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# Evaluation of Mesenchymal Stem Cells in Autoimmune Disease: A Systematic Review and Meta-Analysis

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# EVALUATION OF MESENCHYMAL STEM CELLS IN AUTOIMMUNE DISEASE: A SYSTEMATIC REVIEW AND META-ANALYSIS

by

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## ABSTRACT

Mesenchymal stem cells, also known as multipotential stem/progenitor cells and mesenchymal stem/progenitor cells, are denoted by the acronym MSCs. MSCs can differentiate into multiple lineages from different germ layers *in vitro*, and in limited situations, *in vivo*. MSCs have broad anti-inflammatory and immune-modulatory properties, which is the greatest focus of MSCs in clinical testing. Despite their promise and use in over 950 clinical trials, pivotal questions remain unanswered. Recent clinical trials have demonstrated that when MSCs are harvested from autoimmune patients, they show a reduction in their therapeutic efficacy in comparison to MSCs from a healthy person. Identifying quality MSCs through patient selection is one area that could provide substantial advancements in the basic understanding and clinical application of MSCs. By conducting a systematic review and meta-analysis, our results indicate that MSCs from autoimmune patients possess fundamental differences compared to their healthy counterparts. MSCs from autoimmune patients displayed reduced characteristics of stemness and regenerative properties, such as decreased differentiation potential and proliferation. These fundamental differences, and poor defining criteria, could explain MSCs inability to demonstrate therapeutic effectiveness in clinical trials.

# ACKNOWLEDGMENTS

I want to begin thanking the individual that has been instrumental in my success in the master's program at Missouri S&T, my advisor, Dr. Julie Semon. Without her, I would not have seen the potential within myself. She pushed me beyond what I thought were my limits and helped me learn to look at the bigger picture. I would not be writing this thesis if not for her continuous guidance, support, and understanding. I cannot express in words the gratitude I feel for Dr. Julie Semon. I only hope I can become half the person she is, and I will strive every day to become the scientist she believes me to be.

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# NOMENCLATURE

| Symbol | Description |
|---|---|
| HC | Healthy Control |
| AD | Autoimmune Disease |
| BMSCs | Bone Marrow-Derived Mesenchymal Stem Cells |
| ASCs | Adipose Derived-Mesenchymal Stem Cells |
| d-MSCs | Dermal Derived-Mesenchymal Stem Cells |
| SF-MSCs | Synovial Fluid Derived-Mesenchymal Stem Cells |
| AD-MSCs | Autoimmune Disease Mesenchymal Stem Cells |
| HC-MSCs | Healthy Control Mesenchymal Stem Cells |
| RA | Rheumatoid Arthritis |
| MS | Multiple Sclerosis |
| SS | Systemic Sclerosis |
| SLE | Systemic Lupus Erythematosus |
| ND | No Difference |
| EAE | Experimental Autoimmune Encephalomyelitis |
| wtASCs | Wild-Type Adipose Stem Cells |
| GvHD | Graft Versus Host Disease |
| QA | Quality Assessment |
| IVAT | *In Vitro* Assessment Tool |

# 1. INTRODUCTION

Mesenchymal stem cells, also known as mesenchymal stromal cells or medicinal signaling cells, are commonly denoted by the term: MSCs[1–3]. First isolated 50 years ago from the adherent portion of bone marrow, they are a spindle shaped cell population that can adhere to plastic and maintain a fibroblast-like morphology[1,2,4]. Originally identified in adult bone marrow, MSCs are now sourced from additional tissues, including peripheral blood, umbilical cord tissue and blood, dermal tissue, adipose tissue, and synovial fluid[5–7] (Figure 1.1.).

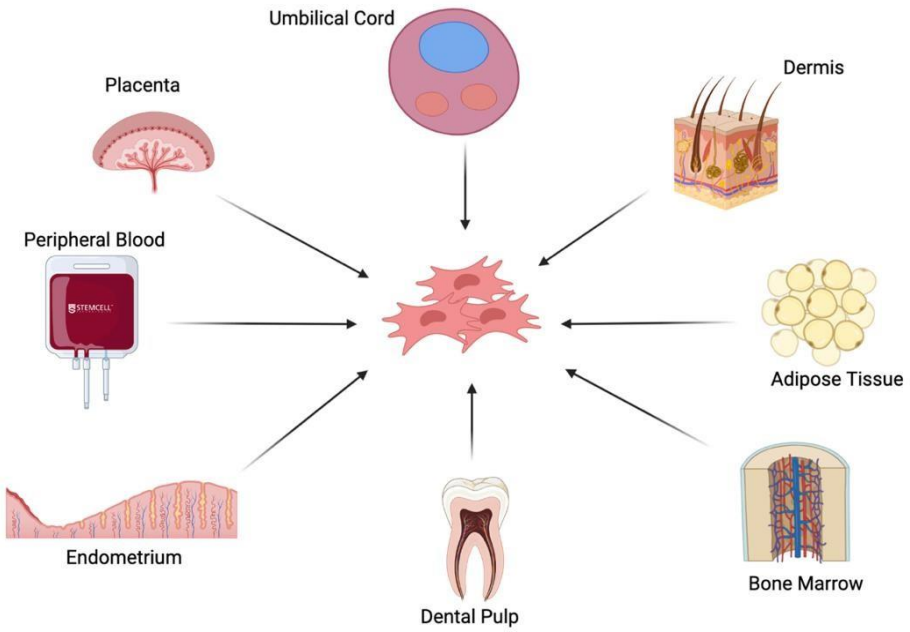


Figure 1.1. Source of MSCs. The most common tissue sources for MSCs include adipose tissue and bone marrow.

MSCs have been studied in over 950 clinical trials throughout the world[8]. Bone marrow derived MSCs (BMSCs), still the most common source of MSCs for clinical

trials, are found along the endosteum and are isolated by aspiration and separation with a ficoll gradient[9–11]. However, BMSCs are an invasive harvest, especially in patients with skeletal disorders[12]. Adipose-derived MSCs (ASCs) are extracted from subcutaneous fat and isolated from the stromal vascular fraction (SVF). They have a similar therapeutic effect of BMSCs, are acquired by a non-invasive harvest, and provide a large number of cells after isolation[12–14]. MSCs can also be acquired from synovial fluid (SF-MSCs) by extracting fluid from the hip or knee joints, or from medical waste popliteal cyst to avoid further injury to patients[15–17]. SF-MSCs have also been shown to have increased chondrogenic and osteogenic potential when compared to BMSCs[15,16,18]. Another source of MSCs is the dermis, usually from a skin biopsy or the foreskin (d-MSCs)[19–21]. Similar to other sourced MSCs, d-MSCs possess wound healing properties, but they also have the ability to differentiate into both neural and mesodermal cells[18,21]. Overall, all MSCs possess similar characteristics that allow them to be therapeutic, such as migrating to damaged tissue, stimulating angiogenesis, engrafting into target tissue, and regulating immune responses[18,22–24].

