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Impact of Normothermic Perfusion enriched with Mesenchymal Stromal Cells on Renal Ischemia/Reperfusion Injury

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Thesis presented in order to obtain the degree of Doctor of Philosophy (PhD)
in Biomedical and Pharmaceutical Sciences

Academic Year 2025-2026



“The important thing is not to stop questioning. Curiosity has its own reason for existing.”

Albert Einstein

“Research is to see what everybody else has seen, and to think what nobody else has thought.”

Albert Szent-Györgyi

Cette thèse porte mon nom, mais elle est le reflet d'une aventure humaine collective que je n'aurais pu mener sans l'aide et la présence de nombreuses personnes, que je tiens à remercier une à une.

Tout d'abord, je remercie le Professeur François Jouret de m'avoir chaleureusement accueillie dans son laboratoire, de m'avoir offert l'opportunité de réaliser ce doctorat et de m'avoir prodigué de précieux conseils tout au long de cette aventure. Merci pour votre disponibilité, pour les sorties au marché de Noël avec le labo et pour ces blagues qui savaient apporter une touche de bonne humeur, quoi qu'il arrive. Je remercie également le Professeur Olivier Detry, tout d'abord pour avoir imaginé ce projet captivant que j'ai pu m'approprier au fil des années. Merci d'avoir été une source d'inspiration, de m'avoir transmis votre passion pour la transplantation, de m'avoir épaulée au quotidien, de m'avoir écoutée et comprise à chaque fois que j'en avais besoin. Merci de m'avoir fait confiance et de m'avoir poussée à donner le meilleur de moi-même. Je vous en serai éternellement reconnaissante.

Je tiens à remercier les membres de mon comité de thèse, le Dr Pauline Erpicum, le Dr Frédéric Baron et Marc Lagny, pour leurs conseils avisés au fil de ces comités et pour les pistes qu'ils m'ont offertes afin d'enrichir mes articles et mon manuscrit. Merci aux membres extérieurs, le Professeur Tom Darius et le Dr Clara Steichen, de m'avoir fait l'honneur de siéger dans mon jury de thèse et de m'avoir accompagnée avec tant de bienveillance.

Un tout grand merci à la « team cochon » — Gaby, Nicholas, Morgan, Olivier, Claire, Clarisse et Marc. Nous savons tous que ce projet n'aurait jamais vu le jour sans la contribution de chacun d'entre vous. Je suis profondément reconnaissante d'avoir fait partie de cette équipe de choc. J'y ai découvert des personnes qui se battent jour et nuit pour sauver des vies et qui, malgré des emplois du temps déjà bien chargés, ont su consacrer du temps à la recherche et à cette thèse. J'ai vite compris que je n'étais pas la seule à être portée par ce projet — nous l'étions tous — et c'est cette énergie collective qui a permis à ce manuscrit de naître. J'ai également découvert un univers à part entière dont j'ignorais l'existence : celui de perfusionniste. Marc et Claire, merci pour votre bienveillance et votre savoir-faire à toute épreuve. Merci de m'avoir guidée et portée, de jour comme de nuit. Merci à Clarisse et Aloïs pour ces moments inoubliables lors des congrès de Valence et d'Athènes, qui m'ont permis d'en apprendre encore davantage sur votre métier si fascinant. Merci à Gaby d'avoir répondu présent chaque fois qu'il le pouvait, d'avoir été là malgré tous les combats qu'il mène — ta force est un exemple pour nous tous. Merci à Olivier, Nicholas et Morgan d'avoir permis de mener ces chirurgies à bien. On dit parfois qu'il est difficile de travailler avec des chirurgiens ; je ne sais pas si j'ai simplement eu la chance de croiser les plus dévoués et les plus généreux, mais ce fut un réel plaisir de travailler à vos côtés. Nicholas, merci pour tes conseils, ta rigueur et ton dévouement sans faille. Tu as été bien plus qu'un collègue : un troisième promoteur dans le travail, un mentor officieux dans les moments difficiles. Je t'en serai longtemps reconnaissante. Nous avons une chance immense de t'avoir parmi nous au laboratoire. Merci également aux nombreux étudiants et assistants qui nous ont prêté main-forte : Sasha, Victoria, Bastien, Ysaline, et j'en oublie certainement... Une mention spéciale pour Walid — compagnon de bibliothèque pendant les études, et complice des longues soirées au labo lors de la mise au point du modèle, où le travail se mêlait aux confidences. Merci à Jean-Paul pour son soutien lors des chirurgies, mais aussi pour être celui vers qui nous nous tournions instinctivement — maître des clés des armoires et de tous les recoins du laboratoire.

Ce travail n'aurait pu voir le jour sans le geste généreux des patients et de leurs familles qui ont consenti au don d'organes à la recherche. C'est grâce à cet acte de confiance et d'altruisme que des projets de recherche comme celui présenté dans ce manuscrit deviennent possibles. Je leur exprime ma plus profonde gratitude et mon respect. Je pense également aux cochons qui ont contribué, à leur manière, à ce projet. Leur sacrifice ne m'a jamais laissée indifférente, et c'est avec beaucoup de respect et de gratitude que je tiens à les honorer.

Je remercie également les « personnes de l'ombre ». Tout d'abord Vincent, du CRAW, merci d'avoir veillé à ce que les expérimentations puissent avoir lieu, même en cas de dernière minute. Merci pour ces messages « je démarre » qui me rassuraient chaque mercredi matin. Merci à Xavier d'avoir répondu à mes appels de détresse durant ces quatre années de thèse, de nous avoir aidés à trouver du matériel — ou à en improviser —, et de nous avoir ainsi fait gagner un temps précieux.

Je remercie les membres du LTCG, et particulièrement Alexandra, Chantal, Pascale et Étienne, pour leur contribution à ce projet. Merci pour les nombreuses réunions et pour avoir été au rendez-vous à chaque expérimentation, même lorsque les délais se faisaient courts.

Merci aux plateformes du GIGA, essentielles à l'obtention des résultats présentés dans ce manuscrit. Merci tout particulièrement à Chantal Humblet, de la plateforme immuno-histo, ainsi qu'à Céline, Alex et Gaëtan, de la plateforme imagerie. Votre disponibilité et vos conseils ont été d'une valeur inestimable tout au long de cette thèse. Merci également à l'équipe du laboratoire d'Étienne Cavalier, et en particulier à Caroline Le Goff et Nunzio, pour les analyses d'iohexol et pour leur sollicitude.

Je remercie chaleureusement tous mes collègues du +5 — Lucia, Jean-Paul, Justine, Pauline, Tiago, Cécile, Prescilia, Elisa et Guillaume — pour tous ces beaux moments partagés ces dernières années. Merci pour les midis accompagnés de fous rires, pour l'appui au quotidien et pour les conseils, tant professionnels que personnels. Un merci tout particulier à Cécile, Presci et Elisa, qui, au-delà d'être des collègues formidables, sont devenues de véritables amies. Je mesure la chance que j'ai eue d'être à vos côtés. Merci de m'avoir aiguillée, consolée, et d'avoir été les rayons de soleil de certaines longues journées. Merci d'avoir été là pour célébrer mes victoires et pour me redonner de l'élan après les coups durs. Vous êtes des femmes remarquables, je vous souhaite tout le bonheur que vous méritez et je serai toujours là pour vous.

Je tiens également à remercier les étudiants que j'ai eu la chance d'encadrer durant cette thèse. Marine, ma première étudiante mémorable, qui s'est révélée au fil de son stage et l'a terminé brillamment. Adélaïde, à la carapace solide mais au cœur tendre, qui ignore encore aujourd'hui à quel point l'encadrer m'a fait grandir. Lucie, Pauline et Lisa, qui ont fait preuve d'une belle motivation et se sont pleinement investies. L'encadrement d'étudiants fut un défi que j'ai relevé avec bonheur et qui m'a permis de m'épanouir bien plus que je ne l'imaginais. Transmettre ma passion pour la recherche médicale et pour ce projet fut une vraie école, autant sur le plan professionnel que personnel.

Je remercie mes amis Khaled et Manon, sans qui je n'en serais pas là aujourd'hui. Nous sommes tous les trois la preuve que l'échec ne définit pas l'avenir d'un étudiant.

Malgré un début de parcours semé d'embûches, nous n'avons cessé d'enchaîner les réussites depuis notre rencontre — la haute école, puis la passerelle, le master et enfin le doctorat. Vous m'avez poussée à dépasser mes limites, vous avez cru en moi bien plus que je ne croyais en moi-même. Merci pour cette présence constante, année après année. Votre vivacité d'esprit et votre persévérance m'ont toujours impressionnée. J'espère que nous continuerons longtemps à fêter nos victoires ensemble.

J'en profite pour remercier également les professeurs de la Haute École Charlemagne — Mme Devillé, Mme Schmeits, Mme Verjans, M. Waroux et M. Ben — qui, malgré les années passées, me témoignent encore aujourd'hui leur appui.

Je remercie également mes « amis de la région » — Chris, Camille, Sofian, Quentin, Manon, Antoine, Claire, Anne-K, Marine et Elisa. Depuis les secondaires, et malgré la distance que j'ai mise entre nous en m'installant dans la région liégeoise, vous avez toujours été d'un grand soutien. Merci pour ces beaux moments partagés et pour ceux qui nous attendent encore, merci pour cette amitié indéfectible. Un merci tout particulier à Jou et Laurine, devenues bien plus que des amies — une famille que je me suis choisie. Ces vacances à l'île de la Réunion avec toi, Jou, étaient bien plus que magiques ; elles m'ont donné la force dont j'avais besoin pour traverser cette dernière année de doctorat. Et ces city trips et soirées entre filles — une bulle de légèreté qui arrivait toujours au bon moment.

Merci également aux amis rencontrés ces dernières années. Inès et Ysaline, venues au labo pour leurs stages de recherche, et vite devenues des coups de cœur et un ancrage précieux durant cette thèse. Jacobine, des midis partagés avec les filles aux restos en tête-à-tête — merci pour cette belle amitié qui a grandi naturellement. Un grand merci aux « Go Muscu » — Alex, Léo, Pauline, et tout particulièrement Camille, Laura et Cyrielle. Merci d'être devenues des indispensables, bien au-delà des murs de la salle. Le sport et ces cours collectifs partagés, ont été pour moi un refuge face à la pression que je m'imposais chaque jour. Merci également à mes coachs, Vincent, Franck et Camille, pour avoir contribué, à leur manière, à l'aboutissement de cette thèse.

Je ne peux que remercier de tout cœur ma famille — mon papa et ma maman — pour m'avoir soutenue tant dans mes études que dans cette thèse. Je sais que je n'ai pas toujours été facile à suivre, mais merci de m'avoir fait confiance même lorsque vous ne compreniez pas totalement mes choix. Merci à mes frères, Tom, Louis et Timon, qui sont aussi mes confidents, mes piliers et mes meilleurs amis. Merci d'avoir été là, même quand l'armée vous envoyait bien trop loin de moi à mon goût. Merci d'avoir été fiers de moi, d'avoir cru en moi, de me faire rire aux éclats et d'être les frères exceptionnels que vous êtes. On dit souvent qu'on ne choisit pas sa famille — mais vous 5, je vous aurais choisis entre mille. Je vous aime.

Enfin, K, merci d'avoir été ma bouffée d'air frais chaque jour de ces quatre dernières années. Merci pour les ravitaillements en Nalu lors de mes longues journées, pour les soirées sushis en l'honneur des articles publiés, pour les longues conversations de fin de journée quand le doute s'installait, merci d'être la lumière qui éclaire mes nuits. Merci de croire en moi et de me porter vers le haut comme personne ne sait le faire. Pour toujours, quoi qu'il arrive.

Je remercie chaque personne ayant contribué, de près ou de loin, à l'aboutissement de cette thèse, mais aussi à faire de moi la personne que je suis aujourd'hui. MERCI !

Résumé

La transplantation rénale représente le traitement de référence de l'insuffisance rénale terminale, offrant une survie et une qualité de vie supérieures à toutes les formes de dialyse. Cependant, la pénurie persistante d'organes contraint la communauté de transplantation à élargir les critères d'acceptation des donneurs — notamment aux donneurs décédés après arrêt circulatoire (DCD) et aux donneurs à critères élargis (ECD) — dont les greffons sont particulièrement exposés aux lésions d'ischémie-reperfusion (IRI). Ces lésions, pivot de la dysfonction précoce du greffon, augmentent le risque de reprise retardée de fonction (DGF).

La perfusion normothermique (NMP) constitue une avancée majeure dans la préservation des organes : en maintenant le rein à température physiologique avec un perfusat oxygéné et enrichi en substrats métaboliques, elle restaure un métabolisme aérobie actif, permet une évaluation dynamique de la viabilité du greffon et ouvre une fenêtre d'intervention thérapeutique. Parallèlement, les cellules stromales mésenchymateuses (MSC) se sont imposées comme une plateforme thérapeutique prometteuse grâce à leurs propriétés immunomodulatrices et régénératives pluripotentes, médiées principalement par voie paracrine via leur sécrétome.

Cette thèse explore la combinaison de ces deux technologies selon un programme de recherche translationnelle en quatre étapes. Dans un premier temps, une revue systématique avec méta-analyse a permis de dresser un état des lieux rigoureux des thérapies à base de cellules souches administrées lors de la perfusion *ex vivo* de greffons rénaux. Sur cette base, un modèle porcin de NMP simultanée des deux reins d'un même donneur a été développé et validé, permettant des comparaisons intra-animales appariées et éliminant la variabilité inter-individuelle. Ce modèle a ensuite servi de plateforme pour évaluer l'administration intra-artérielle de MSC humaines de grade clinique lors de la NMP de reins porcins ischémiques. Enfin, cette stratégie a été transposée à des reins humains déclinés pour transplantation.

Ces travaux établissent les fondements scientifiques, techniques et translationnels de la délivrance intra-artérielle de MSC humaines de grade clinique lors de la NMP comme nouvelle stratégie potentielle d'atténuation des lésions d'IRI en transplantation rénale à partir de donneurs décédés, et ouvrent la voie à une éventuelle application clinique.

Abstract

Kidney transplantation represents the gold standard treatment for end-stage renal disease, offering superior survival and quality of life compared to all forms of dialysis. However, the persistent organ shortage compels the transplant community to broaden donor acceptance criteria — particularly to donation after circulatory death (DCD) donors and extended criteria donors (ECD) — whose grafts are especially vulnerable to ischemia-reperfusion injury (IRI). These injuries, which are central to early graft dysfunction, increase the risk of delayed graft function (DGF).

Normothermic machine perfusion (NMP) represents a major advance in organ preservation: by maintaining the kidney at physiological temperature with an oxygenated, metabolically enriched perfusate, it restores active aerobic metabolism, enables dynamic assessment of graft viability, and opens a window for therapeutic intervention. In parallel, mesenchymal stromal cells (MSCs) have emerged as a promising therapeutic platform owing to their immunomodulatory and regenerative properties, mediated primarily through paracrine signalling via their secretome.

This thesis explores the combination of these two technologies within a four-step translational research programme. First, a systematic review with meta-analysis was conducted to provide a rigorous overview of stem cell-based therapies administered during *ex vivo* perfusion of kidney grafts. Building on this foundation, a porcine model of simultaneous NMP of both kidneys from the same donor was developed and validated, enabling paired intra-animal comparisons and eliminating inter-individual variability. This model then served as a platform to evaluate intra-arterial delivery of clinical-grade human MSCs during NMP of ischaemic porcine kidneys. Finally, this strategy was translated to human kidneys declined for transplantation.

This work establishes the scientific, technical, and translational foundations of intra-arterial clinical-grade hMSC delivery during NMP as a novel potential strategy for attenuating IRI in deceased-donor kidney transplantation and paves the way for eventual clinical application.

List of Abbreviations

A-MSC	Adipose tissue-derived mesenchymal stromal cell
AKD	Acute kidney disease
AKI	Acute kidney injury
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
AUC	Area under the curve
bFGF	Basic fibroblast growth factor
BM-MSC	Bone marrow-derived mesenchymal stromal cell
BPAR	Biopsy-proven acute rejection
CEUS	Contrast-enhanced ultrasound
CIT	Cold ischemia time
CKD	Chronic kidney disease
COX-2	Cyclooxygenase-2
DAMPs	Damage-associated molecular patterns
DBD	Donation after brain death
DC	Dendritic cell
DCD	Donation after circulatory death
DGF	Delayed graft function
DKD	Diabetic kidney disease
DMSO	Dimethyl sulfoxide
DSA	Donor-specific antibody
ECD	Expanded criteria donor
eGFR	Estimated glomerular filtration rate
ELISA	Enzyme-linked immunosorbent assay
ESRD	End-stage renal disease
EV	Extracellular vesicle
FENa	Fractional excretion of sodium
FMN	Flavin mononucleotide
GFR	Glomerular filtration rate
GLMM	Generalized linear mixed model
GMP	Good manufacturing practices
HGF	Hepatocyte growth factor
HLA	Human leukocyte antigen
HMP	Hypothermic machine perfusion
HOPE	Hypothermic oxygenated perfusion
IDO	Indoleamine 2,3-dioxygenase
IFN-γ	Interferon-gamma
IGL-1	Institut Georges Lopez solution

IL-1β	Interleukin-1 beta
IL-1RA	Interleukin-1 receptor antagonist
IL-6	Interleukin-6
IL-8	Interleukin-8
IL-10	Interleukin-10
IL-18	Interleukin-18
INAMI	Belgian national institute for health and disability insurance
IRI	Ischemia-reperfusion injury
IRR	Intrarenal resistance
ISCT	International society for cell and gene therapy
IV	Intravenous
JAK-STAT1	Janus kinase – signal transducer and activator of transcription 1
KDIGO	Kidney disease: improving global outcomes
KDPI	Kidney donor profile index
KIM-1	Kidney injury molecule-1
LC-MS	Liquid chromatography-mass spectrometry
LDH	Lactate dehydrogenase
LN	Lupus nephritis
LTCG	Laboratory of cell and gene therapy (CHU Liège)
MAPC	Multipotent adult progenitor cells
MRI	Magnetic resonance imaging
MSC	Mesenchymal stromal cell
NF-κB	Nuclear factor kappa B
NGAL	Neutrophil gelatinase-associated lipocalin
NK	Natural killer cell
nKSPCs	Neonatal kidney stem progenitor cells
NMP	Normothermic machine perfusion
NMR	Nuclear magnetic resonance
ORS	Organ Recovery System
PAS	Periodic acid-Schiff (staining)
PD-L1	Programmed death-ligand 1
PGE-2	Prostaglandin E2
PNF	Primary non-function
PRISMA	Preferred reporting items for systematic reviews and meta-analyses
PRR	Pattern recognition receptor
QAS	Quality assessment score
RAAS	Renin-angiotensin-aldosterone system
RBC	Red blood cells
RBF	Renal blood flow
RCT	Randomized controlled trial
ROS	Reactive oxygen species

RRT	Renal replacement therapy
SCS	Static cold storage
skGFR	Single kidney glomerular filtration rate
SLE	Systemic lupus erythematosus
sNMP	Subnormothermic machine perfusion
SYRCLE	Systematic review centre for laboratory animal experimentation
TEC	Tubular epithelial cell
TGF-β	Transforming growth factor-beta
TNF-α	Tumour necrosis factor-alpha
Treg	Regulatory T cell
TSG-6	Tumour necrosis factor-stimulated gene 6 protein
U-MSC	Umbilical cord-derived mesenchymal stromal cell
UW	University of Wisconsin solution
VEGF	Vascular endothelial growth factor
WBC	White blood cells
WIT	Warm ischemia time
WJ-MSC	Wharton's jelly-derived mesenchymal stromal cell
YAP	Yes-associated protein
ZO-1	Zonula occludens-1

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Chapter 1 – General Introduction & Aims

Chapter 1. General Introduction & Aims

1. Kidney Transplantation

1.1 — Kidney Function

1.1.1 — Anatomy

The kidneys are paired retroperitoneal organs located on either side of the vertebral column at the level of the twelfth thoracic to the third lumbar vertebra. On gross examination, the renal parenchyma is divided into two distinct zones: the outer cortex, which contains the glomeruli and the proximal and distal tubules, and the inner medulla, organized into pyramidal structures whose apices — the renal papillae — drain urine into the minor calyces, the major calyces, and ultimately the renal pelvis, which connects to the ureter. The vascular architecture of the kidney is highly specialized: the renal artery branches successively into segmental, interlobar, arcuate, and interlobular arteries before reaching the afferent arterioles that supply the glomerular capillary tufts. Venous drainage follows a symmetrical pattern, and the kidneys receive approximately 20–25% of cardiac output at rest, reflecting the large metabolic demands of its filtration function. The functional unit of the kidney is the nephron, of which each human kidney contains approximately 0.7 to 1.5 million.¹ Each nephron consists of a renal corpuscle — comprising the glomerulus and its surrounding Bowman's capsule — followed by a series of tubular segments with distinct anatomical and functional identities: the proximal convoluted tubule, the loop of Henle (with its descending limb, ascending limb, and ascending limb), the distal convoluted tubule, and the collecting duct, which is shared between several nephrons and ultimately drains into the renal papilla (**Figure 1**). The structural organization of the nephron is directly coupled to its functional specialization: each segment expresses a unique complement of transporters, channels, and receptors that collectively execute the kidney's capacity for selective reabsorption and secretion.²

1.1.2 — Physiological functions

Filtration and excretion. The primary function of the kidney is the continuous filtration of blood plasma through the glomerular capillary wall, a process driven by hydrostatic pressure gradient between the glomerular capillary and Bowman's space. This generates an ultrafiltrate of approximately 180 liters per day, the vast majority of which is subsequently reabsorbed along the tubular segments. The glomerular filtration rate (GFR) — defined as the volume of plasma filtered per unit time — is the central parameter used to quantify overall kidney function. In healthy adults, GFR ranges between 90 and 120 mL/min/1.73 m². The filtered load of small solutes such as creatinine, urea, and uric acid is only partially reabsorbed, allowing their net urinary excretion and maintaining plasma homeostasis.

