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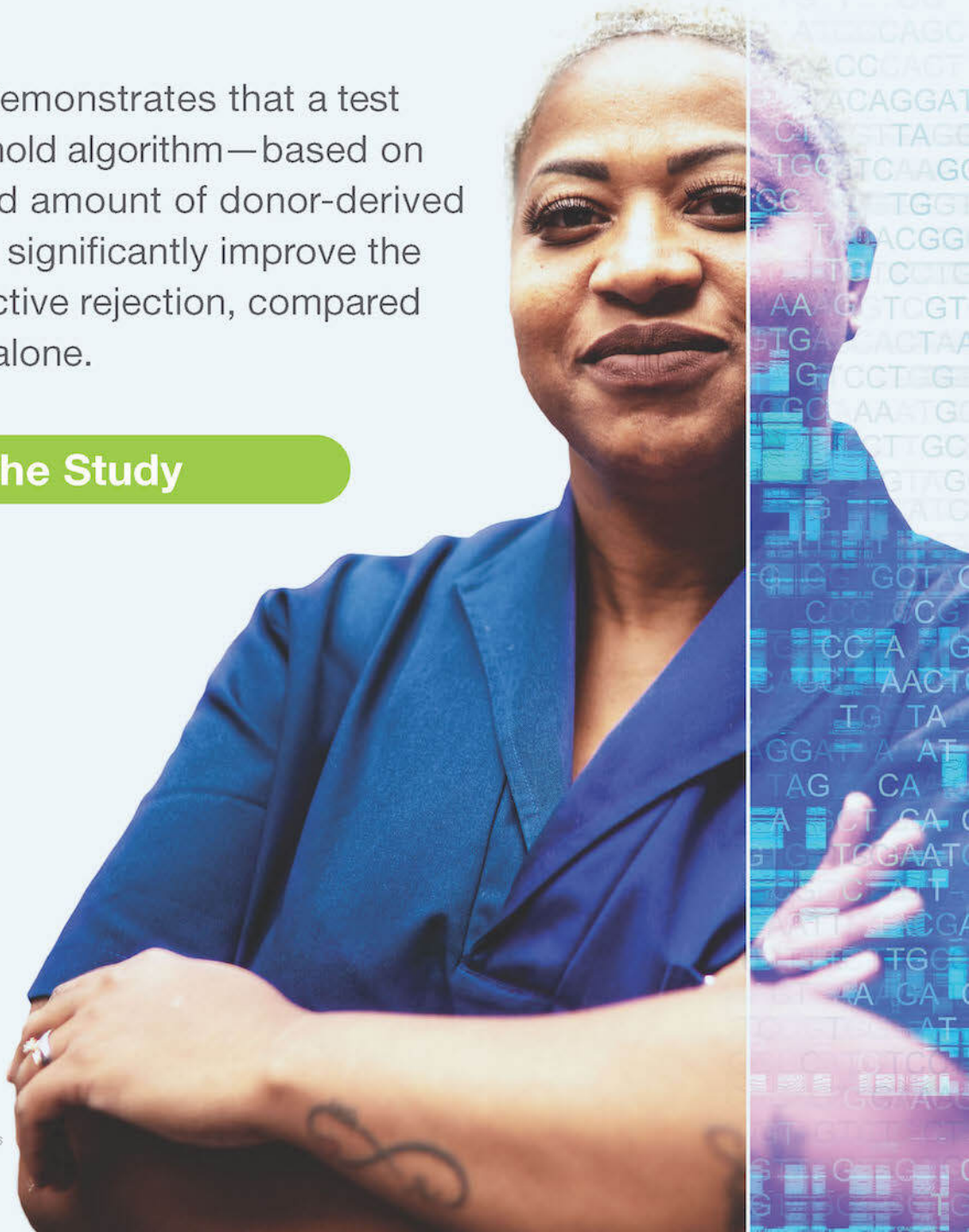

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MINIREVIEW

The current status of stem cell-based therapies during ex vivo graft perfusion: An integrated review of four organs

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Abbreviations: ADAR, adenosine deaminase, RNA-specific; AFC, alveolar fluid clearance; AKT1, v-akt murine thymoma viral oncogene homolog 1; AKT3, v-akt murine thymoma viral oncogene homolog 3; ALP, alkaline phosphatase; ALT, alanine aminotransferase; A-MSC, adipose-derived mesenchymal stromal cell; ANG-1, angiopoietin 1; AOX1, aldehyde oxidase 1; AST, aspartate aminotransferase; ATP, adenosine triphosphate; ATP6V0D2, vacuolar H+-ATPase d2 Subunit; BALF, bronchoalveolar lavage fluid; BCL2L11, BCL2-like 11; BICC1, bicaudal C homolog 1 gene; BID, BH3-interacting domain death agonist; BIRC5, baculoviral IAP repeat-containing 5; BM-MSC, bone marrow-derived mesenchymal stromal cell; BTLA, B- and T-lymphocyte attenuator; BW, bodyweight; CALB1, calbindin 1; CASP2, caspase-2; CASP8, caspase-8; CAT, catalase; CCL11, chemokine (C−C motif) ligand 11; CD40LG, CD40 ligand; CFTR, cystic fibrosis transmembrane conductance regulator; CXCL2, chemokine C-X-C motif ligand 2; CXCL3, chemokine C-X-C motif ligand 3; CXCR4, C-X-C chemokine receptor type 4; CXCR5, C-X-C chemokine receptor type 5; CXCR7, C-X-C chemokine receptor type 7; DCDC2, doublecortin domain-containing protein 2; DHCR24, 24-Dehydrocholesterol reductase; DNM2, dynamin 2; dP/dt_{max}, maximal slope of systolic pressure increment; dP/dt_{min}, maximal slope of diastolic pressure decrement; *E.coli*, *Escherichia coli*; EB, endobronchially; ECD, extended criteria donor; EGF, endothelial growth factor; EPX, eosinophil peroxidase; EV, extracellular vesicle; EVHP, ex vivo heart perfusion; EVLP, ex vivo lung perfusion; FADD, fas (TNFRSF6) associated via death domain; FASLG, Fas ligand; FBS, fetal bovine serum; FGF-1, fibroblast growth factor-1; FGF-2, fibroblast growth factor-2; FLT-3 L, FMS-like tyrosine kinase 3 ligand; FOXG1, forkhead box G1; GCP, Good Clinical Practice; G-CSF, granulocyte colony-stimulating factor; GGT, gamma-glutamyltransferase; GITR, glucocorticoid-induced tumor necrosis factor receptor; GM-CSF, granulocyte-macrophage colony-stimulating factor; GPX, glutathione peroxidase; GPX4, anti-glutathione peroxidase 4; GRB2, growth factor receptor-bound protein 2; GSH, glutathione; GZMB, granzyme B; HA, hyaluronic acid; HGF, hepatocyte growth factor; HIF1A, hypoxia-inducible factor 1-α; HLA-G5, human leukocyte antigen-G5; HLSC, human liver stem cell; HLSC-EV, human liver stem cell extracellular vesicle; HMGB1, high-mobility group box 1; HMP, hypothermic machine perfusion; HO-1, heme oxygenase 1; HO-1/BMMSC, heme oxygenase 1-modified bone marrow-derived mesenchymal stromal cell; HOPE, hypothermic oxygenated perfusion; HSP70, heat shock protein 70; HYOU1, hypoxia upregulated 1; IA, intra-arterially; ICAM-1, intercellular adhesion molecule 1; IDH2, isocitrate dehydrogenase 2; IDO, Indolamine-2,3-dioxygenase; IFN-γ, interferon-gamma; IGF, insulin-like growth factor; IH, intrahepatic; IL, interleukin; IL-1-RA, interleukin-1 receptor antagonist; IP-10, interferon-inducible protein 10; IT, intratracheal; IV, intravenous; KRT19, cytokeratin-19; KRT7, cytokeratin-7; LDH, lactate dehydrogenase; LLL, left lower lobe; MAPC, multipotent adult progenitor cell; MAPK3, mitogen-activated protein kinase 3; MAPK4, extracellular signal-regulated kinase 4; MCP-1, monocyte chemoattractant protein-1; MCP-3, monocyte chemoattractant protein-3; M-CSF, macrophage colony-stimulating factor; MDA, malondialdehyde; MDC, macrophage-derived chemokine; MIP, macrophage inflammatory protein; MP, machine perfusion; MPO, myeloperoxidase; MSC, mesenchymal stromal cell; MSC-CM, mesenchymal stromal cell-conditioned medium; MSC-EV, mesenchymal stem cell-derived extracellular vesicle; mTOR, mechanistic target of rapamycin; MV, microvesicle; MVB, multivesicular body; NDUFS8, NADH dehydrogenase Fe-S protein 8; NGAL, neutrophil gelatinase-associated lipocalin; NK, natural killer; NMP, normothermic machine perfusion; NO, nitric oxide; NOX4, NADPH oxidase 4; NOXO1, NADPH oxidase organizer; PAK1, p21 protein (Cdc42/Rac)-activated kinase; PAP, pulmonary arterial pressure; PAR, pulmonary artery resistance; PBG, peribiliary gland; PCNA, proliferating cell nuclear antigen; PD-1, programmed cell death protein-1; PDHB, pyruvate dehydrogenase beta; PGE2, prostaglandin E2; PTGS2, prostaglandin-endoperoxide synthase 2; PV, portal vein; PVR, pulmonary vascular resistance; RAI, rejection activity index; RANTES, regulated on activation, normal T expressed and secreted; RBC, red blood cell; RML, right middle lobe; RNA, ribonucleic acid; ROS, reactive oxidative species; SDF-1α, stromal cell-derived factor-1α; Serpinb1b, Serine (or cysteine) peptidase inhibitor, clade B, member 1b; SLC16A1, monocarboxylate transporter 1; sNMP, subnormothermic machine perfusion; SOCS3, suppressor of cytokine signaling 3; SOD, superoxide dismutase; SOD2, superoxide dismutase 2, mitochondrial; SOX4, sex-determining region Y-related high-mobility group box transcription factor 4; STC-1, stanniocalcin-1; STIP1, transformation-sensitive protein IEF SSP 3521; Tau-w, time constant of the left ventricular pressure decay; TCL1a, T-cell leukemia/lymphoma 1A; TGF, transforming growth factor; TIMP-1, tissue inhibitor of metalloproteinase-1; TNF-α, tumor necrosis factor-α; TPN, total parenteral nutrition; TPO, thyroid peroxidase; TPVR, total pulmonary vascular resistance; T-reg, regulatory T cell; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; TXNRD2, thioredoxin reductase 2; UC-MSC, umbilical cord-derived mesenchymal stromal cell; UW-MPS, University of Wisconsin machine perfusion solution; VEGF, vascular endothelial growth factor.

