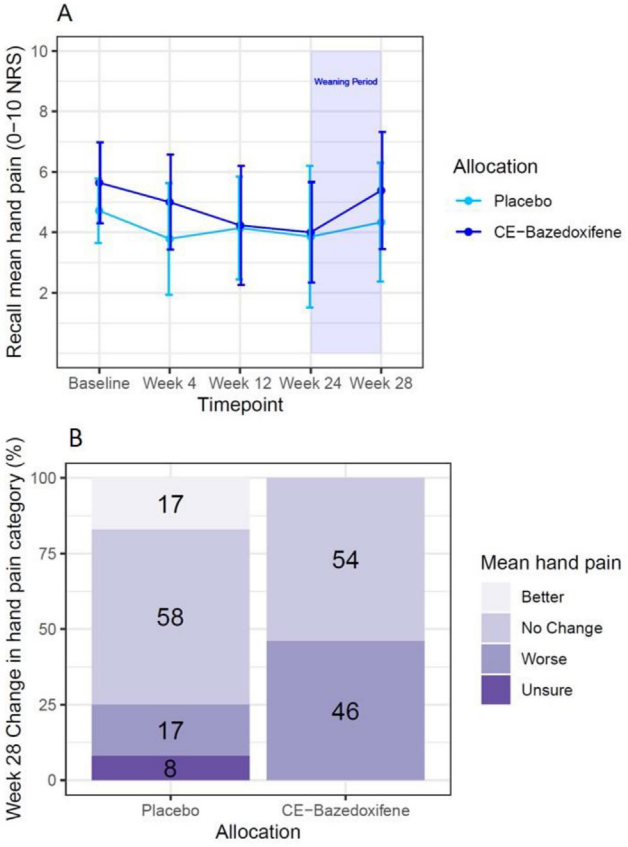
Methods: We recruited females aged 40-65 years and 1-10 years after final menstrual period with definite hand OA and ≥2 painful hand joints across three primary/secondary care sites and from the community. Medical exclusions included those typical for clinical HRT use. Design was parallel group, double-blind 1:1 randomisation of CE-bazedoxifene or placebo, taken orally once daily for 24 weeks, then tapering for 4 weeks before study end at Week 28. Primary feasibility outcomes were rates of eligible participant identification, recruitment, randomisation, retention, compliance, and likelihood of unblinding. Adverse events (AEs) were collected. Secondary clinical outcomes included the anticipated primary outcome in a full trial of mean hand pain over 14 days prior to each visit, scored on a 0-10 numerical rating scale (NRS) where 10 is worst pain possible, as well as hand function, appearance and menopause symptoms. Progression criteria to a full RCT were: (i) recruitment ≥30 participants across all sites in 18 months (or proportionate to time open); (ii) a drop-out rate of ≤30% of randomised individuals; and (iii) acceptability to the majority of participants, including acceptable AE rates. All clinical outcomes were analysed on an intention-to-treat basis. Though not powered to detect a treatment difference, change and treatment effects (the difference in the outcome between the two groups) were indicated with 95% CIs, with all models adjusted for clinical subtype of painful hand joint, study site, and baseline values. The sample size for a full trial was estimated using the standard deviation (SD) of week 24 mean hand pain.

Results: Due to the COVID-19 pandemic, the recruitment window was reduced to 12-15 months. From May 2019 to December 2020, 434 enquiries/referrals were received. Of 96 telephone pre-screens, 35 individuals were potentially eligible and of these, 33 gave consent to participate. Of the remaining, 250/401 (62%) were ineligible, whilst 55/401 (14%) chose not to proceed, with the most common reason being not wanting to take HRT. 28/35 (80% (95%CI 63%,92%)) eligible participants were randomised to study medication. All 28 participants completed all follow-ups with high compliance (100% active, 13/14[93%] placebo) and outcome measure completeness (100%, mean hand pain). All three AE-related treatment withdrawals were on placebo when unblinded. No serious AEs occurred. Participants/investigators were well blinded (participant blinding index 0·50[95%CI 0·25 to 0·75]). All three prespecified progression criteria were therefore met for a full trial. The treatment effect difference over 24 weeks in mean hand pain between active and placebo was -0·71 (95% CI -2·20 to 0·78) (Fig 1A). During tapering/stopping medication, mean hand pain increased by 1·31 points in the active arm compared with 0·17 in the placebo arm, indicating a possible effect of cessation of medication (Fig 1A). Furthermore, 6/13 (46%) participants in the active group reported worsening pain at week 28 compared with week 24, but only 2/12 (17%) were worse on withdrawing placebo (Fig 1B). The sample size for a full trial was estimated as 296 (based on MCID 0·8 on NRS, SD 2.0, 90% power, 10% drop-out, alpha 5%).

