STUDY PROTOCOL

platelet-Rich plasma as a symptom- and disEaSe-modifying Treatment fOR knee ostEoarthritis - the RESTORE trial

SHORT STUDY TITLE

The RESTORE trial

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Sponsor
University of Melbourne

CONFIDENTIAL

STATEMENT OF COMPLIANCE

This document is a protocol for a clinical research study. The study will be conducted in compliance with all stipulations of this protocol, the conditions of ethics committee approval, the NHMRC National Statement on Ethical Conduct in Human Research (2007) and the Note for Guidance on Good Clinical Practice (CPMP/ICH-135/95).

NOTE: Some sections may not apply to your study. You should delete those that are N/A. Delete all guidance text (marked in RED) and margin comments prior to submission.

Contents

Title platelet-Rich plasma as a symptom- and disEaSe-modifying Treatment fOR knee ostEoarthritis - the RESTORE trial
Objectives Primary: To determine whether a series of injections of platelet-rich plasma (PRP) into the knee joint will lead to significantly greater reduction in knee pain and less loss of medial tibial cartilage volume in the knee joint when compared to a series of placebo saline injections at 12 months. Secondary: To determine whether a series of injections of platelet-rich plasma into the knee joint will have significantly greater benefits for other clinical (other pain measures, physical function, quality of life, global rating of change) and MRI structural (effusion, synovitis, cartilage morphology, bone marrow lesions, cartilage defects, meniscal morphology) outcomes compared to placebo injections at 2 (clinical), and 12 months (clinical and MRI).
Study Design Two-group, superiority, randomized, placebo controlled trial
Planned Sample Size 288 participants
Selection Criteria Aged over 50 years with painful knee osteoarthritis and mild to moderately severe structural change on x-rays.
Study Procedures Random allocation to receive either 3 PRP injections or 3 inactive sterile salt water (saline) injections into the knee joint over 3 weeks, with a 2 and 12-month follow up.
Statistical Procedures ***Sample Size Calculation:*** An analysis of covariance adjusted for baseline scores, indicates that 115 participants per arm will have 80% power to detect a 40% reduction in loss of cartilage volume with a two-sided significance level of 0.05 (2.8% in the control group vs. 1.68% in the PRP group, standard deviation of 3.5%, within-participant correlation of 0.5). With this number of participants, we have >99% power to detect the minimum clinically important difference in pain. Allowing for 20% loss to follow-up, we will recruit 144 participants per arm - total 288 participants. ***Analysis Plan:*** Detailed description of the statistical methods is listed in the final Statistical Analysis Plan. Please refer to: https://healthsciences.unimelb.edu.au/__data/assets/pdf_file/0009/3439314/RESTORE_Statistical_Analysis_Plan.pdf
Duration of the study Four years

GLOSSARY OF ABBREVIATIONS

ABBREVIATION	TERM
AE	Adverse Events
AQOL-8D	Assessment of Quality of Life instrument – 8-dimension
DHM	Douglass Hanly Moir (RNSH site blood collection clinic)

HA	Hyaluronic Acid
ICOAP	Intermittent and Constant Osteoarthritis Pain questionnaire
IOP	Imaging@Olympic Park (the UoM site for radiology and treatment)
KL	Kellgren Lawrence (radiographic grading system)
KOOS	Knee Osteoarthritis Outcome Score questionnaire
MOAKS	MRI Osteoarthritis Knee Score
MRI	Magnetic Resonance Imaging
NRS	Numeric Rating Scale
NSAID	Non-steroidal anti-inflammatory drug
OA	Osteoarthritis
PASE	Physical Activity Scale for the Elderly questionnaire
PISCF	Participant Information Statement and Consent Form
PRP	Platelet-Rich-Plasma
PSP	Personal Support Person
RCT	Randomized Controlled Trial
RNSH	Royal North Shore Hospital
SAE	Serious Adverse Events
UoM	University of Melbourne
US	Ultrasound

1. Study Management

1.1 Chief Investigator

Investigator	Contact information
Prof Kim Bennell	P: 03 8344 4135 E: k.bennell@unimelb.edu.au Centre for Health, Exercise and Sports Medicine Department of Physiotherapy School of Health Sciences University of Melbourne VIC 3010

1.2 Principal Investigator

Name	Role	Contact information
Prof David Hunter	Rheumatologist, contributed to the design of the trial, particularly with respect to MRI outcomes and PRP protocols. He will be responsible for overseeing the conduct of the trial in Sydney, will perform the final screening of participants for study entry, be involved with the analysis and interpretation of the results and will contribute to manuscript preparation.	P: 02 9463 1887 E: david.hunter@sydney.edu.au Rheumatology Department 7C Medical Administration Acute Services Building Royal North Shore Hospital St Leonards NSW 2065

1.3 Associate Investigators

Name	Role	Contact information
Prof Rachelle Buchbinder	Rheumatologist and clinical epidemiologist, contributed to the design of the trial particularly with respect to clinical trial methodology and the PRP intervention. She will be responsible for overseeing the conduct of the trial, data interpretation and manuscript preparation, contributing to PRP protocols including training of the doctors in the study PRP protocol and procedures for ensuring fidelity of the PRP intervention.	P: 03 9508 1652 E:Rachelle.buchbinder@monash.edu Suite 41, Cabrini Hospital 183 Wattletree Rd Malvern VIC 3144
Dr Yuanyuan Wang	Clinical epidemiologist, contributed to designing the trial, particularly with respect to the outcome assessing structural change using MRI. She will be responsible for overseeing the MRI data collection and measurements, will be involved in the analysis and interpretation of the results and will contribute to manuscript preparation.	P: 03 9903 0353 E: yuanyuan.wang@monash.edu Department of Epidemiology and Preventative Medicine Monash University 99 Commercial Road Melbourne VIC 3004
Dr Kade Paterson	Trial coordinator, contributed to designing the trial. He has helped develop the study protocol and study documents, will monitor study progress at both sites, will be involved	P: 03 8834 0425 E: kade.paterson@unimelb.edu.au Centre for Health Exercise and Sports Medicine Department of Physiotherapy

	with the interpretation of the results and will contribute to manuscript preparation.	School of Health Sciences University of Melbourne VIC 3010
Dr Jessica Kasza	Biostatistician, contributed to sample size calculations and statistical analysis plan. She will perform the statistical analyses, be involved in the analysis and interpretation of the results and will contribute to manuscript preparation.	P: 03 9903 0597 E: Jessica.Kasza@monash.edu Department of Epidemiology and Preventative Medicine Monash University 99 Commercial Rd Melbourne VIC 3004
Prof Andrew Forbes	Biostatistician, contributed to sample size calculations and statistical analysis plan. He will be responsible for overseeing the researcher who will perform the statistical analyses. He will be involved in the analysis and interpretation of the results and will contribute to manuscript preparation.	P: 03 9903 0580 E: Andrew.Forbes@monash.edu Department of Epidemiology and Preventive Medicine Monash University 99 Commercial Rd Melbourne VIC 3004
Mr Ben Metcalf	Research Scientist, contributed to designing the current trial. He will help develop the study protocol and study documents, monitor study progress at both sites, will be involved with the interpretation of the results and will contribute to manuscript preparation.	P: 03 8344 8127 E: b.metcalf@unimelb.edu.au Centre for Health Exercise and Sports Medicine Department of Physiotherapy School of Health Sciences University of Melbourne VIC 3010
Prof Flavia Cicuttini	Rheumatologist and clinical epidemiologist, contributed to designing the trial, particularly with respect to the outcomes assessing structural change using MRI. She will have a role in the study execution with a particular focus on the collection and measurement of the MRI data including quality control. She will be involved in the analysis and interpretation of the results and will contribute to manuscript preparation.	P: 03 990 30158 E: flavia.cicuttini@monash.edu Department of Epidemiology and Preventive Medicine Monash University 99 Commercial Road Melbourne VIC 3004
Prof Anthony Harris	Health economist, contributed to designing the current trial, particularly with respect to the economic analyses. He will be responsible for performing the health economic analyses, will be involved in the analysis and interpretation of the results and will contribute to manuscript preparation.	P: 03 9905 8380 E: Anthony.Harris@monash.edu Centre for Health Economics Building 75, 15 Innovation Walk Monash University Clayton VIC 3800
Dr James Linklater	Musculoskeletal radiologist, contributed to the design of the trial, particularly with respect to MRI sequences and PRP protocols. He will be responsible for obtaining MRI imaging and administering PRP injections at the RNSH site.	P: 02 4737-3300 E: linklj@bigpond.com Castlereagh Imaging 60 Pacific Highway St Leonards NSW 2065
A/Prof David Connell	Musculoskeletal radiologist, contributed to the design of the trial, particularly with respect to MRI sequences and PRP protocols. He will be responsible for obtaining MRI	P: 03 9420 1700 E: d.connell@iop.net.au Imaging @ Olympic Park 60 Olympic Boulevard Melbourne VIC 3004

