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PRINCIPAL INVESTIGATOR:  Dr. Kathleen E. Rodgers

RECIPIENT:  University of Southern California
Los Angeles, CA 90089

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**Evaluation of MMX1902 as an Oral Treatment for Duchenne Muscular Dystrophy**

Kathleen Rodgers, Ph.D.
E-Mail: krodgers@usc.edu

**Performing Organization Name(s) and Address(es)**
University of Southern California
3720 S. Flower Street Third Floor
Los Angeles, CA 90089-0001

**Sponsoring / Monitoring Agency Name(s) and Address(es)**
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**Distribution / Availability Statement**
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**Abstract**
In the cardiac-focused study conducted, 10-week-old mdx mice were exercised at 15 m/min for 60 minutes 2 times a week for 10 weeks. Four different SQ treatment groups (n = 9/group) were evaluated – wild-type controls and three groups of mdx mice: vehicle (saline) and two MMX1902 doses (1.0, and 2.0 mg/kg/day). DMD-associated cardiomyopathy is a dilated cardiomyopathy and, as such, is marked by increased left ventricular volume and decreased ejection fraction with compensatory tachycardia. Following treatment and exercise, echocardiography showed daily MMX1902 treatment at the 2 mg/kg/day dose to reduce left ventricular end systolic volume, increase ejection fraction, and ameliorate tachycardia resulting in cardiac functional measures comparable to exercised wild-type control mice. Further, embryonic myosin heavy chain (eMHC) staining of the diaphragm showed a significant increase in eMHC positive muscle fibers with MMX1902 treatment, at both doses, supporting the potential for MMX1902 treatment to stimulate and sustain regeneration even in the face of long-term, intensive exercise.

**Subject Terms**
Duchenne Muscular Dystrophy, renin, angiotensin

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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Duchenne Muscular Dystrophy (DMD) is one of the most common and devastating genetic diseases of childhood, affecting approximately 1 in 3500 live male births. We have developed a small molecule agonist of the Mas receptor that has provided benefit in *mdx* mice, an animal model of DMD. This proposal seeks to: 1) test the oral efficacy of MMX1902 in *mdx* mice, 2) assess the ability of MMX1902 to provide benefit with delayed treatment, 3) evaluate the potential of MMX1902 to positively affect cardiac function, 4) optimize the synthesis of MMX1902 and produce GMP material, and 5) establish a complete ADME profile on the molecule.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Duchenne Muscular Dystrophy, renin, angiotensin

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

**What were the major goals of the project?**

*List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.*

- **Specific Aim 1** - Oral MMX1902 Dose Optimization
- **Specific Aim 2** – Delayed Administration of MMX1902
- **Specific Aim 3** – Evaluation of Cardiac Function Following MMX1902 Administration
- **Specific Aim 4** – Small Batch GMP Manufacture of MMX1902
- **Specific Aim 5** – Pre-IND Meeting with FDA

**What was accomplished under these goals?**

*For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.*
• Specific Aim 1 – Preliminary MMX1902 Data: Oral MMX1902 Dose Optimization
In order to better understand the effects of dose and dosing on the efficacy of MMX1902, MMX1902 was studied in non-treadmill *mdx* mouse model where 5 week old mice are treated for 6 weeks. This model is used by contract research organizations to provide powerful pre-clinical data in a cost-effective manner. As this model is both shorter in duration (6 weeks vs. 10 weeks) and does not require laborious treadmilling, we used this model to optimize MMX1902’s oral dosing prior to final dose optimization in the treadmill exercised *mdx* mouse model. In this study, two vehicle dosed control groups (wild-type and B10-*mdx*) and two groups MMX1902 (once or twice daily dosing with 8 mg/kg) were treated daily by oral gavage. An additional group was subcutaneously dosed with MMX1902 at 2 mg/kg and served as a positive control. Histological evaluation of the diaphragms showed a decrease in degenerating fibers and inflammatory loci (Figure 1 A-B). No significant changes were seen in regenerating fibers most likely due to the lower degree of muscle strain and injury in this non-treadmilled model. Additionally, MSC cultures and plasma levels of anti-inflammatory IL-10 points to oral efficacy of MMX1902 point to the benefit of twice daily dose of 8.0 mg/kg (a total daily dose of 16 mg/kg) (Figure 1 C-D). Together, these results demonstrate the oral efficacy of MMX1902 treatment at reducing diaphragm pathology, stimulating bone marrow MSC proliferation, and increasing circulating levels of anti-inflammatory cytokine IL-10.

