Explore 2020 Research (Lay & Scientific Reports)

Funded by:	Australian Rheumatology Association (ARA)
Recipient:	Prof Johannes C Nossent
Intended Department	University of WA- Sir Charles Gairdner Hospital
Project:	***Defining the burden of disease for JIA in WA - a longitudinal population-based cohort study.***

PLAIN LANGUAGE SUMMARY (LAY REPORT):

Since receiving the Arthritis Australia Project Grant in 2021 to study the epidemiological characteristics of Juvenile Arthritis in Western Australia we have undertaken a number of research activities. Firstly, we utilised the Western Australian Rheumatic Disease Epidemiological Registry (WARDER) to identify unique patients aged 15 years and younger with a (first) hospital admission for JIA between 1990 and 2012 to produce measures of JIA hospitalisation rates and admission characteristics (ie for joint injection, infections or disease activity) for that period. Secondly, we used these data to estimate a minimum (ie hospital based) prevalence of JIA in WA. Thirdly we used WA specific PBS prescription data for the TNF inhibiting drugs Enbrel and Adalimumab to quantify the number of JIA patients requiring treatment with TNFi biologicals (the newest disease modifying drugs) in WA since 2003. Fourthly, data on JIA outcomes (need for repeat admissions, joint surgery, mortality and development of comorbid conditions such as diabetes or kidney disease) are currently being extracted/cleaned/analysed. Finally, we have also started the process of data acquisition from another WA health data source to study the association between maternal pregnancy and early life risk factors and JIA development. Results so far are that we have identified 786 unique cases of JIA in WA hospital records with an average age at index hospitalization of 7.6 (±4.4) years, the majority of which were girls (n=465, 59.2%) with 18% % of patients from rural regions and 6.1% (n=48) identified as Aboriginal. JIA was the primary diagnosis in 90.2% (n=709) with approximately 45 % of these admissions required for joint injections. The average annual hospitalisation rate for JIA patients was 7.9 per 100,000 with the rate for boys (6.3) approximately half of the rate in girls 11.2) per 100,000. Surprisingly and despite the advances made in treating JIA since 2000, the JIA admission rate did not change significantly over the study period (annual percentage change (APC): 1.3, p=0.10). Based on these admission data, the average prevalence of JIA over the study period overall was 59.9 per 100,000 children with the rate in girls (89.4) twice the rate in boys (44.5).The prevalence of JIA at the end of the study period was 72.7 overall (52.9 in boys and 114.5 per 100,000 in girls). Data from the PBS database for TNFi use in WA indicated that the use of Enbrel/Adalimumab for JIA increased almost linearly from 2003 and based on the WHO definition of daily dose per 1000 population per day (DDD) TNFi usage reached 0.37 in 2012, indicating TNFi usage by 1 in 2700 children. When combined with the minimum prevalence data form hospital admission this indicates TNFi use by at least 1 in 3 children with JIA.These findings will be presented at national and international scientific meetings in 2022 and a scientific paper is currently being written. With the bulk of the other data collection has been completed, we are aiming to write two more papers on JIA risk factors and outcomes over the next 6 months.

What questions did the grant set out to answer? What problems did you try to solve, or gaps in knowledge did you try to fill? Why is this important?

This grant was to investigate disease characteristics, potential triggers and long-term consequences for children registered with JIA in the Western Australia Rheumatic Disease Epidemiology Registry over the period 1980-2015. The main reasoning behind this is the very limited

Australian data available for these areas with our knowledge mainly extrapolated from overseas studies. Having Australia specific data will allow for better insight and planning of JIA service in (Western) Australia. Particular outcomes of interest for this longitudinal observational cohort were to fill in gaps in our knowledge about the disease prevalence as well as the rates and reasons for hospital admission for JIA and potential changes over time given 2 advances in disease management. services for children with JIA. More specifically, we aim to provide specific data on the rate of joint injections, joint surgery, serious infections, pregnancy frequency and outcomes, cancer development, mental health problems, mortality and direct health care costs seen with JIA. While our research is descriptive and epidemiological in nature, this will provide important Australian first data.

What did you discover during the course of the grant?
1) The minimum prevalence of JIA in Western Australia is 0.12% for girls and 0.05% for boys. While this aligns with data from other countries, it is lower than reported in a study of school children in Perth (0.4%) and Belgium (0.16%).
2) Despite the significant use of biological therapy (at least 1 in 3) by JIA patients, the rate of first hospital admissions for JIA has not decreased since. The reasons behind this discrepancy require further study.
3) Preliminary data suggest that history of rheumatic disease in first degree family members and early life infection are risk factors for JIA development.

Have the findings of the research already benefitted people with musculoskeletal disease? How might the findings inform further research to help sufferers in the future?
Given that our findings are descriptive and epidemiological we have filled a number of knowledge gaps in scientific literature for the Australian setting of JIA. This will benefit patients over time as it can serve to underwrite further research into the clinical, serological and/or laboratory-based aspects of the disease.

Are you planning to continue the research? *Please provide details.*
The grant was used to support a part time Research Associate for collection/extraction and cleaning of WARDER data to obtain a full dataset of longitudinal health data for JIA patients. We now have a fully functional JIA database from which the first results are being produced (posters, presentations, draft manuscript). We will continue investigating a range of health outcomes for patients with JIA in Western Australia and expect this research expected to produce at least 3 more manuscripts on various health outcomes for patients with JIA over a 2-year period.

SCIENTIFIC SUMMARY (SCIENTIFIC REPORT)

What were the main scientific objectives of the grant?
Defining the burden of disease for Juvenile Arthritis in Western Australia by
a) Providing long term Western Australian data of JIA incidence and prevalence based on a large cohort.
b) Identify potential triggering events including the number and type of severe infections and/or physical trauma in the period before JIA development (“look back period”).
c) Determine whether a JIA diagnosis increases the likelihood for serious depression requiring admission and/or ED visits for ancillary issues, e.g. accidental or deliberate intoxications

What were the main scientific achievements of the grant?
To date this funding has supported 2 conference abstracts, 1 poster and 1 manuscript currently under peer review. Another manuscript is being drafted for submission.
JIA prevalence paper: We identified 786 unique cases of JIA in WA with mean age at index hospitalization of 7.6 (sd 4.4) years, female patients the majority (n=465, 59.2%). JIA was primary diagnosis in 90.2% (n=709). 82.2% of patients lived in metropolitan regions and 6.1% (n=48) identified as Aboriginal. Based on these data we estimated hospital-based JIA point prevalence at the end of study (2012) to be 114.5/100.000 for girls (0.12%) and 52.9/100.000 for boys (0.05%). There was no significant change in annual prevalence over the 22 years study period (graph).
JIA TNFi usage data PBS derived data on usage of Enbrel and Adalimumab (TNFi) in WA for the indication of JIA were obtained from Services Australia and expressed as Defined daily dose per 1000 population per day (DDD) as recommended by WHO for drug surveillance studies. TNFi use for JIA in WA increased almost linearly from 2003 onwards (Graph) and DDD reached 0.37 in 2012, indicating TNFi usage by 1 in 2700 children. Aligning this significant use of TNFi in JIA patients (at least 1 in 3) with the stable hospital admission rate mentioned above is currently under further investigation to determine whether there have been changes in the specific admission diagnoses such as infections, joint injections etc over time to explain this curious finding.
JIA risk factors Preliminary data linkage aligning WARDER data with Midwife registry data is underway to determine the impact of maternal disease/life style and early life events on the risk of developing JIA. This subproject is expected to be finalise in 2022.
JIA outcomes As we now have a fully functional JIA database from which the first results are being produced, we expect completion of the study of JIA outcomes (readmission rates for JIA, development of mental health conditions, serious infections, joint surgery and mortality) over the next 12-18 months.

What problems, if any, did you encounter in achieving the project's objectives, and how did you address them?
No major hindrances were encountered other than the time consuming admin process of appointing a suitable Research Assistant and the time need for RA to become familiar with the underlying dataset.

Have you disseminated, or plan to disseminate, the results of this research? Please tell us about:
Meetings/conferences
JIA abstracts were submitted and accepted for the 2022 ARA-ASM and EULAR scientific meetings.

Any other ways in which you may have disseminated the research, including to the public and the media (please provide URLs to relevant press releases or media articles).
Once the full manuscripts for the JIA projects mentioned above become available for the scientific community, we will share the results of our research at educational platforms (social media, online, magazine, seminars) run by the Arthritis & Osteoporosis Foundation of Western Australia. This funding was particularly helpful in establishing a range of research

projects. While the benefits here reflect those that were achieved within 12 months of receiving the funding, the funding will support further research in the next few years.

Funded by:	Australian Rheumatology Association (ARA)
Recipient:	Prof Richard Day
Intended Department	Department of Clinical Pharmacology & Toxicology- St Vincent's Hospital
Project:	***Effect of self-monitoring urate levels of adherence to allopurinol with people with gout.***

Plain language summary (Lay report)

Using the Arthritis Australia 2021 National Research Program Grant, our research study is examining why people with gout do not take their allopurinol as prescribed by their doctor, as well as investigating novel methods of improving allopurinol medication-taking behaviour. Gout is caused by elevated urate concentrations in the blood, and allopurinol helps to control gout by re-ducing the production of urate. An increase in urate concentrations in the blood results in a build-up of urate in the joints, triggering painful gout flares. Consistently taking allopurinol prevents this build-up from happening by keeping urate concentrations below a target (<0.36 mmol/L), and therefore reduces the occurence of painful gout flares. Allopurinol is a safe and effective medica-tion; however, many people with gout struggle to take their allopurinol every day. This leads to poor patient outcomes in the gout community.

