

Efficacy and Broadly Reactive Immunity Induced against Seasonal and Pandemic Strains of Influenza in Variosite Mice

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Variation Biotechnologies, Inc.

Trivalent seasonal influenza vaccines have a number of disadvantages including limited breadth of vaccine-induced immunity and relatively poor efficacy in at-risk populations such as the elderly. We have used a novel technology to develop a synthetic seasonal influenza vaccine designed to provide multi-season protection and address these disadvantages.

Methods We used crystallographic structural data while designing discontinuous B cell epitopes and also considered the location of human T cell epitopes. Our SFV2 vaccine contains 5 immunogens, as either single peptides or cocktails of peptides (Variosite formulations) that contain 16 peptide variants (a total of 35 distinct peptides). These peptide variants account for the antigenic variability present at these epitopes, accounting for past and future antigenic variation of the influenza virus in hemagglutinin (HA) and nucleoprotein (NP). These peptides were entrapped within a liposomal delivery vehicle that was then adsorbed to aluminum hydroxide (NAM-1 adjuvant system). We immunized ferrets intramuscularly on days 0 and 28 with SFV2/NAM1, the NAM1 vehicle lacking the SFV2 antigens, or with commercial influenza vaccine, and 14 days after the final vaccination, animals were challenged with influenza A/Solomon.

Results Peak (day 2) viral load was 1-log lower in animals vaccinated with SFV2/NAM1, and the magnitude and duration of fever was reduced. Efficacy correlated with very high titers of virus-specific serum IgG titers, as well as induction of hemagglutination inhibition (HI) titers. This reactivity extended across many drifted subtypes of influenza, including the recently emerged H1N1/California pandemic isolate.

Conclusions This vaccine and underlying technology represent a novel means of inducing broadly reactive immunity needed to protect against infection with variable pathogens.

Vaccine development and testing at Midwest Research Institute

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Midwest Research Institute

Midwest Research Institute (MRI) is an independent, not-for-profit organization that performs contract research for government and industry. Founded in 1944, MRI has built a reputation for innovation, technical excellence, and problem solving. Today, as one of the nation's leading research institutes, MRI conducts programs in the areas of national security and defense, life sciences, energy, agriculture and food safety, and engineering. With this unparalleled experience working at the intersection of government and life sciences, MRI has developed virus propagation and characterization procedures, animal models, and in vitro assays to support influenza vaccine testing. Recently, MRI has been working with a variety of influenza virus strains, including highly pathogenic avian influenza H5N1, seasonal influenza A and B viruses, and novel viruses such as H1N1 swine influenza virus. In vitro assays include hemagglutination inhibition, virus neutralization, IgG and IgA ELISAs, and proinflammatory cytokines. In vivo study designs include development and implementation of a nose-only bioaerosol inhalation exposure system (NBIES) in order to enable more reliable, reproducible, and quantifiable pathogen exposures. The NBIES allows for better understanding of the pathophysiology of disease, model development, and evaluation of vaccine efficacy. MRI is currently utilizing these capabilities to support development and testing of influenza vaccines; however the technologies have utility for other important public health concerns including anthrax and plague and more common diseases caused by other respiratory pathogens.

From Foot and Mouth to Pandemic Influenza: A Rapid Viral Diagnostic Screening for People and Livestock

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New tools and approaches are warranted for rapid detection of all the known as well as the unknown viral pathogens. Globalization of travel and the transportation of livestock coincident with rapidly emerging and mutant forms of viral pathogens necessitate a rapid and broad viral diagnostic detection capability. A rapid screening test to detect viral infection well before the "signs and symptoms" are evident is highly desirable. Early detection will enable effective quarantine, isolation and timely therapeutic intervention. This capability would enable more effective protection of citizens, travelers and livestock imports and exports. Due to the lack of effective anti-viral treatments and the rapidity of viral mutations there exists a technology gap globally that requires full attention.

The RPS Viral Detectors have filled the gap by commercializing a line of diagnostics for broad screening of viral infection in humans and livestock. The high throughput Viral Detector ELISA is available from RPS now. The rapid point-of-care (POCT) Viral Detector will be available within a few months. Using a simple noninvasive finger stick (drop of blood) as the sample, the RPS Viral Detectors can test for the presence of a viral infection even before the clinical "signs and symptoms" are evident. The Viral Detector POCT takes 10 minutes from sampling to test result.

Decades of research have been conducted on the role of the Mx gene in the human and animal innate immune response to viral infections. Within a few hours after infection, the Mx gene is turned on and the gene product, the Mx protein increases in the blood while the clinical "signs and systems" are not exhibited for a few more days. The RPS Viral Detectors utilize patented anti-Mx antibodies that bind with high affinity for the Mx gene products in the sample.

Reduction of Vesicant Toxicity by Ebselen and a Related Analog

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Vesicants exhibit a wide array of effects on the body including, but not limited to, bone marrow suppression and the blistering of skin. Skin lesions induced by vesicants are prone to infection. Though no effective antidote exists for vesicant poisoning, here we report that the organoselenium drug ebselen (EB-1) exhibits dual efficacy with regard to the toxicity of nitrogen mustard (HN2). It attenuates the toxicity of HN2 in cultured skin cells while inhibiting the growth of several types of microbes including certain types of yeasts and gram positive bacteria. The experiments described here also show that a structural analog of EB (EB-2), like the parent compound, protects skin cells from vesicant toxicity. To characterize the effect of the organoselenium compounds upon HN2 toxicity, skin cells were treated *in vitro* with HN2, a commercially available vesicant, in the presence or absence of EB-1 or the related analog EB-2. Cell viability was then quantified biochemically and microscopically. Our studies have generated important new information concerning the structure-activity relationship of organoselenium compounds as anti-vesicants. The studies shown here may lay the groundwork for using EB or its one of its analogs as an effective treatment for vesicant injury *in vivo*.

