# **A SYSTEMATIC REVIEW OF CLINICAL STUDIES INVESTIGATING MESENCHYMAL STEM CELLS FOR FRACTURE NON-UNION AND BONE DEFECTS**

# A systematic review of literature

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# Abstract

**Background**: Fracture non-union is a significant problem with a wide demographic and massive economic and social elements, as well as the clinical difficulties it presents. Conventional treatments with autograft and allograft bone grafting poses serious difficulties, thus, it is necessary to develop novel techniques with our ever increasing knowledge of bioengineering using natural materials.

**Methods**: Papers were searched from a variety of sources. The search was not limited to English-language studies. Unpublished papers were also searched. Papers had to meet the definition of fracture non-union or bone defect and had to be clinical trials, using human MSCs. The individual merit of the mesenchymal stem cells needed to be noted.

**Results**: The results presented in this review show that the use of mesenchymal stem cells for the treatment of non-union and bone defects is optimistic. Several papers had positive outcomes to report. There is a need for higher level evidence.

**Discussion**: A strong need of clinical results is required to further progress in cell therapy. Launched trials will hopefully provide this information in the near future. If clinical trials are positive, further development of complex tissue engineering techniques may be developed to treat large bone defects. A cost-benefit analysis and economical evaluation to identify the best indications for bone repair cell therapy as a standard procedure is vital.

**Keywords**: Mesenchymal stem cells, MSC, treatment, fracture non-union, ununited fractures, pseudoarthrosis, bone defects

# Introduction

### Fracture Non-union

Fracture non-union poses a massive clinical problem as it is an outcome that is difficult to predict, and is associated with chronic pain and reduced locomotor function. Causes of fracture non-union includes biology associated factors (e.g. poor vascularisation), host associated factors (e.g. vascular disease) and treatment-associated factors (e.g. improper stabilisation).[[1]](#endnote-1) The definition of fracture non-union is not well defined. A 2007 paper defined fracture non-union as a disturbance of normal healing with the expectation that no consolidation will be achieved without focused and accurate treatment.[[2]](#endnote-2) According to the U.S. Food and Drug Administration (FDA), non-union is defined as a fracture for which a minimum of 9 months has elapsed since the injury and for which there have been no signs of healing for 3 months.[[3]](#endnote-3) This definition is, however, not comprehensive as some fractures experience delayed union or post-operative complications, such as infection. As there is no fully accepted definition, most clinicians rely on clinical and radiological examination.[[4]](#endnote-4) The end stage of non-union may be the formation of pseudoarthrosis, or false joint, where a synovium is formed over the fractured bone. Weber and Cech classify non-unions as either hypervascular, which are capable of biological reaction, and avascular, which are not.[[5]](#endnote-5) This classification by Weber and Cech was defined in 1976 and is still being used today, having several distinctions in the categories helps inform the likely outcomes.

Fractures are a massive economic and social burden to the UK. A 2008 paper by Donaldson et al. took a sample representative of the general UK population and estimated the incidence of fractures to be 3.6 per 100 people per year. Lifetime fracture prevalence exceeds 50% in middle-aged men, and 40% in women over the age of 75 years, with a reduced frequency in the non-white population.[[6]](#endnote-6) A 2013 paper by Mills et al. looked at the epidemiology of fracture non-union in adults in the Scottish population, expecting there to be approximately 850,000 new adult fractures each year and a retrospective analysis of this data found the overall incidence of non-union to be 18.4/100,000per annum.[[7]](#endnote-7) Non-union is considered a rare complication of paediatric fractures, with an estimated risk of 1 in 500 per fracture in boy’s aged under 14 and girls of all ages under 19. The risk in boys increases to approximately 1 in 200 per fracture for those aged 15 – 19.[[8]](#endnote-8) In the United States, approximately 5-10% of the 7.9 million fractures created each year in the US fail to achieve bony union.[[9]](#endnote-9) The average cost of treating a non-union is expected to be around £7,000 to £79,000 per person in addition to the indirect costs to the patient, such as loss of earnings, which are hard to quantify.[[10]](#endnote-10)

Large non-unions and bone defects (greater than four to five cm) are most frequently treated with bone grafting. Autologous bone graft is the preferred treatment but the supply of suitable bone is limited and its collection is painful, with a risk of infection, haemorrhage, disability, nerve damage and loss of function. Therefore, it is important to turn our attention towards natural biomaterials and tissue engineering.[[11]](#endnote-11)

For the purpose of this paper, I will be defining non-union as according to the FDA definition and therefore this forms an inclusion criterion.

