONIC FUNDS TRANSFER (EFT) REQUIREMENT]

Authority: This Agreement is issued pursuant to the authority of 10 U.S.C. § 2358, as amended.

COOPERATIVE AGREEMENT SCHEDULE

1. The purpose of this Administrative Modification is to correct the Paragraph 8 Agreement Funding as issued in modification P00003. The future planned funding schedule is adjusted as follows:

FROM:
8. Agreement Funding: This Agreement is incrementally funded in the amount of $7,165,061. The Government's obligation to make payments to the Awardee is limited to only those funds obligated by this Agreement or by modification to this Agreement. Subject to availability of funds and continued satisfactory progress on the Agreement as determined by the Government, the Government agrees to provide funding according to the following schedule:

FY18 Planned: $ 673,723.00 (Base Period)
FY19 Planned: $5,003,544.00 (Base Period)
FY20 Planned: $1,665,394.00 (Base Period)
Total: $5,668,938.00

The Awardee shall notify the AO and AAO in writing promptly whenever the total Agreement amount is expected to exceed the needs of the Awardee for the project period by more than 5%. This notification shall not be required if an application for additional funding is submitted for a continuation award

TO:

8. Agreement Funding: This Agreement is incrementally funded in the amount of $7,165,061. The Government's obligation to make payments to the Awardee is limited to only those funds obligated by this Agreement or by modification to this Agreement. Subject to availability of funds and continued satisfactory progress on the Agreement as determined by the Government, the Government agrees to provide funding according to the following schedule:

FY19 Planned: $4,003,544.00 (Base Period)
FY20 Planned: $1,665,394.00 (Base Period)
Total: $5,668,938.00

The Awardee shall notify the AO and AAO in writing promptly whenever the total Agreement amount is expected to exceed the needs of the Awardee for the project period by more than 5%. This notification shall not be required if an application for additional funding is submitted for a continuation award.

2. All other terms and conditions of Cooperative Agreement HR0011-17-2-0069 remain unchanged and in full force and effect.

FOR THE UNITED STATES OF AMERICA,
DEFENSE ADVANCED RESEARCH PROJECTS AGENCY

(b)(6)

Susan K. Shean
Agreements Officer, Contracts Management Office

4/25/18
(Date)

Agreement No.: HR0011-17-2-0069
PR: **HR0011835421**
Modification: **P00005**
Effective Date: May 23, 2018
DARPA's CFDA Number: 12.910
DODAAC: HR0011

Issued by: Defense Advanced Research Projects Agency (DARPA)
Contracts Management Office (CMO)
675 North Randolph Street, Arlington, VA 22203-2114
Attn: Susan K. Shean, Agreements Officer,
susan.shean@darpa.mil, 703-526-4128

Recipient: Duke University
Office of Research Administration 2200 West Main Street, Suite 710, Durham, NC 27705
Attn.: (b)(6) @mc.duke.edu

Recipient Identification Numbers/Codes:

DUNS: 044387793
CAGE: 4B478
TIN: 56-0532129
Cooperative Agreement Amount: $12,833,999.00

Accounting and Appropriation Data:

SUBCLIN 000101 ACRN AA	
FUND CITE	AMOUNT
012199 097 0400 000 N 20162017 D 1320 BPPPTT 2016.BT-01.CORE.A DARPA 255	$1,491,338.00
SUBCLIN 000102 ACRN AB	
FUND CITE	
012199 097 0400 000 N 20172018 D 1320 BPPPTT 2017.BT-01.CORE.A DARPA 255	$1,500,000.00
SUBCLIN 000103 ACRN AB	
FUND CITE	
012199 097 0400 000 N 20172018 D 1320 BPPPTT 2017.BT-01.CORE.A DARPA 255	$1,000,000.00
SUBCLIN 000104 ACRN AC	
FUND CITE	
012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255	$1,500,000.00
SUBCLIN 000105 ACRN AC	
FUND CITE	
012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255	$1,673,723.00
SUBCLIN 000106 ACRN AC	
FUND CITE	
012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255	**$1,000,000.00**
Total Funding	**$8,165,061.00**

Payment Office Information:

Defense Financial Accounting Services IN VP DAI DARPA
8899 East 56th Street, Indianapolis, IN 46249-0002
DODAAC: HQ0685

[AWARD SUBJECT TO ELECTRONIC FUNDS TRANSFER (EFT) REQUIREMENT]

Authority: This Agreement is issued pursuant to the authority of 10 U.S.C. § 2358, as amended.

COOPERATIVE AGREEMENT SCHEDULE

1. The purpose of this Modification is to provide incremental funding in the amount of $1,000,000 to CLIN 0001 via SubCLIN 000106, ACRN AC, as authorized by PR HR0011835421. As such, Paragraph 8 Agreement Funding is updated as follows:

   8. Agreement Funding: This Agreement is incrementally funded in the amount of **$8,165,061.** The Government's obligation to make payments to the Awardee is limited to only those funds obligated by this Agreement or by modification to this Agreement. Subject to availability of funds and continued satisfactory progress on the Agreement as determined by the Government, the Government agrees to provide funding according to the following schedule:

   FY16 Obligated $1,491,338.00 (Award)
   FY17 Obligated: $1,500,000.00 (Award)
   FY17 Obligated: $1,000,000.00 (P00001)
   FY18 Obligated: $1,500,000.00 (P00002)
   FY18 Obligated: $1,673,723.00 (P00003)
   FY18 Obligated: $1,000,000.00 (P00005)
   FY19 Planned: $3,668,938.00 (TBD)
   FY20 Planned: $1,000,000.00 (TBD)
   Total: $12,833,999.00

   The Awardee shall notify the AO and AAO in writing promptly whenever the total Agreement amount is expected to exceed the needs of the Awardee for the project period by more than 5%. This notification shall not be required if an application for additional funding is submitted for a continuation award.

2. All other terms and conditions of Cooperative Agreement HR0011-17-2-0069 remain unchanged and in full force and effect.

FOR THE UNITED STATES OF AMERICA,
DEFENSE ADVANCED RESEARCH PROJECTS AGENCY

(b)(6)

Susan K. Shean
Agreements Officer, Contracts Management Office

5/23/18
(Date)

Agreement No.: HR0011-17-2-0069
PR: HR0011835421-001
Modification: P00006
Effective Date: July 10, 2018
DARPA's CFDA Number: 12.910
DODAAC: HR0011

Issued by: Defense Advanced Research Projects Agency (DARPA)
Contracts Management Office (CMO)
675 North Randolph Street, Arlington, VA 22203-2114
Attn: Susan K. Shean, Agreements Officer,
susan.shean@darpa.mil, 703-526-4128

Recipient: Duke University
Office of Research Administration 2200 West Main Street, Suite 710, Durham, NC 27705
Attn.: (b)(6) @mc.duke.edu

Recipient Identification Numbers/Codes:

DUNS:	044387793
CAGE:	4B478
TIN:	56-0532129

Cooperative Agreement Amount: $12,833,999.00

Accounting and Appropriation Data: Not applicable to this modification

Payment Office Information:

Defense Financial Accounting Services IN VP DAI DARPA
8899 East 56th Street, Indianapolis, IN 46249-0002
DODAAC: HQ0685

[AWARD SUBJECT TO ELECTRONIC FUNDS TRANSFER (EFT) REQUIREMENT]

Authority: This Agreement is issued pursuant to the authority of 10 U.S.C. § 2358, as amended.

COOPERATIVE AGREEMENT SCHEDULE

The purpose of this Modification is to issue Exhibit B Research Description Document Revision 1 (June 2018) to incorporate administrative changes described below as authorized by PR HR0011835421-001 and to issue Attachment (1) Revision 1 Monthly Financial Status Template to incorporate invoice reporting requirements. As such, the above-numbered agreement is revised as follows:

1. Exhibit B, Research Description Document Revision 1 (June 2018) is issued with the following changes:

a. Throughout the document Animal Testing Tables for "Model FcRn-/- hFcRn Mice" Rationale wording is revised changing bullet wording from "N= 5 per group" to "N>= 10 per group."

b. The paragraph addressing submission of a Monthly Financial Status is changed as shown below to incorporate reference to the new template with invoice reporting requirements and to correct the addressee for the report.

FROM:
The Contractor shall also submit a Monthly Financial Status to provide financial program progress and inform the DARPA Program Manager of any potential cost issues. The financial status shall be prepared using the Attachment (1) template and submitted to kelly.waud.ctr@darpa.mil.

TO:
The Contractor shall also submit a Monthly Financial Status to provide financial program progress and inform the DARPA Program Manager of any potential cost **and invoicing** issues. The financial status shall be prepared using the Attachment (1) **Revision 1** template and submitted to **michael.dalianis.ctr@darpa.mil**.

2. This modification results in no change to the total cost of the Cooperative Agreement and all other terms and conditions of Cooperative Agreement HR0011-17-2-0069 remain unchanged and in full force and effect.

FOR DUKE UNIVERSITY

(b)(6)
(Signature)

(b)(6)
Office of Research Administration
(Name, Title)

07/09/2018
(Date)

FOR THE UNITED STATES OF AMERICA, DEFENSE ADVANCED RESEARCH PROJECTS AGENCY

(b)(6)
Susan K. Shean
Grants Officer
Contracts Management Office

1-11-18
(Date)

Agreement No.: HR0011-17-2-0069
PR: HR0011835421-002
Modification: P00007
Effective Date: December 31, 2018
DARPA's CFDA Number: 12.910
DODAAC: HR0011

Issued by: Defense Advanced Research Projects Agency (DARPA)
Contracts Management Office (CMO)
675 North Randolph Street, Arlington, VA 22203-2114
Attn: Susan K. Shean, Agreements Officer,
susan.shean@darpa.mil, 703-526-4128

Recipient: Duke University
Office of Research Administration 2200 West Main Street, Suite 710, Durham, NC 27705
Attn.: (b)(6) @mc.duke.edu

Recipient Identification Numbers/Codes:

DUNS:	044387793
CAGE:	4B478
TIN:	56-0532129

Cooperative Agreement Amount: $12,833,999.00

Accounting and Appropriation Data: Not applicable to this modification

Payment Office Information:

Defense Financial Accounting Services IN VP DAI DARPA
8899 East 56th Street, Indianapolis, IN 46249-0002
DODAAC: HQ0685

[AWARD SUBJECT TO ELECTRONIC FUNDS TRANSFER (EFT) REQUIREMENT]

Authority: This Agreement is issued pursuant to the authority of 10 U.S.C. § 2358, as amended.

COOPERATIVE AGREEMENT SCHEDULE

The purpose of this administrative Modification is to issue Exhibit B Research Description Document Revision 2 (October 2018) to incorporate changes to the tasking under the Agreement as authorized by PR HR0011835421-002 and to add Exhibit H for the incorporation of ACURO approval letters, as follows:

1. Exhibit B, Research Description Document Revision 2 (October 2018) is issued to accommodate new approaches and pipelines using new equipment and removes tasking and approaches that were less promising from the perspective of the Program Manager:

2. This modification results in no change to the total cost of the Cooperative Agreement and all other terms and conditions of Cooperative Agreement HR0011-17-2-0069 remain unchanged and in full force and effect.

List of Exhibits:

Exhibit A - DARPA Agency Specific Terms and Conditions (September 2017) - 6 pages
Exhibit B - Research Description Document Revision 2 (October 2018) – 19 pages
Exhibit C - Agreement Officer's Representative Appointment Memorandum (September 2017) – 3 pages
Exhibit D – Monthly Financial Status Template (September 2017) – 1 page
Exhibit E – Quarterly Technical Report (September 2017) – 13 pages
Exhibit F – Intellectual Property Assertions (September 2017) – 3 pages
Exhibit G – Monthly Technical Report Power Point Template (September 2017) – N/A
Exhibit H – ACURO Letters:
Amendment to Protocol DARPA-0376.02 (March 5, 2018) – 2 Pages
Amendment to Protocol DARPA-0376.01 (November 9, 2018) – 2 Pages
Amendment to Protocol DARPA-0376.02 (November 9, 2018) – 2 Pages

FOR DUKE UNIVERSITY

(b)(6)

(Signature)

(b)(6)

(Name, Title)

12/27/2018

(Date)

FOR THE UNITED STATES OF AMERICA, DEFENSE ADVANCED RESEARCH PROJECTS AGENCY

(b)(6)

Susan K. Shean
Grants Officer
Contracts Management Office

12.28.18

(Date)

Agreement No.: HR0011-17-2-0069
PR: HR0011835421-002
Modification: P00008
Effective Date: January 4, 2019
DARPA's CFDA Number: 12.910
DODAAC: HR0011

Issued by: Defense Advanced Research Projects Agency (DARPA)
Contracts Management Office (CMO)
675 North Randolph Street, Arlington, VA 22203-2114
Attn: Susan K. Shean, Agreements Officer,
susan.shean@darpa.mil, 703-526-4128

Recipient: Duke University
Office of Research Administration 2200 West Main Street, Suite 710, Durham, NC 27705
Attn.: (b)(6) @mc.duke.edu

Recipient Identification Numbers/Codes:

DUNS: 044387793
CAGE: 4B478
TIN: 56-0532129

Cooperative Agreement Amount: $12,833,999.00

Accounting and Appropriation Data: **REPEATED FOR CLARIFICATON PURPOSES – NO NEW OBLIGATIONS IN P00008**

Accounting and Appropriation Data	AMOUNT
SUBCLIN 000101 ACRN AA 012199 097 0400 000 N 20162017 D 1320 BPPPTT 2016.BT-01.CORE.A DARPA 255	$1,491,338.00
SUBCLIN 000102 ACRN AB 012199 097 0400 000 N 20172018 D 1320 BPPPTT 2017.BT-01.CORE.A DARPA 255	$1,500,000.00
SUBCLIN 000103 ACRN AB 012199 097 0400 000 N 20172018 D 1320 BPPPTT 2017.BT-01.CORE.A DARPA 255	$1,000,000.00
SUBCLIN 000104 ACRN AC 012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255	$1,500,000.00
SUBCLIN **000105** ACRN AC 012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255	$1,673,723.00
SUBCLIN 000106 ACRN AC 012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255	$1,000,000.00
Total Funding	$8,165,061.00

Payment Office Information:

Defense Financial Accounting Services IN VP DAI DARPA
8899 East 56th Street, Indianapolis, IN 46249-0002
DODAAC: HQ0685

[AWARD SUBJECT TO ELECTRONIC FUNDS TRANSFER (EFT) REQUIREMENT]

Authority: This Agreement is issued pursuant to the authority of 10 U.S.C. § 2358, as amended.

COOPERATIVE AGREEMENT SCHEDULE

The purpose of this administrative modification is to clarify that a subCLIN error in modificatons P00003 and P00004 was corrected in P00005, as shown on page 1 and below.

1. Under P00003 and P00004, funding of $1,673,723 was incorrectly issued under subCLIN 000104. This error was corrected to subCLIN 000105 in P00005, as shown below:

P00003 and P00004 INCORRECT

SUBCLIN **000104** ACRN AC
FUND CITE
012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255 $1,673,723.00

P00005 CORRECTED

SUBCLIN **000105** ACRN AC
FUND CITE
012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255 $1,673,723.00

2. This modification results in no change to the total cost of the Cooperative Agreement and all other terms and conditions of Cooperative Agreement HR0011-17-2-0069 remain unchanged and in full force and effect.

