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**TITLE:** The role of an aggrecan 32mer fragment in post-traumatic osteoarthritis

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**14. ABSTRACT**
Recommended to be brief (approx. 200 words) of the main findings during the reporting period.
In this fourth reporting period, we successfully developed an assay to measure the aggrecan 32mer fragment in human and mouse serum. The 32mer has great potential as a novel biomarker for OA pain, as well as a target marker for aggrecanase activity. We have applied for a one-year no cost extension to further develop the assay, testing cohorts of existing human and mouse sera.

Specifically, we will validate the use of plasma 32mer levels as markers for osteoarthritis (pain) in mice at different time points after DMM surgery, and this in wildtype as well as Chloe mice (as negative controls). In addition, we will continue to validate the assay in existing human sera.

| 15. SUBJECT TERMS<br>Aggrecan, cartilage, osteoarthritis, post-traumatic osteoarthritis, immunoassay, 32mer, AF-28, hyperalgesia, destabilization of the medial meniscus, immunotherapy, immunomodulation, pain | | | | | |
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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 also activates neurons that elicit pain. We will test the hypothesis that i) the aggrecan 32mer contributes to the development and pathogenesis of post-traumatic OA and ii) 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.*

| **Specific Aim 1** | **Timeline** | **Site 1** | **Site 2** |
|---|---|---|---|
| **Major Task 1** | Months | Fosang | Malfait |
| Subtask 1: DMM surgeries for Study 1, treatment from time of surgery, timepoints 4, 8, 16 weeks.<br>Experimental groups and numbers/group:<br>Gr. 1 (n=18): Naïve (x 3, for each time point; total n= 54)<br>Gr. 2 (n=18): Sham (x 3, for each time point; total n= 54)<br>Gr.3 (n=18): DMM, untreated (x 3, for each time point; total n= 54)<br>Gr. 4 (n=18): DMM, AF-28 treated (x 3, for each time point; total n= 54)<br>Gr.5 (n=18): DMM, isotype control Antibody treated (x 3, for each time point; total n= 54) | 1-12 | | 33% complete[a] |
| Subtask 2: Pain measures: Study 1.<br>This is done on the mice from subtask 1 in a longitudinal fashion, bi-weekly. | 1-12 | | 100% complete Oct. 2017 |

| | | | |
|---|---|---|---|
| Subtask 3: Embedding and sectioning of hindlimbs; samples will be shipped to Melbourne in batches | 4-24 | 100% complete Oct.2017 | |
| Subtask 4: Staining and histologic scoring of sections for cartilage parameters | 4-24 | 100% complete Oct.2018 | |
| Subtask 5: Staining and histologic scoring sections for bone parameters | 4-24 | 75% complete | |
| Subtask 6: Immunostaining of sections | 4-24 | 100% complete Oct.2018 | |
| Subtask 7: *In vitro* cell culture treated with 32mer +/-AF-28. This task requires 414 wildtype mice per year, for two years. Local ethics approval to harvest tissues from culled mice has been approved and is due for renewal in November 2017. Start time for this task pending ACURO approval | 1-24 | 100% complete Sept. 2018 | |
| Milestone(s) to be Achieved:<br>IACUC/ACURO Approval for in vitro studies | 4 | 100% complete June 2016 | |
| Additional AF-28 and IgG1 isotype control antibody made under contract by CSIRO, Australia | 4 | 100% complete June 2017 | |
| Identify the molecular effects of AF-28 *in vitro* in chondrocytes, synovial fibroblasts, bone cells | 24 | 100% complete Sept. 2018 | |
| Renew approval for IRB#: 3369-04012R3 'Predict OA progression' to provide serum and synovial fluid samples for AlphaLISA assays | 4 | 0% complete Not begun[b] | |
| Renew approval for IRB#: 7939-06-11R1 to provide synovial fluid samples for AlphaLISA assays. | 6 | 0% complete Not begun[b] | |
| **Major Task 2:** | | | |
| Subtask 1: DMM surgeries for Study 2, treatment from 2 weeks after surgery, time-points 4, 8, 16 weeks.<br>Experimental groups and numbers/group:<br>Gr. 1 (n=18): Naïve (x 3, for each time point; total n= 54)<br>Gr. 2 (n=18): Sham (x 3, for each time point; total n= 54)<br>Gr.3 (n=18): DMM, untreated (x 3, for each time point; total n= 54) | 13-24 | | 33% complete[a] |

