Cellular regenerative therapy for acquired non-congenital musculoskeletal disorders

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Stem cells have an inherent capacity to facilitate regeneration; this has led to unprecedented growth in their experimental use in various clinical settings, particularly in patients with diseases with few alternative treatment options. However, their approved clinical use has to date been restricted largely to haematological diseases and epidermal transplantation to treat severe burns. After thorough searching of two databases, this review illuminates the role of stem cell therapy for treatment of musculoskeletal diseases. Research suggests that successful application of stem cells as regenerative mediators is in all likelihood dependent on the ability of endogenous tissue-resident reparative mediators to respond to paracrine signals provided by the applied stem cells. Therefore, an understanding of how the pathological environment influences this process is crucial for the ultimate success of stem cell therapies. The current review presents both the progress and limitations of stem cells as regenerative mediators in the context of musculoskeletal disorders.

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Hype, hope and reflection

The touted transformative potential of stem cells, and their pivotal role in maintaining adult tissue homeostasis, has resulted in medical science shifting from a paradigm of repair to one of regeneration.[1] Regenerative cell therapy aims to add or replace human cells in order to regenerate tissue by using the intrinsic regenerative properties of the delivered cells. As a result, therapies utilising these highly regenerative units have been widely pursued in pre-clinical and clinical trials. Despite this, clinical applications, beyond the accepted utilisation of stem cells in the treatment of haematological diseases, have been relatively unsuccessful. This may be attributed to a number of aspects including poor engraftment and survival, inability of cells to differentiate into the target tissue, and the emerging understanding that, in many cases, an inflammatory or fibrotic host environment with underlying pathology deters the regenerative capacity of applied cells.[1,2] A further complication that hampers progress is that most clinical trials testing cellular regenerative therapies are undertaken in small cohorts and there is variability with regard to the type of stem cell population used, their method of delivery, formulation (combination of cellular and drug therapy), the level of control or randomisation, and even the outcome measures assessed.[1] To aggravate these issues, recent retractions by leading journals of previously lauded stem cell science and its clinical application,[3,4] as well as the proliferation of corrupt clinics exploiting the unrealistic hope of individuals seeking therapies for currently non-treatable diseases, threaten to sabotage this emerging area of regenerative medicine.[1]

It is therefore necessary to re-evaluate the current approach to regenerative medicine such that a realistic framework of expectations, related to developing clinical therapies, is established. Critical aspects to be considered include a clinical understanding of stem cell efficacy (based on data from completed clinical trials) as well as knowledge of the specific cellular microenvironment, and the underlying molecular mechanisms required for the success of cellular regenerative therapy.

Autologous stem cell therapies for non-congenital musculoskeletal disorders

Musculoskeletal disorders (MSDs) include a wide range of conditions that result from either trauma or degeneration and can affect the muscles, joints and skeleton; they include disabling conditions such as arthritis, chronic inflammatory diseases, autoimmune diseases, myopathies and osteoporosis (Table 1).[5]

Results of various clinical trials utilising stem cell therapy to treat MSDs have been published in the peer-reviewed literature (Fig. 1A). These trials are for the most part ongoing and are in a range of different phases, representing both uncontrolled (n=33) and controlled (n=28) trials; of the latter, 19 were randomised. A range of cellular delivery approaches were used including localised injection at the major site of the clinical disorder (therefore at least initially restricting administered cells to the affected area), stem cell infusion (injecting the cells into the systemic peripheral circulation) as well as the introduction of a physiological scaffold (Fig. 1A). Many clinical trials, including the transplantation of stem or stromal cells to stimulate the intrinsic repair mechanisms in the recipient tissue, appear in a comprehensive registry of clinical trials (Fig. 1B).[6] Others, such as autologous chondrocyte implantation (ACI) for the repair of cartilage defects, are available for clinical use.[7] However, in most cases these cellular therapies rely on allogeneic donor tissue. Allogeneic cells (both stromal and stem) sourced from young healthy donors have physiological, metabolic and genetic advantages when compared with those harvested autologously from either older individuals, where degenerative processes may persist, or from patients with underlying inflammatory conditions.[8] In the South African context, finding matching allogeneic donor stem cells for the purposes of regenerative therapy is, however, a challenge; this is due to a lack of resources and skilled personnel in the healthcare sector, poor and inequitable access to affordable cellular therapy and the high burden of disease.[9] In this context, autologous cell therapies may therefore offer a more feasible alternative. There are, however, currently no published study results