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The use of extended criteria donor grafts is a promising strategy to increase the number of organ transplantations and reduce waitlist mortality. However, these organs are often compromised and/or damaged, are more susceptible to preservation injury, and are at risk for developing post-transplant complications. Ex vivo organ perfusion is a novel technology to preserve donor organs while providing oxygen and nutrients at distinct perfusion temperatures. This preservation method allows to resuscitate grafts and optimize function with therapeutic interventions prior to solid organ transplantation. Stem cell-based therapies are increasingly explored for their ability to promote regeneration and reduce the inflammatory response associated with in vivo reperfusion. The aim of this review is to describe the current state of stem cell-based therapies during ex vivo organ perfusion for the kidney, liver, lung, and heart. We discuss different strategies, including type of cells, route of administration, mechanisms of action, efficacy, and safety. The progress made within lung transplantation justifies the initiation of clinical trials, whereas more research is likely required for the kidney, liver, and heart to progress into clinical application. We emphasize the need for standardization of methodology to increase comparability between future (clinical) studies.

KEYWORDS
animal models, immunobiology, ischemia reperfusion injury (IRI), organ perfusion and preservation, regenerative medicine, solid organ transplantation, stem cells, tissue injury and repair, translational research/science

1 | INTRODUCTION

Solid organ transplantation remains the only curative option for patients with end-stage organ failure. However, the existing gap between the need for suitable donor organs and the availability of those organs remains a major challenge. Therefore, various strategies are employed to expand the donor pool. Among these strategies is the increasing use of extended criteria donor (ECD) organs, referring to grafts of older donors, from obese or otherwise comorbid donors and organs from donation after circulatory death. Due to increased susceptibility to ischemia–reperfusion injury (IRI), the use of ECD organs is associated with higher rates of primary nonfunction, delayed graft function and organ-specific complications.[1,2] Despite this, the use of ECD organs has become reality and is inevitable to prevent aggravation of the organ shortage.

Stem cell-based therapies have been proposed as innovative approaches for treatment of IRI, to reduce the use of immunosuppression, and even for induction of long-term immune tolerance. Mesenchymal stromal cells (MSC) and multipotent adult progenitor cells (MAPC) have been reported to participate in repairing tissue or organ injury mainly through their paracrine effects, recruitment of recipient progenitor cells, and stimulation of their proliferation and differentiation (Figure 1).[3,4] Some of the beneficial effects are mediated by the release of extracellular vesicles (EVs). EVs represent a broad class of membrane-enclosed protein and ribonucleic acid (RNA)-containing bodies of cell origin. They can be divided into exosomes, generated by the endosomal pathway, and microvesicles, produced by shedding of the plasma membrane.[5,6] EVs are involved in tissue repair and immune regulation by serving as vehicles for transfer of membrane and cytosolic proteins (Figure 2).[5–7] Isolated EVs could serve as a cell-free tissue regenerative approach, evading the safety concerns about injecting living cells, such as immunogenicity, embolism, and even tumorigenicity.[8]

Early clinical trials showed safety of intravenous MSC infusion, and the first steps toward efficacy studies in humans have been made.[9] Although some encouraging results have been shown, such as increased regulatory T-cell expansion and weaning of immunosuppressive drugs, long-term graft and patient outcomes are still lacking, and therefore, none of these cell therapies is routinely used in organ transplantation.[9] A limitation of the current post-transplantation cell administration is the non-specific delivery of cells to the recipient, rather than to the graft, which may hamper the required immunomodulation through cell interactions or trans-differentiation at the injury site. Also, the administration of cells after transplantation is missing the window of opportunity to repair injured grafts before implantation.

Machine perfusion (MP) is an emerging preservation technique to maintain organs for transplantation in an environment to mimic physiology by circulation of oxygenated perfusate through the organ in hypothermic or (sub)normothermic conditions. The continuous flow permits wash out of toxic metabolites and provides oxygen and nutrients. In addition, in normothermic perfusion conditions, assessment of metabolic and mechanical function under near-physiological circumstances can be performed. The strong advantage of combining stem cell-based therapies with ex vivo MP is to deliver cells or EVs in the right place at the right time, overcoming the loss of valuable cells in the systemic circulation, and it may be attractive to

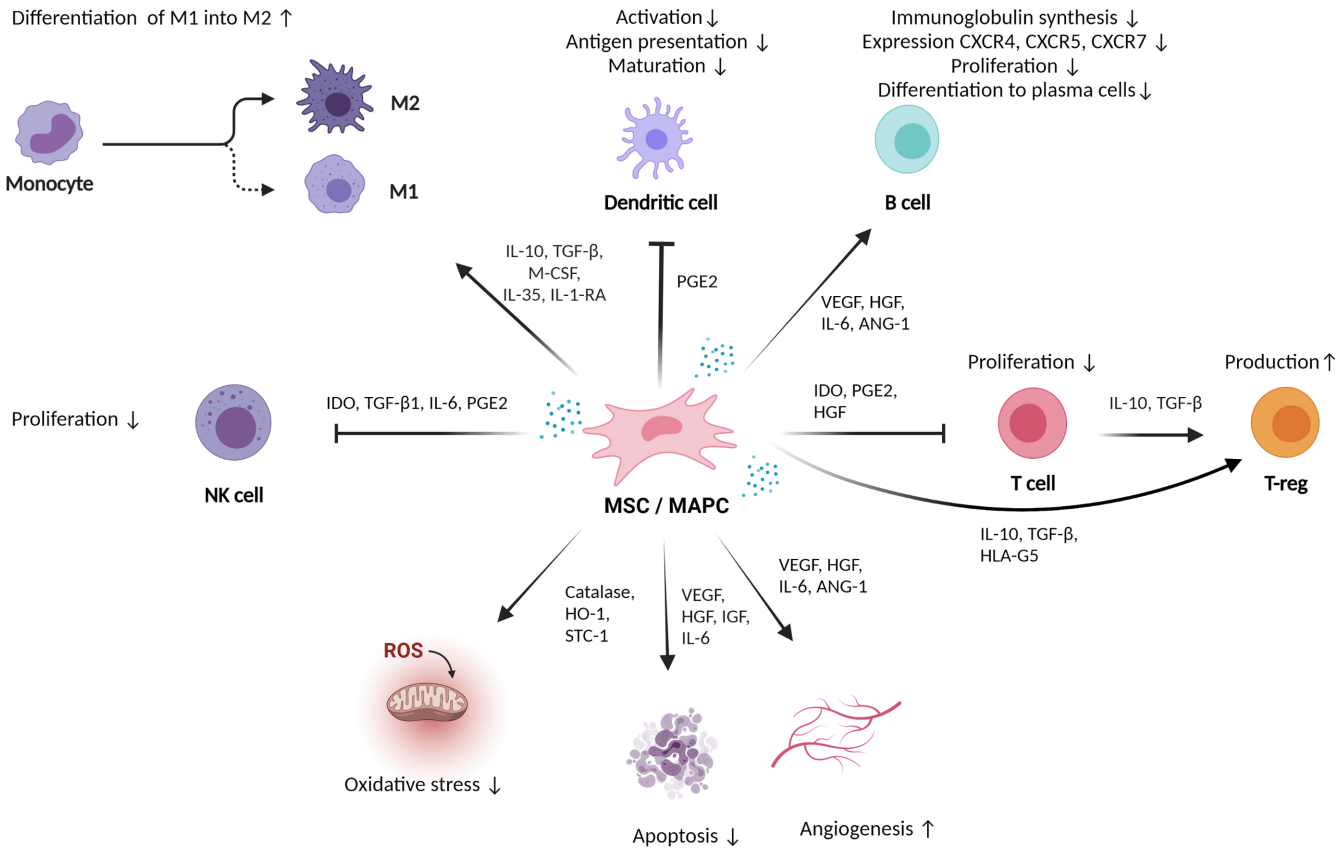

FIGURE 1 Effects of mesenchymal stromal cells (MSCs) and multipotent adult progenitor cells (MAPCs) on modulation of immune response and stimulation of repair. MSC and MAPC exert their effects through paracrine mechanisms and cell-to-cell interactions with immune cells, such as B cells, natural killer (NK) cells, T cells, T-regulatory (T-reg) cells, macrophages, and dendritic cells. Secretion of soluble factors, including chemokines, growth factors, and cytokines contribute to their antiapoptotic, angiogenic, and anti-oxidative properties. ANG-1, angiopoietin-1; CXCR4, chemokine receptor type 4; CXCR5, chemokine receptor type 5; CXCR7, chemokine receptor type 7; HGF, hepatocyte growth factor; HLA-G5, human leukocyte antigen-G5; HO-1, heme oxygenase 1; IDO, indolamine-2,3-dioxygenase; IGF, insulin-like growth factor; IL, interleukin; IL-1-RA, interleukin-1 receptor antagonist; M-CSF, macrophage colony-stimulating factor; PGE2, prostaglandin E2; ROS, reactive oxidative species; STC-1, stanniocalcin-1; T-reg, T-regulatory; TGF-β1, transforming growth factor beta 1; TGF-β, transforming growth factor-beta; VEGF, vascular endothelial growth factor.

apply stem cell-based therapies outside the body from a regulatory and biosafety point of view.