Regardless of their source of origin, MSCs were initially of clinical interest because of their ability to self-renew and replace damaged or diseased tissue by differentiating into multiple lineages, such as osteocytes, chondrocytes, and adipocytes[8,25–27]. However, the current paradigm is that MSCs are useful in clinical applications because they are generally considered non-immunogenic, possess immune modulatory properties, and secrete an extensive array of growth factors and cytokines, which can activate and support endogenous cells[23,28]. Recent studies have focused on the MSC's non-immunogenic and immunoregulatory properties, and how MSCs possess

qualities that make them a prime candidate for therapeutic applications in patients with autoimmune disease (AD)[29–31]. Currently, autoimmune diseases are treated with immunosuppressants to prevent further damaged caused by the patient's immune system[32–34]. MSCs offer an alternative to the traditional therapies by acting on the immune cells, essentially "resetting the deregulated immune system of patients with severe autoimmune diseases"[29–31].

AD remains a growing population of individuals that currently lack a cure, or form of treatment that repairs damage caused by the patient's immune system[32–34]. Approximately 320 million of the world's population has been diagnosed with at least one of the 80 ADs, and its prevalence continues to rise[32,35]. This analysis focuses on five ADs, which are among the list of most commonly diagnosed ADs: multiple sclerosis (MS), psoriasis, systemic lupus erythematosus (lupus), rheumatoid arthritis (RA), systemic sclerosis (SS)[32,33]. Numerous studies have shown evidence, for these ADs in particular, that MSCs possess the characteristics needed to ameliorate these diseases[29–31,36–38].

Despite the potential MSCs have for clinical applications in ADs, the therapeutic efficacy of MSCs in many clinical trials has shown inconsistent results[39–41]. Additionally, pre-clinical results have shown the inherent limitations that must be addressed in order to provide more clinical success of all types of MSCs [39–41]. Studies have demonstrated MSCs derived from patients with ADs are genetically distinct, resulting in inconsistent therapeutic effects between MSCs from AD patients and healthy persons[42–57]. These fundamental differences have been shown to result in discrepancies in clinical outcomes,

increasing the difficulty for interpretation [40,41]. Studies have also shown that murine models using MSC therapy for AD have a reduced therapeutic potential[58] (Figure 1.2.).

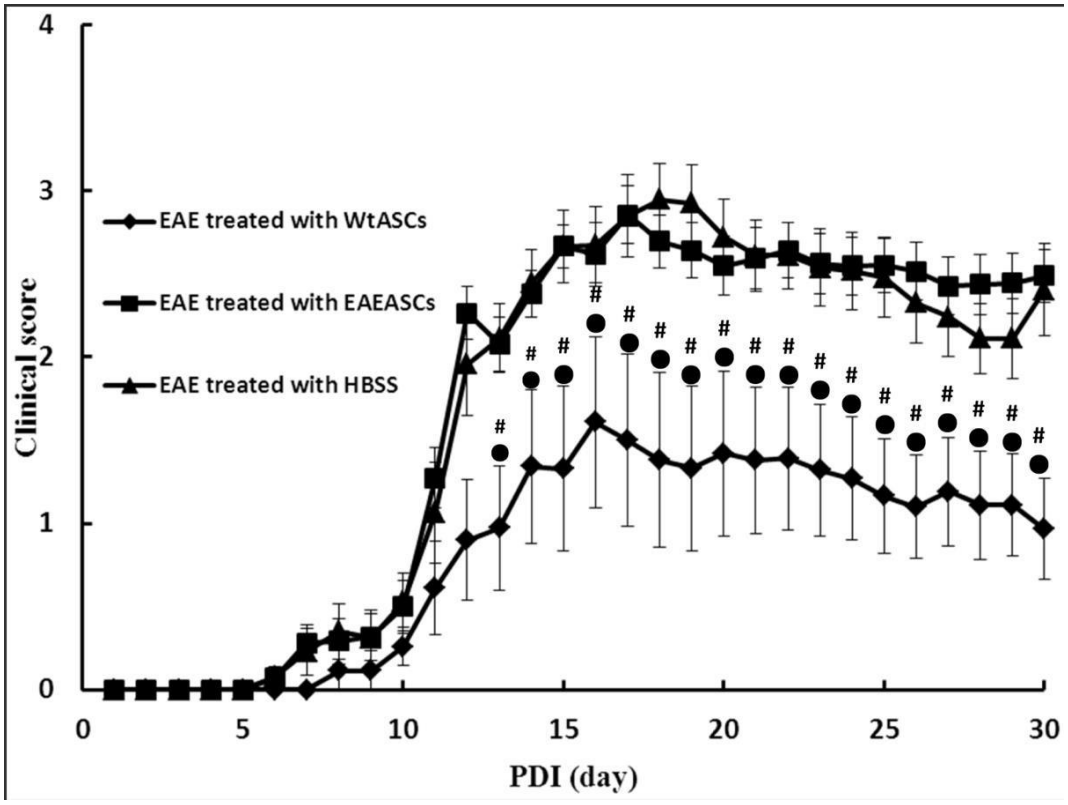


Figure 1.2. Multiple Sclerosis Murine Model. Multiple sclerosis induced murine model treatment with wtASCs and autologous EAE ASCs[58].

With these discrepancies and limitations, investigators are still working to find the optimal source for MSCs. Identifying quality MSCs through patient selection is one area that could provide substantial advancements in the basic understanding and clinical application of MSCs. It is our hypothesis that MSCs from autoimmune patients possess fundamental differences compared to healthy individuals, and thus result in disappointing clinical outcomes. Therefore, the results from this analysis provide further insight on these fundamental differences and impact the criteria used for establishing quality

sources for MSCs. Additionally, this analysis provides a better understanding on the underlying mechanisms behind the pathogenesis of AD.

# 2. MATERIALS AND METHODS

The study abided by the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) set forth by the provided checklist[59].

## 2.1. SEARCH STRATEGY

Three independent investigators performed literature searches from February of 2022 until May 2022 using PubMed and Scopus. The following search terms were utilized in each database: ("mesenchymal stem cell" OR "mesenchymal stromal cell" OR "MSCs" OR "ASCs") AND ("Scleroderma" OR "Psoriasis" OR "Multiple sclerosis" OR "Systemic sclerosis" OR "Rheumatoid arthritis" OR "Lupus"). Weekly updates were provided from Google Scholar, PubMed, and Scopus if any studies were published that matched the search terms.

## 2.2. INCLUSION AND EXCLUSION CRITERIA

Eligibility criteria were determined prior to beginning the searches and applied to studies during abstract and title screening, as well as during the full text assessment. Studies were included if: 1) there was a comparison between MSCs that were isolated from healthy individuals and MSCs isolated from individuals with one of the five autoimmune diseases listed in the search terms; 2) Studies were published and accessible in English; 3) studies included MSCs from any tissue source; and 4) studies were peer-reviewed. Studies were excluded if: 1) MSCs were derived from non-human species; 2)

MSCs were treated with any pharmaceutical agent for the duration of the study; or 3) the article was a review, conference proceeding, or retracted study.

## 2.3. STUDY SELECTION

Three investigators, independently, recorded the number of results produced by each search term. Duplicates between the search terms were removed. Results between the two databases were then combined and duplicates between the two databases were removed. Investigators independently screened titles and abstracts for eligible studies utilizing the predetermined exclusion criteria. Eligible studies were then forwarded to a fourth investigator who removed duplicates between investigators. Eligible studies were subjected to a full text assessment utilizing the same exclusion criteria that was used during the titles and abstract screening. Full text assessment was completed by two investigators and any discrepancies were discussed.