Conclusions: This first study of a RCT of HRT for painful hand OA met its progression criteria, indicating that a full trial of an HRT in this population is feasible and acceptable. Although not powered to detect an effect, there was a trend towards improvement in hand pain on treatment and worsening of hand pain on tapering in the active arm only. This adds to proof-of-concept data in this area, justifying more work.ISRCTN12196200. Funded by Research for Patient Benefit programme, National Institute for Health Research (UK) PB-PG-0416-20023



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ASSESSING ALLOGENEIC CHONDROPROGENITOR MANUFACTURE UNDER GOOD MANUFACTURING PRACTICE (GMP) SERUM FREE AND XENO-FREE CONDITIONS.

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Purpose: Based on the current available literature, Autologous Chondrocyte Implantation (ACI) is considered the gold standard treatment for medium to large chondral lesions, which if left untreated may progress to end-stage osteoarthritis. An allogeneic source of cells could significantly reduce production costs, streamline adoption and transform the current 2-stage procedure to a single step intervention, improving patient access and reducing patient burden. In order to effectively manufacture allogeneic cells under 'Good Manufacturing Practice' (GMP) conditions, alternatives for autologous serum, as currently used in most ACI protocols, must be sought. Hence, this study assessed the manufacture of chondroprogenitors from healthy adult knee articular cartilage, using serum cf. xeno-GMP free reagents.

Methods: Adult chondroprogenitors were isolated from full depth human articular cartilage obtained from a total knee replacement (n=1, aged 78 yrs), healthy cadaveric osteochondral allografts from the knee (n=2, aged 30 and 42 yrs) and talus (n=2; aged 22 and 25 yrs) using selective adhesion to vitronectin. A GMP compliant serum- and xeno-free medium (StemMacs MSC Expansion (Miltenyi Biotech) was compared with standard culture conditions using DMEM/F12 (Gibco) with foetal bovine serum (FBS; Gibco) for the expansion of chondroprogenitors. The chondroprogenitors were characterised in terms of growth kinetics, immunoprofiles (by flow cytometry), chondrogenic pellet forming capacity (as glycosaminoglycan (GAG)/DNA) and histological analysis using the Bern score (0-9; by two independent assessors).

Results: Chondroprogenitors isolated from all donors were successfully expanded in both standard culture media and serum- and xeno-free media up to passage 2. At passage 0-1 there was no significant difference between the doubling time of the chondroprogenitors expanded in either media; however at passage 1-2, chondroprogenitors grown in serum- and xeno-free media had a significantly lower doubling time compared to those grown in standard media (p= 0.048, paired t-test). Growing the chondroprogenitors in serum- and xeno-free media did

not appear to alter the immunoprofiles and, for both media types, all chondroprogenitors were immunopositive (>95%) for chondropotency markers (CD166, CD44, CD151), MSC markers (CD90, CD73 and CD105), integrin markers (CD29 and CD49a) and immunonegative (<3%) for CD19, CD34, and CD45. Furthermore, there were no significant differences between the chondrogenic pellets when assessed biochemically (GAG/DNA content) and histologically.

Conclusions: Our preliminary findings suggest that serum- and xeno-free media can be used to manufacture allogeneic chondroprogenitors in a GMP compliant manner, without negatively impacting on their growth or phenotype.

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SENOPROTECTIVE EFFECTS OF MESENCHYMAL STROMAL CELLS-DERIVED EXTRACELLULAR VESICLES IN OSTEOARTHRITIS

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Purpose: Age is the most important risk factor in osteoarthritis (OA) and is associated with the accumulation of senescent cells that contribute to the functional decline of the joint. Currently, no curative therapeutic options can be provided to OA patients. In this context, the therapeutic effect of mesenchymal stromal cells and their extracellular vesicles (MSC-EVs) has been demonstrated in preclinical models. Here, we aimed to investigate the impact of adipose tissue MSC-EVs (ASC-EVs) on senescence using both *in vitro* and *in vivo* models of OA.

Methods: ASC-EVs were isolated by differential ultracentrifugation of culture supernatants and characterized by size, concentration, morphology and phenotype. Chondrocytes isolated from OA patients were stimulated with IL-1β for 48h and then incubated with ASC-EVs for seven days. Senescence was assessed by SA-βGal activity, phalloidin staining, quantification of gene expression and senescence-associated secreted factors. Synoviocytes isolated from OA patients were treated with ASC-EVs for two days and analyzed by the expression of inflammatory and oxidative stress markers. For *in vivo* analysis, intra-articular injections of ASC-EVs were administrated in a mouse model of collagenase-induced OA at day seven after disease induction. Analysis of gene expression of the total knee joint was performed on days 9 and 14, and histological and confocal analyses of mouse tibias were performed on days 24 and 42.

Results: *In vitro*, ASC-EVs decreased the number of SA-βGal+ chondrocytes, as well as the release of SASP factors such as MMP3, IL-8, and VEGF. Similar findings were observed with synoviocytes. After *in vivo* injections of ASC-EVs in OA mice, the analysis of the total knee joints revealed decreased expression of genes related to senescence, inflammatory and oxidative pathways. We observed improvement in OA score and diminished cartilage degradation in the group of mice treated with ASC-derived EV.

