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TITLE: Adenosine A3 Receptor Antagonists for the Treatment of Neuropathic Pain

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CONTRACTING ORGANIZATION: BioIntervene Inc.

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14. ABSTRACT

Our goal is to develop BIO-205, a highly-selective agonist for the A3 adenosine receptor subtype, as an orally-administered monotherapy analgesic for the treatment of neuropathic pain and as an adjunct for opioid analgesics that increases analgesic efficacy while blocking the opioid's unwanted effects (addiction potential, tolerance, and physical dependency). To reach this goal, the current grant supports drug manufacture, the development of analytic methods to measure the drug in biologic fluids, animal safety and toxicology studies, and the preparation of requisite documentation for an application to the FDA for an Investigational New Drug (IND) designation. In the past year we have completed manufacture of the first (100 g) batch of drug, nearly completed the second batch (750 g), completed development of analytical methods, developed the protocols for the safety/toxicology studies, and have begun drafting the IND documents.

15. SUBJECT TERMS

Addiction, Analgesia, Analgesic tolerance, Drug manufacture, IND, FDA, Opioid, Opioid adjunct, Neuropathic pain, Chronic pain.

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1. INTRODUCTION.

BioIntervene has discovered novel potential analgesic drugs that are not narcotics and are non-addictive. These drugs excite the 3A receptor subtype of adenosine receptor. Unlike currently available analgesic drugs, these compounds are effective against both the usual kinds of chronic pain syndromes (for example, osteoarthritis) but also against the treatment-resistant neuropathic pain syndromes that occur when nerves are injured by disease, toxins or trauma. Our goal is to develop our lead drug candidate, BIO-205, as an orally-administered, monotherapy analgesic for the treatment of neuropathic pain. Moreover, we have found that BIO-205 greatly amplifies the analgesic efficacy of morphine-like drugs while simultaneously blocking the opioid's unwanted effects (the euphoria that promotes addiction, analgesic tolerance, and physical dependency). This suggests that adding BIO-205 to an opioid analgesic will yield superior analgesia while minimizing the potential for opioid abuse. The current grant supports drug manufacture, the development of analytic methods to measure the drug in biologic fluids, animal safety and toxicology studies, and the preparation of the requisite documentation for an application to the Food and Drug Administration (FDA) for an Investigational New Drug (IND) designation. The IND is required before we can test BIO-205's safety and efficacy in man. This Annual Report covers the first 12 months of progress for the grant's 18-month funding period.

2. KEYWORDS.

Addiction, Analgesia, Analgesic tolerance, Drug manufacture, IND, FDA, Opioid, Opioid adjunct, Neuropathic pain, Chronic pain.

3. ACCOMPLISHMENTS.

What were the major goals of this project? As outlined in the Statement of Work, there are 5 Major Tasks supported by this grant. All of the manufacturing and safety/toxicology studies described below are conducted under FDA-mandated Good Laboratory Practices (GLP) guidelines. Safety/toxicology studies in two species are required by the FDA. We have chosen rat and monkey; the dog was found to be unacceptable as it metabolizes the drug differently than mouse, rat, monkey, and man. The Major Tasks and Subtasks are:

Major Task 1: Drug manufacture and analytics.

1.1: Stage I – manufacture 100 g of BIO-205.

1.2: Analysis of Stage I compound.

1.3: Stage II – manufacture 650 g of BIO-205.

1.4: Analysis of Stage II compound.

Major Task 2: Toxicology studies.

2.1: Rat repeated dose, oral dose-range finding and toxicology study (118 rats).

2.2: Rat, 4-week oral dosing toxicology study with 4-week recovery (168 rats).

2.3: Monkey, single oral dose escalation and repeated dose range-finding and toxicology (14 rhesus monkeys).

2.4: Monkey, 4-week oral dosing toxicology study with 4-week recovery (16 rhesus monkeys).

Major Task 3: Safety pharmacology studies.

3.1: CNS safety pharmacology in rats (64 rats).

3.2: Pulmonary safety pharmacology in rats (64 rats).

3.3: Cardiovascular safety pharmacology in monkeys (8 rhesus monkeys).