## 2.4. DATA EXTRACTION

Two investigators extracted relevant data, which consisted of: (1) cell demographics (cell source, isolation location, and passage); (2) donor demographics (number of patients/controls, age, BMI, disease duration, and gender); (3) proliferation rate, assays, duration, and results; (4) differentiation assays, duration, and results; (5) surface antigens (positive markers, negative markers, and thresholds); (6) cell morphology and plastic adherence; (7) angiogenic assays; (8) any other assays performed in the study (microarrays, PCRs, western blots, etc.). Once data was extracted from all

included studies, a third investigator combined both excel files and compared data. Any discrepancies were discussed.

## 2.5. QUALITY ASSESSMENT

Each study was assessed for overall quality and risk of bias. A customized assessment tool called the In Vitro Assessment Tool (IVAT) was created to determine the quality and risk of bias for each study (Table 1). The parameters of IVAT that were used to assess the risk of bias include:

(1) Selection bias

(2) Performance Bias

(3) Detection Bias

(4) Reporting Bias

Questions for each type of bias were applied to each study and responses were recorded in an excel sheet. Based on the responses, the studies were appointed specific points. Once all studies were assessed, these points were totaled and put into a percentage. Studies were assigned a risk of bias based on where their percentage score fell in the predetermined range. No studies were removed following quality assessment.

## 2.6. ISSCR/ISCT ANALYSIS

Image J software (or Fiji) was used to quantify the differences between differentiation images extracted from studies. Images were obtained as JPGs, and relevant data, 1) magnification of images; 2) stain used; 3) cell source; and 4) duration of the assay performed, was recorded. The percent area was then calculated from Image J

and recorded in a separate excel sheet. From the percent area, the index was calculated by setting all control images to 1 and dividing the percent area of the AD-MSC differentiation image by the percent area of the HC-MSC differentiation image. The indexes were then averaged and graphed based on differentiation potential, cell source, and disease.

# 3. RESULTS

## 3.1. STUDY SELECTION

The primary literature searches produced 28,439 potential studies (Figure 3.1.). With these studies, 7,033 were for multiple sclerosis (MS), 8,445 for lupus (SLE) 7,286 for rheumatoid arthritis (RA), 4,175 for systemic sclerosis (SS/Scleroderma), and 1,500 for psoriasis. Duplicates were then removed, resulting in 13,452 studies that were subjected to abstract and title screening utilizing previously mentioned exclusion criteria. There were 12,678 studies that were excluded, resulting in 774 studies that were sent to the primary investigator, where duplicates between independent investigators were removed. There was full-text assessment of 496 studies utilizing the same criteria for the title/abstract screening process. This resulted in 404 studies being excluded, and 92 studies that were subjected to qualitative analysis.

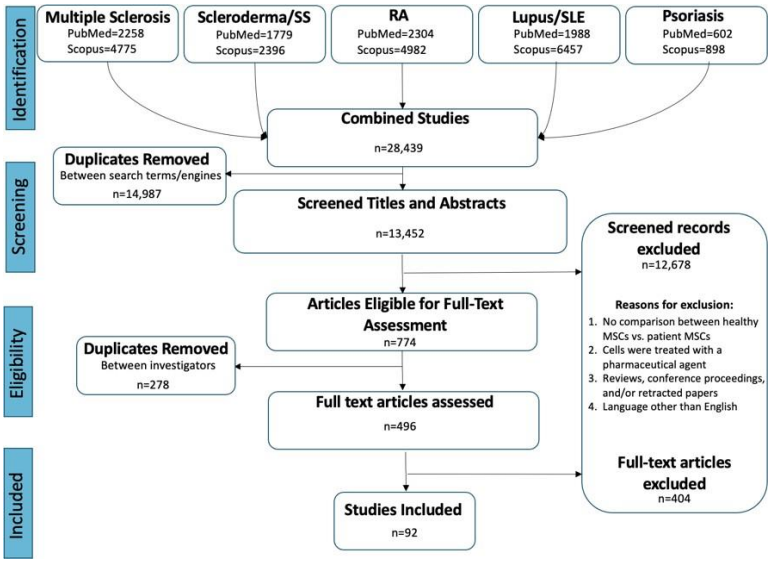


Figure 3.1. Schematic of Search and Study Selection Protocol. A total of 92 studies metinclusion criteria.

## 3.2. STUDY QUALITY

The IVAT was used to determine the quality of studies, as well as determine any risk of bias (Table 3.1.).

Table 3.1. IVAT Criteria. Questions to determine overall quality of each study.

| | Selection Bias | | | Performance Bias | | | Detection Bias | | | Reporting Bias |
|---|---|---|---|---|---|---|---|---|---|---|
| | Was the origin of patient and control MSCs reported? | Were the patient and control MSCs the same source, age/gender-matched, and passage? | Was the duration/stage/ type of the disease reported? | Was a standard culture media used for control treatment? | Were the experiments conducted in triplicate? | Were at least 3 donors evaluated? | Were reliable assays used to assess the outcome? | Were all methods described exhaustively? | Statistical methods were appropriate | Were all the results correspondin g to each method described? |
| ++ | specific location for both patient and control | Control and patient matched on all factors | All patients had both duration and "stage" are reported | Yes, name and source of media | Triplicate for all assays | 3 donors for each assay | All | All | All | All results corresponded to each method |
| + | general location for both patient and control | Control and patient matched on most factors | All patients had either duration or "stage" reported | Yes, name and source not mentioned | Most | Most | Most | Most | Most | Most results corresponded to each method |
| ? | unknown = not reported | Unknown | Unknown | unknown | unknown | unknown | unknown | unknown | unknown | unknown |
| - | specific location for patient; unknown location for control | Control and patient matched on 1-2 factors | Some patients had duration or "stage" reported | FBS, basal media, supplements OR concentration not standard | Some | Some | Some | Some | Some | A couple results coresonded to each method |
| -- | general location for patient; unknown location for control | Control and patient not matched | Duration and "stage" were not reported | Not standard culture media | None | None | None | None | None | No results corresponded to each method |

Most studies produced a moderate to high quality score, with 70 studies being high (scores 21-30), and 19 studies being moderate (scores 11-20) (Figure 3.2.A). Selection, detection, and reporting biases all had high percentages of good quality (100%, 86%, 98%, respectively) (Figure 3.2.B). All bias categories had low percentages of poor quality. Performance bias had a slightly lower percentage of good quality, and higher percentage of unknown (71.5%,27.3%, respectively). This lower score was mostly due to if a study conducted experiments in triplicate which had 56.2% unknown, or not reporting. Half of the questions demonstrated some levels of poor quality, but these percentages of poor quality were relatively low (<14.6%) (Figure 3.2.C).