2 | KIDNEY TRANSPLANTATION

Six publications describe stem cell-based therapy during kidney MP. Detailed findings are described in Table 1. Treatment of rat kidneys with MSCs or its derived EVs during hypothermic machine perfusion (HMP) resulted in a reduction of histopathological renal injury.[10] Both cells and vesicles resulted in higher levels of pyruvate and lower levels of glucose and lactate in the draining perfusate, indicating larger use of provided energy substrates.[10] Brasile et al. infused human MSCs during subnormothermic perfusion (sNMP) of human discarded kidneys.[11] This is the only study that performed a titration experiment, showing good kidney perfusion up to infusion of $100*10^6$ cells. Treatment resulted in a reduced inflammatory response and increased synthesis of adenosine triphosphate (ATP). Pool et al. injected $10*10^6$ human fluorescent-labeled MSCs in porcine kidneys during normothermic machine perfusion (NMP) and showed that a minority of the glomeruli stained positive for MSCs.[12] In a follow-up study, MSC-treated kidneys showed significantly lower levels of renal injury markers and increased concentrations of immunomodulatory cytokines.[13] A third study from this group took the MSC-NMP model one step further, and autotransplanted pig kidneys after NMP, during which pig or human MSCs were injected.[14] MSC treatment did not affect urinary and perfusate levels of neutrophil gelatinase-associated lipocalin (NGAL), or kidney injury assessed by histology during 14 days post-transplantation. However, plasma creatinine on days 2–7 was significantly lower in the NMP + human MSC group, compared to NMP alone. Administration of MAPCs in paired discarded human kidneys during NMP resulted in improved urine output and decreased urinary concentrations of NGAL.[15] Also, MAPCs exerted an anti-inflammatory response and increased microvascular perfusion of the renal medulla and cortex.

Summarizing, MSCs and MAPCs universally showed potential to mitigate renal IRI on a biochemical and histological level. Adipose tissue-derived MSCs were mostly used. Heterogeneity in cell number currently hampers direct clinical translation. MSC concentrations between 10 and $100*10^6$ did not cause adverse effects on kidney flow, but infusion of

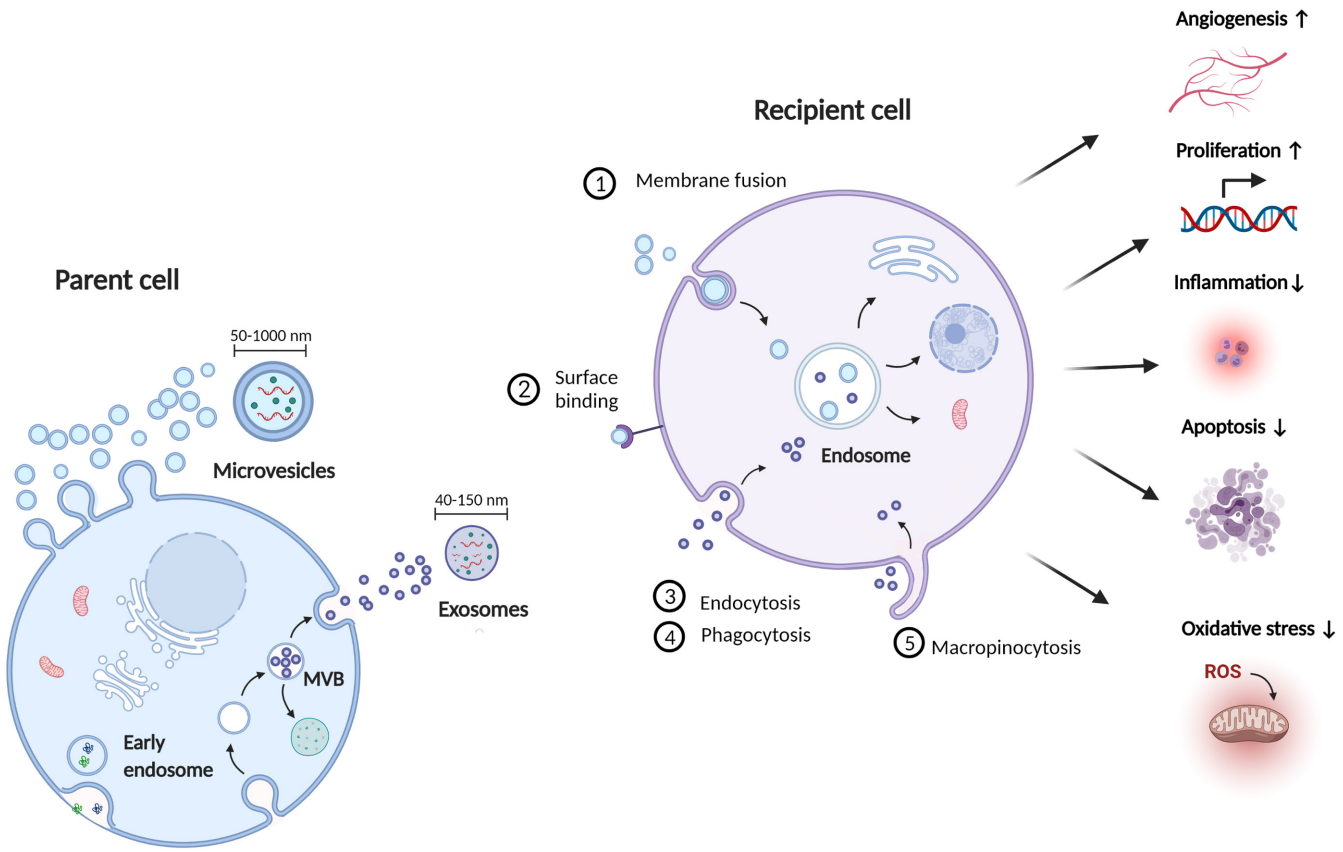

FIGURE 2 Extracellular vesicle (EV) biogenesis, release, and biological mechanisms of internalization by recipient cells. Microvesicles (MVs) are generated by outward budding and fission of the plasma membrane. Exosomes are intraluminal vesicles formed by inward budding of multivesicular bodies (MVBs). MVBs fuse with the plasma membrane to release intraluminal vesicles into the extracellular space. EVs can be internalized through membrane fusion, surface binding, endocytosis, phagocytosis, and micropinocytosis. EV content can be directly transferred to the cellular components (nucleus or trans-Golgi network) or can enter the endosomal system before a biological response is elicited. EVs are involved in physiological processes, including promotion of angiogenesis, stimulation of proliferation, immune regulation, protection against apoptosis, and reduction of oxidative stress. ROS, reactive oxidative species.

$200*10^6$ cells had a negative effect on renal artery flow, increased perfusion pressure, and reduced renal oxygen consumption.[11] A maximum of $100*10^6$ MSC (~$1.25*10^6$ MSCs/kg BW) for a human kidney seems therefore safely tolerated. To standardize outcomes in future research, we propose to express the administered dose in number of cells/100 g of kidney tissue, which would approximate 80×10^6 cells/100 gram kidney for the reviewed studies. All NMP studies administered cells after 1 h of perfusion. None of the studies evaluated the impact of administration prior to normothermic reperfusion on inflammation and functional outcomes. Interestingly, administration in the cold and subnormothermia also showed protective and regenerative effects, but no direct comparison was made between different temperature strategies. Currently, no clinical study is published showing outcomes of kidneys treated with stem cell-based therapies during ex vivo kidney perfusion.

3 | LIVER TRANSPLANTATION

The administration of stem cell-based therapy during liver MP was described in 12 publications. Detailed information of 10 animal and 2 human liver studies is provided in Tables 2A, 2B. Infusion of porcine MSCs in rat livers during NMP improved bile production and ameliorated narrowing of the sinusoidal space.[16] Administration of rat bone marrow (BM) MSCs in rat livers during NMP resulted in improved lactate clearance and bile production compared to NMP alone.[17] Also, treated livers showed less histological injury, reduced hepatocyte apoptosis, and oxidative stress. The same group demonstrated increased bile production and decreased levels of alanine transaminase (ALT) and aspartate aminotransferase (AST) following administration of MSCs. Histology revealed the presence of MSCs in hepatic sinusoids and a lower Suzuki score.[18] Cao et al. increased complexity of the model and transplanted rat livers after NMP, adding naïve rat BM-MSCs or heme oxygenase 1 (HO-1)-transduced BM-MSCs.[19] Administration of either type of MSCs improved graft survival, decreased histological injury, and lowered levels of AST and ALT compared to NMP alone, but HO-1-BM-MSC to a greater extent. In a follow-up study, repair of bile duct injury in transplanted livers following administration of BM-MSCs and HO-1-BM-MSCs during NMP was demonstrated.[20] Treatment promoted the proliferation of residual peribiliary gland

TABLE 1 Pre-clinical studies evaluating the efficacy of stem cell-based therapy in ex vivo kidney perfusion.

