Award Number: W81XWH-16-1-0706

TITLE: The Role of an Aggrecan 32mer Fragment in Post-Traumatic Osteoarthritis

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CONTRACTING ORGANIZATION: University of Melbourne
Australia 3052

REPORT DATE: October 2017

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
October 2017	Annual	30 Sep 2016 - 29 Sep 2017

4. TITLE AND SUBTITLE

The Role of an Aggrecan 32mer Fragment in Post-Traumatic Osteoarthritis

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-16-1-0706

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research
U.
Fort Detrick, Maryland
21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release;

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Recommended to be brief (approx. 200 words) of the main findings during the reporting period.
During this reporting period we completed an 8 week DMM experiment, in the presence and absence of the AF-28 antibody. This experiment revealed a trend for an AF-28-mediated reduction in knee hyperalgesia, but not mechanical allodynia, in mice at 8 weeks post-surgery. This trend was not statistically significant, however, we are looking forward to results from the next time point, at 16 weeks post-surgery. The hind limbs from the 8 week study, from mice with and without AF-28 treatment, have been shipped to Australia and will now be analyzed for changes in cartilage, bone and synovial structure. In addition, we have made good progress on developing an AlphLISA immunoassay for detecting 32mer in sera, and we are not far away from final ethics approval to test sera in this new assay.

15. SUBJECT TERMS
Aggrecan, cartilage, osteoarthritis, post-traumatic osteoarthritis, immunoassay, 32mer, AF-28, hyperalgesia, destabilization of the medial meniscus, immunotherapy, immunomodulation, pain

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
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TABLE OF CONTENTS

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

Aggrecan is a major component of articular cartilage. It is degraded in arthritic disease, causing structural damage, joint failure and pain. In this proposal we focus on a specific aggrecan degradation product, the aggrecan 32mer, and its contribution to the development of osteoarthritis (OA). We have evidence that the aggrecan 32mer promotes catabolic and inflammatory responses in joint tissues, influences bone cell death and bone accrual beneath cartilage and might also activate neurons that elicit pain. We will test the hypothesis that the aggrecan 32mer contributes to the development and pathogenesis of post-traumatic OA and that blocking aggrecan 32mer activity following joint injury with a 32mer-specific monoclonal antibody (AF-28) will be chondro-protective, osteo-protective and will provide effective joint analgesia, leading to healthier joint outcomes. The aims are to 1) determine if and how therapeutic blockade of aggrecan 32mer, using antibody AF-28, can limit or prevent the severity of PTOA following acute knee injury and 2) develop a biomarker assay for detecting the 32mer in human synovial fluids and/or sera.

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

aggrecan, osteoarthritis, post-traumatic osteoarthritis, cartilage, biomarker, bone, pain, joint injury, joint damage, neutralizing antibody

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.

Aim 1, Major Task 1

- Subtasks 1-6: DMM surgeries, staining and scoring of hindlimbs and pain studies for mice treated with AF-28 from time of surgery onwards.
- Subtask 7: *In vitro* culture of cells treated with 32mer +/-AF-28

Milestones Major Task 1

i. IACUC/ACURO Approval for in vitro studies: target date Jan 2017; completed Nov 2016.
ii. Additional AF-28 and IgG1 isotype control antibody antibody made under contract by CSIRO, Australia: target date Jan 2017; completed June 2017.
iii. Identify the molecular effects of AF-28 in vitro in chondrocytes, synovial fibroblasts, bone cells, target date Sept 2018; in progress, 30% complete.
iv. Renew approval for IRB#: 3369-04012R3 'Predict OA progression' to provide serum and synovial fluid samples for AlphaLISA assays: target date Jan 2017; completed Nov 2016.
v. Renew approval for IRB#: 7939-06-11R1 to provide synovial fluid samples for AlphaLISA assays; target date Mar 2017; completed Jan 2017.

Aim 1, Major Task 2

- Subtasks 1-6: DMM surgeries for Study 2, with treatments commencing 2 weeks post-surgery. Commencing in year 2.
- Subtask 7: In vitro culture of cells treated with 32mer +/-AF-28. Listed in error; this subtask is continuing under subtask 7 of Major task 1.
- Subtask 8: DMM surgeries in Pirt-GCaMP3 mice, with treatment from time of surgery, commencing in year 2.