	imaging and administering PRP injections at the UoM site.	
Mrs Jillian Eyles	Trial coordinator, will set up and manage Sydney study site. Will monitor study progress in Sydney, will be involved with the interpretation of the results and will contribute to manuscript preparation.	P. 02 9463 1773 E. jillian.eyles@sydney.edu.au 7C Clinical Admin, Rheumatology Dept School of Medicine, Northern Royal North Shore Hospital University of Sydney, NSW 2065
Ms Vicky Duong	Research assistant, will recruit participants and conduct study assessments in Sydney. Will monitor study progress in Sydney, will be involved with the interpretation of the results and will contribute to manuscript preparation.	P. 02 9463 1896 E. vicky.duong@sydney.edu.au 7C Clinical Admin, Rheumatology Dept School of Medicine, Northern Royal North Shore Hospital University of Sydney, NSW 2065
A/Prof Bing Wang	Senior biochemist, will be responsible for the storage and assessment of the frozen platelet-rich plasma samples at the Melbourne site.	P: 03 990 30648 E: Bing.Wang@monash.edu Department of Epidemiology and Preventive Medicine Monash University 99 Commercial Road Melbourne VIC 3004
Dr Shirley Yu	Rheumatologist, will be responsible for the screening and rating of participant x-rays and blood samples. She will work under the guidance of the principal investigator Prof David Hunter.	P. 02 9463 1887 E. shirleyyu@uni.sydney.edu.au 7C Clinical Admin, Rheumatology Dept School of Medicine, Northern Royal North Shore Hospital University of Sydney, NSW 2065
Dr Win Min Oo	PhD student, will be involved in a substudy looking at the psychometric properties of ultrasound diagnostics in knee osteoarthritis, which will only be performed at the Sydney site. He is experienced in ultrasound diagnostics, and will perform the ultrasounds in this study as well as be involved in the interpretation and manuscript preparation for the substudy.	P. 0451 570 506 E. wioo3335@uni.sydney.edu.au 7C Clinical Admin, Rheumatology Dept School of Medicine, Northern Royal North Shore Hospital University of Sydney, NSW 2065
Mr Matthew Daniels	Medical student, will be involved in a substudy looking at the psychometric properties of ultrasound diagnostics in knee osteoarthritis, which will only be performed at the Sydney site. He is experienced in ultrasound diagnostics, and will be involved in the interpretation and manuscript preparation for the substudy.	P. 0448 989 654 E. mdan7235@uni.sydney.edu.au 7C Clinical Admin, Rheumatology Dept School of Medicine, Northern Royal North Shore Hospital University of Sydney, NSW 2065

1.4 Statisticians

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Dr Jessica Kasza	P: 03 9903 0597 E: Jessica.Kasza@monash.edu Department of Epidemiology and Preventative Medicine Monash University 99 Commercial Rd Melbourne VIC 3004

1.5 Internal Trial Committees

Initially fortnightly and progressing to monthly team meetings will be held between Chief, Principal and Associate investigators via teleconference to discuss overall progress of the trial including report of any adverse events.

1.6 Independent Safety and Data Monitoring Committee

The safety and data monitoring committee will be comprised of:

Dr Rodger Laurent (Rheumatologist, Royal North Shore Hospital)
Dr Jillian Patterson (Statistician, University of Sydney)
Dr Leslie Shrieber (Rheumatologist, Royal North Shore Hospital)

The committee will meet and receive a study report every 6 months. The nominees of the safety and data monitoring committee are not involved in the study, do not have any conflicts of interest and will not benefit in any way from the results of this trial.

1.7 Sponsor

The University of Melbourne

1.7 Funding and resources

NHMRC Project Grant (#1106274)

2. INTRODUCTION AND BACKGROUND

2.1 Background Information

Osteoarthritis, of which the knee joint is the most commonly involved lower limb site, affects around 2 million Australians with conservative projections estimating a 58% increase by 2032 [1]. Knee OA causes substantial pain and physical dysfunction ultimately impairing quality-of-life. The total economic cost of OA in Australia in 2012 was $8.5 billion [1]. This includes $3.75 billion in direct costs, the majority being joint replacement surgery for advanced disease (80,000 Australians in 2012 and increasing at a rate of >5% per annum) [1] and knee arthroscopic surgery (~100,000 Australians annually). The societal and health care burden of OA will continue to rise given population ageing and escalation in obesity. In recognition of the burden, arthritis is a government priority health area.

There is no cure for knee OA and to date, most research has focused on treatments to alleviate pain and prevent functional decline. Recommended drug therapies (such as analgesics and anti-inflammatory agents) and non-drug therapies (such as exercise) have short-term clinical benefits but effect sizes are small to modest at best [2]. Furthermore, these drugs can have adverse events, while uptake and maintenance of exercise is often poor leading to lack of long-term benefit. Intra-articular therapies in clinical use for knee OA include hyaluronic acid (HA, a viscosupplement), and corticosteroids. However, HA is controversial with variable recommendations given across clinical guidelines [3]. In those from the Osteoarthritis Research Society International published in 2014, HA was given an uncertain recommendation due to inconsistent conclusions among meta-analyses and conflicting results regarding safety [4], while those from the UK NICE recommended against their use [5]. Intra-articular steroids are generally recommended although not universally [3], and for short-

term pain relief only given that benefits are limited to a few weeks [4]. As knee OA is typically progressive, with both symptoms AND structural deterioration drivers for joint replacement, identifying efficacious safe treatments that address both is an important objective. Currently, however, there are no approved disease-modifying therapies.

One such biologic therapy is platelet-rich plasma (PRP), an autologous blood product that contains an elevated concentration of platelets. Activation of PRP releases an initial burst then a sustained release of growth factors and other molecules, including platelet-derived growth factor, transforming growth factor-β, type I insulin-like growth factor and vascular endothelial growth factor. These proteins are responsible for a range of critical tissue healing roles such as chondrocyte apoptosis inhibition, bone and vessel remodelling, inflammatory modulation, and importantly, collagen synthesis. Additionally, other bioactive molecules released by platelets, such as fibrin, act as a scaffold and chemo-attractant for further migration of stem and other cells to the damaged tissue. Given the limited repair capacity of articular cartilage, these roles offer a mechanism by which PRP may enhance tissue healing and cartilage regeneration in knee OA.

Although limited randomised controlled trial (RCT) evidence suggests short-term symptomatic benefits of PRP for knee OA, the published studies are at high risk for bias and lack longer-term follow-up, and there are no RCTs investigating the structural effects of platelet rich plasma.

2.2 Research Question

Will a series of injections of PRP into the knee joint lead to a significant improvement in joint symptoms, and slow disease progression, as measured by magnetic resonance imaging (MRI), in people with mild to moderate knee osteoarthritis?

2.3 Rationale for Current Study

Identifying treatments that reduce symptoms and slow disease progression in knee OA is an international research priority as there are no such approved therapies currently available. Outcomes from this study will provide the world's first high quality RCT evidence of the symptomatic and structural benefits of PRP to either support or discourage use of PRP for knee OA. This is important given that several 2014 systematic reviews [6-8] highlight the limited number of RCTs (n=6), the fact that all studies have a high risk of bias and that none have included structural outcomes. This study is also timely because in Australia, MBS items for all PRP injections were removed in Jan 2015 due to increase in costs without evidence of treatment efficacy. Hence our trial will inform the Medical Services Advisory Committee deliberations about PRP and future subsidisations.

Regardless of whether the results support or contradict our hypotheses, they will provide essential information to fill a major gap in the literature and will inform international clinical practice guidelines. No current guidelines make any recommendations about PRP given the lack of evidence. If PRP is found to have a disease-modifying effect, this will have world-wide rapid impact given the lack of such treatments and the fact that PRP is safe and minimally invasive.

3 STUDY OBJECTIVES

3.1 Primary Objective

To determine if a series of injections of PRP into the knee joint lead to significantly greater reduction in knee pain and less loss of medial tibial cartilage volume in the knee joint when compared to a series of placebo saline injections at 12 months

3.2 Secondary Objectives

To determine if a series of injections of PRP into the knee joint will have significantly greater benefits for other clinical (other pain measures, physical function, quality of life, global rating of change) and MRI structural (effusion, synovitis, cartilage morphology, bone marrow lesions, cartilage defects, meniscal morphology) outcomes compared to placebo injections at 2 (clinical) and 12 (clinical and MRI) months.
To conduct an embedded economic evaluation of PRP.