Our study consists of two phases. Phase 1 is observational, whereby people with gout are provided a self-testing device to measure their urate concentrations for 12 months. The device is similar to glucose meters used by people with diabetes. The study explores how empowering pa-tients to track their own urate concentrations influences the way they take their allopurinol. We are also evaluating how the collection of their own urate concentrations can help facilitate discus-sions with their doctor regarding their gout management, specifically allopurinol dosing decisions. Phase 2 consists of semi-structured interviews with people with gout, including people who are either currently or previously prescribed allopurinol. This aims to learn more about the lived expe-riences of people with gout, including what factors influence their allopurinol taking behaviour. Both Phases recruited people with gout across Australia, including in rural and remote regions. This is the first time that self-testing devices have been used by people with gout to monitor their urate concentrations as a way to improve their medication-taking behaviour.

For Phase 1, we have recruited 32 people with gout. Only one participant has withdrawn, due to conflicting time commitments. Most participants are male (93.8%), with half living in metropolitan areas (53.1%). Most participants are approaching the 9-month milestone with three more months until their exit interview is conducted. To date, the average adherence rate is 85.5% (95% CI 76.4,91.6), with urate concentrations decreasing on average by 0.17 mmol/L (95% CI 0.15, 0.20). Most participants now have urate concentrations within the target range (0.33 mmol/L, 95% CI 0.31,0.35). Additionally, in response to sharing the urate concentration data obtained with the de-vice with their general practitioner, four participants have had their dose of allopurinol increased. The exit interview will further explore patient perspectives on using the self-testing device to manage
their gout over the year.

Phase 2 is complete, with 26 participants interviewed. Participants were mainly male (92.3%), over 60 years old (53.8%), living in metropolitan areas (65.4%), who had gout for over 10 years (88.5%), and have never seen a rheumatologist/gout specialist (80.8%). These interviews identified that doc-

tor-patient relationships, an accurate understanding of how allopurinol works,and gout flare experiences influence the gout medication taking behaviour of people with gout.
Phase 2 results have been submitted to a peer-reviewed journal as an invited publication.
Gout is the most common inflammatory arthritis in men, affecting up to 6.8% of Australians.1 Owing to its substantial prevalence, gout represents a significant musculoskeletal disease burden, with the cost of gout to the Australian healthcare is $202 million per year which is 1.5% of total musculoskeletal disease expenditure.2 Given how effective allopurinol is in eliminating the cause of gout (elevated urate concentrations), improving how people with gout take allopurinol repre-sents an achievable means of reducing musculoskeletal disease burden in Australia. This research into patient-led models of care to manage gout informs the design of health services to better manage gout. The ability to self-monitor urate concentrations has improved our participant's gout health literacy, facilitated discussions with their primary healthcare team to optimise their gout medications, improved their urate control and reduced the frequency of gout flares. In light of the impressive improvements in gout control with this patient-led model-of-care, findings from this research provided the pilot data for a recent MRFF application designed to rollout this service in rural and remote regions of NSW and VIC. Arthritis Australia was also a partner in this MRFF application. The funding outcome will be known in May, 2022.
1. Pisaniello HL, Lester S, Gonzalez-Chica D, Stocks N, Longo M, et al. Gout prevalence and predictors of urate-lowering therapy use: results from a population-based study. Arthritis Res. Ther. 2018;20(1):143.
2. AIHW 2019b. Disease expenditure in Australia. Cat. no. HWE 76. Canberra: AIHW. Viewed 12 April 2022.

SCIENTIFIC SUMMARY (SCIENTIFIC REPORT)

Gout is the most common inflammatory arthritis in men, affecting up to 6.8% of Australians.1 Owing to its substantial prevalence, gout represents a significant musculoskeletal disease burden, with the cost of gout to the Australian healthcare approx. $202 million per year, which is 1.5% of total musculoskeletal disease expenditure.2 While allopurinol is safe and effective, target urate attainment (<0.36 mmol/L) is poor and adherence to allopurinol is sub-optimal. Therefore, the main objective of the study funded by the Arthritis Australia grant was to determine whether self monitoring of urate concentrations in people with gout influences their adherence to allopurinol. Secondary objectives were to determine whether self-monitoring of urate concentrations in people with gout facilitates attainment of serum urate concentration targets, obtaining consumer (people with gout) opinions and experiences with self-monitoring of urate concentrations, and to understand the opinions and experience of consumers on approaches to improve adherence to allopurinol.
This study consists of two phases. Phase 1 is observational and proof-of-concept, whereby people with gout (n = 32) were provided with a self-testing device to measure their urate concentrations for 12-months. Phase 2 is a semi-structured interview with people with gout, including people who are either currently or previously prescribed allopurinol. Both Phases recruited people with gout across Australia, including in rural and remote regions. This is the first time that self-testing devices have been given to people with gout to monitor their urate as a behavioural intervention to improve their medication adherence, and gout patients are given the opportunity to suggest strategies to improve adherence.
For Phase 1, we recruited 32 people with gout, with one participant withdrawing from the study due to conflicting time commitments. Participants are mainly male (93.8%), with half living in metropolitan areas (53.1%). Most participants are approaching the 9-month milestone with three months until their exit interview. The exit interview will explore patient perspectives on the effectiveness of a self-testing device as a strategy for improving their adherence and managing their gout. To date, the average adherence rate is 85.5% (95% CI 76.4, 91.6), with urate

concentrations decreasing on average by 0.17 mmol/L (95% CI 0.15, 0.20). Most participants now have urate concentrations within the target range (0.33 mmol/L, 95% CI 0.31, 0.35). Additionally, in response to sharing the urate concentration data obtained with the device with their general practitioner, four participants have had their dose of allopurinol increased.
Phase 2 is complete, with 26 participants interviewed. Most participants were male (92.3%), over 60 years old (53.8%), living in metropolitan areas (65.4%), who had gout for over 10 years (88.5%), and had never seen a rheumatologist/gout specialist (80.8%). These interviews identified that doctor-patient relationships, an accurate understanding of how allopurinol works, and gout flare experiences influence the gout medication taking behaviour of people with gout.
Only the acquisition of equipment was delayed by the COVID-19 pandemic. The study design accommodated for study visits to be conducted remotely via telehealth, to ensure that the developing COVID-19 pandemic did not hamper participant recruitment or ongoing involvement. For example, we purchased a version of the MEMS adherence monitoring caps that have near field communication (NFC) capacity, meaning adherence data can be accessed remotely by study investigators through an app on the participant's phone. We also conducted all study visits over the phone or using video conferencing. One of the study participants was switched from allopurinol to febuxostat during the study by their general practitioner. In response, we altered the study inclusion criteria to allow participants to be prescribed any urate-lowering medication, not just allopurinol. The participant returned to allopurinol a month later.
Findings from this study will be disseminated in peer-reviewed journals. Findings from Phase 2 have already been submitted to the British Journal of Clinical Pharmacology (submitted manuscript attached). Preliminary data from both Phases of the study have been presented at the 2021 Australasian Pharmaceutical Science Association - Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists (ASPA-ASPECT) Joint Conference (abstracts attached).
1. Pisaniello HL, Lester S, Gonzalez-Chica D, Stocks N, Longo M, et al. Gout prevalence and predictors of urate-lowering therapy use -results from a population-based study. Arthritis Res. Ther. 2018;20(1):143.
2. AIHW 2019b. Disease expenditure in Australia. Cat. no. HWE 76. Canberra: AIHW. Viewed 12 April 2022.
Current Results Dissemination (attached)

Conference Posters and Abstracts

Chan J, Coleshill M, Hughes D, Michael T, Spragg J, Aslani P, Day R, Stocker S. Effect of selfmonitoring urate on allopurinol adherence. ASPA-ASCEPT Annual Scientific Meeting. Virtual, 2021.

Spragg J, Aslani P, Coleshill M, Chan J, Michael T, Stocker S. Optimising adherence to allopurinol: gout patients' perspectives. ASPA-ASCEPT Annual Scientific Meeting. Virtual, 2021.

Invited Journal Manuscripts

Spragg J, Michael T, Aslani P, Coleshill M, Chan J, Day R, Stocker S. Optimising adherence to allopurinol: gout patients' perspectives. British Journal of Clinical Pharmacology. Submitted February 2022.

Funded by:	Suzette Gately
Recipient:	Dr Jiao Jiao Li
Intended Department	Kolling Institute, Faculty of Medicine and Health, University of Sydney
Project:	***A novel regenerative therapy for treating Osteoarthritis***

2021 Arthritis Australia Research Grant-in-aid – Final Report
PLAIN LANGUAGE SUMMARY (LAY REPORT)

Brief overview of research

Osteoarthritis is a leading cause of chronic pain and disability, and the most common musculoskeletal reason for hospitalisation in Australia. There is no cure for this disease. A range of non-surgical treatments are used clinically, but only to manage symptoms until joint deterioration proceeds to the extent that a total joint replacement surgery is needed. However, the surgery brings increased risk of complications, and the implant usually last for less than 20 years. An alternative therapy that can provide a cure is urgently needed.

Mesenchymal stem cells have recently brought new hope for treating osteoarthritis. They are a type of stem cell that can be found in the adult bone marrow, which can produce many types of beneficial factors that reduce inflammation and promote the ability of damaged tissues to self-repair. However, direct injection of mesenchymal stem cells into osteoarthritic joints in early-stage clinical trials have not shown consistent benefits or any ability to 'cure' osteoarthritis. Our preliminary investigations suggested that mesenchymal stem cells can 'respond' to a diseased environment, essentially adopting the diseased state of surrounding joint structures when injected into an osteoarthritic joint, and losing their ability to produce beneficial factors.

To solve this problem, in this project we set out to grow mesenchymal stem cells in the laboratory and extract the beneficial factors they secrete, which are housed in nanoscale particles called 'extracellular vesicles'. We created different types of conditions for the mesenchymal stem cells to produce these extracellular vesicles and tested them in an experimental model of a human osteoarthritic joint. We found that when mesenchymal stem cells were grown under different types of conditions, their extracellular vesicles had different types of therapeutic benefits on human osteoarthritic cells. These exciting findings have broad implications on our path to developing a new, effective, and off-the-shelf solution for treating osteoarthritis.