Mesenchymal stem cells overexpressing Extracellular Superoxide Dismutase as a Radiological and Nuclear Threat Medical Countermeasure

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Exposure to high dose of ionizing radiation leads to death, lifespan shortening, cataractogenesis, and carcinogenesis. At present, no approved drugs or therapies are available to treat radiation injuries. Formation of superoxide anion (O_2^-) after ionizing radiation is a major determinant of radiation injuries. Irradiated tissues release O_2^- for days to months after radiation exposure. Extracellular superoxide dismutase (ECSOD) is a potent antioxidant enzyme. Mesenchymal stem cells (MSCs), a subset of bone marrow stem cells, migrate to radiation injured tissues after intravenous administration.

To test our hypothesis that MSCs overexpressing ECSOD have a therapeutic effect for radiation injuries, human and mouse MSCs were transduced with Ad5CMVECSOD, an adenovirus carrying human ECSOD gene, and secretions of high level biologically active ECSOD were detected. Mice were then given 9 Gy total body γ irradiation and 24 hours later a tail vein injection of Ad5CMVECSOD-transduced mouse MSCs (ECSOD-mMSCs), Ad5CMVntlacZ-transduced mouse MSCs (ntlacZ-mMSCs), or phosphate-buffered saline (PBS). Remarkably, 52% of mice in ECSOD-mMSCs treatment group survived for 35 days, whereas only 9% of mice in ntlacZ-mMSCs treatment group and 10% of mice in PBS treatment group survived for 35 days (Abdel-Mageed et al., Blood 2009; 113:1201; Patent "PCT/US09/48754", filed June 26, 2009).

Mice that had survived for 35 days were then monitored for their entire lifespan. Mice in ECSOD-mMSCs treatment group survived 207 days longer than mice in PBS or ntlacZ-mMSCs treatment group. Mice in ECSOD-mMSCs treatment group developed cataracts 39 days later than mice in PBS or ntlacZ-mMSCs treatment group. No tumor development was observed in mice in ECSOD-mMSCs treatment group, whereas large abdominal tumor was found in mice in PBS treatment group.

We predict that the approach described here can be rapidly implemented as a medical countermeasure against radiation in response to a nuclear terrorist attack, nuclear war, and other nuclear/radiological emergency.

**IAI | Radiation Nuclear Medical Countermeasures Research and Development
Program**

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The NIH/NIAID medical countermeasures (MCMs) program for mitigation or treatment of radiation injuries was initiated in 2005 to develop drugs and devices for use following a radiation incident or attack. A broad research and development program was established to investigate a range of radiation effects, and MCMs at different development stages. Significant advances have already been made by researchers in the program developing treatments for syndromes resulting from radiation exposure. These include treatments for hematopoietic (bone marrow and immune reconstitution), gastrointestinal (loss of intestinal crypt structure and breakdown in mucosal wall integrity), lung (pneumonitis and fibrosis) and skin (beta burns and late fibrosis) injuries. In addition, the program has initiated studies on complicated radiation exposure scenarios, such as those involving combined injuries (radiation with burn, wound, infection or other trauma), and internalized radionuclides, as well as studies on combination therapies. Through grants, contracts and inter-agency agreements, the program has established a robust MCM pipeline which includes broad screening, early target identification and mechanistic studies; focused work on MCMs targeting radiation-induced cellular events such as apoptosis and inflammation; and advanced product development. An area of intense work has been the work on the validation of rodent and non-human primate surrogates for screening, evaluation and efficacy of candidate MCMs for hematopoietic and gastrointestinal syndromes and early work on lung radiation animal models. These studies are critical, as they will enable *in vivo* validation of any promising countermeasure, and thus meet the HHS Food and Drug Administration (FDA) "Animal Rule" for licensure. Working closely with the FDA and other HHS partner agencies such as the NIH National Cancer Institute (NCI) and the Biomedical Advanced Research and Development Agency (BARDA), NIAID has supported small and large animal studies on over 125 MCMs, as investigators continue to advance both drugs that are already licensed for different indications and novel compounds toward FDA licensure and potential inclusion in the Strategic National Stockpile.

Inventory of Issues Constraining or Enabling Industrial Involvement in Medical Countermeasure Development

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Introduction: The National Biodefense Science Board (NBSB) Medical Countermeasure Markets and Sustainability Working Group (M&S-WG) is soliciting comment, feedback, and guidance from members of industry, other Government agencies, and the public at large on their working document, *Inventory of Issues Constraining or Enabling Industry Involvement in Medical Countermeasure Efforts*. Recommendations for improvement of the *Inventory* will be considered by the Working Group to strengthen and refine the document.

Background: There exist a variety of limitations and barriers to biotechnology and pharmaceutical companies' involvement in the biosecurity and biodefense efforts of the U.S. Government (USG), most notably medical countermeasure advanced research and development programs coordinated by the U.S. Department of Defense (DoD) and the U.S. Department of Health and Human Services (HHS). Make-up of the medical countermeasure development efforts has been called fragmented, with confusing approaches used. To delineate and simplify the complexities of USG endeavors in medical countermeasure development, and the interactions between Government agencies and private industry, the NBSB M&S-WG assembled an inventory (or grid) of issues. This inventory includes factors that may discourage industry involvement or partnering with the USG in medical countermeasure development efforts, reported constraints to industry involvement, and potential solutions for relief from a particular constraint. The inventory has been catalogued by financial, legislative, scientific, human capital, regulatory, and societal elements.

Purpose: The M&S-WG is pursuing multiple avenues to broadly circulate the inventory in order to acquire as much outside input as possible. In addition to the Working Group's request for review and comment on the inventory, the Working Group also requests participating stakeholders prioritize the elements of the inventory to better inform the Working Group's deliberations when considering potential recommendations.

**Biodosimetry as a Priority for Radiological Public Health Emergencies
of IAI and BARDA**

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In the days following detonation of a 10-kiloton improvised nuclear device (IND) in a major city, more than one million people may request or require evaluation for radiation exposure. No one type of biodosimetry assay will be likely to provide definitive specificity of measurement with the throughput needed to handle so many people. Thus the ultimate solution may be a “system of systems” in which multiple types of screening tools are employed to focus quickly on those (an estimated 100,000 people) who need rapid medical attention based on an absorbed dose of 2 Gray or more. In HHS, the NIH National Institutes of Allergy and Infectious Diseases (NIAID) leads basic research efforts to develop medical treatments and diagnostic tools for CBRN and naturally occurring public health threats. The role of BARDA is the advanced research, development and acquisition of these medical countermeasures for the Strategic National Stockpile. Together NIAID and BARDA are collaborating on the development of rapid, accurate biodosimetry diagnostic tools and assays.