| | | | |
|---|---|---|---|
| Gr. 4 (n=18): DMM, AF-28 treated (x 3, for each time point; total n= 54)<br>Gr.5 (n=18): DMM, isotype control Antibody treated (x 3, for each time point; total n= 54) | | | |
| Subtask 2: Pain measures: Study 2<br>This is performed on the mice from subtask 1 in a longitudinal fashion, bi-weekly. | 13-24 | | 100% complete Oct. 2018 |
| Subtask 3: Embedding and sectioning of hindlimbs | 15-30 | 100% complete Feb.2019 | |
| Subtask 4: Staining and histologic scoring of sections for cartilage parameters | 15-30 | 100% complete April 2019 | |
| Subtask 5: Staining and histologic scoring sections for bone parameters | 15-30 | 60% complete | |
| Subtask 6: Immunostaining of sections | 15-30 | 100% complete April 2019 | |
| Subtask 7: *In vitro* cell culture treated with 32mer +/-AF-28. This task requires 414 mice per year. Ethics approval to harvest tissues from culled mice will be renewed in November 2017. | 1-24 | 100% complete Sept. 2018 | |
| Subtask 8: DMM Surgery in Pirt-GCaMP3 mice, treatment from time of surgery, for 8 weeks.<br>Experimental groups and numbers/group:<br>Gr.1 (n=18): DMM, untreated<br>Gr. 2 (n=18): DMM, AF-28 treated<br>Gr.3 (n=18): DMM, isotype control Antibody treated. | 13-18 | | 0% complete Plan changed[a] |
| Milestone Achieved: renew ACURO Approval for in vitro studies | 18 | 100% complete March 2018 | |
| Milestone(s) to be Achieved:<br>- 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<br><br>- Determine if AF-28 can limit DRG activation in Pirt-GCaMP3 mice following DMM – 8 week time-point | 28 | 100% complete April 2019<br><br>0% complete Plan changed[a] | 100% complete April 2019<br><br>0% complete Plan changed[a] |
| **Specific Aim 2** | | | |
| **Major Task 3** | | | |

| | | | |
|---|---|---|---|
| Milestones to be achieved: Local and HRPO approval to use existing human samples and to collect new human samples, as described in subtasks 2 and 3, below. | 12 | 100% complete Dec. 2018 | |
| Subtask 1 Develop AlphaLISA method for 32mer detection | 1-12 | 50% complete | |
| Subtask 2 Seek approval of local Human Research Ethics Committee to collect synovial fluids from 20 joint replacement patients. | 1-12 | 100% complete Oct.2017 | |
| Subtask 3 Obtain HRPO approval to use existing human samples as follows:<br>1) Sera and synovial fluids from 138 patients with osteoarthritis, collected at Duke University<br>2) Synovial fluids from 11 patients following anterior cruciate ligament surgery, collected at Duke University<br>3) Synovial fluids collected from surgical waste (exempt protocol), collected at Duke University. Number yet to be determined.<br>4) Serum from 49 patients following anterior cruciate ligament surgery, collected at The University of Melbourne | 1-12 | 0% complete Not begun[b]<br><br>100% complete May 2018 | |
| Subtask 4 Screen the cohorts of sera and synovial fluids described in subtasks 2 and 3 using the AlphaLISA method developed in months 1-12. | 12-30 | 100% complete | |
| Milestone(s) Achieved:<br>Establish the alphaLISA method for the detection of 32mer in human synovial fluids and serum<br>Determine if 32mer is a potential biomarker for PTOA pathology by screening cohorts | 1-30 | % complete July 2019 | |
| Write up research findings for publication | 18-36 | 20% complete | |

a. The data from the first timepoint indicated that AF-28 was not a neutralizing antibody, therefore it was considered not good use of resources to continue these experiments.

b. We were unable to obtain local human ethics approval (Melbourne, Australia) to use these samples.