3.3 Ultrasound substudy

A separate study utilising data from the trial aims to determine whether ultrasonography is an effective diagnostic tool in routine osteoarthritis management. Performance metrics of the ultrasonography will be assessed by using the Outcome Measures in Rheumatology Clinical Trials (OMERACT) filter which examines the validity, reliability and responsiveness to treatment. This study will investigate these psychometric properties of ultrasound in osteoarthritis, using only participants from the Sydney site.

4. STUDY DESIGN

4.1 Type of Study

The RESTORE trial is designed as a two-group, superiority, randomized, placebo controlled trial.

4.2 Study Design

A randomized placebo controlled trial will be conducted in Melbourne and Sydney over 4 years. 288 participants aged over 50 years with painful knee osteoarthritis and mild to moderately severe structural change on x-rays will be recruited from the community. Clinical assessments will be performed at baseline and at 2 and 12 months and MRI structural assessments of the knee joint at baseline and 12 months.
The broad aim of this study is to determine whether PRP injected into the knee joint improves symptoms and slows disease progression, as measured by MRI, in people with mild to moderate knee osteoarthritis.

4.3 Number of Participants

We will recruit 144 participants per arm - total 288 participants.

4.4 Study sites

There are four study sites.
Site 1: The University of Melbourne – recruitment site, approximately 144 participants
Site 2: Royal North Shore Hospital – recruitment site, approximately 144 participants
Site 3: Imaging @ Olympic Park – treatment site for participants recruited by the UoM site
Site 4: Castlereagh Imaging - treatment site for participants recruited by the RNSH site

4.5 Expected Duration of Study

Anticipated start date: Late 2016
Anticipated stop date: Late 2020

We anticipate a recruitment period for this study of 2-3 years.

Final primary outcome measures will be collected at 12 months post randomization.

4.6 Primary and Secondary Outcome Measures

Name	Description	Scale	Time-points measured
Primary Outcomes			
Overall pain	Scored on an 11-point NRS for average overall pain in the last week where 0=no pain and 10=worst pain possible.	Ranges from 0 to 10; higher scores indicate worse pain.	Baseline, 2 months (secondary time point) and 12 months (primary time point) *Additionally will be asked at 6 and 9 months to identify trends in pain trajectory.
Medial tibial cartilage volume	A MRI will be performed of the study knee using a 3T whole body system with dedicated extremity coil and a T1-weighted fat suppressed 3D gradient recall acquisition sequence.	Annual % change in cartilage volume.	Baseline and 12 months
Secondary Outcomes			
Walking pain	Scored on an 11-point NRS for average pain on walking in the last week where 0=no pain and 10=worst pain possible.	Ranges from 0 to 10; higher scores indicate worse pain.	Baseline, 2 months and 12 months
KOOS	Knee Osteoarthritis Outcome Score		
Pain	Scored from 9 questions regarding knee pain in the last week.	Ranges from 0 to 100; lower scores indicate worse pain.	Baseline, 2 months and 12 months
Other Symptoms	Scored from 7 questions regarding knee symptoms in the last week.	Ranges from 0 to 100; lower scores indicate worse symptoms.	Baseline, 2 months and 12 months
Function in Daily Living	Scored from 17 questions regarding knee function in the last week.	Ranges from 0 to 100; lower scores indicate worse function.	Baseline, 2 months and 12 months
Function in Sport and Recreation	Scored from 5 questions regarding knee function in the last week.	Ranges from 0 to 100; lower scores indicate worse function.	Baseline, 2 months and 12 months
Knee-related Quality of Life	Scored from 4 questions regarding knee related quality of life in the last week.	Ranges from 0 to 100; lower scores indicate worse quality of life.	Baseline, 2 months and 12 months
Global improvement	Global rating of change		

Improvement in pain	Scored from a 7-point global rating of change Likert scale from “much worse” to “much better” when compared to baseline.	Those “moderately better” or “much better” will be classified as improved.	2 months and 12 months
Improvement in physical function	Scored from a 7-point global rating of change Likert scale from “much worse” to “much better” when compared to baseline.	Those “moderately better” or “much better” will be classified as improved.	2 months and 12 months
Improvement overall	Scored from a 7-point global rating of change Likert scale from “much worse” to “much better” when compared to baseline.	Those “moderately better” or “much better” will be classified as improved.	2 months and 12 months
Quality of life (AQoL-8D)	Health-related quality of life is evaluated with the 35-item Assessment of Quality of Life Instrument (8D version).	Ranges from −0.04 to 1.00; higher scores indicate better quality of life.	Baseline, 2 months and 12 months *Additionally will be asked at 6 and 9 months for the health economic evaluation
ICOAP	Intermittent and Constant Osteoarthritis Pain questionnaire		
Constant knee pain	Scored from a 5-item regarding constant knee pain in the previous week	Ranges from 0 to 100; higher scores indicate worse pain	Baseline, 2 months and 12 months
Intermittent knee pain	Scored from a 6-item regarding intermittent knee pain in the previous week	Ranges from 0 to 100; higher scores indicate worse pain	Baseline, 2 months and 12 months
MOAKS sub-scores	MRI Osteoarthritis Knee Score sub-scores		
Meniscal Morphology	Meniscal Morphology: Any regions with worsening?	Any regions with worsening at 12 months compared to baseline. Yes or No	12 months
Inter-Condylar Synovitis	Worsening in Inter-Condylar Synovitis	Worsening in inter-condylar synovitis at 12 months compared to baseline. Yes or No	12 months
Cartilage Morphology	Change in Cartilage Morphology - number of areas with worsening in thickness	Number of areas with worsening in thickness at 12 months compared to baseline: categorised as 0, 1, 2, or 3+.	12 months
Whole Knee Effusion	Change in Whole Knee Effusion Category	Change in Whole Knee Effusion at 12 months compared to baseline:	12 months

		categorised as Worsen, No Change or Improve	
Bone marrow lesions (BML) score in the medial tibiofemoral compartment	Assessed from the MRIs using Categorical scoring (range 0-3 per region)	Range 0-6. Medial tibia and medial femoral condyle region BML scores are added.	Baseline and 12 months
Cartilage defects score in the medial tibiofemoral compartment	Assessed from the MRIs using Categorical scoring (range 0-4 per region)	Range 0-8. Medial tibia and medial femoral condyle region cartilage defect scores are added.	Baseline and 12 months
Other Measures			
Weight	Will be measured at the first treatment visit and the final MRI visit	Measured in kilograms	Baseline and 12 months
Drug/supplement use	Participants will complete a table detailing a variety pain and arthritis medications and supplements used in the previous month.	Medication and supplements that were taken at least once a week in the past month.	Baseline, 2 months and 12 months
Adverse Events	Any undesirable clinical occurrence in a subject, whether it is considered to be treatment-related or not, that includes a clinical sign, symptom or condition.	Number and type of events.	2 months and 12 months
Success of blinding - participant	Participants will be asked which group they think they are in (PRP or placebo)	Number of participants who correctly identified, incorrectly identified or were unsure of their treatment group.	After the 3rd injection
Success of blinding - doctor	Injecting doctors will be asked which group they think each participant is in (PRP or placebo)	Number of participants whose doctor was correctly identified, incorrectly identified or were unsure of their treatment group.	After the 3rd injection
Use of co-interventions	Participants will be asked to report other treatments that they have undergone during the study for their study knee	Number and type of co-interventions.	2 months and 12 months

Number of injections	Number of injections administered will be reported	Will be between 0 and 3.	Injection appointments 1, 2 and 3
Descriptive Measures			
Height	Will be measured at the first treatment visit and the final MRI visit	Measured in metres	Baseline and 12 months
Body mass index (BMI)	Calculated from the measured height and weight	Reported in kg/m^2	Baseline and 12 months
Age	Age at the time of the baseline assessment.	Reported in years	Baseline only
Gender		Male or female	Baseline only
Duration of symptoms	Participants self-reported duration since their knee symptoms began	Reported in years	Baseline only
Radiographic disease severity	Rated on the Kellgren-Lawrence scale.	Trial participants will be either grade 2 or grade 3.	Baseline only
Knee alignment	Measured from the knee x-ray	Reported in degrees	Baseline only
Current employment	Participants will report their current employment status	The number of participants in current employment	Baseline only
Unilateral symptoms	Participants will be asked whether they experience symptoms in one knee or two.	The number of participants with unilateral and bilateral symptoms	Baseline only
Current symptoms in other joints	Participants will be asked to tick joints from a list that they currently experience symptoms in.	Number of other symptomatic joints per participant.	Baseline only
Expectation of treatment	Scored by the participants answering the effect they think the PRP injections will have on their knee.	Answers are on a 5 point Likert scale from “no effect at all” to “complete recovery”	Baseline only
PainDETECT	A tool designed to detect neuropathic pain. It contains 13 items, most of which of which use a 6-point scale.	Participants will be classified as having a neuropathic pain component is “unlikely”, “ambiguous”, or “likely”.	Baseline only
PASE	Physical activity levels in the last week will be assessed using the Physical Activity Scale for the Elderly (PASE) questionnaire.	Ranges from 0 to 400+; higher scores indicate greater levels of physical activity	Baseline, 2 months and 12 months