FOR THE UNITED STATES OF AMERICA,
DEFENSE ADVANCED RESEARCH PROJECTS AGENCY

(b)(6)

1/4/19

Susan K. Shean
Grants Officer
Contracts Management Office

Agreement No.: HR0011-17-2-0069
PR: **HR0011937768**
Modification: **P00009**
Effective Date: January 10, 2019
DARPA's CFDA Number: 12.910
DODAAC: HR0011

Issued by: Defense Advanced Research Projects Agency (DARPA)
Contracts Management Office (CMO)
675 North Randolph Street, Arlington, VA 22203-2114
Attn: Susan K. Shean, Agreements Officer, susan.shean@darpa.mil, 703-526-4128

Recipient: Duke University
Office of Research Administration 2200 West Main Street, Suite 710, Durham, NC 27705
Attn.: (b)(6) @mc.duke.edu

Recipient Identification Numbers/Codes:

DUNS: 044387793 CAGE: 4B478 TIN: 56-0532129

Cooperative Agreement Amount: $12,833,999.00

Accounting and Appropriation Data:

Accounting and Appropriation Data	AMOUNT
SUBCLIN 000101 ACRN AA 012199 097 0400 000 N 20162017 D 1320 BPPPTT 2016.BT-01.CORE.A DARPA 255	$1,491,338.00
SUBCLIN 000102 ACRN AB 012199 097 0400 000 N 20172018 D 1320 BPPPTT 2017.BT-01.CORE.A DARPA 255	$1,500,000.00
SUBCLIN 000103 ACRN AB 012199 097 0400 000 N 20172018 D 1320 BPPPTT 2017.BT-01.CORE.A DARPA 255	$1,000,000.00
SUBCLIN 000104 ACRN AC 012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255	$1,500,000.00
SUBCLIN 000105 ACRN AC 012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255	$1,673,723.00
SUBCLIN 000106 ACRN AC 012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255 $1,000,000.00	
SUBCLIN 000107 ACRN AD **012199 097 0400 000 N 20192020 D 1300 BPPPTT 2019.BT-01.CORE.A DARPA 255**	**$1,250,886.00**
Total Funding	**$9,415,947.00**

Payment Office Information:

Defense Financial Accounting Services IN VP DAI DARPA
8899 East 56th Street, Indianapolis, IN 46249-0002
DODAAC: HQ0685

[AWARD SUBJECT TO ELECTRONIC FUNDS TRANSFER (EFT) REQUIREMENT]

Authority: This Agreement is issued pursuant to the authority of 10 U.S.C. § 2358, as amended.

COOPERATIVE AGREEMENT SCHEDULE

1. The purpose of this Modification is to provide incremental funding in the amount of $1,250,886 to CLIN 0001 via SubCLIN 000107, ACRN AD, as authorized by PR HR0011937768. As such, Paragraph 8 Agreement Funding is updated as follows:

8. Agreement Funding: This Agreement is incrementally funded in the amount of **$9,415,947.** The Government's obligation to make payments to the Awardee is limited to only those funds obligated by this Agreement or by modification to this Agreement. Subject to availability of funds and continued satisfactory progress on the Agreement as determined by the Government, the Government agrees to provide funding according to the following schedule:

FY16 Obligated $1,491,338.00 (Award)
FY17 Obligated: $1,500,000.00 (Award)
FY17 Obligated: $1,000,000.00 (P00001)
FY18 Obligated: $1,500,000.00 (P00002)
FY18 Obligated: $1,673,723.00 (P00003)
FY18 Obligated: $1,000,000.00 (P00005)
FY19 Obligated: $1,250,886.00 (P00009)
FY20 Planned: $3,418,052.00 (TBD)
Total: $12,833,999.00

The Awardee shall notify the AO and AAO in writing promptly whenever the total Agreement amount is expected to exceed the needs of the Awardee for the project period by more than 5%. This notification shall not be required if an application for additional funding is submitted for a continuation award.

2. All other terms and conditions of Cooperative Agreement HR0011-17-2-0069 remain unchanged and in full force and effect.

FOR THE UNITED STATES OF AMERICA,
DEFENSE ADVANCED RESEARCH PROJECTS AGENCY

(b)(6)

1/10/19

Susan K. Shean
Grants Officer
Contracts Management Office

Agreement No.: HR0011-17-2-0069
PR: HR0011942671
Modification: P00010
Effective Date: August 16, 2019
DARPA's CFDA Number: 12.910
DODAAC: HR0011

Issued by: Defense Advanced Research Projects Agency (DARPA)
Contracts Management Office (CMO)
675 North Randolph Street, Arlington, VA 22203-2114
Attn: Susan K. Shean, Grants Officer, susan.shean@darpa.mil, 703-526-4128

Recipient: Duke University
Office of Research Administration 2200 West Main Street, Suite 710, Durham, NC 27705
Attn.: (b)(6) @mc.duke.edu

Recipient Identification Numbers/Codes:

DUNS: 044387793 CAGE: 4B478 TIN: 56-0532129

Cooperative Agreement Amount: $12,833,999.00

Accounting and Appropriation Data:

Accounting and Appropriation Data	AMOUNT
SUBCLIN 000101 ACRN AA 012199 097 0400 000 N 20162017 D 1320 BPPPTT 2016.BT-01.CORE.A DARPA 255	$ 1,491,338.00
SUBCLIN 000102 ACRN AB 012199 097 0400 000 N 20172018 D 1320 BPPPTT 2017.BT-01.CORE.A DARPA 255	$ 1,500,000.00
SUBCLIN 000103 ACRN AB 012199 097 0400 000 N 20172018 D 1320 BPPPTT 2017.BT-01.CORE.A DARPA 255	$ 1,000,000.00
SUBCLIN 000104 ACRN AC 012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255	$ 1,500,000.00
SUBCLIN 000105 ACRN AC 012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255	$ 1,673,723.00
SUBCLIN 000106 ACRN AC 012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255	$ 1,000,000.00
SUBCLIN 000107 ACRN AD 012199 097 0400 000 N 20192020 D 1300 BPPPTT 2019.BT-01.CORE.A DARPA 255	$ 1,250,886.00
SUBCLIN 000108 ACRN AD **012199 097 0400 000 N 20192020 D 1300 BPPPTT 2019.BT-01.CORE.A DARPA 255**	**$ 1,569,970.00**
Total Funding	**$10,985,917.00**

Payment Office Information:

Defense Financial Accounting Services IN VP DAI DARPA
8899 East 56th Street, Indianapolis, IN 46249-0002
DODAAC: HQ0685

[AWARD SUBJECT TO ELECTRONIC FUNDS TRANSFER (EFT) REQUIREMENT]

Authority: This Agreement is issued pursuant to the authority of 10 U.S.C. § 2358, as amended.

COOPERATIVE AGREEMENT SCHEDULE

The purpose of this Modification is to provide incremental funding in the amount of $1,569,970 to CLIN 0001 via SubCLIN 000108, ACRN AD, as authorized by PR HR0011942671 and to incorporate an Animal Care and Use Review Office (ACURO) letter DARPA-0376 issued June 10, 2019 as Exhibit H.4, as follows.

1. Paragraph 8 Agreement Funding is updated to incorporate the $1,569,970 incremental funding, as shown below in bold:

> 8. Agreement Funding: This Agreement is incrementally funded in the amount of **$10,985,917.** The Government's obligation to make payments to the Awardee is limited to only those funds obligated by this Agreement or by modification to this Agreement. Subject to availability of funds and continued satisfactory progress on the Agreement as determined by the Government, the Government agrees to provide funding according to the following schedule:
>
> FY16 Obligated $1,491,338.00 (Award)
> FY17 Obligated: $1,500,000.00 (Award)
> FY17 Obligated: $1,000,000.00 (P00001)
> FY18 Obligated: $1,500,000.00 (P00002)
> FY18 Obligated: $1,673,723.00 (P00003)
> FY18 Obligated: $1,000,000.00 (P00005)
> FY19 Obligated: $1,250,886.00 (P00009)
> **FY19 Obligated: $1,569,970.00 (P00010)**
> FY20 Planned: **$1,848,082.00** (TBD)
> Total: $12,833,999.00
>
> The Awardee shall notify the AO and AAO in writing promptly whenever the total Agreement amount is expected to exceed the needs of the Awardee for the project period by more than 5%. This notification shall not be required if an application for additional funding is submitted for a continuation award.

2. ACURO letter DARPA-0376 entitled "Duke DARPA Pandemic Prevention Platform (P3)" for protocol A243-17-11 issued June 10, 2019 is incorporated as Exhibit H.4.

3. All other terms and conditions of Cooperative Agreement HR0011-17-2-0069 remain unchanged and in full force and effect.

FOR THE UNITED STATES OF AMERICA,
DEFENSE ADVANCED RESEARCH PROJECTS AGENCY

(b)(6)

8/16/19

Susan K. Shean Date
Grants Officer
Contracts Management Office

Agreement No.: HR0011-17-2-0069
PR: HR0011043382
Modification: P00011
Effective Date: November 6, 2019
DARPA's CFDA Number: 12.910
DODAAC: HR0011

Issued by: Defense Advanced Research Projects Agency (DARPA)
Contracts Management Office (CMO)
675 North Randolph Street, Arlington, VA 22203-2114
Attn: Susan K. Shean, Grants Officer, susan.shean@darpa.mil, 703-526-4128

Recipient: Duke University
Office of Research Administration 2200 West Main Street, Suite 710, Durham, NC 27705
Attn.: (b)(6) @mc.duke.edu

Recipient Identification Numbers/Codes:

DUNS: 044387793 CAGE: 4B478 TIN: 56-0532129

Cooperative Agreement Amount: $12,833,999.00

Accounting and Appropriation Data:

CLIN/ SubCLIN	ACRN	Line of Accounting	Amount
0001/000101	AA	012199 097 0400 000 N 20162017 D 1320 BPPPTT 2016.BT-01.CORE.A DARPA 255	$1,491,338.00
0001/000102	AB	012199 097 0400 000 N 20172018 D 1320 BPPPTT 2017.BT-01.CORE.A DARPA 255	$1,500,000.00
0001/000103	AB	012199 097 0400 000 N 20172018 D 1320 BPPPTT 2017.BT-01.CORE.A DARPA 255	$1,000,000.00
0001/000104	AC	012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255	$1,500,000.00
0001/000105	AC	012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255	$1,673,723.00
0001/000106	AC	012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255	$1,000,000.00
0001/000107	AD	012199 097 0400 000 N 20192020 D 1300 BPPPTT 2019.BT-01.CORE.A DARPA 255	$1,250,886.00
0001/000108	AD	012199 097 0400 000 N 20192020 D 1300 BPPPTT 2019.BT-01.CORE.A DARPA 255	$1,569,970.00
0001/000109	**AE**	**^^^097^2020^2021^^0400^000^^255^D^20602115E00^^^^^1300^00008522^012199^DARPA^BTO -BIOLOGICAL^BPPPTT^2020.BT-01.CORE.^255.00 R&D Cont^^^**	**$1,200,000.00**
		Total Funding	**$12,185,917.00**

Payment Office Information:

Defense Financial Accounting Services IN VP DAI DARPA
8899 East 56th Street, Indianapolis, IN 46249-0002
DODAAC: HQ0685

[AWARD SUBJECT TO ELECTRONIC FUNDS TRANSFER (EFT) REQUIREMENT]

Authority: This Agreement is issued pursuant to the authority of 10 U.S.C. § 2358, as amended.

COOPERATIVE AGREEMENT SCHEDULE

The purpose of this Modification is to provide incremental funding in the amount of $1,200,000 to CLIN 0001 via SubCLIN 000109, ACRN AE, as authorized by PR HR0011043382, as follows.

1. Paragraph 8 Agreement Funding is updated to incorporate the $1,200,000 incremental funding, as shown below in bold:

8. Agreement Funding: This Agreement is incrementally funded in the amount of **$12,185,917.** The Government's obligation to make payments to the Awardee is limited to only those funds obligated by this Agreement or by modification to this Agreement. Subject to availability of funds and continued satisfactory progress on the Agreement as determined by the Government, the Government agrees to provide funding according to the following schedule:

FY16 Obligated	$1,491,338.00 (Award)
FY17 Obligated:	$1,500,000.00 (Award)
FY17 Obligated:	$1,000,000.00 (P00001)
FY18 Obligated:	$1,500,000.00 (P00002)
FY18 Obligated:	$1,673,723.00 (P00003)
FY18 Obligated:	$1,000,000.00 (P00005)
FY19 Obligated:	$1,250,886.00 (P00009)
FY19 Obligated:	$1,569,970.00 (P00010)
FY20 Obligated:	**$1,200,000.00 (P00011)**
FY20 Planned:	**$648,082.00** (TBD)
Total:	$12,833,999.00

The Awardee shall notify the AO and AAO in writing promptly whenever the total Agreement amount is expected to exceed the needs of the Awardee for the project period by more than 5%. This notification shall not be required if an application for additional funding is submitted for a continuation award.

2. All other terms and conditions of Cooperative Agreement HR0011-17-2-0069 remain unchanged and in full force and effect.

FOR THE UNITED STATES OF AMERICA,
DEFENSE ADVANCED RESEARCH PROJECTS AGENCY

(b)(6)

11/6/19

Susan K. Shean — Date
Grants Officer
Contracts Management Office

Agreement No.: HR0011-17-2-0069
PR: HR0011044534
Modification: P00012
Effective Date: January 9, 2020
DARPA's CFDA Number: 12.910
DODAAC: HR0011

Issued by: Defense Advanced Research Projects Agency (DARPA)
Contracts Management Office (CMO)
675 North Randolph Street, Arlington, VA 22203-2114
Attn: Shane C. Lomelin, Grants Officer, shane.lomelin@darpa.mil, 703-526-2771

Recipient: Duke University
Office of Research Administration 2200 West Main Street, Suite 710, Durham, NC 27705
Attn.: (b)(6) @mc.duke.edu

Recipient Identification Numbers/Codes:

DUNS: 044387793 CAGE: 4B478 TIN: 56-0532129

Cooperative Agreement Amount: $12,833,999.00

Accounting and Appropriation Data:

CLIN/ SubCLIN	ACRN	Line of Accounting	Amount
0001/000101	AA	012199 097 0400 000 N 20162017 D 1320 BPPPTT 2016.BT-01.CORE.A DARPA 255	$1,491,338.00
0001/000102	AB	012199 097 0400 000 N 20172018 D 1320 BPPPTT 2017.BT-01.CORE.A DARPA 255	$1,500,000.00
0001/000103	AB	012199 097 0400 000 N 20172018 D 1320 BPPPTT 2017.BT-01.CORE.A DARPA 255	$1,000,000.00
0001/000104	AC	012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255	$1,500,000.00
0001/000105	AC	012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255	$1,673,723.00
0001/000106	AC	012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255	$1,000,000.00
0001/000107	AD	012199 097 0400 000 N 20192020 D 1300 BPPPTT 2019.BT-01.CORE.A DARPA 255	$1,250,886.00
0001/000108	AD	012199 097 0400 000 N 20192020 D 1300 BPPPTT 2019.BT-01.CORE.A DARPA 255	$1,569,970.00
0001/000109	AE	^^^097^2020^2021^^0400^000^^255^D^20602115E00^^^^^1300^000085 22^012199^DARPA^BTO -BIOLOGICAL^BPPPTT^2020.BT-01.CORE.^255.00 R&D Cont^^^	$1,200,000.00
0001/000110	**AE**	**^^^097^2020^2021^^0400^000^^255^D^20602115E00^^^^^1300^000 008522^012199^DARPA^BTO - BIOLOGICAL^BPPPTT^2020.BT-01.CORE.^255.00 R&D Cont^^^**	**$648,082.00**
		Total Funding	**$12,833,999.00**

Payment Office Information:

Defense Financial Accounting Services IN VP DAI DARPA
8899 East 56th Street, Indianapolis, IN 46249-0002
DODAAC: HQ0685

[AWARD SUBJECT TO ELECTRONIC FUNDS TRANSFER (EFT) REQUIREMENT]

Authority: This Agreement is issued pursuant to the authority of 10 U.S.C. § 2358, as amended.

COOPERATIVE AGREEMENT SCHEDULE

The purpose of this Modification is to provide incremental funding in the amount of $648,082 to CLIN 0001 via SubCLIN 000110, ACRN AE, as authorized by PR HR0011044534, and to incorporate an Animal Care and Use Review Office (ACURO) letter DARPA-0376 issued December 23, 2019 as Exhibit H.5 as shown below.