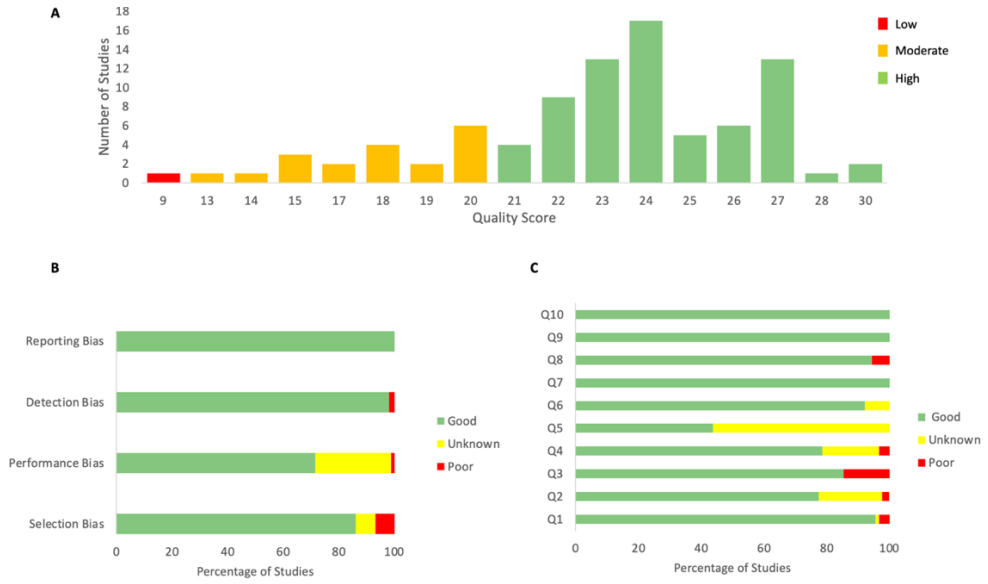


Figure 3.2. Study Quality with IVAT. A) Study quality scores. B) Quality per bias category. C) Quality for each question

## 3.3. STUDY AND SAMPLE CHARACTERISTICS

The demographics of patients and characteristics of the cells analyzed within this study was recorded based on number of studies and sample size.

**3.3.1. Study Demographics.** About 33% of the included studies analyzed MSCs from psoriasis patients, SS and SLE were each about 20% of the studies, where RA and MS each consisted of about 10% of the included studies (Figure 3.3.A). All studies were published between the years of 2000 and 2022, with most studies published in 2013 or later (Figure 3.3.B). The age and gender of patients were commonly reported throughout studies (87.2% and 78%, respectively), but only a small percentage of studies (<10%) reported BMI or race of their patients (Figure 3.3.C). Of the studies that did report gender, 23% of samples and controls were isolated from males, while 55% of samples and 44% of controls were isolated from females (Figure 3.3.D). Lupus studies isolated their samples predominantly from females, with the exception of one male sample[60]. The

other four diseases were isolated from both males and females. The average age of samples analyzed was 37.8 years, while average age of controls was 51.2 year (Figure 3.3.E). Samples from RA had the highest average age, where lupus, psoriasis, and systemic sclerosis all had the lowest average ages (31.2, 35.2, 36.2 years, respectively). Of the few studies that did report BMI, they all isolated MSCs from adipose tissue. The average BMI of samples was 24.8 and for controls was 25.9. Of those who reported race, 48% of samples were isolated from individuals that were Asian, 45.3% from Caucasians, and a small percentage of samples (~1-5%) were from African American or Middle Eastern persons. Controls were reported to be isolated from Asian (77%) and Caucasian (22%) persons.

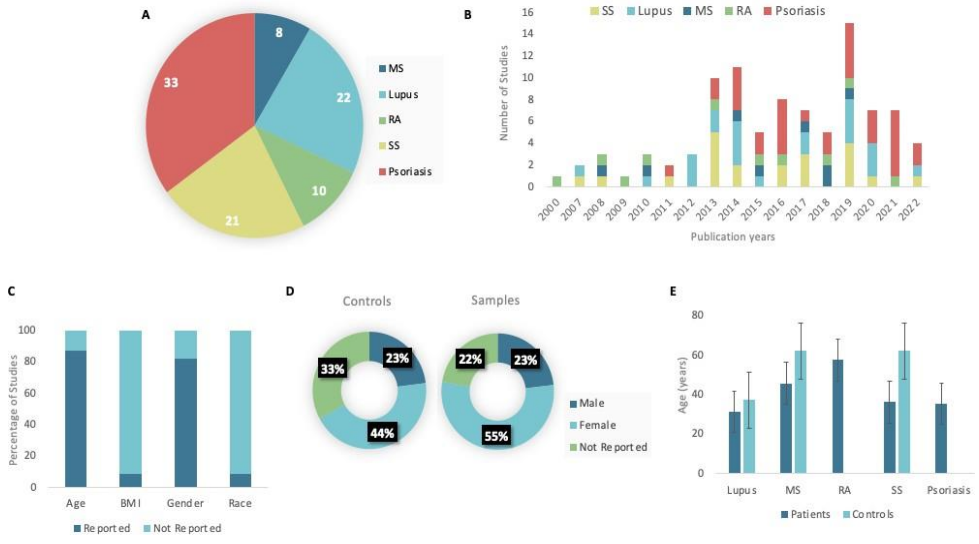


Figure 3.3. Characteristics of 92 Included Studies. A) Studies included for each disease of interest. B) Publication years for each study. C) Percentage of studies that reported demographics of samples. D) Gender of controls and samples. E) Average age of controls and samples.

**3.3.2. Sample Characteristics.** MSCs were reported as being isolated from bone marrow (BMSCs) in 45 of the studies and representing 44.6% of the samples analyzed (Figure 3.4.A and 3.4.C). BMSCs were commonly isolated from the iliac crest, with the smallest percentage isolated from trabecular bone chips (Figure 3.5.). MSCs were also isolated from the dermis (d-MSCs) in 33 studies and representing 34.5% of samples. D-MSCs were commonly isolated from the dermal layer, but specific locations were not reported. MSCs from adipose tissue (ASCs) was reported in 13 studies and represented 12.1% of samples. ASCs were mostly (84.5%) isolated from the subcutaneous layer in the abdomen. MSCs being isolated from synovial fluid (SF-MSCs) was reported in 4 studies and represented 8.8% of samples. SF-MSCs were unanimously isolated from synovial fluid from different joints. Studies from the psoriasis group mainly focused on d-MSCs, with the exception of one study focusing on BMSCs (Figure 3.4.B and 3.4.D). MS Studies only evaluated BMSCs, while studies from lupus, RA, and SS were more diverse in cell source. In total, 1,232 patient samples were analyzed and compared to 1,000 control samples. Samples were mostly (97%) obtained internally (Figure 3.4.E). Controls were also mostly obtained internally (92.6%) but were also purchased from vendors (5%), with the most common vendor being Lonza.

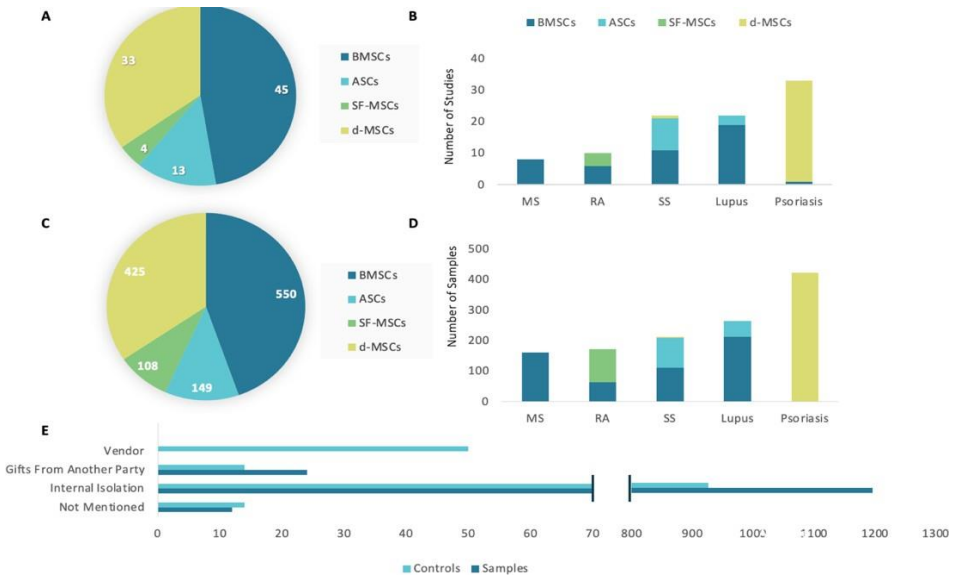


Figure 3.4. Source of Cells. A) Cells sources observed in included studies. B) Cell sources used for each disease of interest. C) Total number of samples analyzed and their cell source. D) Samples cell sources for each disease of interest. E) The process of how samples and controls were obtained.