Presence of knee effusion at baseline	Graded from MRI using MOAKS effusion sub-score	Absent (MOAKS category 0 or 1) or Present (MOAKS category 2 or 3)	Baseline only
Health Economic Evaluation*	*To be reported separately		
Health Care Costs	Asks participants to recall the use of health services in recent months	Costs are estimated for Intervention, Diagnostic, Drugs, Hospital, Medical, Other and Total	From data collected at baseline, 2 months, 6 months, 9 months and 12 months
Quality Adjusted Life Years (QALYs)	Estimated as the area under the curve of preference based quality of life scores	One QALY equates to one year in perfect health	From data collected at baseline, 2 months, 6 months, 9 months and 12 months
Cost Effectiveness ratio	Calculated using predictions of health care costs and QALYs.	Calculated as the ratio of the mean difference in cost to the mean difference in QALYs between the groups	From data collected at baseline, 2 months, 6 months, 9 months and 12 months
World Health Organisation Health Performance Questionnaire (WHO HPQ)	Those in employment will complete a series of questions about their working hours and job performance in the previous 4 weeks	An estimate of the workplace costs of health problems in terms of reduced job performance, sickness absence, and work-related accidents-injuries	From data collected at baseline, 2 months, 6 months, 9 months and 12 months

NRS = Numeric Rating Scale; MRI = Magnetic Resonance Imaging; KOOS = Knee Osteoarthritis Outcome Score; MOAKS = MRI OA Knee Score; BML = Bone marrow lesion; AQOL-8D = Assessment of Quality of Life Instrument (8D version); BMI = Body mass index; QALYs = Quality Adjusted Life Years; WHO HPQ = World Health Organisation Health Performance Questionnaire

5. STUDY TREATMENTS

5.1 Treatment Arms

1) PRP injections into the knee joint
2) Placebo injections into the knee joint

5.1.1 Description

1) PRP injections are made from withdrawing and centrifuging the participant's own blood on the day of treatment
2) Placebo injections are the same volume of saline as the PRP injection.

5.1.2 Dosage and Route of Administration

Platelet-rich plasma injections
Three injections of PRP will be administered to participant's study knees under ultrasound guidance once per week. Three injections is consistent with the majority of RCTs [9, 10], and a recent clinical trial demonstrating the superiority of three PRP injections over one [11].

Placebo (saline) injections
Three injections of saline solution will be administered into the participant's knee under ultrasound guidance once per week.

5.2 Preparation and administration

Preparation and Randomization
Approximately 20mL of blood from the participant's arm (which will be used for the PRP preparation (10mL) as well as plasma sampling (10mL)) will be drawn. Participants will then be randomly allocated into either (1) PRP injections (intervention group) or (2) saline injections (placebo group) using the NHMRC randomisation service. Participants will not be told until the end of the study (12 months after baseline) which group they were allocated to. A research nurse will prepare the injection, and the injecting doctor will not be told which treatment group the participant is allocated to. The injecting doctors are trained musculoskeletal radiologists with extensive experience performing intra-articular PRP injections.

Platelet-rich plasma injections
For the PRP group, approximately 20mL of the whole blood sample will be centrifuged at 1500g for 5 minutes in a separate room to allow extraction of the PRP. A portion of the PRP (4mL) will be withdrawn and saved for analysis, and 5mL withdrawn into a syringe. A 22-gauge needle will be attached to the syringe, and the syringe and needle base will be covered with a patient label to occlude the contents. The nurse will then give the syringe to the injecting doctor who will not know or be able to tell whether the syringe has PRP or placebo (saline). The doctor will then inject local anaesthetic superficially (subcutaneously) at the injection site of the participant's knee before treatment (which is optional for the participant), and then perform the PRP injection into the knee joint under ultrasound guidance. After the third injection, participants will also be asked about which group they believe they are in. Injecting doctors will also be asked which group they believe each participant is in at this time.

Participants will return to the site radiology clinic (IOP or Castlereagh Imaging) for their second injection of PRP approximately one week later, and again for a third and final injection approximately one week after that. The injection process will be the same as the first injection.

Saline injections

The placebo group will have the blood sample taken as per the PRP group to assist group blinding. The research nurse will discard the sample in a separate room, and then prepare a syringe with a saline solution (5mL) and attach a 22-gauge needle. A patient label will be placed over the syringe and needle base to occlude the contents. The nurse will then give the syringe to the injecting doctor who will not know or be able to tell whether the syringe has PRP or saline. The doctor will then inject local anaesthetic superficially (subcutaneously) at the injection site of the participant's knee (which is optional for the participant), and then perform the saline injection into the participant's knee joint under ultrasound guidance. After the third injection, participants will also be asked about which group they believe they are in. Injecting doctors will also be asked which group they believe each participant is in at this time.

Participants will return to the site radiology clinic for their second saline injection approximately one week later, and again for a third and final injection one week after that. The injection process will be the same as the first injection.

Dispensing and Product Accountability

The PRP product is autologous from a withdrawal of the participant's own blood at the same treatment session. We will be storing a small sample of the PRP injectate for analyses so we can measure the concentration of platelets in the sample.

5.3 Measurement of participant compliance

Compliance will be reported as the number of injections administered. Adherence to the injection schedule will be monitored by the Site Coordinators and the staff at IOP and Castlereagh Imaging, who will be responsible for booking participants in for injection procedures.

5.4 Excluded medications and treatments

Participants will be asked to discontinue NSAIDs and other analgesics for knee pain, with the exception of paracetamol for rescue pain relief, from 2 weeks prior to baseline assessment until the 12 month follow up assessment. It will be made clear to potential participants that this is a requirement for study entry through the screening and informed consent process.

6. PARTICIPANT ENROLLMENT AND RANDOMISATION

6.1 Recruitment

Potential participants will be identified by (1) mail out or emails sent to existing databases of volunteers who have participated in previous projects and have consented to be contacted for future projects, (2) flyers or posters placed on the notice boards or in newsletters of local clubs (such as RSL, bowls clubs, university of the 3rd Age, the Men's shed, etc), on waiting room walls in general practices, medical, radiology or private physiotherapy clinics (with their permission), (3) paid ads or free postings on Facebook, (4) local and major newspaper ads or newsletter listings, and (5) radio or TV interviews with investigators.

There are 4 steps in the screening process for this study:

1) Telephone screening: All potential participants need to pass a telephone screening interview with one of the researchers. Some of these participants may have previously completed an initial online

survey to determine their initial eligibility. Participants deemed suitable from the telephone screening will be invited to undergo radiographic and blood screening.
2) Radiographic screening: If participants have a suitable weight bearing knee x-ray from the past 12 months, a stamped addressed envelope will be sent to them to post their x-ray to the researchers for screening. If participants don't have a suitable recent x-ray, they will be invited to have a new knee x-ray taken (at no cost to them).
3) Blood screening: At the same visit as the knee x-ray, participants will be asked to undergo a blood test.
Participants must pass the blood and radiographic criteria to continue to screening stage 4.
4) Physical screening: After passing telephone, radiographic and blood screening, participants will be invited to attend an appointment with the local site coordinator. The site coordinator will perform a physical assessment of the knee.
Participants who pass this step are deemed to be suitable for the study.

