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CRADA FINAL REPORT
for the
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FEASIBILITY STUDY AND PROTOCOL DEVELOPMENT FOR MANUFACTURING OF A VETERINARIAN DRUG USING LOCAL PLANT SOURCES AS RAW MATERIALS

Brian Davison
Life Sciences Division, Oak Ridge National Laboratory

Tanya Kuritz
Chemical Sciences Division, Oak Ridge National Laboratory

Ken Williams
BWXT/Y12

Arthur Sass
Sass & Sass, Inc.

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Abstract

This CRADA was a collaborative effort between the Oak Ridge National Laboratory (ORNL) and Sass & Sass, Inc. It also had involvement with the University of Tennessee-Knoxville (UTK) The CRADA focused on the development and commercialization in the U.S. of the substance developed in Russia with potential veterinary applications. The project addressed validation and further characterization of the lead substance necessary for its commercialization in the U.S. market as a veterinarian biologic and at the commercialization of the product for the Russian market, by the Russian group establishing of sustainability of the Russian research groups.

Statement of Objectives

The objective of the CRADA was to use ORNL capabilities and expertise to assist a local business, (Sass & Sass, Inc.) in validation and set up of a manufacturing protocol for the veterinary biologic discovered in Russia. ORNL's primary role was aimed at the oversight, direction, validation and analysis of the data of research, development, manufacturing, and testing of drug candidate(s) proposed by the Russian side. Sass and Sass's primary roles were to provide effort, materials and equipment for the development and validation studies, and to support the legal and regulatory efforts. The efforts led to characterization of the content and stoichiometry of active ingredients from sources available in both East Tennessee, and in Russia and the NIS.

Benefits to the Funding DOE Office's Mission

As a result of this CRADA and collaboration with the Russian side through the Initiatives for Proliferation Prevention (IPP) program, a sustainable pharmaceutical manufacturing facility has been established in Moscow. This facility is operated by an established research institute (Gamaleya) due to the DOE-IPP funding, The facility business employs former bio-weapons scientists of three Russian institutions formerly involved in weapons programs [Gamaleya Institute of Epidemiology and Microbiology, Zelinskiy Institute of Organic Chemistry, and the Choumakov Institute of Poliomyelitis and Viral Encephalitis]. This is responsive to the DOE international non-proliferation mission of the IPP program.

This business is currently producing and selling the veterinary product. This is responsive to DOE International Non-Proliferation mission.

Technical Discussion of Work Performed by All Parties

Identification of the local plant species and other sources suitable for manufacture. Sass & Sass, Inc. and ORNL carried out bio-prospecting and sourcing in East Tennessee and located locally available resources for manufacturing. ORNL compiled and provided information about relevant botanical diversity of East Tennessee and the U.S. ORNL provided plant identification and location mapping for the plants of interest. Sass & Sass, Inc. secured necessary permits for collection of botanical materials. Sufficient quantities of

plant material were gathered during the CRADA for the initial product and validation efforts.

Validation and feasibility assessment of a scalable manufacturing protocol of the proposed AVICS.

ORNL, Sass & Sass, and the Russians jointly assessed the feasibility of scalable manufacturing of veterinarian drugs which led to the selection of a lead substance – phosphorylated polyprenyls – for further development for the US market. Russian chemists performed isolation of a variety of substances in Russia and at the Sass & Sass, Inc. facility in Oak Ridge, TN at a laboratory scale. Comparison of substances and efficacy and economics of their potential manufacture allowed selection of the method for extraction and confirmed results of the feasibility study that led to the selection of phosphorylated polyprenyls as the lead candidate. Based on the results of this study, the process equipment requirements and protocols were established for both the US and the Russian partners. In compliance with the CRADA, Sass & Sass, Inc. supplied and processed over 100 pounds of dried plant material for the feasibility batch manufacture in the US using process equipment at Sass & Sass, Inc. Russian chemists visited the U.S. and prepared, at laboratory scale, three lots of phosphorylated polyprenyls using the Sass & Sass facility and equipment. ORNL advised on protocols and requirements for quality control and set up methods for the HPLC analysis of the substance. This protocol, along with results and samples of the batches, was provided to the Russian side. The Russian side provided samples of material generated from Russian sources, and ORNL helped Sass & Sass, Inc. with the HPLC analysis of the Russian material. Sass & Sass, Inc. provided a facility, equipment, U.S. raw material and hosted Russian scientists for the extended periods of time required for process development. All manufacturing protocols developed in the Sass & Sass, Inc manufacturing facility in the USA were shared with the Russian facility to expedite set up of the process in Russia.

Oversight and validation of the proof of the biological activity of the drug candidate.

ORNL collected information, summarized results and validated toxicological studies carried out by Russian scientists, who determined LD50 in mice. ORNL actively participated, together with the University of Tennessee, in the development of qualified

animal testing protocols. USDA informed Sass & Sass, Inc. and University of Tennessee that results of the Russian controlled studies in animals, incl. anti-encephalitis activity in mice, parvoviral and other infections in dogs and feline infectious peritonitis in cats were not acceptable for the purposes of regulation in the US. Therefore, Russian scientists participated in the clinical studies in the US using the protocols developed by the College of Veterinary Medicine, University of Tennessee; the animal testing protocols were approved by USDA. Russian scientists were trained in research methods (Real-Time PCR, ELISA etc.) and in animal research compliance issues. The US data showed promise for application of the substance against rhinotracheitis in cats. The data were shared with the Russian side. This was a "go/no-go" point of the project, and the sides decided to continue joint efforts based on the potential use of the substance in the veterinary market.

