



When Origin Matters: Properties of Mesenchymal Stromal Cells From Different Sources for Clinical Translation in Kidney Disease

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Advanced therapy medicinal products (ATMPs) offer new prospects to improve the treatment of conditions with unmet medical needs. Kidney diseases are a current major health concern with an increasing global prevalence. Chronic renal failure appears after many years of impairment, which opens a temporary window to apply novel therapeutic approaches to delay or halt disease progression. The immunomodulatory, anti-inflammatory, and pro-regenerative properties of mesenchymal stromal cells (MSCs) have sparked interest for their use in cell-based regenerative therapies. Currently, several early-phase clinical trials have been completed and many are ongoing to explore MSC safety and efficacy in a wide range of nephropathies. However, one of the current roadblocks to the clinical translation of MSC therapies relates to the lack of standardization and harmonization of MSC manufacturing protocols, which currently hinders inter-study comparability. Studies have shown that cell culture processing variables can have significant effects on MSC phenotype and functionality, and these are highly variable across laboratories. In addition, heterogeneity within MSC populations is another obstacle. Furthermore, MSCs may be isolated from several sources which adds another variable to the comparative assessment of outcomes. There is now a growing body of literature highlighting unique and distinctive properties of MSCs according to the tissue origin, and that characteristics such as donor, age, sex and underlying medical conditions may alter the therapeutic effect of MSCs. These variables must be taken into consideration when developing a cell therapy product. Having an optimal scale-up strategy for MSC manufacturing is critical for ensuring product quality while minimizing costs and time of production, as well as avoiding potential risks. Ideally, optimal scale-up strategies must be carefully considered and identified during the early stages of development, as making changes later in the bioprocess workflow will require re-optimization and validation, which may have a significant long-term impact on the cost of the therapy. This article provides a summary of important cell culture processing variables to consider in the scale-up of MSC manufacturing as well as giving a comprehensive review of tissue of origin-specific biological characteristics of MSCs and their use in current clinical trials in a range of renal pathologies.

Keywords: mesenchymal stromal cells (MSCs), tissue source, good manufacturing practice (GMP), advanced therapy medicinal products (ATMPs), kidney disease, cell therapy, clinical application

INTRODUCTION

According to the World Health Organization 2019 Global Health Estimates, chronic diseases are one of the leading causes of mortality worldwide (1). Amongst them, chronic kidney disease accounts for 11–13% global prevalence (2, 3). Based on the course of the injury, kidney diseases and their spectrum of clinical manifestations are stratified into acute kidney injury (AKI), chronic kidney disease (CKD) and end-stage renal disease (ESRD) (4, 5). Persistent loss of kidney function over time leads to kidney failure and at that stage, the current standard of care includes renal-replacement therapies (RRT) (mainly hemodialysis and peritoneal dialysis), or organ replacement. Both strategies suffer significant drawbacks that underpin the need for new preventive and therapeutic approaches.

Cell-based regenerative therapies have the potential to change the paradigm of conventional clinical care. The use of complex biological entities such as cells to promote tissue regeneration and homeostasis, provides a therapeutic alternative to treat and even cure a wide range of diseases. The current cell-based clinical landscape in kidney disease uses hematopoietic stem cells (HSCs), mesenchymal stromal cells (MSCs), and a wide range of blood-derived cells, such as T cells, natural killer (NK) cells, and dendritic cells (6, 7). Notably, blood cell-based therapies using myeloid and T cells are gaining relevance as cellular immunotherapy products to regulate the immune response after procedures such as kidney transplantation (8, 9).

On the other hand, MSCs, which are considered an advanced therapy medicinal product (ATMPs) under EU regulation, have been extensively investigated during the last decade due to their ability to inhibit inflammation and initiate tissue regeneration. The immunomodulatory and anti-inflammatory effects, *via* interactions with immune cells, together with paracrine secretions of anti-apoptotic, anti-fibrotic and matrix remodeling factors, are the main MSC-mediated mechanisms contributing to kidney protection and regeneration (10–12) (**Figure 1**). The effectiveness of MSCs in the treatment of a variety of nephropathies has been largely investigated in pre-clinical models, showing promising results (13). This has encouraged the translation of their use in clinical settings and currently, several early-phase clinical trials have been completed, and many are ongoing, to explore MSC safety and efficacy in renal transplantation, autoimmune diseases, and organ regeneration, especially in late-stage chronic kidney disease patients (**Table 1**). Nevertheless, the road to their routine use in the clinic is far from being a reality. Results in the clinical arena have highlighted the need for better defined therapeutic products. The intrinsic heterogeneity of MSCs in addition to efficacy and safety needs to be extensively investigated before they become a sustainable and affordable therapy (43–45).

One of the current roadblocks relates to a lack of standardization of manufacturing protocols across laboratories and manufacturing centers, which hinders inter-study comparisons within the field (46) and may have significant effects on cell phenotype and performance (47–49). Heterogeneity within MSC populations is another major obstacle; there is

now a growing body of literature highlighting unique and intrinsic properties according to tissue origin and donor-related features, with characteristics such as sex, age and disease status having shown to affect their properties (50–54). In this regard, although clinical data has provided evidence for the safety of MSCs (55), attention has also been given to the immune compatibility and hemocompatibility of specific MSC infusions, urging the inclusion of HLA mismatch assessment and expression of procoagulant factors within the safety release criteria (44, 46, 56).

MSCs were initially discovered by Friedenstein et al. as a non-hematopoietic population of cells within the bone marrow (BM), that were plastic-adherent, had fibroblastic phenotype, were able to generate colonies *in vitro* and undergo osteogenic differentiation (57, 58). Later, several groups identified their ability to differentiate into other mesodermal lineages such as adipocytes and chondrocytes, and their ability to be sub-passaged and expanded *in vitro* (59, 60). Since then, MSCs have undergone an extensive diversification and cells with similar characteristics have been isolated from nearly every vascularized tissue (61) as a subgroup of pericytes that reside near vessels, contributing to their homeostasis and regenerative processes (62–64). As a summary, MSCs have been obtained from adult tissues such as adipose tissue (AT-MSCs) (65), dental pulp (DP-MSCs) (66) and other dental tissues (67), endometrium (EM-MSCs) (68, 69), menstrual blood (Men-MSCs) (70), peripheral blood (PB-MSCs) (71, 72) and from several perinatal and birth-associated tissues, referred hereafter as perinatal tissue-MSCs (PT-MSCs) including MSCs from amnion membrane (AM-MSCs), amniotic fluid (AF-MSCs), umbilical cord blood (CB-MSCs), placenta (PL-MSCs), umbilical cord tissue (UC-MSCs) and Wharton's jelly (WJ-MSCs) (73–78) (**Figure 2**). It is important to note that placental tissue can be fetal or maternal in origin, and therefore, MSCs derived from the two types of tissue should be individually characterized.

Current studies focus on trying to understand the mechanistic characteristics underlying MSC-like cells and their therapeutic effects with respect to the tissue of origin. To date, little is known about tissue-specific properties being able to predict clinical efficacy. Considering the significant effect that origin may have on functional properties, and possible therapeutic outcomes, it has now been recognized that the choice of cell source should be considered when optimizing manufacturing protocols for particular clinical applications. In addition to this attention to the source of MSCs, efforts should focus on developing more homogeneous manufacturing approaches to reduce inter-study variability and improve the interpretation and comparability of results from different centers, which ultimately will help to advance the field. Nevertheless, it seems plausible that an ultimate consensus or harmonization will not be reached due to reasons such as intellectual property as well as infrastructure and resources available for large-scale production.

In this article we provide a comprehensive review on the origin-specific biological characteristics of MSCs and their use in current clinical trials in a range of renal pathologies, and attempt to identify intrinsic biological characteristics with beneficial effects. We have also reviewed the literature regarding culture

TABLE 1 | Summary of clinical trials in KD using MSC registered at ClinicalTrials.Gov. Search done on 23rd April 2021.

NCT number	Status	Phases	Start date	Cell source	Donor source	Dose frequency (N)	Infusion route	Results	References
Acute Kidney Injury									
NCT00733876	Completed	Phase 1	2008	BM-MSC	Allogeneic	2×10^6 cells/kg body mass $N = 1$	la	No AE or SAE ↓40% hospitalization stay and readmission CKD was stable up to 16 mo follow-up No hemodialysis required	(14–17)
NCT01602328	Completed	Phase 2	2012	BM-MSC ^a	Allogeneic	2×10^6 cells/kg body mass $N = 1$	lv	~Recovery, need for dialysis, 30-day mortality, AE and SAE between treated and control groups	(18)
NCT01275612	Withdrawn	Phase 1	2010	BM-MSC	Autologous	1×10^6 cells/kg $N = 1$	lv	Patients evaluated not meet the primary criterion	(19)
NCT04194671	Not yet recruiting	Phase 1 Phase 2	2020	UC-MSC	Allogeneic	NA $N = 2$, 7 d apart	lv		
NCT03015623	Active, not recruiting	Phase 1 Phase 2	2017	BM-MSC ²	Allogeneic	SBI-101 + 2.5×10^8 vs. 7.5×10^8	Time of hemodialysis		
NCT04445220	Recruiting	Phase 1 Phase 2	2020	BM-MSC ^b	Allogeneic	SBI-101 + 2.5×10^8 vs. 7.5×10^8	Time of hemodialysis		
Sepsis-Induced AKI									
NCT02421484	Completed	Phase 1	2015	BM-MSC	Allogeneic	0.3 vs. 1 vs. 3×10^6 cells/kg body mass $N = 1$	lv	No AE or SAE ~Efficacy between treated and control groups	(20)
NCT03369275	Not yet recruiting	Phase 2	2018	BM-MSC	Allogeneic	3×10^6 cells/kg body mass $N = 1$	lv		
Chronic and End-Stage Kidney Disease									
NCT02966717	Active, not recruiting	Phase 2	2016	BM-MSC	Allogeneic	1×10^6 cells/kg body mass $N = 2$, 2 weeks apart	iv		
NCT02166489	Completed	Phase 1	2014	BM-MSC	Autologous	2×10^6 cells/kg body mass $N = 1$	iv	No AE or SAE ~ Renal function	(21)
NCT02195323	Completed	Phase 1	2014	BM-MSC	Autologous	2×10^6 cells/kg body mass $N = 1$	iv		
NCT03321942	Unknown status	Phase 1	2017	AT-MSC	Autologous	NA	iv		
NCT03939741	Recruiting	Phase 1 Phase 2	2019	SVF	Autologous	1×10^6 in 5 mL $N = 1$	iv		

(Continued)

TABLE 1 | Continued

NCT number	Status	Phases	Start date	Cell source	Donor source	Dose frequency (N)	Infusion route	Results	References
Focal Segmental Glomerulosclerosis									
NCT02382874	Completed	Phase 1	2015	BM-MSC	Autologous	2×10^6 cells/kg body mass $N = 1$	iv		
Atherosclerotic Renovascular Disease									
NCT04392206	Recruiting	Phase 1	2020	AT-MSC	Allogeneic	3 vs. 5×10^6 cells/kg body mass $N = 1$	Time of hemodialysis		
NCT01840540	Completed	Phase 1	2013	AT-MSC	Autologous	1×10^5 vs. 2.5×10^5 cells/kg body mass $N = 1$	ia	↑ Cortical perfusion ↑ Renal blood flow ↓ Tissue hypoxia ↑ GFR 3 mo follow-up	(22)
NCT02266394	Completed	Phase 1	2014	AT-MSC	Autologous	NA	ia		
Diabetic Nephropathy									
NCT01843387	Completed	Phase 1 Phase 2	2013	BM-MSC ^c	Allogeneic	1.5 vs. 3×10^8 cells $N = 1$	iv	Trend to stabilized or improved eGFR	(23)
NCT03288571	Not yet recruiting	Phase 1 Phase 2	2019	UC-MSC	Allogeneic	NA $N = 3$, 2 w apart in each kidney	intra-renal		
NCT04216849	Recruiting	Phase 1 Phase 2	2020	UC-MSC	Allogeneic	1.5×10^6 cells/kg body mass $N = 5$, course of 32 w	iv		
NCT04562025	Recruiting	Phase 1	2020	UC-MSC	Allogeneic	1×10^6 cells/kg body mass $N = 3$, weekly	iv		
NCT02585622	Recruiting	Phase 1 Phase 2	2017	BM-MSC ^d	Allogeneic	0.8 vs. 1.6 vs. 2.4×10^8 cells $N = 1$	iv		
NCT04125329	Recruiting	Phase 1	2020	UC-MSC	Allogeneic	1×10^6 cells/kg body mass $N = 3$, monthly	iv		
NCT03840343	Recruiting	Phase 1	2019	AT-MSC	Autologous	2.5 vs. 5×10^5 cells/kg body mass/ $N = 2$, 3 mo apart	ia		
Lupus Nephritis									
NCT00698191	Completed	Phase 1 Phase 2	2007	BM- MSC/UC- MSC	Allogeneic	1×10^6 cells/kg body mass $N = 1$	iv	↓ Proteinuria ↑ Disease improvement	(24–26)
NCT01741857	Completed	Phase 1 Phase 2	2012	UC-MSC	Allogeneic	1×10^6 cells/kg body mass $N = 2$, 7 d apart	iv	↓ Proteinuria ↑ Disease improvement	(27)
NCT00659217	Unknown status	Phase 1 Phase 2	2008	BM-MSC	Autologous	NA $N = 1$	iv		
NCT01539902	Withdrawn	Phase 2	2012	UC-MSC	Allogeneic	5×10^7 $N = 2$, 7 d apart	iv	~ Remission rates in treated and placebo groups	(28)
NCT03580291	Not yet recruiting	Phase 2	2018	UC-MSC	Allogeneic	2×10^6 cells/kg body mass $N = 2$, 7 d apart	iv		

(Continued)