**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 Tasks 1 and 2*
***Subtasks 1-6:*** *In vivo studies*

Destabilization of the Medial Meniscus (DMM) is a surgical procedure used to induce OA-like joint damage in mouse hind limbs. In major tasks 1 and 2 we used DMM surgery, with or without twice weekly injections of AF-28 antibody, in order to observe the effects of AF-28 on the extent and progression of joint pathology. Ten-week old, male wildtype mice were used for DMM. The control groups included injections of isotype control antibody, or no antibody. The contralateral hindlimbs (left legs) were also included as controls. 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.

Two DMM surgeries are complete.

1. DMM#1: treatment with AF-28 or isotype control commenced one day post-surgery and continued twice-weekly until harvest at 10 weeks post-surgery. Groups were naïve+no treatment (n=10 mice); DMM+no treatment (n=9); DMM+isotype control antibody (n=9); DMM+AF28 antibody (n=10).

2. DMM#2: treatment with AF-28 or isotype control commenced two weeks post-surgery. Injections were twice-weekly and continued until harvest at 16 weeks post-surgery. Groups were naïve+no treatment (n=5 mice); DMM+no treatment (n=10); DMM+isotype control antibody (n=10); DMM+AF28 antibody (n=10).

For histology, knee joints were decalcified and embedded coronally in paraffin. Sections (5µm) were cut through the entire weight-bearing area of the joint. Slides were stained at 25µm intervals with Safranin-O Fast Green. Histologic scoring for cartilage structural damage and aggrecan loss was done according to the OARSI guidelines.

For µCT analyses, images were acquired using a Bruker Skyscan 1272 scanner. Following reconstruction, data were converted and regions of interest (ROI) were delineated using Bruker CTAn. ROIs were traced on the lateral and medial tibial plateaus. Thresholds were determined using the automatic 'OTSU' algorithm. 2D and 3D data were generated for all analyses.

Statistical analyses of the bone and histology studies were done using GraphPad Prism software. Data are reported as mean +/- 95% CI. Initial analysis of variance between groups was done using a one-way ANOVA test. Unpaired *t*-tests were used to determine differences between treatments.

***Results for DMM#1,*** Knee hyperalgesia was assessed at 2, 4, 8 and 10 weeks post-DMM surgery. We reported 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 (**data reported in October 2017 Annual Report**). Treatment with AF-28 from Day 1 had no protective effect on progression of joint damage, as assessed by histology (**data reported in October 2018 Annual Report**). Ten weeks after DMM surgery, mice showed significant cartilage damage in the medial compartment (tibial plateau and the medial femoral condyle), but not the lateral compartment. There was no effect of AF-28 or isotype control antibody on cartilage damage. Proteoglycan loss was significant in the medial and lateral tibial plateau of DMM treated mice, but there was no effect of AF-28 antibody. µCT analyses showed that DMM surgery caused displacement of the medial meniscus and gross deformation of the joint. but did not appear to cause gross changes to bone mineralisation or sub-chondral bone accrual (**data reported in May 2019 Biennial Report)**. Analyses of total bone volume and more

mineralised bone by µCT showed no statistically significant effect of DMM surgery on these parameters, nor any effect of AF-28 antibody (**data reported in May 2019 Biennial Report)**. Analyses of trabecular thickness, spacing and number showed no statistically significant effect of DMM surgery on these parameters, nor any protective effect of AF-28 antibody (**data reported in May 2019 Biennial Report)**. Because we expected DMM surgery to cause subchondral bone accrual and an increase in mineralised bone, CI Malfait will refute/confirm these data by scoring bone parameters via histology.

***Results for DMM#2***, knee hyperalgesia was assessed at 2, 4, 8, 12 and 16 weeks post-surgery. Again, there was no statistically significant effect of AF-28 antibody on knee hyperalgesia, up to 16 weeks post-surgery. There was also no significant effect of AF-28 on mechanical allodynia of the ipsilateral hind paw (**data reported in October 2018 Annual Report**). Histology showed no statistically significant effect of AF-28 antibody on cartilage damage or proteoglycan loss, up to 16 weeks post-surgery **(data reported in May 2019 Biennial Report)**.