1. Paragraph 5 is adjusted as follows to update the Program Managers:

FROM:

5. DARPA Program Manager: The DARPA Program Manager (PM) representing the Government under this Agreement is:

Defense Advanced Research Projects Agency
Biological Technologies Office (BTO)
ATTN.: Colonel Matt Hepburn
675 North Randolph Street, Arlington, VA 22203-2114
Email: Matt.Hepburn@darpa.mil

TO:
5. DARPA Program Manager: The DARPA Program Manager (PM) representing the Government under this Agreement is:

Defense Advanced Research Projects Agency
Biological Technologies Office (BTO)
ATTN.: Dr. Amy Jenkins
675 North Randolph Street, Arlington, VA 22203-2114
Email: Amy.Jenkins@darpa.mil

2. Paragraph 8 Agreement Funding is updated to incorporate the $648,082 incremental funding, as shown below in bold:

8. Agreement Funding: This Agreement is fully funded in the amount of **$12,833,999.** The Government's obligation to make payments to the Awardee is limited to only those funds obligated by this Agreement or by modification to this Agreement. Subject to availability of funds and continued satisfactory progress on the Agreement as determined by the Government, the Government agrees to provide funding according to the following schedule:

FY16 Obligated	$1,491,338.00 (Award)
FY17 Obligated:	$1,500,000.00 (Award)
FY17 Obligated:	$1,000,000.00 (P00001)
FY18 Obligated:	$1,500,000.00 (P00002)
FY18 Obligated:	$1,673,723.00 (P00003)
FY18 Obligated:	$1,000,000.00 (P00005)
FY19 Obligated:	$1,250,886.00 (P00009)
FY19 Obligated:	$1,569,970.00 (P00010)
FY20 Obligated:	$1,200,000.00 (P00011)
FY20 Obligated:	**$648,082.00 (P00012)**
Total:	$12,833,999.00

The Awardee shall notify the AO and AAO in writing promptly whenever the total Agreement amount is expected to exceed the needs of the Awardee for the project period by more than 5%. This notification shall not be required if an application for additional funding is submitted for a continuation award.

3. ACURO letter DARPA-0376 entitled "Duke DARPA Pandemic Prevention Platform (P3)" issued December 23, 2019 is incorporated as Exhibit H.5.

4. All other terms and conditions of Cooperative Agreement HR0011-17-2-0069 remain unchanged and in full force and effect.

FOR THE UNITED STATES OF AMERICA,
DEFENSE ADVANCED RESEARCH PROJECTS AGENCY

LOMELIN.SHANE.C.(b)(6)
Digitally signed by LOMELIN.SHANE.C.(b)(6)
Date: 2020.01.09 11:16:06 -05'00'

Shane C. Lomelin Date
Grants Officer
Contracts Management Office

Agreement No.: HR0011-17-2-0069
PR: HR0011044534
Modification: P00013
Effective Date: February 19, 2020
DARPA's CFDA Number: 12.910
DODAAC: HR0011

Issued by: Defense Advanced Research Projects Agency (DARPA)
Contracts Management Office (CMO)
675 North Randolph Street, Arlington, VA 22203-2114
Attn: Shane C. Lomelin, Grants Officer, shane.lomelin@darpa.mil, 703-526-2771

Recipient: Duke University
Office of Research Administration 2200 West Main Street, Suite 710, Durham, NC 27705
Attn.: (b)(6) @mc.duke.edu

Recipient Identification Numbers/Codes:

DUNS: 044387793 CAGE: 4B478 TIN: 56-0532129

Cooperative Agreement Amount: $12,833,999.00

Accounting and Appropriation Data: Not Applicable to this modification

Payment Office Information:

Defense Financial Accounting Services IN VP DAI DARPA
8899 East 56th Street, Indianapolis, IN 46249-0002
DODAAC: HQ0685

[AWARD SUBJECT TO ELECTRONIC FUNDS TRANSFER (EFT) REQUIREMENT]

Authority: This Agreement is issued pursuant to the authority of 10 U.S.C. § 2358, as amended.

COOPERATIVE AGREEMENT SCHEDULE

The purpose of this modification is to extend Duke University's agreement, at no additional cost to the Government, from March 26, 2020 to December 26, 2020. Paragraph 2 is adjusted as follows:

FROM:

2. Term: The term of this Agreement commences on September 26, 2017, and continues for 30 months, through March 26, 2020.

The Awardee shall make all requests for no-cost period of performance extensions, in writing, to the Agreements Officer (AO) and Administrative Agreements Officer (AAO), no later than 30 days prior to the end of the current period of performance. The AO and AAO are authorized to grant such request, via modification to the Cooperative Agreement, after receiving approval from the Agreement Officer's Representative and DARPA Program Manager.

TO:

2. Term: The term of this Agreement commences on September 26, 2017, and continues for **39 months**, through **December 26, 2020**.

The Awardee shall make all requests for no-cost period of performance extensions, in writing, to the Agreements Officer (AO) and Administrative Agreements Officer (AAO), no later than 30 days prior to the end of the current period of performance. The AO and AAO are authorized to grant such request, via modification to the Cooperative Agreement, after receiving approval from the Agreement Officer's Representative and DARPA Program Manager.

2. All other terms and conditions of Cooperative Agreement HR0011-17-2-0069 remain unchanged and in full force and effect.

DUKE UNIVERSITY

(b)(6)

(Signature)

(b)(6)

(Name, Title)

2/18/2020

(Date)

FOR THE UNITED STATES OF AMERICA,
DEFENSE ADVANCED RESEARCH
PROJECTS AGENCY

LOMELIN.SHANE.C.(b)(6) Digitally signed by LOMELIN.SHANE.C.(b)(6) Date: 2020.02.19 08:45:04 -05'00'

SHANE C. LOMELIN
Grants Officer
Contracts Management Office

02/19/2020

(Date)

Agreement No.: HR0011-17-2-0069
PR: HR0011046821 / HR0011046823
Modification: P00014
Effective Date: April 6, 2020
DARPA's CFDA Number: 12.910
DODAAC: HR0011

Issued by: Defense Advanced Research Projects Agency (DARPA)
Contracts Management Office (CMO)
675 North Randolph Street, Arlington, VA 22203-2114
Attn: Shane C. Lomelin, Grants Officer, shane.lomelin@darpa.mil, 703-526-2771

Recipient: Duke University
Office of Research Administration 2200 West Main Street, Suite 710, Durham, NC 27705
Attn.: (b)(6) @mc.duke.edu

Recipient Identification Numbers/Codes:

DUNS: 044387793 CAGE: 4B478 TIN: 56-0532129

Cooperative Agreement Amount: **$14,520,128**

Accounting and Appropriation Data:

CLIN/ SubCLIN	ACRN	Line of Accounting	Amount
0001/000101	AA	012199 097 0400 000 N 20162017 D 1320 BPPPTT 2016.BT-01.CORE.A DARPA 255	$1,491,338.00
0001/000102	AB	012199 097 0400 000 N 20172018 D 1320 BPPPTT 2017.BT-01.CORE.A DARPA 255	$1,500,000.00
0001/000103	AB	012199 097 0400 000 N 20172018 D 1320 BPPPTT 2017.BT-01.CORE.A DARPA 255	$1,000,000.00
0001/000104	AC	012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255	$1,500,000.00
0001/000105	AC	012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255	$1,673,723.00
0001/000106	AC	012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255	$1,000,000.00
0001/000107	AD	012199 097 0400 000 N 20192020 D 1300 BPPPTT 2019.BT-01.CORE.A DARPA 255	$1,250,886.00
0001/000108	AD	012199 097 0400 000 N 20192020 D 1300 BPPPTT 2019.BT-01.CORE.A DARPA 255	$1,569,970.00
0001/000109	AE	^^^097^2020^2021^^0400^000^^255^D^20602115E00^^^^^1300^000085 22^012199^DARPA^BTO -BIOLOGICAL^BPPPTT^2020.BT-01.CORE.^255.00 R&D Cont^^^	$1,200,000.00
0001/000110	AE	^^^097^2020^2021^^0400^000^^255^D^20602115E00^^^^^1300^000085 22^012199^DARPA^BTO - BIOLOGICAL^BPPPTT^2020.BT-01.CORE.^255.00 R&D Cont^^^	$648,082.00
0001/000111	**AE**	^^^097^2020^2021^^0400^000^^255^D^20602115E00^^^^^1300^000085 22^012199^DARPA^BTO - BIOLOGICAL^BPPPTT^2020.BT-01.CORE.^255.00 R&D Cont^^^	**$500,000.00**
0002/000201	**AF**	^^^097^2020^2021^^0400^000^^255^D^20602115E00^^^^^1300^000085 22^012199^DARPA^BTO - BIOLOGICAL^BCORTT^2020.BT-01.COREC^255.00 R&D Cont^^^	**$420,000.00**
		Total Funding	**$13,753,999.00**

Payment Office Information:

Defense Financial Accounting Services IN VP DAI DARPA
8899 East 56th Street, Indianapolis, IN 46249-0002
DODAAC: HQ0685

[AWARD SUBJECT TO ELECTRONIC FUNDS TRANSFER (EFT) REQUIREMENT]

Authority: This Agreement is issued pursuant to the authority of 10 U.S.C. § 2358, as amended.

COOPERATIVE AGREEMENT SCHEDULE

The purpose of this modification is to 1) extend Duke University's agreement end date, 2) add incremental funding in the amount of $920,000, 3) add CLIN 0002 for COVID-19 Research, 4) incorporate RDD Revision 3 for additional in-scope tasks, as well as to deliniate specific tasks associated with CLIN 0002, and 5) increase value of agreement from $12,833,999, by $1,686,129, to $14,520,128.

1. Extend POP end date from December 26, 2020, to September 25, 2021.

FROM:

2. Term: The term of this Agreement commences on September 26, 2017, and continues for 39 months, through December 26, 2020.

The Awardee shall make all requests for no-cost period of performance extensions, in writing, to the Agreements Officer (AO) and Administrative Agreements Officer (AAO), no later than 30 days prior to the end of the current period of performance. The AO and AAO are authorized to grant such request, via modification to the Cooperative Agreement, after receiving approval from the Agreement Officer's Representative and DARPA Program Manager.

TO:

2. Term: The term of this Agreement commences on September 26, 2017, and continues for **48 months**, through **September 25, 2021**.

The Awardee shall make all requests for no-cost period of performance extensions, in writing, to the Agreements Officer (AO) and Administrative Agreements Officer (AAO), no later than 30 days prior to the end of the current period of performance. The AO and AAO are authorized to grant such request, via modification to the Cooperative Agreement, after receiving approval from the Agreement Officer's Representative and DARPA Program Manager.

2. Paragraph 8 Agreement Funding is updated to incorporate the $920,000 incremental funding, as shown below in bold:

> 8. Agreement Funding: This Agreement is funded in the amount of **$13,753,999.** The Government's obligation to make payments to the Awardee is limited to only those funds obligated by this Agreement or by modification to this Agreement. Subject to availability of funds and continued satisfactory progress on the Agreement as determined by the Government, the Government agrees to provide funding according to the following schedule:
>
> FY16 Obligated: $1,491,338.00 (Award)
> FY17 Obligated: $1,500,000.00 (Award)
> FY17 Obligated: $1,000,000.00 (P00001)
> FY18 Obligated: $1,500,000.00 (P00002)
> FY18 Obligated: $1,673,723.00 (P00003)
> FY18 Obligated: $1,000,000.00 (P00005)
> FY19 Obligated: $1,250,886.00 (P00009)
> FY19 Obligated: $1,569,970.00 (P00010)

FY20 Obligated:	$1,200,000.00	(P00011)
FY20 Obligated:	$648,082.00	(P00012)
FY20 Obligated:	**$420,000.00**	**(P00014 / NEW CLIN 0002 / Tasks 1.5, 2.5 and 4.3)***
FY20 Obligated:	**$500,000.00**	**(P00014 / CLIN 0001)**
Total:	**$13,753,999.00**	

***Recipient invoicing and DFAS payments for Tasks 1.5, 2.5, and 4.3 only are to be billed and paid against CLIN 0002 to ensure accurate funds tracking.**

The Awardee shall notify the AO and AAO in writing promptly whenever the total Agreement amount is expected to exceed the needs of the Awardee for the project period by more than 5%. This notification shall not be required if an application for additional funding is submitted for a continuation award.

3. CLIN 0002 added for RDD Tasks 1.5, 2.5, 4.3 for COVID-related research.

4. Exhibit B, Research Description Document Revision 3 (March 2020) is issued to accommodate new in scope tasks, as well as tasks 1.5, 2.5, and 4.3 for COVID-related research.

List of Exhibits:
Exhibit A - DARPA Agency Specific Terms and Conditions (September 2017) - 6 pages
Exhibit B - Research Description Document Revision 2 (March 2020) – 19 pages
Exhibit C - Agreement Officer's Representative Appointment Memorandum (September 2017) – 3 pages
Exhibit D – Monthly Financial Status Template (September 2017) – 1 page
Exhibit E – Quarterly Technical Report (September 2017) – 13 pages
Exhibit F – Intellectual Property Assertions (September 2017) – 3 pages
Exhibit G – Monthly Technical Report Power Point Template (September 2017) – N/A
Exhibit H – ACURO Letters:
Amendment to Protocol DARPA-0376.02 (March 5, 2018) – 2 Pages
Amendment to Protocol DARPA-0376.01 (November 9, 2018) – 2 Pages
Amendment to Protocol DARPA-0376.02 (November 9, 2018) – 2 Pages

5. All other terms and conditions of Cooperative Agreement HR0011-17-2-0069 remain unchanged and in full force and effect.

DUKE UNIVERSITY

(b)(6)
(b)(6) o=Duke University, ou=(b)(6) Office of Research Administration, email=(b)(6) (b)(6)@duke.eu, c=US
2020.04.02 14:36:30 -04'00'

(Name, Title)

FOR THE UNITED STATES OF AMERICA, DEFENSE ADVANCED RESEARCH PROJECTS AGENCY

LOMELIN.SHANE.C.(b)(6) Digitally signed by LOMELIN.SHANE.C.(b)(6)
Date: 2020.04.03 08:15:01 -04'00'

SHANE C. LOMELIN
Grants Officer, Contracts Management Office

(Date)

(Date)

Exhibit B

Duke University
Research Description Document
"Duke DARPA Pandemic Prevention Platform (P3)"
Revision 3 (January 2020)

The Contractor shall furnish the necessary personnel, materials, facilities, and other services as may be required to manage and complete the contracted effort in accordance with this Research Description Document (RDD) and the schedule set forth herein. The work and services to be performed hereunder shall be subject to the requirements and standards contained in the above titled Contractor's proposal, copies of which are in the possession of both parties. In the event of an inconsistency between the provisions of this Cooperative Agreement and the Contractor's proposal, the inconsistency shall be resolved by giving precedence in the following order: (1) the attachments to the cooperative agreement, and (2) the Contractor's proposal. The contracted effort is unclassified.

(b)(6) Support for the project may not continue without the active direction of the PI. The PI shall keep the DARPA Program Manager, the Agreements Officer Representative (AOR) and the Agreements Officer informed on contract progress through submission of the reports and other deliverables identified in Exhibit A, DARPA Agency Specific Terms and Conditions (July 2016), Paragraph 5. Reports and Reports Distribution, as described below.

Quarterly R&D Status Report - The contractor shall provide quarterly progress reports as applicable, to the addressees identified in Cooperative Agreement Exhibit A, paragraph 5. b) Report Distribution. The purpose of these reports, 5-10 pages in length, is to present a summary of work completed by RDD tasking and milestones met; the advancement in the state-of-the-art on the research and development involved; discuss any problems encountered; update the program schedule, present the program financial status; and discuss remaining work, using the template below:

(i) A description of progress during the current quarterly reporting period. Please do not include extensive discussions of previous results.
(ii) A table with the following headings: Task (including sub-tasks); milestone/ deliverable; planned start date; planned completion date; percent completion; status (before-, on-, or behind schedule).
(iii) A summary of all problems or areas of concern.
(iv) Planned activities and milestones for the next reporting period.
(v) A description of any major equipment purchased or constructed during the reporting period.
(vi) Notification of any changes in key personnel associated with the contract during the reporting period.
(vii) A summary of substantive information derived from noteworthy trips, meetings, or conferences held in connection with the contract during the reporting period.
(viii) Related accomplishments (publications, patents, media coverage) since the last quarterly report.

Final Technical Report - The Final Technical Report and Final Financial Status Report shall address the topics set forth below and be delivered in accordance with Cooperative Agreement Exhibit A, paragraph 5. b) Report Distribution.

