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

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CONTRACTING ORGANIZATION: University of Melbourne
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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Recommended to be brief (approx. 200 words) of the main findings during the reporting period.

In this second reporting period, we achieved three major milestones:

1. We finalized the histological analysis of all knees of the prophylactic experiment performed in year 1. We found no effect of a 10-week prophylactic treatment with AF 28 on cartilage damage or proteoglycan loss. Analyses of bone damage are in progress.
2. We completed a 16-week experiment where mice were treated starting 2 weeks after DMM surgery. This treatment strategy had no effect on pain-related behaviors.
3. We successfully developed an assay to measure the 32-mer fragment in human serum, which has great potential as a novel biomarker for OA/OA pain, as well as a target marker for aggrecanase activity.

Unfortunately, we have also encountered issues that delayed progress and will require substantive changes going forward:

1. The data collected to date suggest that AF-28 is not a neutralizing antibody. Further in vivo experiments are on hold until analysis of the first DMM experiment is complete.
2. PI Fosang has a health issue and we therefore, urgently requested that co-I Malfait can share the role of Co-PI.

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:** 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 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; Completed Sept ’18.
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. Started May 2018 (year 2)- delayed due to delay in ACURO approval (April 2018)
- 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. We have decided not to do these experiments. We have provided the rationale for this decision below.

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; in progress, 50% complete.

ii. Determine whether AF-28 can limit DRG activation in Pirt-GCaMP3 mice following DMM – 8 week time-point; no longer part of the experimental plan. Rationale provided in section 5.

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, 90% 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 Tasks 1 and 2
Subtask 1: *DMM surgeries*
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. Ten-week old, male, C57BL/6 wildtype mice were operated on. 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 began 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 began 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 DMM#1, 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. (**See data reported in October 2017 Annual Report**).

Major Tasks 1 and 2

Subtask 2: *pain measures*

To assess the effects of AF-28 antibody on DMM-induced pain, in 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**).

.

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 (see **Figure 1**). There was also no significant effect of AF-28 on mechanical allodynia of the ipsilateral hind paw **(Figure 2)**.

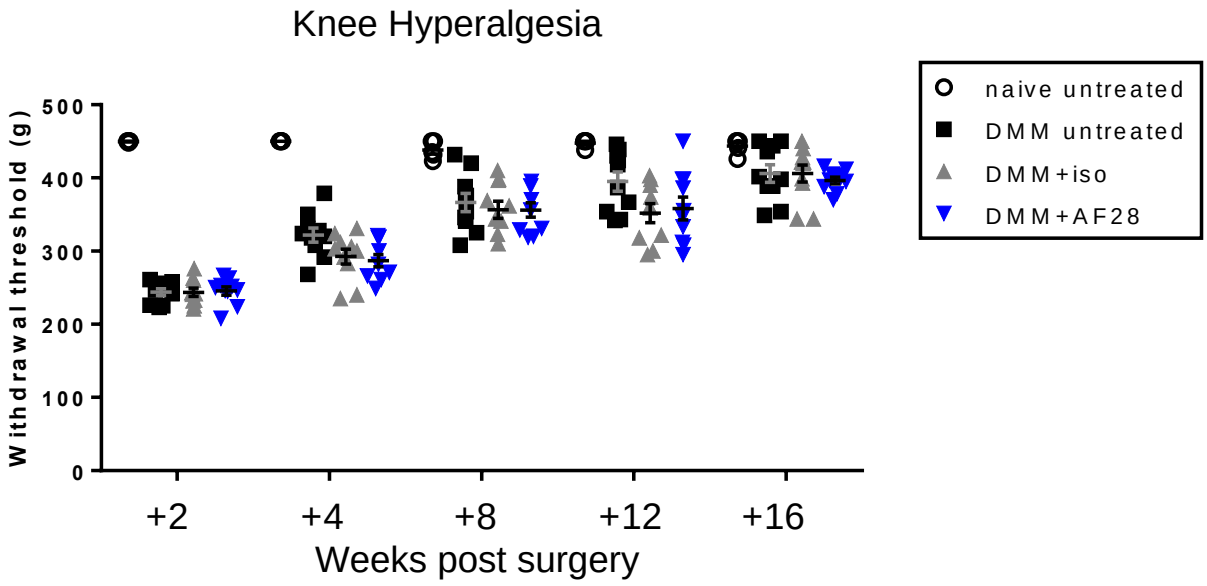


Figure 1. DMM#2. No significant effect of AF-28 antibody on knee hyperalgesia
Data points are mean+/-SEM.