Conclusions: Our *in vitro* and *in vivo* results indicate that ASC-EVs are effective in decreasing senescence likely contributing to improved OA phenotype. Further studies are warranted to characterize the composition of EVs that could be correlated to these observations.

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TRANSCUTANEOUS VAGAL NERVE STIMULATION REDUCES PAIN AND HISTOLOGICAL OSTEOARTHRITIS IN DESTABILIZATION OF THE MEDIAL MENISCAL MOUSE MODEL

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Purpose: Osteoarthritis (OA) is a cartilage degenerative joint disease, characterized by pain, stiffness, swelling, and functional disability affecting millions of people worldwide. Limited treatment options are available. Invasive vagal never stimulation (VNS) is FDA-approved for epilepsy and depression treatment and non-invasive VNS could be an attractive treatment for OA. The vagal nerve activates the cholinergic anti-inflammatory pathway by producing acetylcholine in nerves and non-neuronal cells leading to reduced synovial inflammation and joint pain in mouse models of rheumatoid arthritis. We hypothesized that transcutaneous-VNS will reduce OA pain induced by destabilization of the medial meniscus (DMM) in mice.

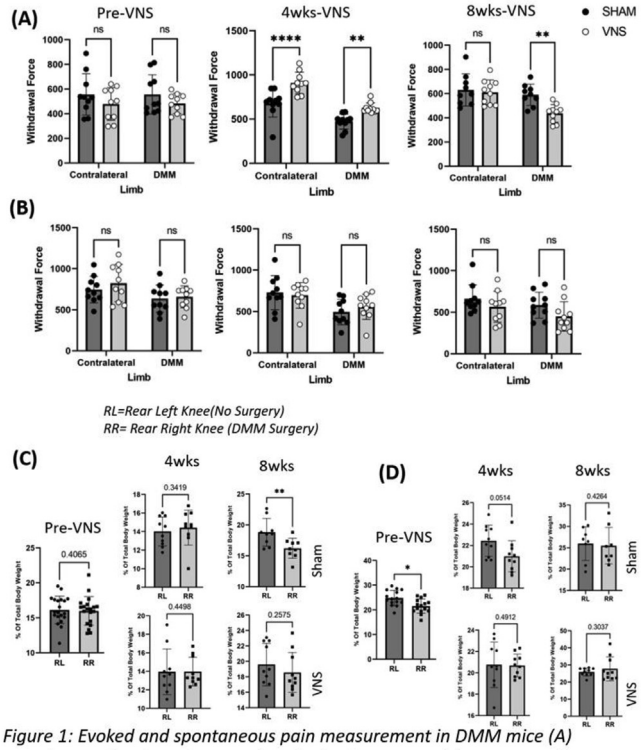

Figure 1: Evoked and spontaneous pain measurement in DMM mice (A) Females: 4 wks of VNS improved evoked pain measured by pressure algometer. (B) Males: VNS did not reduce evoked pain. (C) Females: dynamic weight bearing was improved by 8 wks VNS treatment. (D) Males: dynamic weight bearing trended towards improvement after 4 wks VNS treatment. $*p < 0.05$, $**p < 0.01$ and $****p < 0.0001$

Methods: At 16 weeks of age, male (n=20) and female (n=19) C57BL/6 mice underwent DMM surgery. At 4 weeks post-op, mice were randomly assigned to 10 minutes of VNS or SHAM stimulation for 8 weeks (5 days/week). In a blinded fashion, evoked pain using pressure algometry and spontaneous dynamic weight-bearing pain behavior was assessed post-op at 4, 8 and 12 weeks, corresponding to 0 (pre-VNS), 4 and 8 weeks of VNS or SHAM stimulation. At 12 weeks post-op, joints were sectioned and stained with safranin-O/ fast green for blinded OARSI grading and serum was collected for multiplex cytokine analysis.

Results: In female mice, VNS significantly improved algometer pain at 4 weeks and weight bearing at 8 weeks (Fig.1A&C). These mice had significantly decreased IFN-γ, IL-5, and IL-9 levels at 12 weeks post-op. In male mice, VNS improved weight bearing at 4 weeks (Fig.1D) and decreased GM-CSF and IL-2 levels with increased IL-4 and macrophage inflammatory protein -1β (MIP-1β) levels at 12 weeks post-op. After 8 wks of VNS treatment, male VNS treated mice had significantly lower OARSI scores in the lateral compartment of both femur and tibia compared to SHAM-stimulated mice. No differences were seen in OARSI scores in female mice VNS compared to SHAM-treated mice.

Conclusions: Our study suggest that tVNS improves pain and joint histopathology, and reduces inflammation in DMM mice through the mediation of IFN-γ, IL-5, IL-9, GM-CSF, IL-2, IL-4, and MIP-1β. Important roles of GM-CSF, IFN- γ, IL-2, IL-4 and IL-5 have been seen in another chronic pain condition, fibromyalgia (FM). In animal models of neuropathic pain, IL-4 promotes prolonged analgesia