6.2 Eligibility Criteria

6.2.1 Inclusion Criteria

Participants are eligible for the study if they meet all inclusion criteria below:

Inclusion criteria:
(i) aged>50 years;
(ii) knee pain on most days in the last month;
(iii) tibiofemoral osteophytes on x-ray; and
(iv) A minimum pain score of 4 on an 11-point numeric rating scale for the last week

Exclusion criteria:
(i) Kellgren and Lawrence (KL) grade 1 indicating questionable disease or grade 4 indicating severe disease;
(ii) predominant lateral tibiofemoral disease;
(iii) Hyaluronic acid injection in past 6 months, corticosteroid injection in past 3 months or autologous blood product in the past;
(iv) knee surgery on their most painful knee within past 12 months;
(v) systemic or inflammatory joint disease;
(vi) history of crystalline or neuropathic arthropathy;
(vii) knee joint replacement or high tibial osteotomy on their most painful knee;
(viii) plan for joint surgery in next 12 months;
(ix) other muscular, joint or neurological condition affecting lower limb function;
(x) needle phobia;
(xi) immunosuppression or acute infective processes;
(xii) cancer or other tumour-like lesions;
(xiii) bleeding disorder or receiving anti-coagulation therapy;
(xiv) presence of a warm tense joint effusion;
(xv) platelet count ≤150,000/µL;
(xvi) any other medical condition precluding participation in the study including contraindication to MRI such as pregnancy;
(xvii) be unwilling to discontinue NSAID and other analgesic usage for knee pain, with the exception of paracetamol for rescue pain relief, from 2 weeks prior to baseline assessment until the 12 month follow up assessment;
(xviii) Body mass index (BMI) >40kg/m2 because of problems fitting in to the MRI machine knee coil; and
(xix) Inability to understand written/spoken English.

6.3 Informed Consent Process

All potential participants will receive oral and written information about the purposes, potential risks and process of the study from their site coordinator. According to the latest revision of the World Medical Association Declaration of Helsinki, an informed consent will be obtained from all participants by signing the consent form after understanding the information delivered and before proceeding with the screening visits.

If participants pass the telephone screening process, which will involve a detailed verbal description of the project to ensure that participants are happy to comply with trial procedures, participants will be sent the Participant Information Statement and Consent Form (PISCF) in the post or by email.

Participants will be encouraged to phone their site-coordinator if they have any questions or concerns regarding the contents of the PISCF. After reading the PISCF, if they give their consent to participate, they will sign the consent form along with a witness to their signature. Participants will return the signed consent form to the site coordinator via either the post or by scanning and emailing the document.

At the clinical screening visit with the site co-ordinator, those who chose to scan and email the document will be asked to bring their copy of the consent form. For all participants attending the clinical screening visit, the site coordinator will again go over the study procedures, making sure that each participant has read and understood the contents of the PISCF including the potential risks. It will be made clear that participants will be able to withdraw at this point or up until their trial enrolment and randomization.

Participants will not be permitted to undergo any screening procedures (x-ray, blood test or physical assessment) until the signed consent form has been completed and returned to the site coordinator.

6.4 Enrolment and Randomisation Procedures

At the first treatment appointment, a research nurse will withdraw approximately 20mL of blood from the participant's arm. Participants will be randomly allocated to the platelet-rich plasma injection group or the placebo injection group using a randomization schedule prepared and stored by the NHMRC Clinical Trial Centre and accessed by telephone. Randomization will occur according to a 1: 1 allocation and will be stratified in blocks with varying sizes of 6 or 10, stratified according to site (Melbourne or Sydney) and radiographic disease severity (KL grades 2 or 3). A research nurse at each site will telephone the NHMRC Clinical Trial centre just prior to administration of the first injection to reveal the participant's group allocation. The research nurse will enter the participant's group allocation into a password protected, backed-up folder on a computer server, accessible to only the Chief and Principal Investigators who are not responsible for participant recruitment, data collection or treatment.

6.5 Blinding Arrangements

Participants will not be told until the end of the study (12 months after baseline) which group they were allocated to. A research nurse will prepare the injection in a separate room, and a patient label will be placed over the syringe to occlude the contents. The nurse will then give the syringe to the injecting doctor who will not know or be able to tell whether the syringe has PRP or saline. The study participants will be blinded to group allocation. All clinical and MRI assessments will be conducted by an assessor blinded to treatment allocation.

The same preparation process will be in place for injection appointment 2 and 3, in that blood will be withdrawn from the arm of participants of both groups, which will help with blinding of participants to their group. Unused excess plasma from the placebo group will be disposed of.

6.6 Breaking of the Study Blind

6.6.1 On Study

Group allocation can be immediately unblinded if deemed necessary by the Chief Investigator in the case of any unexpected adverse events related to the study and this is an exclusive decision of the Chief Investigator.

6.6.2 Following Completion of the Study

After the final assessments (12-month follow-up questionnaires and MRI scan), participants will be made aware of whether they received injections of PRP or placebo.

6.7 Participant Withdrawal

6.7.1 Handling of withdrawals and losses to follow-up

If a participant withdraws from the study, they will have their reasons for withdrawal recorded (if they are contactable) and any information provided up to the point of withdrawal will be kept in accordance with intention to treat analyses.

6.8 Trial Closure

Follow-ups in the study protocol are scheduled at 2, 6, 9 and 12 months after study entry. As the final injection for each participant will be administered 2 weeks after study entry, it is unlikely that adverse events will commence after study participation has completed at 12 months.

6.9 Continuation of therapy

Participants will only receive the proposed treatment during the 12-month period of the study, and will not be offered any continuation of the proposed study therapy after completing the study.

7. STUDY VISITS AND PROCEDURES SCHEDULE

Study Flow Chart

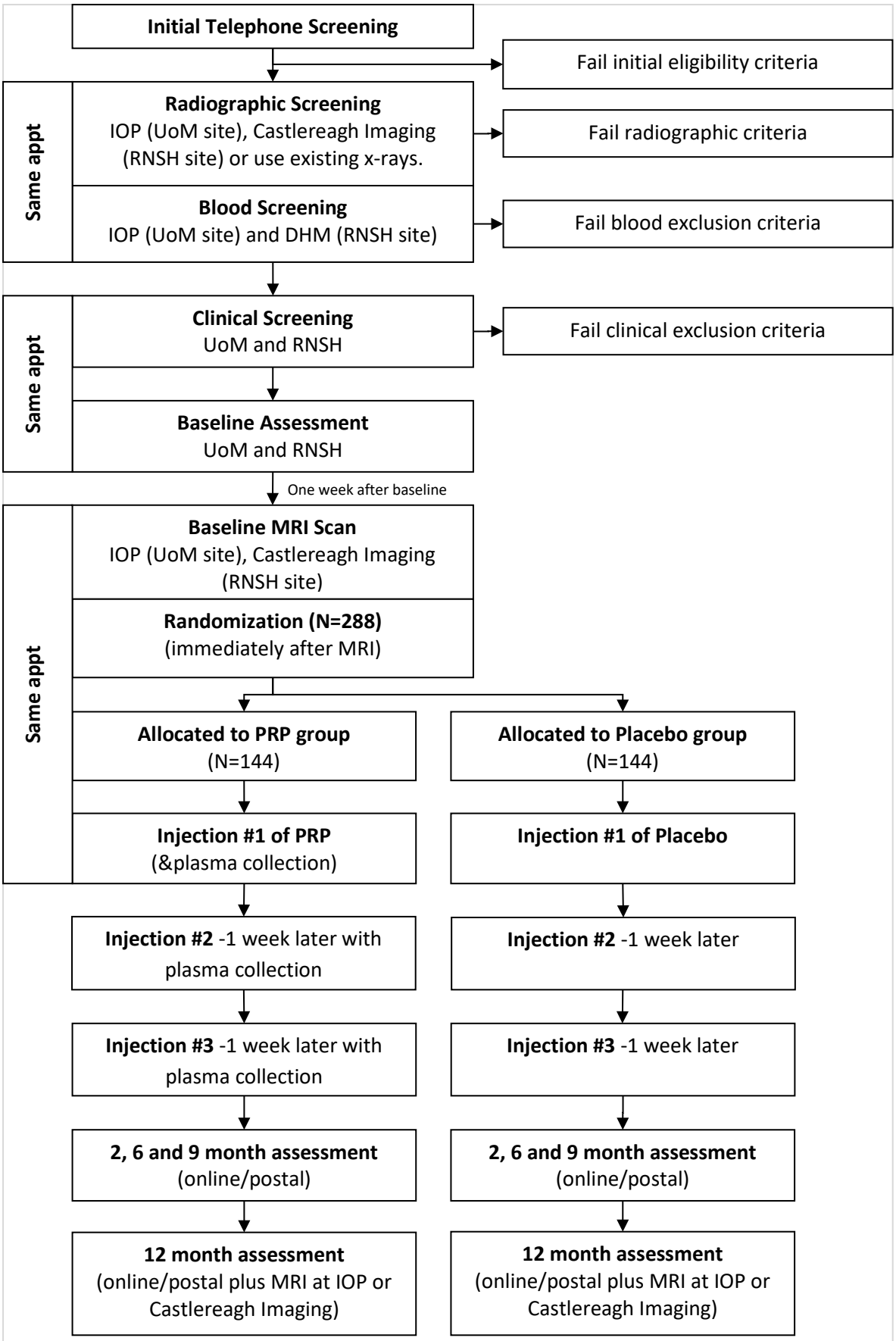
Initial Telephone Screening
Fail initial eligibility criteria
Same appt
Radiographic Screening
IOP (UoM site), Castlereagh Imaging (RNSH site) or use existing x-rays.
Fail radiographic criteria
Blood Screening
IOP (UoM site) and DHM (RNSH site)
Fail blood exclusion criteria
Same appt
Clinical Screening
UoM and RNSH
Fail clinical exclusion criteria
Baseline Assessment
UoM and RNSH
One week after baseline
Same appt
Baseline MRI Scan
IOP (UoM site), Castlereagh Imaging (RNSH site)
Randomization (N=288)
(immediately after MRI)
Allocated to PRP group
(N=144)
Allocated to Placebo group
(N=144)
Injection #1 of PRP
(&plasma collection)
Injection #1 of Placebo
Injection #2 -1 week later with plasma collection
Injection #2 -1 week later
Injection #3 -1 week later with plasma collection
Injection #3 -1 week later
2, 6 and 9 month assessment
(online/postal)
2, 6 and 9 month assessment
(online/postal)
12 month assessment
(online/postal plus MRI at IOP or Castlereagh Imaging)
12 month assessment
(online/postal plus MRI at IOP or Castlereagh Imaging)

Table 1. Outline of the assessment and procedures of the study.