Research questions answered and their importance

Our team's survey of the literature indicates that the current international landscape of using extracellular vesicles derived from stem cells as a treatment for osteoarthritis is limited to less than 20 studies, all in experimental models (cells or small animals) of osteoarthritis. Importantly, all existing studies in this space are limited by a critical challenge: the extracellular vesicles were generated from stem cells grown under randomly selected conditions, and the effects of changing the culture environment of stem cells on the therapeutic efficacy of the resultant extracellular vesicles *have never been investigated*. Currently, these non-optimal stem cell-derived extracellular vesicles need to be injected at high concentration and frequency in experimental animals to demonstrate a beneficial effect on osteoarthritis, on the order of 100 μg of extracellular vesicles weekly or several times weekly in mice. Putting this in context for human therapy, we need to use 60 million stem cells to produce enough extracellular vesicles for a single injection in the human knee joint. This is nowhere near viable for clinical application. This project set out to test whether growing the stem cells in different culture conditions might improve the therapeutic benefits of their

extracellular vesicles on osteoarthritic cells, to essentially 'optimise' the production of extracellular vesicles from stem cells and increase their potential of being used in the future as a clinically viable therapy.

Findings of the study

The key finding from our pilot study was that when different groups of extracellular vesicles derived from mesenchymal stem cells were applied to human osteoarthritic cells, each group of extracellular vesicles had *different effects* on the osteoarthritic cells. The nature of the effects depended on the culture environment of the mesenchymal stem cells. For instance, extracellular vesicles from mesenchymal stem cells grown in an environment that encouraged cell survival induced anti-inflammatory behaviour in osteoarthritic cells. In contrast, extracellular vesicles from mesenchymal stem cells grown in an environment that encouraged cartilage formation induced osteoarthritic cells to produce signals relevant to cartilage repair. These were very exciting results suggesting that this line of research would potentially not only allow us to produce extracellular vesicles from stem cells that had *improved therapeutic benefits* on osteoarthritic cells, but also to tailor the culture environment of stem cells so we can produce extracellular vesicles with *specific and possibly personalised functions*.

During the course of the project, we also made a number of methodological advances. First, we were able to formulate our own in-house culture medium for growing mesenchymal stem cells, which removed our reliance on commercial culture medium. Second, we optimised the method of isolating extracellular vesicles from the culture medium that has been used to grow mesenchymal stem cells, and we can now produce the extracellular vesicles as purified preparations. Third, we found that the biological activity of extracellular vesicles does not diminish after freezing and thawing, which enables their potential future application as an off-the-shelf therapy. All of these findings have important implications in our further work along this line of research, towards developing a new regenerative therapy for osteoarthritis from stem cell-derived extracellular vesicles.

Implications for further research

I presented the project idea and a snapshot of our preliminary findings as part of an invited National Science Week talk "Regenerate and Cure" in 2021. This virtual seminar attracted >50 audience members from across Australia and internationally, many of whom were patients with osteoarthritis or their family members. Although this new therapy will not be immediately available, understanding the drawbacks of current clinical therapies including stem cell injections for osteoarthritis, and seeing hope that there is a promising therapy in development on the horizon, spiked immense interest in the audience and great community engagement. The results from our pilot study provide a pioneering finding in the field that the key to producing efficacious and clinically viable treatment for osteoarthritis perhaps lies in optimising the production of stem cell-derived extracellular vesicles. With further research to test a wider range of conditions for growing stem cells, performing detailed analyses to understand the molecular mechanisms underlying their therapeutic benefits, and testing them in experimental animals with osteoarthritis, we will understand more about the properties of stem cell-derived extracellular vesicles and be able to build them towards a possibly curative clinical therapy for osteoarthritis.

Future directions

Thanks to this pilot project, I have convened an extensive project team consisting of researchers in basic science (across cell and molecular biology, materials science, nanotechnology, and data science),clinicians, and an industry partner to carry our idea forward. This pilot project has built up essential preliminary data to allow us to proceed with nationally competitive grant applications, as well as partnership with industry, to performed more detailed analyses as well as to set up a

therapeutic development platform. Pitching the idea underlying our research, I was the national winner of Falling Walls Lab Australia 2021. I am excited to be leading a multidisciplinary team to carry this research forward, with the hope of bringing a cure for patients with osteoarthritis in the future.

SCIENTIFIC SUMMARY (SCIENTIFIC REPORT)

Objectives of the study

Osteoarthritis is a leading cause of disability in aging individuals. Current clinical therapies only treat symptoms rather than stopping disease progression. The objective of our pilot study was to build up preliminary data towards developing a first-of-its-kind therapy for osteoarthritis – extracellular vesicles (EVs) from mesenchymal stem cells (MSCs). EVs are nanoscale packages that convey biological signals, and MSC-derived EVs have the same functions (e.g., anti-inflammatory, pro healing) as the parent MSCs, but offer significant advantages compared to injecting live cells. The EVs do not trigger an immune response, have more stable characteristics, and can be easily stored and used off-the-shelf. There are only about 20 studies internationally which have tested MSC-EVs in various experimental models of osteoarthritis. Although, the majority of studies have found therapeutic benefits of the MSCEVs in these models, they mostly required prohibitively high concentration and/or frequency of application in cells or small animals (mice or rats) which would be nowhere near viable for further translation into a clinical therapy. We wanted to devise a strategy for bridging this gap. Based on our preliminary investigations, we hypothesised that changing the culture conditions of MSCs might be a breakthrough point to potentially 'customise' their generation of EVs and improve the therapeutic benefits of these EVs.

The scientific objectives of our study were as follows:

(1) Develop and optimise the method of generating different types of MSC-EVs by changing culture conditions of MSCs.

(2) Test the effects of the different groups of MSC-EVs on an *in vitro* human osteoarthritic cell model.

Main scientific achievements

There were several key achievements arising from our pilot study that has built up a solid foundation for carrying this research forward, as follows.

- The MSC-EVs are purified from MSC conditioned medium (medium that has been used to culture MSCs). We found that commercially available culture medium usually contains a high concentration of serum (at least 10%), which contains its own source of EVs and would contaminate our EV preparations. We hence sought to formulate our own serum-free culture media for MSCs that fulfilled different functions, such as growth and differentiation. It was a difficult task particularly to formulate serum-free MSC differentiation medium, but we were able to develop three types of serum-free MSC medium for producing growth, chondrogenic, and osteogenic culture conditions. This was a significant methodological achievement.
- We optimised the method of isolating EVs from MSC conditioned medium by ultra-centrifugation and verified the repeatability of the isolation method. We also confirmed that the isolated MSCEVs can be stored at -80°C for at least 3 months without loss of biological activity. These findings have important implications for practical applications of the EVs, considering that they are developed with the purpose of being eventually applied as a clinical therapy.
- We successfully isolated different groups of MSC-EVs under three different types of culture conditions created using our in-house formulated culture medium.
- We set up an experimental *in vitro* model of an osteoarthritic joint using human synovial fibroblasts isolated from osteoarthritic joints that were discarded from joint replacement surgery. We used this model to test the therapeutic efficacy of our different groups of MSC-EVs.
- We applied the different groups of MSC-EVs to human osteoarthritic cells using the *in vitro* model, comparing against control groups including: cells grown in basal medium only (negative control), cells grown in co-culture with MSCs (positive control), cells grown in EV-depleted MSC

conditioned medium, and cells grown in whole MSC conditioned medium. We analysed cell responses for markers of inflammation, osteogenesis, and chondrogenesis.
We found that the EVs derived from MSCs grown in different types of culture media significantly affected the expression of inflammatory (e.g., MMP-13, IL-8) and differentiation (e.g., SOX9) markers in human osteoarthritic cells. Importantly, the different groups of EVs had distinctly different effects on the osteoarthritic cells, while the EV-depleted MSC conditioned medium did not provide the same effects. These exciting results suggest that (i) MSC-EVs, rather than the rest of the conditioned medium, have significant effects in modulating the behaviour of human OA cells, and (ii) EVs from the same MSC source but generated under different culture conditions can have distinctly different or even 'customised' effects on target cells. The findings of this pilot study have enabled us to build up a bigger project with cross-disciplinary team members to systematically optimise the conditions for culturing source MSCs, to produce MSC-EVs with maximum, disease specific therapeutic effects that can be applied as a clinical therapy for osteoarthritis.

Problems encountered during the project

The project progress was significantly affected by COVID-19. The project team has encountered a total of 10 months of laboratory closure between March 2020 and November 2021 during which no experimental work could be performed. Moreover, CIA Li took up a new academic position during the course of the project and moved from the University of Sydney to UTS in July 2020. Coupled with unpredictable laboratory closures, this created significant challenges to CIA's laboratory access in both her previous and new institutions. After consulting with team members, a decision was made to keep the grant funding at CIA's previous institution (Kolling Institute, University of Sydney) and establish an honorary appointment for CIA with the University of Sydney after the move, to enable completion of the project work.
Again due to laboratory closures, it was difficult to coordinate work across institutions and hence part of the project was re-scoped. The bioprinting aspect of the project could not be performed as the labs used for cell culture and bioprinting respectively were not open at the same time. A compromise was made by using one representative cell type, osteoarthritic human synovial fibroblasts, as the cell model, which previously has been shown by CIA to be representative of inflammatory responses in osteoarthritis. In this project, the synovial fibroblasts exhibited characteristic hallmarks of osteoarthritis and responded to EV treatment, which was sufficient for addressing the project objectives.

