The Evolution of Regenerative Medicine:

Multilineage-Differentiating Stress-Enduring (Muse) Cells as a Novel Therapeutic Frontier

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Introduction

The field of regenerative medicine has made significant advances in recent decades with the sequential discovery and refinement of approaches to promote the body to self-heal injuries and conditions which would, if left alone, heal incompletely or not at all. Tools such as platelet rich plasma (PRP) and mesenchymal stem cells / medicinal signaling cells (MSCs) isolated from tissues such as bone marrow or adipose have enabled rapid progress in the achievement of positive outcomes for thousands of patients. In 2010, a potentially paradigm-shifting discovery was made by Mari Dezawa and colleagues at Tohoko University in Japan of a type of cell found endogenously in the body which, amazingly, was resistant to incredibly harsh treatment, naturally formed small clusters reminiscent of early stage embryonic development, and that possessed the ability to differentiate into multiple cell types [1]. These cells were named multilineage-differentiating stress-enduring cells, or Muse cells for short[1].

Muse cells are found in low abundance in tissues throughout the body, including bone marrow and adipose. Similarly to embryonic stem cells or induced pluripotent stem cells (iPSCs), they bear the cell surface marker SSEA-3, in contrast to other stem-like cells such as MSCs often found in the same compartments [2]. SSEA-3 is not only an indicator of pluripotency, but it actually plays a role (along with FGF-2) in maintaining self-renewal and multilineage potential [3]. This multilineage pluripotency includes the ability to differentiate into all three "germ" layers (ecto-, meso- and endoderm) of tissue. These cells have the ability to home to the site of an injury using sphingosine 1 phosphate (S1P) as a cue. S1P is released by damaged cells and activated platelets upon injury, and creates a gradient to drive a series of inflammatory, immune, and repair-guiding cells to the necessary location [4].

KEY INSIGHT

Muse cells are the only known endogenous stem cell that:

- Resist harsh physiological stress
- Spontaneously home to injury sites via S1P gradients
- Differentiate into all three germ layers
- Maintain a non-tumorigenic profile through culture expansion

Comparison Between MSCs and Muse Cells

As with Muse cells, MSCs are also found in relative abundance in bone marrow and adipose tissue. These cells were originally called mesenchymal stem cells and later renamed medicinal signaling cells upon the discovery that, although they possess the ability to differentiate into different cell types *in vitro*, in the body their important role in tissue maintenance and healing of injury arises from signals they provide to the existing cellular inventory to guide repair rather than participate directly by differentiation [5]. In that sense, they are not true "stem" cells. In contrast, Muse cells can and actively do differentiate into the needed cellular lineages for the injured tissue. This may confer upon these cells the ability to address injuries in tissues which are tough-to-repair owing to low native regenerative potential, such as the heart.

[1] Although it is an acronym, and MUSE is occasionally used, "Muse" is the standard shorthand utilized in the scientific literature and community.

Using Muse Cells as a Regenerative Treatment

When clinicians want to use a particular cell type (e.g., platelets; MSCs) as a component of a treating composition, this can often be done relatively easily by accessing a biofluid such as blood, marrow, or adipose aspirate, sometimes performing some manner of separation to remove unwanted cells, and often

performing a concentrating step to remove unneeded volume. However, Muse cells are at such low concentrations (0.03% of mononucleated cells in marrow [6]; 0.01%-0.2% of cells in blood [7]), that it is impractical to prepare a treating composition with high cellularity simply by concentrating from an endogenous sample. This naturally suggests lab-based culture expansion of the cells.

However, culture-expanded cells often carry a risk of forming neoplasms (cancer) when re-introduced into the body; culture expansion is naturally selecting for cells which retain strong proliferative potential outside of their purely native environment which is one of the classical hallmarks of cancer [8]. Muse cells have the exciting behavior of retaining their pluripotency through culture expansion, and maintaining other markers suggesting low risk of tumorgenicity [1]. This suggests that sample harvesting, isolation (commonly by long term trypsin incubation, see for example [9]), culture expansion, cell sorting using SSEA-3 (perhaps in conjunction with other stem cell markers such as CD105), and delivery in an appropriate dose and formulation might be a new tool for regenerative procedures. As previously mentioned, the ability of Muse cells to migrate to injury sites using S1P gradients suggests that simple intravenous delivery may be sufficient, without the need to directly administer to a specific location; this would be of tremendous benefit if the injury is not readily accessible (such as ischemic stroke in the brain).

Preclinical Data: Building Excitement Around Muse Cells For Regenerative Procedures:

Muse cells have been used to treat a number of injury or disease models in preclinical species, with generally good success. What follows is a noncomprehensive but illustrative list of animal studies performed using Muse cells. The wide array of tissues and organ systems investigated is a direct testimonial to the capabilities and flexibility of Muse cells.