Table 1. Results of search for 'autologous stem cell therapy' AND 'musculoskeletal diseases or conditions' in ClinicalTrials.gov

Condition	Autologous therapy/ intervention	Number of registered studies	Phase								Discontinued	Completed studies
			Early I	I	I/II	II	II/III	III	IV	N/A		
Tendon disorders												
Tendinitis; tendinopathy	MSCs, ADSCs, PRP	*n*=3				2				1		
Autoimmune diseases												
Rheumatoid arthritis; osteoarthritis; degenerative arthritis	MSCs, ADSCs, PRP, CD34$^+$ HSCs, BM, SVF	*n*=48	1	13	18	10	1	1		4	2 withdrawn; 1 terminated	18 (2 with results available)
Cartilage disorders												
Articular cartilage; degenerative discs; osteochondritis; spondylolisthesis	MSCs, BMMNs, PBSCs, BM	*n*=12	2		5	3	1			1	2 withdrawn; 1 unknown status	4 (no results available)
Myopathy												
Myasthenia gravis; compartment syndrome	HSCs	*n*=2		2							1 terminated	1 (no results available)
Necrosis - osteomalacia												
Osteonecrosis; avascular necrosis; osteomalacia; Kienböcks disease	MSCs, BM	*n*=6		1	2	2			1			1 (no results available)
Dystrophy												
Muscular dystrophy; Limb girdle dystrophy	MSCs, BMMNs	*n*=3		3								2 (no results available)
Other												
Pseudo-arthrosis; leg length inequality	MSCs, PRP	*n*=2		1		1					1 suspended	1 (no results available)

MSC = mesenchymal stem cell; ADSC = adipose-derived stem cell; PRP = platelet-rich plasma; HSC = haematopoietic stem cell; SVF = stromal vascular fraction; BM = bone marrow; PBSC = peripheral blood stem cell; BMMC = bone marrow mononuclear cell.
Clinical trial phases: Early I – exploratory, no therapeutic or diagnostic goals; I – safety, adverse effects in healthy volunteers; II – preliminary, safety and effectiveness; III – larger populations, safety and effectiveness, dosages; IV – approval for marketing, safety, efficacy, optimal use.

from trials, utilising autologous stem cells for the treatment of MSDs, that have successfully completed all four phases of clinical trials; this lack has contributed to the rise of extensive direct-to-patient marketing of unproven cellular therapies (Table 1).[1,10]

A search of the US National Library of Medicine's clinical trials database (ClinicalTrials.gov; accessed 25 October 2018) using the search terms 'musculoskeletal diseases or conditions' AND 'autologous stem cell therapy' yielded 80 results. This is, however, not a comprehensive list of ongoing studies as, despite the fact that clinical trials carried out in the United States are required to be registered, compliance is low and many trials are ultimately not registered. However, given that ClinicalTrials.gov is the largest trials registry, the selected 80 studies nevertheless provide an overview of current international trends in cell therapy for MSDs. Of these 80 studies, 4 were excluded based on disease (cancer or genetic disorder) and therefore, for the purpose of the present review, the remaining 76 studies were grouped together based on the type of musculoskeletal condition (Table 1; Fig. 1B). The majority of these clinical trials were performed in the context of autoimmune-related disorders such as rheumatoid arthritis, osteoarthritis and degenerative arthritis (*n*=48), followed by cartilage disorders (*n*=12), necrosis and osteomalacia (*n*=6), non-inherent dystrophy (*n*=3), tendon disorders (*n*=3) and non-inherent myopathy (*n*=2). Most of these studies are, however, in the early trial phases (Phase 1/2) with very little safety and efficacy data available; additionally, more than 60% of these studies focussed on inflammatory auto-immune conditions.