(i) Task Objectives

(ii) Technical Problems
(iii) General Methodology (i.e., literature review, laboratory experiments, surveys, etc.)
(iv) Technical Results
(v) Important Findings and Conclusions
(vi) Significant HardwareDevelopment
(vii) Special Comments
(viii) Implications for FurtherResearch
(vix) Standard Form 298, August 1998

The Contractor shall also submit a MonthlyFinancialStatus to provide financial program progress and inform the DARPA Program Manager of any potential cost issues. The financial status shall be prepared using the Attachment (1) Revision 1 template and submitted to (b)(6) @darpa.mil.

Reports and papers shall be marked using Distribution Statement A: "Approved for Public Release." Papers and articles published as a result of DARPA sponsored research shall include a statement reflecting the sponsorship. In addition, a bibliography of the titles and authors of all such papers are to be included in the Final Technical Report. The cover or title page of each of the above reports or publications prepared, will have the following citation:

Sponsored by:
Defense Advanced Research Projects Agency
Biological Technologies Office (BTO) Program: Pandemic Prevention Platform (P3) Issued by DARPA/CMO under Contract No. HR0011-17-2-0069

Travel is authorized for attendance at the Kickoff Meeting and PI Meetings.

Animal use is anticipated in this effort. The contractor shall obtain all necessary Institutional Animal Care and Utilization Committee (IACUC) approval and demonstrate this approval to the Government prior to beginning experimentation with animals. If animal use is no longer anticipated, or changes significantly from the approved IACUC then the PI must submit a letter stating the discontinuation of animal use for this effort and/or receive appropriate authorization for IACUC changes of previously specified protocols. Unless prior approval by DARPA is given, IACUC documentation must be provided prior to contract award. Absolutely no funds can be used for animal research and testing (including, but not limited to, animal purchase, housing and care) until DoD veterinary approval is granted.

1.0 SCOPE

For each Task Area below, we describe the scientific team, the task area approach, and proposed innovations. Innovation and platform development is expected to occur within an initial 24-month developmental phase with much of the development activities focused on the first 12 months and waning thereafter. A single capability demonstration is planned 24-30 months (with Duke-selected pathogen influenza).

Exhibit B – Research Description Document has been updated in Month 28 (Jan 2020) to reflect deletions and additions based on platform optimization and feedback from DARPA Program Manager/SETA.

Justification of Pathogens (Influenza and Chikungunya virus)

The World Health Organization (WHO) estimates that influenza transmission results in up to 500,000 deaths annually. Furthermore, influenza virus has been estimated to be responsible for an annual economic burden in excess of $87 billion. The current standard of care for influenza prevention is seasonal immunization with a multivalent cocktail of inactivated influenza viruses predicted to be antigenically representative of circulating strains (H1N1, H3N2 and B). The majority of influenza virus vaccine preparations available for human vaccination in the United States are generated via propagation in embryonated eggs using a procedure that has remained virtually unchanged since the mid-1900's. The generation/administration of vaccines in this way **1)** is laborious resulting in a minimum five to six-month lag between virus isolation and vaccine availability **2)** may select for egg-adaptation mutations resulting in a loss of immunogenicity with regard to circulating strains, and **3)** requires sufficient time post- immunization for the development of virus-specific antibodies (~2 weeks). Thus, there is a critical need for fast-acting antiviral countermeasures, such as therapeutic antibodies, that can be rapidly isolated, evolved, manufactured, and safely delivered to at risk individuals. As such, naturally occurring infection with seasonal or a weaponized, highly pathogenic, influenza strain remains a significant global challenge to both civilians and the US military.

Chikungunya virus (CHIKV) is an *Aedes spp.* mosquito-vectored RNA virus and the etiological agent of chikungunya fever and the associated polyarthralgia disease. In recent decades, CHIKV has been responsible for millions of human infections in Africa, the Indian Ocean Islands, India, South America, the Caribbean, and Southeast Asia. Genetic adaptation of CHIKV has facilitated efficient transmission of the virus by *Aedes albopictus* mosquitoes, which have a wide geographic distribution. Epidemic activity of CHIKV has resulted in the emergence of human cases into the United States and Europe with the potential for subsequent localized transmission. As such, CHIKV remains a significant global public health concern and threat to US troops because of the potential for rapid global expansion due to human/mosquito transmission cycles, coupled with the debilitating nature of infection, potential for long-term sequela, and lack of approved vaccines/antivirals. As with influenza virus, there is a critical need for fast-acting antiviral countermeasures against chikungunya virus that can be rapidly isolated, evolved, manufactured, and delivered to at-risk individuals.

Below, we outline the assumptions of expected samples to initiate the viral propagation (TA1), complete antibody evolution (TA2) and produce a countermeasure (TA3) as part of our proposed platform; we have developed a variety of mitigation strategies to ensure we can use a broad array of starting materials. Within the Project Work Description and Requirements section below, we provide clear tasks/subtasks and deliverables for each Task Area, with timelines for each.

Task Area 1 (TA1): Approach/Innovation for On-demand Platform to Grow Virus

We propose a comprehensive P3 virus propagation platform capable of rapid and broad screening of potential culture systems and subsequent down selection for the generation of large-scale virus stocks to support downstream activities in TA2/3. Platform development and optimization will occur at Duke during the Developmental Phase (0-24 months). We will participate in a capability demonstration with influenza (Base).

Virus Propagation – (Task Areas 1.1, 1.2)

A major technical hurdle that must be overcome is the design and implementation of a virus propagation system

with sufficient breadth of susceptibility such that rescue and high titer growth of a diverse range of human viruses (DNA, RNA, enveloped, non-enveloped, etc.) is reasonably assured. Classically, the selection of culture systems for virus rescue/amplification has been predicated on tropism assumptions and knowledge of the virus group of interest. Arboviruses, for example, are inoculated into suckling mice, as well as mammalian and arthropod in vitro culture. Clinical/diagnostic virology laboratories often employ commercially available eukaryotic cells for the isolation and identification of virus in clinical samples. The cells used for viral diagnostics, including primary monkey kidney, human foreskin fibroblast, human embryo fibroblast, rabbit kidney, as well as various cancer/immortalized lines (MDCK, A549, HEp-2, etc.) are chosen because of their broad susceptibility to numerous well-characterized human viruses. Positive cultures are then identified visually (lethality, cytopathic effect (CPE), etc.) or immunocytochemically (ICC; cross-reactive antibodies). These techniques, though effective, are slow and laborious. Furthermore, although cell types used for diagnostics may be susceptible to infection with numerous viruses, they may or may not be competent for the production of sufficient quantities of virus progeny to support antibody discovery, efficacy, and evolution studies. **We therefore propose to develop in TA1 a "Thaw-and-Infect" eukaryotic cell culture array comprised of cell types/lines competent for the isolation and high titer growth of a variety of known and unknown viral isolates**.

Thaw-and-Infect culture array: We will identify appropriate cell types capable of supporting replication and virus stock propagation for all virus families known to induce significant disease in humans. The identified cell types/lines will be curated from appropriate commercial vendors/national repositories (ATCC, BEI Resources, etc.) and amplified (RBL Virology Unit) to generate in-house cryopreserved seed-lots in accordance with vendor recommendations. As possible, we will develop/adapt cell lines to a minimum number of standard growth conditions (medium composition, temperature, atmospheric conditions, etc.) to optimize downstream efficiency of culture array setup and viral growth competency screens. SOPs will be developed for seed stock generation, recovery, and propagation as part of the TA1 virus propagation platform.

We anticipate the final virus culture platform array format to consist of a single or multiple multi-well culture plates. For all cell lines, we will identify a seeding density that results in an infection-appropriate confluence in this format and use this information to generate working frozen stocks of appropriate density that can achieve this confluence within 18-24 h of recovery from cryostasis. The "shelf- life" of working lots of all lines, in terms of percent viability, will be evaluated approximately every 3 months and after cryopreservation. Once we have generated seed and working lots of all relevant cell lines, we will evaluate the ability of the culture array to rescue selected viruses. The focus of effort in the Base agreement will be influenza and CHIKV.

In the event of a public/military health crisis, we recognize that the "virus" could be supplied as: **1)** infectious seed stock of known or unknown sequence/identity, **2)** clinical/surveillance sample from an infected individual, or **3)** viral sequence information without infectious material. However, we envision scenarios 1 and 2 being far more probable (i.e., sponsor-supplied infectious material for subsequent propagation). Therefore, we will evaluate the ability of the Thaw-and-Infect cell array to rescue selected viruses in the following contexts: direct inoculation of infectious seed stock (infectious culture supernatant) and virus "spiked" into an appropriate clinical/surveillance milieu, such as Zika virus in mosquito homogenate or human rotavirus in a fecal suspension.

Because we recognize the potential that during a pandemic outbreak only electronic viral sequence information may be available, we will work with Synthetic Genomics Vaccine, Inc. (b)(6) Task 1.2) to optimize their protocols for the synthesis of error-free viral infectious clone genome for direct transfection Synthesis demonstrations will help to identify areas that can be improved to reduce the time required to synthesize varied virus genomes. Once synthesized, the genomes will be sequence-verified and delivered to Duke for virus rescue. Additionally, we will quantify transfection efficiency of all cell lines in the panel using up to five commercially available transfection reagents and DNA/RNA expressing a standard fluorescent protein.

Using our Thaw-and-Infect cell array platform, we will rapidly identify permissive cell lines and use visual estimation of replication metrics (kinetics of spread and median fluorescence intensity; MFI) to select permissive lines for propagation of large-scale cultures. Briefly, 18-24 h prior to inoculation the cell array will be rescued from cryostasis, re-fed with appropriate growth medium and maintained at standard growth conditions (1 day).

We anticipate the sponsor supplying a minimum of 1.0 mL virus source material, which will be divided evenly for identification and propagation. The virus source material will be uniformly suspended in an appropriate volume of infection medium (~5.0 mL representing a maximum 10-fold dilution), sterile filtered if necessary, and used to directly inoculate our culture array. The inoculum will remain in contact with the cell monolayers for 1-2 h and may involve centrifugation- enhanced inoculation as this has been shown to enhance rescue of some viruses. The inoculum will then be removed, and the cells refreshed with appropriate virus growth medium. Viral replication in the cell culture panel will be imaged every 8-12 h, as described below, using virus sequence-specific Fluorescently-tagged Oligonucleotide Probe (FOPs) hybridization to viral nucleic acid and live cell imaging or monitored more efficiently with a Virus Particle Counter. Virus seed stocks will be harvested at ~72 h from up to n=5 cell lines based on the results of the kinetics/MFI analysis as supernatants/cell lysates (3 days). In addition, culture supernatants will be sent for sequence verification.

Virus detection: Classic methodologies for the visualization of virus infected cells (CPE or ICC) are slow requiring days to weeks for sufficient replication/antigen expression and the availability of appropriate immunological reagents. We propose to significantly expedite this process by instead directly detecting replicating viral nucleic acids via the hybridization of viral sequence-specific (commercially purchased) FOPs in permeabilized live cultures coupled with fluorescent live single cell imaging using a high-throughput multimodal plate reader or by using a Virocyte Virus Particle Counter. During the development phase, and in parallel with our cell culture array curating activities, we will develop/optimize conditions for these detection methodologies using our selected viruses (influenza, chikungunya) and appropriately matched cell culture systems, which we will subsequently include during our cell culture panel qualification tests.

Approximately every 8-12 h following inoculation, the virus growth media will be removed and stored in a matched multi-well plate, followed by reversible permeabilization of the cell monolayers using streptolysin O or comparable reagent. To achieve signal specificity and mitigate background, following permeabilization, we will incubate cultures with tagged oligo-nucleotide pairs capable of fluorescence resonance energy transfer similar as to previously described. We anticipate evaluating n=3-5 probe pairs per virus to determine if there is a preferable target(s) based on virus-specific replication parameters/kinetics. Following completion of this live cell imaging, the monolayers will be washed, virus growth medium returned, and culture incubation continued. Although it may be necessary to incubate propagation cultures for multiple days to obtain peak titers, we anticipate the ability to detect viral nucleic acid using FOPs by 24-48 h post-infection. Once this methodology is fully optimized, we anticipate the ability to rapidly quantify titer (tissue culture infectious dose 50%; TCID50 or focus forming assay; FFA) of in-house generated stocks using readily available commercial reagents (**2 days**).

During the optimization work (0-12 months) new rapid virus particle counting technology became available that could eliminate the process described above with FOPs and accelerate TA1 virus growth assessment. This technology will be incorporated into the TA1 platform workflow and is anticipated to reduce the overall timeline by 20-25%.

Culture scale-up: We will aim to generate virus working stocks of 500 mL per lot at a titer of $\geq 10^6$ FFU/mL to support Plaque Reduction Neutralization Test 90% (PRNT90) assays, fluor-labeling of virus for cell sorts, **w**hole virus ELISA assays and animal model challenge studies**.** We will compare 72 h titers of the top 5 first round cultures down select three candidate cell lines for the generation of large scale working stocks. To decrease the temporal lag between seed lot generation/titration and working lot amplification, we will rescue larger cultures of all five preliminary cell lines from cryostasis while titration plates are incubating/developing. However, only the three "best" lines (based on kinetics of spread, MFI, and titer) will be inoculated to generate the large working lots. Eukaryotic cell culture expansion is the major time-limiting step in large-scale virus propagation. It is standard practice (RBL Virology Unit) to cryopreserve eukaryotic cells at a density between $1x10^6$ and $2x10^6$ cells/mL. Starting with this cell density, we estimate it would take 7-8 days of *in vitro* culture expansion to reach a sufficient cell count ($\geq 1x10^8$) to seed a multi-layer flask (~1,720 cm^2) capable of supporting the growth of a ~500 mL culture. Therefore, to reduce this lag time, we propose to identify conditions for the generation of high density frozen cell stocks (~10^8 cells/mL) similar to as previously described.

For all lines included in the Thaw-and-Infect array, cells will be frozen at various densities (up to 10^8 cells/mL) and the effects of freezing density on viability upon recovery following 7-14 days of cryostasis will be evaluated. If necessary, we will optimize the composition of the freezing medium to maximize the viability of high density cell stocks. Working lots (25 x 1.0 mL aliquots) will be generated for all cell lines at a density that can support virus propagation (up to 500 mL in volume) 24-48 h after sample acquisition, or at the maximal density that does not have a negative impact on post-thaw viability. We will also evaluate the "shelf-life" of working lots of all lines in terms of percent viability every 3 months after cryopreservation.

Prior to inoculation, high density cultures will be rescued from cryostasis, re-fed with appropriate growth medium and maintained at standard growth conditions (1-2 days). The respective virus seed stocks will be uniformly suspended in an appropriate volume of infection medium, sterile filtered if necessary, and used to directly inoculate large cultures. Following an appropriate infection time (1-2 h), the inoculum will then be removed and the cells refreshed with appropriate virus growth medium, and the cultures maintained under standard conditions. Virus yield in large cultures will be monitored every 8-12 hours via rapidly developed quantitative real-timePCR or virus particle counting. Cultures will be harvested upon reaching a copy number of $\geq 10^{10}$ copies/mL (~10^6 infectious units/mL, assuming a 10,000:1 genome to infectious unit ratio) or when less than a 10-fold increase is observed in three subsequent measurements (3-5 days). Working lots of virus will be harvested as cell culture supernatant/lysate and quantified via TCID50 or FFA. Additionally, the genetic diversity/drift of working virus lots relative to source material (or earliest passage for which sufficient material is available to support this analysis) will be evaluated by sequencing.

As mentioned above we will also explore the use of a ViroCyt Virus Particle Counter to monitor virus growth across all aspects of the TA1 platform. This innovative new piece of equipment would be used to count virus particles in culture in order to rapidly evaluate virus growth during virus stock propagation. We will conduct several demo runs to evaluate the ability of this machine to meet our needs. This includes monitoring growth of several different viruses at multiple MOIs, growth media and time points. If this instrument proves to be more efficient at monitoring cell growth, as predicted, we will use this strategy in place of quantitative RT-PCR and FOPs to accelerate viral growth evaluation, saving several hours of time per platform run.

