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# Use of Adult Stem Cells for Orthopedic Regenerative Medicine Applications

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**ABSTRACT:** Musculoskeletal disease is a leading cause of morbidity across the world, associated with pain, immobility, deformities, and in some cases, death. Other factors affecting quality of life include lack of independence, an inability to perform routine tasks, and reduced social interaction. Disease prevalence increases with age and we are living longer. In the coming years, the incidence of disorders affecting the skeleton will rise, causing huge healthcare and socioeconomic burden. Current treatments are typically restricted to pain management followed by end-stage total joint replacement. Cell-based therapies are an appealing biological option in orthopedics, as they may provide long-lasting restoration of skeletal tissue function by exploiting the intrinsic stem cell-like capacity of mesenchymal stromal cells (MSCs) to differentiate into bone and cartilage. Here, we review recent data on the use of MSCs in orthopedics, focusing on clinical trials and discussing the advances made as well as the hurdles to overcome.

**KEYWORDS:** adult stem cell, mesenchymal stromal cell, MSC, osteoprogenitors, orthopedic regeneration

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## 1. Introduction

Interest in the use of mesenchymal stromal cells (MSCs) as cell or tissue transplantations or as therapeutic agents has been fuelled by reports of their seemingly all-encompassing potential for clinical benefit in a wide range of pathologies. Although their use is often limited to case studies or small single center trials and outcomes are rarely reported, MSCs are now being used in a number of diverse clinical trials (www.clinicaltrials.gov). It remains a major challenge for scientists, clinicians, and regulatory bodies to provide sufficient scientific data and understanding to design relevant trials that can be ethically authorized. This concise review will explore some of the most recent uses of MSCs as therapeutics in orthopedic conditions, defining the parameters by which scientific understanding supports their use while discussing their controversial use in non-orthopedic medicine and highlighting the social and ethical issues that have subsequently arisen. We will also define the cells we believe to be the most suitable for orthopedic regeneration and discuss pitfalls in their use through inefficient homing and engraftment.

## 2. Orthopedic Regenerative Medicine

Bone has the intrinsic ability to self-regenerate and heal without scarring.[1] Nevertheless, this biological process can proceed imperfectly or fail altogether resulting in a range of pathological conditions. The ability of MSCs to differentiate into osteoblasts and chondrocytes is the main reason for the optimism emanating from the scientific and clinical communities that MSCs could be used to treat patients in whom bone regeneration is unsuccessful. Several recent reviews have discussed the use of MSCs as therapeutics in orthopedics,[2–5] so in this first section we will concentrate mainly on the latest publications, summarizing the previous work in different clinical conditions and examining the number and phenotype of MSCs used,

where possible, as this is a particularly relevant theme. It is timely to highlight that some in the scientific community have proposed that MSCs should be defined as being plastic adherent cells, have tri-lineage differentiation capacity in vitro, and phenotypically express CD105, CD73, and CD90 in the absence of CD45, CD34, CD14 or CD11b, CD79α or CD19, and HLA-DR.[6] These criteria are likely to be updated in the future as additional and, importantly, more specific markers or methodologies are identified.

**2.1. Non/delayed unions.** Non/delayed unions occur in approximately 5–10% of fractures[7] and despite their regular occurrence, management remains challenging. They are defined as non-bridged areas after 6 months of periosteal and endosteal healing and result in severe functional impairment for patients. The first attempt to treat non/delayed unions with bone marrow-derived cells was made in 1978[8] with Connolly et al subsequently reporting high levels of successful union of tibial fracture in multiple patients following the injection of autologous bone marrow cells.[9,10] Hernigou et al established that a minimum number of bone marrow progenitor cells were likely to be required for a successful union relating therapeutic efficacy to the amount of progenitor cells injected and the number of cells that grafted,[11,12] which has influenced future clinical trials and two recent studies have specifically used MSCs and observed therapeutic benefit (Table 1). Liebergall et al conducted a randomized and prospective trial in 24 patients with distal tibial fractures in which they aspirated iliac crest bone marrow to yield MSCs that they combined with platelet-rich plasma and demineralized bone matrix and injected into fracture sites.[13] The MSC-treated group had no adverse effects and had a faster median union time of 1.5 months compared with 3 months in the control group with magnetic resonance imaging (MRI) evidence of early calcifying callus formation at the site of graft placement. Giannotti et al selected eight patients with non-union limb fractures and isolated MSCs from iliac crest isolates that they then expanded for 10–18 days and induced osteogenic differentiation by 4 days of culture with 50 μg/mL of ascorbic acid and 10 μM hydrocortisone.[14,15] The MSCs were combined with fibrin and implanted into the fracture; all patients recovered their limb function and radiographic healing was observed in all patients. Importantly, this study attempted to characterize their MSCs showing their CD105 and CD90 surface expression with the absence of CD34 and CD45 in addition to the cells' ability to calcify in vitro through von Kossa staining and initiate alkaline phosphatase activity after incubation with osteogenic induction.[14,15] During the study by Liebergall et al, a small amount of the MSC graft was transplanted into immune-deficient mice, but technical difficulties meant that the cellular source of the resulting bone formation could not be determined.[13] Technical advances may still need to be made to show with confidence that fracture union and new bone arose from the transplanted cells; this is a major hurdle to overcome in this and many other clinical trials using MSCs.

