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

PRINCIPAL INVESTIGATOR: Anne-Marie Malfait, MD PhD

CONTRACTING ORGANIZATION: The University of Melbourne, Parkville 3052 Australia

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Anne-Marie Malfait

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The University of Melbourne, PaediPaediPaediatrics
Dept. of Paediatrics
Grattan Street, Parkville 3052
Australia

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14. ABSTRACT

Recommended to be brief (approx. 200 words) of the main findings during the reporting period.
In this fifth reporting period, we extended the activity in the absence of the agreement with the University of Melbourne. We have a fully executed agreement on file now (in place since December 2020). We have received a new plate reader at Rush (Perkin Elmer), necessary for continuing the project (arrived December 2020).
While the administrative transfer was ongoing (and delayed due to the pandemic lockdown), we have tested existing mouse sera for the levels of the aggrecan 32-mer fragment. We have found that these old sera were no longer usable (likely due to instability of the 32-mer), and we will therefore conduct new experiments to generate sera for biomarker development.
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
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:

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:

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

3. ACCOMPLISHMENTS:

What were the major goals of the project?

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. Experimental groups and numbers/group: Gr. 1 (n=18): Naïve (x 3, for each time point; total n= 54) Gr. 2 (n=18): Sham (x 3, for each time point; total n= 54) Gr.3 (n=18): DMM, untreated (x 3, for each time point; total n= 54) Gr. 4 (n=18): DMM, AF-28 treated (x 3, for each time point; total n= 54) 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. 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	

Specific Aim 1	Timeline	Site 1	Site 2
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: 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. Experimental groups and numbers/group: Gr. 1 (n=18): Naïve (x 3, for each time point; total n= 54) Gr. 2 (n=18): Sham (x 3, for each time point; total n= 54) Gr.3 (n=18): DMM, untreated (x 3, for each time point; total n= 54) Gr. 4 (n=18): DMM, AF-28 treated (x 3, for each time point; total n= 54) Gr.5 (n=18): DMM, isotype control Antibody treated (x 3, for each time point; total n= 54)	13-24		33% complete[a]
Subtask 2: Pain measures: Study 2 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	

Specific Aim 1	Timeline	Site 1	Site 2
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. Experimental groups and numbers/group: Gr.1 (n=18): DMM, untreated Gr. 2 (n=18): DMM, AF-28 treated 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: - 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 - Determine if AF-28 can limit DRG activation in Pirt-GCaMP3 mice following DMM – 8 week time-point	28	100% complete April 2019 0% complete Plan changed[a]	100% complete April 2019 0% complete Plan changed[a]

Specific Aim 2	Timeline	Site 1	Site 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: 1) Sera and synovial fluids from 138 patients with osteoarthritis, collected at Duke University	1-12	0% complete Not begun[b]	

Specific Aim 2	Timeline	Site 1	Site 2
2) Synovial fluids from 11 patients following anterior cruciate ligament surgery, collected at Duke University 3) Synovial fluids collected from surgical waste (exempt protocol), collected at Duke University. Number yet to be determined. 4) Serum from 49 patients following anterior cruciate ligament surgery, collected at The University of Melbourne		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: Establish the alphaLISA method for the detection of 32mer in human synovial fluids and serum Determine if 32mer is a potential biomarker for PTOA pathology by screening cohorts	1-30	100% 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?

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 analytes, 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 decided to assay. **Figure 1c** shows 32mer peptide concentrations in mouse serum following DMM surgery: sera were collected at 4 and 16 weeks post-surgery. 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.

During the few weeks early on in 2020 when we were able to work on this project (still in Melbourne), the following experiments were performed (as was outlined in the 2019 annual report):
We assayed sera from DMM and matching sham-operated mice, at time points up to 16 weeks post-surgery. These existing sera were provided by a collaborator, and shipped from Sydney to Melbourne early 2020. The samples were delayed in transit (due to forest fires) and arrived thawed and at room temperature. There were no protease inhibitors in the samples, so we can assume some level of degradation. Results are shown below in Fig. 2, showing that the levels of 32-mer were significantly lower than previously found in mouse sera, most likely due to degradation. Unfortunately, at this point in time the Melbourne lab closed its doors, and all shipments to the US stopped due to the pandemic. Now that the grant has been transferred to Chicago and the plate reader has arrived here, we propose to now continue assay development in Chicago. We will

assay sera available at Ruish form existing studies, including 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 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