Author	Type of kidney	Type of therapy	Timing, dosage, and route administration	Perfusion technique, temperature and perfusate composition	Findings
Gregorini et al (2017)[10]	Rat	Rat MSCs or MSC –EVs	**Timing:** just prior to HMP **Dosage:** $0.3*10^6$ MSCs or MSC- EVs **Scaling dosage:** $2.2*10^6$ MSCs or MSC-EVs/kg BW **Route:** IA	**Perfusion technique:** 4 h HMP **Temperature:** 4°C **Perfusate composition:** Belzer solution	*During perfusion* **Adverse effects:** no evidence of macro/microvascular engorgement or thrombosis on immunohistochemistry **Functional:** LDH↓, MDA↓, glucose↓, lactate↓, pyruvate↑ **Biological:** - **Histological:** renal ischemic injury↓ **Other:** up-regulation of IDH2, NDUFS8, PDHB, CALB1, SLC16A1, ATP6V0D2
Brasile et al (2019)[11]	Human discarded	Human MSCs	**Timing:** "after hemodynamic stabilization", not further specified **Dosage:** $100*10^6$ MSCs **Scaling dosage:** N.S. ~ $1.25*10^6$ MSCs/kg BW (est.) **Route:** IA	**Perfusion technique:** 24 h SNMP **Temperature:** 32°C **Perfusate composition:** acellular medium	*During perfusion* **Adverse effects:** injection of $200*10^6$ MSCs was associated with reduced vascular flow and a significant reduction in the oxygen consumption **Functional:** ATP-synthesis ↑ **Biological:** Eotaxin↓, G-CSF↓, IL-6↓, IP-10↓, MIP-1α↓, MIP-1β↓, RANTES↓,TNF-α↓, MCP-3↓, FLT-3L↓, GM-CSF↓, Fractalkine↓, MDC↓, IL-1β ↓, EGF↑, FGF-2↑, TGF-α↑ **Histological:** mitosis ↑, PCNA↑, no MSC migration to renal parenchyma
Pool et al (2019)[12]	Porcine	Human A-MSCs	**Timing:** after 60 min of NMP **Dosage:** 0.1, 1 or $100*10^6$ A-MSCs **Scaling dosage:** N.S.~0.0008–$0.77*10^6$ MSCs/kg BW (est.) **Route:** IA	**Perfusion technique:** 7 h NMP **Temperature:** 37°C **Perfusate composition:** Williams' Medium E, human albumin, amoxicillin-clavulanate, and autologous, washed, leukocyte depleted RBCs	*During perfusion* **Adverse effects:** no changes in haemodynamics after administration **Functional:** - **Biological:** - **Histological:** MSCs were detected by immunohistochemistry in lumen of glomeruli **Other:** fluorescence showed that only a minority of glomeruli were positive for infused MSCs and most of these glomeruli contained multiple MSCs

(Continues)

TABLE 1 (Continued)

Author	Type of kidney	Type of therapy	Timing, dosage, and route administration	Perfusion technique, temperature and perfusate composition	Findings
Pool et al (2020)[13]	Porcine	Human A-MSCs, BM-MSCs	**Timing:** after 60 min of NMP **Dosage:** $10*10^6$ A-MSCs or $10*10^6$ BM-MSCs **Scaling dosage:** N.S.~$0.077*10^6$ MSCs/kg BW (est.) **Route:** IA	**Perfusion technique:** 2–3 h HMP followed by 7 h NMP **Temperature:** 4°C (HMP), 37°C (NMP) **Perfusate composition:** HMP: UW-MPS NMP: pure erythrocytes, human albumin, sodium chloride, sodium bicarbonate, calcium gluconate, glucose, insulin, mannitol, creatinine, amoxicillin-clavulanate, verapamil, Augmentin, Sterofundin, calcium	*During perfusion* **Adverse effects:** not reported **Functional:** LDH↓, NGAL↓ **Biological:** HGF↑, IL-6↑, IL-8↑ **Histological:** - **Other:** endothelin-1 levels higher in BM-MSC group compared to A-MSC group
Lohmann et al (2020)[14]	Porcine	Porcine A-MSCs or human A-MSCs	**Timing:** after 62 min of NMP **Dosage:** $10*10^6$ A-MSCs **Scaling dosage:** $0.1*10^6$ A-MSCs/kg BW **Route:** IA	**Perfusion technique:** 14 h HMP followed by 4 h NMP **Temperature:** 4–7°C (HMP), 37°C (NMP) **Perfusate composition:** HMP: UW-MPS NMP: allogenic RBCs, human albumin, sodium bicarbonate, calcium gluconate, glucose, amoxicillin-clavulanate, insulin, mannitol, creatinine, verapamil	*During perfusion* **Adverse effects:** MSC administration did not affect perfusion parameters **Functional:** - **Biological:** - **Post-transplant:** - **Histological:** MSCs retained in renal cortex *Post-transplantation* **Adverse effects:** MSC administration did not affect *in vivo* reperfusion **Functional:** - **Biological:** - **Post-transplant:** - **Histological:** -
Thompson et al (2021)[15]	Human discarded	Human MAPCs	**Timing:** after 60 min of NMP **Dosage:** $50*10^6$ MAPCs **Scaling dosage:** N.S. ~ $0.625*10^6$ MSCs/kg BW (est.) **Route:** IA	**Perfusion technique:** 7 h NMP **Temperature:** 36.5°C **Perfusate composition:** cross-matched RBCs, Ringer's solution, mannitol, dexamethasone, sodium bicarbonate, heparin, prostacyclin, glucose, insulin, multivitamins, TPN	*During perfusion* **Adverse effects:** not reported **Functional:** urine output ↑, microvascular perfusion ↑, NGAL↓, Indolamine-2,3-dioxygenase↑ **Biological:** IL-1β ↓, IL-10 ↑ **Histological:** majority of the MAPCs were in the glomeruli in sections of the cortex and around the peritubular space in the sections of the medulla.

Abbreviations: A-MSC, adipose-derived mesenchymal stromal cell; ATP, adenosine triphosphate; ATP6V0D2, vacuolar H+-ATPase d2 Subunit; BM-MSC, bone-marrow derived mesenchymal stromal cell; BW, bodyweight; CALB1, Calbindin 1; est., estimated; FGF-2, fibroblast growth factor-2; FLT-3L, FMS-like tyrosine kinase 3 ligand; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; h, hours; HGF, hepatocyte growth factor; HMP, hypothermic machine perfusion; IA, intra-arterially; IDH2, isocitrate dehydrogenase 2; IL, interleukin; IP-10, interferon-inducible protein 10; kg, kilogram; LDH, lactate dehydrogenase; MAPC, multipotent adult progenitor cell; MCP-3, monocyte chemoattractant protein-3; MDA, malondialdehyde; MDC, macrophage-derived chemokine; MIP, macrophage inflammatory protein; MSC-EV, mesenchymal stromal cell derived extracellular vesicle; N.A., not applicable; N.D., not determined; NDUFS8, NADH dehydrogenase Fe-S protein 8; NGAL, neutrophil gelatinase-associated lipocalin; NMP, normothermic machine perfusion; N.S., not specified; PCNA, proliferating cell nuclear antigen; PDHB, pyruvate dehydrogenase beta; RANTES, Regulated on Activation, Normal T Expressed and Secreted; RBC, red blood cell; SLC16A1, monocarboxylate transporter 1; TGF-α, transforming growth factor-α; TNF-α, tumor necrosis factor-α; TPN, total parenteral nutrition, UW-MPS, University of Winconsin machine perfusion solution.

TABLE 2 Pre-clinical studies evaluating the efficacy of MSC-based therapy in ex vivo liver perfusion.

Author	Type of liver	Type of therapy	Timing, dosage, and route administration	Perfusion technique, temperature and perfusate composition	Findings
Sasajima et al (2018)[16]	Rat	Swine A-MSCs	**Timing:** start perfusion **Dosage:** 0.2 or 1.0*10^6 MSCs **Scaling dosage:** 0.7–3.4 *10^6 MSCs/kg BW **Route:** PV	**Perfusion technique:** 2 h NMP, portal **Temperature:** 37°C **Perfusate composition:** Krebs-Henseleit bicarbonate solution	*During perfusion* **Adverse effects:** not reported **Functional:** bile production ↑ **Biological:** - **Histological:** sinusoidal space narrowing↓, hepatocellular vacuolation↓
Yang et al (2020)[17]	Rat	Rat BM-MSCs	**Timing:** start perfusion **Dosage:** 10–30*10^6 MSCs **Scaling dosage:** 47.6–142.9*10^6 MSCs/kg BW **Route:** PV	**Perfusion technique:** 6 h NMP, portal **Temperature:** 35–38°C **Perfusate composition:** fresh blood, DMEM/F12, FBS, penicillin–streptomycin solution, heparin, insulin, dexamethasone	*During perfusion* **Adverse effects:** not reported **Functional:** bile production↑, lactate clearance↑, MPO-levels ↓, MDA-levels↓ **Biological:** - **Histological:** Suzuki score↓, hepatocyte apoptosis↓, mitochondrial damage↓ **Other:** mitochondrial membrane potential↑
Sun et al (2021)[18]	Rat	Rat BM-MSCs	**Timing:** start perfusion **Dosage:** 10–30*10^6 MSCs **Scaling dosage:** 47.6–142.9*10^6 MSCs/kg BW **Route:** PV	**Perfusion technique:** 4 h NMP, portal **Temperature:** 36–38°C **Perfusate composition:** fresh blood, DMEM/F12, FBS, penicillin–streptomycin solution, heparin, insulin, dexamethasone	*During perfusion* **Adverse effects:** not reported **Functional:** ALT↓, AST↓, bile production↑, MDA-levels↓, GSH↑, ROS↓ **Biological:** - **Histological:** Suzuki score↓, BM-MSC could be colonized in the hepatic sinusoids **Other:** expression level of GPX4↑ and PTGS2↓
Cao et al (2020)[19]	Rat	Rat BM-MSCs and HO-1/BM-MSCs	**Timing:** not specified **Dosage:** 15–30*10^6 MSCs **Scaling dosage:** 65.2–130.4*10^6 MSCs/kg BW **Route:** PV	**Perfusion technique:** 4 h NMP, portal **Temperature:** 36–38°C **Perfusate composition:** fresh blood, DMEM/F12, FBS, penicillin–streptomycin solution, heparin, insulin, dexamethasone	*Post-transplantation* **Adverse effects:** not reported **Functional:** survival time↑, ALT↓, AST↓, ALP↓, GGT↓ **Biological:** IL-1β ↓, IL-6↓, TNF-α↓, HMGB1↓ **Histological:** liver damage↓ **Other:** effects on histology and functional parameters were greater in group of HO-1/BM-MSCs than in BM-MSCs
Tian et al (2021)[20]	Rat	Rat BM-MSCs and HO-1/BM-MSCs	**Timing:** not specified **Dosage:** 15–30*10^6 MSCs **Scaling dosage:** 68.2–136.4*10^6 MSCs/kg BW **Route:** PV	**Perfusion technique:** 4 h NMP, portal **Temperature:** 37–38°C **Perfusate composition:** fresh blood, DMEM/F12, FBS, penicillin–streptomycin solution, heparin, insulin, dexamethasone	*Post-transplantation* **Adverse effects:** not reported **Functional:** survival time↑, ALT↓, AST↓, ALP↓, GGT↓, total bilirubin↓ **Biological:** - **Histological:** apoptosis↓, proliferation PBGs↑, dilatation PBGs, PCNA-levels↑, caspase-3-positive cells↑, VEGF expression in PBG-cells ↑ **Other:** blocking Wnt signaling significantly inhibited the repair effect of MSCs