Stem cell populations residing within the bone marrow have shown the most promise as therapeutic agents and require very little, if any (in cases where stem cells are not affected by underlying disease), *in vitro* manipulation. These include heterogeneous cellular populations such as bone marrow (BM) aspirates, bone marrow mononuclear cells (BMMNs), mesenchymal stem cells (MSCs) and haematopoietic stem cells (HSCs; CD34$^+$). Another heterogeneous cell population, not residing within the bone marrow, which also shows potential

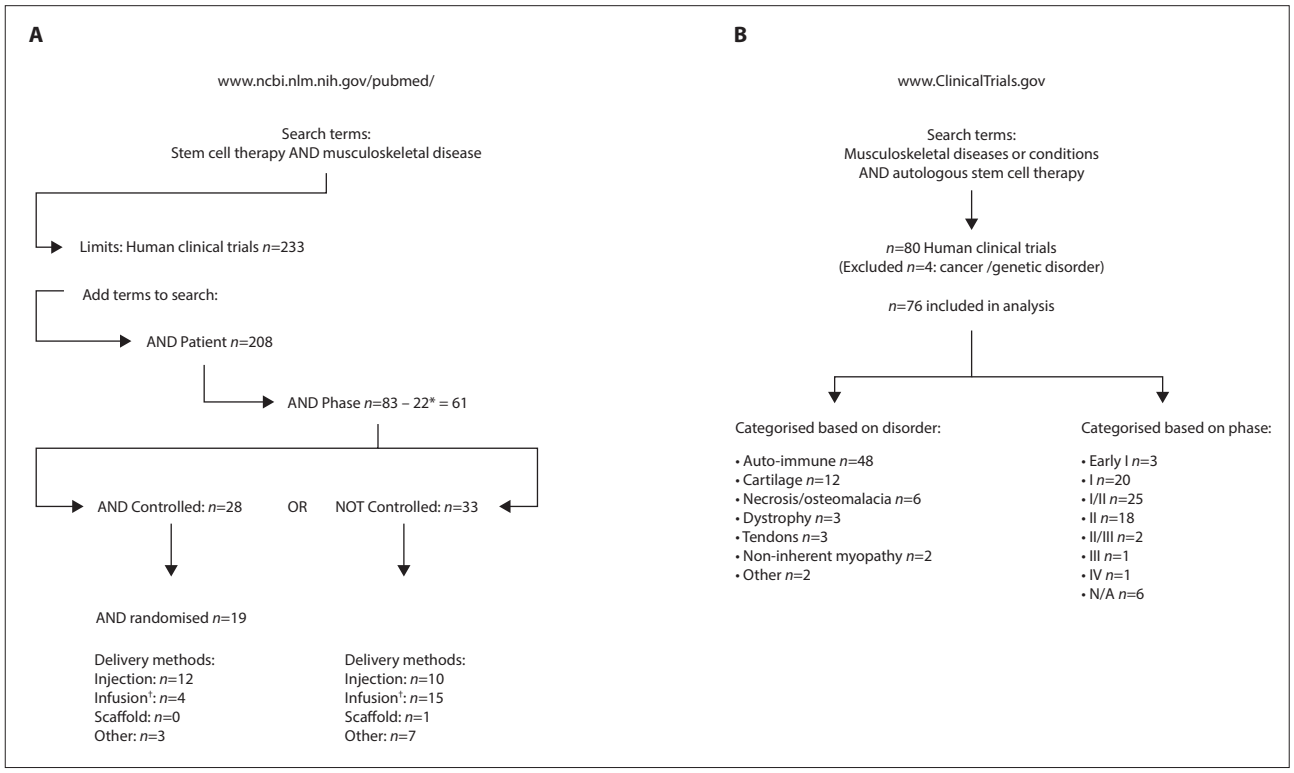

Fig. 1. Search strategy. Two separate search strategy approaches were applied. 1A. Database: www.ncbi.nlm.gov/pubmed; use of the terms 'injection', 'infused' and 'scaffold' did not yield a full breakdown of delivery methods. 1B. Database: www.clinicaltrials.gov; details of search results are presented in Table 1.
**Excluded because either stem cell therapy not mentioned, or musculoskeletal disease not mentioned.*
†Or infused/re-infused: not all published papers sourced have been cited; further selection was based on relevance to text ClinicalTrials.gov, accessed 25 October 2018.

for therapeutic use and is far more easily accessible, is the adipose tissue-derived stromal cell (ADSC) population from the stromal vascular fraction (SVF). However, a potential disadvantage of these ADSCs is the suggested higher rate of adverse events (AEs) when utilised in clinical trials.[11] The two clinical trials with results available (Table 1) were carried out by the same research group, using intra-articular injection of autologous bone marrow-derived MSCs to treat knee osteoarthritis (NCT011832728 and NCT 01586312). Although these studies included a very small number of patients (*n*=12), the outcomes were positive, demonstrating functional improvement, pain relief and improved cartilage quality.[12,13] These results highlight the therapeutic potential of autologous bone marrow MSCs for localised conditions in the absence of systemic pathology.