3.4: *In vitro* hERG inhibition.

3.5: *In vitro* Genotoxicity studies: Ames assay, micronucleus assay, and chromosomal aberration assay.

Major Task 4: Bioanalysis/formulation analysis.

4.1: Rat bioanalytical method validation.

4.2: Monkey bioanalytical method validation.

4.3: Toxicology studies formulation validation.

4.4: hERG study formulation validation.

4.5: Genotoxicology study formulation validation.

Major Task 5: Approvals and prepare IND.

5.1: Submission to ACURO.

5.2: FDA planning meeting.

5.3: Design protocol for Phase I studies.

5.4: Prepare Investigator's Brochure.

5.5: Prepare and submit IND.

What was accomplished under these goals?

Major Task 1: Drug manufacture and analytics. This Task is days away from completion. After consideration of several candidates, we chose to have drug manufactured and analytics developed by WuXi Apptec, a Chinese CRO. WuXi is one of the world's largest CROs for this type of work. Subtask 1.1, preparation of the first 100 g batch of BIO-205, has been completed. During this work, the small-batch (milligrams) synthesis procedure that we began with was improved and we now have a procedure with a significantly higher yield which gave us 150 g of product without any increase in time or cost. Liquid chromatography and mass spectrometry analytics (Crystal Pharmatech and WuXi) confirmed that the drug product has the correct molecular structure and showed that the product contained no significant impurities.

Having established a reliable and efficient large-batch (grams) synthesis procedure, we initiated Subtask 1.3, preparation of 650 g of BIO-205. Manufacturing is complete, with a yield of 1,000 g obtained with no extra time or cost. Analytic confirmation of molecular identity and purity are expected within days. With the completion of Major Task 1 we will be able to proceed with Major Tasks 2-4.

Major Task 2: Toxicology studies & Major Task 3: Safety pharmacology studies. We also chose to contract with WuXi Apptec for the Toxicology and Safety studies. They are inspected regularly by the FDA and by the Association for Accreditation of Laboratory Animal Care (AAALAC) International, which ensures compliance with the animal care and use regulations of the USA. They have a record of success with supplying data accepted by the FDA and have passed repeated FDA inspections.

The WuXi Institutional Animal Care & Use Committee (IACUC) has reviewed and approved the protocols for both rat (Subtask 2.1) and monkey (Subtask 2.3) repeated dose, oral dose-range finding and toxicology studies. These protocols and all documents required were submitted to ACURO on 18 July 2019. The grant requires completion of other toxicology and safety Subtasks, but USA regulations require that animal use protocols specify drug dose, or a

small range of doses. This is not possible until we have completed the dose-ranging and toxicology studies described in Subtask 2.1 and 2.3. Thus, we will have to submit a new ACURO application for the remaining toxicology and safety studies after we have the results from the work specified in the first application.

Subtask 3.4, *In vitro* hERG inhibition, and Subtask 3.5, *In vitro* Genotoxicity studies, use cell-based assays that do not require IACUC protocols or ACURO approval. Contracts have been signed for these studies and they will begin soon.

Major Task 4: Bioanalysis/formulation analysis. Subtasks 4.1 and 4.2, Bioanalytic method validation for rat and monkey, are needed to develop a method for identifying and measuring drug levels in biologic fluids. Thus, this work requires blood and urine that is obtained from animals that are given the drug as part of the Major Task 2 & 3 study protocols. We have already established a liquid chromatography-mass spectrometry analytic method to detect and measure BIO-205 in water and do not anticipate any difficulty adapting this method to blood and urine samples.

Work on Subtask 4.3, Toxicology studies formulation validation, is in progress. We are determining BIO-205's maximum concentration and stability in the liquid formulations that are typically used for similar oily substances (for example, mixtures of alcohol, Tween, PEG, etc.). The challenge here is to develop a formulation that is a fluid that is stable and that has little viscosity such that it can be drawn into a syringe for injection. The FDA requires that we determine the drug's "No Adverse Effect Level" (NOAEL), which is the maximum dose that can be given without a detectable effect on the animal's health. Paradoxically, a problem arises with drugs that are very safe – one must give very high doses (up to an FDA-specified maximum of 1,000 mg/kg) in the search for a dose that produces an adverse event. It can be extremely difficult to get such a large amount of drug into a useable formulation. This is especially true for drugs like BIO-205 that are oily and have little or no aqueous solubility. A similar problem arises when performing the *in vitro* cell-based assays for hERG function (potassium currents in heart muscle cells) and genotoxicity. Note that developing the formulations to be used for toxicology, hERG studies, and genotoxicity studies is likely to be different from the development of the formulation that will be used in the first-in-man studies because the clinical studies will use drug doses considerably below those that evoke an adverse event.