In contrast, high-molecular-weight proteins and blood cells are retained by the size- and charge-selective filtration barrier constituted by the fenestrated glomerular endothelium, the glomerular basement membrane, and the podocyte foot processes with their slit diaphragms.³

Fluid and electrolyte homeostasis. Beyond filtration, the tubular segments of the nephron exercise precise control over the composition and volume of the extracellular fluid. Sodium reabsorption — the single largest energy-consuming process in the kidney — occurs at multiple sites along the nephron through a combination of active transport via the Na^+/K^+ -ATPase at the basolateral membrane and passive co-transport driven by the resulting electrochemical gradient. Fine-tuning of sodium balance and blood pressure is orchestrated by the renin-angiotensin-aldosterone system, a hormonal cascade initiated by renin secretion in response to reduced renal perfusion pressure, low tubular sodium delivery, or sympathetic activation. Renin converts angiotensinogen to angiotensin I, which is then cleaved by the angiotensin-converting enzyme to the biologically active angiotensin II. This effector peptide acts on renal tubules to promote sodium reabsorption and on the adrenal cortex to stimulate aldosterone secretion, which in turn enhances distal tubular sodium retention and potassium excretion. Potassium homeostasis and water balance are equally dependent on the integrity of these tubular mechanisms (**Figure 1**).⁴

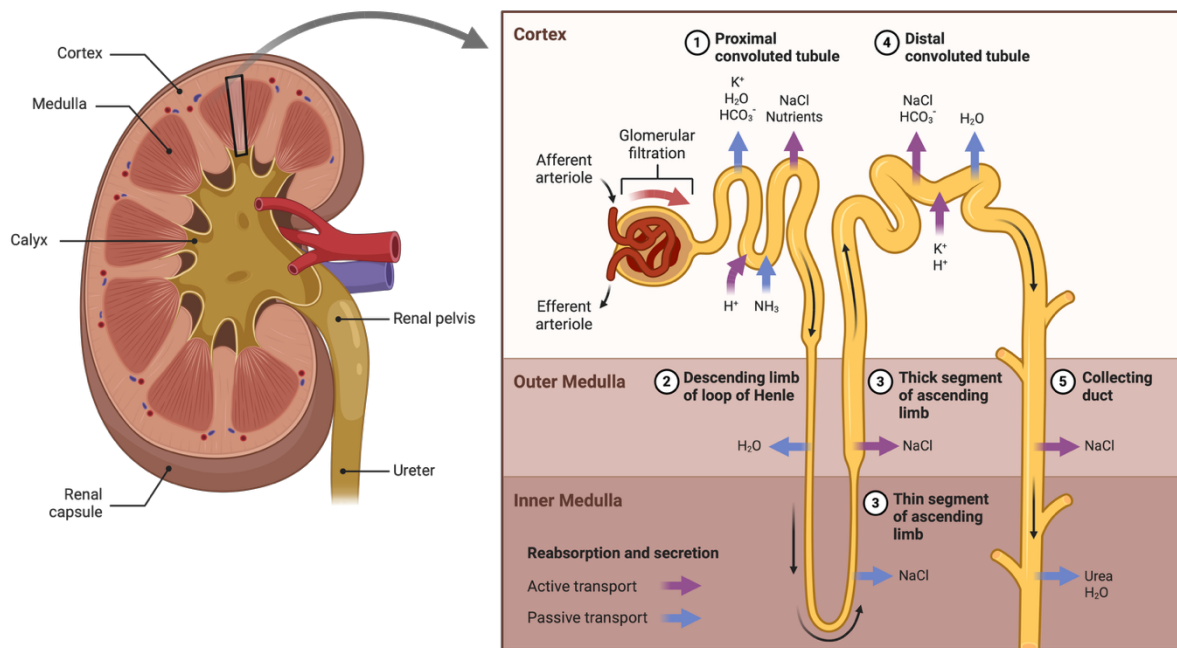


Figure 1. Kidney anatomy and tubular transport functions along the nephron. Left: coronal section of the kidney (cortex, medulla, calyx, renal pelvis, ureter). Right: segmental transport functions of the nephron — ① proximal convoluted tubule (bulk reabsorption of K^+ , H_2O , HCO_3^- , nutrients; H^+/NH_3 secretion), ② descending limb (passive H_2O reabsorption), ③ ascending limb (NaCl reabsorption, water-impermeable), ④ distal convoluted tubule (NaCl , HCO_3^- reabsorption; K^+/H^+ secretion), ⑤ collecting duct (NaCl , H_2O , urea reabsorption under hormonal control). Purple: active transport; blue: passive transport. Created with BioRender.com.

Acid-base balance. The kidney plays an indispensable role in maintaining systemic pH within the narrow physiological range of 7.35–7.45. This is achieved through bicarbonate reabsorption in the proximal tubule and through net acid excretion in the distal nephron and collecting duct, where hydrogen ions are secreted into the tubular lumen and buffered by ammonia and phosphate. In conditions of metabolic acidosis, the kidney increases ammoniogenesis and augments net acid excretion, while in metabolic alkalosis, bicarbonate excretion is increased.⁵

Endocrine functions. In addition to its filtration and transport roles, the kidney operates as a major endocrine organ. Erythropoietin, produced by peritubular fibroblasts in the renal cortex in response to tissue hypoxia, is the principal regulator of red blood cell production.⁶ The kidney is also the site of the final hydroxylation step in vitamin D activation: 25-hydroxyvitamin D is converted to the biologically active 1,25-dihydroxyvitamin D (calcitriol) by the enzyme 1 α -hydroxylase in proximal tubular cells, a process regulated by parathyroid hormone and fibroblast growth factor-23.⁷ Finally, renin secretion itself constitutes an endocrine output of the kidney with systemic consequences far beyond sodium and blood pressure regulation.

1.1.3 — Assessment of kidney function in clinical practice

Serum creatinine and estimated GFR. In clinical practice, GFR is most estimated indirectly through serum creatinine concentration, an endogenous metabolite of muscle creatine that is freely filtered by the glomerulus and minimally secreted by the tubules. While serum creatinine is readily measurable and widely available, its relationship to GFR is nonlinear and substantially influenced by non-renal determinants including muscle mass, age, sex, diet, and ethnicity. Estimated GFR (eGFR) equations — most notably the CKD-EPI 2021 creatinine equation — incorporate these variables to provide a more standardized estimation. However, even validated eGFR equations carry inherent imprecision of ± 15 –30% around the true GFR in individual patients, and perform least reliably at extremes of muscle mass.^{8,9} The addition of serum cystatin C — a low-molecular-weight protein produced at a constant rate by all nucleated cells and freely filtered by the glomerulus — to estimating equations substantially improves accuracy, particularly in populations where creatinine-based estimates are unreliable.^{9,10}

Measured GFR: from inulin to iohexol. When precision is required, direct measurement of GFR using exogenous filtration markers is the appropriate approach. Urinary inulin clearance has historically been considered the gold-standard method, as inulin is freely filtered, neither secreted nor reabsorbed by the tubules, and not metabolized. However, its practical constraints — including the need for continuous intravenous infusion, timed urine collections, and highly specialized laboratory infrastructure — have largely restricted its use to dedicated research settings, and the commercial availability of pharmaceutical-grade inulin has progressively declined. Iohexol plasma clearance has emerged as the most widely adopted practical alternative.

Administered as a single intravenous bolus, this non-ionic iodinated contrast agent is quantified in plasma by high-performance liquid chromatography or tandem mass spectrometry. GFR is derived from its disappearance curve using validated mathematical formulas. The choice between single-sample and multiple-sample protocols has been extensively studied but single-sample strategies using the Jacobsson iterative method at 300 minutes show high concordance with multi-sample results, making them practical for routine use.¹¹ The impact of different area-under-the-curve calculation methods on GFR precision has also been formally evaluated by Pottel *et al.* in 2021.¹² Recognizing the growing need to standardize iohexol-based GFR measurement across centers and countries, the European Kidney Function Consortium published in 2024 a comprehensive consensus document covering all practical aspects of the iohexol protocol.¹³ This consensus explicitly positions iohexol as the preferred non-radiolabelled exogenous marker for measured GFR. Despite the progress achieved with newer race-free and sex-free eGFR equations, Delanaye, Cavalier and colleagues of the CHU of Liege have consistently argued that no estimating equation achieves sufficient individual-level accuracy to replace measured GFR in high-stakes clinical situations.^{14,15}

Urinary biomarkers. Analyses of urine and measurement of urinary biomarkers provide information complementary to GFR estimation, allowing detection of tubular injury independently of filtration loss. Proteinuria — quantified as the urinary albumin-to-creatinine ratio — is a sensitive marker of glomerular barrier dysfunction. In the context of acute kidney injury (AKI), two tubular injury biomarkers have gained particular relevance: neutrophil gelatinase-associated lipocalin (NGAL), expressed in the thick ascending limb and collecting duct and rapidly released into urine within hours of tubular injury, and kidney injury molecule-1 (KIM-1), a transmembrane protein upregulated in dedifferentiated proximal tubular cells following ischemic or toxic injury. Both markers enable earlier detection of AKI than serum creatinine.¹⁶ In the transplant setting specifically, NGAL and KIM-1 in the perfusate and urine during machine perfusion have emerged as early predictors of graft function and delayed graft function (DGF).¹⁷

1.2 — End-Stage Renal Disease

1.2.1 — Definition and staging of chronic kidney disease

Chronic kidney disease (CKD) is defined as a persistent abnormality in kidney structure or function, present for more than three months, with implications for health. The internationally accepted classification system, established by the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines, organizes CKD into a bidimensional staging framework combining GFR categories (G1 to G5, ranging from ≥ 90 to < 15 mL/min/1.73 m²) with albuminuria categories (A1 to A3, from < 30 to ≥ 300 mg/g urinary albumin-to-creatinine ratio) (**Figure 2**).

KDIGO : Prognosis of CKD by GFR and albuminuria categories				Persistent albuminuria categories Description and range		
				A1	A2	A3
				Normal to mildly increased	Moderately increased	Severely increased
				<30 mg/g <3 mg/mmol	30-300 mg/g 3-30 mg/mmol	>300 mg/g >30 mg/mmol
GFR categories (ml/min/1.73 m ²) Description and range	G1	Normal or high	≥90			
	G2	Mildly decreased	60-89			
	G3a	Mildly to moderately decreased	45-59			
	G3b	Moderately to severely decreased	30-44			
	G4	Severely decreased	15-29			
	G5	Kidney failure	<15			

Figure 2. Prognosis of chronic kidney disease (CKD) by GFR and albuminuria categories. The heat map illustrates the risk of CKD progression based on the combination of GFR categories (G1–G5) and persistent albuminuria categories (A1–A3). Green: low risk (if no other markers of kidney disease, no CKD); Yellow: moderately increased risk; Orange: high risk; Red: very high risk. GFR, glomerular filtration rate. Adapted from KDIGO 2024,¹⁸ modified using BioRender.com.

This combined GFR-albuminuria matrix allows stratification of individual risk for CKD progression, cardiovascular events, and mortality, and guides the intensity of monitoring and intervention. Importantly, albuminuria is recognized as an independent predictor of adverse outcomes at every level of GFR, including in the normal range.^{3,19} CKD affects approximately 10–13% of the adult population worldwide and represents a major global health burden. According to the most recent Global Burden of Disease analysis covering 204 countries, approximately 4.59 million people were living with kidney failure requiring replacement therapy in 2023, with prevalence rising steadily over the past three decade.²⁰

The transition from CKD to end-stage renal disease (ESRD) — defined in the 2024 KDIGO guidelines as kidney failure requiring replacement therapy — follows a trajectory that is neither uniform nor inevitable. Key predictors of progression include the underlying aetiology, the degree of proteinuria, blood pressure control, glycaemic status in diabetic patients, and the rate of GFR decline over time. Once GFR falls below approximately 15–20 mL/min/1.73 m², uraemic symptoms typically emerge and dialysis should be considered, although renal replacement therapy generally becomes necessary only when GFR drops between 10 and 15 mL/min/1.73 m².^{18,21}

1.2.2 — Renal Replacement Therapy Options

When kidney function is no longer compatible with life, renal replacement therapy (RRT) constitutes the only life-sustaining option. Three modalities are currently available: haemodialysis, peritoneal dialysis, and kidney transplantation.

Haemodialysis is the most widely used form of RRT worldwide, accounting for most dialysis patients globally. It operates by diffusion of solutes and ultrafiltration of excess fluid across a semipermeable membrane separating the patient's blood from a dialysate solution, typically delivered in three sessions per week of three to four hours each. Vascular access — most commonly an arteriovenous fistula — is a critical component of successful haemodialysis delivery. However, arteriovenous fistula creation and maintenance carry significant morbidity, including aneurysm formation, stenosis, and thrombosis, with surgical revascularization often required over the course of a patient's dialysis career.²²

Peritoneal dialysis uses the peritoneal membrane as the dialysing surface, with fluid instilled and drained through a permanent abdominal catheter. It can be performed at home, either continuously or with automated overnight cyclers, offering patients greater autonomy, flexibility, and freedom from hospital attendance. Compared to haemodialysis, peritoneal dialysis offers several important potential advantages: better preservation of residual kidney function, fewer dietary restrictions, lower risk of blood viral infections, reduced need for erythropoiesis-stimulating agents, and generally lower cost.^{23,24}

Kidney transplantation represents the optimal form of RRT for eligible patients with ESRD, offering superior outcomes across all major clinical endpoints — including patient survival, graft survival, quality of life, and long-term cardiovascular risk — compared to any form of chronic dialysis. The mortality benefit of transplantation over dialysis is substantial and well established, with transplant recipients demonstrating markedly lower long-term mortality rates despite the perioperative risks inherent to transplant surgery and the lifelong requirement for immunosuppression.²⁵ The cost-effectiveness advantage of transplantation over dialysis is also consistently demonstrated, as the initial cost of transplantation is largely offset within two to four years by the elimination of recurrent dialysis costs. These considerations collectively establish kidney transplantation as the best available option but also as the treatment of choice whenever it is medically and logistically feasible.^{18,26}

1.3 — Different Types of Organ Donation

1.3.1 — Living donation

Kidney donors are broadly classified as living or deceased (**Figure 3**). Living donor kidney transplantation represents the optimal form of transplantation, offering several decisive advantages over deceased donor surgery.

Cold ischemia time is virtually absent, graft function is typically immediate, and the elective nature of the procedure allows thorough recipient preparation and optimal surgical planning. Living donation is classified as either living related — between genetically related individuals such as siblings or parents — or living unrelated, including emotionally related pairs such as spouses, friends, and paired kidney exchange programs. Both categories yield superior graft outcomes compared to deceased donor transplantation, with higher rates of immediate graft function, better long-term graft survival, and reduced incidence of DGF.^{27,28}

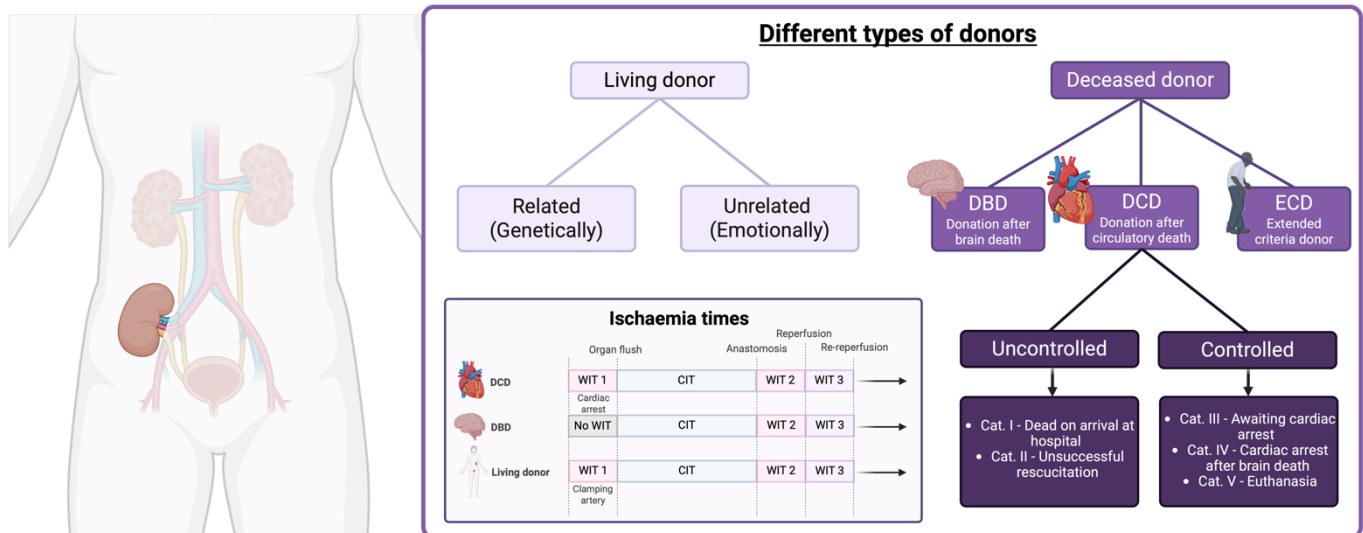


Figure 3. Classification of kidney donors and associated ischaemia times. Kidney donors are classified as living (related or unrelated) or deceased. The inset timeline illustrates the sequence and relative duration of warm ischaemia time (WIT) and cold ischaemia time (CIT) for each donor type. CIT = cold ischaemia time; DBD = donation after brain death; DCD = donation after circulatory death; ECD = extended criteria donor; WIT = warm ischaemia time. Created with BioRender.com.

1.3.2 — Donation after Brain Death

Donation after brain death (DBD) has historically been the primary source of deceased donor kidneys. The concept of brain death was first formally defined by the Ad Hoc Committee of the Harvard Medical School in 1968. Their criteria — including absence of brainstem reflexes, spontaneous breathing, and isoelectric EEG — established the basis for DBD. Brain death triggers a cascade of profound systemic alterations that directly compromise organ quality. The initial autonomic storm — characterized by a catecholamine surge, severe hemodynamic instability, and hypertensive crisis — gives way to cardiovascular collapse, progressive hypotension, and hormonal dysregulation. Simultaneously, the neurological insult activates a systemic inflammatory response with release of pro-inflammatory cytokines, complement activation, and upregulation of endothelial adhesion molecules, collectively priming the organ for enhanced susceptibility to subsequent ischemia-reperfusion injury (IRI) upon transplantation.^{27,29}

1.3.3 — Donation after Circulatory Death

Donation after circulatory death (DCD) refers to organ procurement from donors in whom death is confirmed by the irreversible cessation of circulatory function, as opposed to neurological criteria. The Maastricht classification categorizes DCD donors into four groups: categories I and II (uncontrolled DCD — unexpected cardiac arrest outside or within hospital, respectively), category III (controlled DCD — withdrawal of life-sustaining treatment in a planned setting), and category IV (cardiac arrest in a brain-dead donor). A fifth category, encompassing donors following euthanasia, has been proposed and is exclusively applicable in countries where euthanasia is legally permitted, including Belgium.³⁰ In clinical practice, the vast majority of DCD donation activity in most countries involves controlled category III donors, where the planned nature of the procedure allows for optimized procurement logistics and reduced uncontrolled warm ischemia time (WIT).^{27,31} The fundamental challenge of DCD kidneys lies in their unavoidable exposure to total donor warm ischemia time which is the sum of the agonal WIT and the acirculatory WIT. In the Eurotransplant region, the median cold ischemia time across deceased donor kidney transplants is approximately 16 hours, with warm ischemia time generally maintained below 10–20 minutes.^{32,33} WIT is typically defined as the interval from stop ventilation to start *in situ* cold flush — during which the organ undergoes anaerobic metabolism, ATP depletion, and early ischemic cell death in the absence of effective oxygen delivery. This exposure is qualitatively different and more severe than that occurring in DBD donation, and it directly translates into higher rates of DGF, increased risk of primary non-function, and greater susceptibility to IRI upon reperfusion.^{34,35} According to the Eurotransplant 2024 annual report, DBD donors constituted many deceased donors across all member states, though the DBD/DCD ratio varies substantially between countries. Germany contributed the highest absolute number of donors, while the Netherlands stands out as the country with the highest proportion of DCD donors.³⁶ At the national level, Belgian donation activity has followed an upward trajectory over the past decade, with a particularly marked increase in DCD donation. Total donor numbers reached a combined peak of 422 in 2025 — including 205 DBD and 217 DCD donors, the highest DCD figure recorded over the entire period (**Figure 4**).

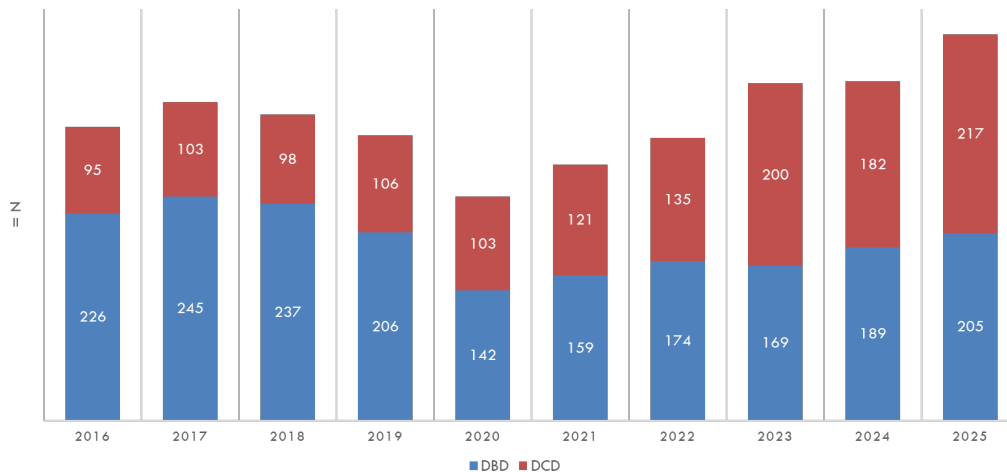


Figure 4. Evolution of Belgian donation activity (DCD and DBD donors). Annual number of DBD and DCD donors in Belgium, 2016–2025. DBD = donation after brain death; DCD = donation after circulatory death. Source: Donor & transplant statistics BTS 2025.