TABLE 1 | Continued

NCT number	Status	Phases	Start date	Cell source	Donor source	Dose frequency (N)	Infusion route	Results	References
NCT03458156	Active, not recruiting	Phase 1	2017	UC-MSC	Allogeneic	1 × 10 ⁶ cells/kg body mass N = 1	iv		
NCT03174587	Completed	Phase 1	2017	BM-MSC	Allogeneic	1, 2 and 3 × 10 ⁶ cells/kg body mass ^a N = 3	iv	No AE Infusion was tolerated	(29)
NCT04522505	Active, not recruiting	Phase 1	2017	BM-MSC	Allogeneic	1, 2, 3 and 10 ⁶ cells/kg body mass ^a N = 3	iv		
NCT04835883	Recruiting	Phase 2	2019	BM-MSC	Allogeneic	2 × 10 ⁶ cells/kg body mass N = 2, 12 d apart	iv		
NCT04318600	Completed	Phase 1	2014	Amniotic- MSC	Allogeneic	1 × 10 ⁶ cells/kg body mass N = 3, monthly	iv		
NCT03917797	Recruiting	Phase 2	2019	UC-MSC	Allogeneic	NA	iv		
NCT03673748	Not yet recruiting	Phase 2	2021	BM-MSC	Allogeneic	1.5 × 10 ⁶ cells/kg body mass N = 1	iv		
NCT02633163	Recruiting	Phase 2	2018	UC-MSC	Allogeneic	1 vs. 5 × 10 ⁶ cells/kg body mass N = 1	iv		
Kidney Transplant									
NCT00659620	Unknown status	Phase 1 Phase 2	2008	BM-MSC	Autologous	NA	iv		
NCT00658073	Completed	Phase 1	2008	BM-MSC	Autologous	1-2 × 10 ⁶ cells/kg body mass N = 2, 24 h and 2 w after Tx	iv	↓ Acute Rejection ↓ Risk of opportunistic infections, ↑ eGFR 1-year follow-up	(30)
NCT00734396	Completed	Phase 1 Phase 2	2009	BM-MSC	Autologous	1 × 10 ⁶ cells/kg body mass N = 1	iv	No AE Resolution of tubulitis without IF/TA in two patients	(31)
NCT00752479	Completed	Phase 1 Phase 2	2008	BM-MSC	Allogeneic	2 × 10 ⁶ cells/kg body mass N = 1, 7 d post Tx	iv	↑ Serum Creatinine > Acute Graft Dysfunction ↑ Regulatory T cells ↓ Memory CD8 ⁺ T cells	(32)
						2 × 10 ⁶ cells/kg body mass N = 1, Tx	iv	↓ Memory CD8 ⁺ T cells ↓ Donor-specific CD8 ⁺ T cell cytolytic response ↑ Expansion of CD4 ⁺ CD25 ⁺ FoxP ⁺ Treg cells	(33)
NCT02012153	Recruiting	Phase 1	2013	BM-MSC	Autologous	2 × 10 ⁶ cells/kg body mass N = 1, 1 d before Tx	iv	↑ Graft function for 5 to 7 years follow-up ↓ CD8 ⁺ T cell in 3 of 4 patients ↓ <i>ex vivo</i> T cell donor-specific cytotoxicity	(34)

(Continued)

TABLE 1 | Continued

NCT number	Status	Phases	Start date	Cell source	Donor source	Dose frequency (N)	Infusion route	Results	References
NCT02492490	Unknown status	Phase 1 Phase 2	2014	SVF	Autologous	1 × 10 ⁶ cells/kg body mass N = 4, 0, 7, 14, 21 d after Tx	iv	↑ CD4 ⁺ CD25 ⁺ FoxP ⁺ Treg cells ↑ Naïve and transitional B cells. 1 patient successfully discontinued immunotherapy with CsA	
NCT02561767	Completed	Phase 1 Phase 2	2015	BM-MSC	Autologous	1 × 10 ⁶ cells/kg body mass N = 4, 0, 7, 14, 21 d after Tx	iv	No AE or SAE ↑ GFR. Renal function stable ↑ B cell levels	(35)
NCT02563366	Unknown status	Phase 1 Phase 2	2015	BM-MSC	Allogeneic	1 × 10 ⁶ cells/kg body mass N = 4, 0, 7, 14, 21 d after Tx	iv		
NCT02490020	Completed	Phase 1	2016	UC-MSC	Allogeneic	iv: 2 × 10 ⁶ cells/kg body mass, 48 h before Tx +/- ia: 5 × 10 ⁶ cells/kg body mass, during Tx N = 2	iv + ia	No AE No MSC engraftment → Post-operative complications → eGFR	(36, 37)
NCT02563340	Unknown status	Phase 1 Phase 2	2015	BM-MSC	Allogeneic	1 × 10 ⁶ cells/kg body N = 4, 2 w apart	iv		
NCT02492308	Unknown status	Phase 1 Phase 2	2014	SVF	Autologous	1 × 10 ⁶ cells/kg body N = 4, 0, 7, 14, 21 d after Tx	iv		
NCT02409940	Completed	Phase 1	2013	BM-MSC	Allogeneic/ Autologous	0.2-3 × 10 ⁶ cells/kg body N = 2, 1 d pre- and 30 d post-Tx	iv	No AE or SAE ↑ Graft function ↑ CD4 ⁺ CD25 ⁺ FoxP ⁺ Treg cells ↓ CD4 ⁺ T cell proliferation	(38, 39)
NCT02565459	Recruiting	Phase 1	2015	BM-MSC	Allogeneic	1 vs. 2 × 10 ⁶ cells/kg body mass N = 1, Tx	iv		
NCT02387151	Completed	Phase 1	2015	BM-MSC	Allogeneic	1.5 - 2 × 10 ⁶ cells/kg body mass N = 2	iv		(40)
NCT02057965	Active, not recruiting	Phase 2	2014	BM-MSC	Autologous	1 vs. 2 × 10 ⁶ cells/kg body mass N = 2, 6 and 7 w after Tx	iv		(41)
NCT03478215	Recruiting	Phase 2	2016	BM-MSC	Autologous	1, 2, and 3 × 10 ⁶ cells/kg body mass ^a N = 1	iv at surgery		
NCT01429038	Completed	Phase 1 Phase 2	2012	BM-MSC	Allogeneic	1.5 vs. 3 × 10 ⁶ cells/kg body mass N = 2, 3 and 5 d post Tx	iv	No MSC engraftment 2 Kidney/MSK HLA MM 1 MSC MM	(42)

MSC From Commercial Entities: ^aAC607 (AlloCure Inc.), ^bSBI-101 plasmapheresis device in combination with MSC (Sentient Biotechnologies Inc.), ^cRexlemestrocel-L (Mesoblast Ltd.), ^dORBCEL-MTM (Orbsen Therapeutics Lt.).

~, Similar; ↑, Increase; ↓, Decrease; <, Dose-escalated study.

AE, Adverse events; AKI, Acute Kidney Injury; AT, Adipose Tissue; BM, Bone Marrow; CsA, Cyclosporin A; d, day; eGFR, estimated Glomerular Filtration Rate; GFR, Glomerular Filtration Rate; HLA, Human Leukocyte Antigen; ia, intra-arterial; IF, Interstitial fibrosis; iv, intra-venous; Kg, Kilogram; MM, Mismatch; mo, month; MSC, Mesenchymal Stromal Cell; NA, Not Available; SAE, Severe adverse events; SVF, Stromal Vascular Fraction; TA, Tubular Atrophy; Treg, Regulatory T cells; Tx, Transplant; UC, Umbilical Cord; w, week; y, year.

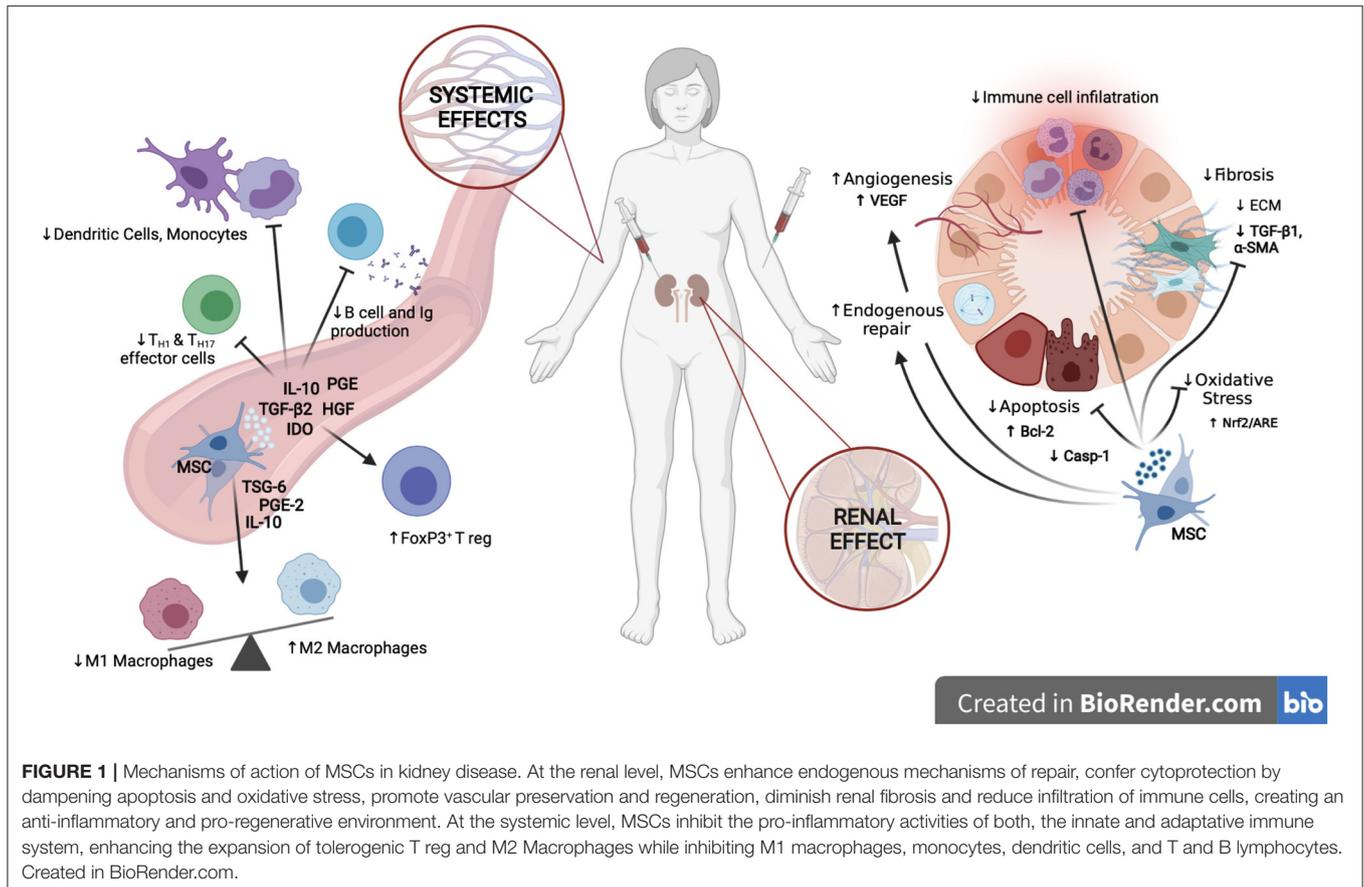


FIGURE 1 | Mechanisms of action of MSCs in kidney disease. At the renal level, MSCs enhance endogenous mechanisms of repair, confer cytoprotection by dampening apoptosis and oxidative stress, promote vascular preservation and regeneration, diminish renal fibrosis and reduce infiltration of immune cells, creating an anti-inflammatory and pro-regenerative environment. At the systemic level, MSCs inhibit the pro-inflammatory activities of both, the innate and adaptive immune system, enhancing the expansion of tolerogenic T reg and M2 Macrophages while inhibiting M1 macrophages, monocytes, dendritic cells, and T and B lymphocytes. Created in BioRender.com.

processing variables that are important to consider during the scale-up and manufacture of the cell product. As part of this study, we conducted a search in the ClinicalTrials.gov database of current registered clinical trials on kidney disease. According to our search, fifty-four clinical trials are being or have been conducted around the world to study the safety and efficacy of MSC-based ATMPs in a variety of renal diseases (accessed in April 2021, <https://clinicaltrials.gov>) (Table 1). We acknowledge that this list may not be exhaustive as it is derived from one well-known clinical trial registry, and it is possible that some other clinical trials may be listed in other national or international registries, which have not been considered in this review. The search includes clinical studies at all different stages (completed, recruiting, or not enrolling). Search terms included: “mesenchymal stem cells,” “mesenchymal stromal cells,” “kidney injury,” “kidney disease,” “kidney transplant,” combined in various modifications with “AND” and “OR.” When possible, information about MSC tissue source, donor (allogeneic or autologous), and cell processing variables such as cell plating densities, passage number, culture media supplements and culture devices for cell expansion, were extracted (Table 2). When available, additional sources of information were retrieved from hand searches of relevant papers and/or websites.