Milestones for Major Task 2

i. Determine if AF-28 has efficacy in limiting PTOA onset or severity on inflammation, cartilage, bone and pain outcomes when administered 2 weeks post-surgery: Commencing in year 2.
ii. Determine whether AF-28 can limit DRG activation in Pirt-GCaMP3 mice following DMM – 8 week time-point: Commencing in year 2.

Aim 2, Major Task 3

- Subtask 1: Develop an AlphaLISA method for 32mer detection.
- Subtask 2: Screen cohorts of sera and synovial fluids described in Milestones 1 and 2 by AlphaLISA.

Milestones for Major Task 3

i. Seek approval of local Human Research Ethics Committee to collect synovial fluids from 20 joint replacement patients. Target date Oct 2017; completed Oct 2017.
ii. Obtain HRPO approval to use existing human samples. Target date Oct 2017; in progress, 50% complete.

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.

Overall Project Aim

Acute joint injury is the most significant risk factor for the development of post-traumatic osteoarthritis (PTOA). Irrespective of the cause of PTOA, the consequences for the joint include synovial inflammation, cartilage destruction, sub-chondral bone accrual, and osteophyte formation. Pain is also a key feature of PTOA and in advanced disease, uncontrolled pain is the major driver for joint replacement surgery. The lack of treatments for PTOA creates an unmet need for effective therapies to treat pain and arrest joint erosion. Our project addresses this need.

Aggrecan is the major proteoglycan in cartilage, and in osteoarthritis (OA) it is degraded by metal-dependent proteinases. We have previously shown that a 32 amino-acid peptide fragment of aggrecan (the 32mer) is pro-inflammatory and pro-catabolic in joint cells, and that the 32mer might mediate cartilage/bone crosstalk. Our collaborators at RUSH University, Chicago, have also discovered that the 32mer activates nociceptors in explant cultures of dorsal root ganglia (unpublished) and that 32mer-deficient mice (Chloe) fail to develop knee hyperalgesia, which is a pain-related behaviour associated

with experimental PTOA in mice. Together, these data suggest that an anti-32mer therapeutic has potential as an early intervention following acute joint injury. Moreover, the 32mer has potential as a biomarker for monitoring the progression of PTOA following joint injury.

We hypothesise that i) the 32mer contributes to the pathogenesis of PTOA and ii) blocking 32mer activity with monoclonal AF-28 following joint injury will be chondro-protective, osteo-protective and will provide effective analgesia, leading to healthier joint outcomes.

The aims of this project are to
1) determine if, and how, therapeutic blockade of aggrecan 32mer using AF-28 can limit or prevent the severity of PTOA and its pain responses in a mouse model of PTOA (the DMM model)
2) investigate the mechanism of 32mer action *in vitro*, in chondrocytes, subchondral bone cells and synovial fibroblasts
3) develop a biomarker immunoassay for the detection of 32mer in human synovial fluid and/or serum.

Major Task 1

Subtask 1: *DMM surgeries for study 1, treatment from time of surgery, time points 2, 4, 8 & 10 weeks.*

Destabilization of the Medial Meniscus (DMM) is a surgical procedure used to induce OA-like joint damage in mouse hind limbs. The first major task *(Subtask 1)* was to use DMM surgery, with or without twice weekly injections of AF-28 antibody, in order to observe the effects of AF-28 on joint pathology. The control groups included injections of isotype control antibody, or no antibody. The test group included injections of AF-28 (10mg/Kg). Naïve (uninjected) mice were also included as a negative control for the effects of surgery. Injections were commenced one day post-surgery. There was no significant difference in the body weights of mice between the different experimental groups with time, other than a trend of injection effect, seen as slightly decreased body weights (**Figure 1**).