1.4 — Organ Shortage

1.4.1 — The supply-demand imbalance

The gap between the number of patients awaiting kidney transplantation and the number of organs available remains the central challenge of transplant medicine. Within the Eurotransplant region alone, 13,570 patients remained active on the waiting list at the end of 2024, with 7,150 organs from 2,181 deceased donors successfully allocated that year. In Belgium specifically, patients still face waiting times of several years and continued clinical deterioration while on dialysis. The consequences are not merely logistical: each additional year on dialysis is associated with progressive cardiovascular morbidity, increased sensitization, and worsening functional status, all of which negatively impact post-transplant outcomes.³⁷

1.4.2 — Strategies to Expand the Donor Pool

Faced with a persistent organ shortage, the transplant community has progressively broadened the criteria for donor acceptance. Expanded criteria donors (ECDs) are defined as deceased donors aged ≥ 60 years, or aged 50–59 with at least two of the following: a history of hypertension, a terminal serum creatinine ≥ 1.5 mg/dL, or death from cerebrovascular cause. These donors carry a significantly higher risk of primary non-function (PNF), DGF, and earlier graft loss compared to standard criteria donors.^{37–39} The growing use of DCD grafts — which, despite higher short-term DGF rates, achieve long-term outcomes comparable to DBD when appropriate preservation strategies are applied — has been formally endorsed as a safe and necessary expansion of the donor pool.^{40,41}

1.5 — Kidney Graft Preservation Techniques

1.5.1 — The fundamental challenge of organ preservation

The removal of a kidney from its donor initiates an unavoidable sequence of ischemic injury whose severity is directly determined by the duration, temperature, and conditions of preservation. During warm ischemia, the cessation of blood flow rapidly depletes cellular ATP stores, causing failure of energy-dependent ion pumps — most critically the Na^+/K^+ -ATPase — and leading to intracellular sodium and calcium overload, mitochondrial dysfunction, and ultimately cell death through necrotic and regulated pathways including ferroptosis and apoptosis.^{42–45} Cold ischemia, while slowing these processes through metabolic suppression at low temperature, does not halt them entirely. Ongoing cellular damage accumulates throughout the preservation period and manifests upon reperfusion as the IRI syndrome — a cascade of oxidative stress, complement activation, innate immune recruitment, and endothelial inflammation. This is the primary driver of DGF and contributes to long-term allograft fibrosis (**Figure 5**).^{46,47} The fundamental goal of organ preservation is therefore to minimize the magnitude of this ischemic insult and to maintain maximal cellular viability between donor procurement and graft transplantation.

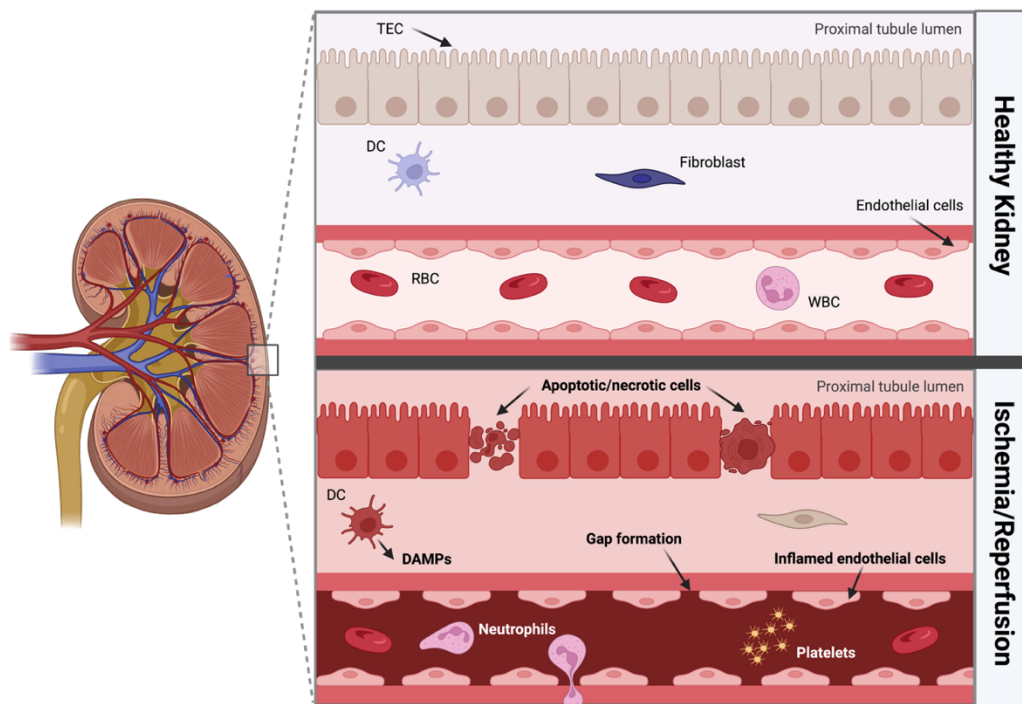


Figure 5. Cellular and vascular consequences of ischemia-reperfusion injury in the kidney. In the healthy kidney (upper panel), TEC maintain brush-border integrity, the interstitium harbours quiescent DC and fibroblasts, and peritubular capillaries carry RBC and WBC under normal flow. Following ischemia and reperfusion (lower panel), TECs undergo apoptosis and necrosis with loss of epithelial integrity, activated DCs release DAMPs, and endothelial injury causes gap formation and an inflamed phenotype. Neutrophil recruitment and platelet aggregation in the microcirculation further impair perfusion and amplify inflammation, collectively driving DGF after transplantation. DAMPs, damage-associated molecular patterns; DC, dendritic cells; RBC, red blood cells; TEC, tubular epithelial cells; WBC, white blood cells. Adapted from Li *et al.* (*Cell Death Discovery*, 2024),⁴⁵ modified using Biorender.com.

1.5.2 — Static Cold Storage

Static cold storage (SCS) remains the most widely used preservation method globally and constitutes the reference standard against which all alternative techniques are evaluated. Its principle is simple: following vascular flushing with a cold preservation solution at the time of procurement, the graft is stored on ice at approximately 4°C, reducing metabolic demand to approximately 5–10% of baseline normothermic activity.^{27,48} Several preservation solutions have been developed, differing in their ionic composition, osmolarity, and colloid content — each designed to counteract specific mechanisms of cold ischemic injury. The University of Wisconsin (UW) solution has long been considered the historical gold standard. The Institut Georges Lopez solution (IGL-1), developed in Lyon (France) and now in current use in Belgium, represents a more recent alternative combining a low-potassium, high-sodium ionic composition with polyethylene glycol as the primary colloid. This is an approach designed to minimize cellular oedema and inflammation while offering practical advantages in terms of viscosity and flushing volume. In a single-centre comparative study, kidneys preserved with IGL-1 demonstrated significantly higher rates of immediate graft function compared to UW-based solutions, with comparable long-term graft survival.⁴⁹ Despite these differences in composition, no preservation solution can fully prevent the cumulative cellular injury that accrues during prolonged cold ischemia.⁵⁰ SCS is straightforward, inexpensive, and logistically simple, but carries fundamental limitations that become critical with high-risk grafts — namely organs with elevated susceptibility to delayed graft function, primary non-function, and inferior long-term graft survival. Its passive nature precludes active cellular repair, real-time functional assessment, or detection of subclinical injury during the preservation period. Cold ischemia time accumulates cumulatively and is directly associated with increasing rates of DGF and primary non-function, particularly for DCD and expanded criteria donor kidneys.^{48,51}

1.5.3 — Hypothermic Machine Perfusion

Hypothermic machine perfusion (HMP) represents the first major technological advance beyond SCS, replacing passive cold storage with continuous perfusion of the kidney at 4–8°C using an acellular preservation solution. By maintaining continuous flow through the renal vasculature, HMP washes out toxic metabolites, provides minimal metabolic substrate supply, reduces microvascular resistance, and prevents the endothelial dysfunction associated with prolonged static cold storage.^{52–54} The evidence base for HMP is now robust and consistent across multiple randomized controlled trials (RCT) and meta-analyses: the most recent Cochrane meta-analysis (22 RCTs, 4,007 participants) confirmed with high-certainty evidence that continuous non-oxygenated HMP significantly reduces DGF compared to SCS in both DCD and DBD settings and improves one-year graft survival.⁵⁵ Economic analyses consistently show that HMP is cost-saving or cost-effective compared to SCS in both American and European healthcare settings.^{55,56}

An important refinement is the addition of active oxygenation to the perfusate — oxygenated HMP (HMPO, also termed HOPE) — which allows modest mitochondrial ATP resynthesis during preservation and attenuates reperfusion injury at implantation.^{57,58} The Cochrane meta-analysis confirmed that oxygenated HMP confers additional benefit over non-oxygenated HMP specifically in high-risk DCD donors over 50 years of age, further improving graft survival, renal function at one year, and acute rejection rates.⁵⁵ The use of oxygenated HMP is increasingly adopted internationally, particularly for marginal DCD grafts, and is now incorporated into national preservation protocols in several countries.

Belgium has been at the forefront of the systematic implementation of HMP at national scale. In September 2022, the Belgian National Institute for Health and Disability Insurance (INAMI) approved full reimbursement of a centralized HMP service for all ECD and DCD kidneys procured and transplanted within the country — making Belgium one of the first countries worldwide to implement nationally reimbursed HMP as standard of care. This national perfusion service is operated by the company Organ Recovery System (ORS), whose trained technicians are present at every procurement, transport the perfusion device, and prepare and manage the machine throughout each donation procedure. In this model, perfusion machines are not allocated to individual transplant centres but are deployed as a mobile, centralized resource. HOPE is applied systematically to DCD donors aged over 50 years within this national protocol. A retrospective analysis of 242 kidney transplantations preserved with continuous HMP during the first year of implementation (October 2022–September 2023) demonstrated excellent outcomes: DGF in 14.4% overall, one-year death-censored graft survival of 96.3%, and patient survival of 98.3%. Critically, the estimated annual cost savings from reduced dialysis requirements reached €3.59 million. Following these results, the INAMI approved a five-year extension of the reimbursement scheme (2025–2030), with planned expansion of the indications to include DBD donors under 50 years with a peak serum creatinine >1.5 mg/dL, combined kidney–other organ transplants, and cross-border HMP exchange with the Netherlands (currently under contractual negotiation).⁵⁹

Machine perfusion parameters measured during HMP — particularly renal vascular resistance and perfusate biomarkers including NGAL and flavin mononucleotide (FMN) — have emerged as useful predictors of post-transplant outcomes, though their role in formal viability-based decision-making remains to be prospectively validated.^{60,61}

1.5.4 — Subnormothermic Machine Perfusion

The recognition that both temperature and perfusion modality independently influence preservation outcomes has motivated exploration of the intermediate temperature range — subnormothermic machine perfusion (sNMP), typically performed at 20–32°C.

At these temperatures, metabolic activity is partially restored — allowing limited functional monitoring and some cellular repair — while avoiding the complexity and oxygen delivery requirements of full normothermia. Moreover, this allow to crucially eliminating the need for RBC-based perfusates with their attendant haemolysis risk.^{62,63} Preliminary preclinical data are encouraging; Abraham *et al.* demonstrated in a porcine DCD autotransplant model that 24 hours of sNMP at 22–25°C with an acellular, portable perfusate significantly reduced post-transplant serum creatinine and blood urea nitrogen compared to SCS.⁶⁴ A direct head-to-head comparison of NMP and sNMP was recently reported by Krüger *et al.* using the fully synthetic, RBC-free perfusate Omnisol in paired porcine kidneys over 6 hours. NMP was associated with better haemodynamic parameters and functional readouts during the first 3 hours, while sNMP demonstrated comparatively stable perfusion and significantly lower injury biomarkers — including lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and NGAL — beyond 3 hours. Creatinine clearance was maintained, suggesting that sNMP may be advantageous for extended preservation windows. Importantly, the synthetic perfusate met oxygen requirements in both temperature regimes without an oxygen carrier, substantially reducing injury markers compared to blood-based NMP protocols from the same group.⁶⁵ More recently, Deffrennes, Hosgood *et al.* reported the first human experience of extended subnormothermic acellular perfusion at 32°C for 6, 12, or 24 hours in 21 declined kidneys: all maintained stable haemodynamic and renal function during perfusion, with injury markers stable up to 12 hours and marginally increasing beyond. sNMP kidneys showed significantly superior functionality and histology at reperfusion compared to matched SCS controls.⁶⁶ Nonetheless, robust clinical trial evidence for sNMP remains absent.

1.5.5 — Normothermic Machine Perfusion

Normothermic machine perfusion (NMP) addresses the limitation of SCS and HMP by perfusing the kidney at physiological temperature (35–38°C) using an oxygenated perfusate — either blood-based or entirely acellular — supplemented with nutrients and metabolic substrates to sustain full aerobic metabolism. This fundamental shift — from passive preservation to active metabolic support — is the defining advantage of NMP over all cold preservation strategies (**Figure 6**).^{67–69}

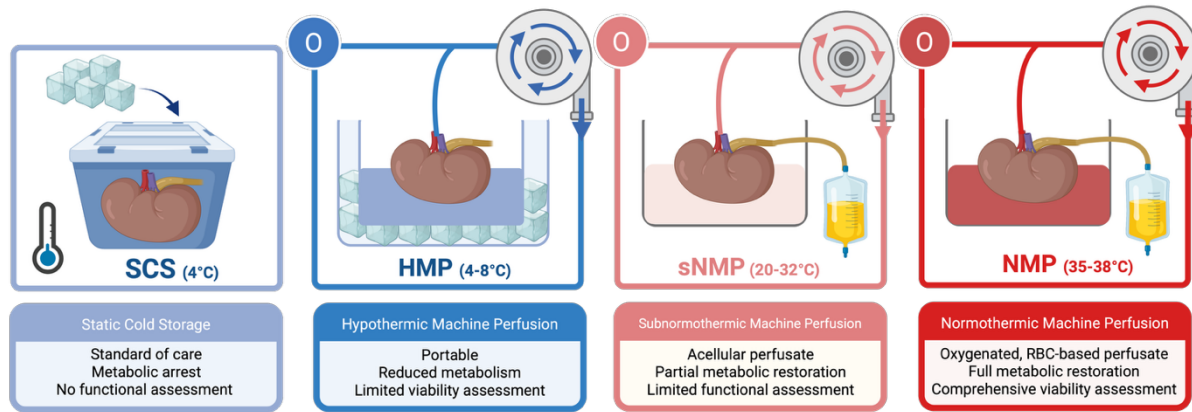


Figure 6. Overview of kidney preservation modalities. From left to right: static cold storage (SCS) at 4°C provides metabolic arrest without active perfusion and remains the current standard of care. Hypothermic machine perfusion (HMP) at 4–8°C adds continuous circulation and enables limited viability assessment. Subnormothermic machine perfusion (sNMP) at 20–32°C partially restores cellular metabolism using an oxygenated acellular perfusate. Normothermic machine perfusion (NMP) at 35–38°C fully restores organ metabolism with an oxygenated RBC-based perfusate, enabling comprehensive viability assessment. O, oxygenation; RBC, red blood cells. Adapted from Van Leeuwen *et al.* (*Front. Med.*, 2022),⁷⁰ modified using Biorender.com.

In practice, NMP requires a dedicated perfusion circuit consisting of a pump, an oxygenator, a reservoir, and a heat exchanger, all working in concert to deliver a continuously oxygenated and temperature-controlled perfusate to the kidney via its renal artery (**Figure 7**). The perfusate — most commonly a red blood cell-based solution supplemented with plasma, nutrients and antibiotics — mimics physiological blood composition as closely as possible, providing the organ with the oxygen and metabolic substrates required to sustain aerobic cellular function. Acellular, synthetic perfusates have also been explored as an alternative, offering greater standardization and logistical simplicity, although their capacity to sustain prolonged normothermic perfusion remains under investigation.^{71,72} Once connected to the circuit, the kidney is progressively rewarmed to 35–38°C, at which point full aerobic metabolism resumes. Mitochondrial respiration is restored, ATP synthesis is reestablished, and the organ transitions from the metabolic suppression of cold storage to active physiological function. This reactivation is evidenced by the resumption of urine production, which represents one of the earliest and most clinically meaningful indicators of organ viability. Perfusion pressure and flow are carefully controlled throughout, typically targeting a renal arterial pressure of 75–85 mmHg. Throughout the perfusion period, the circuit enables continuous monitoring of key functional and biochemical parameters. Urine output, creatinine clearance, electrolyte handling and vascular resistance are collected at regular intervals and interpreted dynamically, providing a real-time physiological portrait of the organ that no static preservation strategy can offer.

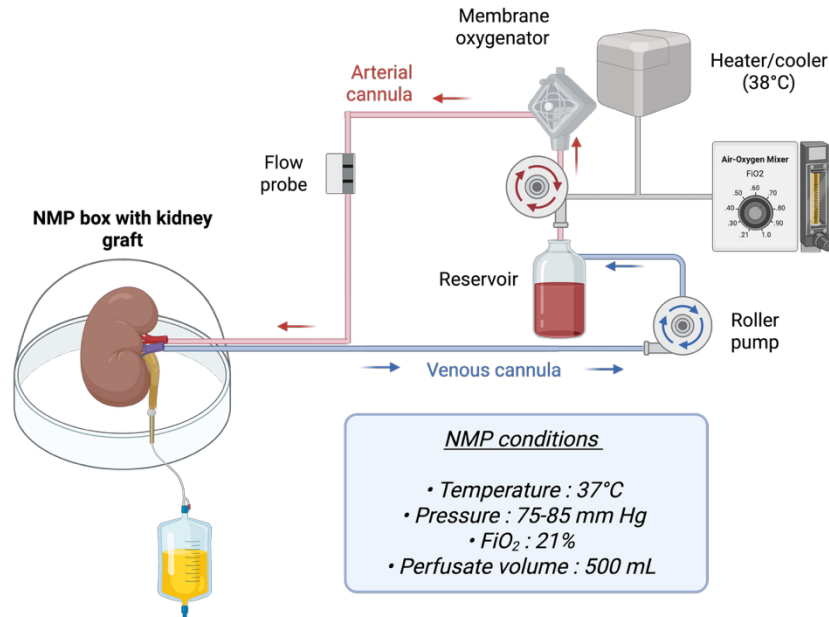


Figure 7. Schematic representation of the normothermic machine perfusion (NMP) circuit. The kidney is placed in a perfusion chamber and connected to the circuit via arterial (red) and venous (blue) cannulas. The blood-based perfusate (500 mL) is actively circulated by a peristaltic pump and oxygenated via an air-oxygen mixer (FiO_2 21%). Perfusion is maintained at 37°C and 75–85 mmHg, mimicking physiological renal perfusion conditions. Urine produced during perfusion is collected for continuous functional monitoring. FiO_2 , fraction of inspired oxygen; NMP, normothermic machine perfusion.

The clinical development of kidney NMP has progressed rapidly, with multiple feasibility studies confirming its safety and logistical practicability. However, despite its biological rationale, RCT data have not yet demonstrated a consistent reduction in DGF rates compared to SCS or HMP, suggesting that the added value of NMP in standard clinical scenarios may be primarily in the domains of assessment and intervention rather than preservation per se.^{40,73,74} Indeed, the most clinically significant application of NMP is viability assessment: functional parameters measured during perfusion — including urine output, creatinine clearance, vascular resistance, and biomarker profiles in the perfusate — allow a dynamic, physiologically grounded evaluation of organ quality that is impossible during cold preservation. This enables more informed decision-making for organs that would otherwise be discarded based on donor characteristics alone.^{75,76} A further frontier of NMP is extended and prolonged preservation. Through recirculation of the produced urine to maintain metabolic homeostasis, perfusion durations of 24 hours and beyond have been achieved with preservation of graft function, opening the possibility of transplanting organs that would previously have been discarded due to logistical time constraints or the need for prolonged assessment.^{77–81}

2. Normothermic Machine Perfusion (NMP)

2.1 — NMP vs. HMP: Physiological Rationale and Comparative Evidence

As detailed in section 1.5.3, HMP achieves its preservation benefit primarily through metabolic suppression, continuous vascular washout, and — in its oxygenated form — partial mitochondrial resuscitation during cold storage. The landmark COMPARE trial, a randomized double-blind paired phase 3 trial conducted by Jochmans *et al.* in 2020, provided the highest-quality evidence to date for the additional benefit of oxygenation during HMP in high-risk grafts.⁵⁷ In this study specifically designed for DCD donors aged 50 years or older, HOPE significantly reduced severe post-transplant complications, improved one-year graft survival, and reduced the relative risk of acute rejection compared to standard non-oxygenated HMP. Renal vascular resistance, continuously monitored during perfusion, reflects microvascular integrity and has been associated with post-transplant outcomes. Soluble markers in the perfusate — including NGAL and FMN released from injured tubular cells — provide molecular correlates of ischemic injury that can inform acceptance decisions, particularly for marginal grafts.⁵⁹ However, none of these parameters constitutes a validated viability threshold with sufficient sensitivity and specificity to support systematic decision-making, and they remain adjunctive tools rather than formal contraindications. The fundamental limitation of HMP — including its oxygenated variant — remains thermodynamic. Structural damage accumulated prior to procurement — whether from prolonged warm ischemia in DCD donors or from donor comorbidity in ECD grafts — cannot be substantially reversed under hypothermic conditions. HMP preserves and partially resuscitates the organ; it cannot functionally evaluate or therapeutically recondition it in any clinically relevant sense.

The comparative landscape of kidney preservation strategies has been substantially clarified by a series of RCT and updated meta-analyses published between 2020 and 2024.^{55,82} The position of NMP in this comparative hierarchy is more nuanced. The largest RCT to date evaluating NMP in kidney transplantation — conducted by Hosgood *et al.* and published in *Nature Medicine* in 2023 — randomized 338 DCD kidneys to conventional SCS or SCS followed by one hour of end-ischemic NMP using an oxygenated red blood cell-based perfusate.⁷⁵ Despite confirming the safety and logistical feasibility of NMP, the trial demonstrated no significant reduction in DGF rates, acute rejection, graft function, or one-year graft survival compared to SCS alone. These findings were attributed to the short duration of perfusion and to the pro-inflammatory properties of red blood cell-based perfusates at normothermia. A subsequent systematic review and meta-analysis incorporating eight NMP studies nonetheless identified a pooled reduction in DGF across all NMP comparisons when longer perfusion durations were included, suggesting a duration-dependent effect. Critically, the same analysis found no significant benefit of short-duration end-ischemic NMP over SCS alone.⁸³

These data collectively support a hierarchical interpretation: continuous HMP is currently the evidence-based standard of preservation for high-risk grafts, while NMP offers additional value specifically as a viability assessment and therapeutic delivery platform.