Why regenerative mechanisms fail

Although the use of a patient's own cells for therapeutic purposes is deemed to be preferable, the success of such autologous stem cell therapy is dependent on the medical history of the patient. Stem cells derived from patients with underlying pathologies such as chronic inflammatory (rheumatoid arthritis), degenerative (ageing, osteoporosis), metabolic (obesity, diabetes) and genetic (osteogenesis imperfecta, muscular dystrophy) disorders, are not as effective in promoting healing as those from healthy individuals.[2,8,14,15] This outcome is due, in part, to numerous pathological alterations in the niche microenvironment that may desensitise endogenous stem cells and alter their characteristics to such an extent that they cease to be regenerative agents.[2,15] For example, in a trial of autologous MSC therapy for osteonecrosis, 81% of patients displayed improvements in symptoms, whereas those without improvements had generally entered the trial at a more advanced stage of their disease, including a more severely hypoxic environment.[16] Despite the initial expansion of MSCs in an optimal *ex vivo* environment with adequate oxygen supply, hips with stage III or IV disease, with poor *in vivo* oxygen supply to the affected area, progressed. Therefore, systemic as well as local conditions may affect the efficacy of stem cell therapy, as stem cells may exhibit functional changes including impaired growth, differentiation and migratory capacity.[2,15,17]

A recent review of the literature, on the negative aspects of a range of diseases, indicates that stem cell dysfunction can occur as a result of genomic instability, telomere attrition, senescence, mitochondrial dysfunction, epigenetic changes or loss of proteostasis.[2] There is, furthermore, mounting evidence that the altered microenvironment, established as a result of the transition from health to chronic injury or disease, not only transforms intracellular characteristics, but also adversely affects cellular communication.[2,18] It has now become accepted that the regenerative potential of stem cells is mediated largely through their release of biologically active molecules, and the subsequent effect of these on target cells. Therefore, an understanding of such paracrine signalling in endogenous stem cell populations is likely to be at the epicentre of progress towards addressing their impaired regenerative capacity in the presence of underlying chronic disease.[19] For therapeutic applications of autologous stem cells, it is therefore essential that either the appropriate stem cell subpopulation (with retained regenerative properties) is used, or that the appropriate *in vitro* manipulation to correct stem cell function is performed prior to application. Such *in vitro* manipulation is not required to be at the level of gene therapy, but may include strategies such as antioxidant pre-conditioning.[18]

Alternatively, allogeneic stem cells from a healthy donor should be considered.

Therapeutic properties of stem cells

The multi-functional properties of stem cell populations include immune-modulatory, anti-inflammatory, growth-promoting and multi-lineage differentiation (bone, cartilage, muscle, adipose tissue) capabilities.[20,21] The paracrine properties of minimally manipulated stem cells are mediated through the release of growth factors, cytokines and microvesicles, the formation of tubules for mitochondria transfer, and/or the release of exosomes (small-sized extracellular vesicles containing miRNAs, lipids and proteins).[22,23] Although significant insight has been gained into the constituents of the secretome over the last few years, the complete secretome of the various populations of stem cells remains to be fully characterised and compared. A reference database for healthy stem cells, once established, can be used to assess patient-specific harvested stem cells prior to transplantation. What complicates this laudable goal is the fact that stem cells are extremely responsive to their microenvironments, and the slightest extracellular change can lead to modifications in stem cell gene expression and secretion of signalling biomolecules.[2,15,18] Hence, conditions for pre-transplantation *ex vivo* assessment will need to be standardised carefully before extensive *ex vivo* expansion under more ideal conditions. The following three sections give a brief overview of which mechanisms are suspected to be crucial for therapeutic success under different disease conditions.