**2.2. Cartilage regeneration.** Cartilage is considered to have minimal capacity to repair and approximately 15% of the world's population suffers from joint diseases.[2] Current treatments include drug therapy, arthroscopy, and joint replacement[16] as well as autologous chondrocyte implantation (ACI).[17] However, ACI requires the extraction of chondrocytes from the patient which causes trauma to healthy articular cartilage. Wakitani et al have published the results of several case studies or clinical trials in which allogeneic MSCs have been delivered into the intra-articular cartilage observing an improved outcome[18–20] (Table 1). Most recently, Vangsness et al conducted a randomized, double-blind, controlled study in 55 patients with partial meniscectomy; allogeneic MSCs were injected superolaterally into the knee 10 days after surgery.[21] There was an evidence of meniscus regeneration and improvement in knee pain in both the MSC-treated groups, where $5 \times 10^7$ or $1.5 \times 10^8$ MSCs were injected, 12 months after administration; with 24% and 6%, respectively, of MSC-treated patients reaching a clinically defined improvement by MRI, compared to none of the vehicle control group. The allogeneic MSCs used in this study were from a commercial source and were reported to have been manufactured under good manufacturing practice (GMP) by scaled adaptation of techniques reported by Pittenger et al[22] and Kebriaei et al.[23] Cell identity and purity, although not specifically defined, "passed established quality-release criteria";[21] strict definition of cells used in clinical trials would be useful for outcome comparisons and strengthen their potential for more wide spread usage.

**2.3. Osteogenesis imperfecta.** Osteogenesis imperfecta (OI) is a congenital skeletal disease characterized by low bone mass and bones are prone to fracture. OI is clinically classified into four types based on disease severity, but additional types of disease caused by recently identified genetic mutations have been described. Horwitz et al were the first to demonstrate the potential of MSCs as therapeutic agents in OI by treating three children with allogeneic bone marrow transplants resulting in increased bone mass and decreased bone fracture.[24] Given the incidence of OI, the number of trials, and the participants within each trial, has always been small. Nevertheless, consistent, if only transient, improvements in bone mass and reduced bone fracture have been observed following MSC transplantation in OI sufferers and Horwitz et al have published further investigations since their initial result in 1999[25–27] (Table 1). Most recently, Götherström et al isolated and expanded MSCs from fetal livers; they were positive for CD29 CD44, CD73, CD105, CD166, and HLA class I, but negative for HLA class II antigens, CD14, CD31, CD34, and CD45 and demonstrated in vitro tri-lineage differentiation capability.[28] Two children with OI were transplanted with MSCs, $5 \times 10^6$ or $3 \times 10^7$ cells/kg, pre-natally at 31-week gestation and again at age 8 or 19 months with $3 \times 10^6$ or $1 \times 10^7$ cells/kg, respectively. MSC transplantations resulted in improved growth velocities and no new fractures. Donor cell engraftment