2.2 — Assessment of Kidney Graft Viability During NMP

2.2.1 — *The unmet need for reliable pre-transplant viability assessment*

The decision to accept or discard a deceased donor kidney for transplantation remains one of the most consequential judgements in clinical transplantation. Current assessment tools encompass donor clinical parameters, pre-implantation biopsy scores, machine perfusion characteristics, and composite risk indices such as the Kidney Donor Profile Index (KDPI). As comprehensively reviewed by Dare *et al.*, none of these tools individually provides sufficient predictive power to reliably distinguish a viable but morphologically compromised graft from a truly non-functional one.⁸⁴ Machine perfusion parameters during HMP — renal resistance, flow, and perfusate biomarkers — correlate with post-transplant outcomes at a group level. But there is a lack of sensitivity and specificity to support individual discard decisions, as demonstrated in large registry analyses in which most recipients of kidneys with "undesirable" perfusion parameters, still achieved acceptable graft function.^{51,56} The consequence of this diagnostic uncertainty is twofold: viable organs are discarded out of caution, while some suboptimal organs are transplanted with poor outcomes — both failures that a reliable viability assessment platform could substantially reduce. NMP addresses this unmet need by restoring near-physiological conditions in the isolated organ, enabling real-time measurement of functional parameters that are simply inaccessible during cold preservation. By re-establishing active metabolism, urine production, and vascular reactivity, NMP transforms the preservation period into a dynamic, standardized test of organ function (**Figure 8**).^{67,83,85}

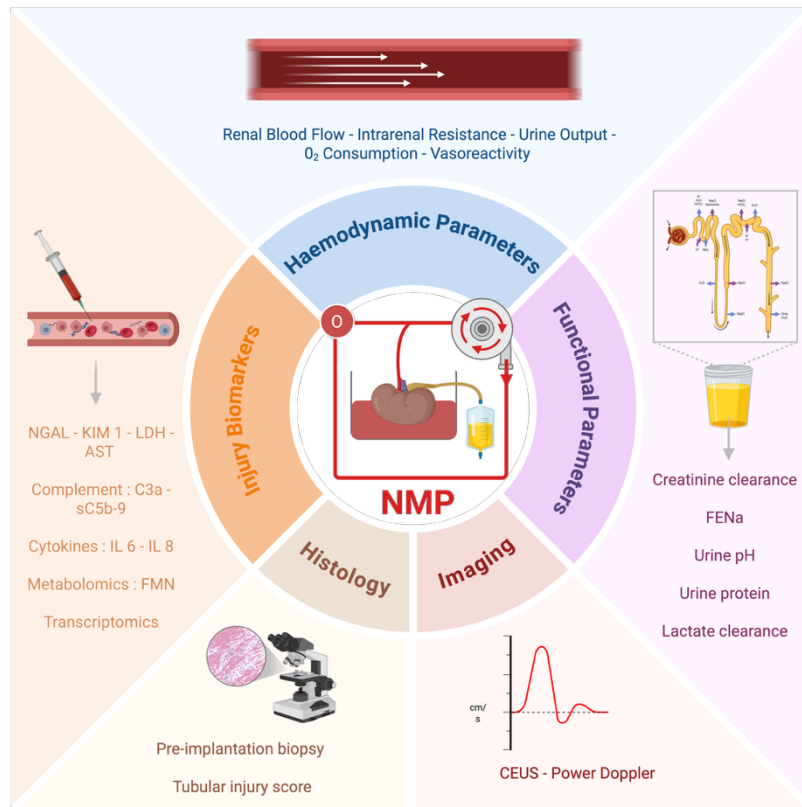


Figure 8. Multiparametric viability assessment of kidney grafts during NMP. During NMP at 35–37°C, kidney graft quality is evaluated through four complementary categories of parameters: haemodynamic parameters, injury biomarkers, functional parameters and morphological/imaging assessment. Integration of these parameters informs the viability decision prior to transplantation. AST, aspartate aminotransferase; CEUS, contrast-enhanced ultrasound; FENa, fractional excretion of sodium; FMN, flavin mononucleotide; KIM-1, kidney injury molecule-1; LDH, lactate dehydrogenase; NGAL, neutrophil gelatinase-associated lipocalin; NMP, normothermic machine perfusion. Created with BioRender.com.

2.2.2 — Hemodynamic and Functional Parameters

Among the parameters evaluated during NMP, renal blood flow and intrarenal vascular resistance are the most immediately accessible and clinically interpretable.⁸⁶ A low vascular resistance and adequate arterial flow reflect preserved microvascular integrity and endothelial function — early indicators of a graft with functional vascular architecture capable of supporting post-transplant perfusion. Conversely, persistently high resistance may indicate microvascular obstruction, thrombosis, or irreversible injury, and has been incorporated as a primary component of viability scoring systems.^{67,87} Urine production during NMP is a direct indicator of active tubular function and has been a central element of the Hosgood-Nicholson scoring system — a practical viability tool that combines flow, urine output, and macroscopic appearance into a reproducible assessment score applicable within the first hour of normothermic perfusion.⁸⁷

Creatinine clearance calculated from simultaneous perfusate and urine creatinine concentrations provides a quantitative estimate of glomerular filtration activity during NMP, offering a more refined evaluation of function than urine output alone. Fractional excretion of sodium (FENa) complements this assessment by reflecting tubular reabsorptive capacity — a function that is among the first to be lost in severe ischemic injury.⁸⁸ The vascular response to physiological stimuli during NMP — vasoreactivity — provides an additional window into endothelial viability and the integrity of pressure-autoregulation mechanisms. Power Doppler ultrasound and contrast-enhanced ultrasound (CEUS) have been explored as adjunctive imaging tools to characterize microvascular perfusion distribution across the renal parenchyma during NMP. This enables spatial assessment of cortical and medullary perfusion heterogeneity that may not be captured by global flow measurements alone.^{89,90}

2.2.3 — Urinary and Perfusate Biomarkers

The urine produced during NMP constitutes a uniquely informative biofluid — reflecting active tubular secretion, glomerular filtration, and renal metabolic activity in real time. Urinary NGAL, is among the most extensively studied NMP biomarkers. In a series of 56 declined human kidneys undergoing *ex vivo* normothermic perfusion, Hosgood *et al.* demonstrated that urinary NGAL — combined with functional perfusion parameters and macroscopic appearance — provided informative assessment of kidney quality, supporting its use as an adjunct to functional parameters.⁸⁷ KIM-1 has also been measured in NMP perfusate and urine, and his level at 6 hours of NMP correlate with donor function parameters.⁸⁸ Beyond classical injury markers, the perfusate composition during NMP provides a window into the immunological and inflammatory state of the organ. Pro-inflammatory cytokines — including TNF- α , IL-6, IL-8, and IL-1 β — are measurable in the perfusate and reflect the ongoing innate immune activation within the graft.^{88,91} Complement activation, a central effector mechanism of ischemia-reperfusion injury, has been documented during both porcine and human kidney NMP: perfusate levels of C3a, C3d, and soluble C5b-9 increase progressively during perfusion and correlate with cytokine levels and with impaired creatinine clearance during porcine NMP. This suggests that complement monitoring could serve both as a viability indicator and as a therapeutic target for complement-directed interventions.⁹² Perfusate proteomic and metabolomic profiling has also identified broader molecular fingerprints of ischemic injury, donor organ quality, and early inflammatory responses, with complement pathway elements, coagulation proteins, and damage-associated molecular patterns (DAMPs) emerging as candidate biomarkers from LC-MS-based analyses.^{93,94} Transcriptomic analyses of kidneys before and after NMP have revealed a pro-inflammatory cellular landscape dominated by activation of innate immune pathways, including upregulation of NF- κ B targets, interferon-stimulated genes, and damage-associated pattern recognition receptor (PRR) signalling. These findings could explain the immunological consequences of transplantation and identify potential targets for therapeutic intervention during the perfusion window.^{95,96}

2.3 — Protocol optimization in kidney NMP

Despite the growing clinical interest in NMP, a fundamental limitation persists: no consensus protocol exists. The composition of the perfusate, the choice of oxygen carrier, the temperature of perfusion, the management of the transition from cold storage, and the target perfusion parameters all vary substantially across centres and studies — making cross-study comparison of outcomes and biomarker thresholds largely unreliable.^{71,97,98}

The dominant clinical approach uses leukocyte-depleted packed red blood cells (RBC) suspended in a nutrient-enriched base medium — typically Ringer's lactate or Williams' Medium E — supplemented with albumin, mannitol, heparin, sodium bicarbonate, and vasoactive agents.^{97,99} This RBC-based approach provides physiological oxygen delivery via haemoglobin and supports metabolic activity over short perfusion periods. However, it carries inherent logistical constraints — RBC crossmatching, availability, storage effects. Progressive haemolysis during perfusion generates free haemoglobin, prooxidative changes, and iron-driven ferroptosis that may offset the preservation benefit.^{100,101} The impact of RBC characteristics on NMP outcomes has been specifically examined by Venema *et al.* in a porcine model, demonstrating that RBC-related variables independently affect kidney function and injury during perfusion, independent of ischaemic history.¹⁰² Whole blood-based perfusion has been explored as an alternative offering a broader complement of plasma proteins and coagulation factors, but introduces additional inflammatory complexity through activated leukocytes and platelets, and has been found to be unsustainable beyond approximately five hours.⁹⁷ Acellular perfusates represent an increasingly attractive alternative. Studies using Williams' Medium E without oxygen carriers have demonstrated adequate short-term viability assessment in discarded human kidneys, with comparable functional parameters and lower logistical burden.¹⁰³ The four-day cell-free preservation protocol by de Haan *et al.* further establishes the superiority of acellular sNMP for prolonged preservation, negating both haemolysis and ferroptosis entirely.⁷⁹

The transition from cold preservation to normothermic perfusion represents an underappreciated source of additional injury. Porcine studies comparing standard cold preflush NMP to direct NMP without prior cold flush have demonstrated that the preflush itself aggravates ischemic injury, resulting in inferior creatinine clearance, higher urinary NGAL, and greater osmotic nephropathy in the preflushed group.¹⁰⁴ Perfusion pressure and flow targets vary between protocols and devices, with most clinical reports using a mean arterial pressure of 75–80 mmHg in a pressure-controlled mode. The choice between pressure-driven and flow-driven perfusion affects the shear stress exerted on the endothelium and the distribution of microvascular perfusion, but rigorous comparative data in human kidneys remain sparse. The absence of a consensus NMP protocol remains a major obstacle to cross-centre comparability of viability criteria and therapeutic outcomes.

2.4 — Clinical Studies and RCT

The first clinical application of *ex vivo* normothermic perfusion to a human kidney was reported by Hosgood and Nicholson in 2011 — a landmark case in which a kidney from a 62-year-old ECD donor was perfused at sub-normothermic temperature (mean 33.9°C) for 35 minutes using a red blood cell-based plasma-free solution immediately prior to implantation, following 11 hours of static cold storage.¹⁰⁵ The same group subsequently published the first formal clinical series in 2013, reporting outcomes of 18 ECD-DBD kidneys undergoing *ex vivo* normothermic perfusion immediately before transplantation — demonstrating a significantly lower DGF rate compared to static cold storage (5.6% vs 36.2%, $p=0.014$).¹⁰⁶ Building on this foundation, Hosgood *et al.* extended the concept to declined DCD kidneys in 2018, introducing a quality assessment score based on macroscopic appearance, renal blood flow, and urine output during NMP — demonstrating the potential to rescue organs that would otherwise have been discarded.¹⁰⁷ The Cambridge-Leicester group progressively scaled the technique to include longer perfusion durations and transplantation of severely declined kidneys deemed untransplantable by conventional assessment, establishing proof of concept for NMP as both a rescue and evaluation tool.

The first North American results of clinical kidney NMP were reported by Mazilescu *et al.* in 2022, confirming international reproducibility of the technique and supporting its broader adoption across transplant programs with different logistical and regulatory frameworks.⁷⁶ A subsequent phase 1 cohort study (NKP1) including 36 patients demonstrated the safety and feasibility of up to 24 hours of NMP prior to transplantation, with 100% 30-day graft survival and one-year eGFR comparable to a matched historical control cohort despite significantly longer total preservation times.⁷³

The largest RCT (Hosgood *et al.*, *Nature Medicine*, 2023) confirmed safety but found no DGF benefit with one hour of end-ischemic NMP, with negative results attributed to short duration and RBC-based perfusate toxicity.⁷⁵ Pooled meta-analysis data suggest a duration-dependent DGF benefit, and single-centre feasibility series including NEXT-Kidney have confirmed logistical practicability — collectively positioning NMP as an assessment and therapeutic platform rather than a brief terminal preservation step.^{74,83}

The critical unanswered question — whether NMP is superior, equivalent, or inferior to HMP in high-risk grafts — is being directly addressed by the ongoing Erasmus MC RCT (NCT04882254), which randomizes ECD-DBD and DCD kidneys to HMP alone or HMP followed by 120 minutes of end-ischemic NMP, with immediate graft function as the primary endpoint.¹⁰⁸ This trial will provide the first head-to-head evidence between the two active perfusion modalities. An overview of the published clinical experience with kidney NMP — from first-in-man feasibility to randomized trials — is provided in **Table 1**.

Table 1. Key clinical studies and randomized controlled trials of NMP in kidney transplantation.

Study	Design	n	Donor type	NMP duration	Primary endpoint	Key results
Hosgood & Nicholson, 2011. ¹⁰⁵	Case report	1	ECD-DBD	35 min (33.9°C)	Feasibility	First-in-man NMP; single kidney (donor age 62) perfused after 11h SCS; successful transplantation
Nicholson & Hosgood, 2013. ¹⁰⁶	Prospective cohort	18	ECD-DBD	63 ± 16 min (34.6°C)	DGF vs SCS control	First clinical series; DGF 5.6% (NMP) vs 36.2% (SCS), p=0.014; all grafts successfully transplanted
Hosgood & Nicholson, 2018. ¹⁰⁷	Prospective 2-phases cohort	Phase 1– 42 Phase 2– 10	Declined DCD	~60 min	Organ rescue / quality assessment score (QAS)	NMP used to assess declined DCD kidneys; QAS based on macroscopic appearance, RBF and urine output; 5/10 declined kidneys successfully transplanted in phase 2
Mazilescu <i>et al.</i> , 2022. ⁷⁶	Prospective cohort	13	DBD (n=6) DCD (n=7)	171 min (44–275 min)	Feasibility · safety	First North American results; DGF 30.8% vs 46.2% (HMP control, p=0.51); no difference in 1-year graft function or survival
Hosgood <i>et al.</i> , 2023. ⁷⁵	Randomized controlled trial	338 (168 SCS / 170 NMP)	DCD	1h end-ischemic	DGF	Safety confirmed; no DGF benefit (NMP 38.2% vs SCS 40.6%, p=0.65)
Hameed <i>et al.</i> , 2025. ⁷⁴ (NEXT-Kidney)	Single-centre feasibility study	—	DCD	Variable	Logistical feasibility	Confirmed clinical practicability; supports NMP as assessment and therapeutic platform rather than brief preservation step
Dumbill <i>et al.</i> , 2025. ⁷³	Phase 1 cohort	36	DBD DCD	Up to 24h	Safety · 30-day graft survival	100% 30-day graft survival; 12-month eGFR 46.3 vs 49.5 mL/min/1.73m ² (p=0.44) despite longer preservation (15.7 vs 8.9h, p<0.0001)
Rijkse <i>et al.</i> , ongoing ¹⁰⁸ (NCT04882254)	RCT	—	ECD-DBD DCD	120 min end-ischemic (after HMP)	Immediate graft function	First head-to-head RCT: HMP alone vs HMP + NMP; results pending

DGF = delayed graft function; ECD = extended criteria donor; DBD = donation after brain death; DCD = donation after circulatory death; eGFR = estimated glomerular filtration rate; HMP = hypothermic machine perfusion; NMP = normothermic machine perfusion; Ph = phase; QAS = quality assessment score; RBC = red blood cells; RBF = renal blood flow; RCT = randomized controlled trial; SCS = static cold storage. Ongoing trial highlighted in yellow — results pending.

3. Mesenchymal Stromal Cells

3.1 — Definition, Characterization, and Production of MSC

3.1.1 — Defining Mesenchymal Stromal Cell

Mesenchymal stromal cells (MSC) were first isolated from bone marrow by Friedenstein in the 1970s as plastic-adherent, fibroblast-like colony-forming cells. They have since become one of the most widely studied experimental cell therapy platforms globally, with over 1,500 registered clinical trials. The fundamental challenge in the field has long been the absence of a universally accepted and consistently applied definition. In 2006, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cell and Gene Therapy (ISCT) proposed a set of minimal criteria intended to standardize MSC identification across laboratories and institutions.¹⁰⁹ These three core criteria require that MSC: (i) adhere to plastic under standard culture conditions; (ii) express CD73, CD90, and CD105 at $\geq 95\%$ of the cell population, while being negative ($\leq 2\%$) for hematopoietic and endothelial surface markers — including CD45, CD34, CD14 or CD11b, CD79 α or CD19, and HLA-DR; and (iii) demonstrate trilineage mesenchymal differentiation potential *in vitro*, specifically toward osteogenic, adipogenic, and chondrogenic lineages under appropriate inductive conditions (**Figure 9**). Nearly two decades later, the ISCT criteria remain widely used but are increasingly recognized as insufficient to capture the full biological complexity of MSC preparations, or to predict their therapeutic efficacy. MSC constitute a heterogeneous population with vastly different functional and immunomodulatory properties — a problem of consequence in the clinical translation of MSC therapies. A scoping review found that only 18% of a sample of 318 preclinical and clinical MSC publications explicitly referred to the ISCT minimal criteria. Viability assays were reported in only 18% of studies; and only 20% included at least one functional assay.¹¹⁰ Clinical studies showed particularly inconsistent reporting of characterization and manufacturing details. This finding helps to explain the heterogeneity of outcomes across MSC clinical trials and underlines the urgent need for updated consensus definitions and reporting standards.

3.1.2 — Sources and Tissue Origin

MSC can be isolated from any vascularized tissue, but the most clinically relevant sources differ substantially in their abundance, accessibility, and functional properties (**Figure 9**).^{111,112} Bone marrow-derived MSC (BM-MSC) represent the historical reference source: they were the first to be described, the most extensively characterized, and remain the primary source used in clinical trials worldwide, including in the solid organ transplantation context. Their isolation requires an invasive bone marrow aspiration procedure typically yielding a low frequency of MSC precursors — necessitating extensive *ex vivo* expansion to reach therapeutic doses.

Adipose tissue-derived MSC (A-MSC) offer a practical alternative: adipose tissue is available in large quantities from liposuction procedures, yields substantially higher numbers of stromal vascular fraction cells per unit volume than bone marrow, and produces MSC with comparable immunophenotypic and functional profiles to BM-MSC. Some source-dependent differences have been demonstrated in differentiation and secretory potential. Umbilical cord-derived MSC (U-MSC) represent a neonatal source with several immunological advantages: they are isolated from perinatal discard tissue without donor risk, express lower levels of HLA class I and are consistently HLA class II negative, proliferate more rapidly, and can be expanded to very large cell numbers from a single donor. Source-dependent differences in immunomodulatory potency, trophic factor secretion profiles, and responses to inflammatory priming have been documented in comparative studies, though no single source has demonstrated consistent clinical superiority.^{113,114}

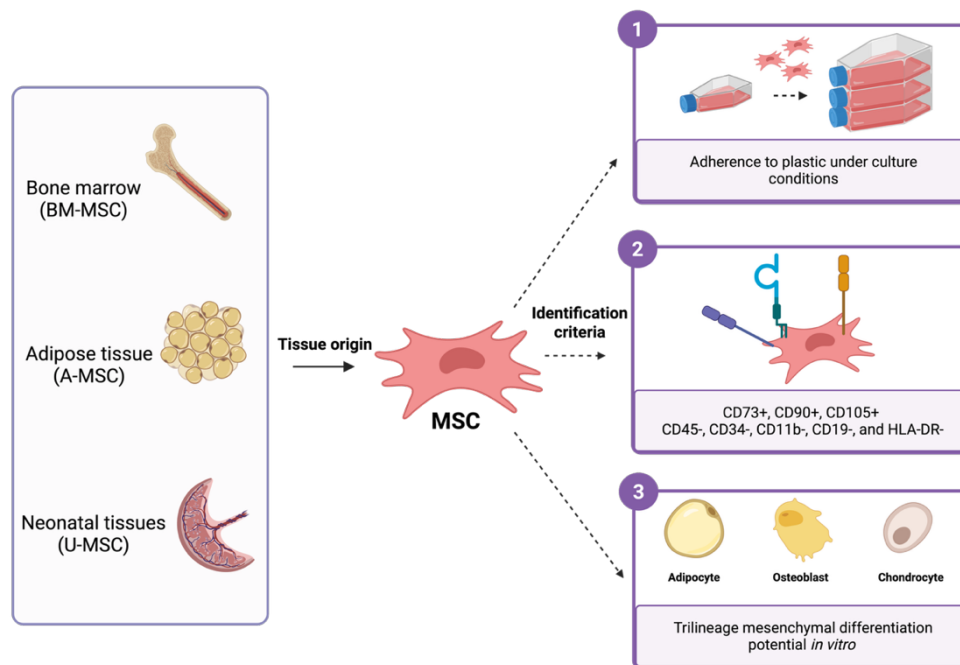


Figure 9. ISCT identification criteria for MSCs and tissue origins. MSCs can be isolated from multiple tissue sources, including bone marrow (BM-MSC), adipose tissue (A-MSC), and neonatal tissues (U-MSC). Regardless of their origin, MSCs must fulfil three minimum criteria as defined by the ISCT: (1) plastic adherence under standard culture conditions; (2) expression of surface markers CD73, CD90, and CD105, with absence of CD45, CD34, CD11b, CD19, and HLA-DR; and (3) capacity for trilineage mesenchymal differentiation into adipocytes, osteoblasts, and chondrocytes *in vitro*. Adapted from Zhou et al. (*Cell & Molecular Immunology*, 2023),¹¹⁵ modified using Biorender.com.