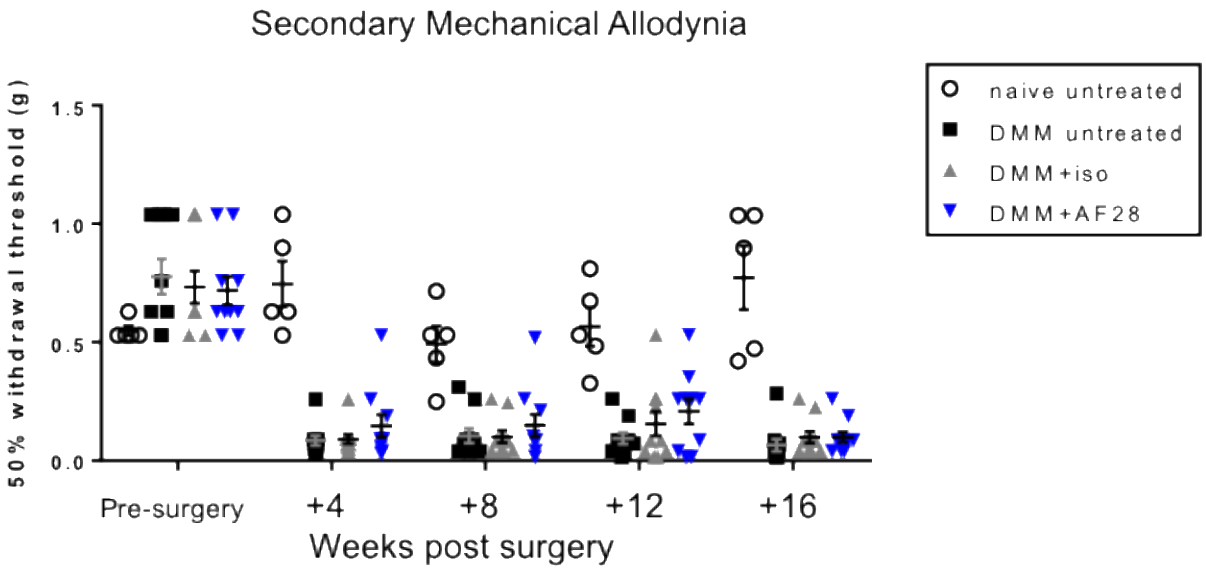


Figure 2. DMM#2. No significant effect of AF-28 antibody on mechanical allodynia
Data points are mean+/-SEM.

Major Tasks 1 and 2

Subtask 3-6:

The fixed hind limbs from DMM#1 and #2 experiments have been shipped to Australia. For DMM#1, the following analyses have been done:

- μCT scanning and image collection
- histology and immunohistochemistry analyses
- scoring of the histology by two blinded investigators, for cartilage, bone and osteocyte pathology. Full data in this report.
- analyses of the μCT images; 90% complete

For the DMM#2, the following has been done:

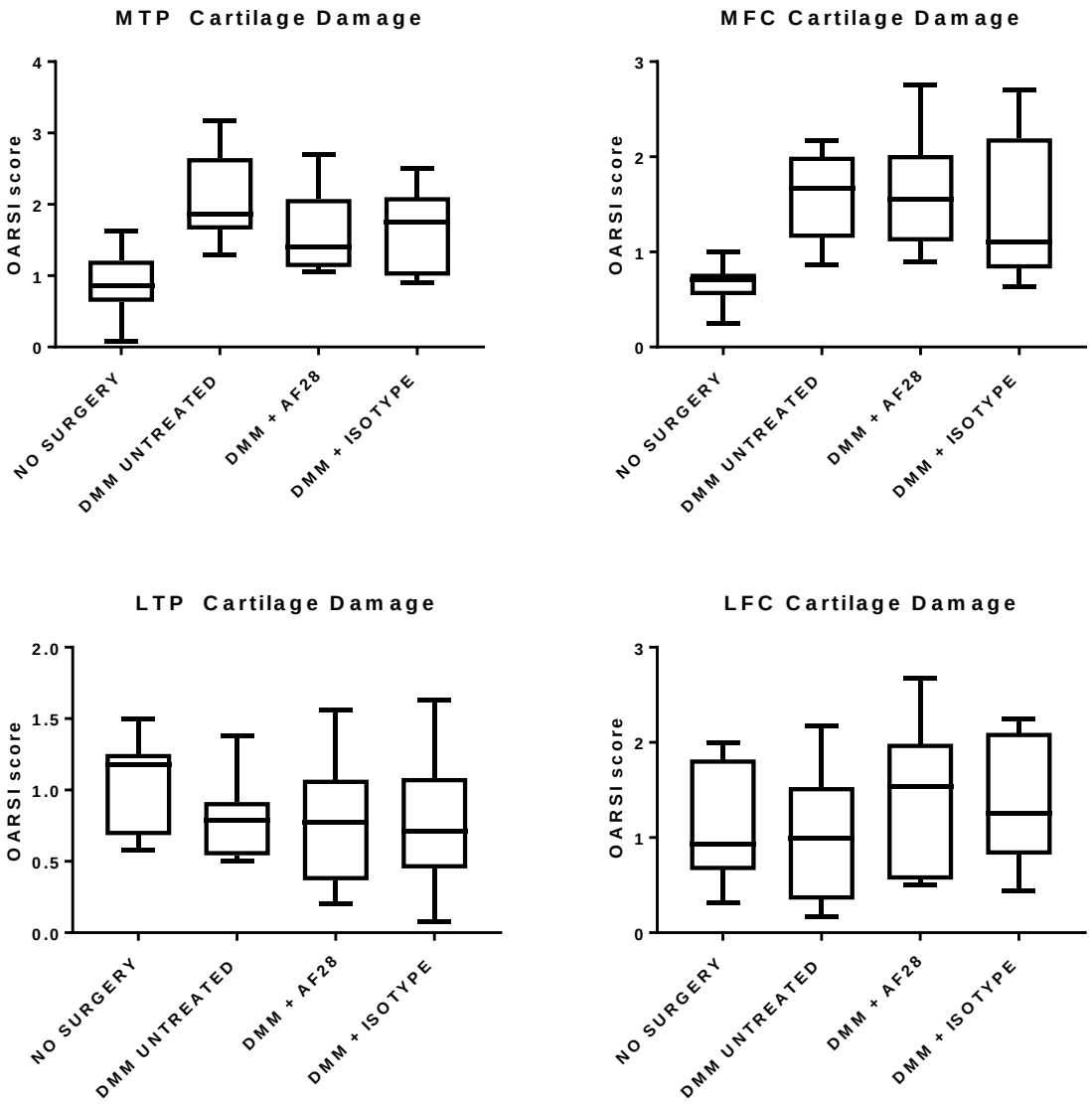
- μCT scanning and image collection

Analyses of μCT images for DMM#2 will begin in November'18. Likewise, histology, immunohistochemistry and scoring for this experiment will begin in November '18.