**Protectan B B a Medical adiation ountermeasure for adical Im rovement of
Miti ation emato oietic and astrointestinal unction in Acute adiation ndrome**

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Cleveland Bio a s, Inc.

Protectan CBLB502 is the first medical radiation countermeasure of its kind demonstrating efficacy against both the hematopoietic (HP) and gastrointestinal (GI) components of Acute Radiation Syndrome (ARS) in rodents and non-human primates whether administered before or after lethal radiation exposure. A single intramuscular injection of CBLB502 given 1-48 hours following exposure of rhesus monkeys to 6.75Gy of total body radiation increases the survival rate from ~20% to 70-80%. While the surviving control monkeys demonstrate multiple signs of radiation sickness in HP (including immune system) and GI, the majority of animals treated with CBLB502 possessed no significant structural abnormalities in their radiosensitive organs, consistent with data obtained in mice. Multiple indications of efficacy of CBLB502 in irradiated monkeys include reduction in severity of and faster recovery from thrombocytopenia and neutropenia when administered up to 72 hours post-exposure in a non-human primate model. These data suggest that CBLB502 offers true protection from radiation induced ARS, rather than just a delay in symptom onset. Significant efficacy was demonstrated in protection of HP and GI systems when CBLB502 is administered up to 24 hours *rior* to radiation exposure indicative of not only radiomitigating but also radioprotective activity of the drug, thus enabling a variety of scenarios of CBLB502 applications. On one hand, it can significantly reduce mortality and decrease incapacitation within the general population if injected more than 24 hours after a radiological or nuclear event. On the other hand, it can be used by military or first responder personnel entering a region devastated by a nuclear detonation or "dirty bomb" radiological device and CBLB502 has strong supporting data to meet the key FDA requirements for drug approval under the Animal Efficacy Rule. Projected stability exceeds two years, with planned studies testing out beyond two years. The manufacturing technology is cost-effective production of over 100,000 doses per manufacturing batch under current Good Manufacturing Practice (cGMP) conditions with additional scale-up possible. This summer, CBLI successfully completed a Phase I human safety trial, with a larger Phase 2 study to begin this fall. CLBI's current development schedule will deliver FDA-approved drug in ready-to-use syringes by both military personnel and civilians within 18 months.

Adenovirus Vectored Protective Antigen Vaccine

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PaxVax Inc.

Current generation Protective Antigen (PA) protein-based anthrax vaccines have significant limitations that impact their potential for use in the civilian population in the post-exposure prophylaxis (PEP) setting. Primary concerns are lack of protective toxin neutralizing antibody levels after a single dose, minimal levels even after two doses, and significant injection site reactogenicity. Secondly, these products require parenteral administration and cold-chain storage and shipping, which complicates product deployment and use. The need for improved vaccines to address these issues is well accepted. PaxVax is working to develop anthrax vaccines for the PEP setting based on a novel replicating Adenovirus serotype 4 (Ad4) viral vector platform. This vector-based approach supports the design and development of an Ad4-PA based vaccine, effective after a single dose, that can be orally administered and produced inexpensively as room-temperature stable capsules or tablets. The vaccine is based on the oral U.S. Military Adenovirus 4 vaccine, which was safely administered to more than 10 million U.S. Military recruits, and demonstrated to be effective at preventing respiratory Ad4 infection.

The PaxVax Ad4 vectored PA-based vaccines were designed using vectors with a deletion in the viral E3 gene region and the human Cytomegalovirus Immediate Early promoter (CMV-IE) to drive transgene expression. Genes encoding PA were designed to support translation of the protein in two forms (1) a soluble protein that is secreted and (2) a form with a glycosylphosphatidylinositol anchor to yield a membrane-associated protein with potentially increased immunogenicity. Infection studies completed in human cells (A549) confirm that the Ad4-PA vectors express high levels of either the secreted or membrane bound PA within the 48 hours, as measured by Western blot and flow cytometry. Studies assessing immunogenicity and protection in a mouse LT challenge model are ongoing, and results will be presented.

A **erical ar on Adsorbent A Potential Agent for Mitigation of Radiation**
Enteritis and Decorporation of Ingested Radiocontaminants

M. S. Harris, J. Bornstein, and K. Anderson

enteric bacteria

Enteric bacteria have been implicated in the pathogenesis of radiation-induced intestinal injury in germ-free and antibiotic-treated mice. Toll-like receptor (TLR) ligands of bacterial origin activate innate and adaptive immune responses and alter epithelial cell function. AST-120 (spherical carbon adsorbent) is an oral, non-absorbed, carbon-based adsorbent with high specific surface area and broad scavenger activity against microbially generated inflammatory mediators, used in Japan (Kremezin®) in over 200,000 patients since 1991. Intraluminal binding of bacterial ligands by AST-120 could prevent radiation enteritis and mitigate injury post-exposure. Studies were conducted to assess the binding of AST-120 to bacterial ligands and its anti-inflammatory potency in animal and human models of enteritis and bacterial dysbiosis. Binding studies were performed at 10mg/dl analyte in the presence of 2g AST-120. SD rats were administered 3% dextran sodium sulfate solution for 7 days, with or without AST-120 4g/kg/day. Human studies were performed in 20 subjects with pouchitis post ileal pouch anal anastomosis, a model of bacterial dysbiosis and enteritis, at 2g TID for 4 weeks. AST-120 potently adsorbed a variety of microbial products affecting immune function, including lipopolysaccharide, a TLR-4 ligand, and formyl-methionyl-leucyl-phenylalanine (fMLP), a potent chemoattractant inducing tissue-destructive oxygen-derived free radicals in phagocytic cells. AST-120 was demonstrated to prevent mucosal injury in the DSS colitis model and to heal inflammation and symptoms in human subjects with pouchitis.

CONCLUSION: AST-120 could offer protection against radiation enteritis. Its established safety profile, lack of systemic availability, oral administration, and easy-to-use sachet make it an ideal agent for military and civilian populations. The recognized binding of heavy metals to activated carbon surfaces raises the possibility that AST-120 could decorporate a variety of water and food-borne radionuclides to prevent injury from these agents.