The TA1 platform strategy will result in generation of a working lot of virus sufficient to begin antibody identification and evolution (TA2) and can be used for further amplification/scale-up as necessary. **This will be achieved as follows (10-15 days):**

- Sequence identification of sponsor provided source material and synthesis of FOPs
- Primary down-selection of n=5 virus culture systems and generation of n=5 putative virus seed stocks (passage +1 stocks)
- Infectious unit quantification of virus seed lots and secondary down-selection of n=3 culture systems for working lot amplification
- Virus working lot propagation (passage +2 stocks), 500 mL culture volume and titer $\geq 10^6$ infectious units/mL

If both virus sequence and virus source material are available at the start then an educated jump into a permissive cell line can reduce this timeline to 6-10 days.

TA1 Analytics: To show progeny viruses are bio-identical to original isolate (or earliest passage for which sufficient material is available to support this analysis), Duke will utilize proteomic analyses to characterize virus isolates and progeny viruses used for subsequent countermeasure development. We have established methods in the DHVI/CGMP Analytics group to evaluate virus protein profiles from samples including SDS-PAGE, western blot and RP-UPLC to resolve and detect individual virus proteins that can be compared between stocks. Furthermore, when paired with mass spectrometry, these methods can verify protein identity, sequence (in containment ABI sequencer), and detect glycosylation and other protein modifications. The Duke proteomics facility (https://genome.duke.edu/cores-and-services/proteomics-and- metabolomics), led by (b)(6) (b)(6) is equipped to support this analysis, as required. Similarly, virus stocks can be evaluated using LC-MS methods as a reliable and reproducible way to identify and quantify their lipid composition . Data analysis will

be supported by the DHVI/DARPA P3 statistics team as needed.

Innovation: Our approaches for virus amplification and quantification are built upon proven classic methodologies. However, we believe the combination of rapid quasi-real-time imaging of replicating viral nucleic acid combined with a culture array comprised of representative cell lines capable of supporting replication of all known human virus families will greatly expedite virus replication/quantification. Specifically, this virus growth platform will **1)** rapidly identify and down-select putative culture systems, **2)** reduce incubation times for virus quantification assays, **3)** reduce the lag time between seed and working stock propagation, **4)** enable quick/accurate multiplicity of infection calculation for optimal growth in subsequent virus preps, and **5)** facilitate rapid turnaround neutralization assays for the support of TA2/TA3 activities.

Task Area 2 (TA2): Approach/Innovation for System to Isolate/Evolve Antibodies

The Duke DARPA P3 TA2 platform assumes two possible sample input scenarios based on real- world pandemic experiences (convalescent PBMC/plasma from an infected human or viral isolate/clinical specimen). Our current and innovative platform approaches to antibody isolation, evolution, screening and candidate selection, accommodating both pandemic sample types, are detailed below. During months 1-12 we made significant progress on our overlapping approaches and with DARPA guidance have down-selected and streamlined for focused development of a final TA2 platform:

Convalescent PBMC sample (Tasks 2.1-2.2)

If a peripheral blood sample from an infected (acute/convalescent) individual is available, we propose a streamlined approach. The (b)(6) laboratories have developed a suite of technologies that currently enable the identification and affinity optimization/evolution of neutralizing antibodies from memory B cells and plasma cells. (b)(6) has converted this approach to high-throughput 384 well plate platform and also built out the entire pipeline within BSL3/Select Agent containment. Together these technology platforms/approaches synergize to provide an unprecedented ability to rapidly isolate human antibodies and provide innovation opportunities to shorten the time of antibody isolation and countermeasure delivery.

The (b)(6) lab (UT Austin) will no longer be developing plasma Ab isolation components of the platform. Rather they will be reduced to a warm-ready consulting/content expert role in support of our isolation and evolution approaches. The (b)(6) lab has world-class approaches and tools for isolating plasma antibodies and for analyzing B cell lineages form NGS. These capabilities may benefit Duke P3 in the future as things evolve.

Isolation Approach: Pathogen-specific Memory B or Plasma Cells (b)(6) Duke): During the 24- month developmental phase, fluorophore-labeled whole virions (provided by TA1 team), or other recombinant protein/VLP hooks will be used to sort influenza and chikungunya, specific memory B cells for antibody isolation. In addition, plasma cells will be sorted as single cells. The VH and VL genes of both single pathogen-specific memory B cells or single plasma cells will be amplified by PCR, and the full Abs will be produced by transient transfections using overlapping PCR. Bulk plasma cell sorting will also allow us to eliminate the need for use of fluorophore-labeled virions for sorting.

Innovation: Currently, memory B or plasma cell sorting and pathogen-specific Ab isolation, specificity identification and Ab production takes ~17 days. We propose to innovate to decrease this time to at most 12 days. Within the first six months we plan to streamline our existing RT-PCR process by combining PC primers used in the nested PCR process. This will reduce the number of PCR plates in our first and second round of PCR. Furthermore, the streamlined process will reduce the number of plates that need to be sequenced (in containment ABI sequencer). Reductions in PCR and sequencing will speed up isolation of candidate countermeasure antibodies.

Evolution Approach:. Improving antibody neutralization potency H/L Chain Swapping (b)(6) (b)(6) Duke): Concurrent with fluorescent whole virus-specific single B cell sorting experiments, unpaired antibody variable heavy (VH) chain gene and variable light (VL) chain gene next generation sequencing (NGS) will be performed. Once neutralizing antibodies have been identified from the whole virus-specific single B

cell sorts, Cloanalyst (PMID 24795717) will be used to probe the NGS dataset for additional clonally related VH and VL chain gene sequences. The (b)(6) laboratory (Duke) will use Antigen Receptor Mutation Analyzer for Detection of Low-Likelihood Occurrences (ARMADILLO) (Pubmed 29861171) to computationally determine clade independent somatic hypermutations that have been strongly selected for by the immune system. Mutation events will be ranked by frequency and up to twenty-two of the most frequent amino acid mutations will be selected. Combinatorial libraries containing all mutation pairings possible (up to 1 x10^6 VH or VL chain gene sequences) will be in-house made and rapidly synthesized on the Synthetic Genomics BioXP3200. VH + VL chain gene pairs will be transiently transfected into mammalian cells. The cells with the highest binding to fluorescent whole virus (TA1) will be sorted for RT-PCR of VH + VL chain genes. These antibodies will be tested in neutralization assays (TA1).

Innovation: Instead of using random mutagenesis this approach relies upon next generation sequencing and computational methods to identify mutations selected by the host immune system. Using the Synthetic Genomics BioXP3200 enables large combinatorial libraries to be rapidly synthesized. Combined with mammalian cell display and fluorescent whole-virus single-cell sorting, synergistic mutations that are likely to increase antibody binding affinity - hence increase neutralization potency – can be identified. The improved neutralizing antibody VH/VL chain gene sequences will be transferred to the TA3 team for pre-production analysis.

Task Area 3 (TA3): Approach/Innovation for Countermeasure Delivery

Messenger RNA (mRNA) is a promising new therapeutic delivery platform. While its application to therapeutic targets including infectious diseases is still in its infancy, work by our team and others has shown that it is potentially transformative. We have established an RNA platform that has the combined benefits of potent delivery, safety and straightforward, rapid production suitable to deliver medical countermeasures.

We have shown that our nucleoside-modified mRNA is a safe and rapid platform for delivery of therapeutic antibodies. Systemic administration of 1.4 mg/kg of nucleoside-modified mRNA encapsulated in lipid nanoparticles encoding the anti-HIV-1 antibody VRC01 resulted in plasma antibody titers of ~170 µg/mL 24 h post-injection in humanized mice. Protective antibody titers were maintained for >1 week following a single administration and were maintained at ~40 µg/mL with repeat administration for over five weeks. Importantly, treatment with half this dose was sufficient to provide full protection from intravenous HIV-1 challenge, demonstrating it is a viable delivery platform for passive immunotherapy. In total, these results show that the current modified-mRNA platform approach meets pre-determined success criteria (criteria 1, 2, 3 and 4). We expect that appropriate Ab evolution to increase potency (TA2) will significantly reduce dosing requirements. Under the Duke DARPA P3 program, we will improve and apply the mRNA platform for delivery of antibodies as effective medical countermeasures for emerging infectious diseases.

RNA Platform for Countermeasure Delivery: We are advancing our RNA platform for a number of clinical applications including therapeutics and vaccines. Scalable GMP processes have been established for mRNA and lipid nanoparticle production in partnership with BioNTech GmbH and Acuitas Therapeutics, respectively. Clinical products using these technology platforms have been safely evaluated in humans (ClinicalTrials.gov identifier: NCT03014089). To facilitate seamless integration into the overall Duke DARPA P3 program, we will transfer these processes and associated release methods to the DHVI CGMP facility. This facility can produce RNA countermeasures at the necessary scale (>1,000 doses) and has the required manufacturing, analytical testing, quality, regulatory and clinical expertise to support the program.

The RNA component of the product is produced *in vitro* from a DNA template (using sequences provided by TA2) through a series of enzymatic reactions utilizing bacterially-expressed enzymes that are commercially available. To initiate the production process, template plasmid DNA (produced in *E. coli* or synthesized on a BioXP) is linearized to allow synthesis of runoff transcripts with the desired 3' end. Alternatively, we may use Rolling Circle Amplification (SGI).

Next, mRNA is synthesized with ribonucleotide triphosphates (rNTPs) by T7 DNA-dependent RNA polymerase. Modified nucleoside triphosphates are included in the reaction mixture for incorporation into the RNA product. The template DNA is then degraded by incubation with DNase. Finally, the mRNA is

enzymatically capped by vaccinia capping enzyme, and the cap1 structure is formed by addition of the enzyme 2'-O-methyltransferase. Incorporation of modified nucleosides, such as pseudouridine and 1-methylpseudouridine, and HPLC purification to remove contaminating double-stranded RNA, increases protein translation while rendering the mRNA immunologically silent. CGMP runs up to three-gram scale have been routinely produced and can be scaled-up further, as needed.

Lipid nanoparticles (LNPs) containing an ionizable lipid have proven to be excellent RNA carriers *in vivo* and are currently in clinical trials for siRNA delivery. Purified RNA will be formulated into lipid nanoparticles using a self-assembly process in which an aqueous solution of RNA at pH 4.0 is rapidly mixed with a solution of lipids dissolved in ethanol. This process is conducted using a two pump system to combine the RNA solution and lipid solution in a controlled reaction using a T-junction connector. The ethanol is then removed from the resulting LNP preparation, and the product is exchanged into final formulation buffer by tangential flow diafiltration. Finally, the LNPs are filtered through a 0.2 μm sterilizing grade filter. This process is readily scalable and has routinely been utilized to produce LNP at the one gram scale.

The production processes for RNA and LNPs are rapid and straightforward and can be conducted in less than a week, which makes the platform uniquely positioned for rapid response. The rate- limiting steps of the process are the production of sufficient plasmid-DNA template encoding the biologic of interest and safety testing of the product. We will evaluate opportunities to make improvements in both of these areas as the program is developed (see platform improvements below).

Innovation: In order to develop an RNA-based medical countermeasure with optimal potency, half-life and ease of use in the field; we will evaluate a number of improvements to the platform during the 24- month developmental phase of the project and implement those that support this objective in the capability demonstrations planned throughout the Base. Potential improvements to be evaluated include the following: mRNA modifications such as 5' cap, optimized 5'- and 3'-UTRs and coding sequence, and different lengths of poly(A)-tail, SMART RNA replicons (Synthetically Modified Alpha Replicon Technology), optimized lipid nanoparticle formulations, improved delivery to utilize less invasive routes of administration, and platform integration improvements. Detailed plans for each improvement area are described below.

Optimize LNP delivery formulation: Our first generation formulation showed that high levels of circulating Ab could be produced with intravenous delivery of RNA/LNPs. Since a simpler route of administration and more potent formulation is desired, we will evaluate alternative delivery routes including both systemic and inhalation to identify an optimal method that will deliver a simpler and more potent product. Formulations that increase circulating Ab titer (i.e., ≥ 20% of peak titer), and/or increase duration of expression (i.e., titer > 10 ug/mL for 45 days post-administration) though a simpler delivery route, will be advanced for integration into pre-clinical and clinical testing. Our collaborators, Acuitas Therapeutics and Arcturus Therapeutics, continue to optimize LNP formulation to increase both liver translation and for use with alternative routes of delivery. We will select the best formulations that have also passed their toxicity tests by the criteria above.

So far, we have delivered modified mRNA-LNPs by the intradermal (i.d.), intramuscular (i.m.), intravenous (i.v.), intraperitoneal (i.p.) subcutaneous (s.c.), and inhaled (i.n.) routes with very high levels of protein translation. Based on these data, we intend to move forward with s.c. administration as the primary route of delivery, since it yields high protein expression, is simple, relatively non-invasive with a straightforward regulatory pathway for clinical use. We will examine delivery of modified mRNA-LNP therapeutics using wild type or FcRn-/- hFcRn mouse model and will select the delivery route based on favorable expression profile, ease of administration, and clear path for clinical implementation (see Animal Testing).

Optimize the RNA ((b)(6) *Duke;* (b)(6) *UPenn;* (b)(6) *SGVI):* The ability of an RNA to be translated at the highest level (peak and duration) determines the dose of that RNA required for a therapeutic effect. Increasing inherent translation of RNA therapeutics reduces the dose required to reach protective Ab concentrations. The optimization of protein translation from RNA, therefore, becomes an important component. The mRNA encoding each new therapeutic Ab developed needs to be optimized, including the cap structure, 5' and 3' untranslated regions (UTRs), coding sequence conformation, nucleoside modifications, and poly(A) tail length, whose optimums can differ for every coding sequence and the target cell type. Such

improvements can increase translation by over 50-fold compared to the wild-type mRNA. As these optimizations are not possible on a 60-day time scale, we propose to perform a formal optimization for the cell types targeted by the delivery route to be used determined above using three existing DHVI human monoclonal antibodies. Our plan is to identify the five best optimizations using these three model mAbs to establish an approach to sequence optimization. Using this platform we can quickly screen/select optimal sequences to deliver countermeasures identified in TA2 for CGMP production.

First, the optimal cap structure (enzymatic, NRcap, "CleanCap (Trilink)", isopropylidene dinucleotide cap analogs, and phosphorothioate caps) and poly(A) tail length will be analyzed for the three model mAb coding sequences using primary cells that translate delivered mRNA for the site of injection followed by *in vivo* delivery. Next, various optimal UTRs and coding sequence optimizations will be analyzed. We have developed a set of plasmids containing a 5' sequence that ensures complete enzymatic capping and 3 different 5' and 2 different 3' UTRs that allows optimization of UTRs for any coding sequence using 6 plasmids. This yields a set of plasmids containing the best UTRs combined with the optimal poly(A) tail length. Two different codon optimizations are cloned into each plasmid and each plasmid is linearized and 3 different mRNAs are made containing Ψ, m1Ψ, or Ψ + m5-cytosine nucleoside modifications. The resulting mRNAs will be used to transfect primary cells that represent the tissues exposed though the selected delivery route (i.e., hepatocytes for IV delivery). A platform including the five best mRNA coding sequences will be defined through testing using model human mAbs. This will allow us to rapidly deploy the best modified mRNA constructs for expression of new human mAbs for delivery as countermeasures once the platform is fully developed.

In addition to improvements in modified mRNA, we will also evaluate whether RNA replicons can increase peak Ab titer and extend Ab expression in vivo. SGVI has developed a self-amplifying RNA vector based on an alphavirus derived from the attenuated TC-83 strain of Venezuelan equine encephalitis virus that can overcome innate immune response shutdown (vector termed SMART: Synthetically Modified Alpha Replicon Technology). Whole body IVIS imaging of mice injected with either SMART or TC-83 replicon RNA expressing luciferase protein revealed that the SMART RNA expressed significantly more luciferase on days 1, 3 and 7 post-injection and remained higher than the TC-83 replicon until day 14. In addition, luciferase was detected at time points out to 28 days post SMART RNA injection demonstrating significant duration of expression.