### Bone defects

Bone defects can be defined as orthopaedic defects that will not heal without intervention. A critical size defect (CSD) is the smallest size tissue defect that will not completely heal over the natural lifetime of an animal.[[12]](#endnote-12) Another definition of this term was described as a segmental bone deficiency 2 – 2.5 times longer than the diameter of the affected bone. Other cofactors, such as anatomical site, associated surrounding soft tissue and metabolic factors characterise a bone defect as critical.[[13]](#endnote-13) Up to now, there is no consensus on the proper definition of CSD in humans. Several animal-based studies have been conducted, but there are few clinical studies focused on managing critical size defects. Current definitions do not appropriately address the dimension of bone.[[14]](#endnote-14)

Significant bone loss is only seen in a small minority of all fractures, but is more common in open fractures. A prospective audit of admissions to the Edinburgh Orthopaedic Trauma Unit between 1988 and 1998 showed fractures with bone loss accounted for 0.4% of all fractures in patients admitted to hospital. When looking at open fractures in isolation, this number jumped to 11.4%.[[15]](#endnote-15) Other factors, besides trauma, capable of creating large bone defects include neoplasms, congenital defects, infection and failed arthroplasty.

Segmental defects in bone remain an ongoing challenge in musculoskeletal care. In segmental defects, the problem involves both absolute and relative size. Their absolute size can be measured and is clear. What is less clear is the impact of relative size of defects in a bone segment (e.g. a 2cm defect in the tibia would be different to a 2cm defect in a clavicle). Current options for bone defects is plentiful, including vacuum therapy, flap techniques, distraction osteogenesis, free vascularised fibula grafts and fibula-pro-tibia transfer.[[16]](#endnote-16)

As much of the available literature treats bone defects as bone tissue that will not heal without intervention, this is the definition that I will be using for paper inclusion in this review.

### Mesenchymal stem cells

The International society for Cellular Therapy (ISCT) proposed a definition of mesenchymal stem cells as cells that adhere to plastic, express specific surface antigens (CD105, CD73, CD90) and not express others (CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA class II), and possess the ability to differentiate into osteoblasts, adipocytes and chondroblasts under standard in vitro conditions.[[17]](#endnote-17) MSCs possess amazing potential; they have been converted into osteoblasts in vitro by incubation with several agents.[[18]](#endnote-18) Thus forming the rationale for using them to treat fracture non-unions and bone defects.

Several animal studies have been conducted. Papers have long shown the benefit of MSCs to increase osteogenesis, demonstrating that bone growth is increased with the use of MSCs more than with fresh bone marrow graft alone.[[19]](#endnote-19)

This review aims to look at patients with un-united fractures or bony defects being treated with mesenchymal stem cells, to see if it results in bony union. Associated risks, complications and improvements to quality of life will also be investigated.

# Methods

The search was conducted using MEDLINE, PubMed, Embase and the Cochrane collaboration. MEDLINE search terms included “Mesenchymal Stem Cells”, “MSCs”, “Treatment”, “Fracture non-union”, “Fractures, ununited”, “Pseudoarthrosis” and “bone defects”. Unpublished studies were searched in the clinical trials registries[[20]](#endnote-20) and the WHO International Clinical Trials Registry Platform (WHO ICTRP).[[21]](#endnote-21) An outline of these trials will be shown in the results section (Table 1).

The search was not limited to English-language studies. Furthermore, reference lists of relevant articles and related citations found by electronic search were hand-searched to identify additional relevant articles. Bias of the articles included was checked according to the CASP checklist and papers were excluded if they were likely to hold substantial bias.

Studies were selected based on the following criteria:

1. Papers had to meet the definition of fracture non-union or bone defect.
2. Papers had to be clinical trials (systematic review of trials, RCT, observational study).

Papers were excluded if:

1. They were conducted on animals, with or without the use of human MSCs.
2. They focused on scientific application, but not the clinical applications of MSCs.
3. They didn’t discuss the individual merit of MSCs (e.g. focused only on marrow transplant, without acknowledging the confounding factors associated with bone marrow grafting compared to just MSCs).