(Continues)

TABLE 2 (Continued)

Author	Type of liver	Type of therapy	Timing, dosage, and route administration	Perfusion technique, temperature and perfusate composition	Findings
Cao et al (2021)[21]	Rat	Rat BM-MSCs and HO-1/ BM-MSCs	**Timing:** after 10 min **Dosage:** $10*10^6$ MSCs **Scaling dosage:** 41.7–$50*10^6$ MSCs/kg BW **Route:** PV	**Perfusion technique:** NMP (duration not described), portal **Temperature:** 36–38°C **Perfusate composition:** fresh blood, DMEM/F12, FBS, penicillin–streptomycin solution, heparin	*Post-transplantation* **Adverse effects:** not reported **Functional:** survival time ↑, ALT↓, AST↓, ALP↓, GGT↓ **Biological:** IFN- γ↓, TNF-α↓, IL-2↓, $CD3^+$↓, $CD68^+$-cells↓, GZMB-expression↓ **Histological:** TUNEL-positive cells↓, RAI-score ↓ **Other:** survival of HO-1/BMMSCs in liver grafts was longer than that of unmodified BMMSCs BM-MSCs increased expression of NKT cell co-inhibitory receptors (CD160, BTLA, PD-1, GITR) and reduced NKT cell expression of IFN- γ
Wu et al (2022)[22]	Rat	Rat BM-MSCs and HO-1/ BM-MSCs	**Timing:** not specified **Dosage:** $10*10^6$ MSCs **Scaling dosage:** 41.7–$50*10^6$ MSCs/kg BW **Route:** PV	**Perfusion technique:** 4 h NMP, portal **Temperature:** 36–38°C **Perfusate composition:** fresh blood, DMEM/F12, FBS, penicillin–streptomycin solution, heparin, insulin, dexamethasone	*Post-transplantation* **Adverse effects:** not reported **Functional:** ALT↓, AST↓ **Biological:** IFN- γ↓, TNF-α↓,IL-4↑, CCL-2↓, CXCL-9↓, CXCL-10↓, $CD4^+$ T-cells↓ **Histological:** RAI-score ↓, hepatocyte apoptosis↓ **Other:** MSCs inhibited dendritic cell maturation, as seen by decreased expression of CD80, CD86, MHCII HO-1/BM-MSCs downregulated genes involved immune response, myeloid dendritic cell differentiation, positive regulation of T cell activation, antigen processing and presentation, and positive regulation of MAPK and ERK cascade
Verstegen et al (2020)[23]	Porcine	Human MSCs	**Timing:** prior to HOPE **Dosage:** 5–$10*10^6$ MSCs/kg **Scaling dosage:** idem **Route:** PV/HA	**Perfusion technique:** 1 h HOPE, portal, followed by 4 h NMP, dual **Temperature:** 10°C (HOPE), 37°C (NMP) **Perfusate composition:** HMP: UW-MPS NMP: autologous pig blood	*During perfusion* **Adverse effects:** not reported **Functional:** - **Biological:** IL-8↑, IL-6↑ **Histological:** - **Other:** no differences in distribution throughout liver between administration routes

TABLE 2 (Continued)

Author	Type of liver	Type of therapy	Timing, dosage, and route administration	Perfusion technique, temperature and perfusate composition	Findings
Laing et al (2020)[24]	Human discarded	Human MAPCs	**Timing:** After 60 min or 240 min **Dosage:** $50*10^6$ MAPCs **Scaling dosage:** not specified; ~$0.67*10^6$ MAPCs /kg BW (est.) **Route:** PV/HA	**Perfusion technique:** 6 h NMP, dual **Temperature:** 37°C **Perfusate composition:** Rhesus-negative RBCs, human albumin, heparin, sodium bicarbonate, calcium gluconate, vancomycin, gentamicin, epoprostenol, aminoplasmal, dextrose	*During perfusion* **Adverse effects:** no effects of cell administration on perfusion parameters **Functional:** - **Biological:** IL-1β↑, IL-4↑, IL-5↑, IL-6↑, IL-8↑, IL-10↑, MCP-1↑, GM-CSF↑, SDF-1α↑ **Histological:** administration via PV resulted in arrested cells in sinusoids, HA-infused cells transmigrated across the vascular endothelium **Other:** increased expression of proteins (HYOU1, GRB2, MAPK4, EGFR, TIMP-1, STIP1, ITGAL, ICAM-1)

Abbreviations: A-MSC, adipose-derived mesenchymal stromal cell; ALP, alkaline phosphatase; ALT, alanine-aminotransferase; AST, aspartate-aminotransferase; BM-MSC, bone-marrow derived mesenchymal stromal cell; BTLA, B- and T-lymphocyte attenuator; BW, bodyweight; CCL2, chemokine (C-C motif) ligand 2; CXCL-9, chemokine C-X-C motif ligand 9; CXCL-10, chemokine C-X-C motif ligand 10; DMEM, Dulbecco's modified Eagle's medium; EGFR, epidermal growth factor receptor; ERK, extracellular-signal-regulated kinase; FBS, fetal bovine serum; GGT, gamma-glutamyltransferase; GITR, glucocorticoid-induced tumor necrosis factor receptor; GM-CSF, granulocyte-macrophage colony-stimulating factor; GPX4, anti-glutathione peroxidase 4; GRB2, growth factor receptor-bound protein 2; GSH, glutathione; GZMB, granzyme B; h, hours; HA, hepatic artery; HMGB1, high-mobility group box 1; HO-1/BMMSC, heme oxygenase 1-modified bone marrow-derived mesenchymal stromal cell; HOPE, hypothermic oxygenated perfusion; h, hour; HYOU1, hypoxia up-regulated protein 1; ICAM-1, intercellular adhesion molecule 1; IFN- γ, interferon-gamma; IH, intra-hepatic; IL, interleukin; LDH, lactaatdehydrogenase; MAPC, multipotent adult progenitor cell; MAPK, mitogen activated protein kinase; MAPK4; mitogen activated protein kinase 4; MCP-1, monocyte chemoattractant protein-1; MDA, malondialdehyde; MPO, myeloperoxidase; MSC, mesenchymal stromal cell; N.A., not applicable; NMP, normothermic machine perfusion; PBG, peribiliary gland; PCNA, proliferating cell nuclear antigen; PD-1, programmed cell death protein-1; PTGS2, prostaglandin-endoperoxide synthase 2; PV, portal vein; RAI, rejection activity index; RBC, red blood cell; ROS, reactive oxidative species; SDF-1α, stromal cell-derived factor-1α; STIP1, transformation-sensitive protein IEF SSP 3521; TGF-β1, transforming growth factor- β1; TIMP-1, tissue inhibitor of metalloproteinases 1; TNF-α, tumor necrosis factor-α; TUNEL, Terminal deoxynucleotidyl transferase dUTP nick end labeling; UW-MPS, University of Winconsin machine perfusion solution.

TABLE 3 Pre-clinical studies evaluating the efficacy of other stem cell-based therapies in ex vivo liver perfusion.