Staining and histologic scoring of sections for cartilage parameters

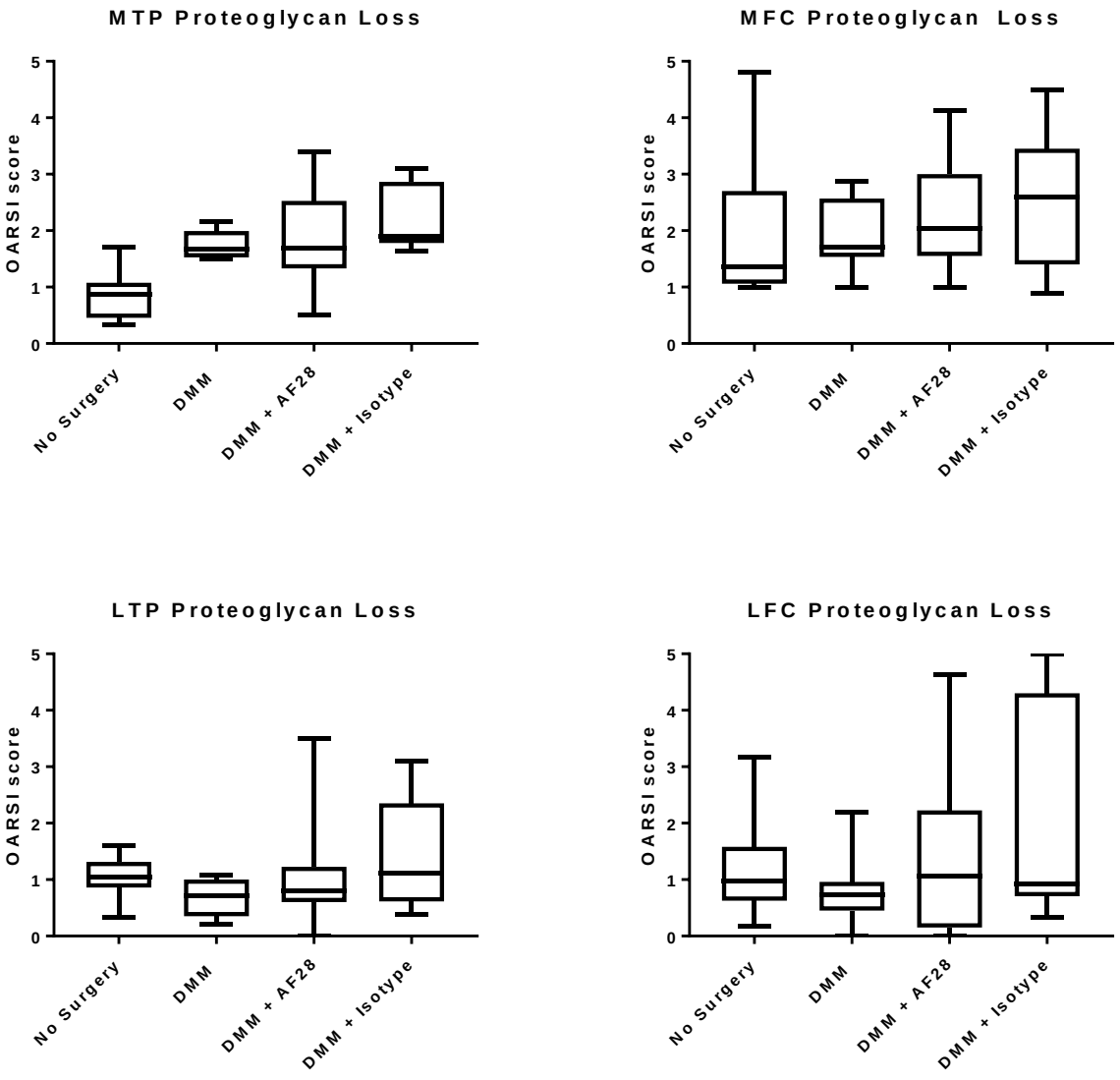
For histology, knee joints were decalcified and embedded coronally in paraffin. 5μm sections were cut through the joint, encompassing the entire weight-bearing area of the joint. Slides were stained at 25μm intervals with Safranin-O Fast Green. Histologic scoring for cartilage structural damage and aggrecan loss was done according to the OARSI guidelines. All four quadrants of the joint; medial femoral condyle (MFC), medial tibial plateau (MTP), lateral femoral condyle (LFC) and lateral tibial plateau (LTP) were scored on multiple sections through the joint by two scorers. Cartilage damage and proteoglycan loss scores for each quadrant of the operated (right) and unoperated (left) legs were graphed.

In DMM#1, prophylactic treatment with AF-28 from surgery to week 8 had no protective effect on progression of joint damage (**Figures 3 and 4**). 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. Proteoglycan loss was significant in the medial and lateral tibial plateau of DMM treated mice, but there was no effect of AF-28 antibody.



MTP: Medial Tibial Plateau
MFC: Medial Femoral Condyle
LTP: Lateral Tibial Plateau
LFC: Lateral Femoral Condyle

Figure 3. DMM#1. Cartilage damage. No significant effect of AF-28 antibody.
Data from the right legs. Error bars show 5-95% confidence interval. Groups were compared using the unpaired t test. In the medial tibial plateau, the difference between cartilage OARSI score in DMM untreated and no surgery was statistically significant (p=0.0002). Likewise, the difference between DMM+AF-28 and no surgery was significant (p=0.0049) and the difference between DMM+isotype and no surgery was significant (p=0.0113). However, there was no significant difference between DMM+AF-28 and DMM untreated, or DMM+isotype and DMM untreated. In the medial femoral condyle, the difference between cartilage OARSI score in DMM untreated and no surgery was statistically significant (p=0.0004); the difference between DMM+AF-28 and no surgery was significant (p=0.0012) and the difference between DMM+isotype and no surgery was significant (p=0.09). There was no significant difference between any of the cartilage OARSI scores for the lateral tibial plateau, or the lateral femoral condyle.