Dissemination of results:

Despite the challenges imposed by COVID-19 and travel restrictions, this project allowed dissemination of findings by CIA Li at several national and international conferences, resulted in numerous conference-related awards, resulted in the publication of 1 review paper and 1 conference abstract, provided data for publications in preparation, and formed the basis for new collaborations and nationally competitive grants. The results of the project were also communicated at numerous community engagement and outreach events throughout 2020-2021. More details are provided below.
Conferences (international)
- Tissue Engineering & Regenerative Medicine International Society (TERMIS) 6th World Congress 2021; 15–19 November 2021; virtual conference.
- 2020 Osteoarthritis Research Society International (OARSI) World Congress on Osteoarthritis; 30 April–3 May 2020; Vienna, Austria. (Cancelled due to COVID-19)
Conferences (national)
- Australasian Wound & Tissue Repair Society (AWTRS) Wound Healing Symposium 2021; November 2021; virtual conference. Won AWTRS best ECR presentation award.
- Pan Pacific Connective Tissue Societies Symposium 2020; co-organised by the Australasian

Wound & Tissue Repair Society (AWTRS) and Matrix Biology Society of Australia & New Zealand (MBSANZ); 24–26 November 2020; virtual conference. Won best ECR presentation award from both AWTRS and MBSANZ.
- 28th Australian Society for Medical Research (ASMR) NSW Scientific Meeting 2020; 13 November 2020; virtual conference. Won best ECR presentation award.

Award recognition

This project contributed to the recognition of my research leadership and achievements through the following awards:
- Winner, Falling Walls Lab Australia 2021. I won the national competition (1st place) among the top 9 finalists from state-based heats, and represented Australia in the global final in Nov 2021. Falling Walls Lab is a world-class research pitch competition, and the Australian final was judged by a distinguished jury including Australia's Chief Scientist, CEO of MTPConnect, and President of Australian Academy of Science.
- Finalist, L'Oréal-UNESCO Australia & New Zealand For Women in Science Fellowship 2021. I was shortlisted as the top 10% of 2021 applicants from across Australia and New Zealand.
- 2021-22 Superstar of STEM, Science & Technology Australia. I was one of 60 Australian women selected as media stars for this acclaimed national program, to promote STEM and act as role models for the public community and younger generation.

Peer-reviewed publications

- Ryan S.T., Hosseini-Beheshti E., Afrose D., Ding X., Xia B., Grau G., Little C.B., McClements L. & Li J.J. (2021). Extracellular vesicles from mesenchymal stromal cells for the treatment of inflammation-related conditions. *International Journal of Molecular Sciences*, **22**(6): 3023. DOI:10.3390/ijms22063023
- Wang G., Xing D., Liu W., Zhu Y., Liu H., Yan L., Fan K., Liu P., Yu B., Li J.J.* & Wang B.* (2022). Preclinical studies and clinical trials on mesenchymal stem cell therapy for knee osteoarthritis: A systematic review on models and cell doses. *International Journal of Rheumatic Diseases*, accepted 9 February 2022. *Joint senior authors. DOI: 10.1111/1756-185X.14306
- (In preparation) Shang V., Li J., Little C.B., Li J.J. Mesenchymal stem cells change inflammatory, osteogenesis, and chondrogenesis gene expressions in response to osteoarthritic cells.

New collaborations

- Prof Gyorgy Hutvagner, School of Biomedical Engineering, UTS
- Prof Jinyan Li, Data Science Institute, UTS
- Prof Kuldip Sidhu, CK Cell Technologies
- A/Prof Jelena Rnjak-Kovacina, UNSW
- Dr Haiyan Lin, RMIT

Nationally competitive grant submissions

This pilot project provided the preliminary data for the following competitive grant submissions:
- NHMRC Ideas Grant in 2022 as CIA.
- ARC Future Fellowship in 2021 as CIA.
- ARC DECRA in 2020 as CIA.
- MRFF Stem Cell Therapies Mission in 2020 as CIA.

Professional contributions

This pilot project helped me establish emerging leadership in osteoarthritis and regenerative medicine that led to the following new professional roles, enabling better communication of my research:
- Osteoarthritis Research Society International (OARSI), Communications Committee (2022–now) & Diversity, Equity, Inclusion (DEI) Task Force (2022–now). Elected internationally.

- Tissue Engineering & Regenerative Medicine International Society (TERMIS), global Editorial Committee (2022–now). Elected internationally.
- Australian & New Zealand Bone and Mineral Society (ANZBMS), Early Career Investigator

Committee (2020–now).
- Australasian Wound & Tissue Repair Society (AWTRS), General Committee (2021–now).
- Matrix Biology Society of Australia & New Zealand (MBSANZ), Leadership Committee (2021–now).

Community engagement and outreach:
I regularly communicate my research and advances in my field to the community, including the following examples over 2020-2021:
- Interview by VJRegenMed during TERMIS World Congress 2021. Interviewed by Video Journal of Regenerative Medicine (VJRegenMed) during the TERMIS World Congress 2021. Spoke on a range of topics including tissue engineering, stem cells, extracellular vesicles, biomaterial scaffolds, osteoarthritis, and bone regeneration. The interview was split into topic segments and published on VJRegenMed's website and social media platforms. VJRegenMed interviews researchers and industry leaders to provide fast, open access scientific and educational information and expert opinion on the key advances in regenerative medicine.
- Invited speaker, "Regenerate and Cure" for National Science Week. Invited by Stanton Library and North Sydney Council to speak on cutting edge technologies in biomedical engineering and my research on stem cell-based therapies. The event was advertised and promoted by National Science Week, UTS, and North Sydney Council. Held virtually on 19 August 2021, this public event captured lots of interest from young (under 20) audiences. >50 attendees.
- Featured in a HubLE Idea video by the International Federation of Musculoskeletal Research (IFMRS), published on the website and social media (September 2020).
- Featured on the Einstein A Go-Go radio program, 3RRR Digital (102.7FM) for 20 PhDs in 20 minutes Postdoc edition, hosted by Dr Shane Huntington, live broadcast on 5 July 2020.
- Invited speaker/Ambassador for high schools (since 2020). I have done >20 school visits (including annually for North Sydney Girls, Sydney Girls, James Ruse) and spoken at >15 school workshops/panels organised through different outreach programs.
I also mentor 3-4 Year 12 students annually for HSC Science Extension. Through these activities, I directly promote a better understanding of my research field and broadly in biomedical engineering to hundreds of school aged students annually.

Funded by:	Arthritis WA
Recipient:	Laura Hutchison
Intended Department	Faculty of Medicine and Health, The University of Sydney
Project:	***Gait Retraining for reducing pain in people with medial knee osteoarthritis***

PLAIN LANGUAGE SUMMARY

a. Give a brief overview of the research you have undertaken, what you hoped to achieve and what you have achieved.

My research project aimed to determine whether gait retraining (changing the participants regular walking pattern) reduces knee pain and the forces inside the knee during walking in people with knee osteoarthritis. Unfortunately, our clinical trial was impacted by the relocation of the Sydney Biomechanics Laboratory and COVID-19. After our new laboratory was installed, we enrolled nine participants into our clinical trial before COVID-19 lockdowns prevented further face-to-face therapy sessions. We ran telehealth sessions with participants already enrolled, however, were unable to enrol new participants into the study as an initial face to face assessment was required. Our original trial has therefore become a pilot trial, which will provide preliminary evidence to inform a larger study commencing shortly.

As we were unable to run our study as planned in 2021, we further refined our upcoming clinical trial protocol based on emerging research. I have also been able to focus on other chapters of my PhD thesis, including the introduction, conducting a systematic review and meta-analysis, finalisation of a pilot study testing a new gait retraining intervention, and write up of our randomised controlled trial protocol manuscript. I have also been able to collaborate with other student research projects in closely related areas and have presented our research at an international conference.

b. What questions did the grant set out to answer? What problems did you try to solve, or gaps in knowledge did you try to fill? Why is this important?

An aspect of my thesis I could work on in 2021 was a systematic review and meta-analysis (a large, systematic analysis of existing literature) on the relationship between knee biomechanics (the way the knee moves and forces it experiences during walking) and knee pain in people with knee osteoarthritis. This is important as pain is often the motivating factor for people with knee osteoarthritis to seek health care, and various biomechanical devices, such as braces, foot orthoses and footwear are used as part of clinical management. However, the relationship between knee biomechanics and pain in people with knee osteoarthritis is unknown with conflicting findings reported in the literature.

I also worked on another systematic review and meta-analysis with a student colleague, which is now published. This study investigated the association between biomechanics during gait with the disease onset and progression of lower limb osteoarthritis. As osteoarthritis has no cure, research is focussing on strategies to slow or stop disease progression, which has potential to delay or prevent costly joint replacement surgeries. Some knee biomechanics are potentially modifiable using devices such as braces, or gait retraining. It may be that by changing knee biomechanics, the disease course of osteoarthritis could be changed.

We also developed and piloted a new gait retraining strategy to be included in our clinical trial that is commencing shortly.

c. What did you discover during the course of the grant?

Our systematic review and meta-analysis on the relationship between knee biomechanics and pain found that people with varus thrust presence (an abrupt outwards “thrust” of the knee during walking) are almost four times as likely to experience pain compared to people without varus thrust. This indicates that in people with knee osteoarthritis, varus thrust should be screened for early in clinical assessment. We also found that the relationship between the load on the inside of the knee during walking and pain varies depending on body mass index (BMI). Therefore, it is important for researchers and clinicians to consider the impact of BMI when using certain interventions that target knee load. I presented these findings recently at an international conference (Osteoarthritis Research Society International 2022 World Congress) and our paper is currently under peer-review.

The systematic review and meta-analysis on the association between biomechanics and the disease onset and progression of lower limb osteoarthritis found that certain gait biomechanics are associated with increased odds of osteoarthritis onset and progression in the knee, and progression in the hip. In particular, there was almost two times increased odds of medial knee osteoarthritis disease progression if participants had varus thrust presence, or higher load on the inside of the knee at baseline. This study was presented at the Congress of the International Society of Biomechanics in 2021 and published in *Osteoarthritis and Cartilage*.

d. Have the findings of the research already benefitted people with musculoskeletal disease?

Although we only had nine people enrolled in our pilot study conducted in 2021, we have received positive feedback from participants regarding the study. Participants average knee pain intensity scores for walking over the past 48 hours reduced by an average of 23 points on a 101-point scale (0 = no pain, 100 = worse pain imaginable) (average 42% pain improvement) at short-term

(approximately six weeks) follow-up, and 27 points (average 50% improvement) at long-term (approximately six months) follow-up. Additionally, as we have published and presented our systematic review and meta-analysis findings at international conferences, clinicians and researchers worldwide may be more aware of biomechanical factors to screen for that are associated with pain and disease progression in people with knee osteoarthritis.

e. How might the findings inform further research to help sufferers in the future?