## *Major Tasks 1 and 2*

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

The aim of the *in vitro* studies was to determine whether AF-28 neutralizes 32mer action in joint cells *in vitro*. In year one, we optimized conditions for isolating and culturing mouse chondrocytes, osteoblasts, osteoclasts and synovial fibroblasts. In the **October 2018 Annual Report**, we reported that isolated chondrocytes and synovial fibroblasts respond to 32mer peptide by increasing their expression of pro-inflammatory and pro-catabolic genes, but that AF-28 antibody did not neutralize these activities. Neither did it block the action of *endogenous* 32mer in cartilage explants. Osteoclasts did not respond to the 32mer and osteoblasts failed to respond to 32mer treatment consistently. We concluded that AF-28 was not a neutralizing antibody. On this basis, we decided to complete the analyses of DMM#2, but not follow on with more DMM surgeries. There have been no further studies done *in vitro* during this reporting period.

## *Major Task 2*

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

Discontinued. See rational in SOW, listed in section 3.

## *Major Task 3*

***Subtasks 1-4:*** *Develop an AlphaLISA assay for 32mer detection*

We have developed a novel immunoassay to detect 32mer in human serum, 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. This assay uses mouse monoclonal AF-28 recognizing the FFG N-terminus, and rabbit polyclonal αEGE recognizing the 32mer C-terminus.

The assay has been optimised for orientation of antibodies, order of addition of antibodies, assay volumes and diluents. We reported in the **May 2019 Biennial Report** that we can detect synthetic

32mer peptide with a 5-log dynamic range of 0.0001-100nM. We can now report that we have assayed sera and synovial fluids for endogenous 32mer peptide from two cohorts of donors with OA to assay.

- Cohort #1 has samples from patients with end-stage OA, presenting for joint replacement surgery St Vincent's Hospital, Melbourne. Sera and synovial fluid samples were collected by us in 2018-2019.
- Cohort #2 included samples from patients with post-traumatic OA as a result of anterior cruciate ligament (ACL) damage, taken 2 year and 4 years post ACL reconstruction. Sera samples were made available to us by a collaborator and in some cases are many years old.

All samples were deglycosylated prior to assay. **Figure 1a** shows the endogenous 32mer concentrations in sera from Cohort #1. The concentration of 32mer peptide in the samples varied from 21pM to 420pM; one sample could not be assayed due to its high lipid content. The results were reproducible on repeat assay. **Figure 1b** shows the endogenous 32mer peptide concentrations in sera from Cohort #2; concentrations of 32mer varied widely, from 40pM to 1430pM. A one-way ANOVA test confirmed that there was no significant difference between the groups. We were surprised to find that all groups, including control groups, had levels of 32mer peptide higher than the levels found in Cohort #1. We wonder whether this is due to the age of the samples. In the next reporting period (one-year extension), we will assay more cohorts in order to determine the expected level of variation of 32mer peptide in human sera, and whether sample freshness impacts on the assay.

The synovial fluid samples from Cohort #1 proved to be too viscous to assay reproducibly. We trialed various methods to reduce the viscosity of the samples, including deglycosylating and diluting in a variety of different diluents. Perhaps with further work we could define conditions suitable for assaying synovial fluids; however, because collecting synovial fluids is an invasive procedure, we think it better to concentrate on producing an assay suitable for sera.

We also assayed mouse sera for 32mer peptide. These experiments were not included in the original SOW, but we had mouse sera available to us from previous non-DOD related research that we wished to test. **Figure 1c** shows 32mer peptide concentrations in mice following DMM surgery: sera were collected at 4 and 16 weeks post-surgery, when mice were 14 and 26 weeks old, respectively. The mean value for 14wk DMM was 3683 pM 32mer, +/- 2694 and the mean value for 26wk DMM was 1474 pM 32mer, +/- 1724 (errors are standard deviation). Comparison of these means by Student's t-test confirmed that there was no significant difference in 32mer peptide concentration between the DMM groups. We did not have matching sera from control mice available, so instead we tested serum from untreated wildtype mice aged 3, 8 and 24 weeks of age. (Note that DMM surgery begins at 10 weeks of age, so the 24 week-old mice were closest in age to DMM mice at 16 weeks post-surgery.) The levels of 32mer peptide were very low in the sera from untreated mice, ranging from 40pM to 180pM, giving us confidence that the assay will be useful for measuring increases in 32mer peptide concentrations as a response to DMM surgery. In the next reporting period we plan to assay sera from DMM and matching sham-surgery mice, at time points up to 16 weeks post-surgery. These serum samples have already been collected and are available to us from a collaborator. We will also assay sera from 'Chloe' mice that have a knockin mutation to aggrecan, such that the 32mer peptide is not generated (1). The sera from Chloe mice will be a useful negative control. If we find significant differences in 32mer peptide in