Commitment to the Creation of Sustainable Influenza Vaccine Production Capacity in the Developing World

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Development of an effective vaccine is considered the cornerstone for controlling a global influenza pandemic. However, there are marked differences between countries regarding their capacities, priorities, and resources to establish seasonal influenza vaccination policies and programs. Building influenza vaccine production capacity in developing countries helps to address a number of critical issues including insufficient availability/access to pandemic vaccines and the need for sustainable approaches to self-sufficiency.

The Biomedical Advanced Research and Development Authority (BARDA) within HHS has supported the accelerated development and production of influenza vaccine for humans FY 2006-2009, with a total of \$28 million provided through WHO to Vietnam, Thailand, Indonesia, India, Mexico Brazil, Egypt, Korea, Romania, Russia and Serbia. In FY 2009, an additional \$7.9 million was provided to Vietnam through PATH. Funds provided through PATH will be used for phase 1 and 2 human clinical trials with vaccine produced in Vietnam.

Through funds provided to the WHO as well as to PATH for clinical trials in Vietnam, BARDA has focused its support on the transfer and development of egg-based vaccine technology. With its support from the Gates Foundation, PATH is focusing on newer emerging technologies that would be affordable to poor and mid income countries.

Building on the WHO's Global Action Plan, there is a need for a comprehensive framework and strategic plan that outlines an assessment of the present state, future vision, and steps necessary to ensure the creation of regionally-based independent and sustainable vaccine production capacity in developing and emerging economy nations through capacity building and technology transfer. HHS plans to host an international stakeholders' workshop in early 2010 in order to provide a forum for discussion and development of a framework or 'roadmap'.

e lo a le adiation Biodosimetr stem

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Medical management of suspected radiation casualties necessitates use of multiple parameter biological-dosimetry assessment. In cases of mass casualties and radiological terrorism military and civilian first-responders need the ability to triage individuals in the field. Multiple parameters presently useful for dose assessment triage include the assessment of radioactivity contamination, clinical signs and symptoms, physical dosimetry, and early-response changes in hematology, blood chemistry, and protein biomarkers.

Efforts to assemble a deployable radiation biodosimetry system are underway and include validation of equipment, establishment of Standard-Operating-Procedures protocols, and development of medical recording worksheets and software tools. Our system includes equipment to measure various radiation blood biomarkers including: amylase activity, C-reactive protein, lymphocytes, and neutrophils. Each of these devices is either small enough to fit in a backpack, or can be packaged and transported via an Anvil case on wheels. Another component of deployable dosimetry includes the equipment protocols and procedures to run the tests. Deployable biodosimetry can also be assisted by a set of tools to aid responders and others responsible for dynamic recording of medical data of suspected irradiated patients. AFRRRI's Biodosimetry Assessment Tool (BAT) and our First-responder Radiological Assessment Triage (FRAT) software can be loaded onto laptops or handhelds, respectively, to rapidly record and assess dose estimations in a field deployable setting. A deployable multi-parameter biodosimetry system will aid the collecting and diagnosing and treating of irradiated individuals in a field deployable environment. [Research was supported by AFRRRI work units RBB4AR and RAB4AL.]

Resolving legal challenges for MCM Preparedness

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Successful creation of a global infrastructure for MCM preparedness requires adept resolution of key legal challenges. If left unresolved, efforts to integrate multi-jurisdictional planning might be impeded. Amid a health crisis, necessary capacities might not operate effectively in the face of legal uncertainties about who is authorized to act, the scope of their authority, and how to protect proprietary and privacy rights. By contrast, resolving legal challenges can build confidence by clarifying responsibilities.

Planning decisions about which MCMs to stockpile and distribute – all these decisions require harmonized assessments of risks and opportunities for MCM development. Yet, no global authority is designated to make such assessments, and inconsistent lexicon and production quality standards impede decision making processes.

From the perspective of private sector MCM producers, legal uncertainties are disincentives to becoming engaged, e.g. approval and funding of new research, protection of intellectual property rights, and potential liabilities for those MCMs' adverse consequences.

Grave legal challenges concern licensing of novel MCMs and authorizing their emergency use. MCMs that are licensed in one country might face legal barriers to their use in another country. Institutional arrangements to harmonize licensing of medicines face persistent questions as to how such arrangements might operate rapidly amid a crisis.

Legal challenges also concern the logistics of an MCM infrastructure: where to locate stockpiles; who may access those stockpiles, how to transport stockpiled MCMs across borders, and how to distribute those MCMs to needy populations. At their destination, a legally empowered command and control system must dispense them safely and securely even as law enforcement is trying to sustain public order.

All these challenges should be resolved now, before a crisis demonstrates the costs of legal confusion.

Simultaneous detection of Enteroviruses and Herpes Simplex Viruses in CSF using the Spartan Real-time Platform

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The Spartan DX-12 ("DX-12", Spartan Bioscience Inc., Ottawa ON) is a new generation real-time PCR instrument, incorporating a rapid 2-temperature PCR cycle, 2-channel detection, and a 12-well sample capacity in a compact and affordable format. These features make it ideal for on-demand, non-batched mono- or multi-plex PCR analyses where speed and convenience are essential. Molecular amplification is the standard for laboratory detection of herpes simplex (HSV) and enterovirus (EnV) in CSF; diagnosis of central nervous system infections caused by these agents is an ideal application of the DX-12. We evaluate the performance of the TrimGen eQ-PCR™ HSV-EnV detection kit ("eQ-PCR", TrimGen Corp, Sparks MD) which detects and differentiates HSV 1 and 2 and EnV in a simultaneously performed single-tube PCR reaction.

Methods The limit of detection of DX-12 eQPCR was determined by testing 3 triplicates of serially diluted stock HSV-1, HSV-2, (ABi Inc., Columbia, MD; 140, 14, 1.4 copies/reaction) and EnV (1, 0.1, 0.01 TCID₅₀). Archived clinical samples tested by fully validated in house monoplex HSV1/2 and EnV PCR assays ("reference PCR"; ABI 7500) were anonymized and tested in by DX-12-eQPCR (HSV n= 29, EnV n=30, HSV/EnV PCR negative CSF n= 20). Results obtained by DX-12 eQPCR were compared with those from reference PCR. Extractions were performed by QIAamp DNA Mini Kit (HSV) and viral RNA Mini Kit (EnV).