Both systematic reviews highlighted the importance of varus thrust as a biomechanical factor of interest in knee osteoarthritis as it is associated with both pain and disease progression. This finding is significant, as varus thrust can be screened for and potentially modified in the clinical setting. However, currently, there is minimal research addressing varus thrust presence clinically, and most biomechanical research regarding knee osteoarthritis has been focussed on medial knee joint load. Therefore, this would be an area for further investigation informed by our findings.

f. Are you planning to continue the research? Please provide details.

As this research forms part of my PhD thesis, it will continue into at least 2023. We are soon commencing our large (postponed) clinical trial to determine whether gait retraining reduces pain and the forces at the inside of the knee during walking. In the future, I also hope to conduct projects regarding gait retraining research translation, and investigation into the effect of gait retraining on osteoarthritis disease progression outcomes using imaging. I am also interested in exploring ways to address varus thrust presence clinically.

PLEASE NOTE THAT MS HUTCHISON HAS SPECIFICALLY REQUESTED THAT THE BELOW NOT BE PUBLISHED ON OUR WEBSITE

"The scientific report contains sensitive information regarding our upcoming clinical trial and per our ethics committee requirements, cannot be made available to members of the public"

SCIENTIFIC SUMMARY (SCIENTIFIC REPORT)

a. What were the main scientific objectives of the grant?

The main scientific objectives of the grant were guided by my PhD thesis aims:

1. To explore the relationship between knee biomechanics during gait and pain in people with medial knee osteoarthritis via a systematic review and meta-analysis
2. To develop and pilot an active placebo gait retraining intervention (one which does not reduce the external knee adduction moment (KAM), a surrogate measure for medial knee load during gait) for use in our randomised controlled trial
3. To evaluate against a placebo the effects of two KAM reducing gait retraining interventions on pain, and medial knee load in people with medial knee osteoarthritis.

b. What were the main scientific achievements of the grant?

1. From a systematic review and meta-analysis of 40 studies, we found that most knee biomechanics during gait were not strongly related to pain in people with knee osteoarthritis. Due to the complexity of assessing pain, and small correlation coefficients and odds ratios between known factors associated with pain in people with knee osteoarthritis, these findings were not unexpected. Of note, we found that when early stance and overall peak KAM outcomes were pooled, there was no correlation between peak KAM and pain. This finding is noteworthy as previously in the field, it was thought that there was a relationship between peak KAM and pain. However, we did find a small negative correlation between *early stance* peak KAM and pain, and a medium positive correlation for the *overall* peak KAM and pain. We found that varus thrust presence was associated with almost four

times increased odds of reporting pain compared to people without varus thrust, indicating that varus thrust presence should be identified early in clinical assessment. Finally, meta-regression revealed that body mass index (BMI) significantly moderated the relationship between peak KAM and pain. For one unit increase in BMI, the peak KAM-pain correlation coefficient decreased by almost 0.1. This may help to explain previously conflicting study findings, and indicates that BMI is important for clinicians and researchers to consider regarding load-reducing interventions. This paper is currently under second review with *Arthritis Care & Research*.

2. We piloted an active placebo gait retraining condition hypothesised not to reduce knee joint loading outcomes that could be used as a placebo condition in our randomised controlled trial. The intervention involved participants walking with a more upright trunk. It was hypothesised that by making alterations to sagittal plane trunk position, frontal plane knee joint loading outcomes would not be affected. Results of piloting on five healthily female participants indicated no change in knee joint loading outcomes when walking with a more upright trunk. Therefore, we have developed a credible placebo gait retraining condition with plausible biomechanical rationale for participants in our randomised controlled trial. This study manuscript is currently being prepared.

3. Unfortunately, due to the Sydney Biomechanics Laboratory being moved and COVID-19 restrictions, we were only able to recruit nine participants into our randomised controlled trial in 2021. Although the protocol was disrupted, and some of the intervention sessions changed to telehealth, participants average knee pain intensity scores for walking over the past 48 hours reduced by an average of 23 points on a 101-point visual analogue scale (0 = no pain, 100 = worse pain imaginable) (average 42% pain improvement) at short-term (approximately six weeks) follow-up, and 27 points (average 50% improvement) at long-term (approximately six months) follow-up. Changes in knee joint biomechanics were as expected. This study has now served as a pilot study to inform the larger randomised clinical trial commencing shortly and will still form a chapter in my thesis.

c. What problems, if any, did you encounter in achieving the project's objectives, and how did you address them?

We were very restricted by the Sydney Biomechanics Laboratory being relocated (therefore not in use for some time), and University/NSW Health policies regarding COVID-19. This meant we were unable to conduct face to face research for most of 2021. We addressed these problems by working on aspects of my thesis that were not impacted by these constraints. We also made some changes to our upcoming trial protocol based on the results of the 9 participants enrolled in our first study. We have changed the primary outcome, from pain to KAM with a resulting decrease in sample size requirements. Hopefully, this means I will be able to submit my thesis close to time. I was also able to work on closely related research projects with another student (information outlined in lay report).

c. Have you disseminated, or plan to disseminate, the results of this research? Please tell us about:

References for peer-reviewed papers that have been published

- D'Souza, N, Charlton, J, Grayson, J, Kobayashi, S, Hutchison, L, Hunt, M & Simic M 2022, 'Are biomechanics during gait associated with the structural disease onset and progression of lower limb osteoarthritis? A systematic review and meta-analysis', *Osteoarthritis and Cartilage*, 30:381-394, https://doi.org/10.1016/j.joca.2021.10.010.

Papers that have been submitted and/or accepted for publication

- Hutchison, L, Grayson, J, Hiller, C, Kobayashi, S, Simic, M 2022, 'The relationship between knee biomechanics and pain in people with knee osteoarthritis: a systematic review and meta-analysis' – under second review in *Arthritis Care & Research*
- Ohashi, T, Grayson, J, D'Souza, N, Hutchison, L & Simic, M 2022, 'Toe-in and toe-out Gait REtraining intervention for people with knee osteoArthritis Trial (The GREAT study): A pilot randomised clinical trial' – under review in *Journal of Biomechanics*

Meetings/conferences at which you have presented this research, or are due to present it

- **Osteoarthritis Research Society International World Congress on Osteoarthritis, Berlin, April 2022. Abstracts:**
    - Hutchison, L, Grayson, J, Hiller, C, D'Souza, N, Kobayashi, S, & Simic, M 2022, 'Is there a relationship between knee biomechanics and pain in people with knee osteoarthritis? A systematic review and meta-analysis, *Osteoarthritis and Cartilage*, 30, S142-S143, https://doi.org/10.1016/j.joca.2022.02.180
    - D'Souza, N, Ohashi, T, Grayson, J, Hiller, C, Hutchison, L, & Simic, M 2022, 'Toe-in and toe-out gait retraining for people with medial knee osteoarthritis: A pilot randomised clinical trial', *Osteoarthritis and Cartilage*, 30, S143-S144, https://doi.org/10.1016/j.joca.2022.02.181
- **Australian Physiotherapy Association "Thrive" Conference, Brisbane, 2021/22 (*we had two abstracts accepted for this conference which was unfortunately postponed and then cancelled due to COVID-19).**
  Accepted abstracts:
    - Ohashi, T, Grayson, J, D'Souza, N, Hutchison, L (presenting author), & Simic, M 2022, 'Toe-in and toe-out Gait REtraining for people with medial knee osteoArthritis Trial (the GREAT study): A pilot randomised clinical trial.
    - D'Souza, N., Charlton, J, Grayson, J, Kobayashi, S, Hutchison, L, Hunt, M & Simic, M 2022, 'Are biomechanics during gait associated with the structural onset and progression of lower limb osteoarthritis? A systematic review and meta-analysis
- **XXVVII Congress of the International Society of Biomechanics, Stockholm, 2021**
  Abstract:
    - D'Souza, N, Charlton, J, Grayson, J, Kobayashi, S, Hutchison, L, Hunt, M & Simic, M 2021, 'Are biomechanics during gait associated with the structural onset and progression of lower limb osteoarthritis? A systematic review and meta-analysis
- **Sports Medicine Australia Conference, Gold Coast, 2022**
    - I am submitting three abstracts to present at this conference regarding my systematic review and meta-analysis, and our pilot study testing (outcome expected July 2022).

Any other ways in which you may have disseminated the research, including to the public and the media

- Press release regarding Nicole D'Souza's systematic review covered by The University of Sydney News
- Channel Nine News aired a segment about our research featuring my supervisor A/Prof Milena Simic, and colleague Nicole D'Souza.

Funded by:	Australian Rheumatology Association (ARA)
Recipient:	Assoc Prof Meilang Xue
Intended Department	Sutton Arthritis Research Laboratory, Royal North Shore Hospital
Project:	***Association of EPCR gene polymorphism with Rheumatoid Arthritis***

INVESTIGATORS: Assoc Prof Meilang Xue, Professor Lyn March and Dr Lara Bereza-Malcolm
Sutton Arthritis Research Laboratory, Level 10, the Kolling Building, University of Sydney at Royal North Shore Hospital, St Leonards NSW 2065

Plain language summary (LAY REPORT)

a. Give a brief overview of the research you have undertaken, what you hoped to achieve and what you have achieved. b. What questions did the grant set out to answer? What problems did you try to solve, or gaps in knowledge did you try to fill? Why is this important? c. What did you discover during the course of the grant?