MSC-BASED THERAPIES IN KIDNEY DISEASES

Disease Overview

Generally, kidney diseases have been subdivided into acute kidney injury (AKI) and chronic kidney disease (CKD), according to the duration of the injury. While AKI is described as an abrupt decline in renal function, CKD emerges after years of progressive and persistent loss of glomerular filtration rate and albuminuria (4, 5). Although they were originally considered two individual entities, it is now clear they share an intrinsic link: maladaptive repair following AKI leads to progressive CKD, and at the same time patients with underlying CKD are more likely to develop AKI resulting in a deterioration in renal function (82). Often asymptomatic, the progressive nature of CKD leads to a vicious cycle of injury that ultimately causes renal failure or end-stage renal disease (ESRD) (83). At the time of late-stage CKD diagnosis, renal function has declined beyond physiological reserve and kidney failure is diagnosed. Despite significant advances in understanding the pathophysiology of AKI and CKD, current therapeutic and pharmacological approaches only offer supportive treatment to handle and manage underlying complications (84). In recent years MSCs and their derived by-products, mainly paracrine signals and extracellular vesicles

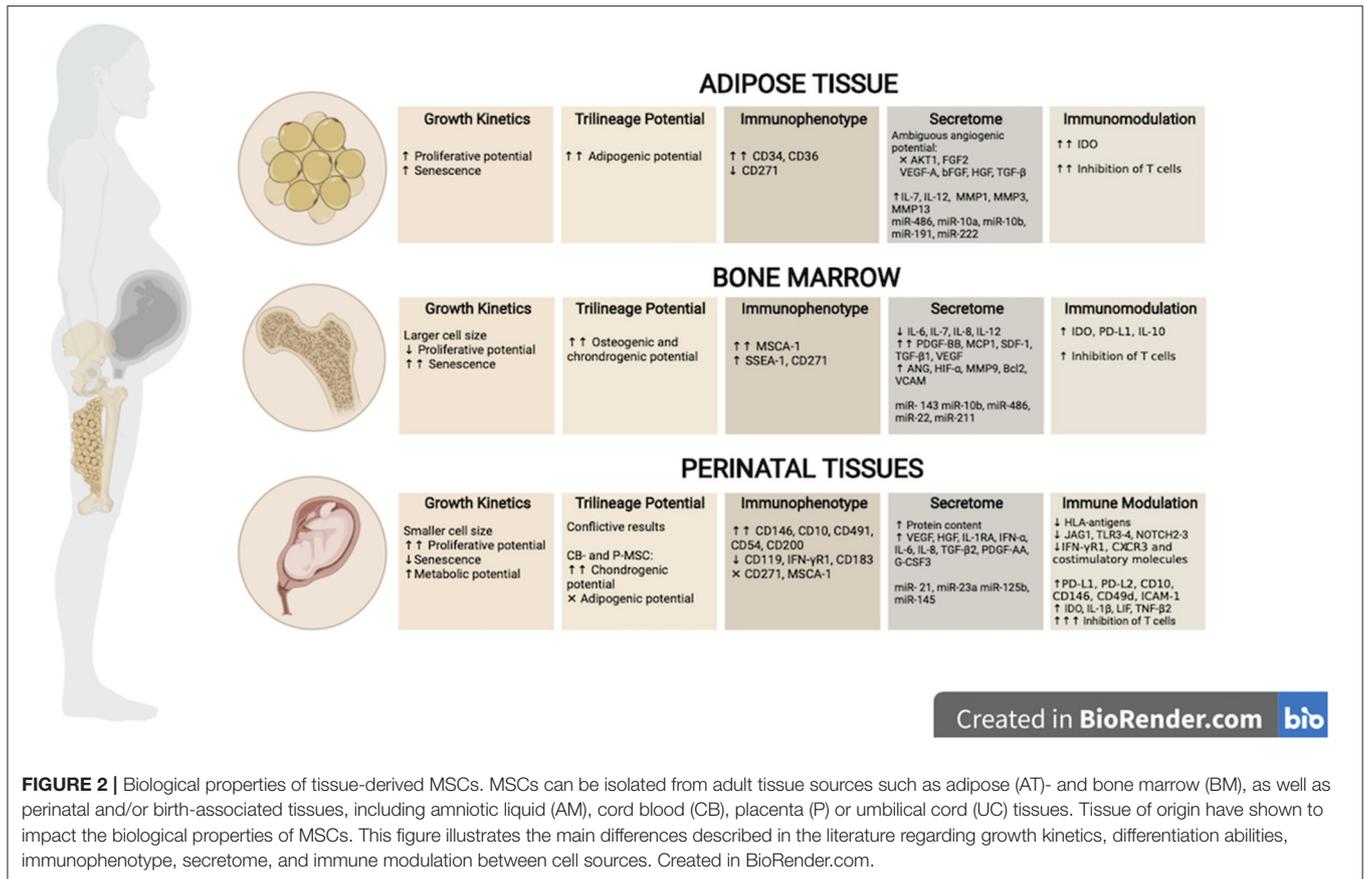


FIGURE 2 | Biological properties of tissue-derived MSCs. MSCs can be isolated from adult tissue sources such as adipose (AT)- and bone marrow (BM), as well as perinatal and/or birth-associated tissues, including amniotic liquid (AM), cord blood (CB), placenta (P) or umbilical cord (UC) tissues. Tissue of origin have shown to impact the biological properties of MSCs. This figure illustrates the main differences described in the literature regarding growth kinetics, differentiation abilities, immunophenotype, secretome, and immune modulation between cell sources. Created in BioRender.com.

(EVs), have emerged as a novel cell-based therapy to treat acute and chronic kidney injury. Herewith, this section reviews the growing body of preclinical and clinical evidence on the potential role of MSCs in recovery after kidney damage.

Mechanisms of Action of MSCs in Kidney Disease

MSCs in Acute Kidney Injury

AKI is considered a severe clinical syndrome in hospitalized patients, with a prevalence of 1–9%, and is especially common among critically ill patients, affecting 45% of patients admitted to the intensive care unit (85). The abrupt decline in renal function is accompanied by an alteration of the homeostasis within the kidney. The decline in glomerular filtration rate results in accumulation of serum creatinine, blood urea nitrogen (BUN), and/or reduction in urine output (85). Along with the original insult, functional disturbances cause a reduction in renal cell mass due to cell death, impairing renal function and facilitating the subsequent progression to fibrosis. Notwithstanding all efforts to manage the associated clinical manifestations, AKI is still considered an independent risk factor for mortality and development of CKD (86). Within the multiple etiologies of renal injury, ischemia-reperfusion injury (IRI) is the most prevalent form of AKI, together with vascular obstructions within the renal circuit (85), and drug-induced nephrotoxicity (87). IRI is also

an unavoidable event during kidney transplantation, limiting graft functionality and increasing the risk of rejection and graft loss (88).

Inflammation plays a central role throughout the process of kidney injury (89). Shortly after the injury, activation of inflammatory pathways induces the recruitment and infiltration of leukocytes such as neutrophils, monocytes, and dendritic cells. T and B lymphocytes have also been linked to contributing to kidney injury. Conversely, regulatory T cells and M2 macrophages are essential in suppressing inflammation, enhancing tissue remodeling and causing repair. However, if uncontrolled, the endogenous mechanisms of tissue repair within the kidney could promote additional damage and irreversible fibrosis (90). Together, the immune system, the ischemic environment and the endogenous mechanisms of repair converge in a complex milieu of profibrotic and proinflammatory cytokines and chemokines. In this context, MSCs have been proposed as powerful candidates to dampen the severity of AKI and promote effective regenerative processes.

Infusion of MSCs in several *in vivo* models of AKI has resulted in improved renal function by decreasing tubular injury, promoting angiogenesis, reducing oxidative stress as well as inflammation, promoting a pro-regulatory and anti-inflammatory phenotype (91–93). The main mechanisms whereby MSCs have been found to elicit such renoprotective effects are related to paracrine signaling and shedding of

TABLE 2 | Summary of MSC culture processing variables publicly available from clinical trials of KD.

NCT number	Cell source	Donor origin	Media supplements	Cell densities		Passage number	Cell culture system	References
				Isolation	Plating			
Acute Kidney Injury								
NCT00733876	BM-MSCa	Allogeneic					2D Flask	(79)
NCT01602328	BM-MSCb	Allogeneic					2D Flask	(18)
NCT03369275	BM-MSCa	Allogeneic	XF				3D Bioreactor	
NCT02421484	BM-MSCa	Allogeneic	XF				2D Flask	(20)
NCT01840540	AT-MSCa	Autologous	5% hPL	100, 000 cells/cm ²	100 – 250 cells/cm ²		2D Flask	(22, 80)
Chronic Kidney Disease								
NCT02166489	BM-MSCa	Autologous	10% FCS			P1-3	2D Flask	(21)
Diabetic Nephropathy								
NCT01843387	BM-MSCa	Allogeneic	10% FCS			P2	2D Flask	(23)
NCT02585622	BM-MSCa	Allogeneic					3D Bioreactor	
Lupus Nephritis								
NCT00698191	BM-, UC-MSCa	Allogeneic	10% FCS	100, 000 cells/cm ²	1, 000 cells/cm ²	P2-P5	2D Flask	(24–26)
NCT01741857	UC-MSCa	Allogeneic	10% FCS	100, 000 cells/cm ²	1, 000 cells/cm ²	P3-P5	2D Flask	(27)
Kidney Transplant								
NCT01429038	BM-MSCa	Allogeneic	10% FCS			P2	2D Flask	(81)
NCT00658073	BM-MSCa	Autologous	HSA			P3-4	2D Flask	(30)
NCT00734396	BM-MSCa	Autologous	10% FCS	160, 000 cells/cm ²	4, 000 cells/cm ²		2D Flask	(31)
NCT00752479	BM-MSCa	Allogeneic	5% hPL	200, 000 cells/cm ²	200 cells/cm ²	12 d in culture	2D Flask	(33)
NCT02492490	SVF	Autologous	10% FCS				2D Flask	
NCT02561767	BM-MSCa	Allogeneic	10% FCS				2D Flask	(35)
NCT02490020	UC-MSCa	Allogeneic	HSA				2D Flask	(36, 37)
NCT02409940	BM-MSCa	Allogeneic/ Autologous	10% hPL	200, 000 cells/cm ²	500, 000 cells/cm ²	P3	2D Flask	(38, 39)
NCT02012153	BM-MSCa	Autologous	5% hPL	200, 000 cells/cm ²	100–200 cells/cm ²	P1	2D Flask	(34)

AT, Adipose Tissue; BM, Bone Marrow; FCS, Fetal Calf Serum; HSA, Human Serum Albumin; hPL, Human Platelet Lysate; SVF, Stromal Vascular Fraction; UC, Umbilical Cord; XF, Xenofree media.

MSC Commercial Names: ^aAC607 (AlloCure Inc.), ^bRexlemestrocet-L (Mesoblast Ltd.), ^cORBCEL-MTM (Orbsen Therapeutics Lt.).

extracellular vesicles (79, 94). MSC-based therapies have been proven to stimulate the regeneration of tubular epithelial cells by increasing intra-renal levels of HGF (95–97) and TSG6 (98), promoting the activation of pro-survival pathways such as AKT/ERK (99); decreasing tubular apoptosis, by upregulating Bcl2 and downregulating Caspase 3 (100), and inhibiting the endoplasmic reticulum stress response (99). Moreover, MSCs help in counterbalancing the oxidative damage by enhancing the activity of free radical scavengers (101), favoring the activation of the Nrf2/ARE pathway (102) and downregulating the expression of NOX2 which are key ischemia-related insults (102). A large part of the beneficial effect of MSCs is related to their interaction with both, the innate and adaptive immune systems. The complement system serves as a key moderator of the immune system and MSCs have been described to interact with this system in a synergistic manner to modulate the host immune response (103). Conversely, in the context of kidney injury, MSCs have been found to inhibit the overactivation of the complement cascade, decreasing serum levels of C5a as well as intra-renal deposits of C3 and C5aR (104, 105). Downregulation of inflammatory cytokines such as TNF α , MMP9, ICAM1,

NF κ B (100, 106, 107) and chemokines such as CX3CL1 (108), CXCL2, and IL6, decreased the infiltration of pro-inflammatory macrophages (109) and effector T cells while promoting the presence of regulatory T cells (110). This “shift” toward an anti-inflammatory profile seems to be, in part, governed by the expression of IL10 (111) and adherence factors such as ICAM1 and VCAM1 (112). The secretion of pro-angiogenic factors [e.g., VEGF, eNOS (113–116)] has been shown to improve capillary rarefaction (107), dampening the ischemic damage and preventing the progression of interstitial fibrosis (108, 110).

Interestingly, *in vitro* experiments have found that small single-stranded non-coding RNA molecules (miRs) contained within EVs produced by BM-MSCs can protect proximal tubular epithelial cells after ischemia by targeting the expression of mRNAs associated with apoptosis, cytoskeleton reorganization, fibrosis, and hypoxia (117), endowing EVs and their miR cargos with interactive roles in the regenerative process.

Recently, a novel mechanism of action has been proposed whereby MSCs could rescue damaged tubular cells by targeting mitochondrial dysfunction and sustaining their energy supply (118), and restoring physiological dynamics (119). Another

consideration in the therapeutic use of MSCs is the use of genetic modification (120–122) as well as pre-conditioning strategies such as hypoxic culture conditions (123–125), and priming of cells (126–128), which have showed superior therapeutic potential compared to that of unmodified controls (129).

MSCs in Chronic Kidney Injury and End-Stage Renal Disease

CKD emerges as the result of continuous kidney damage and scarring mediated by a dysfunctional inflammatory status (130, 131). The perpetuation of the injury is often a result of high blood pressure, nephrolithiasis, and several underlying conditions such as diabetes mellitus (10), systemic lupus erythematosus (132), or glomerular pathologies (133), as well as the development of *de novo* AKI (134). Regardless of the initial insult, the exacerbated renal fibrosis response that occurs throughout the course of the disease induces morphological alterations with physiological and functional consequences (135). Progression to ESRD is, therefore, inevitable.

Paracrine signaling and/or EVs derived from MSCs have been transiently found within the glomeruli and injured tubules, limiting the extent of the injury by alleviating interstitial fibrosis, recruiting leukocytes, and activating intrinsic repair mechanisms that prevent AKI-CKD transition (136–139). Similar effects have been described in several models of established CKD, where cell and cell-free strategies resulted in reduced accumulation of fibrotic tissue as a result of decreased expression of extracellular matrix components and increased capillary density, attenuation of the pro-fibrotic and pro-inflammatory environment, and promotion of M2 anti-inflammatory macrophages (140–142). However, attenuation of inflammation is not always achieved, probably due to differences in treatment time and frequency (143). In these circumstances, “licensing” strategies have proven to be efficient in promoting an early onset of MSC therapeutic effects (128).