3.1.3 — Clinical-Grade Production and Banking

The translation of MSC therapy from experimental models to clinical use requires robust, reproducible, good manufacturing practices (GMP) capable of generating large numbers of functionally validated cells.

Starting from bone marrow aspirates, the standard clinical-grade expansion protocol involves density gradient isolation of mononuclear cells, seeding in culture flasks under controlled atmospheric conditions, serial passaging over approximately 4 weeks, and final harvest at low passage numbers to limit senescence and phenotypic drift. Mean yields from single donor cultures can reach several hundred million cells, enabling production of multiple aliquots for subsequent thawing and infusion.¹¹⁶ Cryopreservation is an essential enabler of allogeneic off-the-shelf MSC banking, allowing stockpiling of tested, validated, ready-to-use cell products that can be deployed without the delay of fresh culture. However, cryopreservation — typically in DMSO-based cryoprotectants at -80°C or in liquid nitrogen — is associated with reductions in post-thaw viability, adhesion capacity, and short-term immunomodulatory function. The Laboratory of Cell and Gene Therapy (LTCG) at the CHU of Liège has reported one of the largest academic allogeneic MSC banking experiences in Europe. Over 68 clinical-grade large-scale MSC cultures were completed over 7 years, with 464 cryopreserved aliquots stored in liquid nitrogen and 314 bags released and infused across 6 clinical protocols with no infusion-related toxicity.¹¹⁶ This experience has since evolved toward full GMP compliance, as MSC have been reclassified as Advanced Therapy Medicinal Products (ATMPs) under European regulatory frameworks. This designation imposes stringent requirements on manufacturing premises, quality control systems, batch release procedures, and post-market traceability.¹¹⁷

3.2 — Immunomodulatory Mechanisms of Action of MSC

3.2.1 — Overview

Early studies of MSC therapy were guided by the hypothesis that their therapeutic effects derived primarily from their capacity to engraft at sites of injury and differentiate into functional tissue-replacing cells. This model was rapidly challenged by the observation that infused MSC have a very short biological half-life and yet exert potent and durable immunomodulatory effects that outlast their physical presence by days to weeks.¹¹⁸ This paradox redirected the field toward a paracrine paradigm: MSC therapeutic effects are now understood to be mediated predominantly by the bioactive molecules they secrete in response to signals from the local microenvironment. This secretome is a remarkably diverse and context-dependent repertoire of soluble mediators, including cytokines, growth factors, lipid mediators, and extracellular vesicles (EVs). Key immunomodulatory molecules constitutively or inducibly secreted by MSC include prostaglandin E2 (PGE-2), transforming growth factor- β (TGF- β), hepatocyte growth factor (HGF), interleukin-10 (IL-10), interleukin-1 receptor antagonist (IL-1RA), indoleamine 2,3-dioxygenase (IDO), tumour necrosis factor-stimulated gene 6 protein (TSG-6), and programmed death ligand 1 (PD-L1), among many others.^{119,120} A critical feature of MSC biology is that quiescent MSC express most of these immunomodulatory mediators at very low or undetectable levels.

Immunosuppressive function must be actively induced — a process termed "licensing" or "priming" — through exposure to inflammatory signals in the local microenvironment. The principal licensing stimuli are Interferon- γ (IFN- γ) and Tumor necrosis factor- α (TNF- α), which activate the JAK-STAT1 and Nuclear Factor-kappa B (NF- κ B) pathways respectively, driving the upregulation of IDO, PGE2, PD-L1, and TSG-6. IL-1 β activates an overlapping but distinct programme via NF- κ B, inducing cyclooxygenase-2 (COX-2), TSG-6, and interleukin-6 (IL-6). This context-dependency is therapeutically important: MSC infused into a non-inflammatory environment may be largely inert, whereas the same cells delivered into a tissue with active IRI encounter precisely the inflammatory cues needed to license their full immunomodulatory repertoire (**Figure 10**).¹²¹

3.2.2 — Effects on Adaptive and Innate Immunity

MSC interact with every arm of the immune system, acting simultaneously on both adaptive and innate effectors to shift the immunological balance from inflammation toward tolerance.¹¹⁹ MSC suppress T cell proliferation and activation through IDO-mediated tryptophan depletion, PGE2, HGF, and TGF- β , inhibiting Th1 and Th17 polarization while simultaneously promoting Treg expansion. B cells, dendritic cells (DC), macrophages, and natural killer cells (NK) are all modulated through overlapping paracrine pathways: MSC induce a tolerogenic phenotype in DC — characterised by reduced co-stimulatory molecule expression and enhanced IL-10 secretion, favouring T cell anergy and Treg induction over effector activation — drive the M1→M2 macrophage shift via PGE2 and TSG-6, and inhibit NK cytotoxicity via HLA-G and IDO (**Figure 10**). A key conceptual point is that immunomodulatory effects are not restricted to viable MSC: apoptotic and heat-inactivated MSC retain significant activity through efferocytosis by monocytes, which are themselves reprogrammed to an anti-inflammatory phenotype.^{118,122}

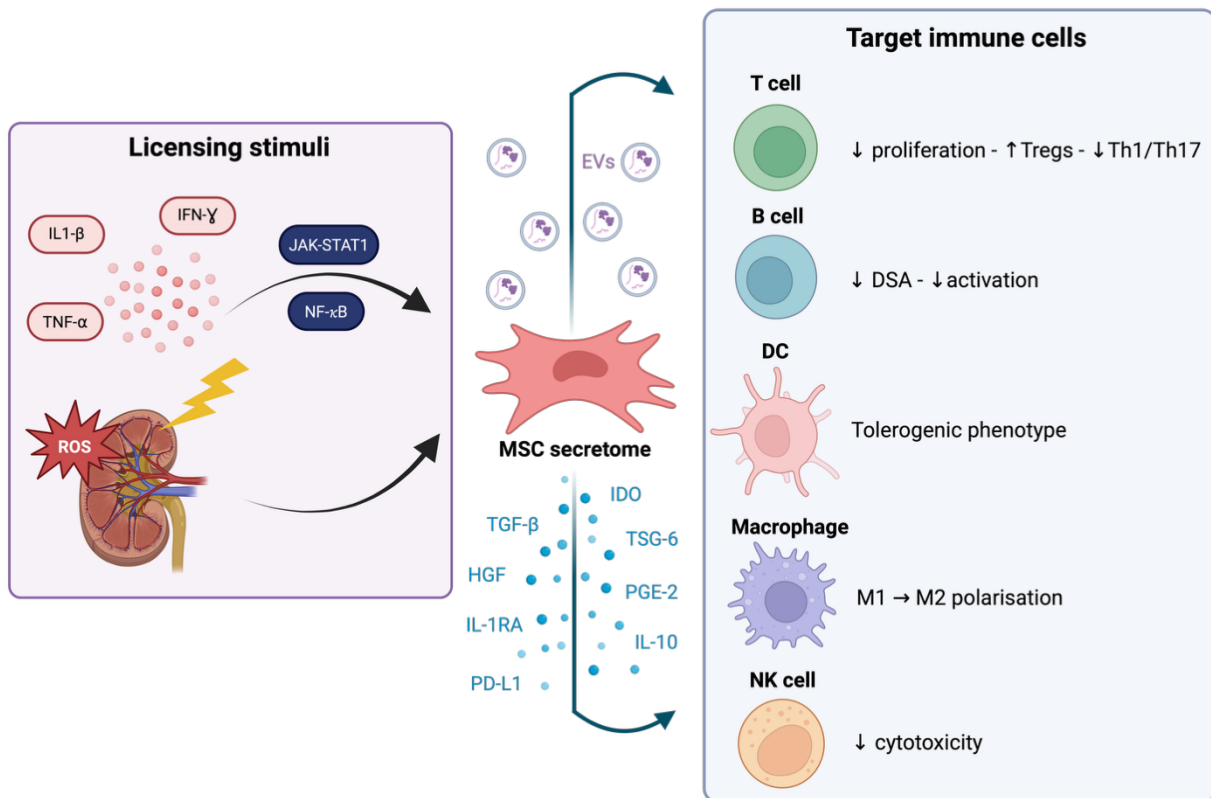


Figure 10. Immunomodulatory mechanisms of MSCs in the context of kidney transplantation. Licensing of MSCs by IRI-derived signals (ROS, IFN- γ , TNF- α , IL-1 β) via JAK-STAT1 and NF- κ B pathways drives the secretion of immunomodulatory mediators and EVs, which collectively suppress T and B cell activation, promote Treg expansion, induce tolerogenic DC, drive M1→M2 macrophage polarisation, and inhibit NK cell cytotoxicity. DSA, donor-specific antibody; DC, dendritic cell; EVs, extracellular vesicles; HGF, hepatocyte growth factor; IDO, indoleamine 2,3-dioxygenase; IFN- γ , interferon-gamma; IL-1 β , interleukin-1 beta; IL-1RA, interleukin-1 receptor antagonist; JAK-STAT1, Janus kinase – signal transducer and activator of transcription 1; MSC, mesenchymal stromal cell; NF- κ B, nuclear factor kappa B; NK, natural killer; PD-L1, programmed death-ligand 1; PGE-2, prostaglandin E2; ROS, reactive oxygen species; TGF- β , transforming growth factor-beta; TNF- α , tumour necrosis factor-alpha; Treg, regulatory T cell; TSG-6, tumour necrosis factor-stimulated gene 6 protein. Adapted from Giacomini *et al.* (*Cell & Molecular Immunology*, 2023),¹²¹ modified using Biorender.com.

3.2.3 — Reparative and Regenerative Mechanisms

Beyond immunomodulation, MSC exert direct reparative effects through trophic signalling — particularly relevant in IRI, where endothelial damage and microvascular dysfunction are the primary pathological targets. Sierra-Parraga *et al.* demonstrated that MSC co-cultured with hypoxia-injured endothelial cells improved wound healing by 45% and fully restored angiogenic tube formation with MSC actively migrating toward injured endothelium via CD29/CD44 interactions.¹²³ Pro-angiogenic factors constitutively secreted by MSC — vascular endothelial growth factor (VEGF), HGF, and basic fibroblast growth factor (bFGF) — further support microvascular repair and cortical reperfusion after transplantation.¹¹³

EVs (Extracellular vesicles) recapitulate many of these effects through microRNA and protein cargo delivery to endothelial, tubular, and immune cells, and represent a promising cell-free therapeutic strategy. While both physical contact and paracrine secretion contribute to MSC reparative effects, the majority can be reproduced by conditioned medium or EVs alone — establishing paracrine signalling as the dominant modality.^{119,123}

3.3 — MSC in Kidney Transplantation

3.3.1 — Preclinical studies

The use of MSC therapy in kidney transplantation required systematic preclinical evaluation of delivery route, timing, dose, and mechanism of action in validated transplantation models.¹²⁴ Foundational evidence for MSC efficacy in renal IRI was established in rodent models with the study of Erpicum *et al.* which demonstrated in a rat bilateral renal clamping model that pre-ischaemic intravenous administration of BM-MSC significantly attenuated kidney injury and modulated renal lipid metabolism, an emerging pathway in IRI pathophysiology.¹²⁵ A few years later, intra-arterial delivery was identified as the preferred route for achieving renal-specific MSC delivery, as it bypasses the pulmonary first-pass effect and concentrates cells at the site of injury. Pool *et al.* first demonstrated in 2019 that infusing MSC into porcine kidneys during *ex vivo* normothermic machine perfusion is technically feasible, with intact MSC detectable in renal cortex after perfusion by immunofluorescence and magnetic resonance imaging (MRI). MSC were predominantly localising to a subset of glomeruli, consistent with the known retention pattern of intra-arterially delivered cells in the perivascular space.¹²⁶ These findings confirmed that NMP provides a viable platform for cellular delivery and that infused cells survive the perfusion circuit and engraft in target renal structures. Sierra-Parraga *et al.* further demonstrated that MSC retained in the renal cortex after intra-arterial infusion maintain their metabolic and immunomodulatory phenotype independently of their metabolic state, providing mechanistic support for the therapeutic relevance of this delivery strategy.¹²⁷ The question of what MSC dose can be safely delivered intra-arterially to the kidney was directly addressed by Munk *et al.* in a dedicated porcine dose-escalation study using MRI-based renal hemodynamic monitoring. Intra-renal arterial infusion of 10×10^6 MSC had no effect on cortical or medullary perfusion, single-kidney GFR, or histology at 14 days. By contrast, infusion of 100×10^6 MSC caused immediate, severe reduction in cortical and medullary perfusion, associated with glomerular microthrombi and signs of an instant blood-mediated inflammatory reaction.¹²⁸ These findings establish 10×10^6 MSC as a safe threshold for intra-renal arterial delivery in the porcine model, defining the practical upper dose boundary for clinical translation.

3.3.2 — Clinical studies

The first clinical application of MSC in kidney transplantation was reported in 2011. In a first pilot, two living-donor kidney transplant recipients received autologous BM-MSC intravenously seven days post-transplant as induction therapy in place of conventional immunosuppression.¹²⁹ Both patients experienced transient acute renal insufficiency attributable to engraftment syndrome — a systemic inflammatory response to MSC lodging in the pulmonary circulation. Based on this finding, the protocol was amended in 2013 to pre-transplant infusion (day –1), and two subsequent patients avoided this complication while exhibiting a pro-tolerogenic immunological profile with expanded circulating Treg and suppressed memory CD8+ T cells.¹³⁰ Long-term follow-up of all four patients confirmed stable graft function over 5–7 years without increased susceptibility to infection or neoplasm, and documented persistent suppression of donor-specific cytotoxicity in three of four patients.¹³¹ In one patient followed over a decade, complete and stable immunological tolerance was achieved, allowing full withdrawal of maintenance immunosuppression — the first such case reported in kidney transplantation treated with remote MSC infusion.¹³²

The pivotal kidney transplantation study reported by Erpicum *et al.* in 2019 concern a phase I–II, open-label, single-centre clinical study in which ten deceased-donor kidney transplant recipients (DBD and DCD) received a single intravenous infusion of third-party allogeneic BM-MSC on day 3 post-transplantation. The intervention was safe and well-tolerated. Compared to historical controls, MSC-treated patients showed improved eGFR trajectories in the first year post-transplant alongside an increased proportion of circulating regulatory T cells — consistent with the pro-tolerogenic immunological shift anticipated from the preclinical evidence.¹³³ These findings represented the first demonstration of safety and immunomodulatory efficacy of allogeneic third-party MSC infusion in deceased-donor kidney transplantation. Beyond the studies already described, the randomized controlled trial by Tan *et al.* demonstrated in 159 living-donor kidney transplant recipients that autologous MSC used as induction therapy reduced acute rejection rates and improved one-year eGFR compared to anti-IL-2R antibody, though these results from a single Chinese centre have not been independently replicated.¹³⁴ The Neptune study further confirmed the safety of HLA-selected allogeneic MSC infused six months post-transplant at reduced tacrolimus exposure, with no acute rejection or graft loss at one year.¹³⁵ The preclinical and clinical evidence base for MSC therapy in kidney transplantation — from rodent IRI models to randomized trials — is summarised in **Table 2**.

Table 2. Overview of key preclinical and clinical studies of MSC therapy in kidney transplantation.

Study	Design	Model / population	n	MSC source · dose · route	Primary endpoint	Key results
Preclinical studies						
Erpicum <i>et al.</i> , 2017. ¹²⁵	Experimental IRI model	Rat (bilateral renal clamping)	35	BM-MSC; IV pre-ischaemic administration	Renal function · tubular injury · lipid metabolism	Pre-ischaemic MSC reduced tubular apoptosis and neutrophil infiltration; modulated renal lipid metabolism
Pool <i>et al.</i> , 2019. ¹²⁶	<i>Ex vivo</i> perfusion study	Porcine	12 A-MSC 10 BM-MSC	A-MSC / BM-MSC; 10 ⁵ –10 ⁷ cells; intra-arterial during NMP	Feasibility · MSC localisation	Intact MSCs detectable by IF and MRI in glomerular capillaries after NMP; only in 10 ⁷ group; circulating MSCs declined steeply to ~10% during perfusion
Sierra-Parraga <i>et al.</i> , 2019. ¹²⁷	<i>Ex vivo</i> NMP study	Porcine	—	BM-MSC; intra-arterial	MSC phenotype under NMP conditions	MSC retained in renal cortex maintain metabolic and immunomodulatory phenotype independently of metabolic state
Munk <i>et al.</i> , 2022. ¹²⁸	Porcine dose-escalation study	Porcine	n=6/group	Allogeneic A-MSC; 10×10 ⁶ vs 100×10 ⁶ cells; intra-renal arterial infusion	Safety · renal perfusion · skGFR · histology	10×10⁶ MSC: safe, no hemodynamic change, normal skGFR and histology at 14 days 100×10⁶ MSC: immediate severe ↓ cortical/medullary perfusion, ↓ skGFR and tissue inflammation at 14 days
Clinical studies						
Perico <i>et al.</i> , 2011. ¹²⁹	Pilot study (phase I)	Living-donor KTx recipients	2	Autologous BM-MSC; IV day +7 post-Tx	Safety · feasibility	Both patients developed engraftment syndrome (pulmonary first pass)
Perico <i>et al.</i> , 2013. ¹³⁰	Pilot study (amended protocol)	Living-donor KTx recipients	2	Autologous BM-MSC; IV day -1 pre-Tx	Safety · immunological profile	No engraftment syndrome; expanded circulating Tregs and suppressed memory CD8+ T cells
Perico <i>et al.</i> , 2018. ¹³¹	Long-term follow-up (5–7 years)	Living-donor KTx recipients	4	Autologous BM-MSC	Long-term graft function · immune monitoring	Stable graft function at 5–7 years; no infection or neoplasm excess; persistent donor-specific cytotoxicity suppression in 3/4 patients

Chapter 1. General Introduction & Aims

Study	Design	Model / population	n	MSC source · dose · route	Primary endpoint	Key results
Casiraghi <i>et al.</i> , 2020. ¹³²	Case report / long-term follow-up	Living-donor KTx recipient	1	Autologous BM-MSC; IV pre-Tx	Tolerance induction	First reported case of complete, stable operational tolerance with full IS withdrawal after remote MSC infusion in kidney transplantation
Tan <i>et al.</i> , 2012. ¹³⁴	RCT	Living-donor KTx recipients	159	Autologous MSC; IV induction therapy vs anti-IL-2R antibody	Acute rejection · 1-year eGFR	Lower acute rejection and improved 1-year eGFR vs anti-IL-2R antibody
Epicum <i>et al.</i> , 2019. ¹³³	Phase I–II open-label study	Deceased-donor KTx recipients	10	Third-party allogeneic BM-MSC; IV day +3 post-Tx	Safety · eGFR · Treg expansion	Safe and well-tolerated; improved eGFR trajectory vs historical controls; expanded circulating Tregs
Dreyer <i>et al.</i> , 2020. ¹³⁵	Phase Ib non-randomized trial	Deceased-donor KTx recipients	10	HLA-selected allogeneic MSC; 2×1.5×10 ⁶ /kg IV; 6 months post-Tx + reduced tacrolimus	Safety · BPAR · graft loss at 1 year	No BPAR or graft loss at 1 year; stable renal function; safety of allogeneic HLA-selected MSC

A-MSC = adipose tissue-derived mesenchymal stromal cell; BM-MSC = bone marrow-derived mesenchymal stromal cell; BPAR = biopsy-proven acute rejection; eGFR = estimated glomerular filtration rate; HLA = human leukocyte antigen; IF = immunofluorescence; IL = interleukin; IRI = ischaemia-reperfusion injury; IV = intravenous; KTx = kidney transplantation; MRI = magnetic resonance imaging; MSC = mesenchymal stromal cell; NMP = normothermic machine perfusion; RCT = randomized controlled trial; skGFR = single kidney glomerular filtration rate; Treg = regulatory T cell.

4. Combining NMP and MSC Therapy

4.1 — NMP is the ideal platform for cell therapy delivery

The concept of using NMP as a delivery platform for cellular therapy emerges from a convergence of two limitations that each technology alone cannot overcome. Intravenous MSC delivery is fundamentally constrained by pulmonary first-pass entrapment, which prevents most cells from reaching the kidney. NMP as a standalone preservation technique restores metabolic activity and enables functional assessment, but does not by itself address the immunological, inflammatory, or endothelial injury that conditions post-transplant outcomes. The combination of these two modalities addresses both limitations simultaneously. Unlike SCS — where the organ is metabolically arrested and inaccessible — the NMP window provides a controlled, reproducible, and time-limited opportunity to deliver therapies directly to the graft with defined exposure conditions.^{136,137} The intra-arterial access of the NMP circuit provides direct entry into the renal vasculature ensuring that the entire cell dose is directed injected into the target organ. Temperature, arterial pressure, flow rate, and circuit composition can all be standardised, providing a degree of pharmacokinetic control for cellular delivery that is simply unattainable with systemic administration. The first delivery of cellular therapy to a human kidney during NMP was reported by Thompson *et al.*¹³⁸ In five paired human kidney perfusions, multipotent adult progenitor cells (MAPC) cells delivered intra-arterially improved urine output, reduced NGAL and shifted the cytokine profile toward IL-10 and IDO upregulation — establishing human proof-of-concept for *ex vivo* cell delivery via the NMP circuit.

4.2 — Current Evidence: What Has Been Done?

The systematic investigation of MSC therapy during kidney NMP has been pioneered by the international MePEP (Mesenchymal cells during Porcine Ex vivo Perfusion) consortium — a collaboration between Erasmus MC Rotterdam, Aarhus University Hospital, UMCG Groningen, and the University of Oxford. The conceptual foundation was articulated by Sierra-Parraga *et al.* in a study which presented the scientific rationale for combining MSC and NMP in kidney transplantation and described the porcine DCD autotransplantation model as the appropriate preclinical platform for testing this combination.¹³⁶ Building on this framework, Pool *et al.* investigated the effect of human BM-MSC and A-MSC added to the NMP circuit of ischemically injured porcine kidneys. MSC-perfused kidneys showed lower perfusate levels of LDH and NGAL compared to vehicle controls, alongside elevated secretion of HGF, IL-6, and IL-8. This indicates active MSC licensing by the inflammatory microenvironment of the ischemic graft and an immunomodulatory paracrine response.¹³⁹ Critically, in a control experiment without a kidney in the circuit, MSC secreted minimal levels of these mediators — confirming that the injured organ itself provides the inflammatory licensing signals that activate MSC function.