# Results

## Effect of concentration of MSCs

Mesenchymal Stem Cells have long shown promise in the treatment of fracture non-union and bone defects. This was first illustrated by Healey et al., who showed promising outcomes in eight patients with primary sarcomas treated by extensive en bloc resections and reconstruction using internal fixation who developed delayed unions or non-unions. These patients were then treated with injection of autogenic bone marrow in situ. Bone formation was noted in seven patients after injection. Union was achieved in five of the patients. This good outcome, achieved under difficult clinical conditions, sparked interest among clinicians regarding autogenic bone marrow grafting as a potential treatment option.[[22]](#endnote-22)

In 1986, Connolly JF et al. carried out a 15-year experiment to follow up patients who had been treated using percutaneous marrow injection to fix a non-union bone defect of their tibia. The sample size was 100 patients and the study concluded that marrow graft have potential for treating this type of injury. This method was shown to be more efficient when compared to the previous standard – open iliac crest grafting. When investigated further, it was shown that the quantity of MSCs injected was positively correlated with the quality of healing.[[23]](#endnote-23), [[24]](#endnote-24)Patients whose fractures did not heal received fewer than 1000 MSCs per mL and fewer than 30,000 MSCs in total, whereas those whose fractures healed received significantly higher MSC concentrations and counts, with a mean of 1500MSCs per mL and 54,000MSCs in total, in a volume of 20 ml, demonstrating a dose-response relationship.[[25]](#endnote-25)

## Case reports

These results were corroborated by Hernigou et al. in 2005. 60 patients with non-infected atrophic non-union of the tibia were evaluated. Marrow was aspirated, concentrated in a cell separator and roughly 20cm3 was injected into non-union sites. The number of progenitor cells that were transplanted was estimated by counting the fibroblast colony-forming units. A positive correlation between the volume of mineralised callus at four months and the number and concentration of fibroblast CFU in the graft was observed. 7 of these patients did not achieve union. In all these cases, both the concentration and the total number of stem cells injected were significantly lower than in patients with bony union (average of 2579 progenitors/cm3). In the 53 that achieved union, there was an average of >1500 progenitors/cm3. In the 7 that did not, the concentration of progenitors was significantly (P=0.001) lower, with 634 progenitors/cm3. However, the fracture gap was reduced, measured by radiograph and computerised tomography (CT). The effect of this paper was reduced as mesenchymal stem cells were not the only transplanted cells. There were also mononuclear cells, alongside other cells, that exhibited osteogenic and angiogenic properties, which would have affected the clinical outcome. There was no placebo cohort.[[26]](#endnote-26)

A case that demonstrates the efficacy of MSCs above other therapies was shown by Bajada et al. A case report detailing a nine year tibial non-union resistant to six previous surgical interventions. They used autologous bone marrow stromal MSCs expanded to 5x106 cells after three weeks of tissue culture, followed by a combination of calcium sulphate in pellet form along with MSCs. Bony fusion occurred after two months.[[27]](#endnote-27) In a similar case (where previous therapeutic interventions have not been successful), MSCs derived from the periosteum were injected into a patient with atrophic non-union of the distal femur after correction osteotomy. The authors established that autologous MSCs cultivated on a three-dimensional matrix made of collagen can encourage bone regeneration in complicated cases of non-union. The advantage of this method is based on the boundless availability of cells that produce osteoinductive transplants after dissection of a small amount of periosteal tissue.[[28]](#endnote-28) Even more individual cases were carried out in 2007, by Maracci et al., with less promising results. Four patients were selected with an age range of 16 to 41 years. Patients were selected after having more conventional surgical therapies, which failed. Bone marrow stromal cells were isolated by their adherence to plastic, and seeded onto porous ceramic scaffolds. Patients 1 and 3 were healed, although a specific time frame is not mentioned. Patient 2 showed complete integration between the implant and host bone after 7 months. Patient 4 required a second surgery after 7 months to fit a cortical allograft in order to improve mechanical stability of the implant. At 2.5 year follow-up, the integrity of the bone implant was maintained. With time, the implants revealed a progressive appearance of cracks and fissures indicative of some bioceramic disintegration, however, the implants were completely integrated with the host bone. There was no control group in the study. The scaffold used was a 100% hydroxyapatite porous ceramic, which was not resorbed after more than 7 years post-implantation- crucial to note as the lack of resorption could compromise the biomechanics of the new bone. No major adverse effects were observed, however what would be defined as a major adverse event was not set out by the paper.[[29]](#endnote-29)