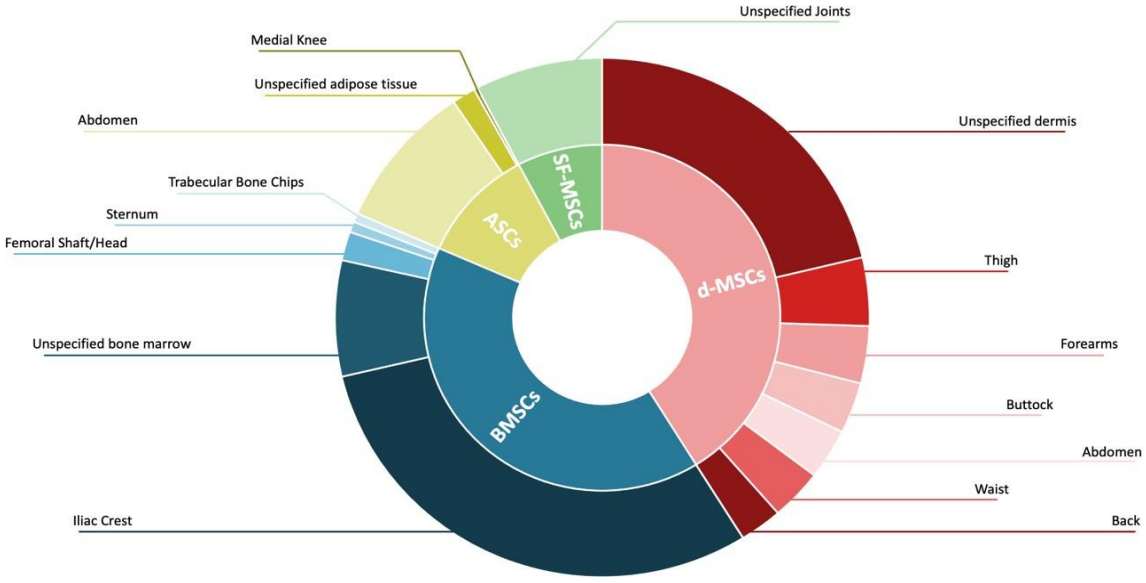


Figure 3.5. Anatomical Locations of Patient Tissue.

### 3.4. ISSCR/ISCT REPORTING AND ANALYSIS

As the aim of our study is to compare MSCs from autoimmune patients to MSCs from normal, healthy persons, it is important to identify all the samples as MSCs. ISCT states that for an MSC to be considered an MSC, it must meet five criteria: adhere to plastic , have a fibroblast-like appearance, self-replicate, have appropriate surface antigens, and differentiate into bone, fat, and cartilage[61]. Proliferation was reported in 40.2% of studies (Figure 3.6.A). More than half the studies (55.4%) reported morphology of their samples. Although only 19.6% of studies reported adhesion properties, adhesion is inherent among all assays in each paper. Differentiation into bone, fat, and cartilage was reported in only 31.5% of studies. However, an additional 22.8% of studies showed differentiation into two tissue types, most commonly adipocytes and osteocytes. Out of all 92 studies, only six reported all five required criteria, with 11 not following ISCT guidelines for MSc identification (Figure 3.6.B). For identification, MSCs must be positive for CD105, CD73, and CD90 and negative for CD45, CD34, CD14/CD11b, CD79α/CD19, and HLA-DR [61]. Over half the studies (59.8%) followed the ISCT criteria regarding surface antigens. However, 18.5% did not follow ISCT standards.

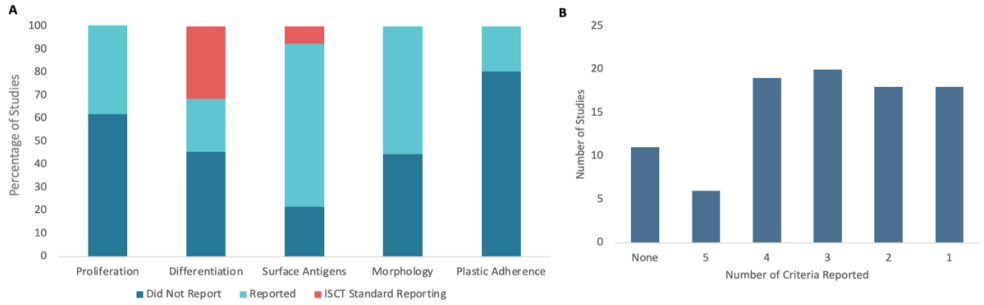


Figure 3.6. ISSCR/ISCT Reporting. A) Percentage of studies that reported each ISSCR/ISCT criteria. B) Number of studies and number of criteria reported for each

**3.4.1. Surface Antigens.** From all the studies that reported surface antigens, the occurrence of the ISSCR/ISCT required surface antigens was recorded (Figure 3.7.A). With the exception of the studies that reported following ISSCR/ISCT standards, others did report at least one of the required surface antigens for both positive and negative. Most of the required markers were reported in over half the studies, with CD79α or CD19 only being reported in 15.2% of studies. The ISSCR/ISCT also provide a threshold that should be used when assessing what antigens are positive versus negative in cells. If the presence of the antigen is ≥95%, it is considered positive. If the presence of the antigen is less than or equal to ≤2%, it is considered negative. The negative cutoffs ranged from <0.1% to <9%, with 48 studies not mentioning their threshold for negativity (Figure 3.7.B). The positive cutoffs ranged from >10% to >95%, with 57 studies not mentioning their threshold for positivity (Figure 3.7.B).

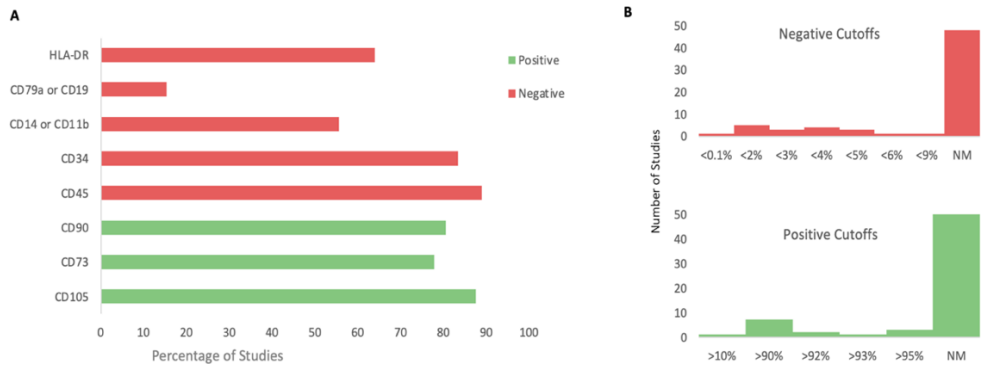


Figure 3.7. Surface Antigens and Cutoffs. A) Percentage of studies that reported standardsurface antigens. B) The positive and negative cutoffs used to determine surface antigen positivity and negativity.