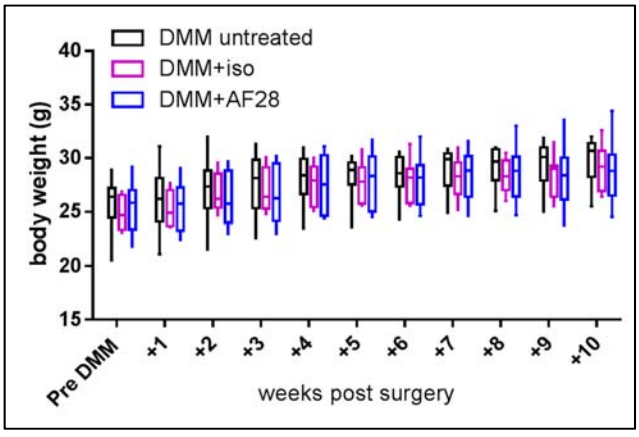

Figure 1. Mouse body weights

Major Task 1

Subtask 2: *pain measures for study 1 (above)*

To assess the effects of AF-28 antibody on DMM-induced pain, knee hyperalgesia was assessed at 2, 4, 8 and 10 weeks post-DMM surgery. The results in **Figure 2** and **Table 1** show that although there was no significant effect of AF-28 antibody on knee hyperalgesia at any time during the experiment, there was a trend for AF-28 to protect against hyperalgesia at 8 and 10 weeks post-surgery.

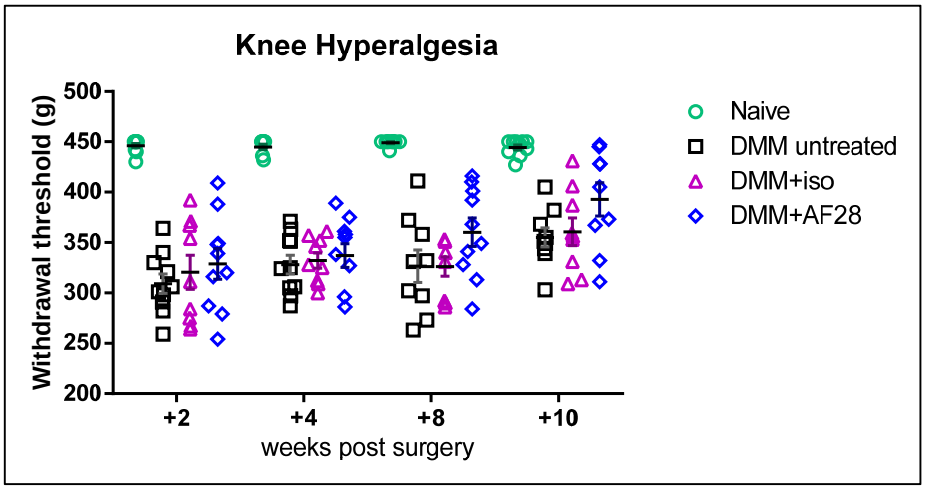

Figure 2. Trend of protection late for knee hyperalgesia

Table 1. There is a trend for AF-28 to protect against knee hyperalgesia at 8 weeks.

Treatments	P value summary at 8 weeks	P value summary at 10 weeks
Naïve vs DMM untreated	<0.0001	0.0001
Naïve vs DMM + isotype	<0.0001	0.0001
Naïve vs DMM + AF-28	<0.0001	0.0068
DMM untreated vs DMM+ iso	>0.9999 ns	0.9865 ns
DMM untreated vs DMM + AF-28	0.1423 ns	0.0910 ns
DMM + isotype vs DMM+ AF-28	0.1361 ns	0.1874 ns

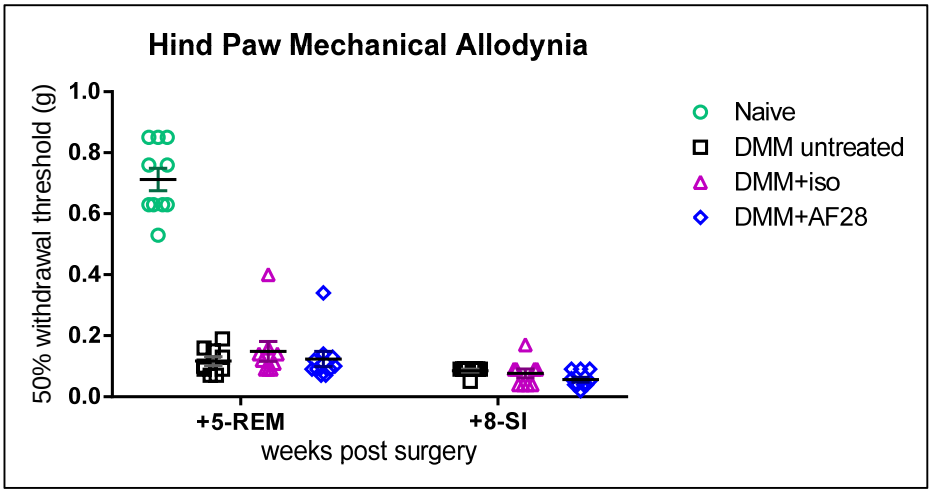

Figure 3. No trend for mechanical allodynia of the ipsilateral hind paw

There was no significant effect of AF-28 antibody on mechanical allodynia of the ipsilateral hind paw **(Figure 3)**. The surgeries and analyses to examine pain readouts at 16 weeks post DMM will commence in the second year of this project.