Major Task 5: Approvals and prepare IND. As required for an Investigational New Drug submission to the FDA, we are preparing the Investigator Brochure (IB) and the study protocols for the Phase I first-in-man safety trials.

Owing to the importance of the IB in maintaining the safety of human subjects in clinical trials, and as part of their guidance on good clinical practice (GCP), the FDA has regulatory codes and guidances for authoring the IB. The IB summarizes all available information about the new drug to be tested, including data about its pharmacology, safety, toxicology, manufacture, and formulation. The IB contains a "Summary of Data and Guidance for the Investigator" section, of which the overall aim is to "provide the investigator with a clear understanding of the possible risks and adverse reactions, and of the specific tests, observations, and precautions that may be needed for a clinical trial. This understanding should be based on the available physical, chemical, pharmaceutical, pharmacological, toxicological, and clinical information on the investigational product(s). Guidance should also be provided to the clinical investigator on the recognition and treatment of possible overdose and adverse drug reactions that is based on previous human experience and on the pharmacology of the investigational product". We have prepared a draft IB that is current up to the initiation of the work performed for this grant and will update this as grant-supported and other studies are completed.

Obtaining an IND will allow us to perform the FDA-mandated Phase 1 first-in-man studies whose purpose is to determine the highest dose of BIO-205 that can be given without producing a serious side-effect. While we will have similar data for rats and monkeys, but human responses may differ and must be checked. As is usual for such trials, we will test healthy volunteers. The Phase 1 protocol is under development.

An ACURO submission was made on 18 July 2019 for dose-ranging and toxicology study protocols and associated documentation for WuXi Animal Care and Use Committee protocols 773-0001-TX (rat) and773-0002-TX (monkey).

An FDA planning meeting is anticipated early in 2020. Its primary purpose is for an informal review of our Phase 1 study protocol.

What opportunities for training provided? Nothing to report – not applicable.

How were the results disseminated to communities of interest? Nothing to report – not applicable.

What do you plan to do during the next reporting period? We will begin the initial dose-ranging and toxicology studies in rat and monkey as soon as we receive ACURO approval. Successful completion of these studies will allow us to specify the doses to be used in the ACURO approval application for the remaining toxicology and safety studies.

Studies to develop drug formulations for the toxicology/safety studies, hERG studies, and genotoxicity studies is continuing. This is routine work and no problems are expected.

hERG and genotoxicity studies will begin soon. We have preliminary data obtained with BIO-205 that was not manufactured per FDA guidelines (and thus cannot be submitted for an IND) that indicates that our compound will not have adverse effects in these assays.

The draft Investigator Brochure will be updated as appropriate. Development will continue for the protocol for the Phase 1 clinical trials.

4. IMPACT.

Nothing to report – not applicable.

5. CHANGES/PROBLEMS.

Changes in approach. None.

Actual or anticipated problems or delays. It has been more than 3 months since our submission for ACURO approval of the initial animal work. The drug needed for these studies will be available within a few days. *Additional ACURO delay will have a significant impact on the grant's progress.*

Changes with impact on expenditures. None.

Significant changes in use or care of animals, biohazards, etc. None.

Significant changes in use or care of human subjects. Not applicable.

Significant changes in use or care of animal subjects. None.

6. PRODUCTS.

Publications, conference papers, presentations. None.

Website or other internet. None.

Technologies or techniques. Significant improvement to the synthesis protocol for BIO-205.

Inventions, patent applications, licenses. None.

Other products. Approximately 1,150 mg of BIO-205 in a form suitable for FDA-mandated animal toxicology and safety testing.