**3.4.2. Morphology and Plastic Adherence.** Of the studies that reported cell appearance, 87% reported that autoimmune MSCs were capable of presenting a fibroblast-like morphology similar to the healthy controls. Of the 12.9% studies that reported autoimmune MSCs presented abnormal morphologies, with the cells being larger, flatter, and having longer podia (Figure 3.8.).

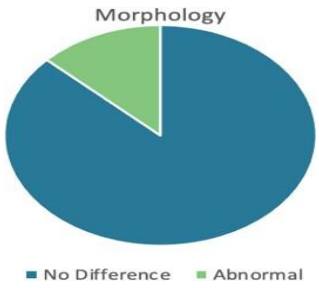


Figure 3.8. Abnormal Morphology of AD-MSCs. Results from included studies.

Some studies further evaluated morphological differences by evaluating cytoskeleton and organelles. Autoimmune MSCs had irregular actin distribution, being disorganized and condensed on the edge of the cytoplasm[52]. Additionally, autoimmune

MSCs were shown to have disorganized cell structure with dilated and distorted ER, swollen mitochondria, condensation of chromatin, increased protein aggregates in ER human, and apoptotic features[62,63]. Unsurprisingly, abnormal morphology was associated with senescence, increased cell size, and cytoplasmic granularity with increased passage[64,65].

**3.4.3. Proliferation.** The 40.2% of studies that reported proliferation utilized a variety of methods to determine the proliferative abilities of their MSCs (Figure 3.9.B). The majority of studies (46%) measured cell growth, mainly evaluated by population doublings and trypan blue (Figure 3.9.A). The rest of the types of evaluation were evenly divided between measuring colony forming units (CFU), analyzing the cell cycle (including ki67 and BrdU), and enzymatic assays such as MTT and CCK kits. The most common duration of these experiments was 12-15 days, with 1-3 days and 18+ days occurring the least (Figure 3.9.C). There were 10 studies that did not report how long they conducted their assays. The majority of studies (64%) showed that MSCs from autoimmune patients had a decreased proliferative ability at some point, with 7% initially decreased then reaching rates similar to controls, 4% initially showing no difference then decreasing, and 53% reporting a consistent decreased proliferative capacity. The only reports of an increased capacity of proliferation came from psoriasis studies, and psoriasis had the most discrepancies in terms of proliferation. All MS studies declared there was no difference in proliferative abilities between autoimmune MSCs and healthy MSCs. Lupus studies demonstrated that MSCs began to grow at similar rates to healthy MSCs, but then began to decline. Conversely, cells from both SS and psoriasis patients were also shown to have decreased proliferative rates comparing to healthy MSCs but

were able to reach a point where they demonstrated similar growth rates (Figure 3.9.E and 3.9.F). The source of MSC did not seem to influence results, with all evaluated MSCs reported in multiple categories (Figure 3.9.D).

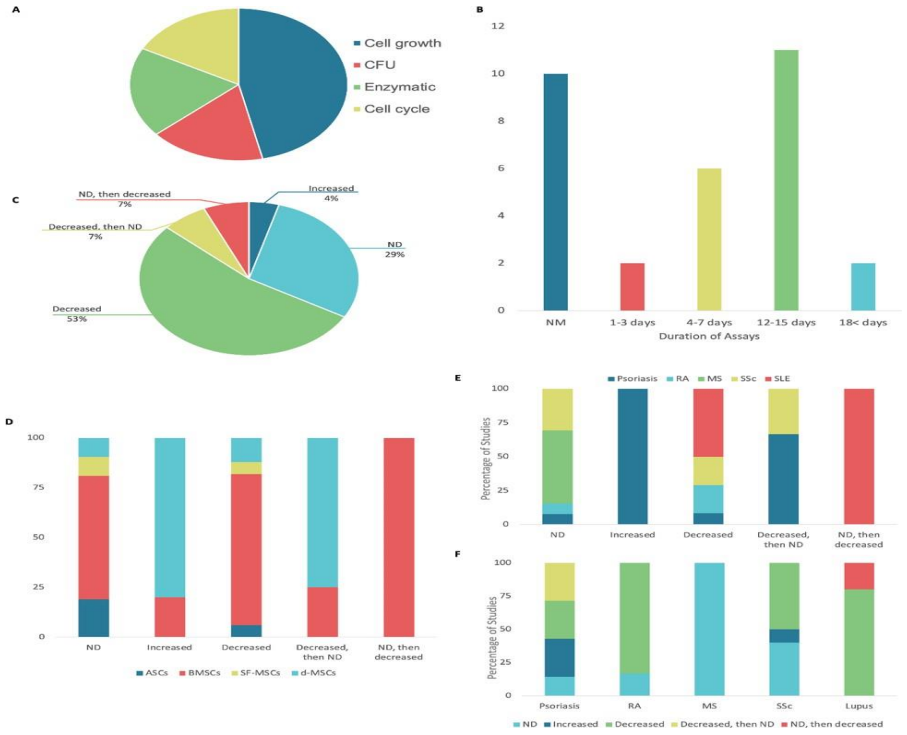


Figure 3.9. Proliferation of AD-MSCs. A) Proliferation assays that were used and percentage of studies that utilized each method. B) Duration of proliferation assays performed. C) Overall results for proliferative rates of AD-MSCs. D) Results of assays and cell source. E) Results of assays and disease type. F) Percentage of each study from each disease type, and overall proliferation outcomes.

**3.4.4. Differentiation.** Of the 54.3% of studies that reported differentiation potential, only 31.5% reported potential into bone, fat, and cartilage. Based on the image analysis, AD-MSCs demonstrated no significant difference in chondrogenic potential compared to HC-MSCs (Figure 3.10.A). Though there is a trend showing decreased osteogenic potential, the p-values calculated did not reflect any significance. However,

there were large variances in terms of standard deviation for both osteogenic and adipogenic potentials. This led to differentiation potential being evaluated by cell source to observe any dependency. Both ASCs and BMSCs did not have any data regarding chondrogenic differentiation, but did show a reduced osteogenic and adipogenic potential compared to HC-MSCs (Figure 3.10.B). SF-MSCs demonstrated a significant increase in chondrogenic and osteogenic potential, and a reduction in adipogenic potential. As for d-MSCs, they demonstrated a significant reduction in osteogenic potential, while their adipogenic and chondrogenic potential was similar to that of HC-MSCs. MSCs isolated from RA patients appeared to show a significant increase in both chondrogenic and osteogenic potential, with a significant decrease in adipogenic potential (Figure 3.10.C). While psoriasis showed a significant reduction in osteogenic potential, with adipogenic and chondrogenic potential similar to that of HC-MSCs. Lupus and SS demonstrated a significant reduction in adipogenic potential, with MS also showing reduced adipogenic potential. Overall, AD-MSCs appeared to have the most reduction in adipogenic potential, followed by osteogenic potential, while chondrogenic potential was not significantly different compared to HC-MSCs.