Results The lower limits of detection of the DX-12 eQPCR for HSV-1 and for HSV-2 were 14 copies/reaction, and for EnV was 0.1TCID₅₀/reaction. The lower limits of detection of reference PCR for HSV-1 and HSV-2 were 14 copies/reaction and for EnV, 0.1 TCID₅₀/reaction. There was 99% ((78/79) agreement between eQPCR and reference PCR for all clinical samples tested (HSV n=29, EnV n=30, negative HSV/EnV CSF n= 20).

Conclusion The TrimGen HSV-EnV eQPCR assay run on the Spartan DX12 platform, performed with the same sensitivity as conventional monoplex PCR assays for these viruses in CSF. The combination of the Spartan DX-12 and TrimGen eQPCR assay provides a convenient, easy to use, accurate, and time-efficient system for the detection of two important and commonly-encountered viral agents of central nervous system infection.

Assessments u ortin B Medical ountermeasure Ac uisition

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Section 3(c)(2) of the Project BioShield Act of 2004 (P.L. 108-276) requires the Secretary of Homeland Security to make determinations of chemical, biological, radiological, and nuclear agents that are material threats to the U.S. population sufficient to affect national security. It also authorizes the Secretary of Health and Human Services to determine the public health consequences stemming from these threats and recommend countermeasures against such threats. If suitable medical countermeasures do not already exist, this process can culminate in a joint DHS-HHS recommendation to the President (Director of Office of Management and Budget) to authorize investment of the BioShield special reserve fund.

At least two DHS Assessments support a given Material Threat Determination (MTD) – S&T Terrorism Risk Assessments: quantitative, end-to-end risk assessments that consider threat, vulnerability, and consequences for thousands of scenarios and integrate the findings of the intelligence and law enforcement communities with input from the scientific, medical, and public-health communities, and Population (formerly Material) Threat Assessments: in-depth studies of single plausible, high consequence scenarios on specific agents used to estimate the potential number of exposed individuals, their exposure levels, and if applicable, the contaminated area.

DHS S&T, DHS Office of Health Affairs and HHS Biomedical Advanced Research and Development Authority (BARDA) and other members of the Public Health Emergency Medical Countermeasure Enterprise collaboratively consider the results of these assessments to inform further modeling and setting of CBRN medical countermeasure requirements.

AEOL 10150: A Broad Spectrum Medical Countermeasure MCM to Treat Radiation or Chemical Induced Injuries

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AEOL 10150 is a broad-spectrum catalytic antioxidant specifically designed to neutralize reactive oxygen and nitrogen species. The neutralization of these species reduces oxidative stress, inflammation, and subsequent tissue damage via signaling cascades induced by radiation or chemical exposure.

The compound represents a broad spectrum MCM with proof of principle data in post exposure models for Acute Radiation Syndrome (GI and Lung), mustard gas and chlorine gas. Our approach to mitigation of organ-specific damage induced by acute exposure to potentially lethal doses of radiation and chemical agents is based on the hypothesis, and supporting data, that these stressors initiate a pro-inflammatory cascade that disrupts organ integrity and function in a radiation or chemical dose- and time-dependent fashion. This common mechanism of action permits analysis of efficacy in multiple models of organ-specific damage. Research is focused on use of validated animal models and research design compliant with the criteria of the Food and Drug Administration (FDA) Animal Rule and development of a clear path to licensure by the FDA.

To this end we have conducted studies of AEOL 10150 in collaboration with Duke University, the University of Maryland and a sponsored Research Consortium sponsored by the National Institute of Allergy and Infectious Diseases within the National Institutes of Health (NIH) focused on major organ-specific sub-syndromes of the ARS and delayed effects of acute radiation exposure establishing proof of principle. Current studies are ongoing in non-human primates and mice to both confirm efficacy and to establish optimal dosing and to expand the treatment window.

In the chemical area, with the guidance and support of NIH CounterACT, Aeolus has conducted studies in collaboration with National Jewish Health, the University of Colorado and Lovelace Respiratory Research Institute that have established proof of principle of the compound as a MCM for mustard gas and chlorine gas exposure. Current studies are ongoing in rodents to confirm efficacy and to establish optimal dosing and to expand the treatment window.

Adenovirus Vectored Oral Pandemic Influenza Virus Vaccine

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PaxVax, Inc.

Infection of poultry species by pathogenic H5N1 influenza viruses continues to be documented in Asia. Transmission of H5N1 influenza to humans is rare, with only 400 documented human cases; however the fatality rate is approximately 60%. As such, preparation for a H5N1 influenza pandemic continues to be a major focus of the WHO, CDC and commercial vaccine manufacturers. There are limited supplies of injectable H5N1 influenza virus vaccines stockpiled in developed nations but these are of the traditional type produced using egg-based manufacturing at high cost and requiring cold-chain storage and transport. The most at-risk populations in Asia cannot afford these vaccines and thus the need for improved H5N1 vaccines remains.

To address these issues PaxVax is developing influenza virus vaccines for use globally based on a novel replicating Adenovirus serotype 4 (Ad4) viral vector platform. This vector-based approach supports the design and development of H5 hemagglutinin (HA)-based vaccines that can be orally administered, may be effective after a single dose and which can be produced inexpensively as room temperature stable capsules, tablets or liquid forms, for pediatric use. The most advanced product is a vaccine designed using an Ad4 vector with a minimal deletion in the viral E3 gene region into which a gene encoding a modified form of HA from H5N1 A/Vietnam/1194/2004 influenza was inserted. Infection studies completed in human cells (A549) document the efficient expression and correct conformation of the HA protein. Immunogenicity studies in a mouse model demonstrated the vaccine to be immunogenic, even in mice with pre-existing immunity induced by injection with wild type Ad4, inducing antibodies and cellular responses at levels predicted to be protective. The vaccine is currently being evaluated clinically in a U.S.-based Phase 1 trial.