Major Task 1
Subtask 3-6:

The fixed hind limbs from the DMM experiment above have been shipped to Australia for

- μCT analysis to measure subchondral bone accrual and osteophyte formation
- histology and immunohistochemistry analyses
- scoring by two blinded investigators, for cartilage, bone and osteocyte pathology.

Major Task 1
Subtask 7: *In vitro culture of cells treated with 32mer +/-AF-28*

The *in vitro* analyses to date have included 32mer treatment of i) mouse cartilage explants, and ii) chondrocytes isolated from mouse knee cartilage, in the presence and absence of AF-28 antibody. The readout for these experiments included increased expression of pro-inflammatory and pro-catabolic genes. The results confirmed that isolated chondrocytes, but not chondrocytes embedded in a cartilage matrix, respond to 32mer peptide *in vitro*. The results also showed that there was no effect of AF-28 antibody on the expression of pro-inflammatory or pro-catabolic genes, under any conditions tested. We know from previous work that immunoglobulins are freely permeable through cartilage matrix. Accordingly, in the next reporting period we will investigate the possibility that AF-28 antibody can block the effects of *endogenous* 32mer (as opposed to *exogenous* 32mer) produced in response to inflammatory mediators such as example, IL-1α.

One limitation of our *in vitro* experiments is the concentration of antibody needed to match or exceed the physiological concentrations of 32mer *in vivo*. The concentration of aggrecan in cartilage is approximately 25μM, and we find that 30μM 32mer reproducibly promotes an inflammatory/catabolic response *in vitro*. Accordingly, concentrations of antibody greater than 30μM are required for *in vitro* experiments aimed at blocking 32mer activity; this is a high concentration of antibody for an *in vitro* experiment.

Subtask 7 of Major task 1 also proposed an analysis of 32mer activity and AF-28 blockade in other cell types present in joints. During the last reporting period we spent time developing and optimizing the experimental conditions for culturing osteoblasts and synovial fibroblasts. In the next reporting period we will examine the effects of 32mer, with and without AF-28 treatment, on osteoblast and synovial fibroblast in cell culture, and we will analyse osteocyte health in relation to 32mer and regions of marked aggrecan loss.

Milestones for Major Task 1

i) IACUC/ACURO Approval for *in vitro* studies: Achieved
ii) Additional AF-28 and IgG1 isotype control antibody made under contract by CSIRO, Australia: Achieved
iii) Identify the molecular effects of AF-28 *in vitro* in chondrocytes, synovial fibroblasts, bone cells: In progress
iv) Renew approval for IRB#: 3369-04012R3 'Predict OA progression' to provide serum and synovial fluid samples for AlphaLISA assays. Achieved
v) Renew approval for IRB#: 7939-06-11R1 to provide synovial fluid samples for AlphaLISA assays. Achieved

Major Task 2

Subtasks 1-6: *DMM surgeries for Study 2, treatment from 2 weeks after surgery* Commencing in year 2.

Subtask 7: *In vitro culture of cells treated with 32mer +/-AF-28* Listed in error; this subtask is continuing under subtask 7 of Major task 1.

Subtask 8: *DMM surgeries in Pirt-GCaMP3 mice, treated from time of surgery, for 8 weeks.* Commencing in year 2.

Milestones for Major Task 2

i) Determine if AF-28 has efficacy in limiting PTOA onset or severity on inflammation, cartilage, bone and pain outcomes when administered 2 weeks post-surgery: Commencing in year 2.

ii) Determine whether AF-28 can limit DRG activation in Pirt-GCaMP3 mice following DMM – 8 week time-point: Commencing in year 2.