**Table 1.** Recent orthopedic clinical trials using MSCs as therapeutics.

| AUTHOR (YEAR) | DISEASE | STUDY TYPE | PATIENT NUMBER | MSC SOURCE | ROUTE | MSC NUMBER | OUTCOME |
|---|---|---|---|---|---|---|---|
| Quarto et al 2001[70] | Non-union | Case series | 3 | Autologous bone marrow | Scaffold loaded | na | Full recovery of limb function |
| Liebergall et al 2013[13] | Non-union | RCT | 12 (+12 controls) | Autologous MSC [+PRP and DBM] | Injected into fracture site | $1 \times 10^8$ | Reduced union time (3 to 1.5 months) in MSC-treated group compared to control |
| Giannotti et al 2013[14,15] | Non-union | Case series | 8 | Autologous MSC | Fibrin clot embedded | na | Successful bone union |
| Wakitani et al 2002[18] | Cartilage repair | Case series | 12 (+12 controls) | Autologous MSC | Collagen gelated | $1.3 \times 10^7$ | Clinical improvement not significantly different but arthroscopic and histological grading scores higher in MSC-treated group than controls |
| Wakitani et al 2004[19] | Cartilage repair | Case series | 2 | Autologous MSC | Collagen gelated | $5 \times 10^6$ | Improved clinical symptoms; cartilage repair |
| Wakitani et al 2007[20] | Cartilage repair | Case series | 3 | Autologous MSC | Collagen gelated | $5 \times 10^6$ | Improved clinical symptoms; cartilage repair |
| Vangsness et al 2014[21] | Cartilage repair | RCT | 18 + 18 (low or high MSC group + 19 controls) | Allogeneic MSC | Injected into knee | $5 \times 10^7$ or $1.5 \times 10^8$ | Increased meniscal volume and decreased pain in both cell-treated groups compared to control. Lower MSC number was more effective |
| Horwitz et al 1999[24] | OI | Case series | 3 | Sibling bone marrow | IV | $6 \times 10^8$ NC | Increased bone mineral content, growth acceleration and reduced fracture |
| Horwitz et al 2001[25] | OI | Case series | 3 (+2 controls) | Sibling bone marrow | IV | $6 \times 10^8$ NC | Growth acceleration in cell-treated group |
| Horwitz et al 2002[26] | OI | Case series | 6 | Allogeneic bone marrow MSCs | IV | $1 \times 10^6$ then $5 \times 10^6$ | Growth acceleration |
| Le Blanc et al 2005[27] | OI | Case report | 1 | Fetal liver-derived MSC | IU | $6.5 \times 10^6$ | MSC engraftment and osteoblastic differentiation |
| Götherström et al 2014[28] | OI | Case series | 2 | Fetal liver-derived MSC | IV | $6.5 \times 10^6$ & $4.2 \times 10^7$ pre- and post-natally OR $4 \times 10^7 + 8.8 \times 10^7$ | Lack of new fractures and improved growth and mobility; lower MSC dose was more effective |
| Emadedin et al 2012[71] | OA | Case series | 6 | Autologous MSC | Injected into knee | $2 \times 10^7$ | Increased cartilage thickness, increased repair tissue and decreased subchondral edema |
| Orozco et al 2013[29] | OA | Phase I/II trial | 12 | Autologous MSC | Injected into knee | $4 \times 10^7$ | Improved cartilage quality with improved OA algofunctional indices |
| Wong et al[30] | OA | RCT | 28 (+28 controls) | Autologous MSC | Injected into knee | $1.46 \times 10^7$ | Improved short-term clinical parameters and cartilage compared to control |

**Abbreviations:** OI, osteogenesis imperfecta; OA, osteoarthritis; RCT, randomized control trial; PRP, platelet-rich plasma; DBM, demineralized bone matrix; IU, intra-uterine transplantation; IV, intra-venous; na, not available; NC, nucleated cells.

by fluorescence in situ hybridization demonstrated low but irrevocable evidence of MSC engraftment up to 9 months after transplantation suggestive of a cellular regenerative, rather than a paracrine, effect. This was the first observational study of pre- and post-natal transplantation of the same allogeneic MSCs with long-term follow-up in OI. The authors concluded that the methodology was safe and probably efficacious, but further clinical studies would be required. The major hurdle to overcome the use of MSCs as therapeutic agents in OI is perhaps one that cannot be overcome, and that is its incidence: the low number of OI cases means that clinical trials will only ever be in small numbers; evidence from case studies must be very convincing for more extensive use.