A case series by Jäger et al. looked at the clinical outcomes of 10 patients with volumetric bone deficiencies treated with MSCs. The study involved patients that had bone marrow aspirates harvested and bone marrow concentrate injected into patients with pseudoarthrosis, bone cysts or revision endoprosthetic bone defects. Unfortunately the study does not state which patients had which diagnosis, so although some do not meet the inclusion criteria for this review, they could not be lifted out of the study, which we considered worthy of including. Clinical success was defined by reduced or absence in pain combined with new bone formation at the transplantation site. The paper shows that the harvesting procedure for MSCs does not significantly increase operative time and that it is not associated with significant complications or side effects. All 10 patients showed roentgenological bone formation within follow up. 70% of patients showed complete bone healing.[[30]](#endnote-30). The study was limited by a small sample size, and variation in the presenting diagnosis. Conversely, Quarto et al. reported the use of cultured BM MSCs combined with hydroxyapatite blocks to fill large bone defects (4-7cms). Three patients were selected for this study and had osteoprognitor cells placed on macroporous hydroxyapatite scaffolds. External fixation was provided for mechanical stability for a mean of 8.5 months (6-13). All patients recovered limb function and reported no problems with the implants at a year follow-up. Radiographs and CT revealed callus formation and integration at the surfaces with the host bone[[31]](#endnote-31).

## Randomised Controlled Trials

An RCT by Dallari et al. looked at the effect of platelet gel, or platelet gel and bone marrow stromal cell (BMSC) supplementation with bone grafts. Patients were selected as they were undergoing high tibial osteotomy to treat genu varum, with an opening defect of >1cm on the medial side. Patients were randomised into group A (lyophilised bone chips and platelet gel), group B (lyophilised bone chips and platelet gel and BMSCs) and group C (lyophilised bone chips). Six weeks after surgery, the osseointegration process was increased in the treatment groups (A+B) compared to the control group (C). In addition, the osseointegration process was significantly better in Group B than in Group A, showing the beneficial effect of BMSCs alone. At 6 weeks, group A had extensive bone formation throughout the defect. Group B had more active bone remodelling than group A. This study, however, was underpowered.[[32]](#endnote-32)

Liebergall made a paper that is a prospective, single centre randomised controlled clinical study aimed at assessing the safety of implanting human MSCs for the repair of distal tibial fractures, which tend towards delayed or non-union. This study evaluated the safety and efficacy of early minimally invasive intervention (MII) in treating these fractures. 24 patients were split into randomly selected intervention and control groups. Bone marrow MSCs were aspirated and mixed with demineralised bone matrix and injected into the fracture site. All fractures healed within 12 months, but there was a significant decrease in fusion time in the intervention group, with reduction in median time from 3 to 1.5 months (blinded and unblinded) and a mean reduction of 4.0 months – 2.2 months (unblinded, P<0.03). However, when the evaluators were blinded, there was no significant decrease in mean reduction fusion time (P<0.06). In addition, neither of the clinical assessments (short form 12, visual analogue scale) indicated a significant difference. Three cases of delayed union occurred in the control group, but only one in the intervention group. [[33]](#endnote-33)

## The importance of vascularisation + the microenvironment

Kitoh et al. showed that bone transplant is unsuccessful if there isn’t adequate soft tissue and vascularisation.[[34]](#endnote-34) Several papers have shown that the microenvironment surrounding the bone defect is of crucial importance[[35]](#endnote-35),[[36]](#endnote-36) and so an interesting paper by Warnke et al. addresses this aspect clinically, looking at blood supply in the growth of bone. They used a 56 year old man as a bioreactor. A titanium mesh was loaded with hydroxyapatitie blocks that were coated in BM MSCs. The latissimus dorsi muscle was used to insert the scaffold, which was nourished by the thoracodorsal artery. The scaffold was removed at 7 weeks and inserted into the mandible, to heal a significant mandibular defect due to a resection for floor of mouth cancer. In the first 6 months he had regained masticatory function and aesthetic form. At 7 – 13 months follow up, bone formation varied between parts of the mandible replacement, with areas or adequate formation surrounded by areas with minor growth. Despite this, stability was provided, allowing for full function of the replacement. Unfortunately, the external titanium scaffold fractured and the titanium mesh was exposed. Infection occurred and biopsy revealed necrotic bone inside the mandible replacement. This area was curetted and filled with bone chips. Infection re-occurred. The patient died 15 months after mandible replacement of MI. His family refused post-mortem. [[37]](#endnote-37)