Major Task 3

Subtasks 1: *Develop an AlphaLISA assay for 32mer detection*

AlphaLISA immunoassay for detecting 32mer

We are developing a new immunoassay to detect 32mer in human serum and synovial fluid, using proprietary AlphaLISA technology (from PerkinElmer). AlphaLISA assays incorporate a biotinylated anti-analyte antibody (our analyte is 32mer) which binds to streptavidin-coated donor beads, while another anti-analyte antibody is conjugated to AlphaLISA acceptor beads. In the presence of 32mer the beads are brought into close proximity, resulting in a chemiluminescent light emission at 615nm, proportional to the amount of analyte present in the sample.

Mouse monoclonal AF-28 recognising the FFG N-terminus, and rabbit polyclonal αEGE recognising the 32mer C-terminus are used in this assay. Both antibodies have been i) labelled with biotin and, separately, ii) conjugated to AlphaLISA acceptor beads in order to test which of the two combinations gives the best configuration for the assay. Biotinylated αEGE in combination with αFFG conjugated to acceptor beads provided the greatest sensitivity in the assay.

The dynamic range, signal and sensitivity of the assay is influenced by the order in which reagents are added. We have tested empirically for the optimal order and combination of reagents, and found that the highest sensitivity is achieved as follows:

- Incubate the 32mer analyte/sample with AF-28 conjugated acceptor beads for 2 hours.
- Add biotinylated αEGE antibody and incubate for a further hour.
- Add streptavidin-coated AlphaLISA donor beads and incubate for 30 minutes.
- Read sample absorbance at 615nm on the PerkinElmer Enspire plate reader.

Other assay parameters, including titration of the AlphaLISA beads and testing a range of assay buffers have been optimized to reduce background and increase the dynamic range of the assay, as follows:

- Foetal bovine serum is the most suitable diluent for quantitating 32mer in serum samples.
- The total assay volume has been scaled down to 2.5µL of sample, with no loss of sensitivity.

We have established assay conditions to detect a dynamic range of 0.008 - 30nM 32mer **(Figure 4).**

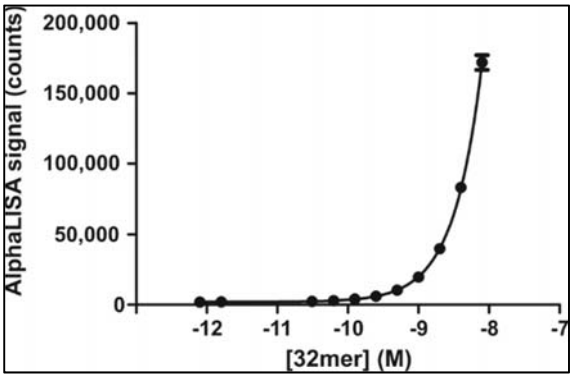

Figure 4. 32mer AlphaLISA has a dynamic range of 0.008 - 30nM

While waiting for HRPO approval to use patient sera and synovial fluids, we have begun work using sera from patients with juvenile idiopathic arthritis to optimize the assay; these sera are available to us from previous, non-DOD funded research. We have discovered that in order to detect 32mer signal above the level of interfering serum molecules, sera must be diluted at least 1:1000, then 'spiked' with a known concentration of 32mer, to bring the total 32mer concentration (sample + spike) to within the range of the standard curve. The known concentration of the spike is then subtracted from the total detected, to derive the concentration of 32mer in the sample. This principle of assay design was published previously by our collaborator, Prof Virginia Kraus (Duke University). We have also overcome an early problem with sample reproducibility by replacing our standard laboratory pipettes with positive displacement pipettes that are better-suited to viscous samples such as sera.

We next determined the optimal diluent for the assay, and the extent of dilution required to achieve assay linearity (R^2 value >0.995), that provides 70-130% assay linearity. We found that optimal linearity was achieved in foetal calf serum (FCS) with $R^2 = 0.994$, with 96% recovery of 32mer in two-fold dilutions. In a follow-up "spike and recovery" experiment we determined that optimal recovery of spiked 32mer was achieved with a spike value of 8nM 32mer. We have subsequently increased the assay volume to 50µL, in order to improve assay reproducibility.