Author	Type of liver	Type of therapy	Timing, dosage, and route administration	Perfusion technique, temperature, and perfusate composition	Findings
Sampaziotis et al (2021)[26]	Human discarded	Human cholangiocyte organoids derived from galbladder	**Timing:** not specified **Dosage:** $10*10^6$ organoid cells **Scaling dosage:** N.D. **Route:** IH bile duct	**Perfusion technique:** Up to 100 h NMP, dual **Temperature:** 37°C **Perfusate composition:** RBCs, colloid solution, cefuroxime, heparin, sodium bicarbonate, calcium gluconate, TPN, insulin, prostacyclin	*During perfusion* **Adverse effects:** no duct dilatation or obstruction **Functional:** bile pH↑, bile volume↑ **Biological:** - **Histological:** organoids engrafted in biliary compartment **Other:** organoids expressed biliary markers (KRT7, KRT19, CFTR, GGT) and upregulated intrahepatic markers (SOX4, BICC1, DCDC2)
Rigo et al (2018)[28]	Rat	Human HLSC-EVs	**Timing:** after 15 min **Dosage:** $500*10^6$ HLSC-EVs/g liver **Scaling dosage:** N.A. **Route:** PV	**Perfusion technique:** 4 h NMP, portal **Temperature:** 37°C **Perfusate composition: fresh blood**, Williams E medium, glucose, penicillin, streptomycin, glutamine, heparin, bicarbonate	*During perfusion* **Adverse effects:** not reported **Functional:** ALT↓, LDH↓ **Biological:** - **Histological:** Suzuki score↓, hepatic apoptosis↓ **Other:** RNA overexpression of HIF1A and TGF-β1↓
De Stefano et al (2021)[29]	Rat	Human HLSC-EVs	**Timing:** within 15 min **Dosage:** 500 or $2500*10^6$ HLSC-EVs/g liver **Scaling dosage:** N.A. **Route:** PV	**Perfusion technique:** 6 h NMP, portal **Temperature:** 37°C **Perfusate composition:** human RBC concentrate, Williams E medium, glucose, penicillin, streptomycin, glutamine, heparin, bicarbonate	*During perfusion* **Adverse effects:** perfusion parameters were homogeneous in the study groups **Functional:** vascular resistance↓, bile production↑, ALT↓, AST↓, pH self-regulation↑, phosphate↓ **Biological:** - **Histological:** necrosis↓, PCNA-index↑, HLSC-EVs were present in hepatic tissue in both treatment groups **Other:** significant differences in vascular resistance, bile production, histological necrosis, and PCNA-index were only found in high dose group

Abbreviations: ALT, alanine-aminotransferase; AST, aspartate-aminotransferase;BICC1, bicaudal C homolog 1 gene; CFTR, cystic fibrosis transmembrane conductance regulator; DCDC2, doublecortin domain-containing protein 2; est., estimated; g, gram; GGT, gamma-glutamyltransferase; h, hours; HIF1A hypoxia-inducible factor 1-α; HLSC-EV, human like stem cell derived extracellular vesicle; IH, intra-hepatic; KRT7, cytokeratin-7; KRT19, cytokeratin-19; LDH, lactaatdehydrogenase; MAPK4, extracellular signal-regulated kinase 4; N.A., not applicable; N.D., not determined; NMP, normothermic machine perfusion; PCNA, proliferating cell nuclear antigen; PV, portal vein; RBC, red blood cell; SOX4, sex-determining region Y-related high-mobility group box transcription factor 4; TGF-β1, transforming growth factor β1, TPN, total parenteral nutrition.

cells (PBGs), inhibited cell apoptosis, and increased the proportion of pluripotent cells in PBGs. The last two rat NMP studies demonstrated that transplanted MSCs alleviated acute rejection following transplantation through the inhibition of dendritic cell maturation and reduction of natural killer cells.[21,22] Also, MSCs infusion reduced hepatocyte apoptosis and concentrations of cytokines related to acute rejection. The effects of HO-1 transduced MSCs on functional and histological parameters were greater than that of naïve BM-MSCs in all previous mentioned studies.[19–22] Verstegen et al. investigated the administration of human-labeled MSCs in pig livers during HMP, followed by NMP.[23] Tracking showed homogenous delivery and conservation of infused cells in the liver during NMP, either through injection in the portal vein or hepatic artery. Laing et al. studied the effects of the delivery of fluorescent-labeled MAPCs in human discarded livers during NMP.[24] They demonstrated that administration did not have an effect on flow rates or resistance and that hepatic artery-infused cells transmigrated across the vascular endothelium, homing in the liver parenchyma. Injection of MAPCs via the portal vein showed arrested cells in the sinusoidal space. Perfusate analysis revealed that MAPC cells secreted immunomodulatory factors. These factors were detected much better when cells were injected after 1 h, but almost absent if cells were injected after 4 h of NMP.

Summarizing, all studies using MSCs and MAPCs showed beneficial effects, without reported safety issues or adverse effects. Absolute cell numbers used in different species ranged from 10 to $30*10^6$ cells. Normalization of the dose to recipient body weight shows a range from 0.2 to $143*10^6$ MSCs/kg bodyweight (BW).[16–18] For future dosing, we propose to standardize stem cell-based therapy protocols to inject between 5 and $10*10^6$ cells per 100 g liver, based on efficacy and safety profiles shown in previous studies. Also concerning timing, a wide variety is found between protocols. Interestingly, cell injection during cold perfusion showed effect, more specific results were found by infusion shortly after reperfusion, and no effects were found after 4 h of NMP, clearly indicating a window of opportunity.

On the route of administration, two studies showed increased cytokine production after arterial injection, where Laing et al. also demonstrate better integration in the liver parenchyma.[23,24]

A special cell type that specifically has been used for liver perfusion is hepatic organoids. Organoids are three-dimensional cellular clusters that can be derived from somatic (pluripotent or stem) cells and that reflect the functionalities of the tissue of the origin.[25] Sampaziotis et al. infused cholangiocyte organoids in the intrahepatic bile ducts of human discarded livers during NMP.[26] The fluorescent-labeled organoids engrafted in the biliary tree, expressed region-specific biliary markers, and showed physiological repair by increased bile pH and bile production. Organoids were not detected in the perfusate during up to 100 h of perfusion, indicating that cells remained anchored in the biliary compartment. Organoid culture is however currently not compatible with clinical application and will need to be developed Good Clinical Practice (GCP) compliant, before clinical application can be considered.

Human liver stem-like cells (HLSCs) have been characterized as a MSC-like cell population derived from adult liver cells with immunomodulatory properties and differentiating capabilities.[27] Administration of HLSC-derived extracellular vesicles (HLSC-EVs) in rat livers during NMP showed successful internalization of HLSC-EVs in hepatocytes.[28] Treated livers showed less histological injury and lower concentrations of AST and LDH at the end of perfusion compared to controls. In a follow-up study, HLSC-EVs improved pH regulation and lowered levels of transaminases.[29] Only high-dose administration decreased vascular resistance and increased bile production. Although attractive as cell-free approach, the use of EVs will need maturation and standardization, before clinical application is feasible.

4 | LUNG TRANSPLANTATION

Seven studies assessed the biological effects of MSCs/MAPCs (Table 3A) or MSC-EVs (Table 5) during ex vivo lung perfusion (EVLP). The optimal administration route and dosage for MSCs was assessed in porcine lungs.[30] Poor survival of MSCs after endobronchially (EB) administration of MSCs was demonstrated, whereas intravascular administration resulted in uptake and sustained retention of cells in the lung parenchyma.[30] Intravascular injection of $5*10^6$ MSCs/kg BW led to optimal parenchymal concentration of human vascular endothelial growth factor (VEGF) and decrease in the pro-inflammatory interleukin-8 in the perfusate, compared to controls.[30] In a porcine lung transplantation model, treatment with MSCs during EVLP resulted in anti-apoptotic and anti-inflammatory effects.[31] After transplantation, treated lungs showed increased lung tissue hepatocyte growth factor (HGF) levels, reduced lung tissue wet-to-dry weight ratio, and a lower acute lung injury score compared to controls. IV administration of human MSCs resulted in better preservation of rat lungs by protecting against oxidative stress and inducing anti-inflammatory effects.[32] MAPCs injected in the bronchus also exerted immunoregulatory effects on pulmonary IRI, comparable to MSCs, as seen by decreased concentrations of neutrophils and pro-inflammatory cytokines in the bronchoalveolar lavage supernatant.[33] Three studies investigated the effects of MSC-EV administration during EVLP (Table 3B). In a murine model, Stone et al. showed attenuation of lung dysfunction and injury after administration of either MSCs or MSC-EVs.[34] Treatment protected from edema and neutrophil infiltration in pulmonary tissue. In another lung IRI model, IV administration of MSC-EVs in rat lungs resulted in reduced vascular resistance, in line with the increased concentration of secreted nitric oxide (NO). EVs restored pulmonary content of ATP and increased expression of genes involved in resolution of inflammation and oxidative stress.[35] Park et al. studied the effects of MSC-EVs in discarded human donor lungs, injured by *E.coli* bacteria.[36] EV-treated lungs showed an increase in alveolar fluid clearance (AFC) and decrease in endothelial permeability to protein, as well as a lower bacterial load and neutrophil count in the injured alveolus.

TABLE 4 Pre-clinical studies evaluating the efficacy of MSC-based therapy in ex vivo lung perfusion.