To select the RNA improvements to be incorporated into the overall Duke DARPA P3 platform for capability demonstration, we will evaluate RNA construct designs. Head to head evaluations of the two most potent RNA designs encoding model mAbs will be first evaluated *in vitro* the relevant human primary cell substrate followed by *in vivo* studies in wild-type or FcRn-/- hFcRn mice to evaluate pharmacokinetics and in ferrets as a Flu disease challenge model (see Animal Testing, **Table 1**). The selected platform improvements will then be prepared for incorporation into development runs and scaled up for use in capability demonstrations and clinical trial material production.

Platform Integration Improvements ((b)(6) *Duke;* (b)(6) *SGVI):* We will also evaluate platform improvements aimed at seamlessly integrating RNA production into the overall response and shortening timelines for delivery. The two areas with the most significant opportunity for improvement are reducing the time from Ab sequence to template DNA and the time for product release. We will work to address these bottlenecks using proven new technologies from SGVI (*in vitro* gene assembly, BioXP) and others (rapid sterility testing). Details of the approaches are below. Heavy and light chain Ab genes can be engineered into RNA very rapidly. The process consists of designing overlapping oligos, synthesis of error corrected genes, assembly of the genes into the RNA vector and synthetically amplifying the vector DNA. The amplified DNA can then enter the RNA production process as a critical reagent. Time from Ab gene sequence to template DNA is less than one week; construction of multiple RNA vectors can occur in parallel, so the requirement for more than one construct does not increase time to generate. We will compare performance of synthetically assembled DNA to the traditional plasmid DNA approach in year one to verify resulting RNAs are of identical quality and potency using the proposed analytical release panel and *in vivo* testing. If selected, the synthetic process would be scaled up to support capability demonstrations and GMP clinical trial lot production.

Animal Testing: Animal studies in support of this program could include a number of animal models including

wild-type, FcRn-/- hFcRn mice, IFN-α/βR-/- mice, ferrets, rabbits and non-human primates (NHPs), as well as other appropriate animal models based on the DARPA-provided challenge virus (**Table 1**).

Table 1: Proposed Animal Models for Duke DAPRA P3 Platform Development Phase		
Model	**Purpose**	**Rationale**
WT or FcRn-/- hFcRn Mice	• RNA comparisons • LNP formulation • Dosing optimization • Route optimization	• Used for pK studies with human IgG • N >= 10 per group; 35 day study duration • Reduced induction of anti-human IgG responses
Ferret	• Human Influenza challenge studies	• Clinical symptom/disease model of seasonal influenza virus infection, • N=10 per group (control, countermeasure + challenge) • 1° Endpoints: weight loss, clinical score
Capability Demonstrations / Integration Phase		
WT or FcRn-/- hFcRn Mice	• Dosing study for pre- clinical data	• Used for pK studies with human IgG • N=10 per group, 12 groups for dose finding • Reduced induction of anti-human ○ IgG responses
Ferret	• Human Influenza.challenge studies (Capability Demonstration #1)	• Clinical symptom/disease model of seasonal influenza virus infection • N=10 per group (control, countermeasure + challenge) • 1° Endpoints: weight loss, clinical score,
Non-Human Primate	• Determine countermeasure peak concentration • Determine countermeasure variability (<10%)	• Large animal studies suited to replicating human physiology • N=5 per group (control, countermeasure + challenge); 60 day study • Endpoint: TBD

Studies during platform development phase are planned to assess *in vivo* pharmacokinetics (pK) and potency. The kinetics of the countermeasure *in vivo* will be determined by ELISA using population pK analysis of multiple animals. We will also conduct challenge studies during development as needed.

Studies during the capability demonstrations will assess countermeasure concentration using multiplex Luminex bead-based assays to determine peak human antibody levels and half-life. We will also determine the inter-NHP antibody concentration variance with the expectation that it will not exceed 10%. Appropriate animal models will be selected for the capability demonstration challenge studies (see **Table 1**) and model-specific (animal model and virus) quantifiable metrics will be defined prior to challenges studies (i.e., death, temperature, weight loss, etc.).

Task Area 4: Approach/Innovation for Platform Integration

Across the 30-month program we will develop a fully-integrated end-to-end platform that can start with unknown samples from a viral outbreak and be prepared to produce an efficacious and safe CGMP medical countermeasure scalable to 20,000 doses within 60 days (see Figure 2, below). To achieve successful platform integration, all task areas will work together harmoniously to ensure work is proceeding in an efficient manner. Having activities centered at DHVI with contributions from subcontractors will be key to this efficiency.

TA1 will initiate with viral propagation at the Duke RBL. We are confident our TA1 team will have the

ability in the end-to-end platform to rapidly identify unknown viral pathogens. Furthermore, the "Thaw and Infect" approach coupled with methods to rapidly quantify virus developed by (b)(6) will enable viral propagation. TA1 and TA2 may be initiated concurrently. For TA2, we have proposed multiple, innovated approaches through the Base that can be used regardless of source samples. Furthermore, by centering isolation/evolution strategies at Duke, we can have concurrent activities to yield the most potent antibodies without compromising time. While the antibodies are evolved/isolated, the TA2 analytics teams will be preparing for binding and neutralization testing.

While TA1 and TA2 proceed, TA3 will initiate preparation for CGMP production. Once the top five Ab sequences are selected, the TA3 teams at UPenn/SGVI will verify optimal vector sequence and design in parallel with final potency evaluation. Once potency and vector optimization is complete the Duke team will initiate RNA production of the best RNA mAb countermeasure candidate. LNPs will then be produced to encapsulate the RNA and the RNA:LNP product will undergo fill/finish and release testing will be done. Concurrent to these activities the regulatory/clinical and/or animal study teams will be preparing for subsequent human or animal studies.

Our approach is unique in that we are combining the best of academia and industry to move pandemic preparedness forward with established platform methodologies and novel innovative approaches to accelerate medical countermeasure development and delivery. Process development and capability demonstration work progressing through the three Task Areas above will be coordinated and synergized by a well-established administrative team of forward leaning project managers, financial/contracts managers, regulatory experts, clinical investigators, etc.

Background: Biologic countermeasures, such as monoclonal antibodies (mAbs), are a rapid/effective means of controlling and containing outbreaks of emerging pathogens where no licensed therapeutic or vaccine is available. However, standard paradigms for their production and delivery limits their use as a first- line prophylactic or therapeutic, since the process for their identification, optimization and production can take months to years. The Duke DARPA Pandemic Prevention Platform (P3) team seeks to apply its experience, innovations, cutting-edge research portfolio, and in-house CGMP manufacturing capabilities to greatly expedite mAb countermeasures for future pandemics. The fully integrated platform will be a major advancement in rapid pandemic countermeasure development and will address the significant global challenge pandemic outbreaks have on both civilian and military populations.

The Duke University DARPA P3 team will be centrally based at the Duke Human Vaccine Institute (DHVI), a 220+ person organization for bench-to-bedside research, including a 38,000 gross sq.ft. BSL-2/3 NIAID-built regional biocontainment facility, in-house CGMP manufacturing facility, and clinical trials capabilities, including the capacity for first-in-man phase I clinical trials at the Duke-NIAID Vaccine and Treatment Evaluation Unit (VTEU). The DHVI has, for the past decade, carried out innovative basic and translational research through highly effective national and international collaborations in the fight against emerging infectious diseases. For the DARPA P3 initiative, Duke investigators have partnered with outstanding collaborators with RNA vaccine and therapeutic platforms and manufacturing expertise (b)(6) UPenn, and (b)(6) Synthetic Genomics Vaccines Inc.). This team is unique because: a) it has been working together for 12+ years; b) members are highly respected leaders in their fields; and c) the team can work quickly and expeditiously in an atmosphere of trust and selfless collaboration.

This multi-disciplinary team has the expertise, novel technology and ability to collaborate to attain the DARPA P3 goals. Duke's approach to meeting these goals is to focus on developing platform approaches drawing upon experience in growing a variety of different viruses, performing single-cell Ab isolation and evolution, and developing rapid and efficient RNA CGMP capabilities eliciting active and passive antibody protection.

Goals and Impact: The Duke DARPA P3 program will combine world-class expertise in virology, immunology and CGMP manufacturing to create a fully integrated platform capable of responding to a viral pandemic within 60 days. Within each Task Area, we identify the current state of the art of the platform proposed and how to improve performance and efficiency of the process. In doing so, we have struck a balance between innovation and taking reasonable risks to achieve workable solutions given the short timeframe. Our approach is unique in

that we are combining the best of academia innovation and industry production to move pandemic preparedness forward. Once the platform is developed, we will seek to publish advances to disseminate this important knowledge, as well as commercialize the platform to ensure a global impact to address the significant challenge pandemic outbreaks have on both civilian and military populations.

Our overall strategy (Base) to develop a fully integrated platform that will start with a viral isolate and peripheral blood from an outbreak subject and produce an efficacious and safe current good manufacturing practice (CGMP) medical countermeasure scalable to 20,000 doses within 60 days. Shown are our task area-specific objectives and deliverables. Duke DARPA P3 innovations include novel methods for identifying and culturing known/unknown viruses; creating innovative reagents to isolate protective antibodies; novel computational mAb engineering/evolution platforms; the use of nucleoside-modified mRNA and viral replicons to safely express therapeutic antibodies; and rapid RNA manufacturing and lipid nanoparticle formulation for active and passive antibody delivery. Assembly of existing platform technologies with these low and moderate to high risk innovative approaches will enable the integrated Duke DARPA P3 consortium to ensure product delivery that rises above the current state of the art in the development of pandemic medical countermeasures.

2.0 PROJECT WORK DESCRIPTION AND REQUIREMENTS

Task Area 1: On-Demand Platform to Grow Virus

The goal of the work in Task Area 1 will to be to develop methods to support viral propagation, so that virus can be used for downstream Tasks (i.e., whole virus sorts, ELISA, neutralization assays). Within Task Area 1, there are two subtasks (Task 1.1 and Task 1.2). The **updated (mod. Oct 2018)** task area approach, deliverables, and timeline are provided in the below.

1.1. Virus Propagation System Approach (Duke, (b)(6)

- "Thaw-and-Infect" eukaryotic cell culture array comprised of cell types/lines competent for the isolation and high titer growth of a variety of known and unknown viral isolates.
  - Identify optimal seeding/freezing density for infection-appropriate confluence between 24-36 hours following recovery from cryostasis.
  - ~25 aliquots per cell line.
  - Final format will consist of a single or multiple multi-well culture plates.
- Viral propagation monitored using Fluorescently-tagged oligonucleotide probe (FOPs) hybridization to viral nucleic acid, quantitative real-time PCR or Virus Particle Counter.
- Analysis of virus stocks to ensure quality
- High density frozen cell stocks of the "Thaw and Infect" culture array capable of supporting virus culture scale up.
  - Cell lines chosen based on influenza and CHIKV susceptibility in literature
  - Identify optimal conditions to generate high density frozen cell stocks (up to 10^8 cells/mL) that can support virus propagation (up to 500 mL culture volume) 24-48 hours following recovery from cryostasis.
  - ~25 aliquots per cell line.
  - Final format will be multi-layer flask.
- The "shelf-life" of working lots of all cell lines, in terms of percent viable recovery, will be evaluated for the first 12 months, then annually thereafter.
- Evaluate the ability of the Thaw-and-Infect culture array to rescue influenza virus (ss segmented -RNA) and CHIKV (181/25 vaccine strain; ss +RNA).
- Quality control - Progeny viruses will be sequenced (in containment ABI sequencer) to monitor drift. As directed by DARPA we will utilize proteomic and other biochemical analyses to characterize virus isolates (lipid, protein, and carbohydrate) using established methods in the DHVI/CGMP Analytics group, including SDS-PAGE, western blot and RP- Furthermore, when paired with mass spectrometry, these methods can verify protein identity, sequence, and detect glycosylation and other protein modifications.

1.1 Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
1.1.1	• Initial development of "Thaw and Infect" eukaryotic cell culture array reduced panel capable of supporting influenza virus and CHIKV propagation.	0-6
1.1.2	• Continued development of "Thaw and Infect" eukaryotic cell culture array full panel.	6-18
1.1.3	• FOPs specific for influenza virus detection/titration or alternative Virus Particle Counter approach • FOPs specific for CHIKV detection/titration or alternative Virus Particle Counter approach • Optimized methods/data report	6-12
1.1.4	• Quality-controlled seed stocks of influenza virus to support DARPA P3 tasks • Quality-controlled seed stocks of CHIKV to support DARPA P3 tasks	9-15
1.1.5	• High density frozen cell stocks of 15 cell lines in "Thaw and Infect" culture array capable of supporting Flu and CHIKV propagation within 24-48 hours after thaw.	9-37
1.1.6	• Quality-controlled working stocks of influenza virus to support DARPA P3 tasks • Quality-controlled working stocks of CHIKV to support DARPA P3	12-18

1.2. Viral Infectious Clone Genomes Approach (SGVI, (b)(6)

- SGVI will demonstrate their ability to rapidly synthesize error-free viral infectious flu genomes should a situation arise where sponsor can only provide viral sequence and not infectious source material.
- Overlapping oligonucleotides composing the Flu Hemagglutinin and Neuraminidase genes will be pooled, ligated and amplified by PCR. Amplified PCR products will then be error corrected using enzymatic methods.
- Next promoter ant terminator regions will be added by assembling into linearized plasmid encoding these elements and the final gene expression construct will be amplified.
- Once synthesized, the HA and NA genes will be sequence-verified and delivered to Duke along with plasmids encoding the other 6 Flu genes for virus rescue.
- Quality Control - Progeny viruses will be sequenced (in containment ABI sequencer) to monitor drift and ensure that the progeny are bio-identical to traditionally produced flu. . As directed by DARPA we will utilize proteomic and other biochemical analyses to characterize virus isolates using established methods in the DHVI/CGMP Analytics group, including SDS-PAGE, western blot and RP-UPLC to resolve and detect individual virus proteins that can be compared between stocks.

1.2 Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
1.2.1	• One Influenza virus infectious clone genome	0-12

1.3 Expansion of HD Cell Line Stocks

- Generate HD stocks of the remaining 15 cell lines would be made (≥ 25 aliquots) to ensure full array is ready and transferable

USRTK v DARPA / 22cv7377(DoD 21-L-0004) / 0103

- Each aliquot would be sufficient to seed 2 x 5-layer 875 cm^2 cell culture flasks
- Cells would be ready for infection within 48 hours
- For each cell line, density testing would be performed to identify optimal freeze media and density based on viable recovery after thaw

1.3 Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
1.3.1	• Expand High density frozen cell stocks to 30 cell lines for the "Thaw and Infect" culture array capable of supporting Flu and CHIKV propagation within 24-48 hours after thaw.	31-48

1.4 Virus Specific Impacts on Viral Growth platform

- Run additional virus types through the platform to de-risk virus-dependent differences and make it virus-independent
- We would utilize ~3 different viruses from different families
- This would strengthen the platform, add to knowledge base by assessing virus growth kinetics and cell susceptibility by qPCR along an infection time course
- This would also help to de-risk the platform by allowing us to identify potential differences with viruses other that influenza and CHIKV
- We have noted higher assay variability with flu than with CHIKV (FISH neut assay)
- Some viruses require cell disruption for harvest, which would affect the virus growth monitoring by qPCR

1.4 Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
1.4.1	• Virus specific impacts on Viral Growth platform	31-48

1.5 Coronavirus Disease (COVID 19) - (NEW CLIN 0002)

- This task covers any work outlined in 1.1-1.4 performed for the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Task Area 2: System to Isolate and Evolve Antibodies

The goal of the work in Task Area 2 will to be to isolate neutralizing antibody to Flu, CHKV, and other viruses from acute/vaccinated/convalescent PBMC, improve the antibody ~100-fold in function by *in vitro* antibody evolution and transfer antibody sequence to Task Area 3 for production and delivery optimization/testing. Within Task Area 2, there are two subtasks (**Tasks 2.1 and 2.2**). The task area approaches, deliverables and timelines are provided below.