Development of the pilot manufacturing procedure

Russian chemists visited the U.S. and worked with Sass & Sass, Inc. to establish a manufacturing process for pilot scale (ca. 100 L final form) manufacturing of the substance using resources provided by Sass & Sass, Inc. During this task, Russian chemists assisted with scaling up of the manufacturing process to a pilot scale and ORNL helped with establishment of standard quality assurance/quality control (QAQC) procedures. This task has also involved acquisition and installation of pilot scale extraction and synthesis equipment, and preparation and qualification of the facilities for regulatory approvals and inspections in both Russia and in the U.S. The complete process has been set up at Sass & Sass, Inc. in compliance with the USDA requirement outlined in 9 CFR, which requires that batches of material used for testing be produced at the final manufacturing site. The Standard Operating Procedures were validated. ORNL delegations attended process demonstration at Sass & Sass Inc.

Analysis of the potency and chemistry of the substance and development of QC procedures

This task was performed by ORNL and utilized analytical chemistry expertise. The analysis of the substance was carried out by HPLC, TLC, UV-spectroscopy, CD-spectrometry and, in collaboration with Sass & Sass, Inc., by chromatography and Nuclear Magnetic Resonance. The purity of the reference standard manufactured through the pilot process at

Sass & Sass, Inc. and other critical parameters were determined by ORNL. These results were critical for the deposition of the reference standard with USDA and NIST. The protocols for manufacturing and analytical testing were shared with the Russian side. Developed by ORNL and Sass & Sass, standardized procedures and protocols for QC and potency testing were accepted by USDA.

Oversight and validation of toxicological and pharmacological characterization of the substance

As ORNL possesses no infrastructure to support this special area of research, the experiments were performed under a BWXT Y-12 subcontract with the College of Veterinary Medicine at UTK under Dr. Legendre, Director, Small Animal Clinic and Professor of the College of Veterinary Medicine. Similar experiments. Experiments were performed outside the CRADA at the NIS institutes in Russia. During this task, Russian scientists analyzed safe dosages and studied the fate and the pattern of the substance accumulation in different organs. ORNL collected, summarized, and analyzed raw data generated through the experiments and provided conclusions and recommendations. The significance of this task for Sass & Sass, Inc. was diminished due to the lack of acceptance of the Russian data by USDA.

Oversight and direction of U.S. regulatory requirements-compliant blind tests of the substance in the experimental viral infections of companion animals

This task required specific expertise and regulatory permissions that ORNL lacks in order to gain USDA regulatory approval. However, the UTK College of Veterinary Medicine has all required expertise and is licensed for animal experimentation with threatening viral infections. Therefore, all animal tests were performed at UTK. Participation of the Russian scientists in the controlled clinical studies was not warranted, since the Russian regulatory system requires a different set of procedures. The Russian scientists carried out the studies according to Russian requirements, in Russia. ORNL participated in experimental protocol development along with UT and Sass & Sass, Inc. ORNL provided necessary oversight and data validation, and collected and processed some raw data. Sass and Sass, Inc. supported and led the U.S. legal and regulatory efforts. ORNL provided guidance materials based on the USDA regulatory submission to the Russian side in order to assist with their

submission to the Pharmacological Committee of Russia. Participation of the Russian scientists in the U. S. controlled clinical studies was not warranted, since Russian regulatory system requires different set of procedures. The Russian scientists carried out the similar studies according to Russian requirements, in Russia.

Study of the mode of action of the substance and identification of drug targets in vivo and in vitro

The mode of action and immune response of the organism was evaluated using blood samples collected from experimental animals in Russia. Immunologic status of animals was assessed by ELISA analysis of cytokines in blood.

ORNL carried out in vitro studies, independent of the work in Russia, using cell cultures without the participation of the Russian scientists. ORNL identified potential molecular targets of the substance and filed an Invention Disclosure.

Oversight and validation of testing of the substance in animal models in Russia

Russian scientists were provided hands-on compliance training at UTK. However, Russian regulatory authorities have established a different set of procedures required for animal testing.

The field work was supervised by Dr. Legendre, Director, Small Animal Clinic and Professor of the College of Veterinary, UTK who visited Russia to provide his on-site guidance for animal testing procedures and requirements. USDA did not consent to use of any animal data generated in Russia for U.S. domestic regulatory purposes.

Subject Inventions (As defined in the CRADA)

ORNL Disclosure 1438C has been filed for the potential mechanism of action of the substance. Sass & Sass elected rights in the invention and is currently pursuing patent protection.

Commercialization Possibilities

Polyprenyl phosphates are in the last stage of development by Sass & Sass, Inc. USDA is regulating the substance as a veterinary biologic and the facility as veterinary biologic manufacturing facility under 9 CFR. Sass & Sass, Inc. is proceeding with commercialization and USDA licensure.

Plans for Future Collaboration

None, at this time.

Conclusions

All parties were successful in meeting the objectives of this endeavor. Selected technical results are positive on the impacts on animal health. Phosphorylated polyprenyls have an extremely low toxicity (tested in mice and cats). Phosphorylated polyprenyls protect animals (in vivo) in the following experimental infections: tick-borne encephalitis (Russian data), herpes (US and Russian data), hepatitis and influenza (Russian data). Phosphorylated polyprenyls has been successfully tested for the treatment of clinical distemper cases in dogs. Encouraging results were achieved in general animal health protection in testing in U.S. animal shelters.

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1. **B.H. Davison**, 5700, MS-6164
2. **T. Kuritz,** 4500N, MS-6194

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2. **C.K. Williams,** BWXT-Y12, 1099 COM, MS-8260
3. **R.G. Ball,** BWXT-Y12, 1099 COM, MS-8260
4. **Arthur Sass,** Sass & Sass, Inc., Oak Ridge, TN