I IAI Radiation Biodosimetry Research and Development Programs

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The National Institute of Allergy and Infectious Diseases (NIAID) has initiated several programs that support research and development of biodosimetry techniques and devices. In the event of a mass-casualty nuclear/radiological event, such as a terrorist attack or nuclear incident, the radiation exposure amount received by each individual will need to be quickly determined and it will be imperative to rapidly establish which victims have been exposed to radiation and those who have not been exposed. The degree of medical attention required and subsequent course of treatment of exposed victims largely depends on the dose of radiation received. Several devices currently being examined for the use after such an event include, but are not limited to a high-throughput, minimally invasive, robotic biodosimetry workstation that uses automated versions of two well-established manual assays (γ -H2AX foci and micronucleus formation), a hand-held electron paramagnetic resonance (EPR) dosimeter using samples from teeth and nails to measure radiation exposure, an automated, dicer analysis system for cytogenetic biodosimetry, a portable biodosimeter based on radiation-induced metabolomics expression, a biodosimetry tool using a fully integrated biochip and a micro/nanofluidic cartridge that can perform whole-blood microarrays for gene signatures of radiation injury; and hand-held lateral flow diagnostics for urine and/or saliva, to detect and measure radiation-induced protein biomarkers. These and other projects currently supported by NIAID's biodosimetry development initiative are further defining radiation-specific biomarkers of damage, and program awardees continue to develop these advanced technologies for field use in the case of a radiological incident.

An Effective and Versatile Multivalent Vaccine Platform for Pandemic Influenza and Biodefense

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Antigenic variance has proved exceptionally problematic to the development of effective vaccines against the multiple strains and subtypes of dangerous pathogens. In response to the need to develop vaccines against global health and biodefense threats, we have developed the Complex Adenoviral Vector vaccine system (CAAdVax), a vaccine technology for multivalent antigen presentation. This non-replicating vector has the capacity to synthesize multiple antigens *de novo* in the recipient, thereby inducing a robust immune response that mimics natural infection. The unique multivalent nature of CAAdVax allows simultaneous protection against multiple viral strains/subtypes, enhancing efficacy. It has been successfully used to elicit protective responses against lethal agents such as H5N1 Avian Influenza, H1N1 Pandemic Influenza, Ebola, Marburg, Dengue, Rift Valley Fever, and Chikungunya viruses in animal models. These CAAdVax vaccines induce a broad spectrum of adaptive immune responses, including potent neutralizing antibodies and cellular immunity. These responses are rapidly induced and sustained. In challenge experiments, vaccinated animals of the appropriate model for pathogen challenge, including non-human primates, have shown protection against multiple subtypes of Ebola, Marburg, dengue H5N1 avian influenza and H1N1 influenza viruses. Furthermore, we have shown efficacy in animals when the vaccine is administered intranasally or subcutaneously, in addition to the more traditional intramuscular route. In GLP-toxicology and biodistribution studies, we have demonstrated repeated high-dose vaccine administration of CAAdVax is safe and well tolerated. Currently, our Ebola, Marburg, and Dengue vaccines are approaching Phase I clinical development, and the CAAdVax platform is being used to develop promising new vaccines against global health and biodefense threats.

Pharmacokinetics of different forms of PEGylated recombinant BChE in homologous Macaques in Multiple Doses and Routes of Delivery

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Currently, native butyrylcholinesterase (BChE) is the leading pre-exposure treatment candidate for OP toxicity due to its potent bioscavenging ability and circulatory stability. Any recombinant (r) form of BChE will need to exhibit the same high potency, high plasma retention time (120-200 hrs at 3 mg/kg) and a lack of immunogenicity. Pharmacokinetic (PK) profiles and analysis are used to indicate the periods after administration that such biologics are likely to exhibit optimal benefit or protection and may differ considerably depending on the glycosylation, post-translation modification, oligomerization and immunogenicity of the bioscavenger as well as the animal model and the route of delivery. In the case of the tetrameric or monomeric PEG-rBChE, as therapeutic treatments, the route of delivery greatly effects PK parameters (AUC, T_{1/2}, C_{max}, T_{max}, MRT) and thus bioavailability and efficacy. The results of dose response kinetics and immunogenicity studies using both monomeric and tetrameric forms delivered by SC, IM and IV routes following multiple injections in homologous macaques (Ma) will be discussed.

In addition, the pharmacokinetics of clearance of macaques following two injections with 5mg/kg of either PEG-rMaBChE (homologous model) or PEG-rHuBChE (heterologous model) 4-6 weeks apart and by different routes has been compared. Since multiple injections of PEG-rMaBChE in homologous macaques does not induce an anti-BChE antibody response, this homologous model is excellent for evaluating PEG-rMaBChE delivery options to optimize plasma stability while the heterologous model demonstrates the negative effects of immunogenicity of PEG-rHuBChE on bioavailability; providing important animal PK and safety data for the development of a potential human treatment.

Animal Models to Evaluate the Efficacy of Medical Countermeasures for Select Agents and Infectious Materials

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Crucial to the development of prophylactic and therapeutic products for treatment of human diseases are well characterized animal models. The models need to reflect the pathology, pathophysiology, and clinical symptoms of the human diseases or intoxications and are essential to the licensure of the medical countermeasures. They are even more critical for those diseases where efficacy testing is not feasible or ethical in humans. Battelle has developed and can provide animal models predictive of human disease and prophylaxis or treatment responses using a wide range of common laboratory species including mice, rats, hamsters, rabbits, ferrets, swine, goats, and nonhuman primates. Many of these animal models have been developed specifically for licensure of medical countermeasures under circumstances where approval may be based upon evidence of effectiveness obtained from studies done in animals under the Animal Efficacy Rule, 21 CFR 314.600-650 (drugs) and CFR 601.90-95 (biologicals). To date, we have evaluated the efficacy of countermeasures for bacterial agents (*Bacillus anthracis*, *Yersinia pestis*, *Ranunculus tularensis*), viral agents (highly pathogenic avian influenza (H5N1), pandemic influenza, Venezuelan equine encephalitis (VEE), monkeypox, West Nile), toxins (botulinum), and chemical agents (sarin, soman, VX, sulfur mustard). The safety and security requirements mandate that studies be performed in a BioSafety Level 3 (BSL-3) laboratory or chemical surety facility. In addition, pivotal studies need to be performed under FDA Good Laboratory Practice (GLP) regulations. These studies often include a large number of toxicity/disease endpoints using routine and sophisticated technologies such as telemetry, clinical pathology (hematology, serum chemistry), immunophenotyping (flow cytometry), cytokine analysis, transcriptomics, immunological response assays, evaluation of circulating toxin levels, and/or traditional anatomic pathology and histology. Examples of animal models and the study endpoints to evaluate the efficacy of vaccines and/or therapeutics for anthrax, botulinum toxin, and nerve agent countermeasures will be presented.