Liver

- **Liver fibrosis in mouse [10];** Muse cells homed to carbon tetrachloride-damaged liver and subsequently differentiated into functional hepatocytes.

Cardiovascular

- **Acute myocardial infarction in minipigs [11];** Acute myocardial infarction in minipigs [11]; compared to vehicle treatment, Muse cells administered intravenously led to smaller infarct size, significantly greater left ventricle ejection fraction and fractional shortening, and significantly smaller left ventricle end-systolic and end-diastolic dimensions, without causing any measured arrhythmias. Histology revealed that the Muse cells homed to the infarct border and were found expressing cardiac and vascular endothelial markers.

Gut

- **Intestinal epithelium damage in rat in vitro model [12];** Intestinal epithelium damage in a rat in vitro model [12]; Muse cell treatment provided significantly greater protection and anti-inflammatory responses compared to bone marrow MSCs.

Skin

- **Skin wounds in rats** [13]; Muse cells injected around the edges of wounds were observed to incorporate into the cellular environment and differentiate.
- **Atopic dermatitis in mice** [14]; treated mice had visibly reduced dermatitis, improved associated behavior (scratching), reduced inflammatory signaling, and improved wound healing and keratinocyte proliferation.
- **Diabetic skin ulcers in mice** [15]; diabetic immunocompromised mice had evidence of Muse cells incorporated into the wound site and significantly accelerated wound healing compared to treatment with non-Muse cells expanded from human adipose MSCs.
- **Epidermolysis bullosa in mice** [16]; intravenously injected human Muse cells homed to the site of skin injury in type XVII collagen knockout mice, deposited human type VII and XVII collagen, reduced the spread of the injury, and suppressed hair loss and development of gray hair compared to a vehicle treatment.

Neurology

- **Subacute spinal cord injury in rats [17];** after a mid-thoracic spinal cord contusion, rats treated with human Muse cells showed an improvement in hindlimb motor function, smaller cystic cavities, and larger numbers of preserved distal 5-HT fibers than vehicle treated animals. After a treatment at 20 weeks with diphtheria toxin to specifically ablate human cell functions, these effects were reversed, suggesting that the (most likely differentiated) Muse cells were playing continuing critical roles.
- **Intracerebral hemmorhage in rats [18];** after intravenous delivery of Muse cells, rats had improved motor performance after only 2 days compared to vehicle or intravenous human menstrual blood derived endometrium stem cells (MenSCs). MenSCs showed improvement after 3 days but the extent of improvement was superior with Muse cell treatment. Using histology of brain sections, both MenSCs and Muse cells were able to reduce inflammatory cell infiltration in the injured region, with Muse cells had a significantly greater reduction.

Urology

- **Interstitial cystitis (rat) [19]:** Treated rats did not have increased urination frequency or decreased bladder capacity and did not develop nociceptive behavior after a 0.2 N HCl challenge compared to vehicle and non-Muse cell treated groups.
- **Erectile dysfunction following cavernous nerve injury (rat) [20]:** 28 days after intravenous infusion, Muse cell treated animals showed a significant positive response in intracavernous pressure and arterial pressure after pelvic nerve electrostimulation compared to vehicle or MSC infused groups.

Clinical Studies with Muse Cells

A number of clinical trials using culture-expanded Muse cells have been performed, employing the allogeneic product CL2020, developed by the Life Science Institute (a Mitsubishi Chemical Group subsidiary). Note: development of CL2020 was discontinued in 2023 due to shifts in business strategy, though the underlying science remains actively pursued.

A number of clinical trials using culture expanded Muse cells have been performed. These studies these have used an allogenic Muse cell product, CL2020, developed by the Life Science Institute, a subsidiary of the Mitsubishi Chemical Group. In 2023, the development of CL2020 was stopped, in part because of shifts in Mitsubishi Chemical Group's pharmaceutical business strategy.

- 3 subjects treated for ST-elevation myocardial infarction [21]; left ventricle ejection fraction and wall motion score index showed marked improvement.
- Open label non controlled trial in epidermolysis bullosa [22]; statistical improvement in ulcer size and patient reported pain were seen at early times after a single administration of Muse cells.
- Randomized placebo-controlled trial of allogenic Muse cells in subacute ischemic stroke [23]; positive responses (modified Rankin Scale (mRS) scores ≤2 at week 12 compared to a baseline of ≥3 at baseline) were seen in 40% of the treated patients (n=25) compared to 10% of vehicle treated (n=10).
- Phase II, open trial of allogenic Muse cells in amyotrophic lateral sclerosis (ALS) [24]; demonstrated safety but questionable efficacy (note that the trial only included 5 subjects).
- Nonblinded single arm study of cervical traumatic spinal cord injury [25]; 10 patients were treated and statistically significant improvement in motor scores, activity, and quality of life scores improved from baseline following a single IV dose.
- A pilot study of neonatal hypoxic-ischemic encephalopathy (HIE) [26]; 9 neonates were given a single Muse cell intravenous dose along with therapeutic hypothermia. All subjects survived, and 67% had normal scores in the Kyoto Scale of Psychological Development at 78 weeks compared with 33% in a registry of neonates in Japan born with HIE and treated with therapeutic hypothermia.