**2.4. Osteoarthritis.** Osteoarthritis (OA) is already the most common form of arthritis; it is chronic, painful, and debilitating and its incidence is increasing worldwide with

an aging population. Symptomatic management rather than disease-modifying treatments have been relied upon but recent clinical trials suggest MSCs have the potential to be useful as therapeutics in OA (Table 1). Most recently, Orozco et al administered $4 \times 10^7$ bone marrow-derived MSCs, phenotyped as strongly positive for CD90 and CD166; moderately positive for CD105, CD106, and KDR; and negative for CD34, CD45, and HLA-DR, to 12 conventional treatment-unresponsive OA patients.[29] The patients were monitored for up to 12 months and had significantly reduced and lasting pain relief with a modest improvement in life quality. Importantly, cartilage improvement monitored by MRI was noted in 11 of 12 patients, suggesting that MSC administration was effective at both management of OA symptoms and partial disease modification. In a larger randomized control trial, Wong et al injected MSCs, phenotyped to the International Society for Cell Therapy defined standards,[6] and cultured with hyaluronic acid or hyaluronic acid only controls, intra-articularly into the knees of 28 patients per group in conjunction with microfracture and medial opening-wedge high tibial osteotomy.[30] A number of clinical outcome parameters were used to demonstrate both initial and long-term improvement in knee functionality and cartilage repair in the cell-treated group[30] providing good evidence for the use of MSCs as therapeutics in OA. Nevertheless, the cartilage improvement was seen in only 11 of 12 patients in the study by Orozco et al and this improvement varied between patients,[29] suggesting a degree of donor-dependent effect that needs to be better understood for optimal treatment strategy and successful outcome.

**2.5. Cell phenotype, number, and administration.** While we have attempted to concentrate on discussing bone marrow-derived MSCs and purposefully highlighted publications in which the cells had been phenotyped, substantial ambiguities persist regarding the mode of isolation, handling, identity, function, and administration of MSCs for therapeutic benefit. Indeed, contention arises even in the naming of the cells. From mesenchymal *stem* cells[22,31] to mesenchymal *stromal* cells[32] and some in the field now refer to the cells capable of generating only skeletal tissues (bone, cartilage, and fat, rather than broader mesenchymal cell types) as skeletal stem cells.[33] MSCs only have induced pluripotent stem cell qualities after the transduction of a small number of reprogramming factors,[34,35] but MSCs (or perhaps more accurately, a cell subset within MSCs) have multipotent stem cell properties, specifically for skeletal tissue formation in vivo.[36,37] As such, the term skeletal stem cell does have merit, but has not been widely adopted by the community endeared to the term "MSC." Nevertheless, the MSCs used in many of the clinical trials rarely have their differentiation capability confirmed in vitro prior to administration, which would surely strengthen any in vivo responses in the absence of the ability to track and monitor differentiation. It is encouraging to note that there are exceptions.[14] Similarly, the concept of Giannotti et al for pre-induction of MSCs along the osteogenic lineage deserves more thorough investigation.[14]

MSCs are a heterogeneous population and pre-induction of an osteogenic phenotype or identification of cells within the total population predisposed toward the osteogenic (or chondrogenic) lineage should benefit the orthopedic regeneration potential of MSCs. As reviewed by Via et al,[38] the age of the donor and source of MSC may have some influence on their differentiation capability. Osteogenic differentiation capability has been reported to decrease in bone marrow MSCs with increasing donor age,[39,40] whereas the situation appears to be more complicated in adipocyte-derived MSCs in which age has been shown to increase,[41] decrease[42,43] or have no effect on osteogenic differentiation.[44,45] Osteogenic and chondrogenic differentiation has been reported to be highest in synovium-, periosteum-, and bone marrow-derived MSCs compared with adipose- and muscle-derived cells, whereas adipogenic differentiation is greatest in adipose- and synovium-derived cells.[46] This leads to the possibility of being able to cherry-pick the most suitable source and donor age for the desired therapeutic application. Alternatively, if the most appropriate cell could be enriched or isolated from the heterogeneous population, then this should increase the efficacy of the treatment, reduce the number of cells that are required, and increase the therapeutic success. Osteoprogenitors are typically present in bone marrow aspirates at approximately 0.005% of total nucleated cells[47] and their enrichment may be required to increase successful regenerative capacity.[12,48,49] Dawson et al[47] showed that size exclusion filtration facilitated by acoustic agitation could enrich osteogenic precursors in bone marrow aspirates, increasing the osteogenic and chondrogenic potential of the aspirate, and improving the cell seeding into a graft. Furthermore, distinct MSC subsets can be defined by expression of specific cell surface markers.