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| --- | --- | --- | --- | --- | --- | --- |
| Title | Database searched | Identifier | Sponsor | Intervention | Primary Outcome | Status |
| Bone regenerative medicine using allogeneic bone marrow derived mesenchymal stem cells secretome | ICTRP | JPRN-UMIN000011290 | Nagoya University Hospital | MSC-CM Group | No complications and side effects will be reported throughout the study | Recruiting participants |
| Mesenchymal Stem Cells; Donor and Role in Management and Reconstruction of Nonunion Fracture | Clinical Trials.gov | NCT01626625 | Indonesia University | Stem cells and hydroxyapatite | Healing of fracture; radiological union | Unknown |
| Use of Autologous Bone Marrow Derived Mesenchymal Stromal Cells in Combination With Platelet Lysate Product for Human Long Bone Nonunion Treatment, A Phase 2-3 Clinical Trial | Clinical Trials.gov | NCT02448849 | Royan Institu*t*e | Percutaneous injection | Clinical union within 3 months | Recruiting participants |
| Mononucleotide Autologous Stem Cells and Demineralized Bone Matrix in the Treatment of Non Union/Delayed Fractures | Clinical Trials.gov | NCT01435434 | Hadassah Medical Organization | Sepax is a tool to standardize adult stem cell. Ignite ®ICS injectable scaffold manufactured by Wright Medical Technology | clinical and radiological bony union at 3 months and 6 month | Not yet open to recruit |
| Allogenic Mesenchymal Stem Cell for Bone Defect or Non Union Fracture (AMSC) | Clinical Trials.gov | NCT02307435 | Indonesia University | Mesenchymal stem cells | Percentage of cell that live divided by total number of cells | Recruiting participants |
| Treatment of Atrophic Nonunion Fractures by Autologous Mesenchymal Stem Cell Percutaneous Grafting | Clinical Trials.gov | NCT01429012 | University Hospital of Liege | Mesenchymal stem cells | Safety of Mesenchymal Stem Cells injection in nonunion fractures | Not yet recruiting |
| The Efficacy of Mesenchymal Stem Cells for Stimulate the Union in Treatment of Non-united Tibial and Femoral Fractures in Shahid Kamyab Hospital | Clinical Trials.gov | NCT01788059 | Emdadi Kamyab Hospital | Injection of the mesenchymal stem cell in non- union site | clinical and radiological union at 1 month to 6 months | Completed |
| Treatment of Non Union of Long Bone Fractures by Autologous Mesenchymal Stem Cell | Clinical Trials.gov | NCT01206179 | Royan Institute | Cell injection | Radiological progression of bone fusion | Completed |
| Injection of Concentrated Autologous Bone-marrow (IMOCA) and Bone Union of Open Tibial Shaft Fracture: Randomized Study to Assess Efficiency of IMOCA in Addition to Standard of Care. | Clinical Trials.gov | NCT00512434 | University Hospital, Tours | Osteosynthesis | Proportion of patients requiring secondary intervention because of delayed union or nonunion within twelve month post fracture. | Completed |
| Autologous Stem Cell Therapy for Fracture Non-union Healing | Clinical Trials.gov | NCT02177565 | Robert Jones and Agnes Hunt Orthopaedic and District NHS Trust | Carrier plus in vitro expanded autologous BMSCs | Radiological assessment of new callus and fracture bridging | Completed |