sera from DMM and sham-surgery and/or control mice, then we potentially have a method for correlating endogenous 32mer levels in sera with knee hyperalgesia in DMM-treated mice. Development of a robust assay to measure 32mer levels in the serum will lead to a biomarker of aggrecanase activity in the serum, which could be developed for use in clinical trials that target ADAMTS-5 (or in all DMOAD trials). In addition, it is possible that this assay could be developed as a biomarker for pain associated with OA. The currently proposed experiments on existing samples will lay the foundation for future prospective studies in preclinical models as well as in human cohorts.

(1) Little, CB., Meeker, CT., Golub, SB., Lawlor, KE., Farmer, P., Smith, SM. & Fosang, AJ. (2007) Blocking aggrecanase cleavage in the aggrecan interglobular domain abrogates cartilage erosion and promotes cartilage repair *J. Clin Invest* 117, 1627-1636

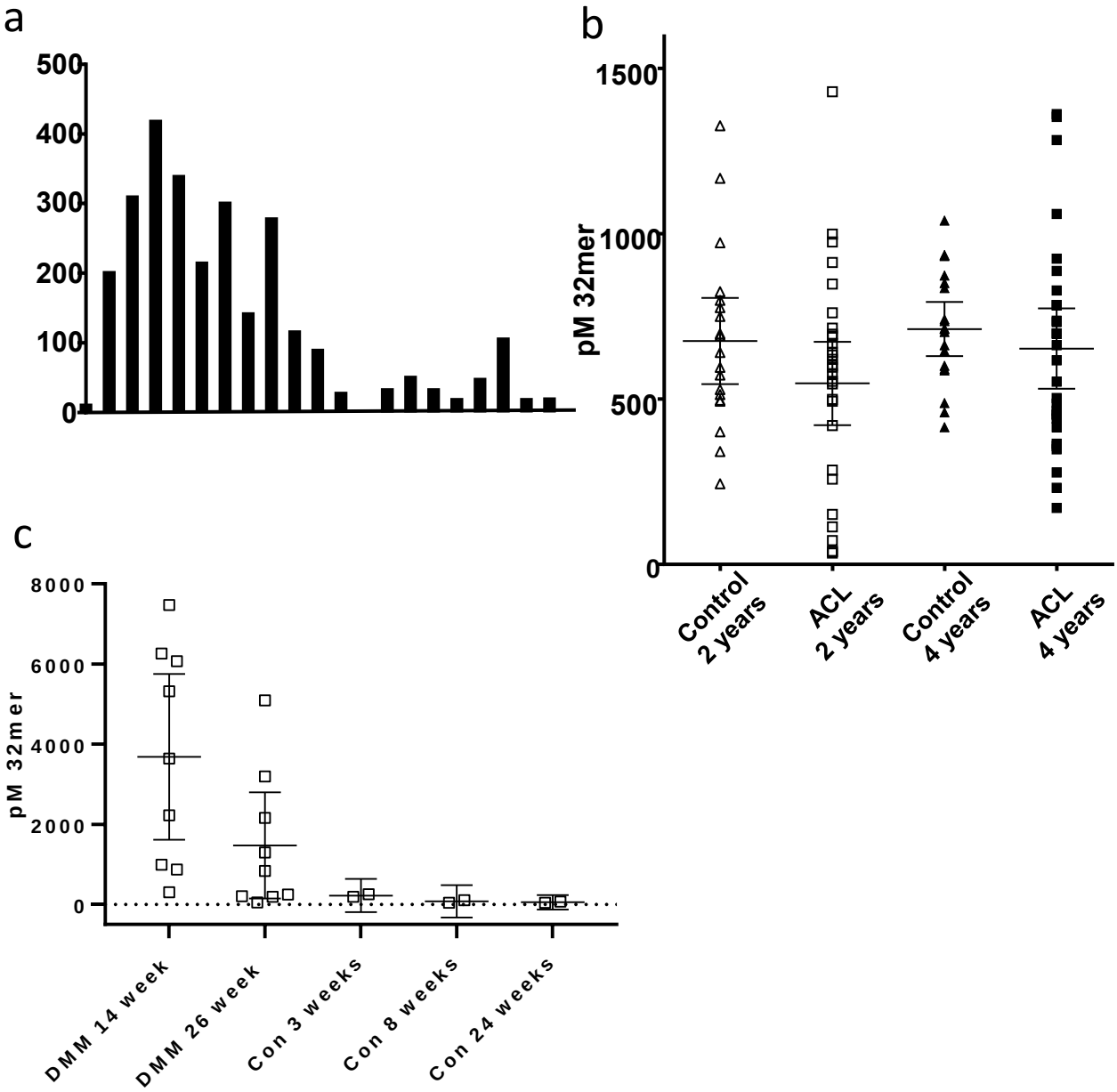


**Figure 1 Concentrations of 32mer in human and mouse sera**

a. Sera from Cohort #1; patients with end-stage OA requiring joint replacement. Each column is an assay of serum from one patient.
b. Sera from Cohort #2; patients with anterior cruciate ligament (ACL) damage, taken 2 year and 4 years post ACL reconstruction. Control sera are matched for age, activity level and anthropometric characteristics. Each data point is an assay of serum from one patient.
c. Sera from mice challenged with a surgical model of OA (DMM), taken at 14 and 26 weeks post-surgery. Control (Con) sera are from mice aged 3, 8 and 24 weeks old. Each data point is an assay of serum from one mouse.

Values in a and b are a mean of triplicate AlphaLISA readings. Values in c are means of triplicates (DMM) and quintuplicates (controls). All values have been normalised to zero, where zero is the concentration of 32mer peptide in normal human serum diluent. Error bars in b and c are +/- 95% CI.

**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 for this reporting period (Oct 2018-Oct 2019)

**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 for this reporting period.

**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.*

We have a one-year no cost extension (Oct 1, '19 – Sept 30, '20) to do the following work.

*Task 1:* Implement 32-mer assay at Rush

This task will require K. Last to travel to Chicago and stay in the Malfait lab for 2-3 weeks. The Malfait lab will seek approval to purchase a Perkin Elmer AlphaLISA plate reader.

*Task 2:* Do further in vitro validation of the 32mer assay at Rush.

*Task 3:* Use the 32mer assay to detect 32mer levels ex vivo in sera from DMM vs sham mice: time-points up to 16 weeks post-surgery, including Chloe mice as negative control.