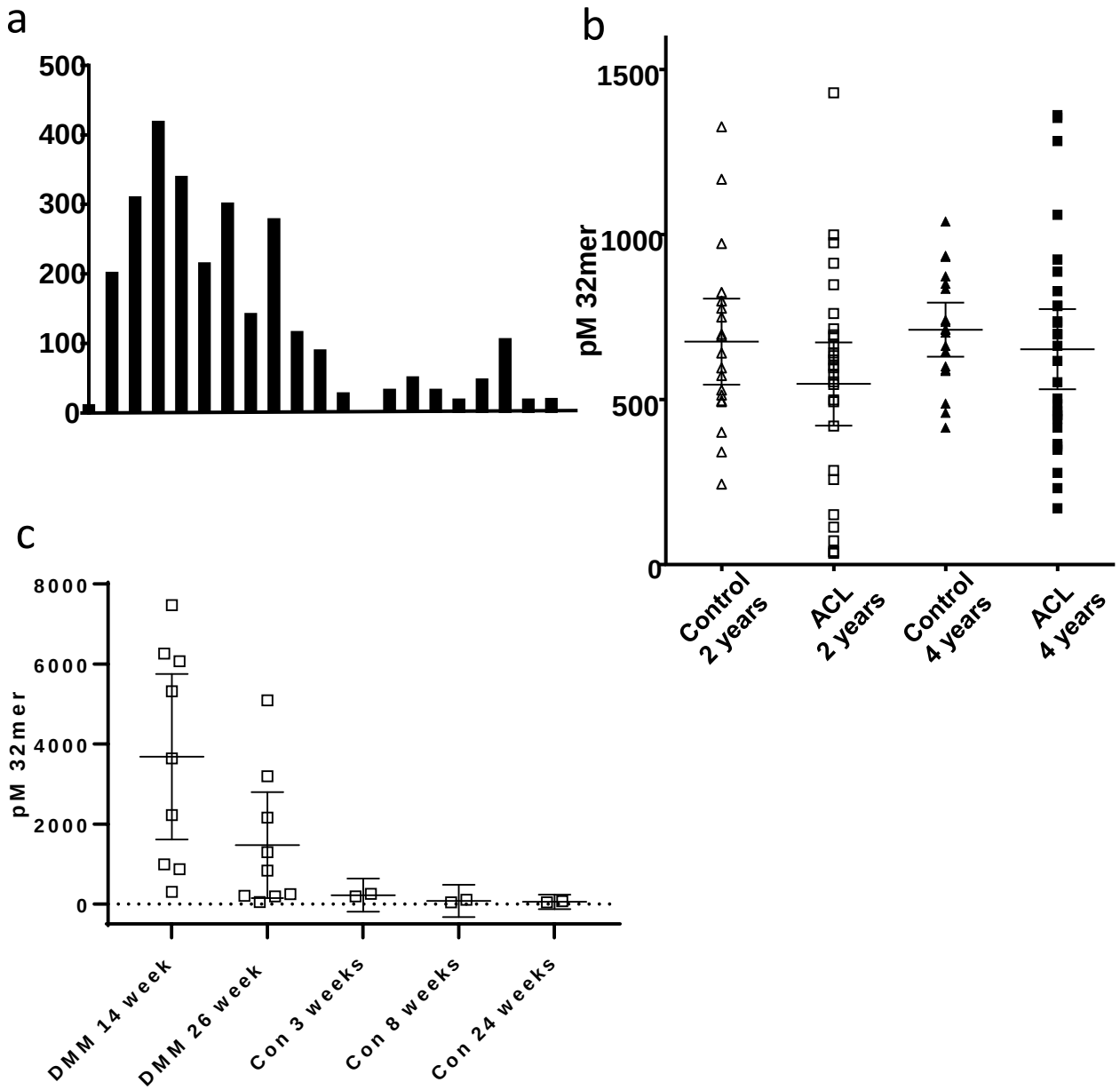

Figure 1 Concentrations of 32mer in human and mouse sera

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.