7. PARTICIPANTS & OTHER ORGANIZATIONS

What individuals have worked on this project?

Name	Gary J. Bennett, PhD	Daniela Salvemini, PhD
Project role	PI	Consultant
Nearest person month worked	12 months	1 month (137.5 hrs)
Contribution to project	Initiation and supervision of all CRO contract work; review and approval of all work and work reports; all financial and reporting tasks.	Review of contracts, CRO progress reports, safety and toxicology protocols, analytics development and draft Investigators Brochure and Phase I study protocols.
Funding support	None other than this grant	Not applicable.

Has there been any change in the active other support during this reporting period of the PI or senior/key personnel? Nothing to report.

What other organizations were involved as partners? None.

8. SPECIAL REPORTING REQUIREMENTS.

Collborative awards. None.

Quad chart. Attached.

9. APPENDICES. None.

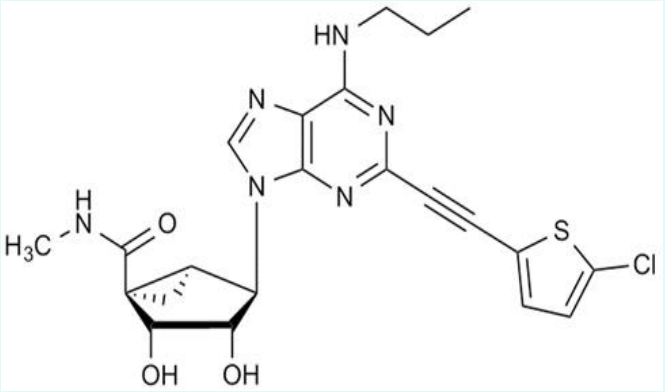
Adenosine 3A Receptor Antagonists for the Treatment of Neuropathic Pain
Log #: BA150673; Award #: W81XWH1810744

PI: Gary J. Bennett, PhD **Org:** BioIntervene Inc. **Award Amount:** $1,881,234,000.

Study/Product Aim(s)

- Major task 1: Manufacture 750mg of BIO-205.
- Major task 2: Conduct toxicology trials in rat and monkey.
- Major task 3: Conduct safety trials.
- Major task 4: Prepare documents for FDA submission for an Investigational New Drug (IND) designation.

Task	Mo.	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15	M162	M17	M18
1.1 Analytics	2																		
1.2 Make 100g batch	9																		
1.3 Prepare primary ref. standard	1																		
1.4 Characterize GLP Lot and standard	2																		
1.5 Prepare and characterize 650 g batch	4																		
2.1 Rat MTD*	1																		
2.2 Rat 7d tox*	1																		
2.3 Monkey MTD*	1																		
2.4 Monkey 7d tox*	1																		
2.5 Rat 5-wk	2																		
2.6 Monkey 5-wk	2																		
3.1 hERG	1																		
3.2 cardiovasc. telemetry	1																		
3.3 CNS safety	1																		
3.4 Respir. safety	1																		
3.5 Genotox studies	1																		
4.1 FDA mtg	1																		
4.2 Protocols for PI studies	3																		
4.3 Identify CROs for PI	3																		
4.4 Prepare Invest. Brochure	6																		
4.5 Submit IND	1																		



BIO-205, a highly selective agonist for the A3 subtype of adenosine receptor and a potential new analgesic drug.

Goals/Milestones achieved:

1. Receipt of batch 1 and batch 2 of BIO-205 from manufacturer.
2. Approval of initial Animal Care and Use Committee protocols for initial dose-ranging toxicology and safety studies from the CRO and submission of application to ACURO. Approval pending.

Comments/Challenges/Issues/Concerns

- Drug manufacture on schedule and on budget.
- Initial animal safety and toxicology studies awaiting ACURO approval – submitted on 18 July 2019 and still no action as of 28 October 2019..

Budget Expenditure to Date

- Actual Expenditure: $874,187 (consulting: $34,375; PI salary+benefits: $86,112; WuXi phase 1 mfg: $144,600; WuXi phase 2 mfg: $583,300; Crystal PharmaTech analytics: $25,800).

Updated: 28 October 2019 (Annual report).