Rheumatoid arthritis (RA) is currently not curable. Identification of RA at initial presentation and treatment at an earlier stage can greatly reduce long-term disability and joint damage, even exert a curative effect for a proportion of patients. However, current diagnostic criteria are not sensitive enough to identify early RA, and although a number of treatments are available, each of them shows a significant non-response rate in patients. Research in RA is increasingly focused on the discovery of biomarkers that can lead to RA early diagnosis and reduction of non-response rates. Therefore, predicting the likelihood of treatment response and RA severity based on DNA/protein testing would be of great patient benefit. Specific gene mutations in an anticoagulant factor, endothelial protein C receptor (EPCR), have been identified as risk factors for cardiovascular diseases. Cardiovascular diseases have been recognized as the main cause of mortality among RA patients with the risk of these diseases estimated at least 50 % greater than that in general population. But what is the status of mutation and expression of this gene in RA has never been investigated. In this project, by identifying specific gene mutations and expression of EPCR in a RA cohort we may be able to provide a new predictive marker for RA and its complications. This biomarker may be used by clinicians for better disease management. In addition, innovative therapeutic interventions to prevent disease progression and improve the overall mobility of RA patients may be developed.

In this study, we found that when compared to normal healthy individuals, RA patients showed a lower frequency of a specific EPCR gene mutation, immune cells from the patients with RA expressed higher levels of EPCR. Due to small numbers of patients, we did not find the correlation between EPCR mutation and cardiovascular disease in these patients.

d. How might the findings inform further research to help sufferers in the future?

The knowledge gained from this project will likely lead to improved therapies to patients with rheumatoid arthritis by facilitating the diagnostic/predictive markers or novel target in RA, improve the overall outcomes of RA patients.

e. Are you planning to continue the research?

Yes, we are continuing this line of research, by recruiting a large cohort of RA patients to validate the current data, and investigate other mutations associated with this gene, and whether these mutations or the expression of this gene can be used a predictive/early diagnostic marker in RA.

SCIENTIFIC SUMMARY

a. ***The scientific objectives***.

Aims: To investigate: 1) EPCR single nucleotide polymorphism (SNP) rs867186-AA, AG and GG phenotypes in RA patients and healthy controls, 2) EPCR levels in the circulation and peripheral blood mononuclear cells (PBMC), and their association with EPCR SNP rs867186- AA, AG and GG phenotypes and disease activity.
Significance: Mutation of EPCR SNP rs867186 is associated with an increased risk of cardiovascular diseases (CVD) and venous thrombosis in general population. CVD is the main cause of mortality among RA, but this mutation is not investigated in RA. This project will determine whether there is an increased EPCR SNP rs867186 mutation and whether this mutation associated with EPCR levels in the circulation and PBMC, and RA disease activity. This work may provide a predictive marker for CVD and venous thrombosis for RA patients and facilitate the development of innovative therapeutic interventions to prevent disease progression and improve the overall mobility of RA patients.
To achieve these objectives, human peripheral blood was collected and PBMCs isolated from blood. DNA was extracted from blood and EPCR SNP rs867186 was detected by PCR and followed by Sanger sequencing at The Australian Genome Research Facility (AGRF). The EPCR on the cell surface was detected by flow cytometry and soluble EPCR by enzyme-linked immunosorbent assay.

b. Main achievements we obtained so far.

1) We found 5 individuals with SNP rs867186 out of 30 RA patients (16.7%), and 8 individuals with SNP rs867186 out of 20 HCs (40%), soluble EPCR levels were nearly 100% more in the individuals with EPCR SNP rs867186 when compared to the individuals without this EPCR SNP.
2) In RA patients, soluble EPCR was inversely correlated with inflammatory markers ESR and CRP, indicating that soluble EPCR may be anti-inflammatory in RA.
3) In healthy individuals, PBMC expressed very low levels of EPCR. In RA patients, there were 20 times more CD3 T cells expressing cell surface EPCR when compared to cells from healthy individuals. Similarly, EPCR was expressed on B cells from RA PBMC, but was almost undetectable in normal cells.
4) There was a significant positive correlation between soluble EPCR and EPCR SNP rs867186 SNP. There was no correction between cell surface and soluble EPCR in plasma in either RA patients or healthy individuals.
5) The higher DAS28 score, the higher EPCR levels on T cell and B surface.

c. Any problems?

Yes, we experience significant delay in patient recruitment due to Covid restriction.

d. Dissemination of results:

Results from current study indicate that EPCR may be a potential biomarker and effector in RA. This project has provided novel data that will create a competitive basis for larger grant applications (NHMRC Ideas has been lodged based on the preliminary data generated from this project).

Funded by:	Australian Rheumatology Association (ARA)
Recipient:	Dr Ross Penglase
Intended Department	Sutton Arthritis Research Laboratory, Royal North Shore Hospital
Project:	***Immune reconstitution following Autologous Haematopoietic Stem Cell Transplantation for Systemic Sclerosis (Scleroderma)***

Plain language summary

The generous grant from Arthritis Australia enabled an in-depth description of immune system recovery after autologous haematopoietic stem cell transplantation for Systemic Sclerosis, using two 18 colour flow cytometry panels.

Systemic sclerosis is a severe and potentially lethal autoimmune disease that causes considerable disability. The main organ involved is the skin, presenting as progressive hardening and thickening of the skin. Patients may also develop severe heart and vascular disease, joint and muscle disease, gastrointestinal problems and fibrosis or scarring of the lungs. To date, there have been few treatment options, however stem cell transplantation has recently been shown to be effective and can result in long term remission from disease. Stem cells are taken from a patient's own blood (autologous stem cells) and used to 'grow' a new immune system. It is hypothesised that this new immune system will no longer 'attack' a patient's own body.

We set out to describe some of the changes in the immune system after stem cell transplantation, using a method called flow cytometry. This involves labelling a cell with up to 18 different cell-surface markers and then passing these cells through a machine to identify which markers are present on each cell. This permitted identification of 10+ primary cell types with many more subpopulations of such cells. Immune cells were drawn from each patient before and up to two years post transplantation. The generous grant from Arthritis Australia covered the reagents and materials required to perform these experiments.

Firstly, we wanted to see if the thymus gland reactivated post-transplantion. This gland sits in the chest and is involved in the 'schooling' of T cells. We hypothesised that reactivation of the thymus is involved in long term remission. Our results showed that after transplantation, this gland recovers activity and repopulates the immune system with both regular T cells and T cells involved in regulation of the immune cells, termed T regulatory cells. These cells have a key role in dampening immune responses and limiting autoimmune disease. Our study is the first to show renewed production of these cells from the thymus after transplantation.

Secondly, after transplantation, these T regulatory cells carry markers that show an improved ability to suppress and regulate other cells. These cells emerge early after transplantation and persist after two years, particularly in those who respond to treatment. These cells also carry markers that allow them access to the skin and other diseased organs.

The two panels allowed identification of many more cell types. For example, we demonstrated that subsets of natural killer (NK) cells and total B cells expand post-transplantation.

While stem cell transplantation is effective, it is not for all patients with systemic sclerosis; if patients have already suffered too much organ damage, particularly to the heart, lungs or gut, then the treatment is considered too high a risk. Therefore, there is a key unmet need to extend effective but safer therapies to all patients with systemic sclerosis. One answer to this is to try and manipulate

immune cells with a modified transplant, or without a transplantation at all. T regulatory cells are an attractive candidate to manipulate with the aim of dampening the autoimmune response. The data gained from this project will be invaluable to develop ways of targeting T regulatory cells.

In the future, these data will be correlated with other clinical and laboratory data collected as part of my PhD. These data will be presented as a thesis and hopefully published in the coming 12-24 months.

SCIENTIFIC SUMMARY

What were the main scientific objectives of the grant?
The main objective of the grant was to describe the kinetics of key immune system populations in systemic sclerosis patients treated with autologous haematopoietic stem cell transplantation. To achieve this, two, eighteen colour panels were designed to allow characterisation of cell populations and key surface markers at single-cell resolution, including populations from the adaptive and innate immune systems. On particular focus was on T regulatory cells, including identification of key subpopulations.

What were the main scientific achievements of the grant?
The first finding of interest pertains to T cells of thymic origin. It is postulated that autologous haematopoietic stem cell transplantation induces long-term remission in part by reactivation of the thymus gland and subsequent export of tolerogenic T cell populations (CD4 cells identified as recent thymic emigrants (RTEs), based on cell surface expression of CD31 and CD45RA). Our study is the first to demonstrate thymic recovery of CD4+ Tregs post AHSCT through identification of Treg RTEs. This may suggest a role for thymic recovery of Tregs in the maintenance of long term-remission seen with AHSCT.

Both CD4+ conventional (Tconv) and regulatory (Treg) cells exhibited an early proportional shift from a naïve to memory phenotype, with an expansion of central (CM) and effector memory (EM) populations.

In our cohort we determined that the absolute number of Tregs did not change after AHSCT, however, reflective of the heterogenous nature of Tregs, we noted a number of key changes in Treg subpopulations. Firstly, we identified an expansion of functionally mature and highly suppressive Tregs (HLADR+) early after transplantation (3 months) that persisted out to 24 months. There was an expansion of Tregs that expressed markers permitting 'homing' to the cutaneous compartment, suggestive that more regulatory cells can access affected tissues post AHSCT.

Analyses also demonstrated significant shifts in CD8 T cell, B cell, NK cell and dendritic cell populations. In particular, there was an expansion of putatively immunosuppressive CD56hi NK cells. We did not detect any significant changes in circulating basophils or monocytes.

What problems, if any, did you encounter in achieving the project's objectives, and how did you address them?
As is the nature of large flow cytometry panels, robust optimisation was required to achieve workable panels and accurate results. This process was interrupted by the COVID-19 pandemic in 2020, when laboratory shutdown and availability of reagents became an issue.

Have you disseminated, or plan to disseminate, the results of this research?

These results have been presented the 2021 American College of Rheumatology Convergence (online meeting) in poster format. These data will form part of my PhD thesis and hopefully a subsequent publication in 2022/2023.

Funded by:	Leanne Stafford Award (funded by Australian Rheumatology Association- ARA)
Recipient:	Shi Nan Luong
Intended Department	Centre for Rheumatology Research, Division of Medicine, University College of London
Project:	***Investigating the relationship between autoimmune rheumatic disease and pregnancy outcomes***

SCIENTIFIC SUMMARY WILL BE SENT BY END OF JUNE 2022.