	Prior to Baseline	Baseline	Injection Visits 1 / 2 / 3	2 Month Survey	6/9 Month Survey	12 Month Follow-up
Telephone Screening	X					
Informed Consent	X					
Screening Blood Test	X					
Physical Screening	X					
Height and Weight		X				X
About yourself		X				
painDETECT		X				
Treatment Expectation		X				
NRS pain measures		X		X	X	X
KOOS		X		X		X
Medications		X		X		X
PASE		X		X		X
ICOAP		X		X		X
AQol-8D		X		X	X	X
Health Costs Data & WHO HPQ		X		X	X	X
PRP or Saline Injection			X			
PRP Sample			PRP group only			
Adverse Events			X	X		X
Co-interventions				X		X
Success of Blinding (participant and doctor)			After Visit 3 Only			
Global Rating of Change				X		X
Radiology/Imaging						
Knee X-ray	X					
MRI		X				X
Ultrasound*		X				X

*RNSH site only

8. CLINICAL AND LABORATORY ASSESSMENTS

Procedures

X-ray screening

If a participant has not had suitable weight bearing x-rays of their knee in the past 12 months, they will firstly be asked to attend IOP (UoM site) or Castlereagh Imaging (RNSH site) to undergo a knee x-ray. If participants do have their own recent suitable knee x-ray, they will be posted a stamped return envelope to post the x-rays to their site coordinator. The Principal Investigator will assess their x-ray and if they are not suitable the reasons will be explained to the participant. If the Principal Investigator is unavailable, CI Bennell or AI Buchbinder or Cicuttini will assess x-rays. If both of their knees are deemed suitable for the study, the most painful knee will be the study knee, and if they pass the remainder of the screening they will undergo injections on that knee only.

Blood screening

Following the knee x-ray (at the same visit), participants will provide a blood sample (for the RNSH site participants this will be at Douglass Hanly Moir, within short walking distance from the x-ray at Castlereagh Imaging. For the UoM site, this will be at IOP). This test is to determine if the participant has a suitable platelet count, and that they are medically healthy. The Principal Investigator will assess the blood test results. If the Principal Investigator is unavailable, AI Buchbinder or Cicuttini will assess blood test results. If they are not suitable the reasons will be explained to the participant, and if they are not deemed to be medically healthy, the blood test results will be provided to the participant and they will be advised to visit their GP with this information.

Physical screening

After being deemed suitable based on the x-ray and blood screening, an appointment will be made for the participant to attend either the UoM or the RNSH to meet with the site coordinators for final stage of screening. The site coordinator will assess the participant's knee to ensure the absence of an effusion, and that skin integrity around the knee is not compromised. Baseline questionnaires will also be completed at this visit.

Baseline questionnaire

If the participant passes the physical screening, they will complete a detailed questionnaire on a computer or on paper with a pen at that visit. The questionnaire will include demographic information and questions about participant's knee pain and symptoms, the medications they take, their physical activity levels, quality of life and a detailed list of their use of health services over the previous 3 months. After completing the questionnaire, an appointment will be made for the participant to undergo an MRI scan (on a different day) at IOP or Castlereagh Imaging.

Baseline MRI scan

At the MRI appointment, the staff will again confirm that the participant is suitable to have an MRI scan (e.g. don't have a pacemaker, defibrillator or have any unsecured metal in their body). Scans will be performed at IOP (UoM site) or Castlereagh Imaging (RNSH site). All knee MRI scans taken as part of the study will be screened by a radiologist and participants will be notified about any non-osteoarthritis features that are deemed important to be brought to the attention of their GP (for example fractures or tumours). At this appointment, they will also have their height and weight measured.

Platelet rich plasma sample

For those in the platelet rich plasma group, the nurse who prepares the injections will aliquot a small portion of the PRP to analyse. The sample will be coded and sent to an established pathology practice (accredited with National Association of Testing Authorities (NATA) and the Royal College of Pathologists of Australasia (RCPA)) in Melbourne or Sydney to determine the platelet concentration and then the sample will be destroyed.

Participants in the PRP group at the Melbourne site will also have a further 3mL of PRP frozen and saved for further analysis. Frozen samples will firstly be stored in a freezer at IOP. Approximately every fortnight, samples stored at IOP will be transported to Monash University (in Melbourne) where they will be stored in a freezer. Upon completion of the study, these frozen samples will be tested in a batch. The samples will be assessed for the concentration of specific cytokines and growth factors. These may include (but not limited to) TGF-B1 and PDGF, IRAP and IL-1B, MMP-9 and IL-6.

Ultrasound evaluation

Participants from the RNSH site will also undergo an ultrasound evaluation of their knees at baseline and at 12 months. The ultrasound will be performed after the MRI scans, before randomization. A

doctor experienced in the assessment of osteoarthritic knees with ultrasound will perform the assessment at Castlereagh Imaging.

Follow-up questionnaires

At the end of months 2 and 12, participants will be sent by email or post (depending on choice) a follow-up survey similar to that they completed at baseline in order to assess pain, function, medication use, physical activity, quality of life, health service usage, adverse effects, co-intervention use and global rating of change. At the end of months 6 and 9, participants will be sent a shorter survey that will ask participants about pain, quality of life and health service usage. As part of the survey distribution to participants, the researchers will update participants on study recruitment and progress in order to keep participants engaged in the trial over the 12 months.

Follow-up MRI scan

At the same time as participants complete the 12 month follow-up questionnaire, the researchers will contact them to organise another MRI scan of their study knee at the same radiology clinic as baseline. Participants will also have their height and weight measured at this appointment.

9. ADVERSE EVENT REPORTING

The risks for participants involved in this study are minimal as there is no systemic treatment involved. However, due to theoretical concerns related to the use of PRP therapy, adverse events will be monitored. To ensure the safety of participants, symptoms suspected to be related to the proposed therapy will be assessed by the Principal Investigator (Prof David Hunter) and, if necessary, the treatment will be discontinued and further medical evaluation will be arranged. All adverse events will be reported.

Any risks to participant are likely to be minor and transient. We have a number of procedures in place to minimise these risks and effectively manage any adverse events should they occur. In contrast, knee OA is a chronic and painful condition for which there are few treatments that effectively reduce symptoms, and there is no treatment that slows disease progression. Expected benefits of platelet rich plasma of reduced pain and/or structural deterioration would make a significant and lasting impact upon participant's well-being and their quality of life. Furthermore, we also expect the placebo group to gain benefit from the treatment in terms of pain, as placebo effects are well documented in treatment of knee OA, particularly for knee injection therapies [12]. The risks of study participation are listed below:

Radiation

As part of everyday living, everyone is exposed to naturally occurring background radiation and receives a dose of about 2 millisievert (mSv) each year. The additional effective dose participants will receive from entering this trial is approximately 0.01 mSv. At this dose level, no harmful effects of radiation have been demonstrated as any effect is too small to measure. Studies suggest any risk is minimal.

Blood samples

Participants with a needle phobia will be excluded during screening. The sample will be taken by the nursing staff at IOP and Douglass Hanly Moir, who are experienced in taking blood samples. The nursing staff will also give advice in order to minimise bruising that may occur in some people.

Knee injections

Participants with a needle phobia will be excluded during screening. The injections in the study will be provided by doctors (radiologists) who are experienced in the administration of PRP injections into

the knee. Before each injection they will offer to inject a local anaesthetic superficially around the knee joint to minimize any pain from the treatment injection. The PRP or placebo injection will be guided by an ultrasound machine to ensure it is injected into the correct part of the joint. There is a risk of infection in the knee from the injections, but this is thought to be very low [8].