Chemokine receptor expression has been studied in MSCs,[50,51] CXCR4 and its ligand CXCL12 are thought to play a significant role in MSC homing, cells strongly expressing the receptor may thus have an increased potential to target damaged sites. On average, less than 1% of MSCs express CXCR4 but populations highly expressing CXCR4 can be identified and enriched and may represent a subset primed for homing.[52] Rapidly dividing MSCs can be distinguished from slowly/non-dividing MSCs by lack of expression of vascular cell adhesion protein (VCAM)-1 and fibromodulin (FMOD); VCAM-1/FMOD double-positive MSC have significantly lower colony forming unit than the double-negative population.[53] There was no difference in osteogenic potential between the two MSC subsets but the study authors noted a trend toward decreased adipogenic potential in the slowly dividing VCAM-1/FMOD double-positive subset.[53] Later, subsets with more specifically defined lineage potential have been identified. Using antibodies raised against a specific epitope of CD56 not expressed on natural killer cells, 39D5, and W8B2 against human mesenchymal stem cell antigen (MSCA)-1, Battula et al have identified the MSC subset MSCA-1+/CD56+, which has no adipogenic potential.[54]

This subset had osteogenic capacity but was particularly efficient for differentiation along the chondrogenic lineage, compared with the MSCA-1+/CD56– subset, which had the expected tri-lineage capacity but was fivefold less efficient at chondrogenic differentiation.[54] The MSCA-1+/CD56+ population might therefore be an attractive population for cartilage regeneration. Data from our laboratory suggest that within the heterogeneous population exist spontaneously differentiating osteogenic progenitors; work to identify markers for this population that might be useful in non/delayed unions, OI, and OA are underway. Therapeutic studies with sorted cells have not, to our knowledge, been undertaken but it may allow for the use of fewer, more focused, cells.

The number of cells administered to patients and the route of administration appear to be important factors. However, the dose is restrained by the fact that cellular senescence occurs with time in culture, thus applying a limiting pressure on the amount of cells that can be expanded for clinical use. It has been calculated that the minimal cell dose necessary for achieving clinical benefit in some animal models is $4 \times 10^7$ cells/kg, which would equate to $2.8 \times 10^{11}$ cells per 70 kg human;[55] an unfeasible amount to produce, and such numbers may also induce side effects. It is likely that administered cell numbers will need to be tightly regulated due to the possible effects on the recipients' coagulation system and the reported potential tumorigenicity of MSCs and/or their effects on recipients' tumors.[56] In recent orthopedic clinical trials, the cell number used has been in the range of millions/kg (Table 1) and indeed, of the few trials in which different doses were administered, increasing dose did not appear to increase clinical benefit.[28] Perhaps the dose is not the most critical factor. Factors affecting MSC localization or the level of their engraftment might outweigh the actual dose. Engraftment efficiencies in the study by Götherström et al were between 0.003% and 7.4%[28] and other studies have detailed, using detection of retroviral markers transduced into MSCs or assessment of allogeneic cell content, similarly low levels of engraftment (1–2%) could evoke clinical improvement.[11,12,26,27,57]

Another factor to consider is the route of administration, which will affect levels of engraftment and thus the amount of cells required to be administered. The vast majority of intravenously (IV) injected MSCs into rodents leads to rapid clearance and lung accumulation, causing embolism and rodent death.[58–60] IV MSC administration in humans during clinical trial has, to our knowledge, largely been reported to have no significant deleterious effects to the patients but one recent case study highlights a reason for concern. A 41-year-old person reported to hospital with chest pain; computed tomography revealed a consolidation at the subpleural area of the right upper lobe, multiple lung emboli, and pleural effusion consistent with a diagnosis of pulmonary embolism and infarct. Investigation revealed the patient had undergone three IV injections of adipose-derived MSC for cervical herniated intervertebral disc; while his parents, who had undergone five similar IV injections for treatment of knee OA, also had evidence of multiple lung emboli.[61] Intra-articular injection might offer a safer, more-directed alternative.[62] In a recent study, 15% of the intra-articular injected adipose-derived MSCs engrafted and were detectable for a month after injection, with 1.5% remaining for 6 months.[63] Use of scaffolds may offer further control to localize a greater proportion of cells and allow a self-renewing pool of engrafted MSCs.[64] Natural and synthetic scaffolds can offer biocompatibility and biodegradability and allow cell penetration and tissue impregnation allowing oxygen and nutrient exchange and studies are ongoing within the scientific community to optimize these parameters. One such example was reported by Kuroda et al. They embedded autologous bone marrow MSCs into a collagen gel, which was transferred to an articular cartilage defect in a knee; after 1 year, the patients' clinical symptoms had improved and there was evidence that the MSCs had differentiated into chondrocytes.[65]