Lessons without Borders

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Disasters occur more frequently and often cause greater destruction internationally than disasters in the United States. However, the global disaster response and humanitarian assistance community generally does not share information with the domestic emergency management community in the United States. The Lessons without Borders (LwoB) Initiative seeks to leverage the internationally-derived lessons learned and promising practices to inform and hopefully improve our U.S. resiliency efforts and our domestic preparedness and response.

LwoB partners with and strategically engages international experts, Governments, and other organizations that deal with disasters around the world, including:

- 1) Natural events, such as earthquakes, hurricanes, and tsunamis;
- 2) Man-made events, such as, terrorist actions like the London bombings; accidental events like industrial chemical releases; and
- 3) Emerging diseases, such as, pandemic influenza.

The LwoB provides an information-sharing architecture and a discussion platform that seeks to

- a) Try to assimilate specific “lessons” that can or should be “learned” from the international event; and
- b) Codify these lessons learned or promising practices into the human experience for future use whenever similar circumstances arise.
- c) Provide a venue for constantly updating international experiences using “wiki” technology for specific lessons learned such that lessons can be contextualized and applied appropriately.

The LwoB uses an operational analysis methodology to develop alternative courses of action to solve problems and issues in domestic health and human security post disaster. This methodology provides the executive decision support process with an operationally defined problem, solution alternatives, and methods for comparing alternatives.

Through the LwoB, the dissemination of lessons learned and promising practices enhances the U.S. Department of Homeland Security’s mission to support other federal, state, local, and tribal Governments and the private sector. LwoB analysis and output is communicated to the decision support process, policy and planning efforts, and the DHS Lessons Learned Information Sharing (LLIS) program. Additionally, these valuable lessons learned and promising practices are made available to our international LwoB partners.

Therapeutic Research Program Broad Spectrum of Inhibitors Protecting Mice against Ricin

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The French Interministerial Research and Development Program against RNBC threats has launched a series of projects in search of inhibitors against various B agents including botulinum toxins, ricin, type three secretion systems (plaque), and large clostridial toxins. The program also develops therapeutic antibodies against toxins and viruses. Finally, two projects propose novel vaccine approaches against plague and anthrax, intended to overcome the drawbacks of existing solutions.

We used cell-based high-throughput screening to identify chemical inhibitors of cell intoxication by ricin. This approach was intended to select molecules protecting cells against the toxin by interacting with a cellular target. Two molecules named Retro-1 and Retro-2 were found, which also protect cells against Shiga-like toxins (produced by uro-hemolytic *E. coli* strains, a significant public health threat worldwide) and cholera toxin. Another molecule named compound 20 protected cells against diphtheria toxin in addition to ricin.

To gain access to their cytosolic target, ribosomal RNA, ricin and Shiga-like toxins traffic through the retrograde route from the plasma membrane to the endoplasmic reticulum, via endosomes and the Golgi apparatus. We showed that Retro-1 and Retro-2 selectively block retrograde toxin trafficking at the early endosomes-TGN interface, without affecting compartment morphology or other trafficking steps. They do not affect endogenous retrograde cargos, demonstrating an unexpected degree of selectivity and lack of toxicity. Accordingly, one of the compounds clearly protects mice from lethal nasal exposure to ricin (1 LD₉₀). In addition, compound 20, which acts at a different level in the cell, also gives some protection against ricin in mice.

Our work discovers the first small molecules that show efficacy against ricin in animal experiments, and identifies the retrograde route as a potential therapeutic target.

Summary of Biological Samples for Biological Dosimetry Assessment
Joint IAEA PAHO and International Exercise
IAEA

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The exercise tested current existing capabilities for safe and expeditious international transport of samples subjected to biological dosimetry assessment. Blood samples were shipped from Peru – Instituto Peruano de Energía Nuclear, Biological Dosimetry Laboratory to participating laboratories in 13 countries within the Latin American Biological Dosimetry Network and the IAEA Response Assistance Network (Argentina, Brazil, Chile, Finland, France, Germany, Hungary, Japan, Mexico, Spain, Turkey, Uruguay, USA).

The means to evaluate the successful shipment of the samples were: a) the ability to timely send/receive the samples, b) conformity or nonconformity of the sample conditions for biological dosimetry purpose at the time of its reception, c) the mitotic index evaluation after 48 hours of culture. Additionally, TLD badges and temperature data loggers provided by IEC/IAEA were used to determine the adequacy of the transport conditions during the shipments. IEC/IAEA was the main financial contributor supporting the cost of the shipments. Financial support was also provided by the Pan American Health Organization (PAHO) and the World Health Organization (WHO).

The culturing protocol for the blood samples shipped in the exercise (non irradiated blood samples obtained through venipuncture and placed in vacutainers containing lyophilized Lithium or Sodium heparin) was based on the ISO and IAEA guidance on biological dosimetry and cytogenetic analysis publications. The shipment had to be performed in line with the WHO guidance and regulations and all permits and shipping documents had to be perfected according to the international shipping requirements (UN 3373 class transport). National regulations and custom requirements had also to be met as applicable.

According to the previous experience gained in international assistance missions coordinated and delivered by IAEA, the sender of the samples was encouraged to explore the possibility that samples could be shipped through the UNDP office in Peru. IAEA IEC assisted in preparation of the exercise also by contacting and requesting support of the UNDP offices and Competent Authorities (in the sense of the Emergency and Assistance Conventions) in the participating countries.