**Table 1.** Clinical trials related to mesenchymal stem cells. Cited in the clinical trials registries searched.

# Discussion

Bone tissue is able to regenerate more predictably than any other tissue in the body. Bone autograft was the safest and most effective grafting procedure because it uses the patient’s own bone cells. However, bone autograft is limited in quantity and represents an additional surgical intervention, with associated complications.[[38]](#endnote-38) The use of stem cells and tissue engineering is therefore essential in treating these pathologies. Bone marrow mesenchymal stem cells have a strong osteogenic potential and are easily obtained by culturing iliac crest aspirates. Bone marrow MSCs are taken from the patient, isolated due to their adherence to plastic, cultured and differentiated into a suitable number, applied to a synthetic scaffold and expanded in vitro on this scaffold and then grafted[[39]](#endnote-39). The results presented in this review show that the use of mesenchymal stem cells for the treatment of non-union and bone defects is optimistic. Several papers had positive outcomes to report, however, there is a dire need for higher level evidence and more research into the environment in which the stem cells are used.

Literature is sparse, especially high level evidence. A 2008 review of literature revealed that there has been no study providing level 1 evidence regarding tissue engineering, bone marrow aspirates, demineralised bone matrices or gene therapy in humans.[[40]](#endnote-40) Clinical trials are difficult to conduct as there are significant ethical and regulatory issues. Substantial effort is required to launch clinical trials, supported only by preclinical arguments and data. Cell therapy is considered an advanced therapy by European legislation[[41]](#endnote-41), where cells are considered ‘engineered’ if they have been subjected to substantial manipulation and are not intended to be used for the same function in the transplanted location as from where they were harvested. At the moment, only autologous MSCs are used for cell therapy. Bone marrow cell concentration of MSCs has been shown as a safe therapy, with no risk of cancer, by Hernigou et al. on 1873 patients.[[42]](#endnote-42) Tarte et al. also researched the risk of neoplastic changes and found no evidence of harmful or neoplastic changes of cultured MSCs as the result of two national trials.[[43]](#endnote-43) However, the immunomodulating effects of MSCs and their ability to maintain the survival of associated cells warrants caution and is a barrier to the conduct of clinical trials.

Bone repair may also be influenced by a number of factors – the defect site, vasculature, and patient’s age. The successful use of mesenchymal stem cells for bone repair in clinical studies has excited many. However, a better understanding of the role of stem cells in bone repair is necessary for more consistent success in the application of treating bone defects and fracture non-union.[[44]](#endnote-44)

Important variations in the number of MSCs are observed in patients and may be a limit of the technique. Tissue engineering is probably the solution to bring a regular number of cells to the patient. It combines MSCS, scaffolds and pro-osteogenic molecules in order to form hybrid constructs.

The scaffold used to culture MSCs may require more investigation. Research by Bajada et al. has shown that calcium sulphate works as an effective scaffold. Hydroxyapatite showed mixed results. Maracacci showed that hydroxyapatite was not resorbed after more than 7 years post-implantation, which compromised the biomechanics of the new bone. However, Warnke et al. used hydroxyapatite as a scaffold and reported no problems related to this. However, they did report that bone growth was inconsistent throughout the graft, but this was not attributed to the scaffold. Further studies may be needed.

To conclude, results from studies about clinical applications of mesenchymal stem cells have been overwhelmingly promising, but further evidence is needed. A strong need of clinical results is required to further progress in cell therapy. Launched trials will hopefully provide this information in the near future. If clinical trials are positive, further development of complex tissue engineering techniques may be developed to treat large bone defects. A cost-benefit analysis and economical evaluation to identify the best indications for bone repair cell therapy as a standard procedure is vital, if MSCs are shown as safe and efficacious from current clinical trials.[[45]](#endnote-45)

## future direction

Table 1 shows clinical trials that are soon to take place, or have recently been completed. Research is looking at a deeper molecular level to aid the treatment of non-union and bone defects. The results show that bone growth requires more than just stem cells; the support of an osteoinductive environment is essential.[[46]](#endnote-46) Recent research has shown the importance of isolated factors such as bone morphogenic proteins, which have been used clinically to enhance and accelerate bone repair. Other growth factors, such as TGF-β and IGF may stimulate fracture repair and minimise the rate of non-union, and research is being conducted in these areas.[[47]](#endnote-47) Wiesmann et al. found that mechanical stimulation and other biophysical stimuli appear to be critical factors for the proliferation and differentiation of bone cells and for the formation of both bone mineral and structure.[[48]](#endnote-48)

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