Participants will be advised that if they experience discomfort, stiffness or swelling it should slowly decrease over the first few days. The doctors will advise a cold compress or paracetamol (e.g. Panadol) for pain relief, or paracetamol combined with codeine (e.g. Panadeine) should participants require something stronger after the procedure. Anti-inflammatories can affect the function of the platelets thus participants will be advised to avoid these medications for the duration of the trial. Participants will be advised that if they experience persisting severe pain or an adverse reaction following the injection, they should contact their site coordinator. Finally, we will have a data and safety monitoring committee to monitor data and participant safety.

MRI evaluations

During the initial eligibility phone screen, participants will be questioned regarding contra-indications to MR imaging, including claustrophobia. By means of standard MRI compatibility checklist, screening of contra-indications to MRI will be repeated prior to the imaging procedures at IOP (UoM site) or Castlereagh Imaging (RNSH site). In case of the presence of absolute MRI contra-indications, participants will be excluded from the study and will not undergo the MR imaging procedures. Along with MRI appointment details, participants will receive an MRI information leaflet and a reminder of requirements of safe imaging procedures (ie removal of belts, jewellery, watches, coins, etc.). During the MR imaging procedures, participants will be given headphones to account for the noise that is inherently associated with MR imaging.

Confronting questionnaires

Some of the outcomes questions that people will be asked to answer involve items that some people might find uncomfortable. These questionnaires will cover topics about their quality of life, psychological well-being and their ability to cope with living with osteoarthritis. We will advise people in the PISCF regarding this issue so people are aware before study entry.

Use of placebo

Participants in the placebo group will be told that improvements from participation in a placebo treatment are common and can be due to the positive effects of seeing a health professional and being in the treatment environment, as well as improvements in thoughts and emotions. To minimise any upset, we will speak to participants on the phone about the effects of placebo treatment and also send some information in the mail.

9.1 Definitions

9.2 Assessment and Documentation of Adverse Events

Adverse events (AE) are defined as any untoward medical occurrence in a clinical trial participant which do not necessarily have a causal relationship with the treatment. AEs will be collected via self-report during the 2 and 12 month data collection. Adverse events that may be expected as part of the interventions or usual care that do not need to be reported to the HREC include: transient increase in pain, stiffness and swelling. Follow-up assessments at months 2 and 12 months will include questioning about any adverse events they believe may be related to the study invention, including their nature, how long they lasted for and what action if any they took (eg. taking medication or seeing a health professional).

9.3 Eliciting Adverse Event Information

Participants will be advised to report any serious adverse events to the site coordinators as soon as they can by telephone, which will be documented. Any adverse events reported by telephone or in questionnaires will be reported to the Chief and Principal Investigators who will be responsible for deciding what action if any is needed on a case by case basis. All reported adverse events will be reported as blinded data to the designated Data Safety and Monitoring Committee for this trial in six monthly study reports.

9.4 Serious Adverse Event Reporting

9.4.1 SAEs

Serious adverse events (SAE) are defined as any untoward and unexpected medical occurrence that:

1. Results in death,
2. Is life-threatening
3. Requires hospitalisation or prolongation of existing inpatientś hospitalisation,
4. Results in persistent or significant disability or incapacity,
5. Is a congenital anomaly or birth defect
6. Any other important medical condition which, although not included in the above, may require medical or surgical intervention to prevent one of the outcomes listed.

Any adverse events deemed to be serious by the Chief and Principal Investigators will be reported to the NSLHD HREC within 72 hours of becoming aware of them. They will also be reported as blinded data to the designated Data Safety and Monitoring Committee for this trial.

9.4.2 SUSARs

Any SUSAR events deemed to be serious by the Chief and Principal Investigators will be reported to the NSLHD HREC within 72 hours of becoming aware of them.
They will also be reported as blinded data to the designated Data Safety and Monitoring Committee for this trial.

9.5 Specific Safety Considerations (Eg. Radiation, Toxicity)

To minimize radiation exposure, we will not expose those who have had a recent knee x-ray of their own to a new x-ray.

As the x-ray in this study is part of the screening process we cannot definitely say how many people will need to be screened to achieve our goal of 288 participants. We also cannot predict definitely how many volunteers will have their own existing knee x-ray who will not need to have a knee x-ray for this study. An educated estimation would be that these unpredictable quantities may balance out to approximately 288 participants that will require a new knee x-ray for this study as part of the screening process.

10. STATISTICAL METHODS

10.1 Sample Size Estimation

Primary outcomes will be the 12-month change in (i) overall average knee pain over the last week measured using an 11-point numeric rating scale with terminal descriptors 'no pain' (score 0) and 'worst pain possible' (score 10); and (ii) medial tibial cartilage volume. The minimum clinically important difference to be detected in OA trials is a change in pain of 1.8 (out of 10 units) [13]. Using control group data from our 12-month NHMRC-funded RCT in mild to moderate knee OA, we assume a between-participant standard deviation (SD) of change in pain of 2.4 and baseline to 12-month correlation in scores of 0.29 [14]. We wish to find a 40% reduction in amount of cartilage loss with platelet rich plasma. Based on our data [15], slowing the rate of cartilage loss by this amount will delay need for knee replacement. Assuming that PRP reduces the rate of cartilage loss by 40%, this is likely to double the time to joint replacement. For example, if rates of cartilage loss are constant over time it can be estimated that patients treated with PRP will take approximately 30 years to progress from the earlier stages of knee OA (when 20% of cartilage is lost [16]) to end stage knee OA (when 60% of cartilage is lost [15]) compared to controls (15 years). For medial tibial cartilage volume, we anticipate the control group to lose 2.8% (SD 3.5%) of cartilage volume in 12-months [14], with a conservative baseline to 12-month correlation in scores of 0.50. These assumptions, together with an analysis of covariance adjusted for baseline scores, indicates that 115 participants per arm will have 80% power to detect a 40% reduction in loss of cartilage volume with a two-sided significance level of 0.05. With this number of participants, we have >99% power to detect the minimum clinically important difference in pain. Allowing for 20% loss to follow-up, we will recruit 144 participants per arm - total 288 participants.

10.2 Population to be analysed

See section 6.2: Eligibility criteria.

10.3 Statistical Analysis Plan

Detailed description of the statistical methods is listed in the final Statistical Analysis Plan. Please refer to:

https://healthsciences.unimelb.edu.au/__data/assets/pdf_file/0009/3439314/RESTORE_Statistical_Analysis_Plan.pdf

10.4 Interim Analyses

It is not anticipated that any interim analyses will be performed in this study.

11. DATA MANAGEMENT

11.1 Data Collection & Storage

Identifiable data:

- Initial online screening information will be stored within a website (surveygizmo and REDCap) and accessible only by password to the site coordinators.
- Phone and clinical screening forms and study consent forms: will be completed on paper by the site coordinators and stored in a locked filing cabinet accessible only to the site coordinators.
- Details of people screened will be stored electronically in a Microsoft Access database, accessible only to the site coordinators stored securely on password-protected servers.
- X-ray, MRI and ultrasound images will be stored electronically at the radiology clinics involved in the study and securely stored and subject to the normal confidentiality guidelines adhered to at

the clinics. Once electronically transferred to the researchers, the site coordinators will export de-identified images, label with appropriate subject codes and store securely on password-protected servers.

- For the x-ray screening assessments, the Principal Investigator will complete a screening form in Google Forms accessible only to him and the site coordinators by password. Data from eligible participants will be exported to Microsoft Excel and stored securely on password-protected servers.
- Blood and platelet-rich plasma test results will be stored electronically at the radiology clinics involved in the study and securely stored and subject to the normal confidentiality guidelines adhered to at the clinics. Once electronically transferred to the researchers, data from eligible participants will be exported to Microsoft Excel and stored securely on password-protected servers.

Re-identifiable/coded data:

- Questionnaires: may be completed on paper or electronically, and will contain only participant codes, and no identifying information. Paper copies will be stored in locked filing cabinets, separate from a cabinet containing any identifiable data and only accessible to the researchers. Electronic copies will be stored in the surveygizmo and REDCap website, accessible only to the site coordinators by password protection. Data from within surveygizmo and REDCap will eventually be exported to Microsoft Excel and other statistical packages used by the researchers for analyses and stored securely on password-protected servers.
- Data from MRI assessments: researchers responsible for the assessment of MRIs will use a Matlab and/or OsiriX software package for image assessment which exports quantitative outputs to a secure Microsoft Excel file which is stored securely on password-protected servers.
- At the UoM and RNSH, all computer based files will be stored on secure and backed-up servers, accessibly only to the researchers using a password. All radiology images (x-ray, MRI and ultrasound) and blood test results will be transferred electronically from the radiology or pathology clinics to the Chief and Principal Researchers using existing systems at the clinics as they will be listed as the referring doctors on the referral forms for these assessments. These systems require the Chief and Principal Researchers to log in to secure websites for access. All identifiable paperwork (screening forms and consent forms) will be stored in a locked filing cabinet accessible only to the site coordinators.