Author	Type of lung	Type of therapy	Timing, dosage, and route administration	Duration EVLP and perfusate composition	Findings
Mordant et al (2016)[30]	Porcine	Human UC-MSCs	**Timing:** During second hour **Dosage:** 50, 150, 300*10^6 MSCs **Scaling dosage** 1.7, 5, 10*10^6 MSCs/kg BW **Route:** EB/IV	**Duration EVLP:** 12 h **Perfusate composition:** Steen solution, heparin, cefazolin, methylprednisolone	*During perfusion* **Adverse effects:** IV administration of 300*10^6 MSCs (10*10^6 MSCs/kg BW) was associated with an increase in PVR **Functional:** - **Biological:** VEGF ↑, IL-9 ↓ **Histological:** retention of MSCs in lung parenchyma after IV-administration, no detection of MSCs after EB-administration **Other:** intravascular administration of 150*10^6 MSCs (5*10^6 MSCs/kg BW) was the optimal tolerated dose. bronchial fluid is not optimal for survival of MSCs
Nakajima et al (2019)[31]	Porcine	Human UC-MSCs	**Timing:** after 120 min **Dosage:** 150*10^6 MSCs **Scaling dosage:** 4.4*10^6 MSCs/kg BW **Route:** IV	**Duration EVLP:** 12 h **Perfusate composition:** Steen solution, heparin, cefazolin, methylprednisolone	*During perfusion* **Adverse effects:** not reported **Functional:** peak airway pressure↓ **Biological:** HGF↑, IL-18↓, IL-4↑, IFN- γ↓, cleaved caspase-3 ↓ **Histological:** TUNEL-positive cells ↓, MSCs were found in capillaries and alveolar interstitium at end EVLP *Post-transplantation* **Adverse effects:** not reported **Functional:** - **Biological:** lung tissue HGF level↑, lung tissue TNF-α ↓, **Histological:** acute lung injury score↓, T-cell infiltration↓
Pacienza et al (2019)[32]	Rat	Human UC-MSCs	**Timing:** 150 min prior start **Dosage:** 1*10^6 MSCs **Scaling dosage:** 3.5*10^6 MSCs/kg BW **Route:** IV	**Duration EVLP:** 1 h **Perfusate composition:** Steen solution	*During perfusion* **Adverse effects:** not reported **Functional:** loss of lung compliance↓, protein carbonyls↓, SOD ↓, CAT↓ **Biological:** - **Histological:** lung injury score↓, MPO-activity↓, MPO-positive cells↓ recruitment neutrophils↓
Martens et al (2017)[33]	Porcine	Human MAPCs	**Timing:** start of perfusion **Dosage:** 150*10^6 MAPCs **Scaling dosage:** 3.6*10^6 MAPCs/kg BW **Route:** airway	**Duration EVLP:** 6 h **Perfusate composition:** acellular albumin containing dextran solution	*During perfusion* **Adverse effects:** not reported **Functional:** - **Immunological:** TNF-α ↓, IL-1β ↓, IFN-γ↓, neutrophils ↓ **Histological:** -

Abbreviations: BALF, bronchoalveolar lavage fluid; BW, bodyweight; CAT, catalase; est., estimated; EVLP, ex vivo lung perfusion; h, hours; HGF, hepatocyte growth factor; EB, endobronchially; IFN-γ, interferon-gamma; IL, interleukin; IT, intratracheal; IV, intravascular; kg, kilogram; LLL, left lower lobe; MAPC, multipotent adult progenitor cell; MPO, myeloperoxidase; MSC, mesenchymal stromal cell; N.A., not applicable; N.S., not specified; PVR, pulmonary vascular resistance; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-α; TUNEL, Terminal deoxynucleotidyl transferase dUTP nick end labeling; UC-MSC, umbilical cord derived MSC; VEGF, vascular endothelial growth factor.

TABLE 5 Pre-clinical studies evaluating the efficacy of MSC-EVs therapy in ex vivo lung perfusion.

Author	Type of lung	Type of therapy	Timing, dosage, and route administration	Duration EVLP and perfusate composition	Findings
Stone et al (2017)[34]	Mouse	Human UC-MSCs /EVs	**Timing:** start perfusion **Dosage:** $3*10^6$ MSCs or EVs **Scaling dosage:** N.D. **Route:** IV	**Duration EVLP:** 1 h **Perfusate composition:** Steen solution, heparin, cefazolin, methylprednisolone	*During perfusion* **Adverse effects:** not reported **Functional:** lung compliance↑, PAP↓ **Biological:** - **Histological:** neutrophil infiltration↓, wet to dry ratio↓, lung injury↓ **Other:** -
Lonati et al (2019)[35]	Rat	Human BM-MSC-EV	**Timing:** after 60 min **Dosage:** 122800 $*10^6$ EVs **Scaling dosage:** $423448*10^6$ EVs/kg BW **Route:** IV	**Duration EVLP:** 3 h **Perfusate composition:** in house perfusion fluid without anti-inflammatory drugs	*During perfusion* **Adverse effects:** not reported **Functional:** TPVR↓, pulmonary ATP-content↑, NO↑, HA↑, lactate↓, glucose↓, **Biological:** CXCL2↑, CXCL3↑, perfusate leucocytes ↓ **Histological:** internalization of EVs within lung cells **Other:** upregulation of genes involved in resolution of inflammation (IL-10, PTGS2, IL-1-RA, SOCS3) and reduction of oxidative stress (SOD2 and HSP70)
Park et al (2019)[36]	Human discarded injured by *E.coli*	Human BM- MSC-EV	**Timing:** after 60 min **Dosage:** 200 µl or 400 µl ($4.7 \pm 0.2 \times 10^7$ EVs/100 µl) **Scaling dosage:** N.D. **Route:** IV	**Duration EVLP:** 6 h **Perfusate composition:** DMEM, bovine serum albumin, fresh human blood	*During perfusion* **Adverse effects:** not reported **Functional:** AFC ↑ **Biological:** - **Histological:** alveolar bacterial load↓, lung protein permeability ↓, influx of neutrophils↓

Abbreviations: AFC, alveolar fluid clearance; ATP, adenosine triphosphate; BM-MSC, bone-marrow derived mesenchymal stromal cell; CXCL2, chemokine C-X-C motif ligand 2; CXCL3, chemokine C-X-C motif ligand 3; DMEM, Dulbecco's modified Eagle's medium; *E.coli*, *Escherichia coli*; EVLP, ex vivo lung perfusion; h, hours; HA, hyaluronic acid; HSP70, heat shock protein 70; IL-1-RA, interleukin-1 receptor antagonist; IV, intravascular; kg, kilogram; MSC, mesenchymal stromal cell; MSC-EV, mesenchymal stromal cell derived extracellular vesicle; N.D., not determined; NO, nitric oxide; PAP, pulmonary arterial pressure; PTGS2, prostaglandin-endoperoxide synthase 2; SOCS3, suppressor of cytokine signaling 3; SOD2, superoxide dismutase 2, mitochondrial; TPVR, total pulmonary vascular resistance; UC-MSC, umbilical cord derived mesenchymal stromal cell; µl, microlitre.

TABLE 6 Pre-clinical studies evaluating the efficacy of stem cell-based therapy in ex vivo heart perfusion

Author	Type of heart	Type of therapy	Timing, dosage, and route administration	Perfusion technique, temperature, perfusate composition	Findings
Korkmaz-Icöz et al (2019)[37]	Rat	Rat BM-MSC-CM	**Timing:** start perfusion **Dosage:** N.S. **Scaling dosage:** N.A. **Route:** intracoronary	**Perfusion technique:** 5 h HMP, isolated Langendorff model **Temperature:** 4°C **Perfusate composition:** Custodiol solution	*Post-transplantation* **Adverse effects:** not reported **Functional:** rebeating time↓, left ventricular systolic pressure↑, $dPdt_{max}$↑, rate pressure product↑, dP/dt_{min}↑, Tau-w ↓ **Biological:** - **Histological:** - **Other:** upregulation of anti-oxidative genes (SOD2 and selectin E) and genes involved in the PI3K/Akt pathway (TCL1a, AKT1, AKT3, MAPK3, PAK1, FOXG1, mTOR, ADAR) Downregulation of genes related to oxidative stress (Serpinb1b, DNM2, TXNRD2, EPX, GPX, MPO, DHCR24, TPO, NOX4, NOXO1, and AOX1), apoptosis (BIRC5, CD40LG, FADD, BCL2L11, CASP2, FASLG, BID, CASP8, AKT1), and pro-inflammatory cytokine (TNF, CCL11) and interleukin (IL-9, CD40LG) genes.

Abbreviations: ADAR, adenosine deaminase, RNA-specific; AKT1, v-akt murine thymoma viral oncogene homolog 1; AKT3, v-akt murine thymoma viral oncogene homolog 3; AOX1, aldehyde oxidase 1; BCL2L11, BCL2-like 11; BID, BH3-interacting domain death agonist; BIRC5, Baculoviral IAP repeat-containing 5; BM-MSC-CM, bone marrow derived-mesenchymal stromal cell-conditioned medium; CASP2, caspase-2; CASP8, caspase-8; CCL11, chemokine (C–C motif) ligand 11; CD40LG, CD40 ligand; DHCR24, 24-Dehydrocholesterol reductase; DNM2, dynamin 2; $dPdt_{max}$, maximal slope of systolic pressure increment; $dPdt_{min}$, maximal slope of diastolic pressure increment; EPX, eosinophil peroxidase; FADD, fas (TNFRSF6)-associated via death domain; FASLG, Fas ligand (TNF superfamily, member 6); FOXG1, forkhead box G1; GPX, glutathione peroxidase; HMP, hypothermic machine perfusion; IL-9, interleukin-9; MAPK3, mitogen-activated protein kinase 3; MPO, myeloperoxidase; mTOR, mechanistic target of rapamycin (serine/threonine kinase); N.A., not applicable; N.S., not specified; NOX4, NADPH oxidase 4; NOXO1, NADPH oxidase organizer 1; PAK1, p21 protein (Cdc42/Rac)-activated kinase 1; Serpinb1b, Serine (or cysteine) peptidase inhibitor, clade B, member 1b; SOD2, superoxide dismutase 2, mitochondrial; Tau-w, time constant of the left ventricular pressure decay; TCL1A, T-cell leukemia/lymphoma 1A; TNF, tumor necrosis factor; TPO, thyroid peroxidase; TXNRD2, thioredoxin reductase 2.