Several studies have also explored MSC therapies in chronic scenarios where renal damage is being perpetuated by underlying pathologies, predominantly autoimmune nephritis caused by systemic lupus erythematosus (SLE) and microvascular complications of diabetes mellitus, commonly referred to as diabetic nephropathy (DN). In both scenarios, preclinical models have described the usefulness of MSCs in ameliorating the pathogenic manifestations albeit through different mechanisms due to the different nature of the insults. MSCs in preclinical models of lupus nephritis (LN) have been shown to act by suppressing the activation of the humoral and cellular immune response, evoking a systemic pro-tolerogenic milieu (144–146). Besides regulating leukocyte infiltration and inhibiting proinflammatory cytokines, beneficial actions in DN models have been also attributed to the reduction of systemic biochemical alterations and reducing renal levels of oxidative stress, apoptosis, and fibrosis while promoting renal regeneration (147–150).

MSCs in Kidney Transplantation

One of the most relevant clinical settings where MSCs have shown potential beneficial effects is renal transplantation. In murine models of kidney transplantation (KTx), infusion of

autologous (151, 152) or syngeneic (153, 154) MSCs induced graft tolerance and recipient survival. The achievement of a pro-tolerogenic environment was, in part, mediated by the production of indoleamine 2, 3 dioxygenase (IDO), crucial in generating regulatory FoxP3⁺ T cells (112, 151). The effect was increased when BM-MSCs were licensed with the pro-inflammatory cytokine IL17A (152, 155). However, administration of MSCs was found to only elicit a tolerogenic response and enhanced graft survival when administered following graft transplantation (112, 153, 154).

Comparable effects have been reported in rodent models where single (156, 157), and multiple (158) administrations of MSCs resulted in significant improvement in graft function and attenuated expression levels of pro-inflammatory cytokines (156–158). Licensing with TGFβ1 (159) or genetic modifications to overexpress CXCR4 (160) enhanced the immunosuppressive abilities and showed an increased induction of regulatory T cells and anti-inflammatory cytokines. Beneficial effects have also been reported in attenuating cellular infiltration and tubular damage due to chronic graft rejection (156, 161, 162). In contrast with this favorable preclinical evidence, other studies have reported that administration of MSCs and their derived EVs did not exert similar beneficial effects (163, 164), highlighting the impact of timing and synergistic immunosuppressive strategies to ensure robust therapeutic effects.

Clinical Translation of MSC Therapies in Kidney Disease

Promising preclinical results, described above, have led to early-phase clinical studies that investigate the safety and efficacy of MSC-based therapies in a wide range of renal pathologies. Based on data compiled from studies registered on clinicaltrials.gov (accessed in April 2021), a total of 54 been registered since 2008. The main results of our search are summarized in **Figure 3** and expanded in **Table 1**, which present an overview of the clinical use of MSCs in kidney disease highlighting heterogeneity in terms of tissue source and product development characteristics. Results from this search showed that MSCs have been most commonly used to improve outcomes of kidney transplant procedures (31.5%), targeting either induction of allograft tolerance or minimizing the use of immunosuppressive drugs. Other trials have focused on the renoprotective potential of MSCs in lupus nephritis (24%), AKI (14.8%), diabetic nephropathy (13%), and CKD/ESRD (16.7%) (**Figure 3A**). Nevertheless, MSC therapies for these conditions have yet to reach later stage clinical trials and market authorization (**Figure 3B**).

Results from our literature search have also highlighted the great heterogeneity within the field in terms of donor and tissue source, mode of cell delivery and cell dose (**Table 1**). During the last decade, BM-MSCs have been the predominant cell source in clinical trials (7). However, clinical MSC products have greatly diversified in the past decade, with equal use of BM-, AT-, and PT-MSC products in clinical trials (44, 165). In the kidney disease clinical arena, the use of BM-MSCs remains the predominant source (58%), although in the past 10 years a diversification

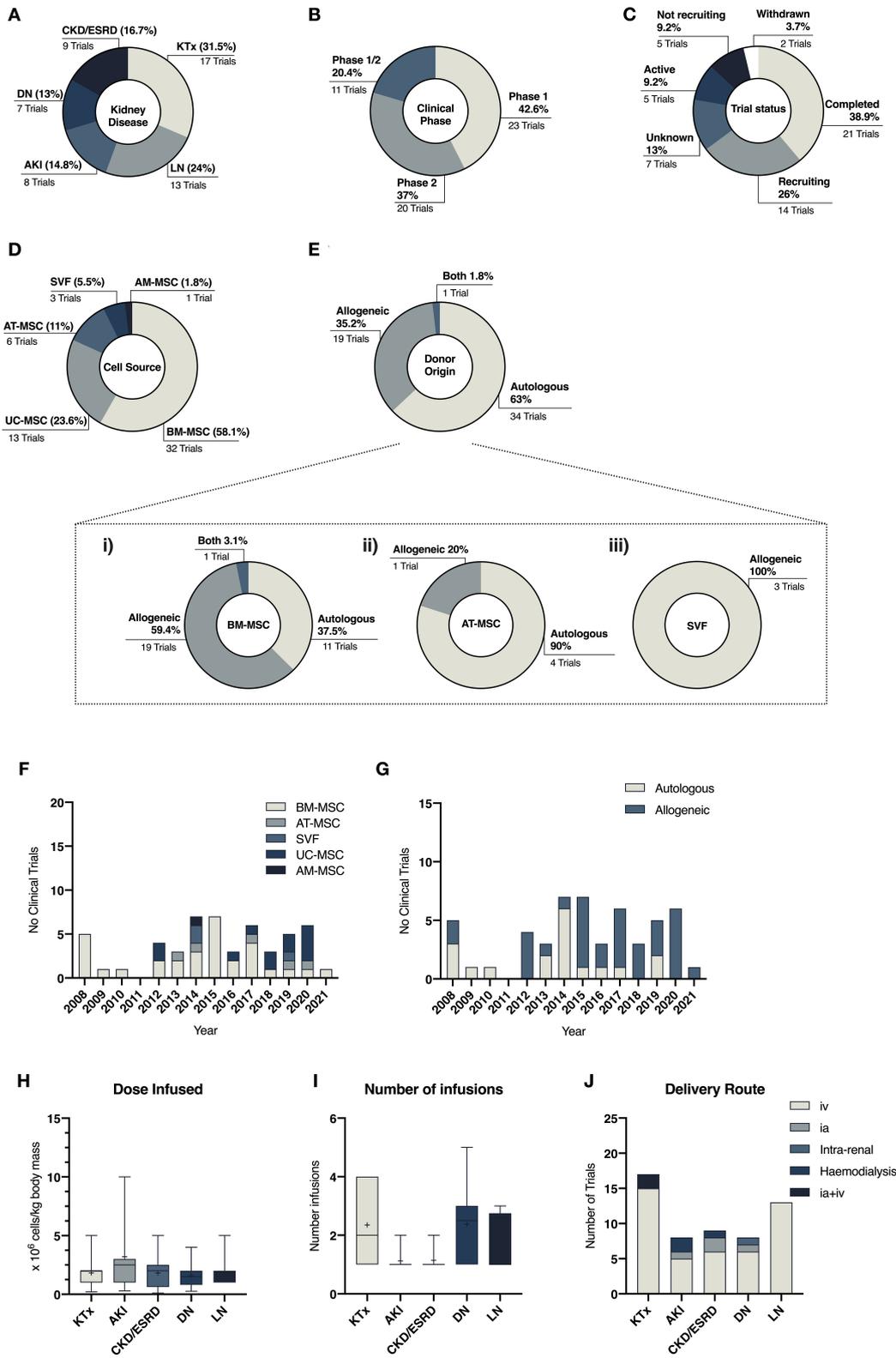


FIGURE 3 | Descriptive data related to clinical trials in kidney diseases comparing the number of trials per disease (A), clinical phase of the studies (B) and their status (C). (D,E) illustrate the heterogeneity of cell and donor source across all studies, while (F,G) depicts the change of cell and donor source preferences over the years. (H–J) illustrate protocol differences across different disease settings related to dose of MSC infused (H), frequency of infusions (I) and choice of delivery route (J).

on the use of different MSC sources have also been noted (**Figures 3D–G**).

Most trials have used intravenous delivery (**Figure 3J**), despite studies which have shown that the majority of MSCs are trapped within the pulmonary circuit (166, 167). Some studies have explored the combination of intra-arterial delivery, to facilitate homing of the cells within the kidney, and intravenous infusion (35, 36). Nevertheless, in both cases, hemocompatibility and levels of procoagulant tissue factor (TF/CD142) should be considered to avoid the onset of the instant blood-mediated inflammatory reaction (IBMIR), as it is an important aspect for the safety and efficacy of these therapies (168). Although clinical protocols have added anti-thrombotic drugs to ensure the safety of MSC products (169), several studies have reported that TF/CD142 expression varies between MSC tissue origin and is highly impacted by culture processing as well as cell-dosage (44, 168, 170). Given the procoagulant nature of MSCs, safety characterization is of utmost importance and even more so in patients undergoing organ replacement therapies that carry the burden of strong immunosuppressive regimes and their side effects such as increased cardiovascular risk (56, 171).

With the increasing presence of allogeneic therapies in the past years (**Figure 3G**), greater considerations should also be placed on the potential impact of MSC immunogenicity and the generation of alloreactive immune responses, as little is known of its long-term clinical implications (172, 173). Despite the presence of extensive evidence showing anti-donor cellular and humoral immune responses following administration of allogeneic MSCs (174, 175), presence of donor-specific human leukocyte antigen (HLA) antibodies has been minimally considered previously (172). This will represent a major risk when repeated injections are being included in therapeutic procedures for potentially pre-sensitized patients such as those undergoing kidney transplantation (56, 173).

Finally, another aspect to consider is whether MSCs are delivered as culture-adapted or “fresh” cells, with optimal metabolic fitness, or cryobanked “off-the-shelf” cells, which are thawed immediately prior to transplantation. While this is an important aspect for efficacy and safety of MSC therapies, results from our search, and others (176), have reported that the method of cell delivery (fresh vs. cryobanked), is often omitted or not clearly stated in manuscripts. The tendency to use cryobanked “off-the-shelf” cells has increased over time (48, 176), most likely due to the logistic advantages of this approach. However, controversy revolves around the use of banked products, with studies demonstrating reduced therapeutic potential, loss of functionality and increased susceptibility to trigger prothrombotic events (177–181), while others have showed minimal impairment of cell viability and fitness (182, 183). In this context, clinical potency has been linked with the concept of metabolic fitness and product viability at the time of infusion (184, 185) making it critical to develop manufacturing methods to rescue cryopreserved cells and restore cell functionality (186). Intriguingly, other studies have demonstrated that apoptotic or dead MSCs, and therefore less metabolically active, confer therapeutic benefits by enhancing the host innate immune response (187–189). Gaining better mechanistic insights behind

the benefits that MSC therapies elicit, may it be due to viable cells, their derived by-products or rather by immune activation through dying/dead cells holds the key to elicit better therapeutic outcomes (186, 190).

Kidney Transplantation

Based on their immune-privileged characteristics, MSCs have been administered in conjunction with RRT to promote graft tolerance and control the host immune system with hopes of enhancing the withdrawal or minimization of immune-suppressive therapies and enhancing organ function. Initial results from a pilot study published by Perico et al. revealed the importance of timing of cell delivery. Autologous infusion of BM-MSCs in two patients seven days after undergoing KTx from a living related donor caused a severe decline in renal function and humoral and cellular acute rejection (32). The post-surgery subclinical inflammatory environment upon which MSCs were transplanted seemed to favor the development of a pro-inflammatory phenotype that could have contributed to an early graft dysfunction (154). Pretransplant administration did not result in impaired graft function, highlighting the paramount relevance of protocol optimization (33). Moreover, it showed a pro-tolerogenic graft environment supported by reduced effector CD8⁺ T cells and expanded regulatory CD4⁺FoxP3⁺ T cells that led to stable graft function after long-term follow-up (34). In one patient, long-lasting counterbalance of regulatory/effector T cells and increased presence of B cells allowed the successful discontinuation of the use of ciclosporin A and tapering of the dose of immunosuppressive drugs (34).

Several other studies have provided further insights into the applicability of MSC in kidney transplant from living related (31, 35, 36, 38) and deceased donors (36, 191). Using kidneys from brain or cardiac deceased donors would potentially increase the number of transplant recipients and meet the growing need for kidney grafts (192). However, these procedures are associated with a higher incidence of early graft dysfunction and acute rejection as prolonged ischemic time exerts an adverse event on graft survival (193, 194). Recently, the combinatorial infusion of UC-MSCs before and during surgery in recipients of deceased donor grafts was proven to be safe and resulted in no adverse clinical events. However, no significant benefit was seen in terms of reduction of postoperative complications, survival rates and graft function (36, 37). A larger study would possibly facilitate a full assessment of improvement in delayed graft function, as a lower incidence was seen in the MSC treated group (36). In a much smaller trial, third party MSCs were infused in five kidney transplant recipients from deceased donors 3–5 days after the procedure. The 6-month safety interim report revealed no graft rejection but some degree of immunization against the shared kidney and MSC donors (191).

Despite the absence of treatment-related serious adverse events in the studies described so far, a side effect of MSC systemic immunosuppressive activity was reported in a small Phase I study, where three out of six patients developed opportunistic viral infections after MSC-infusion (31). Yet, in a much larger study involving 156 patients, inoculation of BM-MSCs resulted in a significantly decreased risk of opportunistic

infections (30). Although no neoplasia-related events have been described in KTx, this stresses the importance of carefully monitoring MSC preparations and monitoring infused patients, particularly in elderly and chronically immuno-suppressed patients with an increased risk to develop tumors and infections.