2.1. Optimize Antibody Isolation from Peripheral Blood Cells and Plasma

- ***Down-selected Isolation Approach.*** *Pathogen-specific Memory B or Plasma Cells (Duke,* (b)(6) : Fluorophore-labeled whole virions (provided by TA1 team) or recombinant labeled protein will be used to sort influenza, chikungunya, and other virus specific memory B cells for antibody H/L chain isolation. In addition, plasma cells may be sorted as single cells. The VH and VL genes of both single pathogen-specific memory B cells or single plasma cells will be amplified by PCR, and the full Abs will be produced by transient transfections using innovative and rapid overlapping PCR.
  - mAb candidates will be produced in small batches and progress through high-throughput binding assays (e.g. whole virus ELISA) and be down-selected for pathogen neutralization in collaboration with TA1 to establish baseline potency prior to handing off to evolution team.

2.1 Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
2.1.1	• Pathogen-specific antibody sequences from PBMCs from Flu-infected subjects using single memory B cell sorts or bulk plasma cells	0-6
2.1.2	• Pathogen-specific antibody sequences from PBMCs from CHKV-infected or other virus-infected subjects using single memory B cell sorts or bulk plasma cells	6-12
2.1.3	• Method for sorting antigen-specific B cells using whole viruses (TA1)	6-12
2.1.4	• Pathogen-specific antibody sequences from PBMCs from Flu and CHKV-infected/vaccinated subjects using single B cell sorts using whole viruses	6-12

2.2. Optimize In Vitro Antibody Evolution

- ***Down-selected and Modified Evolution Approach.*** H/L Chain Swapping *(Duke,* (b)(6) (b)(6)
  - We have down-selected an Ab evolution scheme focused on H/L chain swapping and augmentation of the H/L chain pool form NGS.
  - Concurrent with fluorescent whole virus-specific single B cell sorting experiments, unpaired antibody variable heavy (VH) chain gene and variable light (VL) chain gene next generation sequencing (NGS) will be performed.
  - Once neutralizing antibodies have been identified from the whole virus-specific single B cell sorts, Cloanalyst (PMID 24795717) will be used to probe the NGS dataset for additional clonally related VH and VL chain gene sequences.
  - The (b)(6) laboratory (Duke) will use Antigen Receptor Mutation Analyzer for Detection of Low-Likelihood Occurrences (ARMADILLO) (Pubmed 29861171) to computationally determine clade independent somatic hypermutations that have been strongly selected for by the immune system.
  - Mutation events will be ranked by frequency and up to twenty-two of the most frequent amino acid mutations will be selected. Combinatorial libraries containing all mutation pairings possible (up to 1 x10^6 VH or VL chain gene sequences) will be in-house made and rapidly synthesized on the Synthetic Genomics BioXP3200.
  - VH + VL chain gene pairs will be transiently transfected into mammalian cells. The cells with the highest binding to fluorescent whole virus (TA1) will be sorted for RT-PCR of VH + VL chain genes. These antibodies will be tested in neutralization assays (TA1).

2.2 Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
2.2.1	• Whole virus binding ELISA and neutralization assays for Flu and CHKV	0-18
2.2.2	• Evolved antibody sequences from Flu-infected subjects	12-18
2.2.3	• Evolved antibody sequences from CHKV-infected or other virus-infected subjects	18-24

2.3 Duke DARPA P3 platform Binding Assays and DNA Plasmids for Antibody *In Vitro* Transcription

- Perform ELISAs/Binding assays on samples to support pre-clinical IND-enabling animal studies in TA3
- Provide low-endotoxin Duke DARPA P3 antibody (H/L) plasmids in support of In Vitro Transcription to generate RNA for pre-clinical IND-enabling animal studies in TA3
- Perform ELISA/Binding assays on samples to support pre-clinical IND-enabling animal studies subtask 2.4

USRTK v DARPA / 22cv7377(DoD 21-L-0004) / 0105

2.3 Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
2.3.1	• Duke DARPA P3 platform ELISAs/Binding Assays	31-48
2.3.2	• DNA plasmid templates for Antibody *In Vitro* Transcription	31-48

2.4 Expansion of P3 Platform to Support Isolation of Antibodies from Rare Antigen-Specific Memory B Cells

To identify rare antigen-specific memory B cell subpopulations from peripheral blood, we propose to use our limiting dilution memory B cell culture system. In this method, memory B cells are not pre-selected with antigen-specific hooks, which miss low frequency events. Briefly, we will isolate IgG-positive memory B cells using magnetic-activated cell sorting and culture individual memory B cells at limiting dilution in 96-well tissue culture plates using a stimulation cocktail that promotes cell proliferation and differentiation into antibody-secreting cells. At the end of stimulation, we will measure binding of each individual culture supernatant against up to 8 antigens (including total IgG levels) either via high-throughput ELISA or bead-based flow assay. ELISA will be used for single component antigens whereas the bead-based flow assay will be used for reactivity against whole virions provided by TA1.

Clonally expanded cells are preserved and cells from cultures with confirmed positivity for antigen-binding are fed into the P3 pipeline for isolation of Ig sequences. We will test up to 14,400 individual memory B cells per donor sample. A notable advantage over classic direct PCR approaches is that isolation of Ig gene rearrangements and production of recombinant antibodies occur after functional screening of the starting material.

To increase robustness of the TA2 pipeline we will run proof of concept studies during the expanded performance period to address the following:

- Determine feasibility of approach to isolate monoclonal antibodies from up to two individuals vaccinated against influenza approximately one year prior to sample collection using vaccine strain-matched HA as detection.
- Determine feasibility of approach to isolate monoclonal antibodies from up to two healthy individuals with low titer long-term protective memory against common pathogens to which exposure likely occurred many years prior to sampling (either documented natural exposure or through vaccination). Examples of antigens suitable for this scope include tetanus toxoid, measles, mumps, rubella and CMV.
- In both studies, serum titers will be assessed if matched sera are available.

2.4 Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
2.4.1	• One or more flu-specific monoclonal antibody sequence from a human vaccinated against influenza approximately one year prior to sample collection.	34-40
2.4.2	• One or more pathogen-specific monoclonal antibody sequence from a human with low titer long-term protective memory against a common pathogen	41-48

2.5 Coronavirus Disease (COVID 19) - (NEW CLIN 0002)

- This task covers any work outlined in 2.1-2.4 performed for the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Task Area 3: Deliver Medical Countermeasures

To develop an RNA-based medical countermeasure with optimal potency, half-life and ease of use in the

USRTK v DARPA / 22cv7377(DoD 21-L-0004) / 0106

field; we will evaluate a number of improvements to the platform during the 24-month performance period and implement those that support this objective in a pre-clinical capability demonstration using ferrets and NHPs. Potential improvements to be evaluated include mRNA modifications, SMART RNA replicons (Synthetically Modified Alpha Replicon Technology), and optimized lipid nanoparticle formulations. Within Task Area 3, there are five subtasks (**Subtasks 3.1 - 3.5)**. The task area approaches, deliverables and timelines are provided below.

3.1. Optimize RNA Platform Approach (Duke, UPenn, SGVI)

- We will develop modified RNA using model antibodies to deliver optimal expression.
- Parameters to be evaluated include the following: mRNA modifications such as 5' cap, optimized 5'- and 3'-UTRs and coding sequence, and different lengths of poly(A)-tail. We will develop SMART RNA replicons (Synthetically Modified Alpha Replicon Technology), evaluating 5' cap, UTR improvements as well as replicon improvements. Included in this effort we will compare template DNA derived from standard plasmid minipreps or with synthetic DNA produced by SGVI's gene assembly method (BioXP).
- Using DHVI model influenza monoclonal antibody sequences UPenn will optimize mRNA modifications; SGVI will develop RNA replicon platform; Duke will perform head-to- head-comparison studies in wild type and/or FeRn-/-hFcRn line 32 Tg mice (T32).

3.1 Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
3.1.1	• Optimize RNA for in vivo production of model antibodies	0-9
3.1.2	• Evaluation of modified mRNA and RNA replicon platforms in cell based in vitro models	0-9
3.1.3	• Head-to-head comparison of modified mRNA and RNA replicon platforms in wt or FcRn-/- hFcRn line 32 Tg mice (T32)	9-12

3.2. Optimize LNP formulation Approach

- Our first generation formulation showed that high levels of circulating Ab could be produced with intravenous delivery of mRNA/LNPs. We have evaluated delivery of mRNA-LNPs by the intradermal (i.d.), intramuscular (i.m.), intravenous (i.v.), intraperitoneal (i.p.) subcutaneous (s.c.), and inhaled (i.n.) routes with very high levels of protein translation.
- Based on these data, we intend to move forward with IM, SC and IV administration.
- Formulations that increase circulating Ab titer (i.e., ≥ 20% of peak titer), and/or increase duration of expression (i.e., titer > 10 ug/mL for 45 days post-administration) in mice experiments will be advanced for integration into the final capability demonstrations in Ferrets and NHPs.

3.2 Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
3.2.1	• Evaluate potential RNA formulations with current LNP providers (eg Acuitas, Arcturus for packaging /stabilizing RNAs	0-9
3.2.2	• Test formulations in small animal models (Mice) including wt and FcRn-/- hFcRn Mice	9-24

3.3. Platform Transfer to DHVI-GMP

- At the end of the effort described in Tasks 3.1 and 3.2, we will deliver an optimized process for production of RNA encoded antibodies and an effective system for delivery. For this task, the RNA and LNP production platforms will be implemented at DHVI allowing future options for development and clinical trial material production (to be conducted in DHVI's GMP facility.

3.3 Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
3.3.1	• Platform transition to DHVI (Duke) - Method / Transfer Package	6-15
3.3.2	• Produce countermeasure material for flu ferret and NHP in vivo studies	12-34*

* The time to produce the countermeasure is not expected to take this duration. However, the timeline must align with 2.2.3 and 2.2.4, which is extending the time period.

3.4. Approach to Challenge/pK Animal Studies in Ferret

- After development and down selection of the best RNA and LNP candidates in small animal experiments using model antibodies, we will develop and deliver a Flu countermeasure to Ferrets as a relevant disease model.
- Studies are planned to assess in vivo pharmacokinetics (pK) and potency.
- The kinetics of the countermeasure in vivo will be determined by ELISA using population pK analysis of multiple animals. We will also conduct virus challenge study.
- Studies during this animal capability demonstration will assess countermeasure concentration using multiplex Luminex bead-based assays to determine peak human antibody levels and half-life.

3.4 Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
3.4.1	• In vivo pK RNA/LNP data for Flu counter-measure • Reproducibility data on RNA/LNP for Flu counter-measure	18-24
3.4.2	• In vivo protection capability of Flu counter-measure in ferret challenge model	18-24

3.5. Approach to pK studies conducted in NHP with developed countermeasure

- After testing the selected Flu countermeasure in Ferrets as a relevant disease model, studies will be done to assess in vivo pharmacokinetics (pK) in NHPs as a suitable animal model for human countermeasure expression.
- The kinetics of the countermeasure in vivo will be determined by ELISA using population pK analysis of multiple animals.
- Studies during this capability demonstration will assess countermeasure concentration using multiplex Luminex bead-based assays to determine peak human antibody levels and half- life.
- We will also determine the inter-NHP antibody concentration variance with the expectation that it will not exceed 10%.

3.5 Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
3.5.1	• In vivo pK RNA/LNP data for Flu counter-measure • Reproducibility data on RNA/LNP for Flu counter-measure	18-39

3.6 Development and Testing of IM Delivery of mRNA-LNP countermeasure

- LNP vendor (Acuitas) will formulate model anti-Flu Ab RNA in a panel of at least 5 new LNP formulations for relative in vivo screen for optimal IM expression in mice via IM route (versus IV

USRTK v DARPA / 22cv7377(DoD 21-L-0004) / 0108

route).

3.6 Deliverables and Timelines

Subtask	Deliverable	Timeline (months)
3.6.1	• In vivo pK RNA/LNP data for IM delivered Flu counter-measure • Reproducibility data on RNA/LNP for IM delivered Flu counter- measure	31-48

Task Area 4: Platform Integration

4.1. Capability Demonstration #1 Approach

- Capability demonstration performed with influenza virus at Duke.
- Virus received by Task Area 1 team at Duke.
- Initiate propagation of virus in thaw and infect arrays to develop virus for isolating antigen-specific B cells and for testing antibody neutralization/binding.
- Virus will be provided to Task Area 2 team.
- PBMCs received by Task Area 2 team at Duke.
- Duke will work on antibody isolation from B cells.
- Antibody sequences will be provided to UPenn and/or SGVI for RNA modification.
- Duke will make final RNA sequence selected and enclose the RNA in an LNP (produced at Duke).
- Resulting countermeasure will be tested in vivo in the Ferret Flu challenge model.

4.1 Deliverables and Timeline

Subtask	Deliverable	Timeline (months)
4.1.0	• Data Summary and Report, and Countermeasure	24-30

4.2 Program Management and Platform Integration

- Coordinate generation of mRNA-LNP for NHP study between Duke, Acuitas and Bioqual
- Oversee the design and implementation of NHP study
- Oversee progress of HD cell stocks and expansion of Viral growth platform
- Oversee memory B cell culture subtask, identify and procure samples, and coordinate antibody isolation with TA2 pipeline team
- Coordinate and ensure team prepared for hand off of plasmids (TA2-TA3) and animal samples (TA3-TA2)
- Preparation of monthly progress and quarterly reports
- Verbally or written updates and communication with DARPA PM as needed
- Monitor and ensure project stays within budget proposed
- Pre-IND package preparation upon DARPA direction

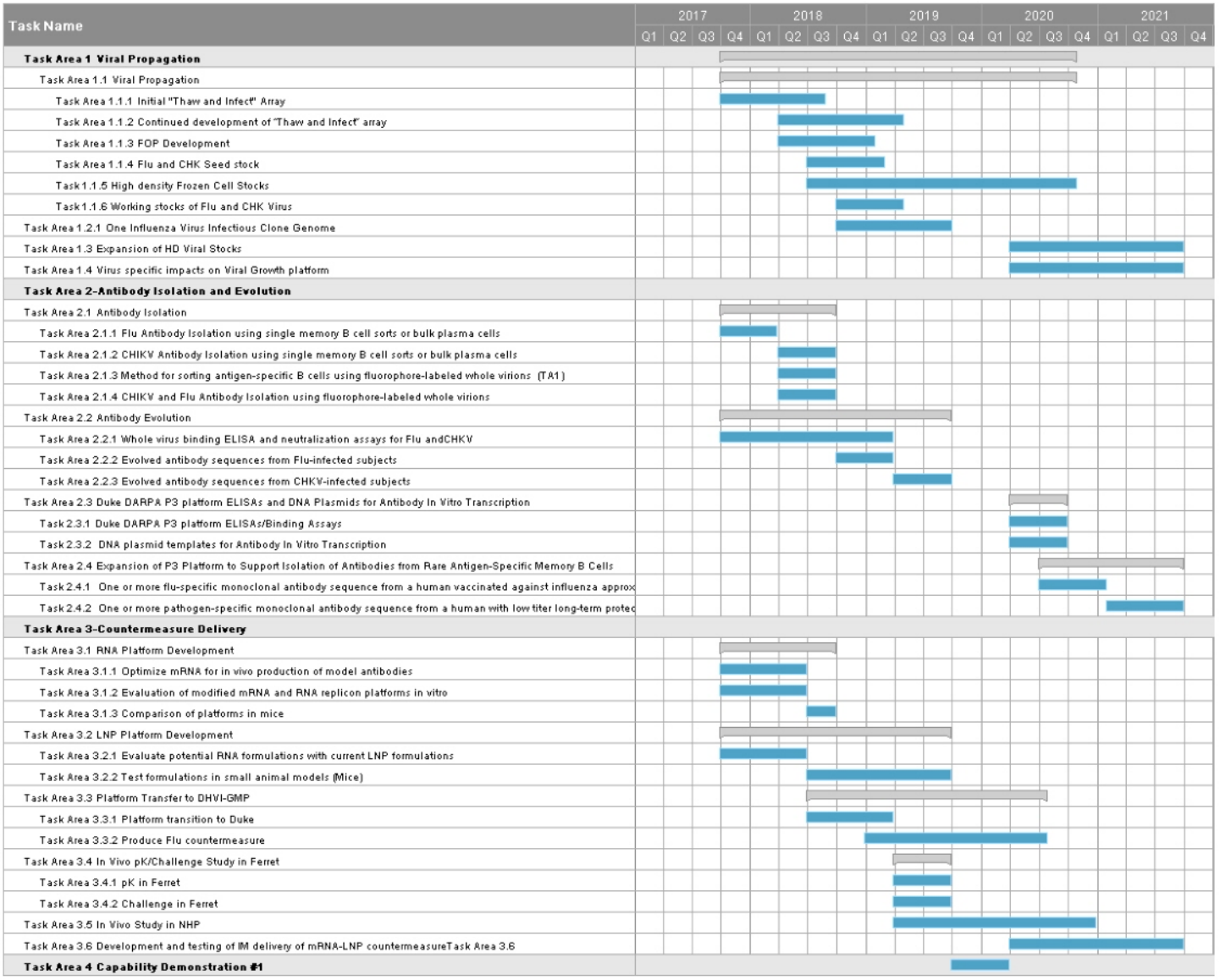
4.2 Deliverables and Timeline

Subtask	Deliverable	Timeline (months)
4.1.0	• Required reports, invoices and supporting documentation	31-48