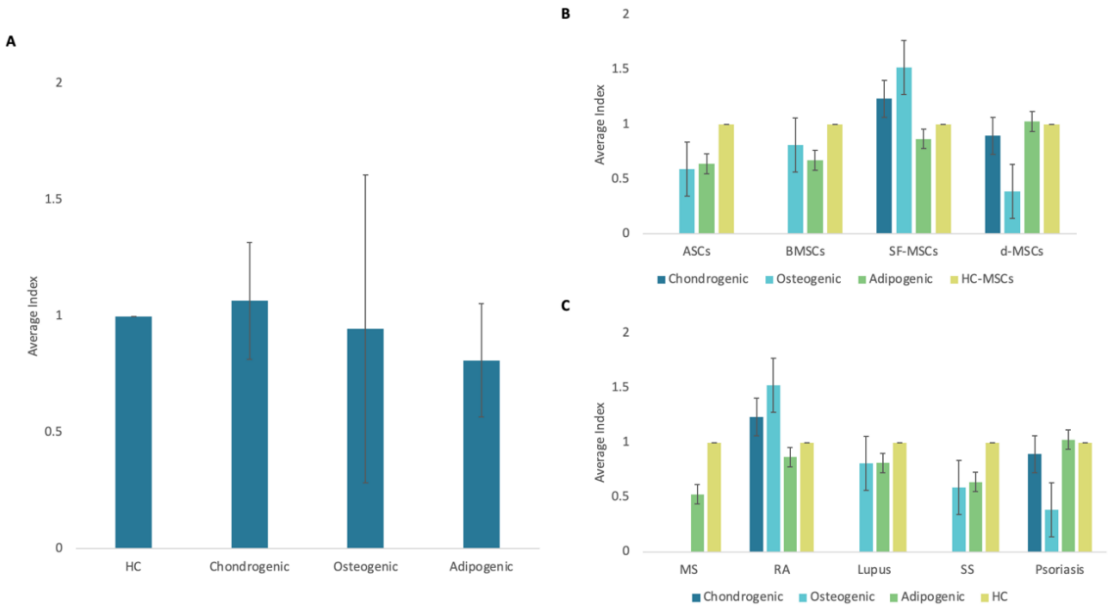


Figure 3.10. Differentiation Potential of AD-MSCs. A) Overall results of differentiation potential into chondrocytes (n=4), osteocytes (n=8), and adipocytes (n=9) of AD-MSCs. B) Differentiation potential of cell source from AD. C) Differentiation potential of each AD.

### 3.5. OTHER PHENOMENON

In addition to the traditional proliferation assays, senescence was also evaluated, typically with a beta-galactosidase staining (Figure 3.11.). While Velier et al., 2019 reported no difference between healthy controls and AD-MSCs, ten studies reported that they experienced an accelerated, or increased, senescence *in vitro*[56,57,64–73]. There were also dissimilarities regarding telomerase activity. Though Nie et al., 2010 shows that AD-MSCs have telomerase activity, others showed that they had an inactive telomerase and shortened telomeres[48,56,64,65,67,74]. Multiple studies from lupus also demonstrated that AD-MSCs were arrested in the G1 phase[69–72]. Based on other reports, AD-MSCs were also found to have an increase in apoptosis[48,63,68]. While Sun et al., 2007 showed that AD-MSCs had a normal karyotype, others showed that AD-MSCs had more DNA double-

stranded breaks and a greater percentage of DNA damage in the DNA tail compared to healthy controls[50,51,75]. Studies also found that the cellular bioenergetics were altered in autoimmune MSCs, as well as dysfunctional mitochondria[54,68]. AD-MSCs also demonstrated a reduced capacity for migration and invasion when compared to healthy controls[50,76,77]. In HUVEC proliferation assays, AD-MSCs were able to simulate HUVEC proliferation at a similar rate of healthy MSCs, but AD-MSCs increased the migration of HUVECs and increased tube formation when co-cultured with HUVECs when compared to healthy controls[78–80]. AD-MSCs demonstrated upregulation of ROS levels compared to healthy MSCs[68,75]. As for clinical effectiveness, only one of the included studies compared AD-MSCs to healthy controls *in vivo*. AD-MSCs demonstrated no therapeutic potential in a murine model of RA. The model demonstrated worsening symptoms following MSC administration[67].

Table 3.2. Phenomenon Altered in AD-MSCs.

| Phenomenon | AD-MSCs | References |
|---|---|---|
| Senescence | ↑ | 56,57,64–73 |
| Telomere Length | ↓ | 48,56,64,65,67,74 |
| Telomerase Activity | ↑ | 48,56,64,65,67,74 |
| Apoptosis | ↑ | 48,63,68 |
| ROS Levels | ↑ | 68,75 |
| DNA Damage | ↑ | 50,51,75 |
| Migration/Invasion | ↓ | 50,76,77. |

## 3.6. DIFFERENTIALLY EXPRESSED MOLECULES

AD-MSCs were found to have 413 differentially expressed molecules that consisted of genes, proteins, and miRNA

Table 3.3. Differentially Expressed Molecules Between AD-MSCs and HC-MSCs.

| Disease Occurrence | Molecules |
|---|---|
| 5 | *IL-6* |
| 4 | *TGF-β* |
| 3 | *HGF, p21, VEG-F, CCL2* |
| 2 | *18 Molecules* |
| 1 | *389 Molecules* |

The molecule that was found to be consistently upregulated in all five diseases was IL6 (Figure 3.11.). IL6 is a proinflammatory cytokine that plays a role in the pathogenesis of chronic inflammation and autoimmunity[81]. The next molecule that was found upregulated in four of the diseases was TGF-$\beta$, and this molecule is known to be a tumor suppressor by having antiproliferative and pro-apoptotic effects[82]. There were four molecules found in at least three of the diseases of interest: HGF, p21, VEG-F, and CCL2. These molecules were found to be expressed differently in each disease, with the exception of p21 being upregulated in all three diseases it was reported in.

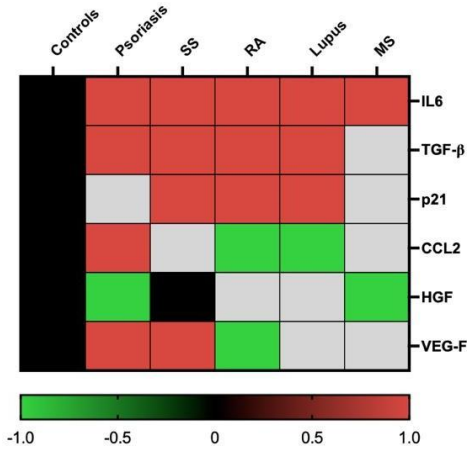


Figure 3.11. Differentially Expressed Molecules in AD-MSCs.

# 4. DISCUSSION

MSCs have shown beneficial effects in clinical trials for many disease outcomes, including GvHD and Crohn's disease. However, there are discrepancies on whether MSCs from autoimmune patients are as therapeutic in the clinic compared to MSCs from healthy persons[39–41]. With an abundance of preexisting *in vitro* studies analyzing MSCs from AD patients, we aimed to utilize the systematic review and meta-analysis approach.