To date, a total of eight clinical trials have been completed and results published, while nine more are yet to be completed or with no publicly available results (**Figure 4**). The main differences between studies can be seen between the cell source and dose regimen, as well as infusion timing and frequency (**Figure 4**). So far, BM-MSCs have been the choice of starting material in fourteen studies (82.3%), six using autologous (43.75%) and eight using allogeneic cells (37.5%); only two studies used SVF from autologous fat tissue and one from UC-MSCs (**Figure 4**). On review of the published literature, no conclusions can be drawn to determine differences in clinical outcomes on the tissue source.

Finally, although we have previously discussed the effects of timing, the rationale for administration weeks after surgery seems to be directed toward generating a pro-tolerogenic environment that would help in easing the withdrawal or tapering of immunosuppressive drugs (34). Results from current ongoing studies looking at whether MSCs in combination with mTOR inhibitor everolimus can be used for tacrolimus withdrawal may be able to shed light on the use of MSCs as a long-term effective immune-suppressive strategy (40, 41).

Acute Kidney Injury

Limited attempts with contradictory results have resulted from the exploration of the safety and efficacy of MSCs in recovering renal function after post-cardiac surgery AKI. An exploratory phase I trial studied the safety and feasibility of infusing allogeneic BM-MSCs in patients with several underlying comorbidities at high risk of developing AKI after open-heart surgery (14, 15). Outcomes from the first five patients showed that prevention infusion of MSCs was safe, averted postoperative decline in renal function, and decreased time of hospitalization and rates of readmission. Moreover, patients with underlying CKD had stable renal function and no disease progression after 16 months follow-up (16).

These encouraging results contrast with those from a recently published randomized, double-blind, phase II study with subjects undergoing cardiac surgery with evidence of early AKI development. Administration of commercial allogeneic MSCs (AlloCure Inc.) after AKI development did not improve the time of renal function recovery, rates of adverse events, need for dialysis or 30-day mortality (18). However, the authors recognized that infusion in an overwhelming status of the disease could have hampered the potential benefits. Further studies should aim to determine whether more favorable effects could be seen in prevention studies, such as the trial by Tögel and Westenfelder described above, rather than interventional studies when MSCs are administered after AKI onset (195).

It is well-known that sepsis, among other pathologies, can lead to the development of AKI in critically ill patients (196). A phase I study explored the safety and tolerability of administered allogeneic BM-MSCs in nine patients with septic shock (20). No infusion-associated or serious adverse events were detected,

and no AKI outcomes reported. A follow-up phase II study (NCT 03369275) will further examine the efficacy of MSCs in this context.

As the regenerative medicine field evolves, new strategies are being developed to combine the use of cell-based therapies with cutting-edge biomedical devices (197–199). In this context, a phase I study is looking at the safety and tolerability of a biologic/device combination product called SBI-101 (Sentien Biotechnologies, Inc. USA). It combines a plasmapheresis device with allogeneic BM-MSCs and is designed to regulate inflammation and promote tissue repair. Two experimental cohorts using a low and high dose of MSC will be tested in AKI patients receiving continuous renal replacement therapy (NCT03015623). Furthermore, a second phase I/II trial (NCT04445220) aims to explore the use of this same device in COVID-19 patients that develop AKI.

The limited and contradictory clinical data available on the use of MSCs in AKI as well as the lack of mechanistic results challenge the possibility of drawing therapeutic roadmaps to guide the use of stromal cells in this context. Most studies related to AKI have used allogeneic BM-MSCs, emphasizing the relevance of “off-the-shelf” therapies in acute settings, where immediate therapy is needed (**Figure 4**). The exception is a phase I study that aimed to explore the use of autologous BM-MSCs for cisplatin-induced AKI in patients with solid organ cancer (NCT01275612). Unfortunately, none of the screened patients met the primary criterion of acute renal failure, and the study was withdrawn.

In terms of cell product preparations, illustrated in **Figure 4**, limited data is available; intravenous administration or infusion through the left carotid or femoral artery are the preferred delivery option whereas cell doses are inconsistent within studies (**Figure 4**). Timing and frequency of infusion, patient selection, guided by more sensitive biomarkers (200), and cell preparation, are some of the concerns that will have to be addressed in future preclinical and clinical studies to establish reliable therapeutic strategies.

Chronic Kidney Injury and End-Stage Renal Disease

Autosomal dominant polycystic kidney disease (ADPKD) is a genetic disease characterized by progressive formation and enlargement of cysts in multiple organs that have a critical effect on kidneys. The infusion of autologous BM-MSCs in a small cohort of ADPKD patients was safe and well-tolerated albeit did not improve renal function (21).

Atherosclerotic renovascular disease is the most common cause of secondary hypertension and leads to deterioration of renal function due to insufficient vascularization and ischemia (201). Current treatments based on blood flow restoration have proven unsuccessful to recover kidney injury upon damage (202). Results from a phase I study showed increased cortical perfusion and decreased renal hypoxia after infusion of autologous AT-MSC, suggesting a beneficial effect of MSCs through amelioration of the inflammatory environment and enhancement of angiogenic properties (22).

Interestingly, not only MSCs but also their by-products are being tested for CKD. In 2016, Nassar et al. administered MSC-derived extracellular vesicles from CB-MSCs in patients

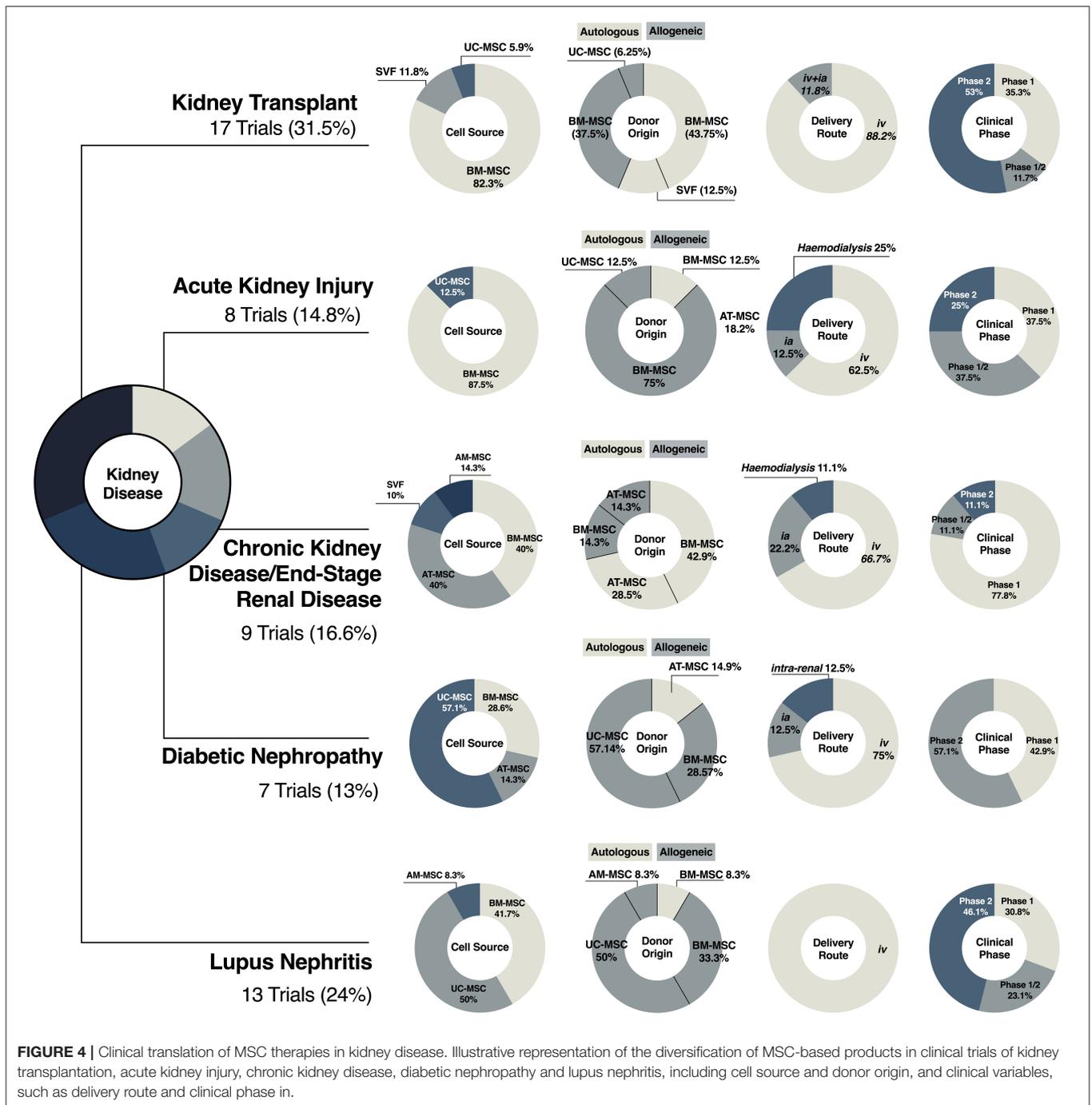


FIGURE 4 | Clinical translation of MSC therapies in kidney disease. Illustrative representation of the diversification of MSC-based products in clinical trials of kidney transplantation, acute kidney injury, chronic kidney disease, diabetic nephropathy and lupus nephritis, including cell source and donor origin, and clinical variables, such as delivery route and clinical phase in.

with CKD. Intravenous infusion resulted in significant improvement of renal function and increase in blood levels of immunoregulatory cytokines (203). Although preliminary, this study opens the window for novel strategies based on EVs derived from cultured MSCs with the potential of developing cell-free therapies (204).

In the context of CKD and ESRD, there is a tendency toward the use of autologous therapies, either from BM or AT. Although it has been recognized that MSC potency could be affected by disease status, several studies have

supported the use of autologous strategies in CKD patients (205–207). Moreover, the use of allogeneic material is less desirable in patients likely to undergo renal replacement, as immune responses against donor antigens following MSC infusion have been documented (175). Overall, clinical protocols agree on administration route, frequency, and cell dosage (Figures 3H–J, 4). It should be considered that underlying patient characteristics, such as disease stage, and the intrinsic complexity of chronic diseases could be reducing any therapeutic benefit.

Diabetic Nephropathy

Diabetic nephropathy (DN) is the most common cause of end-stage renal failure (208). In a multicentre, randomized, placebo-controlled, dose-escalation study conducted in Australia, the safety of two doses of allogeneic BM-MSCs product rexlimestrocel-L (Mesoblast Inc.) was tested in a cohort of patients with advanced DN. No adverse events related to the treatment were reported and patients showed trends of renal function improvement after 12 weeks (23). A similar phase I study conducted in 18 different centers across the USA in patients with type 2 diabetes, showed a decrease in glycated hemoglobin 12 weeks after infusion of the highest dose (209). Although the same MSC preparation and doses were used, improvement in glycaemic control was not observed in the Australian cohort. Both studies demonstrate that infusion of MSCs in diabetic patients is safe and well-tolerated; however, their results enhance the idea that therapeutic outcomes elicited by MSC transplantation may be largely influenced by the disease stage. Results from ongoing trials may be able to confirm the suggestive effects of MSCs in restoring renal function and potentially ameliorating biochemical alterations in DN patients.

Of interest, trials related to DN present a higher heterogeneity in choice of tissue source (five trials using UC-, two BM- and one AT-MSCs) but not in donor origin, with 85% of the trials using allogeneic sources, most likely because the impact of DM on MSCs is still under evaluation (210, 211) (Figure 4). Compared with other trials, protocols have included higher cell doses, with dose-escalation studies looking at fixed doses, and a range of administration frequencies, ranging from a single intravenous injection up to five doses over 32 weeks (Figure 3I).

Lupus Nephritis

Systemic lupus erythematosus is an auto-immune disease characterized by the loss of immune tolerance against self-antigens that affects tissues throughout the whole body (132). Lupus nephritis (LN) is the most common clinical manifestation (212). Considering the ability of MSCs to promote a tolerogenic environment and modulate the immune system, 13 clinical trials have explored the safety and efficacy of BM- or UC-MSCs in LN patients. In this particular scenario, therapies are mainly based on allogeneic cell products (Figure 4) due to impaired immune-modulatory properties and increased senescence in patient-derived BM-MSCs (213).

Results from successful trials identified serum levels of IFN γ as a predictive biomarker of MSC therapeutic efficacy. IFN γ have also been shown to stimulate the levels of IDO (214) and to have a critical role for UC-MSCs in regulating the innate immune system through up-regulation of tolerogenic dendritic cells (145).

However, conflicting results have been published reflecting the heterogeneity among SLE and LN patients, as well as the added challenge of intrinsic confounding factors such as different degrees of disease severity and treatment regimens. While a single intravenous administration of allogeneic BM- (24) and UC-MSCs (25) was proven to evoke clinical improvement and disease remission over time (215), other studies failed to reproduce the aforementioned results (26).

To date, there are 6 more registered trials where allogeneic BM- or UC-MSCs infusion have been or are being tested in safety and efficacy exploratory studies. Based on our search, similar therapeutic regimens—cell dose, frequency, and infusion route (Figures 3I, 4)—have been or are being explored, with limited success when trying to increase infusion frequencies (28, 215) and adjust cell dose (29). Unfortunately lack of details about product preparation limit further inter-study comparisons.