At the time of submission of this abstract, the shipping of the blood samples was completed and the IAEA IEC received a number of TLD / temperature data loggers from the receptor laboratories. The reading of the TLD and temperature data loggers is in progress at IAEA. The coordinating laboratories (Argentina and Peru) are receiving the results of the mitotic index evaluation and a final report containing the information provided by all participants and analysis of the transport conditions as reflected by the IAEA TLD / temperature data logger readings is to be prepared. Conclusions drawn in the exercise will contribute to the enhancement of the capability to timely and properly ship biological samples in international assistance missions.

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Nanotherapeutics is currently developing multiple inhaled and nasal products against bacterial and viral biothreats using its proprietary particle engineering technologies. NanoGENTTM gentamicin sulfate inhalation powder packaged in a multi-dose inhaler is in preclinical development is being developed as a local antibiotic treatment following exposure of *ersinia estis* and *rancisella tularensis*, as well as other respiratory infections. The NanoFOVIRTM program recently began to develop an inhaled version of the injectable antiviral drug, cidofovir, for non-invasive, post-exposure prophylaxis and treatment of the Category A bioterrorism agent smallpox (*Variola ma or*), as well as other viral infections in the airways. In comparison to systemic delivery, inhalation is less invasive, does not require a healthcare worker to administer, and could present fewer side effects compared to injection. Finally, the GelVac nasal vaccine powder system using GelSite®, a novel plant polysaccharide polymer, has been tested in various vaccine formulations for administration by the nasal route and injection, including an in-situ gelling nasal powder influenza vaccine which is entering Phase 1 clinical studies. This novel plant polysaccharide (GelSite® polymer) and its gelling property to encapsulate vaccine/adjuvant formulation as gel particles has demonstrated (1) enhanced the immune response, (2) reduction in the antigen and/or adjuvant dose, and (3) elimination of the boosting dose through a combination of several factors including sustained antigen release, improved antigen uptake, and improved antigen stability.

The NanoGENTTM program is funded in whole or in part with Federal funds from the NIH National Institute of Allergy and Infectious Disease National Institutes of Health (NIAID) and the Biomedical Advanced Research and Development Authority (BARDA), within the U.S. Department of Health and Human Services (HHS), under Contract No. HHSN27220070030C. The NanoFOVIRTM program is funded in whole or in part with Federal funds from the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH) under Contract No. HHSN272200900015C.

Ant ra Vaccine ulfillin t e ation s eeds

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Intentional release of aerosolized *B. anthracis* spores as a biological weapon remains a major national security concern. Once inhaled, spores germinate into bacteria that multiply and secrete toxins, The resulting disease has a fatality rate of 45-90%. Preventative countermeasures may be utilized prior to *B. anthracis* exposure or after exposure, but prior to disease development. BioThrax[®] (Anthrax Vaccine Adsorbed) produced by Emergent BioSolutions Inc. (EBSI), is the only FDA-licensed anthrax vaccine for the pre-exposure protection of individuals at high risk of anthrax exposure and works by stimulating the immune system to produce antibodies against toxins secreted by the anthrax bacteria. Recognizing the importance of proactively immunizing military personnel at risk for anthrax exposure, DoD began an immunization program in 1998 resulting in administration of over 9 million doses of BioThrax to >2.3 million persons. Pre-exposure vaccination may protect against antibiotic resistant engineered *B. anthracis* strains. Because antibiotics are not effective against anthrax toxins or dormant spores, and since inhaled spores may persist for prolonged periods of time prior to germination, optimal post-exposure prophylaxis (PEP) utilizes a combination of antibiotics and anthrax vaccine. Although not licensed for PEP, BioThrax was used under IND for PEP following the 2001 U.S. anthrax-letter attacks. Recognizing the need to provide post-exposure protection to at least 25 million civilians, the U.S. Government has called for the inclusion of 75 million doses of anthrax vaccine in the Strategic National Stockpile (SNS). In response, EBSI has delivered 30 million doses of BioThrax to the SNS, has markedly increased its production capacity, and has built a new state-of-the-art manufacturing facility. Recently FDA has approved significant labelling changes including dosage reduction, IM injection, as well as granting a 4-year shelf life. BioThrax plays a central role in U.S. bio-preparedness programs.

Development of an Attenuated Smallpox Vaccine for Research

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Introduction The attenuated smallpox vaccine LC16m8 was developed in Japan by Dr. Hashizume over 30 years ago. Currently, this LC16m8 vaccine has been stockpiled as a potent countermeasure against bioterror attacks with smallpox. We would like to report our latest research outcomes on the LC16m8 vaccine.

Methods To evaluate the safety and efficacy of LC16m8, using Lister or Dryvax as a comparator, various non-clinical studies were conducted. Further, in order to evaluate the safety and immunogenicity of LC16m8 in human, a Phase I/II clinical trial was conducted in the United States.

Results In the Monkey Neurovirulence Study, although fatal cases were observed in the Lister or Dryvax inoculated animals, no deaths or abnormalities, like paralysis, were observed in the LC16m8 inoculated animals. The Skin Proliferation Study in Rabbits showed that LC16m8 caused a markedly diminished dermal reaction. The SCID Mouse Intraperitoneal Inoculation study showed that LC16m8 had a higher safety profile than that of Lister. The GLP Single/Multi Rabbit Toxicity studies revealed that there were no significant cases with LC16m8 treated animals. The Monkey Intravenous Challenge Study with Monkeypox, where no deaths were observed in the LC16m8 immunized animals, showed that the efficacy of LC16m8 was comparable to that of Dryvax. In Phase I/II clinical trial, seroconversion rate of LC16m8 vaccinated subjects was comparable to that of those vaccinated with Dryvax. Also, there were no significant cases in the clinical trial.

Conclusion

- Monkeypox virus (MPXV) Challenge Study was completed.
- GLP Single/Multi Rabbit Toxicity study was completed.
- Phase I/II clinical study was completed.
- Current GMP manufacturing facility with BLA level was constructed (Annual capability: approximately 80 million doses).
- Process validation was completed.
- Stability study for 5 years was completed.

KAKETSUKEN concludes that the Technology Readiness Level (TRL) is seven.