The feasibility of MSC delivery into porcine kidneys during NMP was confirmed, along with preliminary evidence of biological activity. In the most comprehensive preclinical study of *ex vivo* MSC delivery during NMP, Lohmann *et al.* demonstrated in a porcine DCD autotransplantation model that intra-arterial MSC infusion during NMP is safe and feasible. Y-chromosome tracking confirmed cortical retention after reperfusion, but the study showed no improvement in post-transplant creatinine clearance, renal plasma flow, or fibrosis markers over 14 days. The absence of a functional benefit was attributed to the short follow-up, the autotransplant model's inability to capture alloimmunomodulatory effects, and a potentially insufficient dose or suboptimal timing relative to the kinetics of IRI-driven inflammation.¹⁴⁰ Recently, Vallant *et al.* demonstrated in a translational study spanning rodent, porcine, and human kidneys that MSC can be successfully delivered during HMP. MSC were traceable within kidney tissue after HMP, and modulation of IL-1 β levels was observed, though no functional benefit was demonstrated during subsequent mimicked reperfusion. These results underscore the importance of temperature-dependent MSC licensing and suggest that normothermic rather than hypothermic conditions may be required for full MSC activation.¹⁴¹

4.3 — Identified Gaps and Unmet Needs

The systematic review and meta-analysis presented in Chapter 3 (Navez *et al.*, *medRxiv*, 2025) provides a rigorous appraisal of the existing evidence base and its inherent, confirming that no consistent functional benefit has yet been demonstrated and that the field lacks the standardised protocols required to draw reliable conclusions.⁹⁸ A central obstacle is the absence of a validated, reproducible NMP protocol specifically designed for cell therapy testing. The published studies differ substantially in perfusion modality (some using NMP alone, others a combined HMP+NMP strategy) in the type and source of cells delivered (BM-MSC, A-MSC, MAPC, EVs), in warm and cold ischemia times, and in the animal models used, with widely varying degrees of baseline injury. This heterogeneity makes cross-study comparison essentially impossible and prevents the field from building cumulative knowledge toward clinical translation. Future studies should use clinically representative ischemia conditions — standardised warm and cold ischemia times reflecting actual DCD and DBD procurement scenarios — and should include both porcine and human kidney models with adequately powered and comparable experimental groups. The cell therapy itself must be delivered using clinical-grade MSC preparations that could realistically be used in humans, rather than research-grade cells whose characteristics may differ substantially. Finally, the NMP protocol — including perfusate composition, pressure targets, duration, and urine management — must itself be validated and standardised before being used as a delivery platform. Indeed, the perfusion protocol directly conditions the biological environment in which MSC are expected to act. Developing and validating such an integrated experimental framework — combining a reproducible, clinically applicable NMP protocol with a GMP-grade

MSC delivery strategy across both porcine and human kidney models — is the foundational scientific objective of the original research presented in the following chapters.

5. Objectives

The research presented in this thesis was designed to address the gaps identified above through a stepwise translational programme, organised around five complementary objectives.

Objective 1 aims to systematically synthesise the existing experimental evidence on stem cell-derived therapies combined with machine-perfused kidney transplants, through a systematic review and meta-analysis following PRISMA guidelines. By pooling and critically appraising all published studies across cell types, perfusion modalities, and animal models, this objective provides the formal scientific foundation that motivates and contextualises the subsequent original research — and quantifies the degree of protocol heterogeneity that currently prevents meaningful cross-study comparison.

Objective 2 aims to develop and validate a standardised, clinically applicable porcine NMP model specifically designed for cell therapy testing. A central feature of this model is the simultaneous perfusion of both kidneys from each donor animal, allowing direct paired comparison within the same biological unit and thereby eliminating inter-animal variability as a confounding factor in subsequent comparative studies involving cell therapy. Combined with clinically representative ischaemia conditions — defined functional warm and cold ischaemia times — this paired perfusion strategy provides the methodological foundation for rigorous, unbiased inter-group comparisons within a reproducible NMP protocol.

Objective 3 evaluates, within this validated porcine model, the safety, feasibility, and biological effects of intra-arterial clinical-grade MSC delivery during NMP of ischemic porcine kidney grafts. This objective directly tests the core hypothesis of this thesis — that *ex vivo* intra-arterial MSC delivery during the NMP window can attenuate IRI-associated damage and modulate the immunological profile of the graft — through functional, biomarker and histological endpoints assessed during perfusion.

Objective 4 translates the paired NMP approach to discarded human kidney grafts, characterising MSC distribution and functional impact during perfusion under conditions directly applicable to clinical transplantation. This objective aims to generate human-relevant mechanistic and safety data in a translational *ex vivo* setting — an essential step towards evaluating the combined NMP+MSC strategy in the human organ context prior to any consideration of clinical implementation.

Together, these four objectives form a coherent translational arc — from evidence synthesis, through mechanistic validation, to human organ proof-of-concept — designed to establish the scientific, technical, and regulatory foundations for the potential implementation of intra-

arterial MSC delivery during NMP as a new strategy to improve outcomes in deceased-donor kidney transplantation.

Part I. State of the Art: A Systematic and Narrative Synthesis

Chapter 2. Narrative Review - Mesenchymal/Stromal Cell Therapy in Kidney Diseases: An Update

Published as:

Navez M, Detry O, Jouret F. Mesenchymal/stromal cell therapy in kidney diseases: an update. *Curr Opin Nephrol Hypertens*. April 1, 2026.

Abstract

This review synthesizes advanced preclinical and clinical studies published over the past 18 months evaluating mesenchymal stromal cells (MSCs) and MSC-derived products in kidney diseases. We focused on the translational relevance of MSC-based therapies against ischemia–reperfusion injury (IRI) and toxic acute kidney injury (AKI), as well as chronic kidney disease (CKD) progression in diabetic kidney disease (DKD) and lupus nephritis (LN).

The renoprotective effects of MSC-based therapies are highly dependent on (i) the timing of administration and (ii) the local pathological microenvironment. In IRI and AKI, therapeutic efficacy is confined to specific exposure windows and is driven by early modulation of mitochondrial dysfunction, inflammation, and cell death. In DKD, MSCs from multiple sources consistently improve albuminuria, renal function, and structural damage through anti-inflammatory, anti-fibrotic, autophagy-restoring, and ferroptosis-inhibiting mechanisms. This nephroprotection appears to be largely independent of the glycaemic control. In LN, immune-contextual conditioning critically shapes the phenotypes of MSCs and MSC-derived extracellular vesicle (EVs), with optimized or engineered products outperforming the naïve approaches. In contrast, hypertension-related kidney disease illustrates how chronic ischemia and vascular remodelling limit MSC efficacy unless underlying the hemodynamic stress is corrected.

Across diverse settings of acute and chronic kidney injury, MSC-based therapies act primarily as modulators of early pathogenic cascades rather than curative interventions for advanced damage. Their efficacy critically depends on timing, disease context, and micro-environmental conditioning. Increasingly, cell-free strategies based on EVs offer scalable and potentially safer alternatives, supporting the translational development of context-adapted and combinatorial strategies.

1. Introduction

Renal ischemia–reperfusion injury (IRI) represents a central determinant in the pathophysiology of acute kidney injury (AKI), with a direct impact on both early and long-term outcomes of kidney recovery.¹⁴² By initiating cascades of mitochondrial dysfunction, innate and adaptive immune activation, tubular epithelial cell death, endothelial injury, and maladaptive repair, IRI frequently drives the transition toward acute kidney disease (AKD) and chronic kidney disease (CKD).¹⁴³ Despite major advances in the understanding of the IRI pathophysiology^{144,145} and in the development of immunomodulatory strategies, no targeted therapy is currently available to effectively and positively modulate the pathological processes interconnected along the IRI cascade. Over the past decade, mesenchymal stromal cells (MSCs) and their cell-free derivatives, particularly extracellular vesicles (EVs), have emerged as promising therapeutic platforms owing to their pleiotropic immunomodulatory, cytoprotective, and pro-regenerative properties. Rather than acting through direct tissue replacement, MSC-based approaches exert broad paracrine effects on mitochondrial homeostasis, inflammatory signalling, immune cell phenotyping, cellular senescence, and fibrotic remodelling.^{146–148} This review synthesizes recent preclinical and clinical evidence published over the past 18 months, highlighting (i) how MSCs and MSC-derived products attenuate AKI in conditions of ischemic, toxic, metabolic, and/or immune-mediated insults, including kidney transplantation, and (ii) how MSCs and MSC-derived products slow down CKD progression in cases of diabetic kidney disease (DKD) and lupus nephritis (LN). The therapeutic window and stage-dependent limitations of MSC-based strategies across the spectrum of kidney disease are summarized in **Figure 1**.

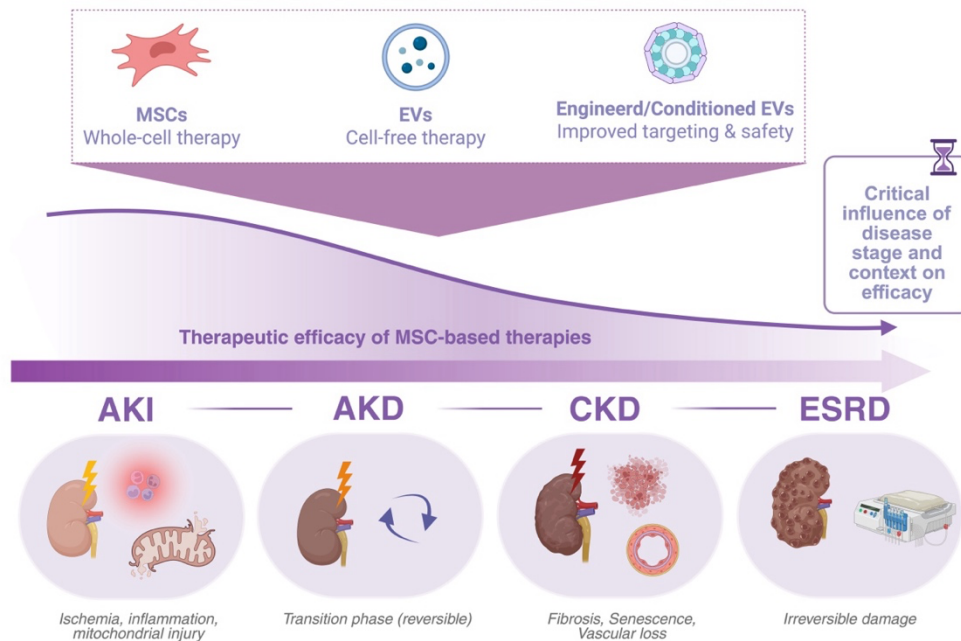


Figure 1. Disease stage and microenvironment shape the therapeutic efficacy of MSC-based strategies. MSC-based therapies show maximal efficacy at early stages of kidney injury, including acute kidney injury (AKI) and the reversible phase of acute kidney disease (AKD), where ischemia, inflammation, and mitochondrial dysfunction predominate. As disease progresses toward chronic kidney disease (CKD) and end-stage renal disease (ESRD), increasing fibrosis, senescence, and vascular loss impose irreversible constraints that limit therapeutic benefit, despite advances in cell-free and engineered MSC-derived products. Created with biorender.com.

2. Ischemia–Reperfusion Injury and Kidney Transplantation

Preclinical studies provide mechanistic information to explain how MSC-based strategies mitigate both acute and chronic consequences of IRI. Exosomes derived from Wharton’s jelly MSCs exert potent renoprotection by delivering high levels of miR-19b, which suppresses GSK3 β signalling and stabilizes pyridoxine metabolism through PDXK preservation, thereby reducing tubular apoptosis in *in vivo* transplanted kidneys and *in vitro* ischemia–reoxygenation models. Optimization of MSC culture conditions further enhances therapeutic potential.^{149,150} Hypoxia-preconditioned three-dimensional MSC spheroids produce exosomes enriched in miR-210, with increased vesicle yield and superior ability to reduce reactive oxygen species (ROS) accumulation, apoptosis, and migration defects after hypoxia–reoxygenation.¹⁵¹

Clinical studies provided essential insight into the feasibility, safety, and immunological impact of MSC-based therapies in kidney transplantation.^{133,152} In recipients with chronic active antibody-mediated rejection, repeated infusions of allogeneic bone marrow–derived MSCs were globally well tolerated, with no acute infusion reactions and only one opportunistic infection reported.^{153*} Importantly, renal functional decline was attenuated, as reflected by an improvement of the estimated glomerular filtration rate (eGFR) slope from a steep decline before treatment to near stability following MSCs therapy.

This functional stabilization was accompanied by reductions in proteinuria and donor-specific antibody (DSA) mean fluorescence intensity, suggesting partial control of ongoing graft injury. Immunophenotyping revealed expansion of CD4⁺CD25⁺CD127^{low} regulatory T cells and CD8⁺CD45RA⁺CCR7⁻ effector cells, together with a reduction in CD8⁺CCR7⁺ memory subsets, consistent with early immunomodulatory effects.^{153*} A complementary study using mass cytometry further demonstrated that within hours of MSCs infusion, highly proliferative CD11b⁺CD11c⁺CD38⁺CD39⁺Ki-67⁺ B- and T-cell subsets expand in the circulation, highlighting the rapid and dynamic immune reprogramming induced by MSCs therapy.¹⁵⁴ Conversely, donor-specific MSCs administered repeatedly in both human and nonhuman primate studies failed to induce durable tolerance and did not result in detectable chimerism. Furthermore, these cells were in some cases associated with *de novo* DSA formation or reversible rejection episodes.¹⁵⁵ Collectively, these findings indicate that MSCs are biologically active and generally safe in the transplant setting, but MSCs should not be considered as stand-alone tolerance-inducing agents.¹⁵⁶

Parallel advances in EV engineering highlight the importance of microenvironmental conditioning. EVs generated under hypoxic conditions display altered transcriptional and microRNA profiles that translate into enhanced anti-apoptotic and pro-proliferative effects on renal tubular epithelial cells compared with normoxic EVs, both *in vivo* and *in vitro*.¹⁵⁷ Beyond the acute injury, several MSC-derived EVs specifically prevent the chronic sequelae of IRI (**Figure 1**). Human umbilical cord MSC-derived exosomes enriched in miR-29a-3p protect against interstitial fibrosis and vascular rarefaction by directly targeting COL1A1 in fibroblasts and TNFR1 in endothelial cells.¹⁵⁸ Complementing these findings, MSC-derived EVs attenuate tubular epithelial senescence through coordinated regulation of Bax/Bcl-2-dependent apoptosis and Ras-pERK-Ets1-p53-mediated senescence pathways. When combined with senolytic agents such as dasatinib and quercetin, these EVs yield additive benefits in reducing tubular atrophy and chronic interstitial fibrosis.¹⁵⁹

Beyond EVs, innovative cell-free platforms have been developed to overcome limitations associated with whole-cell therapy. Mesenchymal membrane particles generated through controlled membrane extrusion reproduce key anti-fibrotic actions of MSCs while avoiding issues related to cell size, pulmonary trapping, and viability. In murine unilateral IRI models and in human induced pluripotent stem cell-derived kidney organoids exposed to hypoxia and inflammatory stimuli, these particles significantly reduced TGF- β signalling, COL1A1 expression, and α -SMA-positive myofibroblast activation without significantly altering immune cell infiltration, pointing toward a predominantly epithelial and stromal mechanism of action.¹⁶⁰ Combination strategies further amplify MSC efficacy by targeting complementary pathogenic pathways.

In experimental renal IRI, adipose-derived MSCs combined with melatonin synergistically improved renal function, reduced histological injury, suppressed pro-apoptotic Bax and caspase-3 expression, and restored anti-apoptotic Bcl-2 levels, illustrating the therapeutic value of pairing MSC-based products with agents targeting oxidative stress and mitochondrial dysfunction.¹⁶¹

In line with these experimental observations, we had explored MSCs as a strategy of ischemic conditioning rather than as a primary immunosuppressive intervention.¹⁶² Experimental studies demonstrated that MSCs administration prior to renal ischemia markedly attenuates AKI, reduces inflammatory infiltration and tubular apoptosis, and limits subsequent tissue damage. Transcriptomic analyses further revealed that MSCs pretreatment induces metabolic reprogramming toward lipid catabolism, with activation of the PPAR- α pathway and reduced lipid peroxidation, identifying metabolic modulation as a key mechanism underlying protection against renal IRI.¹²⁵

The translational potential of MSC- and EV-based strategies is further supported by the development of a porcine model of paired kidney *ex situ* normothermic machine perfusion.^{98*} The low intra-individual variability observed in this model enables one kidney to serve as a direct comparator for the other, providing a robust and clinically relevant platform to evaluate therapeutic interventions against IRI during machine perfusion prior to transplantation.¹⁶³

3. Toxic Acute Kidney Injury

In AKI models, mitochondrial safety consistently emerges as a central mechanism underlying MSC-mediated renoprotection. MSC therapy restores mitochondrial membrane potential, enhances PGC-1 α -driven mitochondrial biogenesis, normalizes the balance between fusion and fission proteins, and reduces mitochondrial ROS accumulation. These effects converge on suppression of NLRP3 inflammasome activation and pyroptotic cell death, thereby limiting tubular injury. Importantly, mitochondrial stabilization is associated with long-term antifibrotic effects, demonstrating that MSCs influence both the acute and reparative phases of AKI.^{164**}

EV-based strategies further refine these protective effects by improving tissue targeting and pharmacodynamics. Adipose-derived MSC small EVs (AMEVs), when surface-modified with ϵ -polylysine–PPD polymers, exhibit enhanced renal tropism and prolonged retention, attenuate epithelial–mesenchymal transition, inhibit mTOR signalling via miR-100 enrichment, and significantly reduce post-ischemic fibrosis.^{165*}

The importance of model selection and MSC source is illustrated by strain-dependent differences in cisplatin-induced AKI severity, where CXCL1 emerged as a robust injury biomarker enabling comparison of MSC potency across sources. Notably, umbilical cord-derived MSCs displayed stronger anti-inflammatory activity than bone marrow or adipose-derived MSCs.¹⁶⁶

MSC-derived exosomes also exhibit substantial reparative capacity in human-relevant systems. In a three-dimensional microfluidic proximal tubule model subjected to hypoxic injury, bone marrow MSC-derived exosomes restored epithelial polarity, as evidenced by ZO-1 upregulation, improved barrier integrity, rebalanced nutrient transport, and stimulated epithelial proliferation, albeit within a defined dosing window, underscoring the importance of pharmacodynamic precision.¹⁶⁷ Mechanistically engineered EVs provide even more targeted benefits. Exosomes enriched in miR-127-3p suppress aberrant autophagy by inhibiting ATG5 and ATG7 through modulation of the KIF3B–Hedgehog pathway, revealing a novel regulatory axis in AKI.¹⁶⁸ Similarly, hUCMSC-derived small EVs enriched in miR-13896 promote anti-inflammatory macrophage polarization by inhibiting the TRADD/NF- κ B signalling axis, thereby limiting cisplatin-induced tubular apoptosis and inflammatory signalling.¹⁶⁹

Translational relevance is reinforced by large-animal studies. In a minipig model of cisplatin-induced AKI, ultrasound-guided subcapsular MSC injection improved renal functional recovery, and identified the kynurenine–aryl hydrocarbon receptor–NF- κ B/NLRP3 axis as a key inflammatory pathway modulated by MSC therapy.^{170*} Complementarily, EVs released by hypoxia-preconditioned adipose-derived MSCs preserved mitochondrial function and redox homeostasis during IRI by maintaining mitochondrial membrane potential, respiratory capacity, ATP production, and antioxidant defences through activation of the Nrf2/HO-1 pathway.^{171,172} In infectious injury, MSCs complement antimicrobial therapy by enhancing ciprofloxacin's effect, reducing bacterial burden, and suppressing TLR4/MyD88/TRAF6/NF- κ B signalling in acute pyelonephritis.¹⁷³ In parallel, MSC-derived EVs enriched in tumor necrosis factor- α –induced protein 6 (TSG-6) exert potent immunomodulatory effects by promoting M2 macrophage polarization and regulatory T-cell induction, leading to marked suppression of renal inflammation and fibrosis and limiting AKI-to-CKD progression (**Figure 1**).^{174–176}

4. Diabetic Kidney Disease

A large body of preclinical evidence supports the therapeutic potential of MSCs and MSC-derived products in DKD, where metabolic stress, chronic inflammation, and progressive fibrosis converge to drive podocyte loss and CKD.^{177,178} Furthermore, recent observations have highlighted the ambiguous role of hypoxia in the development and progression of DKD.¹⁷⁹

Multiple studies demonstrate that MSC therapy directly preserves podocyte integrity—an early and critical determinant of proteinuria—by reducing apoptosis, restoring slit diaphragm proteins such as nephrin and podocin, and limiting foot process effacement in both type 1 and type 2 diabetic models.^{180–182} Accordingly, intravenous, intra-arterial, or repeated systemic administration of bone marrow–, umbilical cord–, placenta–, or adipose-derived MSCs consistently reduced albuminuria, improved renal function, and attenuated mesangial expansion, glomerular basement membrane thickening, and interstitial fibrosis, even in the absence of significant improvement in glycaemic control.^{181,183,184}

At the mechanistic level, multiple studies converge on the potent anti-inflammatory effects of MSCs in the diabetic kidney. MSC therapy suppresses the expression of key proinflammatory cytokines, including tumor necrosis factor- α , interleukin-6, interleukin-1 β , and interleukin-18, and inhibits major inflammatory signalling pathways implicated in DKD progression, such as NOD2, PKC/NF- κ B/STAT3, and MAPK.^{183,185,186} Immunomodulation further contributes to renoprotection, as MSCs promote macrophage polarization toward an anti-inflammatory M2 phenotype, thereby reducing glomerular inflammation and limiting podocyte injury in hyperglycaemic environments.¹⁸³ In parallel, fibrosis-specific mechanisms have been identified, with MSC therapy downregulating profibrotic mediators such as interleukin-11 and transforming growth factor- β , reducing collagen I and III deposition, α -smooth muscle actin expression, and epithelial–mesenchymal transition, ultimately limiting renal scarring.^{187–189}

Beyond whole-cell therapy, several studies emphasize the central role of EVs as key mediators of renoprotection in DKD. These vesicles deliver bioactive cargos, including microRNAs and regulatory proteins, that inhibit mammalian target of rapamycin signalling, restore autophagic flux, stabilize cytoskeletal proteins such as Talin-1, and suppress podocyte epithelial–mesenchymal transition.^{190,191} Restoration of autophagy emerges as a unifying mechanism across multiple DKD models, mediated through miR-99b-5p/mTOR, SIRT1/FOXO1, or SIRT1/TGF- β /Smad pathways, and is shown to be essential for limiting podocyte loss and renal fibrosis.^{181,184,188,190}

More recently, ferroptosis has been identified as a novel pathogenic process contributing to podocyte injury and progressive fibrosis in DKD. Several studies demonstrate that MSCs inhibit ferroptotic cell death by regulating SLC3A2 expression, suppressing Smad2/3-driven METTL3-dependent m6A modification, and preserving protective downstream targets such as sphingosine-1-phosphate receptor 1. Through these mechanisms, MSC therapy reduces lipid peroxidation, inflammatory activation, and extracellular matrix accumulation in diabetic kidneys.^{186,192} Additional complexity is introduced by evidence that MSCs can directly rescue mitochondrial dysfunction in injured podocytes via M-Sec–dependent tunnelling nanotubes, enabling the transfer of healthy mitochondria and restoration of cellular metabolism and survival.¹⁹³

Finally, combinatorial therapeutic strategies appear to further enhance MSC efficacy in DKD. Co-administration of MSCs with renin–angiotensin system blockers, sodium–glucose cotransporter 2 inhibitors, vitamin D supplementation, calorie restriction, or brown and white adipose tissue transplantation resulted in improved glucose handling, reduced oxidative stress, reinforcement of renoprotective RAAS signalling, and superior preservation of podocyte structure and renal function compared with monotherapy.^{180,188,194} Collectively, these studies depict MSC-based therapy as a multifaceted intervention in DKD, acting through coordinated modulation of inflammation, immunity, autophagy, fibrosis, ferroptosis, mitochondrial homeostasis, and paracrine signalling, thereby providing a strong experimental rationale for its continued translational development.