Autoimmune-related disorders

The potent immune-modulatory properties of MSCs in particular can be harnessed to treat autoimmune-related MSDs such as rheumatoid arthritis, osteoarthritis and degenerative arthritis. It has been shown that MSCs can prevent auto-immunity by inhibiting lymphocyte surveillance of tissues and T cell proliferation.[24,25] In addition to its effect on lymphocytes, the anti-inflammatory properties of MSCs function to alleviate persistent inflammation. Intra-articular injection of adipose-derived stem cells has been shown to be safe and efficacious in a number of recent clinical trials, particularly involving patients with osteoarthritis; despite the injection site being intra-articular, these cells may mediate their anti-inflammatory effects by increasing the number of circulating regulatory T cells and reducing the number of classically activated monocytes, thereby promoting a more anti-inflammatory global immune cell phenotype.[26-28] The MSC secretome contains various anti-inflammatory cytokines and RNAs that can modulate effector cell function.[21,23,25] The paracrine signalling of MSCs has been shown to stimulate the release of interleukin 10 (IL-10) from macrophages, and promote a macrophage phenotype switch from a destructive pro-inflammatory (M1 polarised macrophages) to a pro-regenerative anti-inflammatory (M2 polarised macrophages) phenotype.[29] This phenotype switch is a crucial step in any regenerative process, as it facilitates the transition from the inflammatory phase to the proliferative and remodelling stages of healing. Unfortunately, the anti-inflammatory ability of endogenous MSCs becomes compromised when they are exposed to chronic inflammation for extended periods,[2,15,18] thus limiting the effectiveness of autologous therapy under these conditions. Various strategies to 'precondition or re-programme' MSCs *in vitro*, in order to optimise cellular efficacy prior to transplantation, are currently being investigated in pre-clinical models. This includes the potential use of a combination of antioxidants (*N*-acetylcysteine and ascorbic-2-phosphate)[18] as well as other bio-active molecules to either mimic inflammatory stimuli or hypoxia.[30]

Tissue damage: tendons, non-inherent myopathies, acute injuries

The growth and regenerative properties of stem cells are relevant for tendon disorders, non-inherent dystrophy, myopathies and acute injuries. Skeletal muscle regeneration is complex and involves an intricate network of growth and extracellular matrix factor, cytokine and myogenic regulator factor signalling to regulate the activity of both tissue-resident muscle-specific stem cells (satellite cells) as well as non-myogenic progenitors.[31] When endogenous repair mechanisms fail, for example with unresolved inflammatory myopathies, these cells are either unable to facilitate repair or the parent pool of adult progenitors may have become depleted. In such instances, the inflammatory environment may be so severe that stem cells applied as therapy may fail to facilitate regeneration. However, the secretome of harvested exogenous stem or stromal cells can be applied to stimulate a reinstatement of intrinsic tissue repair mechanisms. Stem cell-derived growth factors that may mediate this include vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), insulin-like growth factor-1 (IGF1), transforming growth factor beta (TGFβ), basic fibroblast growth factor (bFGF) and the cytokines/chemokines granulocyte monocyte colony-stimulating factor (GM-CSF), monocyte chemotactic protein-1 (MCP1), interleukin-6 (IL6) and leukaemia inhibitory factor (LIF).[32] These represent a transient source of pro-differentiation signals for myoblasts and fibroblasts, known to reduce cellular apoptosis, promote survival, limit fibrosis and promote angiogenesis within the injured area.[31,32]

Cartilage and bone disorders

In the context of cartilage and bone disorders, researchers rely on the growth-promoting and multi-lineage differentiation capacity of stem cells to regenerate damaged tissue, whilst the immune-modulatory paracrine functions prepare the microenvironment for healing.[33] In the context of fracture healing, the crosstalk between macrophages and MSCs is crucial for all three stages of bone healing, namely haematoma formation, inflammation, granulation tissue and callus formation.[34] Under normal conditions, macrophages regulate the recruitment and differentiation of MSCs via osteo-inductive molecules such as oncostatin M (OSM), prostaglandin E2 (PGE2) and bone morphogenic protein-2 (BMP2). MSCs also have an immunosuppressive impact on macrophages; this is mediated through the paracrine release of PGE2 and inducible nitric oxide synthase (iNOS) by MSCs.[34] Excessive M1 macrophage activity and impaired MSC function can, however, hamper osteogenesis and bone formation in certain disorders. Following regeneration, the structural and mechanical integrity of bone and cartilage is essential for regaining optimal strength and function. Biomaterials and scaffolds thus form key components of the therapeutic strategy and function as a delivery method for MSCs in these disorders. In addition to providing structure, bio-engineered scaffolds with a specific topography, porosity and the ability to release osteo-inductive factors can dictate the differentiation of stem cells into a specific lineage.[35]