These studies form an exciting body of data around the potential for Muse cells to treat a wide array of serious conditions, often without standards of care giving satisfactory outcomes. More work will be needed to refine the understanding of the ideal patient characteristics, dose (both level and regimen), and beneficial co-therapies, but for such a novel treatment to already have demonstrated pilot-level successes in the clinic is a cause for great optimism going forward.

> **KEY INSIGHT**
> Across all published human clinical trials with Muse cells, no tumorigenicity has been reported. Clinical efficacy has been maintained for at least 52 weeks — suggesting durable engraftment without immune rejection.

Safety Profile: Immunogenicity and Tumorigenicity

Immunogenicity

Muse cells, like many other stem cells, have a generally immunopriveleged phenotype. However, Muse cells go beyond the standard tricks of having low HLA expression, with three mechanisms contributing to minimized immune response.

- **First,** they have a low expression of MHC class II receptors, which are largely responsible for the helper T cell response critical for initiating a rejection [27].
- **Second,** they express HLA-G, a special non-classical MHC class I receptor, which binds inhibitory receptors on many immune cells (e.g., T cells, NK cells, dendritic cells) [27].
- **Third,** Muse cells have a stronger expression of signaling molecules such as TGF-β and IL-10, which can stimulate regulatory T (Treg) cells and promote a generally anti-inflammatory and protective environment [12].

In clinical settings, Muse cell-mediated efficacy has been maintained for at least 52 weeks, suggesting that administered cells had not been rejected for at least that duration [23].

Tumorigenicity

Culture expanded MSCs have a tendency to undergo spontaneous mutations which were shown to lead to increased tumor formation in preclinical models [28]; the stress of rapid division can exacerbate this tendency [29]. To build confidence that culture expanded Muse cells will not have this risk, it is helpful to examine the properties of these cells in culture. Critically, Muse cells maintain low telomerase activity [30]. High telomerase activity is found in virtually all cancers [31], and is believed necessary for "immortalizing" the cancer cells and maintaining their unchecked replicative potential. Muse cells also maintain expression of genes such as ataxia-telangiectasia mutated (ATM) kinase, which are involved in sensing DNA damage to trigger cell cycle arrest or apoptosis to eliminate cells undergoing mutation [32], to ensure that only Muse cells most similar to their native form are able to persist and replicate in culture.

A systematic review in 2025 of all human clinical trials with Muse cells found that across all studies, no tumorgenicity was reported [33].

Discussion and Conclusions

The field of regenerative medicine has advanced rapidly in recent decades, following a pattern of growth factor-mediated therapies like platelet rich plasma that primarily serve to signal to other cells that repair is necessary, to cell-based therapies like MSCs derived from bone marrow or adipose that directly guide (but do not become part of) the repair. Muse cells appear poised to be the next step in that evolution: the administration of cells that can directly participate in the repair by becoming the needed cellular components of the damaged tissue. This can allow for treatment of tissues such as heart that historically have limited healing potential even when augmented with existing regenerative medicine procedures, or potentially for more severe injuries where the remaining tissue structure is insufficient for the guidance provided by MSCs on its own to affect a proper repair. The excellent observed safety profile in clinical and preclinical experience, and the emerging understanding of the biology underpinning this safety, can give trial designers and participants some confidence that limited risks are involved in pursuing novel applications.

An important area for further investigation is the long-term performance of allogenic versus autologous Muse cells. While all studies to date suggest that Muse cells maintain an immune privileged profile when dosed from allogenic sources (or even from a different species), it is possible that after more time than has been investigated, any diminishment of the mechanisms discussed conferring the immune privilege may ultimately lead the immune system to reject the differentiated Muse cells and reduce or eliminate the beneficial response (as was seen when Muse cells were selectively eliminated in the rat spinal cord injury model discussed earlier).

While more work is needed to identify the best applications, doses, and co-therapies to achieve the best possible results, the work to date with Muse cells reinforces the exciting and perhaps central role they will play in the further evolution of the field of regenerative medicine. Muse cells may very well represent the "holy grail" of regenerative medicine: a treatment with an admirable safety profile with potent capabilities to identify and repair a wide range of serious conditions.

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