5. Lupus Nephritis

Preclinical and translational studies investigating MSC-based therapies in systemic lupus erythematosus (SLE), particularly LN, revealed that their therapeutic efficacy is tightly linked to immune modulation, regulation of cell death, and control of renal fibrosis. In this immune-driven context, optimized or “trained” MSC-based approaches consistently outperform naïve MSCs in controlling disease progression. In a pristane-induced murine lupus model progressing to end-stage kidney disease, treatment with a trained MSC-derived product significantly improved survival, stabilized proteinuria, and halted glomerulosclerosis. These effects were associated with profound immune reprogramming, including reductions in Th17 polarization, plasmacytoid dendritic cells, interferon- γ , interleukin-17A, B-cell activating factor, and anti–double-stranded DNA antibody levels, highlighting the importance of MSC functional optimization to overcome disease-associated immune dysregulation.¹⁹⁵

Metabolic and hypoxic preconditioning strategies further enhance MSC therapeutic efficacy in LN. Dimethyloxallyl glycine–pretreated human umbilical cord MSCs exhibited superior anti-inflammatory and anti-fibrotic effects compared with unconditioned cells, resulting in marked reductions in proteinuria, inflammatory cytokine expression, and renal fibrosis. These benefits were mechanistically linked to inhibition of the transforming growth factor- β /Smad2/3 signalling pathway, a central driver of fibrotic remodelling in LN.¹⁹⁶

Beyond fibrosis, emerging evidence implicates inflammatory cell death pathways as key contributors to LN pathogenesis. Caspase-4/5/11–mediated non-canonical pyroptosis was shown to be activated in macrophages from patients with SLE and murine models of LN, directly contributing to podocyte injury and renal dysfunction. MSC administration effectively suppressed pyroptotic signalling through galectin-3 and interleukin-10–dependent mechanisms, resulting in significant protection of podocyte structure and renal function in both experimental and clinical settings.¹⁹⁷

In parallel, ferroptosis has also emerged as a critical mechanism of podocyte loss in LN. MSC therapy reduced lipid peroxidation and restored antioxidant defenses by activating the Nrf2/heme oxygenase-1/glutathione peroxidase 4 axis, stabilizing podocyte actin cytoskeleton organization and improving cell viability *in vivo* and *in vitro*.¹⁹⁸ Importantly, MSCs themselves can be functionally altered by the lupus microenvironment. Bone marrow–derived MSCs from lupus-prone mice exhibited a proinflammatory phenotype driven by aryl hydrocarbon receptor activation and Hippo–YAP signalling, leading to enhanced Th17 responses and exacerbation of renal pathology. These effects were reversible through pharmacological inhibition of the AhR or YAP pathways, underscoring the need for careful MSC characterization and conditioning prior to therapeutic use.¹⁹⁹

Cell-free approaches further refine MSC-based immunomodulation in LN. EVs derived from MSCs, particularly those primed with disease-conditioned immune cell media, significantly improved survival, reduced autoantibody production, normalized macrophage polarization toward anti-inflammatory phenotypes, and ameliorated renal histopathology in lupus-prone mice. These findings highlight the importance of immune-contextual conditioning of EVs cargo and support the development of tailored cell-free therapies for immune-mediated kidney disease.²⁰⁰

6. Hypertension-Related Kidney Disease

Hypertension-related kidney disease provides a complementary perspective on the context dependency of MSC efficacy. In this particular setting, chronic hemodynamic stress, vascular remodelling, and ischemia impose intrinsic constraints on regenerative therapies. Studies using human adipose-derived MSCs demonstrated that cells isolated from patients with advanced hypertensive kidney disease exhibit reduced responsiveness to hypoxic preconditioning, with only modest improvements in proliferation, angiogenic signalling, and senescence markers compared with MSCs from healthy donors. These findings suggest that disease-induced intrinsic dysfunction may limit the efficacy of autologous MSC-based strategies in hypertensive nephropathy.^{201**}

In experimental renovascular hypertension, MSC therapy alone failed to reverse established renal dysfunction or structural damage. In contrast, the combination of MSC administration with renal artery revascularization normalized renal function, reversed proteinuria, reduced the number of ischemic glomeruli and atrophic tubules, and improved overall parenchymal architecture.²⁰² These results underscore that correction of the underlying hemodynamic insult is a prerequisite for effective MSC-mediated renal repair and highlight the importance of integrating regenerative therapies into multimodal treatment strategies.

7. Conclusion

Across diverse models of renal injury—including ischemia–reperfusion, toxic exposure, DKD, and immune-mediated nephropathies—a coherent therapeutic paradigm emerges in which MSC-based approaches act primarily as modulators of early pathogenic events rather than as curative interventions for advanced disease. Their efficacy depends critically on timing, disease context, and microenvironmental conditioning, as well as on the choice between whole-cell and cell-free delivery platforms (**Figure 2**). Increasingly, paracrine mechanisms mediated by EVs and other cell-free derivatives offer scalable and potentially safer alternatives to MSC transplantation. Continued refinement through preconditioning, molecular engineering, and rational combination strategies will be essential to translate these promising approaches into effective clinical therapies for kidney diseases and kidney transplantation.

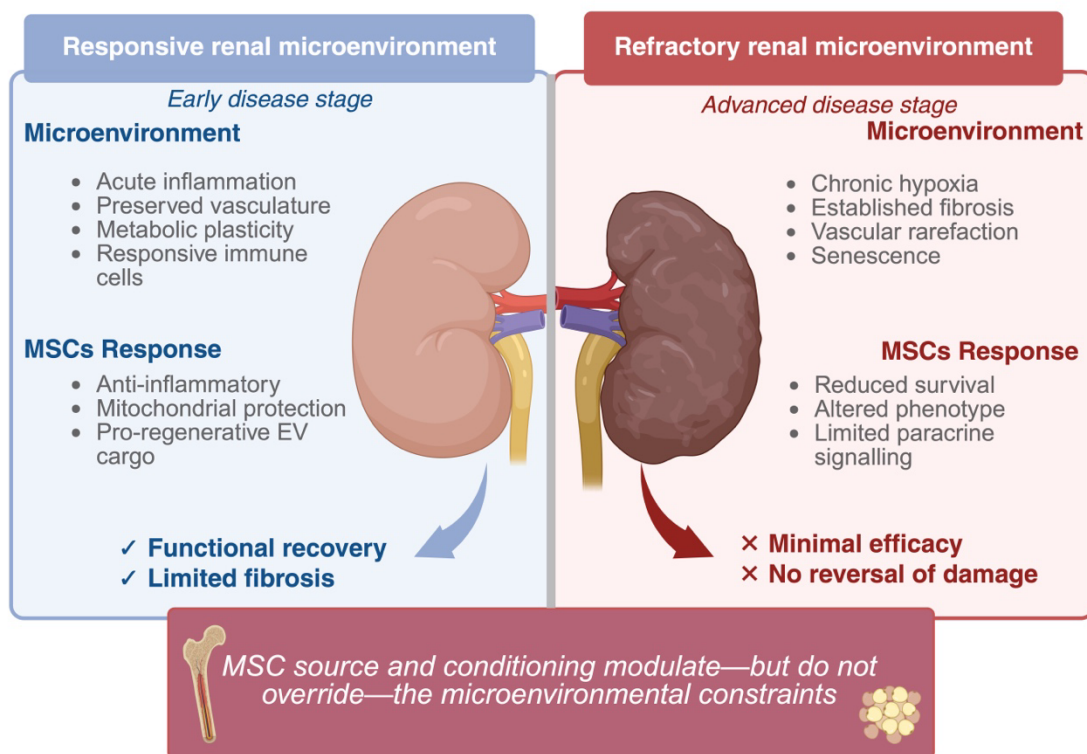


Figure 2. The responsiveness of the renal microenvironment shapes MSC therapeutic efficacy. MSC-based therapies are effective in biologically responsive renal microenvironments characterized by acute inflammation, preserved vasculature, metabolic plasticity, and immune responsiveness, enabling anti-inflammatory, mitochondrial-protective, and pro-reparative paracrine effects that support functional recovery and limit fibrosis. In contrast, refractory microenvironments dominated by chronic hypoxia, established fibrosis, vascular rarefaction, and cellular senescence constrain MSC survival and function, resulting in minimal therapeutic benefit without reversal of established damage. The sources and conditioning approaches of MSCs may modulate their efficacy, but do not overcome the microenvironmental constraints. Created with Biorender.com.

8. Key points

- MSC-based therapies primarily modulate early pathogenic processes in kidney injury rather than reversing established chronic damage
- Therapeutic efficacy is critically dependent on timing of administration and the local microenvironment
- In AKI and IRI, MSCs and MSC-derived EVs confer protection through early regulation of mitochondrial dysfunction, inflammation, and regulated cell death
- In DKD and LN, MSC efficacy relies on context-specific immunomodulatory, anti-fibrotic, and metabolic mechanisms, often independently of the systemic disease control
- Cell-free strategies based on extracellular vesicles offer scalable, safer, and more controllable alternatives to whole-cell MSC therapy, supporting their translational potential

Chapter 3. Systematic Review and Meta-analysis - From Preservation to Regeneration: Stem Cell-Derived Therapies in Machine-Perfused Kidney Transplants – A Systematic Review and Meta-analysis

Published as:

Navez M, Dos Santos Barata E, Maes N, et al. From Preservation to Regeneration: Stem Cell-Derived Therapies in Machine-Perfused Kidney Transplants - A systematic Review and Meta-analysis. Kidney Blood Press Res. Published online June 5, 2026.

Abstract

Ischemia-reperfusion injury (IRI) remains a critical determinant of graft outcomes in kidney transplantation, contributing to delayed graft function and reduced long-term survival. Machine perfusion has emerged as a dynamic preservation strategy offering a therapeutic window to condition organs prior to implantation. The integration of stem cells and extracellular vesicles (EVs), known for their immunomodulatory and cytoprotective properties, into perfusion protocols represents a novel and potentially synergistic approach. However, the evidence base remains limited and heterogeneous.

This systematic review and meta-analysis evaluated the therapeutic potential of stem cell-based interventions during machine perfusion, following PRISMA guidelines. PubMed, Embase, and Scopus were searched for experimental studies using stem cells or EVs during hypothermic or normothermic machine perfusion in animal or discarded human kidneys. Outcomes included renal function, injury biomarkers, inflammation, and histology.

Nine studies were included, seven in meta-analysis. Several reported reductions in inflammatory cytokines (IL-6, IL-1 β) and biomarkers (NGAL) following stem cell or EV administration. However, meta-analysis showed no significant effects on creatinine clearance (SMD: 0.00; 95% CI: -0.54 to 0.55), urine output (SMD: 0.54; 95% CI: -0.46 to 1.55), or NGAL (SMD: -1.68; 95% CI: -5.60 to 2.25). Stem cell retention was limited, mechanisms of action remain incompletely understood, and only one study assessed post-transplant function.

Despite potential immunomodulatory and cytoprotective effects, consistent functional benefits were not demonstrated. Standardized studies incorporating transplant models and long-term outcomes are needed to clarify therapeutic potential and optimize delivery strategies.

1. Introduction

Despite advances in kidney transplantation in recent years, the demand for donor organs continues to significantly exceed supply, resulting in prolonged waitlist durations.^{25,203,204} The ongoing shortage of organs has led to significant efforts to expand the donor pool and increase graft use, driving the development of innovative preservation techniques such as machine perfusion (MP).^{205–208} Although widely used, conventional static cold storage has limitations, such as ischemia-induced cellular damage, suboptimal graft recovery, and high organ discard rates. To overcome these challenges, alternative preservation modalities such as hypothermic and normothermic machine perfusions have been introduced.^{53,163,209,210} These dynamic preservation techniques aim to improve organ viability by providing continuous oxygenation. Normothermic perfusion additionally supports active metabolism by delivering nutrients and metabolic substrates, while clearing of waste products throughout the preservation period.^{75,76,211} Simultaneously, regenerative medicine has become a promising complement to organ preservation, with stem cell-based therapies introducing new strategies to reduce ischemia-reperfusion injury and promote graft repair mechanisms.^{133,138,148,212–214} Specific stem cell populations, along with their bioactive derivatives such as extracellular vesicles (EVs), display powerful regenerative and immunomodulatory capacities that hold promise for enhancing kidney graft preservation and function.^{147,163,215,216} Although mesenchymal stromal cells (MSCs) represent the most frequently studied cell population in this context, other progenitor cell types and stem cell-derived products have also been investigated and were therefore included in this review. Combining these cell-based approaches with advanced perfusion technologies may improve transplant outcomes and bridge the gap between organ supply and demand. This systematic review investigates the therapeutic potential of stem cell-derived products within machine perfusion systems, specifically focusing on their ability to reduce renal ischemia-reperfusion injury and enhance creatinine clearance. The current body of literature on this topic remains highly heterogeneous, notably in terms of perfusion modalities, timing of interventions, analytical methodologies, and types of cellular products. This variability underscores the importance of a comparative analysis of existing studies to elucidate the relative advantages and limitations of each approach. By synthesizing current evidence, our systematic review and meta-analysis aim to provide insights into emerging strategies that could improve kidney graft preservation and post-transplant outcomes.

2. Methods

2.1 — Search Strategy

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.^{217,218} A comprehensive literature search was performed in PubMed, Embase, and Scopus on 25 February 2025, using the concepts “stem cell”, “machine perfusion”, and “kidney”, with additional perfusion and stem cell-related terms. The detailed search strategy is presented in **Table S1** and further described in Supplemental Methods.

2.2 — Study Selection and Data extraction

Studies investigating the effect of stem cell-based therapies combined with MP vs. MP alone on graft function or injury in animal models or patients undergoing kidney transplantation were considered. Studies were included if they met the following criteria: (1) published in English or French, (2) original research articles, (3) focusing on kidney transplantation, (4) conducted in a machine perfusion model, and (5) investigating stem cell-based therapies. Studies were excluded if they did not meet these criteria or were unavailable in full-text format. Details are in the Supplemental Methods.

2.3 — Risk of Bias and Quality Assessment

The Systematic review center for laboratory animal experimentation (SYRCLE's) risk of bias tool for animal studies was used to evaluate study methodologies, with quality assessment of the reported articles based on animal experiments (details in the Supplemental Methods).²¹⁹ For studies on human organs, methodological quality was assessed using the National Institutes of Health (NIH) scoring tools.

2.4 — Statistical Analysis

Renal function was assessed using creatinine clearance during perfusion, after perfusion or after transplantation, and mean urine output during perfusion. Renal blood flow and fractional excretion of sodium were also considered. Renal injury was assessed through Neutrophil Gelatinase-Associated Lipocalin (NGAL) levels. For each outcome, when available, means and standard deviations were extracted for control and treatment groups, along with sample sizes (details in the Supplemental Methods). All analyses were performed using the metafor package in R (v4.3.0; R Core Team, Vienna, Austria).

Although substantial methodological heterogeneity was anticipated across studies—including differences in experimental models, perfusion protocols, and cellular products—a meta-analysis was performed to provide an exploratory quantitative synthesis of available functional outcomes. Funnel plots were generated to explore potential publication bias for the outcomes included in the meta-analyses.

3. Results

3.1 — Study Selection

A total of 1,862 studies were identified through the database search (Pubmed, Embase and Scopus). After removal of duplicates identified by Covidence (n=681), 874 studies were screened with titles and abstracts.²²⁰ During full-text review, 110 of the 119 articles were excluded for the following reasons: five did not involve kidneys, 40 were review articles or conference abstracts, 53 did not involve machine perfusion, and 12 did not involve stem cell-based therapy or derivatives. Thus, nine experimental studies met the predefined inclusion criteria and were included in the qualitative analysis of this systematic review.^{126,138–141,221–224} Of these, four studies reported sufficient results for aggregated data analysis on the effect of MSCs treatment during MP on creatinine clearance, five on urinary output and three on NGAL levels. The study selection process is summarized in the PRISMA flow diagram (**Figure S1**).

3.2 — Quality and risk of bias assessment

Risk of bias was mostly “unclear” for animal studies due to insufficient methodological reporting, particularly in relation to selection bias (**Figure S2, Tables S2 and S3**). Specifically, all studies failed to clearly report on the method of allocation sequence generation and concealment. Baseline group similarity was reported in only one study,¹⁴⁰ while adjustment for potential confounders remained largely unaddressed. Performance bias was variably reported: random housing of animals is not applicable to most included studies, as they were conducted ex vivo rather than involving live animals.^{126,139,141,221,222} Nevertheless, only one study explicitly reported blinding of caregivers and investigators, while the others did not provide sufficient information for assessment.¹⁴⁰ Detection bias was poorly mitigated, with limited reporting on random outcome assessment and assessor blinding. Due to a lack of reporting on the handling and justification of missing data, attrition bias was judged unclear in the majority of studies, except for two studies.^{140,222} Reporting bias was considered low in studies with available protocols or comprehensive outcome reporting.^{126,139,140,221,222} Other sources of bias were minimal across studies, with most publications demonstrating freedom from confounding factors such as unit of analysis errors or funding-related influence.^{126,139–141,221,222} For human studies, the overall quality was better, most often “good” or “not reported” (**Figure S2, Table S4**).

3.3 — Study Characteristics

Nine experimental studies met the inclusion criteria, all exploring stem/progenitor cell therapies delivered during ex vivo kidney machine perfusion. **Table 1** and **Figure 1** summarize key parameters: species, model design, sample size, and ischemia times.

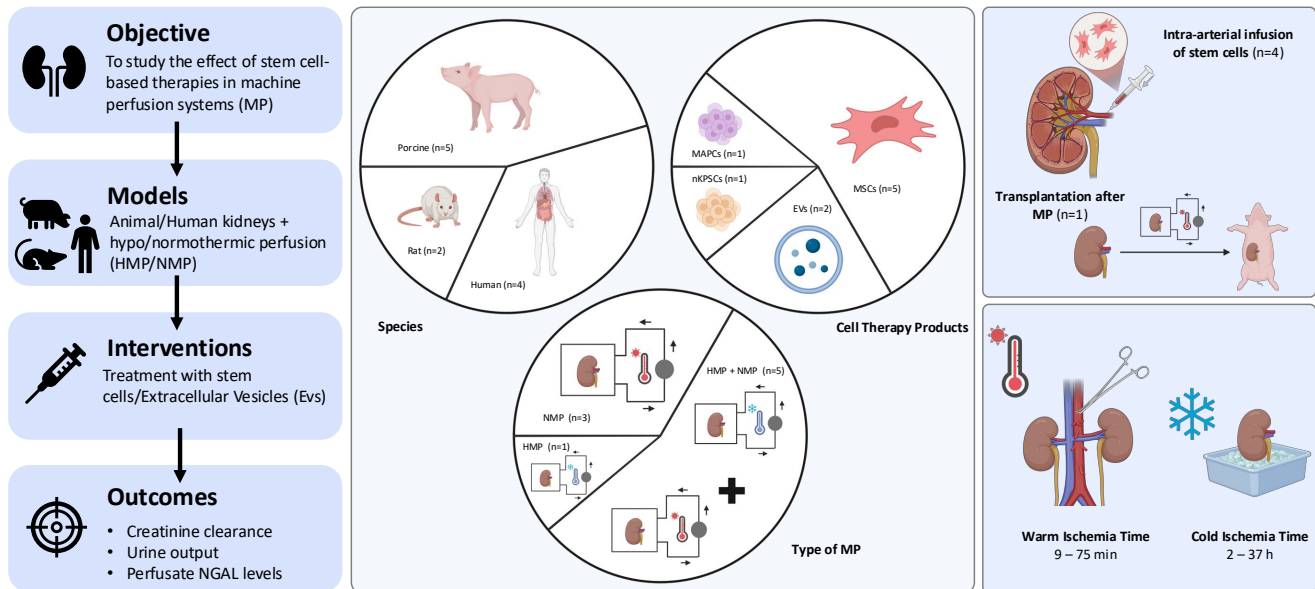


Fig 1. Overview of experimental designs investigating stem cell-based therapies in kidney machine perfusion models. This schematic summarizes key aspects of 9 translational studies evaluating the effects of stem cell-derived therapies in ex vivo kidney perfusion systems. (Left) The primary objective is to assess how stem cells or extracellular vesicles (EVs) influence kidney function using animal (porcine, rat) and human kidneys subjected to hypothermic (HMP) and/or normothermic (NMP) machine perfusion. Reported outcome measures include creatinine clearance, urine output, and perfusate neutrophil gelatinase-associated lipocalin (NGAL) levels. (Center) Studies vary in species used (porcine, rat, human), cell therapy products applied [multipotent adult progenitor cells (MAPCs), kidney progenitor cells (nkPSCs), mesenchymal stromal cells (MSCs), or EVs], and type of machine perfusion (MP) strategy. (Right) Delivery approaches include intra-arterial infusion of stem cells, and organs are subjected to variable warm ischemia times (9–75 min) and cold ischemia times (2–37 h) before perfusion.