Orthopedic clinical trials using MSCs need to be further optimized with regards to the issues discussed in this section as well as being designed for larger groups of patients with stricter randomization and blindness, but the scientific and clinical evidence support these studies.

**2.6. Use of MSCs in non-orthopedic conditions and clinical trial ethics.** MSCs have been reported to differentiate in vitro to a number of different cells outside of the mesenchymal lineage and the cells also have immunomodulatory properties, which are likely to explain any observed clinical effects in non-mesenchyme settings since there is no in vivo evidence for the ability of MSCs to differentiate outside of the mesenchymal lineage. Therefore, these effects may well be attributed to non-progenitor functions.[66] Nowbar et al recently published an interesting and thought-provoking study investigating the outcomes of clinical trials in which autologous bone marrow MSCs were used in an attempt to enhance left ventricular myocardial ejection fraction as a therapeutic option for ischemic heart disease.[67] The authors examined 133 reports from 49 trials and found a significant association, with unknown reason, between the number of factual discrepancies in trial reports and the reported increase in ejection fraction. There were only five trials with no discrepancies and these trials showed no increase in ejection fraction (–0.4%). Trials with 1–10 discrepancies reported a 2.1% increase, those with 11–20 discrepancies reported 3.0%; 21–30 discrepancies showed 5.7% improvement; and five trials with over 30 discrepancies showed a mean effect size of 7.7%. The authors concluded that avoiding discrepancies in clinical trials was difficult and discrepancies may not be errors per se, but these analyses, and those reported previously,[68] cast doubt on multiple studies reporting the therapeutic effectiveness of MSCs for myocardial heart disease. It remains possible that the immunomodulatory effects of MSCs can exert a clinically beneficial effect but further understanding and proper

ethical oversight are required to avoid patient exploitation[69] and patient harm.[61]

## 3. Summary

It is clear that the use of MSCs and related cell types in orthopedics offers clinical benefits to patients with musculoskeletal disease. Hurdles that must be overcome before they are more widely used are issues of cell identity, cell number, cell delivery, cell engraftment, and further evidence of tissue regeneration directly from the transplanted cells rather than any symptomatic effects. Nevertheless, new, more effective treatments are required to meet the burgeoning healthcare problem so the clinical pull must maintain pace with the biological push. MSC properties are donor-dependent; better biomarkers of potency and true mesenchymal *stem* cell identity are required. *Intra*-donor variation also exists. A single donor-derived heterogeneous MSC sample will contain cells of mixed potency. Within-donor selection of appropriate MSC-subtypes is likely to improve efficacy for specific orthopedic conditions. In situ techniques where the host cells are targeted for reactivation, rejuvenation, and regeneration hold promise. This may be achieved by the local delivery of specialized scaffolds, biomolecules, and pharmaceuticals to the disease site and could represent a cost-effective regenerative medicine route, more closely aligned with conventional clinical procedure. Cell-based therapies have a long history of use and there are parallels here with emergence and development of bone marrow hematopoietic stem cell transplants. There are lessons to be learnt and refinements to be made, but with a willing ensemble of scientists, clinicians, and regulatory authorities, the use of MSCs in orthopedics may too become accepted medical practice.

## Author Contributions

Conceived the concept: JMF and PGG. Wrote the first draft of the review: JMF. Contributed to the writing of the manuscript: PGG. Jointly developed the structure and arguments for the review: JMF and PGG. Made critical revisions and approved final version: JMF and PGG. All authors reviewed and approved of the final manuscript.

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