BIOLOGICAL PROPERTIES OF MSCs DERIVED FROM DIFFERENT TISSUE SOURCES

The term “MSC” is nowadays used as an umbrella term that encompasses a variety of progenitor cells retrieved from a number of different tissues. This diversity has generated significant interest in further investigating the properties of MSCs derived from different in this review we have described the pre-clinical and clinical use of MSCs in kidney disease in which the cells have most often been harvested from bone marrow, adipose tissue and umbilical cord. It is not clear what is the rationale for use of MSCs from a particular source and if specific properties of the cells based on tissue of origin would suggest the superiority of one. In this section, we provide a comprehensive description of the biological and functional characteristics of MSCs reported in the literature depending on their tissue source to reflect further on this consideration (Figure 2).

Cell Morphology

MSCs are widely known for exhibiting a common spindle-shaped morphology, notably at the early stages of *in vitro* culture. Although this is the axiom from different starting tissues, cell size and morphology have been shown to vary between adult and younger sources, with perinatal-derived MSCs being relatively smaller and BM-MSCs cultures bearing heterogeneous populations (216–218). Morphological differences have also been reported between UC- and AM-MSCs even when the same genetic background was shared (216). Culturing conditions can also influence cell shape, as morphological changes have been attributed to different media compositions (219) and the use of specific supplements and growth factors (220). Besides altering lifespan, aging also influences cell morphology: older, senescent cells have a larger diameter (54, 221, 222). Interestingly, differences in cell size at early passage have been linked with differential expansion potential and senescence levels (223).

Growth Kinetics

MSC growth is characterized by an initial lag phase, where cells attach to the growing surface, followed by a log phase when cells undergo exponential growth by mitotic division. Finally, cells reach a plateau phase in which mitotic division continues but at a slower rate, as cell division is inhibited by cell-to-cell contact. This *in vitro* growth pattern continues at every passage until the hallmarks of replicative senescence start to appear, such as an increase in cell size, cell cycle arrest, interruption of mitotic divisions and accumulation of cellular debris and

stress fibers (224). For clinical and experimental purposes, MSCs must undergo *ex vivo* culture expansion to generate sufficient cell numbers. However, long-term culture expansion (or *in vitro* aging) has been shown to reduce the replicative lifespan and prompt the onset of senescence (54, 225, 226). This is an important fact, as it may limit the usefulness of these cells in cases where a high degree of *ex vivo* expansion is needed such as that required for achievement of clinical therapeutic doses. Thus, in a “space race” to discover which is the best MSC source for clinical applications, the ability to withstand longer periods in culture before reaching the onset of senescence is considered to be advantageous.

Many studies have now been performed that compare the expansion potential of MSCs obtained from different tissue sources, using culture parameters such as passage number, cumulative population doubling (CPD) and doubling times (DT) to describe cellular aging. When comparing the proliferation of MSCs harvested from different tissue sources, BM-MSCs have been shown to exhibit slower proliferation rates, with DT ranging from 40 to 60h depending on the culture conditions, and earlier appearance of senescence markers in relatively early passages (between passage 6 and 7) (217, 227–231). In contrast, AT-MSCs have shown faster proliferation rates (DT of 20 to 45h) as well as the ability to sustain a longer time in culture (up to passage 8) without any signs of senescence (217, 230, 232–234). These differences were still evident when comparing proliferation and differentiation capacity of AT- and BM-MSCs harvested from the same individual, although significant degrees of donor-to-donor variability was observed (232–234). Variables such as donor, age, sex, and disease status may have a significant effect on MSC characteristics (50, 51, 53, 235), which may discourage the use of adult sources as therapeutic agents while favoring MSCs obtained from birth-associated tissues (231). In general, these cells have exhibited higher proliferative kinetics with lower CPD over time (217, 230, 236–243), often related to lower expression of senescence-associated markers or later onset of senescence (229, 244), as well as upregulation of cell cycle-related genes and DNA damage response and repair (245, 246). These studies reflect the intrinsic heterogeneity between MSC populations in growth kinetics. Individual populations may also contain cells at different stages of differentiation and/or different proportions of highly proliferative cells. These variables have also been shown to vary from donor-to-donor (219).

Determining novel predictive biomarkers of therapeutic potency is of utmost importance before clinical usage, and viability and metabolic fitness have been recently proposed as potency qualities (184). Metabolic status is affected after long-term *in vitro* expansion, and it can reflect differential stemness behavior (242, 247–249), as well as cell immune functionality (250). Overall, considering the need to generate enough number of cells, the proliferative and metabolic characteristics of AT- and UC-MSCs may favor their use over BM-MSCs (251, 252).

Tri-lineage Differentiation Potential

The ability to undergo *in vitro* differentiation toward mesodermal lineages is, probably, the most differential property to biologically identify MSCs (253). Several culture-differentiating conditions

have been reported to demonstrate the ability of MSCs to differentiate into adipocytes, osteoblasts and chondroblasts *in vitro*. Reports on tri-lineage differentiation potential have been inconsistent across different laboratories, and this may be due to the diversity of in-house protocols, culture conditions and media supplements, or the divergence in the cell preparations (219) and *in vitro* aging (54). Moreover, studies have reported a strong “tissue memory” effect, believed to be mainly driven by epigenetic factors (252, 254, 255). For instance, BM-MSCs present enhanced osteogenic and chondrogenic differentiation while AT-MSCs are usually more readily able to exhibit adipogenic differentiation (234, 241, 251).

Conflicting data however exists surrounding PT-MSCs as they have shown a heterogeneous potential to undergo mesodermal differentiation (241, 256, 257). Kern et al. reported that CB-MSCs could not differentiate toward adipocytes, similar findings were also reported for PL-MSCs (238). Other investigators, besides confirming the low adipogenic potential of CB-MSC (258), have reported higher osteogenic (247, 259) and chondrogenic potential (257, 260). Differences in identical genetic background perinatal MSC sources have also been described, with strikingly inconsistent results reported from AM-MSCs (216). Finally, similar observations have been reported for UC-MSC, with some studies suggesting higher adipogenic and osteogenic abilities (243), whereas others stated reduced differentiation compared with adult sources (240, 245).

While the field moves toward cell-free therapies (261) and mechanisms of action are mainly driven by paracrine and immunomodulatory effects (176), assessing the degree of commitment toward mesodermal lineages to determine the most effective and suitable source for cell therapy may have less relevance. However, in other circumstances understanding how these differences affect the biology of MSCs could be an attractive avenue to study biological changes occurring throughout fetal development and adulthood (258), as well as to help define therapeutic strategies where use of MSCs is heavily influenced by such differentiation, such as bone and cartilage regeneration (262, 263).

Cell Surface Markers

MSCs are not a homogeneous population but rather an amalgamation of different subpopulations bearing different cell surface markers. Currently, a “true” marker for MSCs does not yet exist, which makes MSC identification challenging. In an attempt to unify MSC identification and characterization, in 2006 the Mesenchymal and Tissue Stem Cell Committee of the ISCT proposed a panel of minimal surface antigens to define human MSC (253). Within this criterion, they defined that at least 95% of the stromal population should express CD105, CD73, and CD90, and lack ($\leq 2\%$) the expression of CD45, CD34, CD14 or CD11b, CD79alpha or CD19, and HLA class II. The negative markers are commonly used to confirm the absence of contaminant cells in MSC preparations such as hematopoietic progenitors, endothelial cells, leukocytes, and co-stimulatory molecules. The vast majority of studies have reported comparable immunophenotypic profiles that follow ISCT criteria regardless of source, although with moderate donor variability (219, 246,

264). In some cases, extended culture has been seen to reduce the expression of CD105 (54) and UC-MSCs have demonstrated lower (<95%) CD90 and CD105 expression (217).

However, the ISCT criteria do not uniquely identify stromal cells, as the proposed markers are also expressed in other connective tissue cells (265). Therefore, broader flow cytometry panels have been designed to best identify MSCs beyond the minimal criteria. Most of the protocols include the assessment of CD29, CD44, CD59, CD140b, CD166, TLR4, and PDL, commonly expressed (>95%) in human MSCs; and CD93, CD133, CD243, CD235, and SSEA1, with no or very low expression levels in human MSCs. Expression of other markers such as CD71, CD146, CD106, and CD274 has been shown to be heterogeneous, and in some cases correlates with donor age (53). Adhesion molecules such as CD44 (hyaluronic acid receptor) or CD29 (integrin β 1 receptor) are highly expressed in human MSCs and have been recently proposed to be included in characterization panels (266). However, expression of markers such as CD146, another key adhesion molecule, can vary between sources, being highly expressed in UC-MSCs compared with BM-MSCs (230, 237, 240, 264) and subcutaneous AT-MSCs (267). Other markers found to be increased in UC-MSC preparations are CD10, CD49d (integrin α 4), CD54 (ICAM1), (240) CD200, and PDL2, whereas CD119, IFN γ R1 and CD183 (CXCR3) are under-expressed (264). An additional marker with functional relevance that has been shown to vary greatly between sources is the coagulation factor III or tissue factor (TF/CD142) (268), with increased levels being described in AT- and PT-MSCs compared with those of BM-MSCs populations (170, 218, 269, 270).

Other researchers have investigated whether surface markers such as CD271, SUD2, MSCA1, CD34, and CD44 could serve to selectively enrich MSC populations. Differences between sources led to different selection efficiency and changes in biological properties. For instance, only CD34 was able to successfully isolate AT- and BM-MSCs, and interestingly the positive sorted populations showed greater proliferative capacity, increased osteogenic potential and HGF expression (271). Due to their perivascular origin, higher levels of CD34 and CD36 have been reported in AT-MSCs, albeit their expression decreases early after isolation (230, 234, 239). On the other hand, CD271 has been reported to be absent from MSC preparations in other studies (246, 272). Other differentially expressed markers are SSEA4 (higher in BM- and UC-MSC), MSCA1 (absent in UC-MSC, highly expressed in BM-MSC) and CD271 (high in BM-, low in AT-, absent in UC-MSC) (230, 271, 273). Nevertheless, it still remains unclear whether differences in MSC surface markers are correlated with therapeutic activity or potency (266).

Secretome Profile

It is now well-accepted that the therapeutic effects of MSCs are primarily mediated by their ability to interact and respond to environmental stimuli releasing soluble factors and EVs (274). The ability to sense changes is also translated *in vitro*, where cell culture conditions (219) or exposure to licensing strategies (275) can impact the secretome, highlighting plasticity and ability to adapt and respond to surroundings (274, 276).

The so-called MSC secretome is composed of small molecules, chemokines, cytokines, growth factors, as well as EVs (277, 278). The literature has shown striking differences in the composition of MSC-secretome depending on the cell source. Moreover, variable results between studies add to the heterogeneity, further challenging the process of deciphering “true” biological properties that relate to therapeutic actions. It also makes it challenging to choose a specific MSC source to best align with the pathophysiology of the target disease.

Soluble Factors

MSCs have been reported to secrete large amounts of pro-angiogenic, pro-proliferative, anti-apoptotic, anti-inflammatory, anti-fibrotic and matrix-remodeling soluble factors. Several studies have shown that perinatal sources of MSC have a more diverse and protein-abundant secretome, with a more complete pro-angiogenic array (244, 246, 256, 279). Although some studies failed to detect differences in functional studies (256, 279), others have shown *in vitro* superior abilities of UC- and BM-MSCs in inducing angiogenic phenotypes (246). UC-MSCs have also exhibited greater abilities to induce vessel-like structures than maternal sources of MSCs, through enhanced secretion of HGF and VEGF (280). However, a potential confounding factor in these studies is the combination of maternal and fetal cells within PL-MSC preparations, which could be limiting their angiogenic properties (280).

In contrast with studies reporting that AT-MSCs had a weaker angiogenic secretome, lacking central molecules such as AKT1 and FGF2 (246), others have demonstrated *in vitro* and *in vivo* angiogenic potential of AT-MSC preparations in a model of hindlimb ischemia, due to the secretion of VEGFA, TGF β , bFGF and HGF, well-known factors of endothelial cell survival, proliferation, and migration (251, 281, 282).

The secretome of UC-MSCs has been reported to be enriched with anti-inflammatory cytokines such as IL1RA and IFN α , pro-inflammatory cytokines such as IL6 and IL8; and mitogenic factors such as HGF, TGF β 2, PDGFAA and GCSF (240). BM-MSCs, on the other hand, while secreting lower levels of IL6, IL7, IL8, and IL12, have been reported to secrete higher concentrations of PDGFBB, MCP1, SDF1, TGF β 1, and VEGF (232, 240, 251, 283), exhibiting a stronger anti-inflammatory profile that increased upon exposure to hypoxic conditions, together with the expression of other angiogenic and anti-apoptotic factors such as ANG, HIF α , MMP9 and Bcl2 (284, 285). Increased levels of VCAM1 in the BM-MSC cytokine profile have been related to better angiogenic paracrine activity (275, 286).

AT-MSCs contain large amounts of IL7 and IL12 together with several metalloproteinases (MMP1, MMP3, and MMP13) and extracellular matrix components (240). Interestingly, expression of different MMPs between AT- and BM-MSCs has been previously reported, accounting for different mechanisms to promote angiogenesis (287).

Donor-to-donor variability and heterogeneity of MSC populations make it difficult to define a “secretome profile” specific for each tissue source of MSCs. Another layer of complexity relates to the use of cell culture supplements during *in vitro* expansion containing growth factors which may also

affect the secretome (246). Ultimately, dissecting the secretome of each specific MSC preparation may provide insights of their advantages in any given pathology (e.g., superior angiogenic secretome identified in BM- and UC-MSC preparations might make them an optimal source for ischemic disorders).

Extracellular Vesicles and miRs

In recent years, EVs have been proposed as a potential mechanism of therapeutic benefit of MSCs. EVs are lipid bilayer-delimited particles released by cells into the extracellular space carrying within them a range of cargos: subcellular components such as mitochondria, proteins, lipids, microRNAs (miRs), messenger RNAs (mRNAs) and transfer RNAs (tRNAs). Their roles have been described in multiple physiological and pathological process and are considered a mechanism of cell-to-cell signaling (288). MSCs secrete microvesicles (MVs) and exosomes, and both have been widely explored as cell-free alternatives to their cellular counterparts. Cell-free therapies, if able to recapitulate therapeutic efficacy of whole-cell preparations, offer several advantages due to a higher safety profile, lower immunogenicity, potential to bypass the lung trapping effect, and potential inability to induce neoplastic processes (289). It has also been described that EVs suppress pro-inflammatory processes, reduce oxidative stress and fibrosis in several *in vivo* models (290, 291).