MTP: Medial Tibial Plateau
MFC: Medial Femoral Condyle
LTP: Lateral Tibial Plateau
LFC: Lateral Femoral Condyle

Figure 4. DMM#1. Proteoglycan loss. No significant effect of AF-28 antibody.
Data from the right legs. Error bars show 5-95% confidence interval. Groups were compared using the unpaired t test. In the medial tibial plateau, the difference between proteglycan loss in DMM untreated and no surgery was statistically significant ($p<0.0001$). Likewise, the difference between DMM+AF-28 and no surgery was significant ($p=0.0023$) and the difference between DMM+isotype and no surgery was significant ($p<0.0001$). There was no significant difference between DMM+AF-28 and DMM untreated; but a significant difference between DMM+isotype and DMM untreated ($p=0.303$). There was no significant difference between any of the proteoglycan loss scores for the medial femoral condyle. In the lateral tibial plateau, there was a significant difference between DMM untreated and no surgery ($p=0.0316$) and DMM+isotype and DMM untreated ($p=0.0452$). There was no significant difference between any of the proteoglycan loss scores for the medial femoral condyle or the lateral femoral condyle.

μCT analyses of bone parameters

μCT scanning and image collection is complete for DMM#1 and DMM#2. The processing of the images for DMM#1 is almost complete; regions of interest have been defined and thresholding is underway. We will report the data in the next reporting period.

Major Tasks 1 and 2

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

In the first year of the project, we spent time developing and optimizing the experimental conditions for culturing osteoblasts (bone-building cells), osteoclasts (bone-resorbing cells), osteocytes and synovial fibroblasts. We already had expertise culturing chondrocytes and cartilage explants. In year 2, we tested which cell types respond to the 32mer, and whether this response was neutralized by AF-28.

Chondrocytes

We tested the response of mouse cartilage explants and isolated chondrocytes from mouse knee cartilage to the 32mer, in the presence and absence of AF-28 antibody (**reported in October 2017 Annual Report**). The results confirmed that isolated chondrocytes, but not chondrocytes embedded in a cartilage matrix, respond to 32mer peptide *in vitro by* increasing expression of pro-inflammatory and pro-catabolic genes. 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 further investigated the possibility that AF-28 antibody might be more effective at blocking *endogenous* 32mer (rather than *exogenous* 32mer) produced in response to an inflammatory insult such as IL-1α. The results (**reported in the April 2018 Biannual Report**), showed that the addition of AF-28 antibody at concentrations of up to 1.2μM did not reduce the concentration of IL-1-induced MMP-13 released into the medium, suggesting that either endogenous 32mer produced by cartilage explants is not neutralized by AF-28 antibody, or that an additional binding partner might be required.

Synovial Fibroblasts

Synovial fibroblasts respond to 32mer by upregulating expression of pro-catabolic and pro-inflammatory molecules, as previously published[1]. However, AF-28 antibody was unable to neutralize the 32mer activity (data not shown).

Osteoblasts

Gene expression in osteoblasts was not affected by 32mer–treatment (data not shown). Due to the lack of response, AF-28 was not tested in these cells.

Osteoclasts

We investigated the effect of the 32mer on the *in vitro* differentiation of bone-marrow macrophages to osteoclast-like cells, in response to RANKL and M-CSF treatment. The 32mer,

[1] Lees SJ, Golub SB, Last K, Zeng W, Jackson DC, Sutton P, et al. Bioactivity in an aggrecan 32mer fragment is mediated via Toll-like receptor 2. Arthritis Rheum 2015; 67: 1240-1249.

but not the scrambled control peptide, appeared to inhibit the formation of large multinucleated TRAP positive cells Expression of IL-10, CCL2 and TNF genes were increased (**data reported in the April 2018 Biannual Report)**. We have since repeated the experiment and have not been able to reproduce the data. Because osteoclasts do not respond consistently to the 32mer, we have not tested AF-28 in these cells.

Conclusion Thus far we have been unable to block exogenous or endogenous 32mer activity with monoclonal AF-28 antibody in cultured mouse chondrocytes, or in vivo (one experiment only). In light of these findings, we will continue analyzing the mouse limbs collected from DMM#2, but we do not deem that there is any value in doing more DMM surgeries.

Major Task 2

Subtask 8: *DMM surgeries in Pirt-GCaMP3 mice, treated from time of surgery, for 8 weeks.*
Discontinued. See rational in section 5.

Major Task 3

Subtask 1: *Develop an AlphaLISA assay for 32mer detection*
We have developed 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.

This assay uses mouse monoclonal AF-28 recognising the FFG N-terminus, and rabbit polyclonal αEGE recognising the 32mer C-terminus. 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.