Lay summary for Shi-Nan Luong

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease that occurs when the body's overactive immune system attacks its own organs. My research examines the important area of SLE and pregnancy. SLE tends to affect women of childbearing age, and improvement in treatment has resulted in more women with lupus considering pregnancy. However, management of these patients during pregnancy can be fraught with complications of their SLE or pregnancy itself. We do not completely understand the relationship in lupus between the overactive immune system and pregnancy complications and are unable to predict which patients are most likely to improve or get worse in pregnancy. Consequently, there is often a great deal of uncertainty amongst doctors on how to best manage patients with lupus during pregnancy. There is also a lack of accurate information for lupus patients regarding the impact of pregnancy on their disease, and effect of their disease on their pregnancy.

My research project aimed to explore this area of unmet need and involved two parts:
1) To perform an combined analysis of existing studies (a systematic review and meta-analysis) on the fertility side effects of the medication cyclophosphamide, and the benefits of using another medication (called gonadotrophin releasing hormone agonists) in preventing cyclophosphamide-induced infertility in women of child-bearing age with SLE.
2) To identify immune cell numbers/markers (using a laboratory technique called flow cytometry) and blood metabolites in pregnant patients with SLE, and the relationship with pregnancy complications and quality of life.

With the support of Arthritis Australia, I was based at University College London/University College London Hospital, which is renowned for its clinical expertise and research in SLE and other autoimmune rheumatic diseases. During my time in the UK, I published my work on cyclophosphamide and gonadotrophin releasing hormone agonists. I also presented this research at the British Society of Rheumatology Annual Conference and European League Against Rheumatism Annual European Congress.

For the second part of my project, I recruited pregnant lupus patients, non-pregnant lupus patients and healthy pregnant patients from University College London Hospital. Clinical information was collected on lupus disease activity; pregnancy history; pregnancy complications; medications and

birth outcomes. I also asked patients about their health-related quality of life, using questionnaires such as Short-Form 36 and Lupus Quality of Life.

Blood samples were collected for the study of metabolites (called metabolomics) and white blood cells (using the technique flow cytometry). I performed flow cytometry experiments, looking at specific types of white blood cells called regulatory T cells, which are important in a normal pregnancy as they prevent the mother's immune system from recognising the foetus as a "foreign body" and rejecting the foetus from her body. In pregnant women with lupus, there are fewer regulatory T cells, and these T cells do not function normally. Alterations in these cells may be the reason why miscarriages and premature labour occur more frequently in pregnant patients with lupus. My work also involved looking at whether blood biomarkers, such as types of cholesterol or proteins, have an impact on pregnancy outcomes or SLE disease activity.

This project is currently in the data analysis stage, and results will be compared between the three patient groups. Results will also be compared to other patient groups, such as those with rheumatoid arthritis, as part of the larger UK-wide PRINT study (Pregnancy in Rheumatic disease Investigation NeTwork study).

There is lack of knowledge surrounding lupus in pregnancy, and patients value research into the mechanisms underlying lupus. My research project aims to bridge this information gap by exploring the ways in which the immune and metabolic system breaks down in lupus. I hope my study will translate into increased understanding for patients, health care professionals, and the public about the relationship between lupus, adverse pregnancy outcomes and quality of life. This will help improve health outcomes in an area of unmet need.

One outcome of this study would be a greater understanding of the immunometabolic relationship between lupus and pregnancy. Unlike many other studies, patients with severe lupus who fall pregnant have been included, making it more relevant to everyday clinical practice. My research will hopefully allow us to predict which patients are more likely to flare or experience complications during pregnancy, and allow us to provide more accurate, individualised treatment to patients. It will also allow patients to make more informed decisions about the timing and management of their disease in pregnancy.

Patients have been directly involved through exploring quality of life from their perspective. I hope to gain insight into the impact of lupus and discover what is most important to patients. This is a novel and significant area to capture in pregnant patients with lupus, as it will allow us to evaluate the effectiveness of treatment. It provides patients with an opportunity to directly engage in their treatment, and ultimately improve their adherence to management plans. I am aiming for results of this study to be used to create key educational resources for patients and the public, as patients have expressed a keen desire for more information in this area.

This research will form the basis of my PhD thesis. My research is being continued through ongoing collaboration with my rheumatology colleagues at University College London. With the support of Arthritis Australia, I have gained invaluable clinical experience and research skills, particularly in the area of lupus and obstetric medicine. I hope to apply these skills to my everyday clinical practice, and continue to be involved in research projects with my colleagues both in Australia and in the UK. In the future, I would like to establish a dedicated rheumatology-obstetric service in Australia.

Lastly, I would like to thank Arthritis Australia for giving me this invaluable career opportunity.

Funded by:	Australian Rheumatology Association (ARA)
Recipient:	Dr Sultana Monira Hussain
Intended Department	School of Public Health and Preventive Medicine- Monash University
Project:	***Topical Cortisosteroid and hand osteoarthritis***

PLAIN LANGUAGE SUMMARY (LAY REPORT)

a. Give a brief overview of the research you have undertaken, what you hoped to achieve and what you have achieved.

Hand osteoarthritis is very common in the community, causing pain, disability and reduced quality of life. There is no effective treatment for hand osteoarthritis, thus it is an international research priority. Joint swelling (inflammation) is present in 30% of patients with hand osteoarthritis and is associated with pain and joint damage, representing a potential target for reducing pain and improving patient outcomes. Oral corticosteroids are effective in treating inflammation and reducing pain in hand osteoarthritis but are associated with significant side effects including diabetes and osteoporosis. Intra-articular injections of corticosteroids are effective but difficult to administer and their effect lasts for less than a month. Topical corticosteroids are safe, inexpensive and commonly used for skin conditions, with the potential to reduce inflammation; but their effect in hand osteoarthritis has not been examined in a clinical trial. We conducted a clinical trial to test whether topical corticosteroid might reduce pain and improve function in those with painful hand osteoarthritis. If we find that topical corticosteroids are safe and effective, this will provide patients with hand osteoarthritis an effective treatment to relieve pain and improve function.
We have completed enrolment of 106 participants and follow-up assessments. Data analysis is under way and the study findings will be summitted for publication by December 2022 and made available to the study participants after the publication is accepted (likely 2023).

b. What questions did the grant set out to answer? What problems did you try to solve, or gaps in knowledge did you try to fill? Why is this important?

This project aims to assess, using a parallel-group randomized controlled design, the effect of topical corticosteroid (Diprosone OV ointment) administered 3 times daily compared to placebo (plain paraffin ointment) in reducing pain and improving function over 6 weeks in participants with hand osteoarthritis.

There is no effective treatment for hand osteoarthritis to reduce symptoms and improve function. Oral corticosteroids have been shown to be effective in pain relief in those with hand osteoarthritis but are associated with significant adverse effects including diabetes and osteoporosis. Intra-articular injections of corticosteroids are effective but the effects often last less than one month and are technically difficult and often performed under ultrasound guidance which adds significantly to the cost, inconvenience and timeliness of treatment. Topical corticosteroids are safe, inexpensive, and have the potential to reduce inflammation, which might provide a potential treatment for hand osteoarthritis. If topical Diprosone OV ointment administered 3 times daily is found to be more effective than placebo in reducing pain and improving function in participants with hand osteoarthritis, this would provide patients with hand osteoarthritis a safe, inexpensive treatment to reduce the burden of disease in the community.

c. What did you discover during the course of the grant?

We started the clinical trial in October 2020. Due to the COVID-19 pandemic and lockdowns in Melbourne, the study procedures have been significantly affected. Although telehealth has been used for study visits and data collection, the enrolment and having hand x-ray have been challenging and delayed. We have been able to recruit 106 participants and completed follow-up assessment in May 2022. We are now in the stage of establishing database and data analysis. We anticipate to release the study findings to the participants in 2023.

d. Have the findings of the research already benefitted people with musculoskeletal disease? How might the findings inform further research to help sufferers in the future?

The protocol paper for this project has been published [Topical corticosteroid for treatment of hand osteoarthritis: study protocol for a randomised controlled trial. BMC Musculoskelet Disord. 2021;22:1036]. The research data are currently being analysed and manuscripts of study findings will be submitted for publication in international peer-reviewed journals and presented in international and national conferences so that the findings can be disseminated to the research community. This research provides evidence for the efficacy of topical corticosteroids in hand osteoarthritis. If found effective, this can be immediately translated into clinical practice, providing patients with hand osteoarthritis an effective treatment that relieves pain and improves function. The results will inform clinical guidelines internationally.

e. Are you planning to continue the research? Please provide details.

Yes. If topical corticosteroid is found to be effective, we plan to examine better methods for delivery of the medication in the affected hand joints. We found that participants were very enthusiastic about the study but found that the ointment was rather messy to use. This work will be done in collaboration with the Monash University School of Pharmacy.

Acknowledgment
Arthritis Australia

SCIENTIFIC REPORT

Application from: School of Public Health and Preventive Medicine, Monash University, for a 2020-2021 **Arthritis Australia Project Grant**
Subject: Topical Corticosteroid and hand osteoarthritis
Awarded: Project Grant funded by Australian Rheumatology Association

a. What were the main scientific objectives of the grant?

The aim of the study was to assess, using a parallel-group randomized controlled design, the effect of topical Diprosone OV ointment administered 3 times daily compared to placebo (plain paraffin ointment) in reducing pain and improving function over 6 weeks in participants with hand osteoarthritis.
The hypothesis was, topical Diprosone OV ointment administered 3 times daily would be more effective than placebo in 1) reducing pain (visual analogue scale) and 2) improving function (assessed using validated questionnaires) over 6 weeks in participants with hand osteoarthritis.

b. What were the main scientific achievements of the grant? Your answer should be at least 200 words.