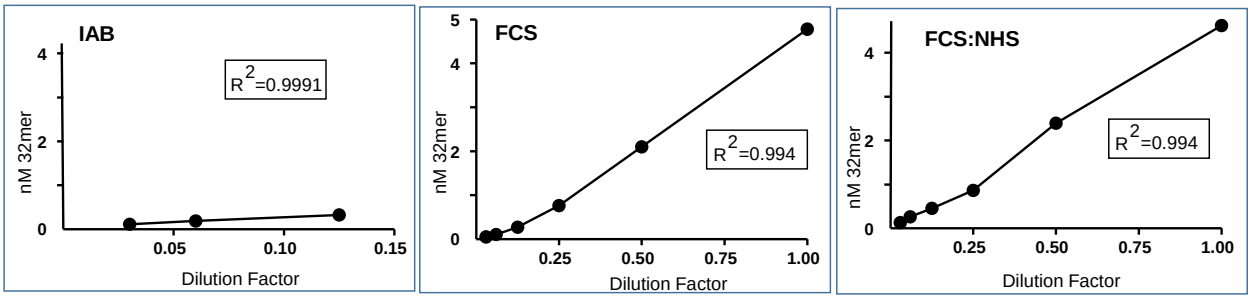

Figure 5. Standard curves generated in IAB, FCS or FCS:NHS to determine linearity

Major Task 3

Subtask 2: *Screen cohorts of sera and synovial fluids described in Milestones 1 and 2 by AlphaLISA.* Pending completions of milestone 1 and milestone 2 below.

Milestone 1: *Seek approval of local Human Research Ethics Committee to collect synovial fluids from 20 joint replacement patients.* Pending.

Milestone 2: *Obtain HRPO approval to use existing human samples.* Pending.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

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.

Nothing to report

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.

Aim 1
Mouse hind limbs from the first DMM experiment have been sent to Australia for micro CT analyses ahead of sectioning for histology and immunohistochemistry.

Aim 2
Work on developing the AlphaLISA assay for the 32mer will continue.
DMM surgeries for major task 2 will begin.

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).

Nothing to report

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.

Nothing to report

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.*

Nothing to report

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.

No changes 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.

The work assaying human sera and synovial fluids for the 32mer (Aim 2) has been delayed by the need for human research ethics approval from multiple institutions. Our HRPO contact advised us to seek approval with the University of Melbourne for a project that combines the use of existing samples from Duke University (Durham, NC), from A/Prof Adam Bryant of the University of Melbourne, and new samples from St Vincent's Hospital in Melbourne. We now have approval from St Vincent's Hospital to collect new samples, and the combined application to the University of Melbourne for all samples is submitted. Once we have University of Melbourne approval, we can then re-apply to the HRPO for final approval for the use of human samples. We hope to have HRPO approval within the next six months.

Changes that had a significant impact on expenditures
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

No changes to report

Significant changes in use or care of vertebrate animals.

No changes 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).*

Name: Amanda Fosang
Project Role: Principal Investigator
Researcher Identifier: ORCID ID 0000-0002-5523-5427
Nearest person month worked: 1
Contribution to project: Supervision of research assistants and administrative officer.

Name: Sue Golub
Project Role: Research Assistant
Researcher Identifier: ORCID ID 0000-0002-0249-0483
Nearest person month worked: 12
Contribution to Project: Laboratory work, including cell and tissue culture, histology, qPCR analyses.

Name: Karena Last
Project Role: Research Assistant
Researcher Identifier: ORCID ID 0000-0002-4396-8404
Nearest person month worked: 7
Contribution to Project: Laboratory work, including establishing and validating the AF-28 immunoassay and managing the mouse breeding program.

Name: Heather Stanton
Project Role: Administrative Assistant/Research Officer
Researcher Identifier: ORCID ID 0000-0002-3427-5614
Nearest person month worked: 7
Contribution to Project: Budgeting, report drafting, managing ACURO and HRPO compliance, drafting of animal and human ethics protocols.

Name: Professor Anne-Marie Malfait (Rush University)
Project role: Collaborator and Animal Experimentalist
ORCID ID: 0000-0003-1428-0384
Nearest person month worked: 1
Contribution to project: Supervision of the DMM experiments

Name: Ms Shuhan Yu
Project role: Research Assistant
ORCID ID: n/a
Nearest person month worked: 3
Contribution to project: Animal work for the DMM experiments

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.*

CSIRO Protein Production Facility
Parkville, Australia
Dr Tim Adams from CSIRO produced the AF-28 antibody for us under contract.

University of Melbourne, Dept of Microbiology
Parkville, Australia
Dr David Jackson from the University of Melbourne synthesized and purified mouse 32mer for us.

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.

No appendices