We reported previously that the dynamic range, signal and sensitivity of the assay is influenced by the order in which reagents are added. We tested empirically for the optimal order in which reagents should be added and determined conditions to achieve the highest dynamic range, signal and sensitivity. We 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 (**see October 2017 Annual Report).** 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 increased the assay volume to 50µL, in order to improve assay reproducibility.

While waiting for HRPO approval to use patient sera and synovial fluids, we began 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 discovered that in order to detect 32mer signal above the level of interfering serum molecules, sera must be diluted and then 'spiked' with a

known concentration of 32mer, to bring the total 32mer concentration (sample + spike) to within the range of the standard curve. In the **October 2017 Annual Report**, we reported that we needed to dilute sera samples 1:1000, for an assay with a dynamic range of 0.008 - 30nM 32mer. In year two, we purchased normal human serum from a commercial source to use as a negative control, allowing us to further optimise the assay conditions. We have now established a method for analysing serum samples that are diluted 1:4. The improved serum assay now detects a dynamic range of 0.001-100nM 32mer in samples which have been spiked with 1nM 32mer.

In year two we also sourced a commercial sample of non-arthritic human synovial fluid and used it to further improve the detection of 32mer in synovial fluid. Synovial fluid was first deglycosylated to remove chondroitin sulphate and keratan sulphate glycosaminoglycan side chains; this improved viscosity and antibody binding, thereby improving assay reproducibility. Incubation times were increased to overnight to improve sensitivity. The assay recovery varied significantly depending on the diluent type, highlighting the importance of choosing diluents that are similar to the sample type tested. Since normal synovial fluid (the optimal assay diluent) was not available to us in large enough quantity for repeated use, we determined that an assay diluent of normal human serum: foetal bovine serum (1:1) was suffient, because it required the lowest dilution of sample, provided the best analyte recovery, and retained assay sensitivity
.
To conclude: the AlphaLISA assay is now fully optimised and ready to use with human sera and synovial fluid samples.

In year 2 we obtained local ethics approval to collect and analyse human synovial fluids from joint replacement patients at St Vincent's Hospital, Melbourne, Australia. We already had local approval to use human sera samples from the University of Melbourne; these samples were collected prior to the start of this project by a collaborator. We rolled both human ethics protocols into one protocol to be administered by the University of Melbourne, which was approved by the University in May 2018. The application to the HRPO for approval has had the initial administrative review (email to PI Fosang on 24th October) and is pending some documentation from us.

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.
Professional Development in Safe Radiation Practices- Ionising Radiation, University of Melbourne, Nov 2017, Karena Last, Suzanne Golub.

Professional Development in the Operation and Safe-use of the Bruker micro-Computed Tomography (µCT) Skyscan 1272, User Certificate obtained, Dec 2017, Karena Last, Suzanne Golub.
"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.

Professional Development in the use of Bruker software: Dataviewer, CTAn, April 2017, Karena Last and Heather Stanton. Further training in CTVox and CTVol, October 2018, Heather Stanton. Trained by Dr Nicole Walsh.

Professional Development in the analyses of µCT scans of mouse knees, Karena Last and Heather Stanton, April 2017. Trained by Dr Nicole Walsh.

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
Micro CT analyses for DMM#1 and #2will be completed. Sectioning for histology and immunohistochemistry for DMM#2 will be done, and scoring completed.

Aim 2
AlphaLISA assays for the 32mer in human sera will be completed.

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.

See points made in 'actual or anticipated problems'

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.

1. 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 were unable to obtain local approval for the use of samples from Duke University, because samples from surgical waste that were considered exempt at Duke University (and without documented evidence of consent) were not considered exempt in Australia. We now have approval from St Vincent's Hospital to collect new samples, and approval from the University of Melbourne for the combined protocol. We have applied to the HRPO for final approval, which is pending documentation to be provided by us (correspondence in October 2018).
2. The data collected to date suggest that AF-28 is not a neutralizing antibody. Therefore, further in vivo experiments are on hold.
3. We had a delay due to delayed ACURO approval (only granted in April 2018). We flag that Co-I Malfait might request a no cost extension.
4. Importantly, PI Fosang has a major health issue and we therefore request that Co-I Malfait be appointed co-PI.

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."

<u>Example:</u>

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, 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: 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.

As mentioned above, we request that Dr. AM Malfait take over as co-PI of the proposal, due to health issues of the PI. Dr. Malfait is the most suitable candidate for taking over leadership of this project.

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