4.3 Coronavirus Disease (COVID 19) - (NEW CLIN 0002)

- This task covers any work outlined in 4.2 performed for the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Overall Timeline



Agreement No.: HR0011-17-2-0069
PR: HR0011048245
Modification: P00015
Effective Date: August 6, 2020
DARPA's CFDA Number: 12.910
DODAAC: HR0011

Issued by: Defense Advanced Research Projects Agency (DARPA)
Contracts Management Office (CMO)
675 North Randolph Street, Arlington, VA 22203-2114
Attn: Shane C. Lomelin, Grants Officer, shane.lomelin@darpa.mil, 703-526-2771

Recipient: Duke University
Office of Research Administration 2200 West Main Street, Suite 710, Durham, NC 27705
Attn.: (b)(6) @mc.duke.edu

Recipient Identification Numbers/Codes:

DUNS: 044387793 CAGE: 4B478 TIN: 56-0532129

Cooperative Agreement Amount: **$22,168,900**

Accounting and Appropriation Data:

CLIN/ SubCLIN	ACRN	Line of Accounting	Amount
0001/000101	AA	012199 097 0400 000 N 20162017 D 1320 BPPPTT 2016.BT-01.CORE.A DARPA 255	$1,491,338.00
0001/000102	AB	012199 097 0400 000 N 20172018 D 1320 BPPPTT 2017.BT-01.CORE.A DARPA 255	$1,500,000.00
0001/000103	AB	012199 097 0400 000 N 20172018 D 1320 BPPPTT 2017.BT-01.CORE.A DARPA 255	$1,000,000.00
0001/000104	AC	012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255	$1,500,000.00
0001/000105	AC	012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255	$1,673,723.00
0001/000106	AC	012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255	$1,000,000.00
0001/000107	AD	012199 097 0400 000 N 20192020 D 1300 BPPPTT 2019.BT-01.CORE.A DARPA 255	$1,250,886.00
0001/000108	AD	012199 097 0400 000 N 20192020 D 1300 BPPPTT 2019.BT-01.CORE.A DARPA 255	$1,569,970.00
0001/000109	AE	^^^097^2020^2021^^0400^000^^255^D^20602115E00^^^^^1300^000085 22^012199^DARPA^BTO -BIOLOGICAL^BPPPTT^2020.BT-01.CORE.^255.00 R&D Cont^^^	$1,200,000.00
0001/000110	AE	^^^097^2020^2021^^0400^000^^255^D^20602115E00^^^^^1300^000085 22^012199^DARPA^BTO - BIOLOGICAL^BPPPTT^2020.BT-01.CORE.^255.00 R&D Cont^^^	$648,082.00
0001/000111	AE	^^^097^2020^2021^^0400^000^^255^D^20602115E00^^^^^1300^000085 22^012199^DARPA^BTO - BIOLOGICAL^BPPPTT^2020.BT-01.CORE.^255.00 R&D Cont^^^	$500,000.00
0002/000201	AF	^^^097^2020^2021^^0400^000^^255^D^20602115E00^^^^^1300^000085 22^012199^DARPA^BTO - BIOLOGICAL^BCORTT^2020.BT-01.COREC^255.00 R&D Cont^^^	$420,000.00

0002/000202	**AG**	^^^097^2020^2021^^0400^000^^255^D^20602115E00^^^^^1300^00008 522^012199^DARPA^BTO - BIOLOGICAL^BCAPTT^2020.BT-01.COREC^255.00 R&D Cont^^^	**$7,648,772**
		Total Funding	**$21,402,771.00**

Payment Office Information:

Defense Financial Accounting Services IN VP DAI DARPA
8899 East 56th Street, Indianapolis, IN 46249-0002
DODAAC: HQ0685

[AWARD SUBJECT TO ELECTRONIC FUNDS TRANSFER (EFT) REQUIREMENT]

Authority: This Agreement is issued pursuant to the authority of 10 U.S.C. § 2358, as amended.

COOPERATIVE AGREEMENT SCHEDULE

The purpose of this modification is to 1) add incremental funding in the amount of $7,648,772, 2) incorporate Exhibit A, DARPA Specific Terms and Conditions (July 2020) to incorporate Article 15 for the Continuation of Essential Performer Services and Article 16 for Protection of Human Subjects, 3) incorporate RDD Revision 4 for additional in-scope tasks, as well as to deliniate specific tasks associated with CLIN 0002, and rename the title of Exhibit B, thereby designating all tasks under the agreement as Mission Essential Services, 4) increase value of agreement from $14,520,128, by $7,648,772, to $22,168,900, and 5) amend Exhibit H to include ACURO approval DARPA-0376.01, datedJuly 29, 2020.

1. Paragraph 8 Agreement Funding is updated to incorporate the $7,648,772 incremental funding, as shown below in bold:

8. Agreement Funding: This Agreement is funded in the amount of **$21,402,771.** The Government's obligation to make payments to the Awardee is limited to only those funds obligated by this Agreement or by modification to this Agreement. Subject to availability of funds and continued satisfactory progress on the Agreement as determined by the Government, the Government agrees to provide funding according to the following schedule:

FY16 Obligated:	$1,491,338.00	(Award)
FY17 Obligated:	$1,500,000.00	(Award)
FY17 Obligated:	$1,000,000.00	(P00001)
FY18 Obligated:	$1,500,000.00	(P00002)
FY18 Obligated:	$1,673,723.00	(P00003)
FY18 Obligated:	$1,000,000.00	(P00005)
FY19 Obligated:	$1,250,886.00	(P00009)
FY19 Obligated:	$1,569,970.00	(P00010)
FY20 Obligated:	$1,200,000.00	(P00011)
FY20 Obligated:	$648,082.00	(P00012)
FY20 Obligated:	$420,000.00	(P00014 / NEW CLIN 0002 / Tasks 1.5, 2.5 and 4.3)*
FY20 Obligated:	$500,000.00	(P00014 / CLIN 0001)
FY20 Obligated:	**$7,648,772**	**(P00015 / CLIN 0002 / Tasks 3.7, 5.1, and 6.1)***
Total:	**$21,402,771.00**	

***Recipient invoicing and DFAS payments for Tasks 1.5, 2.5, 3.7, 4.3, 5.1, and 6.1 only are to be billed and paid against CLIN 0002 to ensure accurate funds tracking.**

The Awardee shall notify the GO and AGO in writing promptly whenever the total Agreement amount is expected to exceed the needs of the Awardee for the project period by more than 5%. This notification shall not be required if an application for additional funding is submitted for a continuation award.

2. Exhibit A, DARPA Specific Terms and Conditions (July 2020) is issued to incorporate Article 15 for the

Continuation of Essential Performer Services and Article 16 for Protection of Human Subjects.

3. Exhibit B, Research Description Document Revision 4 (July 2020) is issued to add additional in scope tasks for COVID-related research and rename the title, thereby designating all tasks under the agreement as Mission Essential Services.

4. Exhibit H, ACURO notice DARPA-0376.01 dated, July 29, 2020, for protocol A242-17-11 is incorporated as Exhibit H.5.

List of Exhibits:
Exhibit A - DARPA Agency Specific Terms and Conditions (July 2020) - 6 pages
Exhibit B - Research Description Document / Mission Essential Performer Services Plan Revision 4 (July 2020) – 11 pages
Exhibit C - Agreement Officer's Representative Appointment Memorandum (September 2017) – 3 pages
Exhibit D – Monthly Financial Status Template (September 2017) – 1 page
Exhibit E – Quarterly Technical Report (September 2017) – 13 pages
Exhibit F – Intellectual Property Assertions (September 2017) – 3 pages
Exhibit G – Monthly Technical Report Power Point Template (September 2017) – N/A
Exhibit H – ACURO Letters:
Amendment to Protocol DARPA-0376.02 (March 5, 2018) – 2 Pages
Amendment to Protocol DARPA-0376.01 (November 9, 2018) – 2 Pages
Amendment to Protocol DARPA-0376.02 (November 9, 2018) – 2 Pages
Amendment to Protocol DARPA-0376.02 (June 10, 2019) – 2 Pages
Amendment to Protocol DARPA-0376.01 (July 29, 2020) – 2 Pages

3. All other terms and conditions of Cooperative Agreement HR0011-17-2-0069 remain unchanged and in full force and effect.

DUKE UNIVERSITY

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Office of Research Administration,
email=
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2020.08.10 17:01:17 -04'00'

(Signature)

(b)(6)

(Name, Title)

8/7/2020
(Date)

FOR THE UNITED STATES OF AMERICA, DEFENSE ADVANCED RESEARCH PROJECTS AGENCY

LOMELIN.SHANE.C. (b)(6)
Digitally signed by LOMELIN.SHANE.C. (b)(6)
Date: 2020.08.11 10:39:51 -04'00'

SHANE C. LOMELIN
Grants Officer, Contracts Management Office

(Date)

Agreement No.: HR0011-17-2-0069
PR: **HR0011049509**
Modification: **P00016**
Effective Date: September 23, 2020
DARPA's CFDA Number: 12.910
DODAAC: HR0011

Issued by: Defense Advanced Research Projects Agency (DARPA)
Contracts Management Office (CMO)
675 North Randolph Street, Arlington, VA 22203-2114
Attn: Shane C. Lomelin, Grants Officer, shane.lomelin@darpa.mil, 703-526-2771

Recipient: Duke University
Office of Research Administration 2200 West Main Street, Suite 710, Durham, NC 27705
Attn.: (b)(6) @mc.duke.edu

Recipient Identification Numbers/Codes:

DUNS: 044387793 CAGE: 4B478 TIN: 56-0532129

Cooperative Agreement Amount: **$22,168,900**

Accounting and Appropriation Data:

CLIN/ SubCLIN	ACRN	Line of Accounting	Amount
0001/000101	AA	012199 097 0400 000 N 20162017 D 1320 BPPPTT 2016.BT-01.CORE.A DARPA 255	$1,491,338.00
0001/000102	AB	012199 097 0400 000 N 20172018 D 1320 BPPPTT 2017.BT-01.CORE.A DARPA 255	$1,500,000.00
0001/000103	AB	012199 097 0400 000 N 20172018 D 1320 BPPPTT 2017.BT-01.CORE.A DARPA 255	$1,000,000.00
0001/000104	AC	012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255	$1,500,000.00
0001/000105	AC	012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255	$1,673,723.00
0001/000106	AC	012199 097 0400 000 N 20182019 D 1320 BPPPTT 2018.BT-01.CORE.A DARPA 255	$1,000,000.00
0001/000107	AD	012199 097 0400 000 N 20192020 D 1300 BPPPTT 2019.BT-01.CORE.A DARPA 255	$1,250,886.00
0001/000108	AD	012199 097 0400 000 N 20192020 D 1300 BPPPTT 2019.BT-01.CORE.A DARPA 255	$1,569,970.00
0001/000109	AE	^^^097^2020^2021^^0400^000^^255^D^20602115E00^^^^^1300^000085 22^012199^DARPA^BTO -BIOLOGICAL^BPPPTT^2020.BT-01.CORE.^255.00 R&D Cont^^^	$1,200,000.00
0001/000110	AE	^^^097^2020^2021^^0400^000^^255^D^20602115E00^^^^^1300^000085 22^012199^DARPA^BTO - BIOLOGICAL^BPPPTT^2020.BT-01.CORE.^255.00 R&D Cont^^^	$648,082.00
0001/000111	AE	^^^097^2020^2021^^0400^000^^255^D^20602115E00^^^^^1300^000085 22^012199^DARPA^BTO - BIOLOGICAL^BPPPTT^2020.BT-01.CORE.^255.00 R&D Cont^^^	$500,000.00
0002/000201	AF	^^^097^2020^2021^^0400^000^^255^D^20602115E00^^^^^1300^000085 22^012199^DARPA^BTO - BIOLOGICAL^BCORTT^2020.BT-01.COREC^255.00 R&D Cont^^^	$420,000.00

0002/000202	AG	^^^097^2020^2021^^0400^000^^255^D^20602115E00^^^^^1300^00008 522^012199^DARPA^BTO - BIOLOGICAL^BCAPTT^2020.BT-01.COREC^255.00 R&D Cont^^^	$7,648,772
0001/000112	**AE**	^^^097^2020^2021^^0400^000^^255^D^20602115E00^^^^^1300^00008 522^012199^DARPA^BTO -BIOLOGICAL^BPPPTT^2020.BT-01.CORE.^255.00 R&D Cont^^^	**$439,641**
		Total Funding	**$21,842,412.00**

Payment Office Information:

Defense Financial Accounting Services IN VP DAI DARPA
8899 East 56th Street, Indianapolis, IN 46249-0002
DODAAC: HQ0685

[AWARD SUBJECT TO ELECTRONIC FUNDS TRANSFER (EFT) REQUIREMENT]

Authority: This Agreement is issued pursuant to the authority of 10 U.S.C. § 2358, as amended.

COOPERATIVE AGREEMENT SCHEDULE

The purpose of this modification is to provide FY20 incremental funding in the amount of $439,641 for the Duke University Agreement in support of the Pandemic Prevention Platform.

1. Paragraph 8 Agreement Funding is updated to incorporate the $439,641 incremental funding, as shown below in bold:

8. Agreement Funding: This Agreement is funded in the amount of **$21,842,412.** The Government's obligation to make payments to the Awardee is limited to only those funds obligated by this Agreement or by modification to this Agreement. Subject to availability of funds and continued satisfactory progress on the Agreement as determined by the Government, the Government agrees to provide funding according to the following schedule:

FY16 Obligated:	$1,491,338.00	(Award)
FY17 Obligated:	$1,500,000.00	(Award)
FY17 Obligated:	$1,000,000.00	(P00001)
FY18 Obligated:	$1,500,000.00	(P00002)
FY18 Obligated:	$1,673,723.00	(P00003)
FY18 Obligated:	$1,000,000.00	(P00005)
FY19 Obligated:	$1,250,886.00	(P00009)
FY19 Obligated:	$1,569,970.00	(P00010)
FY20 Obligated:	$1,200,000.00	(P00011)
FY20 Obligated:	$648,082.00	(P00012)
FY20 Obligated:	$420,000.00	(P00014 / NEW CLIN 0002 / Tasks 1.5, 2.5 and 4.3)*
FY20 Obligated:	$500,000.00	(P00014 / CLIN 0001)
FY20 Obligated:	$7,648,772.00	(P00015 / CLIN 0002 / Tasks 3.7, 5.1, and 6.1)*
FY20 Obligated:	**$439,641.00**	**(P00016)**
Total:	**$21,842,412.00**	

The Awardee shall notify the GO and AGO in writing promptly whenever the total Agreement amount is expected to exceed the needs of the Awardee for the project period by more than 5%. This notification shall not be required if an application for additional funding is submitted for a continuation award.

2. All other terms and conditions of Cooperative Agreement HR0011-17-2-0069 remain unchanged and in full force and effect.

FOR THE UNITED STATES OF AMERICA,
DEFENSE ADVANCED RESEARCH
PROJECTS AGENCY

LOMELIN.SHANE.C.(b)(6) Digitally signed by LOMELIN.SHANE.C.(b)(6)
Date: 2020.09.23 08:40:37 -04'00'

SHANE C. LOMELIN
Grants Officer, Contracts Management Office

9/23/2020

(Date)