Towards a safe and efficacious cellular therapy

When considering the implementation of stem cell therapies in a clinical setting, due consideration should be given to existing

international guidelines that inform proper clinical trial and regulatory processes. Adverse effects (AEs), such as the long-term tumorigenicity of infused stem cells, remains a concern, especially since very few long-term follow-up data are available. In 2016, Centeno *et al.*[11] demonstrated that MSC-based percutaneous stem cell injections for the treatment of knee, hip, ankle/foot, hand/wrist, elbow, shoulder or spine disorders, did not increase the risk for developing neoplasms in 2 372 orthopaedic patients in the first 2 years post injections. However, a total of 325 adverse events were reported, of which 36 were classified as serious. The most common AE was post-procedure pain, with other less frequent AEs including neurological, vascular, allergic, immune, cardiac, infection and skin abnormalities. The authors indicated that bone marrow injections yielded the fewest AEs, and that the rate of AEs increased if other cell populations such as adipose tissue-derived stem cells were used or if the cells were cultured or manipulated *in vitro* prior to use.[11] Indeed, regulations guiding the application of adipose tissue-derived stem cells, and the definition of 'minimally manipulated', are currently a topic of debate.[36]

In an attempt to promote progress and optimise the clinical use of cell therapy for MSDs, a consensus was reached at the 2018 American Association for Orthopaedic Surgeons and National Institute of Health (AAOS/NIH U-13) conference, that osteoarthritis is the MSD with the most urgent need for clinical trial development.[37] This urgency is due to the substantial and progressive morbidity associated with osteoarthritis. To improve the accuracy of monitoring and standardisation, a first step was taken to create a framework for cell therapy, and clinical trial recommendations were made that included the establishment of high-quality patient registries and biorepository-linked registries from both institutions and physicians that offer stem cell therapy.[37] Despite this progress towards establishing a relevant and consistent regulatory framework, it is clear that stem cell therapy is not yet a mature therapeutic option.

Summary and way forward

Despite the rapid growth in stem cell research over the last 20 years, and the expansion of our knowledge of these regenerative cells, disappointingly little clinical progress has been made. Significant extended therapeutic use, beyond standard practices available since the 1960s, remains elusive. What has, however, become increasingly clear is that, if autologous stem cells are to be used successfully in the field of regenerative medicine, the metabolic milieu that signals cellular changes and the mechanisms that execute the endpoint of improper stem cell function, must be identified and addressed as part of the regenerative strategy. With this knowledge, it will be possible to generate a conducive microenvironment which can ensure that these cells, whether from a healthy or pathological setting, can be guided towards a pro-regenerative phenotype. Subsequent controlled clinical trials can then determine the efficacy of this therapeutic approach, prior to release to the public.

In South Africa, the control and use of stem cells is regulated by the National Health Act (NHA); however, relevant guidelines, which would assist medical professionals, have yet to be made public by the National Department of Health.[38] In the absence of these, professional bodies such as the South African Transplantation Society (SATS) and the South African Stem Cell Transplantation Society (SASCTS) have established their own set of guidelines, which medical professionals can consult. Practitioners should be aware that approved stem cell therapies are currently limited to the treatment of blood and immunological disorders and should inform their patients accordingly. There are serious risks related to the use of allogeneic cells derived either from unknown sources or retrieved in the absence of Current Good Manufacturing Processes (CGMP); furthermore, their classification as 'minimally manipulated' is required when utilised autologously. Recent guidelines related to the latter can be found on the website of the Federal Drug Administration (FDA; https://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Tissue/). Finally, patients should be advised to participate only in registered clinical trials approved and regulated by accredited institutions and ethical committees.

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