• Specific Aim 2 – Delayed Administration of MMX1902
During the course of our studies, it became clear that MMX1902 was capable of maintaining muscle function not restoring function as the delayed administration study design would probe. However, as DMD is a lifelong disorder, it was equally crucial to assess the robustness of MMX1902’s effects on muscle function and diaphragm pathology. Therefore, using the same treadmill exercised *mdx* mouse model as our pilot study, 10 week old *mdx* and wild-type mice were treated daily and exercised for 30 minutes at 15 meters/min 3x/week for 20 weeks instead of the 10 weeks used in our pilot. After 20 weeks of treadmill exercise and treatment, muscle performance was assessed using the TREAT-NMD treadmill acceleration protocol ([DMD_M.2.1.003](#)). The 2.0 mg/kg dose of MMX1902 significantly improved running distance before exhaustion compared to vehicle treated *mdx* mice (Figure 2A). The 2.0 mg/kg dose of MMX1902 also significantly decreased circulating levels of pro-inflammatory cytokine IL-6 and increased bone marrow MSC populations (Figure 2B-C). Finally, all doses of MMX1902 given either orally or by SQ, significantly increased regeneration and decreased degeneration and inflammation in H&E stained diaphragm sections (Figure 2D-F). Together, these data point to the ability of MMX1902 to produce lasting effects on muscle performance and progenitor populations.
Specific Aim 2 – Delayed Administration of MMX1902 (cont.)

- Specific Aim 2 – Delayed Administration of MMX1902 (cont.)
  
  **Figure 2.** (A) After 20 weeks of treadmill exercise and treatment, muscle performance was assessed using the TREAT-NMD treadmill acceleration protocol. The 2.0 mg/kg dose significantly improved running distance compared to vehicle treated mdx mice. (B) Plasma samples showed a significant decrease in circulating pro-inflammatory cytokine IL-6 with SQ MMX1902 treatment. (C) At necropsy, bone marrow was collected, cultured, and mesenchymal stem cell (MSC) counts were recorded showing a significant increase with RASRx1902 treatment. Histological evaluation of H&E stained diaphragms showed a significant difference compared to vehicle treated mdx mice in measures of (D) regeneration and (E) degenerating fibers and (F) loci of inflammation with MMX1902 treatment. * = P ≤ 0.05, *** = P ≤ 0.001, and **** = P ≤ 0.0001 in comparison with vehicle control.

Specific Aim 3 – Evaluation of Cardiac Function Following MMX1902 Administration

In our last report, we detailed the effects of MMX1902 on cardiac function in the intensive treadmill exercise model. In the cardiac-focused study conducted, 10-week-old mdx mice were exercised at 15 m/min for 60 minutes 2 times a week for 10 weeks. Four different SQ treatment groups (n = 9/group) were evaluated – wild-type controls and three groups of mdx mice: vehicle (saline) and two MMX1902 doses (1.0, and 2.0 mg/kg/day). DMD-associated cardiomyopathy is a dilated cardiomyopathy and, as such, is marked by increased left ventricular volume and decreased ejection fraction with compensatory tachycardia. Following treatment and exercise, echocardiography showed daily MMX1902 treatment at the 2 mg/kg/day dose to reduce left ventricular end systolic volume, increase ejection fraction, and ameliorate tachycardia resulting in cardiac functional measures comparable to exercised wild-type control mice. Further, embryonic myosin heavy chain (eMHC) staining of the diaphragm showed a significant increase in eMHC positive muscle fibers with MMX1902 treatment, at both doses, supporting the potential for MMX1902 treatment to stimulate and sustain regeneration even in the face of long-term, intensive exercise. New to this progress report is the completion of the histological evaluation of diaphragm pathology by H&E staining. Evaluation of the diaphragm revealed a decrease in degenerating fibers and inflammatory loci (Figure 2A-B) demonstrating that even in the face of intense exercise MMX1902 is able profoundly increase diaphragm health further indicating the respiratory benefits of MMX1902 treatment.
Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. “Training” activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. “Professional development” activities result in increased knowledge or skill in one’s area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

During the course of these study, the team working on this study, Kevin Gaffney, Michael Weinberg, and Josh Dorst, all increased their familiarity with animal handling and increased or improved their skills at immunohistochemistry (IHC), plasma protein level analysis, biomarker discovery, primary cell culture, and H&E quantification. These advances are evidenced in the data outlined in the previous section.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report
What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state “Nothing to Report.”