These samples have already been collected by a collaborator and are available for us to use.

*Task 4:* Determine if 32mer is a potential biomarker for PTOA pathology by screening cohorts.

a. We have access to human synovial fluid and serum specimens for these studies from subjects with knee OA who have undergone arthroscopic procedures or total knee replacement, as well as specimens from non-arthritic human organ donors. These specimens were collected as part of IRB approved repositories and utilized in previous studies. Remaining specimens are de-identified, although diagnosis, age, and gender are available. The use of these specimens falls under the criteria for exemption listed in section 46.10 of the "Code of Federal Regulations for Protection of human subjects (45 CFR46, category number 4)."

*Task 5:* Write up biomarker findings (murine and human)

**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 made in this reporting period.

**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.*

PI Fosang's laboratory closed on 30 September 2019, when Prof Fosang retired. In this reporting period, PI Fosang and her staff (Karena Last and Heather Stanton) devoted time to publishing papers, closing human and animal ethics projects and emptying the laboratory. Prof Fosang is now an emeritus professor at the University of Melbourne. She has non-DOD funds available to pay Karena and Heather for a further few months to finish outstanding work. During this time, Karena will travel to the Rush University to transfer the 32mer assay to Prof Malfait's laboratory.

The focus of the laboratory work will now be at Rush University for the period of the extension. Prof Malfait is now sole PI on the grant.

**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: 9
Contribution to Project: Laboratory work, including cell and tissue culture, histology, qPCR analyses, arthritis scoring.

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, µCT scanning, arthritis scoring.

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. µCT analyses.

Name: Anne-Marie Malfait (Rush University)
Project role: Principal Investigator and Animal Experimentalist
ORCID ID: 0000-0003-1428-0384
Nearest person month worked: 1
Contribution to project: Supervision of 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.*

PI Prof Amanda Fosang retired on September 30, 2019. PI Prof. Malfait will be sole-PI for the one year period of the extension.

**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.*

None for this reporting period.

## 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**