Systematic reviews and meta-analyses provide an evidence-based medicine (EBM) approach that compiles higher levels of evidence from preexisting studies[83,84]. These methods of analyses provide clinicians and scientists with conclusions that will ultimately allow them to make calculated healthcare decisions that can improve clinical outcomes. However, the informativeness and quality of the analysis is only as good as the quality of the studies collected[83–85]. With an increasing trend of publications of *in vitro* studies, a quality assessment (QA) tool that can best determine the quality and risk of bias of these studies is crucial[83,85]. Though there are many QA tools that are used in systematic reviews and meta-analyses (Cochrane, AMSTAR, JBI, etc.), there is not a consistent QA tool for *in vitro* studies[86]. Herein this study, we introduced a QA tool that would benefit analyses looking to analyze *in vitro* studies: *In Vitro* Assessment Tool (IVAT). IVAT will allow clinicians and scientists to assess the overall quality and risk of bias of their pooled studies, resulting in more impactful conclusions. IVAT will select for quality studies to improve clinical outcomes, and specifically for this analysis, provide results that will assist in defining therapeutically effective MSCs.

While there are no criteria for defining a therapeutically effective MSC, the ISSCR/ISCT devised a set of standards to define an MSC in 2006. While there was an update in 2019, that updated focused on the variation in nomenclature of cells. Though this criterion has now been around for over seventeen years, results presented in this analysis indicates few studies are meeting the ISSCR/ISCT criteria. This lack of reporting may be one reason there are inconsistencies among the field for the last 20 years. Phinney et al., reported that even with MSCs increasing use in clinical trials to treat GvHD and Crohn's disease, the efforts made to provide efficacious therapies using MSCs is moving at a slow pace[87]. This staticity is due, in part, to the high variability found across facilities in regards to methods that assess MSC identity[87]. Pittenger et al., reported that it is necessary that each cultivation step is closely monitored to ensure consistency and reproducibility in both research and use in therapeutics[8]. Herein this study, we demonstrate that improvements can be made to further reduce the variability seen within the field, as well as provide a better understanding of the fundamental differences of AD-MSCs that could be contributing to poor therapeutic outcomes. Though the majority of our included studies showed that MSCs from autoimmune patients can differentiate into bone, fat, and cartilage, they do not do so with the same magnitude and ability of MSCs from healthy donors. Despite this variation in differentiation ability, there is not a current level of acceptable differentiation when choosing a cell to use in the clinic. Therefore, resulting in cells that have reduced, or altered, differentiation potential that could be contributing to the overall reduced therapeutic efficacy. AD-MSCs demonstrated a reduced differentiation potential in both adipogenic and osteogenic lineages. MSCs' therapeutic efficacy is partially dependent on their differentiation potential, which allows

them to integrate and stimulate the damaged endogenous tissue[41,88,89]. With AD-MSCs having reduced differentiation potential, this could be due, in part, to their inability to integrate into target tissue [22,90]. Along with reduced differentiation potential, AD-MSCs also demonstrated a decrease in their proliferative rates compared to HC-MSCs. Some studies have demonstrated a correlation between proliferation and differentiation potential[91,92]. This could explain the reasoning for AD-MSCs to demonstrate decreased proliferative rates, as well as reduced differentiation potential. Proliferation of MSCs is another mechanism underlying the therapeutic effectiveness of these cells. Since AD-MSCs experience decreased proliferative rates, this could be effecting the overall stemness and regenerative potential of MSCs[41,93]. This could affect MSCs' ability to replenish the endogenous tissue that has been damaged, as well as produce optimal yields of cells that would be needed for administration[22,88,91,94]. AD-MSCs also demonstrated alterations in other phenomenon such as increased senescence, increased apoptosis, increased DNA damage, increased ROS levels, shortened telomeres, and decreased migration/invasion capabilities. All of these demonstrate that AD-MSCs are experiencing some magnitude of stress within their niche. Since AD-MSCs show increased DNA damage, this could predispose MSCs to increased senescence and shortened telomeres, thus resulting in apoptotic events to occur[95,96]. This dysregulation can reduce the stemness and regenerative properties of MSCs affecting their overall therapeutic efficacy[96,97].

Overall, AD-MSCs demonstrate fundamental differences that could be playing key roles in their ineffectiveness in the clinic. These fundamental differences show an overarching theme of reduced stemness and regenerative properties that are vital to MSC

therapies (Figure 4.1.). Further investigation of these differences could provide insight on the mechanisms underlying the qualities that make MSCs ineffective in the clinic, as well as the pathogenesis of ADs. Furthermore, AD-MSCs were found to have 413 differentially expressed molecules that consisted of genes, proteins, and miRNAs. The only molecule consistent amongst all five diseases was IL6. This molecule is known to play a major role in chronic inflammation and autoimmunity[81]. This could mean that AD-MSCs are already showing inflammatory properties that would reduce the efficacy for autoimmune disease therapies. Though there was only one molecule consistent amongst AD-MSCs, investigation of pathways that the other molecules are involved in could provide more information on how MSCs are affected in AD. Not only could these pathways be investigated within AD, but could also be compared to MSCs from older and obese patients[91,92,95,98,99]. Lastly, designing criteria that is better equipped to identifying therapeutically effective MSCs will allow for more consistency amongst the field, and thus improve the quality amongst clinical outcomes.

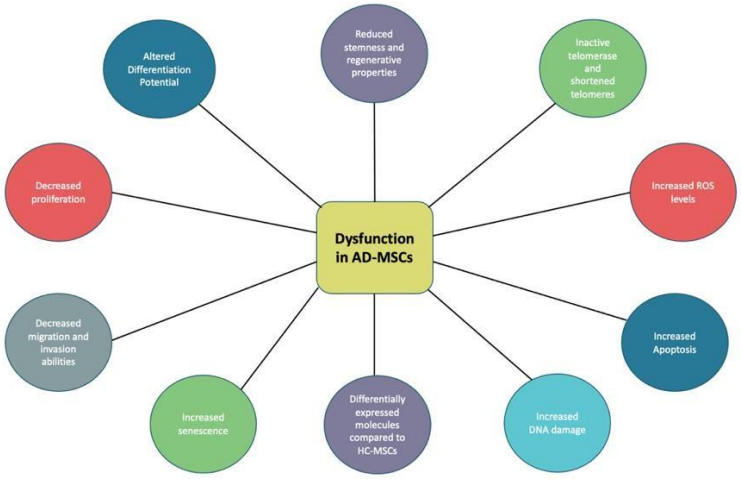


Figure 4.1. Dysfunction in AD-MSCs. Fundamental differences found in AD-MSCs when compared to HC-MSCs.

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# VITA

Hailey Nicole Swain earned her bachelor's in biological sciences from Missouri University of Science and Technology (Missouri S&T), Rolla, MO in May 2021. She began the master's program in environmental and applied biology at Missouri S&T in the following semester, fall of 2021. Hailey worked as a graduate teaching assistant for the molecular genetics, general biology, and cell biology labs. She received the graduate research funding award from the biological sciences department at Missouri S&T in the spring of 2022. She also presented her research at the Graduate Research Symposium and Center for Biomedical Research in spring of 2022. Hailey also received a second-place monetary honorarium for her presentation at the 8$^{th}$ Midwest Conference on Cell Therapy and Regenerative Medicine at Kansas University in fall of 2022. Hailey then completed her degree program honoring her with a master's degree in applied and environmental biology from Missouri S&T in May of 2023.