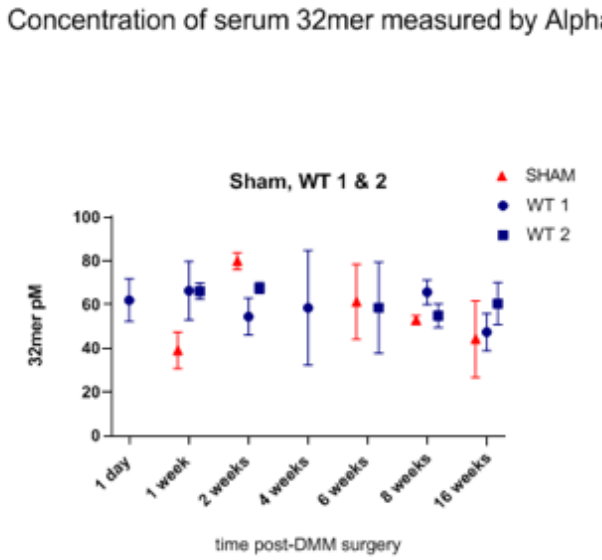

Fig. 2: Sera from mice challenged with sham or DMM surgery, taken at different time points after surgery (1-16 weeks). WT1= DMM experiment 1, WT 2= DMM experiment 2. Mean of 3 mice per group.

What opportunities for training and professional development has the project provided?

Nothing to report for this reporting period.

How were the results disseminated to communities of interest?

Nothing to report for this reporting period.

What do you plan to do during the next reporting period to accomplish the goals?

We had requested 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)

Note: Tasks 1-5 were not started due to two major obstacles: (1) the pandemic-associated lockdown of labs and travel suspension, prohibiting us from acquiring the Perkin Elmer plate reader, and prohibiting shipment of essential reagents and samples from Australia to Chicago (in addition to a temporary closure of all labs); (2) the significant delay in setting up the agreement with the University of Melbourne.

In December of 2020, the transfer was completed.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to report

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

As described above, we incurred major obstacles. (1) the pandemic, and the associated lockdown of labs and prohibition of travel, and prohibiting us from acquiring the Perkin Elmer plate reader; (2) the delay in setting up the agreement with the University of Melbourne.

In December of 2020, the transfer was complete. The plate reader arrived at Rush early December 2020.

Actual or anticipated problems or delays and actions or plans to resolve them

PI Fosang's laboratory closed on 30 September 2019, when she retired. The DoD award remains in Melbourne but the subaward needed to be transferred to Rush University in Chicago- this was completed in Dec 2020. The PI is Anne-Marie Malfait. The plans for the next year (until Sept 30 2021) are to develop the 32 mer assay in murine and human samples.

Changes that had a significant impact on expenditures.

Nothing to report compared to last reporting period. Funds were released, and a Perkin Elmer plate reader was purchased for the laboratory of the new PI. This plate reader and the funds will now be used for developing the 32-mer biomarker assay.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

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:

- **Publications, conference papers, and presentations**

  Journal publications.

  Nothing to report

  Books or other non-periodical, one-time publications.

  Nothing to report

  Other publications, conference papers, and presentations.

  Nothing to report

- **Website(s) or other Internet site(s)**

  Nothing to report

- **Technologies or techniques**

  Nothing to report

- **Inventions, patent applications, and/or licenses**

  Nothing to report.

- **Other Products**

  Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Karena Last
Project Role: Research Assistant
Researcher Identifier: ORCID ID 0000-0002-4396-8404
Nearest person month worked: 0.5
Contribution to Project: Laboratory work, including establishing and validating the AF-28 immunoassay, testing of existing mouse sera.

Name: Heather Stanton
Project Role: Administrative Assistant/Research Officer
Researcher Identifier: ORCID ID 0000-0002-3427-5614
Nearest person month worked: 0.5
Contribution to Project: Assist with transfer of project and data from Melbourne to Chicago.

Name: Anne-Marie Malfait (Rush University)
Project role: Principal Investigator and Animal Experimentalist
ORCID ID: 0000-0003-1428-0384
Nearest person month worked: 0.2
Contribution to project: Supervision of transfer- Communication between Rush and Melbourne- Preparation of report.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

PI Prof Amanda Fosang retired on September 30, 2019. PI Prof. Malfait is now PI for period of the no cost extension.

What other organizations were involved as partners?

None for this reporting period

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES:

No appendices