Currently, there is limited data available on head-to-head comparisons of the paracrine benefits of different sources of MSC. We have only been able to identify a few studies reporting differential compositions and therapeutic effects of EVs derived from different sources. Exosomes derived from EM-MSCs enriched with miR-21 have been shown to confer superior cardioprotection after myocardial infarction over that of AT- or BM-MSC (292). Furthermore, a higher content of angiogenic-related cargos in EVs from AT-MSCs, compared to BM-MSCs, has been shown to promote wound healing (293). Similar findings have been attributed to the presence of miR-125a in AT-MSCs exosomes (294) and found to be enhanced by hypoxia priming (295). Albeit limited, recent data has described higher yields of particles secreted by AM-MSCs than BM-MSCs with similar size distribution, morphology, and immunophenotype (296).

Additional studies exploring the cargo within EV preparations from various sources have also reported beneficial effects. Exosomes secreted from BM-MSCs have been found to activate signaling pathways related to wound healing and angiogenesis (297–300) while their miRNA “repertoire” has been linked with anti-fibrotic, anti-apoptotic, pro-angiogenic and pro-proliferative properties (301) and the modulation of the native immune system (302). Exosomes originated from UC-MSCs have been found to contribute to wound healing (303) and reduce renal fibrosis after ischemic events by increasing capillarity density, reducing cell apoptosis, and restoring mitochondrial dynamics through miR-30b/c/d (116).

Despite the growing body of literature studying exosomes and their cargo in several settings, minimal evidence has been reported trying to underpin the molecular mechanism of action. Ferguson et al. investigated the biological processes modulated

by exosomal miRs and found that targeted pathways were related to Wnt signaling, TGF β and PDGF signaling, proliferation and apoptosis (301). Similarly, the expression profile of miRs in MSC-EVs derived from different sources lacks consistency. Although several studies have compared the expression between EVs and their parental MSC [reviewed by Qiu et al. (304)], limited studies have explored differences in miRs produced by different tissue-derived MSCs. To our knowledge, only one study has investigated the full RNAome derived from AT- and BM-MSC exosomes (305).

Akin to what we have described in the MSC field, EV isolation techniques lack standardization and generate variable products that can yield substantial differences. The use of serum or human platelet lysate supplements or serum-free conditions challenges the direct comparison of the relative contribution of EVs derived from MSC and other non-EVs factors. In a recent study, Whittaker et al. reported that soluble factors, that were non-EV molecules, were essential and sufficient to stimulate angiogenesis and wound healing *in vivo* (306). Their results concluded that most isolation techniques generate heterogeneous preparations containing other bioactive molecules that might mislead the attribution of therapeutic benefits.

Future studies defining the properties of miRs and exosomes will help in better understanding their biological functions and implications in cell-free therapies.

Immunomodulatory Properties

The immune system plays a central role in tissue recovery after injury. MSCs interact extensively with the immune system and promote an anti-inflammatory and pro-regenerative environment that favors injury resolution and, ultimately, tissue repair (19, 307, 308).

Many studies have demonstrated the ability of MSCs to modulate the activation, proliferation, and function of various immune cells such as T and B lymphocytes, natural killer cells (NK), dendritic cells, macrophages, and neutrophils. Such activities rely on the plasticity (309) of MSCs to produce cytokines in response to the different stages of the inflammatory process (310) and researchers are now investigating whether MSC immunomodulatory properties are influenced by their tissue of origin. Nevertheless, results from these studies are rather diverse and it is challenging to make adequate conclusions.

Some studies have compared the immunomodulatory properties of perinatal MSC, mainly UC-MSCs and CB-MSCs, with adult tissue sources (AT- and BM-MSCs). Overall, MSCs derived from perinatal tissues have the lowest expression of HLA antigens (HLA-DMA, HLA-DPB1 and HLA-DR) and immune-related genes (JAG1, TLR4, TLR3, NOTCH2, and NOTCH3) (243), together with decreased amounts of IL1 α , IL6, IL8, (244) and increased IDO, IL1 β , LIF, and TNF β 2 in their secretome (311). UC-MSCs also have the most prominent inhibitory effects on T cell proliferation, in both co-culture and trans-well mixed lymphocyte reaction (MLR) *in vitro* assays, followed by PL-MSCs, AT-MSCs and BM-MSCs (243). Other studies however have shown greater inhibition of allogeneic T cell proliferation by either BM-MSCs *via* increased expression

of PDL1, IL10, and TGF β 1 (230, 241), or AT-MSCs, which have been shown to secrete higher levels of IDO (243, 312).

Key adhesion molecules and other immunological markers such as CD10, CD146, CD49d, ICAM1 (CD200), and PDL2 are also increased in WJ-MSC preparations, together with decreased presence of IFN γ R1, CXCR3 and other costimulatory molecules such as CD80, CD86, and CD40 (264, 313). In a recent *in vivo* study performed by Tago et al., AM-MSCs and not BM-MSCs were able to reduce local inflammation and PD1⁺CD8⁺T cell proliferation when delivered into a murine model of GvHD (314). In addition, PDL1-enriched EVs derived from UC-MSCs have been proven to be the mechanism whereby UC-MSC-EVs enhance immunosuppression (315).

In line with what has been briefly described, Mattar et al. have also highlighted the intrinsic heterogeneity of MSCs, where *in vitro* data also might not relate to the complex *in vivo* situation (316). The inflammatory context is defined by a variety of cell types and stimulating factors that are determined to influence and “license” MSCs which may adapt and change their interactions with the immune system as a result (310). Therefore, future studies should aim to decipher if similarities/disparities of *in vitro* results correlate to similar *in vivo* functions and whether biological properties can help to define cell performance, providing rationale for the use of one particular cell source for any given disease.

CONSIDERATIONS FOR THE GMP-PRODUCTION OF HUMAN MSCs FOR KIDNEY DISEASE

The regulatory agencies in the European Union (EU), United States of America (USA) and Asia, have adapted regulatory pathways to accelerate patient access to advanced therapies such as ATMPs. However, the legal frameworks for ATMPs, as well as the criteria to be met to define a product as such, differs across these regions (317–319). In Europe, MSC-based ATMPs are governed by the EU Regulation 1294/2007/EC and Directive 2009/120/EC, and its manufacturing must be compliant with European current good manufacturing practice (cGMP) guidelines (EudraLex Volume 4, Part IV). These ATMP-specific regulations have been put in place to ultimately ensure the safety and wellbeing of patients, as single alterations in the bioprocess hold the potential to alter the final product with potential risk to the patient. For this reason, in the absence of proof of product comparability, regulatory authorities are prompted to require further re-validation, which in the worst-case scenario have resulted in pre-clinical data being invalidated and clinical trial approval requiring re-authorization. Thus, optimal manufacturing variables must be considered and identified during early stages of development, as changes in the bioprocess workflow later in the translational pathway may have a significant long-term impact on the success of the therapy with time and cost consequences but also a significant time-to-market delay (320, 321). In addition, having full control of the process is crucial for ensuring consistent production and quality standards in terms of safety, identity, and potency, and

this control can only be guaranteed by having in place systems of quality assurance (QA) and quality control (QC) across all stages of the bioprocess. While guidelines and some common criteria now exist for ATMP developers to follow, consistent clinical-grade production of MSCs is not yet achieved due to a lack of standardization and harmonization of manufacturing processes. Critical parameters in MSC manufacturing include the source of the starting material, and culture processing conditions, such as seeding density, passage number, media supplements and culture-expansion devices, among others (322, 323). These parameters have been shown to be highly variable among manufacturing centers and laboratories worldwide (47–49), increasing the number of variables, as illustrated in **Figure 5**, that should be considered when carving therapeutic approaches in specific clinical settings such as KD.

Tissue Origin

For many years BM has been the predominant cell type in clinical trials for kidney disease (**Figure 3D**) and others (47, 48, 324). One of the main advantages of using BM-MSCs is the ability to use them in autologous settings without triggering anti-donor immunoreactions. However, donor-related parameters such as age, disease severity and presence of comorbidities should be considered as they have been shown to affect MSC characteristics (53, 235, 325, 326). Recent attention has been given to donor gender, as there is now increasing evidence of gender influencing MSC properties such as growth kinetics, paracrine secretion and *in vivo* therapeutic potential (52, 53, 327, 328). Another disadvantage of using BM-MSCs is the need for large amounts of raw material to allow for extensive *ex vivo* cell expansion to obtain clinical doses, as these cells constitute a rare population (only 0.001–0.1%) within the whole bone marrow fraction (329). Finally, BM collection requires an invasive bone harvest procedure, which is accompanied by pain, risk of infection and other limitations such as patient’s comorbidities that can render this procedure unsuitable.

Alternative sources such as AT have been considered for many years. Subcutaneous AT has been shown to be an abundant source of AT-MSCs, with a yield of MSC precursors 500 times higher than from an equivalent amount of BM (330). AT-MSCs can be used both in autologous and allogeneic settings, which is advantageous. In addition, AT can be easily accessible as it is discarded as medical waste in many operations, which would be useful in allogeneic settings. In autologous settings, AT is harvested by less invasive procedures than BM, such as liposuction. However, like in BM sources, the therapeutic efficacy of autologous MSC therapies may be limited by the intrinsic impact that the disease, age, and gender may have on MSCs characteristics (50, 51).

Thus, in the past few years, more consideration has been given to the potential use of allogeneic MSC therapies due to the hypoimmunogenic phenotype of these cells (331). Perinatal and birth-associated tissues have become an attractive source of allogeneic MSCs for many reasons, the main being that this material is considered medical waste and discarded every day in hospitals worldwide. Also, these MSCs are obtained from

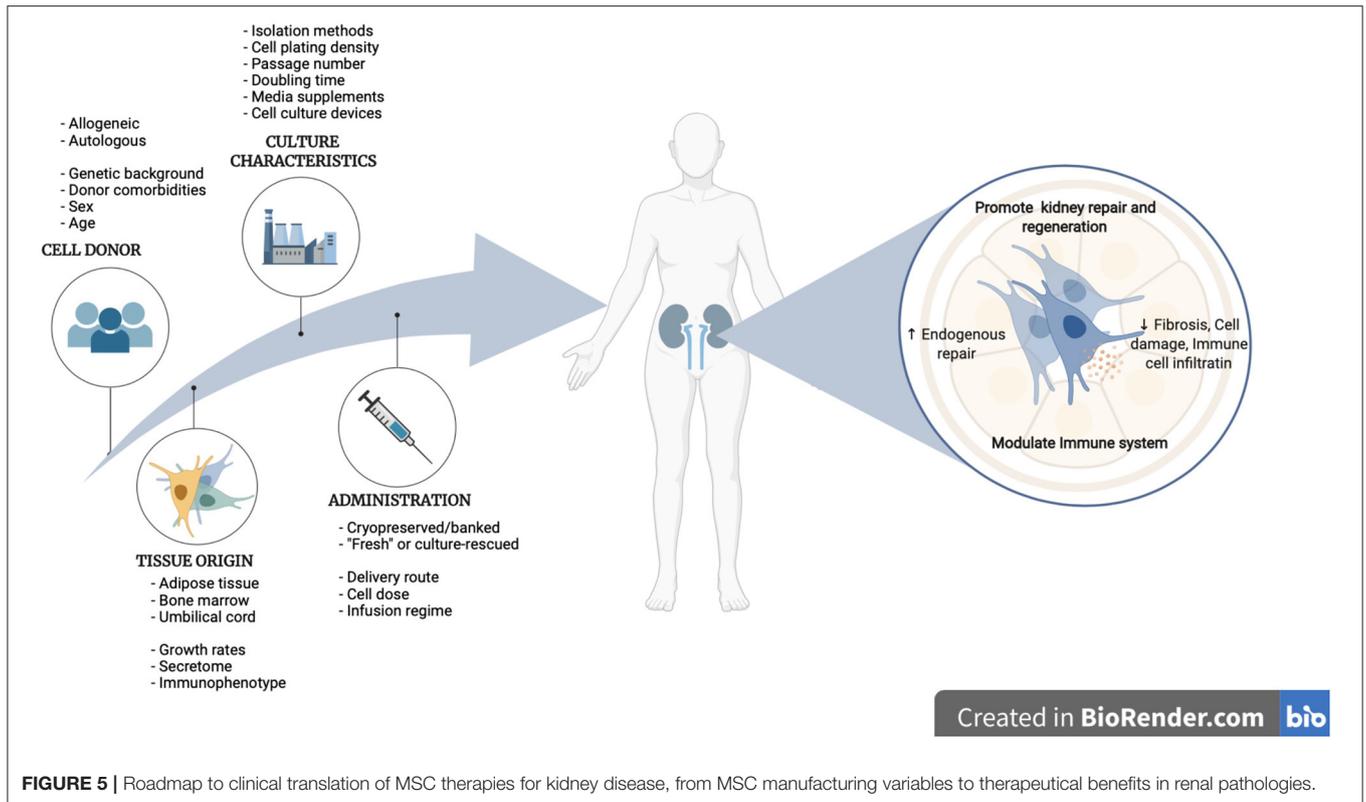


FIGURE 5 | Roadmap to clinical translation of MSC therapies for kidney disease, from MSC manufacturing variables to therapeutical benefits in renal pathologies.

the youngest donors possible (neonates), removing donor age-related confounding effects. Perinatal tissue sources such as UC have reported isolation rates ranging from 0.2–1.8% (216), and these cells have also been shown to have significantly higher proliferation rates, compared to MSCs isolated from adult tissues (217, 237, 241).