Overall, studies regarding the application of MSCs in lungs during EVLP universally demonstrate an anti-inflammatory effect, with absolute cell numbers applied ranging from 1 to 300x10^6 cells. Injection up to 5*10^6 MSCs per kg recipient BW did not result in changes in pulmonary vascular resistance (PVR) and seem to be safe from a rheological point of view, while 10*10^6 cells per kg BW caused increased PVR.[30] To increase homogeneity and comparability, without jeopardizing safety, we would advocate to standardize dosing around 40 × 10^6 cells/100 g lung tissue for future studies. Intravascular cell administration was shown superior over endobronchially injected cells.[30] The optimal timing of cell administration was not subject of direct comparison in any of the studies, but beneficial effects were demonstrated with cell injection prior to the start of EVLP, directly at perfusion and between 1 and 12 h after the start of EVLP.[30–33] No clinical trials have been published on the effects of MSCs or MAPCs on lung tissue inflammation or transplant outcomes. All studies administrating MSC-EVs during EVLP showed anti-inflammatory effects and reduction of lung injury. No adverse effects were reported in the MSC-EVs studies. So far, also no clinical trial was conducted with MSC-EVs during EVLP.

5 | HEART TRANSPLANTATION

No studies were performed using MSC or MAPC during ex vivo heart perfusion. One animal study investigated the effect of intracoronary delivery of MSC-conditioned medium (MSC-CM) during rat HMP on the early phase after transplantation (Table 4).[37] MSC-CM-treated grafts showed improved systolic contractility and diastolic relaxation by regulation of genes involved in anti-oxidative stress, apoptosis, and inflammation. So far, no clinical trials assessed the effects of stem cell-based therapy injection during ex vivo cardiac perfusion.

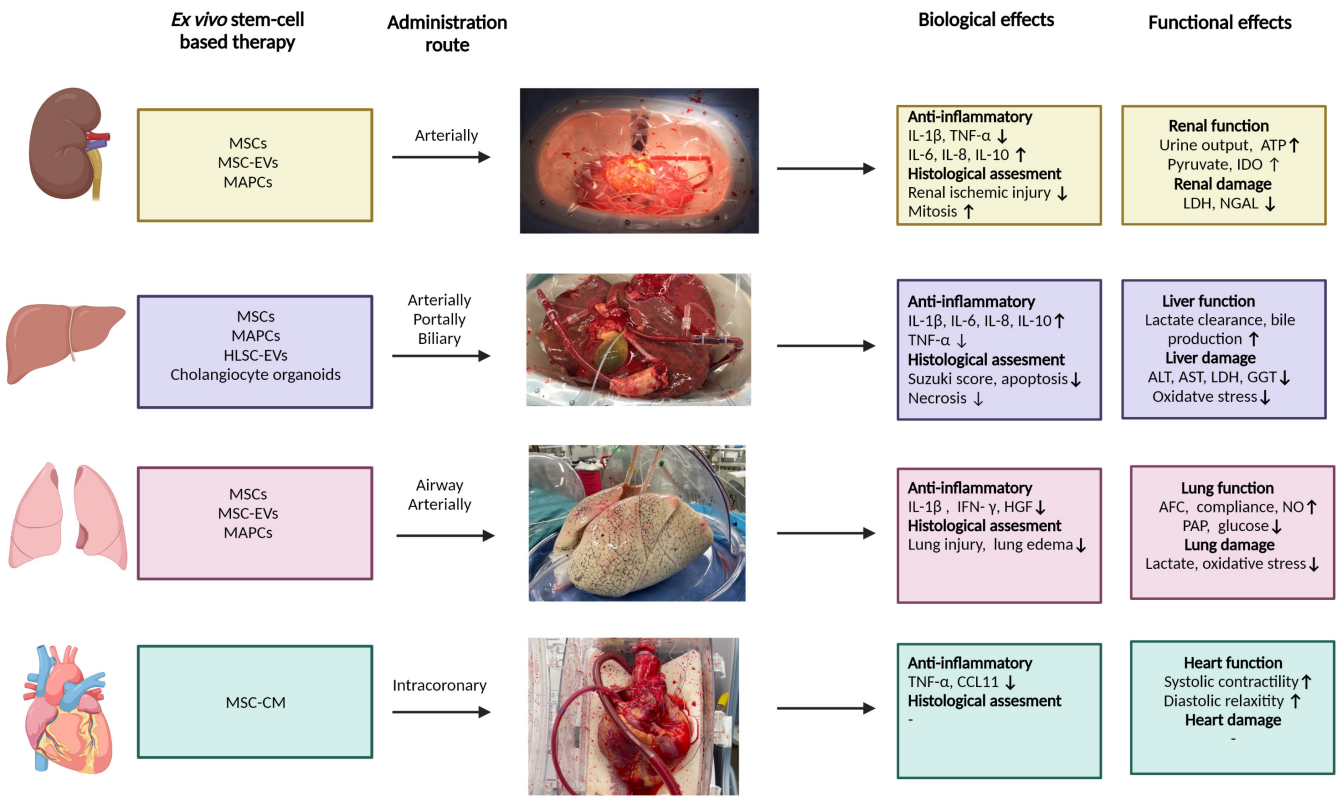

FIGURE 3 Schematic illustrating of effects of administrated stem cell-based therapies in ex vivo organ perfusion models. AFC, alveolar fluid clearance; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ATP, adenosine triphosphate; GGT, gamma-glutamyltransferase; HGF, hepatocyte growth factor; HLSC-EV, human-like stem cell-derived extracellular vesicle; IDO, indoleamine 2,3-dioxygenase; IFN- γ, interferon-gamma; IL, interleukin; LDH, lactate dehydrogenase; MAPC, multiadult progenitor cell; MDA, malondialdehyde; MSC, mesenchymal stromal cell; MSC-EV, mesenchymal stromal cell-derived extracellular vesicle; NGAL, neutrophil gelatinase-associated lipocalin; NO, nitric oxide; PAP, pulmonary arterial pressure; ROS, reactive oxidative species; TNF-α, tumor necrosis factor-α.

6 | CONCLUSION

The significant advancements in MP of the lung, liver, kidney, and heart allow for the increased utilization of marginal donor grafts for patients with end-stage organ diseases. With MP, an ex vivo platform to administer regenerative therapies has become reality.

We believe that all organs could benefit from the administration of stem cell-based therapies prior to transplantation. The inherent immunoregulatory and regenerative properties of MSCs and EVs make them excellent universal candidates to deliver during ex vivo MP. Organoids are effective to support organ-specific repair and function.

Clinical trials should answer the question whether the decreased early inflammatory response will mitigate rejection and improve long-term graft function following transplantation. We believe that ECD organs may potentially benefit the most from stem cell-based therapies, because these grafts are more prone to IRI. Today, only MSCs are clinical grade manufactured, which makes them the most suitable candidates to start clinical trials now.

An important issue that needs to be addressed is the dosage of cells injected. We found a profound difference of 1500-fold between the lowest and highest cell count described, making comparisons in effectivity nearly impossible. We also demonstrated heterogeneity in reporting formats; there were absolute cell numbers, cells per kilogram bodyweight, and cells per gram of organ tissue. The transplant community definitively should standardize cell infusion concentrations in upcoming clinical trials, to make comparisons among future studies possible. We, therefore, propose to report cell dosage in an uniform manner as administered cells per 100 g of donor organ weight. Also, heterogeneity in temperature and perfusate composition was demonstrated among the included studies. Sierra Parraga et al. assessed the effect of perfusion fluid on survival, viability, and functionality of MSCs in vitro,[38] showing reduced capacity of human MSCs to adhere to endothelial cells, reduced metabolic activity, and increased oxidative stress, whereas survival was not affected. There is little known about the effect of the variations in perfusion composition on the survival and functionality of stem cell-based therapies. A consensus meeting addressing these issues could pave the road to a more uniform trial design and make study results better comparable.

In lung transplantation, the optimal dosage, route of administration, and post-transplant outcomes for MSC injection have been

elucidated in porcine perfusion models. Safety and efficacy was confirmed, creating momentum for clinical trials to solidify the therapeutic potential of MSCs in human perfusion models. The first step toward randomized trials assessing meaningful recipient outcomes would be to initiate a human dose-escalation phase 1 trial.

In the other fields, clinical trials are still preliminary, as essential information from pre-clinical studies is scattered or missing. In abdominal transplantation, beneficial biological and functional effects of MSCs are uniformly reported in animal studies, but dose–response studies and supportive short-term transplant outcomes are lacking. One study assessed post-transplant outcomes following kidney autotransplantation, but could not demonstrate any improvement in kidney function or injury in the follow-up period.[14] We propose to complete dose–response studies in porcine models before targeted research in discarded or transplantation models can be pursued. EVHP is currently in the earliest stage of development; only one study reported the potential of stem cell-based therapies during cardiac MP.[37] However, lessons learned from other organs can be used to explore the therapeutic potential and close the gap.

More than a decade has passed since the first pre-clinical study with stem cell-based therapy during ex vivo organ perfusion was performed.[39] Now, the administration of regenerative therapies during MP should progress into clinical practice to develop into a real-world therapeutic option. Technological developments in organ perfusion have already opened up the possibility to extend the therapeutic window to treat poor-quality organs with protective and regenerative stem cell-based therapy even for several days.[11,40] It would be revolutionary to repair marginal grafts that were initially declined after viability assessment in an ultimate effort to make them suitable for transplantation. Treatment and repair of injured organs may further unlock new areas of regenerative medicine and help the community to battle one of the greatest challenges at this moment: the ever-increasing shortage of suitable donor organs.

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DISCLOSURE

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DATA AVAILABILITY STATEMENT

There were no new data created.

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