Tissue samples:

Frozen platelet-rich plasma samples (Melbourne site only) will be stored at IOP and Monash University in a lockable deep freezer using non-identifying subject codes. Only staff at these centres will have access to these samples. The Chief Investigators will be responsible for informing staff at these sites about the management and control of tissue sample access.

11.2 Data Confidentiality

No information which could lead to the identification of a participant will be included in the dissemination of results. Only fully non-identifiable data will be presented when disseminating results. All collected data will also be stored in a locked cabinet throughout the course of the study.

11.3 Study Record Retention

All samples will be destroyed after analysis, or 15 years after the completion of the study, whichever comes first. Data will be retained for 15 years consistent with clinical trial recommendations outlined in section 2.1.1 of the National Health and Medical Research Council's "Australian Code for the Responsible Conduct of Research".

12. ADMINISTRATIVE ASPECTS

The trial will be registered with the ISRCTN registry following ethical review and before trial commencement, under the name "platelet-Rich plasma as a symptom- and disEaSe-modifying Treatment fOR knee ostEoarthritis - the RESTORE trial"

12.1 Independent HREC approval

University of Melbourne HREC project #1647671.1

12.2 Amendments to the protocol

12.3 Protocol deviations

12.4 Participant reimbursement

Participants will receive all imaging procedures (x-ray, MRI and ultrasound), blood testing procedures, treatments and any postage costs for x-rays or surveys as part of the study at no cost to themselves. All travel expenses to attend imaging, treatment or assessment appointments are at the cost of the participants. Car parking will be provided to participants at no cost for visits to IOP, Castlereagh Imaging and the UoM. Participants will have to cover their own parking expenses if they require parking for visits to the RNSH.

Participants who complete the 12-month follow-up assessments (questionnaires and MRI scan) will go into a draw to win one of two iPads (one per recruitment site). Participants will only be informed of this when invited to undergo the follow-up assessments, and not prior to undergoing treatment.

12.5 Financial disclosure and conflicts of interest

RegenLab will be providing the equipment for the injection procedure, including needles, syringes and tubes. RegenLab will have no involvement in the design of the study, the analysis of the data, its interpretation or the preparation and submission of the manuscript as the researchers are committed to an unbiased examination of the effect of PRP injections. This has been explained in the Participant Information Statement provided to participants.

13. USE OF DATA AND PUBLICATIONS POLICY

We will publish a protocol paper prior to completion of the trial. The main trial will be published in a general medical journal or rheumatology/osteoarthritis journal. It is anticipated that a health economic evaluation would be published as a separate paper to that of the main trial findings. Another paper(s) will outline the moderator analyses. Authorship will align with ICMJE authorship recommendations.
Statistical code will be made available from Professor Forbes and Dr Kasza.
Data will be made available from Professor Bennell.

The results will also be disseminated through avenues such as professional organisations, media, and consumer organisations.

14. REFERENCES

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15. APPENDICES

List of Attachments included:

Title of Form (file name)	Description	Date	Version Number
RESTORE NSLHD HREC cover letter.docx	Cover letter to NSLHD for this application	27/10/2016	1
RESTORE_NEAF.pdf	Study NEAF application form	26/10/2016	1
RESTORE Two Page CVs.docx	Two page CVs for each listed researcher	30/08/2017	1.3
RESTORE - NSLHD Template - Methods of Payment - New submissions.pdf	Method of payment form.	24/10/2016	1
1106274 Bennell - final result.pdf	NHMRC grant result	Nov 2016	1
UoM HREC approval letter.pdf	Approval letter for the RESTORE study from the University of Melbourne HREC.	18/10/2016	1
RESTORE_NSLHD_PISCF.doc	Study PISCF – master copy without site specific information	30/08/2017	1.5
RESTORE advertisements.docx	Study advertisement material – master copy without site specific information	19/12/2016	1
RESTORE placebo debriefing letter.doc	The letter we will send to those people in the Placebo group after participation - master copy without site specific information.	19/12/2016	1
RESTORE questionnaire booklet.docx	Study questionnaire – master copy without site specific information. Document includes sections relevant to only some data collection time-points.	19/12/2016	1
RESTORE recruitment website plan.docx	Planned content for the recruitment website – master copy without site specific information	30/08/2017	1.3
RESTORE screening forms.doc	Phone and physical screening form – master copy without site specific information	19/12/2016	1
RESTORE Ionizing Radiation Module_Melbourne.pdf	Ionizing Radiation Module	11/11/2016	1
RESTORE Medical Physicist Report_Melbourne.pdf	Medical Physicist Report for the use of Radiation	8/11/2016	1
RESTORE Ionizing Radiation Module_Sydney.pdf	Ionizing Radiation Module	14/11/2016	1
RESTORE Medical Physicist Report_Sydney.pdf	Medical Physicist Report for the use of Radiation	9/11/2016	1
RESTORE Victorian Specific Module.pdf	The Victorian Specific Module	12/12/2016	1
NSLHD HREC response - RESP16286.docx	Response letter to HREC review	19/12/2016	1

RESTORE - NSLHD Template - Methods of Payment - Amendments.docx	Method of payment form.	30/08/2017	1
RESTORE Body Tissue and Genetic Research Module2.docx	The Victorian Specific Body Tissue and Genetic Research Module	30/08/2017	1.2

RESTORE PROTOCOL AMENDMENTS

DATE	NATURE OF AMENDMENT	REASON FOR AMENDMENT
07/08/2017	Removal of the sentence stating that an ultrasound scan will be performed prior to blood samples being taken before treatment. [pages 21 and 22]	The sentence was erroneous as an ultrasound scan is only performed once at each visit (immediately prior to and during the injections).
07/08/2017	Clarity around the gauge (22-gauge) of the needle used for the knee injections was added to the methods. [pages 21 and 22]	This was previously not stated.
07/08/2017	The use of a local anaesthetic injection immediately prior to the knee injection was made optional for patients, instead of required. [pages 21 and 22]	This change was made to reflect the normal practice of the Radiologists performing the injections for the study.
7/08/2017	Further information added to the description of the placebo injection preparation, clarifying that the blood samples are discarded in a separate room to ensure blinding. [page 22]	This was previously not stated.
07/12/2017	Addition of Dr Shirley Yu, to the list of Associate Investigators. [page 10]	Dr Yu, a Rheumatologist, was brought on the team to work under the guidance of the principal investigator Prof David Hunter and will be responsible for the screening and rating of participant x-rays and blood samples.
07/12/2018	Change in the description of the centrifuge setting for PRP preparation from 3500rpm to 1500g. [page 21]	Due to their being different models of centrifuges at the Melbourne and Sydney sites, having a consistent revolutions-per-minute (rpm) between sites, results in a different level of force applied to the blood tubes during spinning. Stating the centrifuge settings in 1500g is a way to make the forces between sites consistent. 1500g equates to 3500rpm at the Sydney site and 3300rpm at the Melbourne site.
18/01/2018	Addition of a Sensitivity Analysis in the Statistical Analysis Plan, that will exclude patients randomized prior to the 7th of December 2017. [now located on page 11 of the Statistical Analysis Plan document]	As prior to the 7th of December 2017, blood samples were spun with the centrifuge at a speed of 3500rpm at the Melbourne site, which equates to approximately 1680g (higher than the intended centrifugal force of 1500g). The

		effect of the higher centrifuge speed is unknown.
01/03/2018	Specific examples of cytokines and growth factors that will be assessed from PRP samples was added to the list of assessments. [page 28]	Specific cytokines and growth factors had previously not been named.
24/07/2020	Removal of platelet count in 'Other Measures'. [page 18]	Due to the nature of the commercial product chosen, including the characteristics of the blood collection tubes required for the commercial kits, it is not possible to obtain accurate measures of platelet characteristics for each sample. As such, we will no longer report platelet counts as was included in the 'Other Measures' section of the protocol.
03/08/2020	Section 10.3 'Statistical Analysis Plan' section has been revised to now refer to the updated Statistical Analysis Plan document. [page 32]	This change was made as the final detailed statistical methods are outlined in the separately published Statistical Analysis Plan document.