We started the clinical trial in October 2020. Due to the COVID-19 pandemic and lockdowns in Melbourne, the study procedures have been significantly affected. Although telehealth has been used for study visits and data collection, the enrolment and having hand x-ray have been challenging and delayed. We have been able to recruit 106 participants and completed follow-up assessment in May 2022. We are now in the stage of establishing database and data analysis. We anticipate to release the study findings to the participants in 2023.

c. What problems, if any, did you encounter in achieving the project's objectives, and how did you address them?

We started the recruitment of study participants for this clinical trial in October 2020 after obtaining ethics approval and prospective registration of the clinical trial in the ANZCTR. Due to the COVID-19 pandemic and lockdowns in Melbourne, the study procedures have been significantly affected. Although telehealth has been used for study visits and data collection, the enrolment and having hand x-ray performed to assess participants' eligibility for the study have been challenging and delayed. Despite these issues, we have completed study recruitment of 106 participants and completed follow-up assessment in May 2022, with retention rate over 80%. We cleaned the database and commenced data analyses. We anticipate to complete the statistical analysis by September 2022 and submit the manuscript by December 2022.

d. Have you disseminated, or plan to disseminate, the results of this research? Please tell us about:
- **References for peer-reviewed papers that have been published (please provide pdf copies of papers if possible)**
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Intended Department	school of Public Health and Preventive Medecine- Monash University
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Arthritis Australia

SCIENTIFIC REPORT

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Subject: Topical Corticosteroid and hand osteoarthritis
Awarded: Project Grant funded by Australian Rheumatology Association

b. What were the main scientific objectives of the grant?

The aim of the study was to assess, using a parallel-group randomized controlled design, the effect of topical Diprosone OV ointment administered 3 times daily compared to placebo (plain paraffin ointment) in reducing pain and improving function over 6 weeks in participants with hand osteoarthritis.
The hypothesis was, topical Diprosone OV ointment administered 3 times daily would be more effective than placebo in 1) reducing pain (visual analogue scale) and 2) improving function (assessed using validated questionnaires) over 6 weeks in participants with hand osteoarthritis.

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Acknowledgment
Arthritis Australia

Funded by:	Arthritis South Australia
Recipient:	Prof Anthony Purcell
Intended Department	Department of Biochemistry - Monash University
Project:	***Are Hybrid pepide antigens pathogenic in rheumatoid arthritis***

Plain language summary (Lay report)

a. Give a brief overview of the research you have undertaken, what you hoped to achieve and what you have achieved.

We examined a new class of protein antigens that potentially form pathogenic targets of T cell mediated immunity in rheumatoid arthritis (RA). This stemmed from an extrapolation of studies in type 1 diabetes that have shown that "hybrid peptides" consisting of a piece of insulin and a piece of another protein generate novel and highly reactive antigens for T cells. We hypothesized that similar hybrid peptides are generated from candidate autoantigens in RA forming potent stimulators of T lymphocytes and driving damaging immune responses in RA.
Moreover, we examined their presence in synovial fluid (the fluid that baths the joint) from affected joints. Our hypothesis was directly tested using new tools developed in our laboratory that facilitate the identification of these hybrid peptides using mass spectrometry. A large number of candidate peptides were identified including novel modified peptides, autoantigen derived peptides as well as spliced peptides. These peptides are currently being tested for T cell reactivity using blood from healthy volunteers and RA patients. If proven correct this information could revolutionize our understanding of pathogenic T cell responses in RA and provide new avenues for immunotherapeutic development.

b. ***What questions did the grant set out to answer? What problems did you try to solve, or gaps in knowledge did you try to fill? Why is this important?***

The major question we addressed was if hybrid peptide antigens are presented by RA associated HLA-DR4 molecules and if they are immunogenic. In addition to spliced peptides we also accumulated considerable data for other more conventional classes of peptide antigens.

c. ***What did you discover during the course of the grant?***

We discovered around 2% of HLA-DR4 peptides are spliced in origin – that is they represent hybrid sequences that contain sequences derived from more than 1 protein. A number of these hybrid peptides contain segments from joint specific proteins making them of potential disease relevance. These peptides are now being tested for T cell reactivity using HLA-DR4+ patient and healthy donor derived T cells.

d. Have the findings of the research already benefitted people with musculoskeletal disease? How might the findings inform further research to help sufferers in the future?

The outcomes of immunogenicity studies may inform future treatment strategies.

e. Are you planning to continue the research? Please provide details.

We are planning to finalise the initial immunogenicity screening studies and use this data to apply for additional funding (e.g. NHMRC Ideas grant, Arthritis UK, etc.).

Scientific summary (Scientific report)

a. What were the main scientific objectives of the grant? / b. What were the main scientific achievements of the grant?

Aim 1: *To examine the proteome of synovial fluid for the presence of hybrid peptides derived in part from established autoantigens in RA*

We used synovial fluid from anti-citrulline peptide antibody positive (ACPA+ n=5)) and rheumatoid factor positive (RF+) donors. Thus, we will examine the proteomes of ACPA+ RF+ donors (n=5), as well as control synovial fluid isolated from osteoarthritis patients (n=1). Synovial fluid was digested with a combination of proteases to produce tryptic, chymotryptic and elastase digested peptides. Each digest was pre-fractionated using a high pH reversed phase fractionation and subjected individually to high resolution LC-MS/MS. Our analysis did not reveal a high prevalence of hybrid peptides within synovial fluid, consistent with other studies of cellular proteomes (1), suggesting that "pre-formed" hybrid peptides are not abundant in synovial fluid.

Aim 2: *To understand the contribution of spliced peptide antigens in the HLA-DR4 immunopeptidome*

We extended our earlier studies that used the monoallelic T2.DRB1*04:01 cell line to monocyte derived dendritic cells (MoDC) expressing HLA-DRB1*04:01 and B-lymphoblastoid cell lines homozygous for HLA-DRB1*04:01 (the two most important antigen presenting cells in RA pathogenesis). For the DR4+ MoDCs we also fed these cells with a cocktail of RA-associated autoantigens (fibrinogen, vimentin and type II collagen) or synovial fluid. For each condition, on average, we obtained ~ 4000 HLA-DR4 bound peptides with around 2-5% spliced peptides. These data have yielded around 200 candidate hybrid peptide antigens containing segments of fed autoantigens or other relevant autoantigen sequences (see Figure 1 for examples).

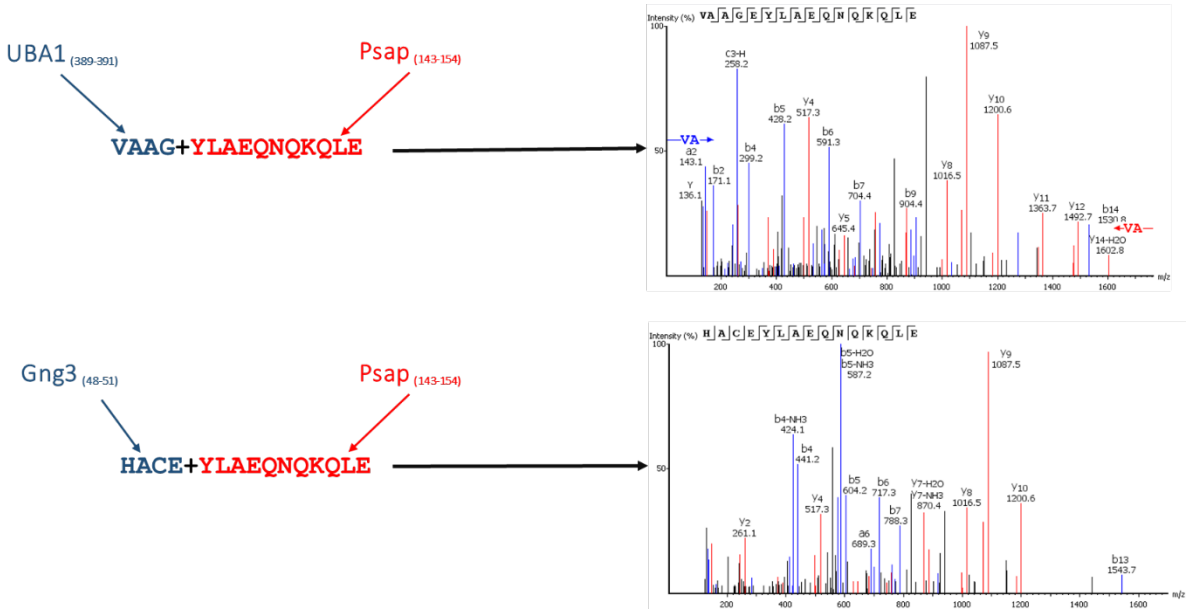

Figure 1: Examples of hybrid peptides identified in the HLA-DRB1*04:01 immunopeptidome containing a common pre-saponin (Psap) C terminal peptide segment and variable N-terminal segments from UBA1 (Ubiquitin Like Modifier Activating Enzyme 1) and Gng3 (G Protein Subunit Gamma 3).

Aim 3: *Preliminary immunological studies of candidate hybrid peptide antigens*

Due to limitations in collection of blood Aim 3 has been delayed. We are currently screening 22 candidate peptides in CD4 T cell assays using PBMCs from healthy donors in the first instance for production of IFN-γ and TNF-α.

c. What problems, if any, did you encounter in achieving the project's objectives, and how did you address them?

1. Access to patient bloods has been hampered by the pandemic. We anticipate obtaining PBMCs through our clinical collaborators in the next few months.

2. We also investigated for the presence of spliced peptides from synovial biopsies. Although a number of peptides were identified, including several citrullinated peptides the spectral quality was not sufficient for the identification of splice peptides. This is due to the small amount of material obtained and the paucity of HLA-DR4+ APC in the biopsy. A solution to this will come from our recently developed peptidePCR approach that involves peptide barcoding of biopsy derived peptides and inclusion of peptide standards to increase confidence of identification of spliced peptides (2).

d. Have you disseminated, or plan to disseminate, the results of this research?

The results of our study have not been reported yet. Obviously, conference attendance was not feasible until very recently due to the pandemic. On-going immunogenicity studies will provide the last data for preparation of manuscripts and conference presentations in 2023.

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