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

During the next reporting period, we have set out to advance the following Specific Aims:

• **Specific Aim 1 - Oral MMX1902 Dose Optimization**
  We are currently carrying out a 10 week oral dose escalation study based on the results in the non-treadmill model described above. We are planning and have the data analysis of this study completed for the next reporting period.

• **Specific Aim 4 – Small Batch GMP Manufacture of MMX1902**
  In order to produce large quantities of MMX1902 under GMP manufacturing conditions, the synthesis of key intermediates needs to be produced on kg scale. Kemxtree Research Laboratories has begun the large-scale synthesis of a key intermediate. Work is underway at Kemxtree to delivery 1 kg of this late stage intermediate by November 31, 2017. Following this work, we will begin the final scale up of MMX1902 under **GMP or GLP** and the stability of MMX1902 will be tested using an accelerated stability protocol. This stability data and batch records along will be used to support filing of the pre-IND briefing package and subsequent pre-IND meeting with FDA.

• **Specific Aim 5 – Pre-IND Meeting with FDA**
  Following successful completion of **Specific Aims 1 - 4**, a pre-IND briefing package will be assembled for submission to FDA and a pre-IND meeting will be held regarding evaluation of MMX1902 treatment in patients suffering from DMD. We will have our pre-IND meeting with the FDA prior to the next reporting period.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

**What was the impact on the development of the principal discipline(s) of the project?**
If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

• **Specific Aim 1 - Oral MMX1902 Dose Optimization**
  This study confirms the oral efficacy of MMX1902 and sets the stage for our ongoing oral dose escalation in treadmill exercised *mdx* mice.

• **Specific Aim 2 – Delayed Administration of MMX1902**
  This study illustrates and confirms the long-term efficacy of MMX1902 and demonstrates its potential to treat this condition over a Duchenne muscular dystrophy patient’s life.

• **Specific Aim 2 – Evaluation of Cardiac Function Following MMX1902 Administration**
  The results from this study compliments those from **Specific Aim 2** showing MMX1902’s ability to maintain efficacy in the face of very intense exercise.
What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

The data obtained demonstrated the oral efficacy of MMX1902 and the robustness of its effects in the face of long-term and intense exercise. Based on this data and literature on natural peptide activator of our receptor of interest, our molecule has the potential to treat patients with cardiac maladies beyond patients with Duchenne muscular dystrophy.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- transfer of results to entities in government or industry;
- instances where the research has led to the initiation of a start-up company; or
- adoption of new practices.

Nothing to Report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- improving public knowledge, attitudes, skills, and abilities;
- changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or
- improving social, economic, civic, or environmental conditions.

Based on our preliminary cardiac efficacy data, our program shows the potential to increase muscle performance and reduce cardiac and diaphragm pathology, a major cause of mortality in patients with Duchenne muscular dystrophy.

5. CHANGES/PROBLEMS: The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, “Nothing to Report,” if applicable:
Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to Report

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution?
committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals.

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state “Nothing to Report.”

- Publications, conference papers, and presentations
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

  Nothing to Report

Books or other non-periodical, one-time publications. Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to Report

Other publications, conference papers, and presentations. Identify any other publications, conference papers and/or presentations not reported above. Specify the
status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.

Nothing to Report

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

Nothing to Report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - data or databases;
  - biospecimen collections;
  - audio or video products;
  - software;
  - models;
  - educational aids or curricula;
  - instruments or equipment;
  - research material (e.g., Germplasm; cell lines, DNA probes, animal models);
  - clinical interventions;
new business creation; and
other.

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate “no change.”

Example:

Name: Mary Smith
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID): 1234567
Nearest person month worked: 5

Contribution to Project: Ms. Smith has performed work in the area of combined error-control and constrained coding.
Funding Support: The Ford Foundation (Complete only if the funding support is provided from other than this award).
Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state “Nothing to Report.”

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed. Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner’s contribution to the project (identify one or more)
- Financial support;
- In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);
- Facilities (e.g., project staff use the partner’s facilities for project activities);
- Collaboration (e.g., partner’s staff work with project staff on the project);
- Personnel exchanges (e.g., project staff and/or partner’s staff use each other’s facilities, work at each other’s site); and
- Other.
8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to https://ers.amedd.army.mil for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Nothing to Report