Overall, results from our search showed that the predominant source of MSCs used in clinical trials in renal pathologies is BM (58.1%), followed by UC (23.6%) and AT (11%) (**Figure 3D**). Also, autologous MSC therapies are predominant (62.5%) over allogeneic. Similar trends have been also observed in other studies (47, 48, 324). Interestingly, in renal pathologies, BM-MSCs have been mostly used in allogeneic settings (59.4%), while AT-MSCs have been used mostly in an autologous manner (80%) (**Figures 3Ei–iii**).

Culture Processing Characteristics

Based on ongoing clinical trial data in kidney disease, human MSCs are transplanted at typical doses of 1–2 million cells/kg and often not exceeding 10 million cells/kg (**Table 2**). Doing a basic dose extrapolation, for an 80 kg person the estimated human MSC doses per patient would range between 80 and 800 million cells per patient. Thus, the generation of clinical doses of MSC requires large-scale *ex vivo* cell expansion and having an optimal scale-up strategy for MSC manufacturing is critical for ensuring product quality while minimizing costs and time of production, as well as avoiding potential risks. The following are some key variables in the cell culture bioprocess to consider when designing MSC therapeutics.

Cell Plating Density

Cell plating density is a key parameter to ensure adequate expansion rates while maintaining stemness properties (332). The literature suggests that plating densities, both at isolation and subculturing, can influence functional and molecular characteristics of the MSCs (49, 219, 333), and yet it is something not well-standardized across laboratories. There are contradictory reports regarding the optimal subculturing seeding densities. Generally, higher plating densities (i.e., >5000 cells/cm²) have resulted in reduced proliferation rates, most likely due to contact inhibition by confluency and the need for continuous premature passaging (332), which is known to critically affect the proliferation rate of MSCs (242, 333–337). Also, the log phase has a longer duration in cells plated at low densities, and therefore more population doublings occur due to a longer exponential growth phase (338). Thus, finding the optimum seeding density for a maximal expansion of therapeutic MSCs while being cost-effective is crucial (339). Some studies have recommended using very low seeding densities when subculturing, as such required for clonal selection (i.e., <500 cells/cm²), as it has been shown to result in the highest cell proliferation rates (339–342). Other studies have used slightly higher densities between 2,000 and 4,000 cells/cm² (49, 206, 343). The disadvantage of using very low seeding densities (i.e. <100 cells/cm²) for a clinical-scale production of MSCs is the large surface area required to culture therapeutic doses of MSCs, which is not feasible when using 175 cm² flasks, due to the need for large incubator occupancy, a sizable amount of lab reagents and increased handling times. Plating densities of 1,000 cells/cm²

have been considered reasonable, as this density still allows for a high number of harvested cells (243, 342, 344). However, often more cost/labor compromises are undertaken with most current clinical trials using plating densities of over 3,000 cells/cm² (48). In the kidney disease clinical trial arena, a mix of low and high plating densities have been reported, ranging from 100 cells/cm² to 500,000 cells/cm² (Table 2).

Cell plating densities at the isolation phase have also shown similar outcomes in clinical trials. Sotiropoulou et al., have shown that initial plating density of bone marrow mononuclear cells (BM-MNCs) had a great impact on the size of the MSC-enriched population derived, with the maximum number of adherent cells at P0 obtained when using lower plating densities (<25,000 cells/cm²) compared to high plating densities (>50,000 cells/cm²), with 1,000 cells/cm² being the optimal condition (341). But similar challenges are encountered here, where large surface areas may be needed for the initial plating. For instance, given that up to 1×10^8 BM-MNC are commonly obtained, $\sim 600 \times 175$ cm² would be needed to seed 1×10^8 BM-MNC at 1,000 cells/cm², which is not practical or cost-efficient. Indeed, the most common seeding densities used in clinical trials are $1.5\text{--}1.6 \times 10^5$ cells/cm², followed by 1×10^6 cells/cm² (48). In our search on clinical trials for kidney disease, seeding densities at isolation have been reported to be $1\text{--}2 \times 10^5$ cells/cm² (Table 2). One approach to reducing the plating surface area at isolation would be cell enrichment by prospective immunoselection using antibodies directed against specific cell surface markers to obtain a more homogeneous, pure, and well-defined functional subset of MSC subpopulation. For instance, some markers that have been used to purify distinct subsets of MSCs include CD146 (345), CD271 (346, 347), Stro-1 (348) and CD362 (349), which have shown properties such as having greater paracrine immunomodulatory and anti-inflammatory properties (188, 345, 349), increased osteogenic commitment (346, 347), and higher production of cardiovascular-relevant cytokine production (348).

Passage Number

MSCs are an adherent cell population and have normal growth inhibition when confluent. This has led to the use of successive passages for obtaining a large amount of MSCs. Passage number, which refers to the number of times cells have been sub-cultured, is often recorded as an indicator of cellular age. Cell expansion requires enzyme dissociation and cell subculture, and while the evaluation of the optimal cell confluence may vary among operators, a 70–80% confluence is recommended to be reached before detachment (323). In general, passage numbers from 1 to 5 are commonly used in clinical trials (48, 324) and we have also found similar trends in our search (Table 2). The use of low passage MSCs for therapy is currently preferred to higher passages due to the impact that extended passaging has in decreasing the cell proliferation rates and increasing senescence times (242, 333–337). Long-term culture has also been shown to affect other properties of MSC such as immunosuppressive activity (242, 335), trilineage differentiation (333, 334, 337), *in vivo* therapeutic potential (333, 337, 350), and have also been shown to increase genomic instability although not to

induce *in vivo* tumorigenicity (336, 337). Also, the advantage of transplanting MSC at earlier passages over late passages was demonstrated clinically in patients with GVHD, where 1-year survival rates were higher in those patients that received MSCs at passages 1–2 (75%) compared to those that received later passage MSCs (passage 3–4) (350). Effects of passage number in combination with cryopreservation cycles have also been described to impact the safety profile of MSC products, with cells cultured for extended times triggering stronger prothrombotic events compared with cells cultured for shorter times and “fresh” cells (44, 168). For these reasons, regulatory agencies have recognized the importance of cellular age tracking during the manufacturing process, as well as the need for karyotypic analysis as a product release criterion. While passage number has been traditionally used for cellular age tracking, this is largely dependent on the specific seeding and harvest density conditions, and therefore it is challenging to make comparisons between studies. Population doubling level (PDL), which refer to the total number of times the cells have doubled during *in vitro* culture, is, therefore, a more robust and accurate parameter to define cellular aging. An upper limit of PDL, before culture ceases to replicate, must be defined for each product. The literature suggests a maximum number of cell population doubling to be between 15 and 30 (333), although this may be influenced by the cell type (i.e., UC-MSCs showed higher proliferation rates and later senescence than AT- and BM-MSCs) (230, 242, 243, 351) and the culture processing characteristics (219).

Media Supplements

The most common basal media employed in current MSC expansion protocols are DMEM or α MEM (48), although α MEM has shown to be more suitable for both isolation and expansion of MSCs (341, 352). MSCs however require media supplements such as serum and/or growth factors to be added to the basal medium for optimal MSC growth. Most expansion protocols, especially at laboratory-scale and in early phase clinical trials, have used fetal calf serum (FCS) with 10% being the standard concentration used for MSC expansion (48). Basic fibroblast growth factor (b-FGF), at a final concentration of 1–2 ng/ml, is also added to the basal media to enhance the proliferation rate of cultured cells while maintaining the multilineage differentiation potential (353, 354). Nevertheless, the use of FCS for large-scale production of clinical doses of MSCs is not a viable option (355). Limitations in the availability of the raw material are a major cost driver and represent a current bottleneck. Also, due to the batch-to-batch variability, FCS lots must be carefully tested to ensure optimal MSC expansion rates and trilineage differentiation potential. In addition to this, there are current regulatory challenges associated with the use of FCS to produce clinical-grade MSCs due to the risk of inter-species cross-contamination, and regulators urge the development of xenogeneic-free compositions. Considerations to using human-derived blood components such as human platelet lysate (hPL), often at a final concentration of 5–10%, have been given (47, 356). While hPL has been shown to have comparable growth factors and cytokines to FCS to support MSC growth (355), it poses some important challenges (357). HPL can be derived from autologous

collections, but it does not represent a good commercial model. Large scale, allogeneic, off-the-shelf pools are easier to standardize, have less lot-to-lot variation, is more economical to produce and therefore represents a better commercial model. Nevertheless, while hPL collections can be obtained from up to 100 different individuals, the size of pools is a current issue and a topic of debate. Recently, regulators have expressed their concern about the increased risk of transmission of infectious agents in large pooled hPL products. The *European Pharmacopeia* have recommended the limitation of pooled donations unless sufficient methods for inactivation or removal of viruses are applied during the production, although it does not give specific recommendations to the pool size (Chapter 5.2.12). However, representatives of the German Federal Regulatory Authority specified the restriction to a maximum of 16 donors (358). This imposes many challenges for ATMP developers and commercial entities, who must fast adapt their products to the constantly evolving regulatory framework. Overall, a consensus is needed to ensure the quality and safety of hPL supplements regarding the source of platelet concentrate, donor- and lot- variability, manufacturing processes and minimum release criteria (357).

Due to the concerns mentioned above, the development of new xenogeneic-free, chemically defined formulations is urgently needed. Chemically defined media (CDM) are generally composed of basal media to which supplements of known composition (i.e., growth factors, hormones, attachment factors, binding proteins, and vitamins) are added (320). An ideal MSC media should contain chemically defined constituents preferably of recombinant human origin that support the isolation and culture expansion of human MSCs obtained from different tissue sources while maintaining MSC phenotypic characteristics, morphology, and mechanism of functional benefit. Ideally, it should also support the attachment of MSCs without coating. Extensive testing is however required to ensure these media fulfill MSC requirements, but when successful, this type of media will have the potential to enhance batch-to-batch consistency in the cell manufacturing process and will therefore represent a more cost-effective and risk-reduced approach. To date, 10% FCS continues to be the most common media supplement employed in clinical trials for kidney disease, although some consideration has been given to the use of xenogeneic-free media such as hPL (5 or 10%), human serum albumin (HSA) and a CDM (Table 2). Currently, some commercial and non-commercial CDM formulations have been investigated (359–363), however, there is still limited availability of some of these media for large-scale manufacturing at GMP quality level.

Cell Culture Devices

Currently, a complete closed system that allows MSC acquisition, expansion, and delivery at the bedside, is not yet available. GMP conditions have been mainly achieved using laminar airflow cabinets to perform the main steps in the bioprocess such as culture inception, medium changes, subculturing and packaging. Traditionally, scale-out of MSC manufacturing has been achieved using 2D monolayer cultures using multilayered flasks (Corning® CellSTACK and Nunc™ Cell Factory™) of 1 to 40 levels, and surfaces ranging from 636 cm to 25, 440 cm.

However, this is not an optimal system for large-scale production of therapeutic doses, as it is labor-intensive, requires significant manual handling, and is not cost-effective (320, 364). These are also static systems, which lack real-time process monitoring of culture conditions, and are more susceptible to batch-to-batch variation due to a non-homogeneous environment within layers (320). Alternatively, GMP-compliant, closed, automated, high-volume cell expansion systems offer great advantages, as they enable the real-time monitoring of process variables such as pH, pO₂, pCO₂, metabolite accumulation or presence of contaminants, and hence it guarantees a homogeneous distribution of culture environment and ensures a culture process under well-controlled and reproducible conditions and production of quality MSCs for clinical use. A variety of bioreactors are available including stirred tank bioreactors with microcarriers (365, 366), rocking motion (367), disposable fixed bed (368), and hollow fiber-based continuous perfusion bioreactors (369, 370). The surface area from these bioreactors ranges from 21, 000 cm²/unit to up to 3, 750, 000 cm²/unit, and they all offer distinct advantages and limitations that must be considered (320, 364, 371). In our search, an overwhelming majority of clinical trials have used 2D culture conditions, with only 1 study considering a 3D bioreactor, the Quantum Cell Expansion System (Terumo BCT) (NEPHSTROM clinical trial, NCT02585622) (Table 2).

CONCLUSIONS AND FUTURE PERSPECTIVES

While the field of cell-based therapies evolves, the selection of particular MSC types in specific clinical conditions remains to be elucidated. In the past decades, BM has been the preferred source of MSCs used in clinical trials of kidney disease, but recently allogeneic sources have emerged as strong candidates in the clinical research arena. Ideally an allogeneic, “off-the-shelf” MSC product would be preferred, especially for acute kidney disease settings where delivery time is crucial. We hypothesize that, the use of MSCs may be rationalized by the intrinsic origin-specific properties which may make one cell type more advantageous for a specific disease condition. Overall, the high proliferative capacity, the stronger immunosuppressive effects and hypo-immunogenetic properties of UC-MSCs paired with their allogeneic nature makes them ideal to be used in an “off-the-shelf,” large-scale, universal production model. Although it is likely that the choice of MSC type may be driven by intellectual and/or industrial property on isolation methods, protocols and/or reagents in addition to issues of functional and biological superiority, considerations should be also given to the safety of this therapies, in particular accounting for differences in immune and hemocompatibility characteristics. Considering this constellation of variables, robust clinical guidelines and well-characterized therapeutic products are urgently needed to deliver safer, effective, and potent MSC therapies to improve clinical outcomes. This will require a greater understanding of the biology of MSCs from different tissue sources along with an alignment with disease pathophysiology coupled with

consideration and standardization of the cell manufacturing variables reviewed in this article.

AUTHOR CONTRIBUTIONS

SC-C, CS-N, and TO'B wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Conflict of Interest: TO'B is a founder, director and equity holder in Orbsen Therapeutics Ltd.

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