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Table 1. Overview of the nine included studies summarizing species, groups, model and ischemia times

Study	Species	N (groups)	Model	Warm Ischemia Time (WIT)	Cold Ischemia Time (CIT)
Lohmann et al, 2021 ¹⁴⁰	Porcine	4 groups of n=7	HOPE (14h) + NMP (4h)	75 min	120 min
Vallant et al, 2024 ¹⁴¹	Human Porcine Rodent	2 groups of n=3 2 groups of n=6	HMP (4h) + NMP (2h)	25 min	24h
Rampino et al, 2022 ²²⁴	Human discarded kidneys	2 groups of n=5	HOPE (4h) + NMP (4h)	Not indicated	16-37h
Oliveira Arcolino et al, 2022 ²²³	Human discarded kidneys	2 groups of n=3	NMP (6h)	9-18min	17h
Thompson et al, 2021 ¹³⁸	Human discarded kidneys	2 groups of n =5	NMP (7h)	Not indicated	13-36h
Pool et al, 2019 ¹²⁶	Porcine	4 groups of n= 3	NMP (7h)	30 min	3,5-5h
Burdeyron et al, 2024 ²²¹	Porcine	3 groups of n=5-6	HMP (24h) + NMP (5h)	32 min	/
Pool et al, 2020 ¹³⁹	Porcine	4 groups of n=4	HMP (2-3h) + NMP (7h)	20 min	/
Gregorini et al, 2017 ²²²	Rat	3 groups of n=5	HMP (4h)	20 min	/

Four studies employed porcine kidneys^{126,139,140,221}, three human discarded kidneys^{138,223,224}, one a rat model²²², and one included all three.¹⁴¹ Porcine kidneys were exposed to controlled warm ischemia (20-75 minutes) via renal artery clamping or circulatory arrest (n=6 studies).^{126,139–141,221,222} In human studies, warm ischemia was uncontrolled, reported in one case as 9-18 minutes.²²³ Most studies used static cold storage, with durations ranging from 2h to 37h (n=6 studies).^{126,138,140,141,223,224} Perfusion protocols varied: typically, kidneys underwent Normothermic Machine perfusion (NMP, at ~37°C) either alone (n=3 studies)^{126,138,223} or following an initial phase of hypothermic machine perfusion (HMP) or oxygenated hypothermic perfusion (HOPE) (n=5 studies).^{139–141,221,224} A single study investigated HMP alone.²²² NMP duration ranged from 2 to 7 hours, while HMP/HOPE phases lasted 2-24 hours. Only one study included transplantation and 14-day follow-up.¹⁴⁰ Cell types investigated were MSCs from adipose tissue (n=1 study),¹⁴⁰ bone marrow (n=2 studies)^{141,222} or both (n=2 studies)^{126,139} (**Table 2**). One study tested EVs from MSCs as a cell-free therapy alternative.²²² Others used neonatal kidney stem progenitor cells (nKSPCs)²²³, multipotent adult progenitor cells (MAPCs)¹³⁸ or EVs from porcine urine progenitor cells (UPCs) or MSCs.^{221,224} Most studies compared cell-based therapy to machine perfusion alone (n=8 studies),^{138–141,221–224} with additional groups such as porcine vs. human MSCs (n=1 study),¹⁴⁰ adipose-derived MSCs (A-MSCs) vs. bone marrow-derived MSCs (BM-MSCs) (n=1 study),¹³⁹ or EVs combinations (HMP/EVs + NMP vs. HMP/EVs + NMP/EVs, n=1 study)²²¹.

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Cell dosing varied: two studies used labelled cells at different concentrations (n=2 studies),^{126,141} three used 10 million cells based on safety data;^{139,140,223} two used arbitrary doses (50 million and 3 million).^{138,222} EVs doses were either arbitrarily set (28.5×10^9 EVs)²²⁴ or based on secretion from cultured cells (6.5×10^9 EVs from 12 million cells).²²¹ Administration was mostly via the renal artery during perfusion, often 60 min after NMP initiation;^{126,138–140,221} some studies did not specify timing but followed a similar approach.

Despite model and protocol heterogeneity, studies shared outcome measures: perfusion parameters, renal function (e.g., urine output, creatinine clearance), and cytokine levels. Mechanistic insights were obtained via biochemical, histological, and immunological analyses, including injury biomarkers (e.g., NGAL, IL-6, IL-10), cytokine profiling, advanced imaging (Magnetic Resonance Imaging (MRI), Contrast-Enhanced Ultrasound (CEUS)), cell tracking, and transcriptomics.

Table 2. Overview of the nine included studies summarizing stem cell type, administrated doses, routes of administration and measured outcomes.

Study	Cell therapy products	Dose	Administration	Measured Outcomes
Lohmann et al, 2021 ¹⁴⁰	A-pMSCs/ hMSCs	10×10^6 cells	Intra-arterial infusion after 62 min of NMP	Perfusion characteristics, metabolic activity, creatinine clearance, NGAL, IL-6 and IL-10 levels, Y-chromosome detection
Vallant et al, 2024 ¹⁴¹	BM-rMSCs/hMSCs	1×10^6 cells 3×10^6 cells 5×10^6 cells	Not indicated	Perfusion characteristics, creatinine clearance, IL1- β and NGAL levels, localisation of MSCs in kidneys, MHC-1 detection
Rampino et al, 2022 ²²⁴	EVs (MSCs)	$28,5 \times 10^9$ EVs	Not indicated	Renal morphology, ischemic damage, Tubular cell proliferation index and mitochondrial distress, HGF and VEGF tissue levels, apoptosis (caspase 3)
Oliveira Arcolino et al, 2022 ²²³	nKSPCs	10×10^6 cells	Intra-arterial infusion 5-10 min before NMP	IL-6, IL-10 and NGAL levels, localisation of stem cells in kidneys, human mixed lymphocyte reaction, cytokines analyses
Thompson et al, 2021 ¹³⁸	MAPCs	50×10^6 cells	Infusion after 60 min of NMP	CEUS, EL-8, KIM-1 and NGAL levels, cytokines analyses, MAPCs tracking in kidney tissue
Pool et al, 2019 ¹²⁶	BM-MSCs/A-MSCs	1×10^5 cells 1×10^6 cells 1×10^7 cells	Intra-arterial infusion after 60 min of NMP	Histology, localisation of stem cells in kidneys, MRI
Burdeyron et al, 2024 ²²¹	EVs (UPCs)	$6,5 \times 10^9$ EV	Infusion after 60 min of NMP or 30 min of HMP	Perfusion characteristics, biochemical analysis, cytokines analyses, metabolomic, histology and RNAseq
Pool et al, 2020 ¹³⁹	BM-MSCs/A-MSCs	10×10^6 cells	Intra-arterial infusion after 60 min of NMP	Perfusion characteristics, cytokines analyses, NGAL and KIM-1 levels, histology
Gregorini et al, 2017 ²²²	BM-rMSCs/EVs (MSCs)	3×10^6 cells	Not indicated	Histology, biochemical assays

3.4 — Composition of the perfusate

Table 3 summarizes the composition of the perfusate used during HMP and NMP, highlighting substantial heterogeneity in both base solutions and pharmacologic additives. Among the perfusion devices used across the included studies, two employed an adapted pediatric cardiopulmonary bypass system.^{138,223} Four studies utilized a custom-made device based on the LifePort® system (Organ Recovery Systems, Itasca, IL, USA).^{126,139,140,221} One study used the VitaSmart® device from Bridge to Life (London, UK),²²⁴ another employed the RM3 system (Waters Medical Systems, Rochester, MN, USA),¹⁴¹ and the remaining study did not specify the device used.²²² Most HMP strategies employed University of Wisconsin-based machine perfusion solutions (UW-MPS, Bridge to Life Ltd., Columbia, SC, USA), typically perfused at 4°C and low pressures ranging from 25 to 50 mmHg (n=5 studies).^{139–141,222,224} Exception included the use of KPS-1® (Organ Recovery System, Itasca, IL, USA) for one study.²²¹ Notably, HMP perfusates were acellular and not supplemented with pharmacologic additives beyond the base solution. In contrast, NMP protocols consistently incorporated oxygenated, red cell-based perfusates, either with autologous whole blood (n=1 study)¹⁴¹ or packed red blood cells (n=7 studies).^{126,138–140,221,223,224} The perfusate was enriched with additives targeting metabolic support (e.g., glucose, insulin, calcium gluconate, sodium bicarbonate), antimicrobial prophylaxis (e.g., amoxicillin-clavulanate, cefuroxime), and vascular modulation (e.g., verapamil, mannitol). Most protocols operated at physiological temperatures (36–37°C) and perfusion pressures between 70 and 120 mmHg, with oxygenation ensured via carbogen (95% O₂/5% CO₂) at flow rates of 0.2–0.5 L/min. While the core components were similar, differences emerged in the complexity of additives—ranging from basic formulations (e.g., creatinine, glucose, antibiotics) to more elaborate mixtures including multivitamins, Nutriflex® (B. Braun Melsungen AG, Melsungen, Germany) and dexamethasone.¹³⁸ Overall, NMP protocols displayed a convergence toward physiologically active, cell-rich perfusates with targeted metabolic and pharmacologic supplementation.

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Table 3. Overview of included studies summarizing perfusion settings

Study	Device	HMP		NMP		
		Solution	Parameters	Perfusate	Additives	Parameters
Lohmann <i>et al</i> , 2021. ¹⁴⁰	Custom-made device based on LifePort system (Organ Recovery System)	500 ml cold University of Wisconsin machine perfusion solution (UW-MPS)	• 4-7°C • 0,1 L/min 100% oxygen • 25 mmHg	• 250 ml 5% human albumin solution • 170 ml allogeneic erythrocytes	• Sodium bicarbonate • Glucose • Insulin • Calcium gluconate • Mannitol • Creatinine • Amoxicillin-clavunate • Verapamil	• 37°C • 0,5 L/min carbogen (95% O₂/ 5% CO₂) • 70 mmHg
Vallant <i>et al</i> , 2024. ¹⁴¹	RM3 (Waters Medical Systems)	500 ml UW-MPS	• 4-6°C • 40-50 mmHg	• 500 ml autologous whole blood • 500 ml of 0.9% saline solution	• Heparin • Cefuroxime • Creatinine	• 36-37°C • 0,2 L/min carbogen (95% O₂/ 5% CO₂) • 100 mmHg
Rampino <i>et al</i> , 2022. ²²⁴	VitaSmart device (Bridge to Life)	UW-MPS	• 4°C • 25-30 mmHg	One unit of HBOCs	Non-Reported	• 20-25°C • 60 mmHg
Oliveira Arcolino <i>et al</i> , 2022. ²²³	Adapted paediatric cardiopulmonary bypass technology	/		Oxygenated red cell-based solution	Non-Reported	• 36°C • 75 mmHg
Thompson <i>et al</i> , 2021. ¹³⁸	Adapted paediatric cardiopulmonary bypass technology (Medtronic)	/		• 1 unit crossed-matched packed red blood cells • 400 ml Ringer's solution	• Sodium bicarbonate • Heparin • Glucose • Nutriflex infusion • Insulin • Multivitamins	• 36.5°C • 75 mmHg

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Table 3. Overview of included studies summarizing perfusion settings

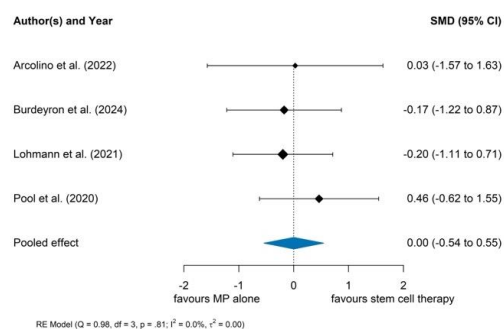
					• Mannitol • Dexamethasone	
Pool <i>et al</i> , 2019. ¹²⁶	Custom-made device based on LifePort system (Organ Recovery System)	/		• 350 ml pure red blood cells • 500 ml Williams’ Medium E	• Albumin • Amoxicillin-clavunate	• 37°C • 120/80 mmHg
Burdeyron <i>et al</i> , 2024. ²²¹	LifePort perfusion machine (Organ Recovery System) for HMP / Custom-made device for NMP	1 L KPS-1	• 4°C • 35 mmHg	• Autologous blood cells • Human albumin solution	• Sodium bicarbonate • Glucose • Calcium gluconate • Creatinine • Amoxicillin-clavulanic acid • Insulin • Verapamil	• 37°C • 75 mmHg • 0,5 L/min carbogen (95% O₂/ 5% CO₂)
Pool <i>et al</i> , 2020. ¹³⁹	Kidney Assist Transport device (Organ Assist) and custom-made device based on LifePort system (Organ Recovery System)	UW-MPS	• 4°C • 25 mmHg	• 288 ml pure red blood cells • 100 ml human albumin solution • 300 ml sodium chloride	• Sodium bicarbonate • Glucose • Calcium gluconate • Creatinine • Amoxicillin-clavulanate • Insulin • Verapamil • Augmentin	• 37°C • 110/70 mmHg • 0,5 L/min carbogen (95% O₂/ 5% CO₂)
Gregorini <i>et al</i> , 2017. ²²²	Non-Reported	UW-MPS	• 4°C	/ /		

3.5 — Functional Outcomes

All studies showed no significant differences in perfusion-related parameters such as renal blood flow, intrarenal resistance, and oxygen consumption between treated and control groups.^{126,138–141,221,223,224} Notably, one study reported enhanced cortical and medullary perfusion in MAPCs-treated kidneys after 4 hours of NMP, as measured by CEUS—a non-invasive imaging technique that evaluates microvascular blood flow in real time.¹³⁸ Creatinine clearance during perfusion and fractional sodium excretion were generally similar across groups, though some studies observed modest improvements. For example, one study reported improved creatinine clearance in kidneys perfused with A-MSCs although this difference was not statistically significant.¹³⁹ Two studies observed increased urine output in treated kidneys,^{138,223} but only one reported a statistically significant difference.¹³⁸ One study reported kidney function measurements over a 14-day period following transplantation, demonstrating significant elevated levels of plasmatic creatinine in control groups compared to the HOPE and NMP+hMSCs groups.¹⁴⁰ However, others found no significant differences in renal function endpoints after machine perfusion, including urine production.

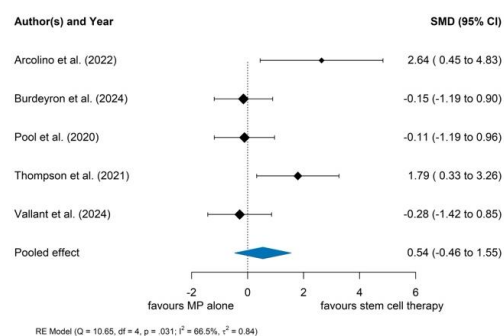
Meta-analysis on aggregated results concerning creatinine clearance was performed on four studies reporting creatinine clearance during NMP,^{139,140,221,223} and one reporting post-HMP values.¹⁴¹ The pooled effect size was not significant (SMD: 0.00; 95% CI: –0.54 to 0.55), indicating no measurable impact. Heterogeneity was low ($I^2 = 0.0\%$, $\tau^2 = 0.00$), and Cochran's Q test did not suggest significant variability between studies ($p = 0.81$). Results from Lohmann et al., who measured creatinine clearance at day 14 post-transplantation, were consistent with the findings during perfusion (**Figure 2**).¹⁴⁰ Mean urine output during perfusion was reported in five studies, which were all included in the meta-analysis (**Figure 3**).^{138,139,141,221,223} The pooled analysis yielded an SMD of 0.54 (95% CI: –0.46 to 1.55), with no statistically significant difference between treated and control groups. Heterogeneity was moderate ($I^2 = 66.5\%$, $\tau^2 = 0.84$), and Cochran's Q test indicated significant between-study variability ($p = 0.031$). In summary, the aggregated results do not demonstrate a consistent link between stem cell-based interventions and functional improvement during perfusion. Meta-analyses with standardized mean differences revealed non-significant pooled effects of stem cell or stem cell-derived product on renal blood flow and fractional sodium excretion with low heterogeneity, as detailed in Supplementary Results (**Figure S3, S4**). Funnel plots were generated to explore potential publication bias and are presented in Supplementary **Figure S5**.

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Study	Control group		Treated group		Effect		Weight
	N _c	Mean _c ± SD _c	N _t	Mean _t ± SD _t	MD (95%CI)	SMD (95%CI)	
During Perfusion							
Arcolino et al. (2022)	3	7.56 ± 9.32	3	7.81 ± 4.24	0.25 (-11.3 to 11.8)	0.028 (-1.57 to 1.63)	11.6 %
Burdeyron et al. (2024)	5	0.63 ± 0.64	12	0.52 ± 0.55	-0.10 (-0.75 to 0.54)	-0.17 (-1.22 to 0.87)	27.2 %
Lohmann et al. (2021)	7	0.97 ± 0.83	14	0.80 ± 0.83	-0.17 (-0.92 to 0.58)	-0.20 (-1.11 to 0.71)	36.0 %
Pool et al. (2020)	5	2.68 ± 0.37	10	4.40 ± 4.21	1.72 (-0.90 to 4.35)	0.46 (-0.62 to 1.55)	25.2 %
Pooled effect						0.00 (-0.54 to 0.55)	100 %
Heterogeneity: Q=0.98, df=3, p=0.81, I²(95%CI)=0.00(0.00 to 75.8%), τ²(95%CI)=0.00(0.00 to 1.01)							
Post-reperfusion							
Vallant et al. (2024)	6	2.59 ± 0.42	6	2.40 ± 0.44	-0.19 (-0.68 to 0.30)	-0.41 (-1.55 to 0.74)	
Day 14 after transplant							
Lohmann et al. (2021)	14	48.5 ± 15.9	14	50.0 ± 8.33	1.50 (-7.90 to 10.9)	0.11 (-0.63 to 0.86)	

Fig 2. Creatinine Clearance During Perfusion: Meta-Analysis of Standardized Mean Differences. This figure summarizes the results of four studies reporting creatinine clearance during machine perfusion.^{221,223,225,226} The pooled effect size is not statistically significant, with the standardized mean difference (SMD [95% CI]) estimated at 0.00 (−0.54 to 0.55), indicating no effect of stem cell or stem cell-derived product administration on creatinine clearance during perfusion. Heterogeneity across studies was low ($I^2 = 0.0\%$), with Cochran’s Q test indicating no significant heterogeneity ($p = 0.81$), and between-study variance $\tau^2 = 0.0$. Results from Lohmann *et al.* (2021), assessing creatinine clearance 14 days post-transplantation, and from Vallant *et al.* (2024), reporting outcomes post-reperfusion are analyzed separately. These findings are consistent with those observed during perfusion.



Study	Control group		Treated group		Effect		Weight
	N _c	Mean _c ± SD _c	N _t	Mean _t ± SD _t	MD (95%CI)	SMD (95%CI)	
Arcolino et al. (2022)	3	824 ± 245	3	1448 ± 105	624 (322 to 926)	2.64 (0.45 to 4.83)	12.6 %
Burdeyron et al. (2024)	5	211 ± 194	12	180 ± 201	-31 (-236 to 174)	-0.15 (-1.19 to 0.90)	23.4 %
Pool et al. (2020)	5	165 ± 120	10	152 ± 106	-13 (-137 to 111)	-0.11 (-1.19 to 0.96)	23.0 %
Thompson et al. (2021)	5	960 ± 189	5	1436 ± 281	476 (180 to 773)	1.79 (0.33 to 3.26)	18.7 %
Vallant et al. (2024)	6	510 ± 224	6	446 ± 190	-64 (-299 to 171)	-0.28 (-1.42 to 0.85)	22.3 %
Pooled effect						0.54 (-0.46 to 1.55)	100 %
Heterogeneity: Q=10.7, df=4, p=0.031, I²(95%CI)=66.5(0.00 to 97.1%), τ²(95%CI)=0.84(0.00 to 14.2)							

Fig 3. Mean Urine Output During Perfusion: Meta-Analysis of Standardized Mean Differences. This figure presents the pooled analysis of five studies reporting mean urine output during machine perfusion.^{138,141,221,225,226} The overall effect of stem cell or stem cell-derived product administration on urine output is not statistically significant, with a standardized mean difference (SMD [95% CI]) of 0.54 (−0.46 to 1.55). Assessment of heterogeneity revealed moderate variability across studies ($I^2 = 66.5\%$), with Cochran’s Q test confirming the presence of heterogeneity ($p = 0.031$) and a between-study variance τ^2 of 0.84. These results suggest that stem cell-based interventions during machine perfusion do not significantly influence urine output.

3.6 — Injury Markers

Biomarker-based assessment of kidney injury yielded conflicting results. Several studies evaluated NGAL and Kidney Injury Molecule-1 (KIM-1) as primary markers. Three reported decreased NGAL levels in urine or perfusate after treatment with nKSPCs, MAPCs, or MSCs, suggesting potential renoprotection.^{138,139,223} Conversely, other investigations failed to detect significant differences in NGAL or KIM-1 levels between treated and control groups.^{140,141,221} One study noted a progressive rise in plasma NGAL with progressive reduction thereafter although not reaching basal levels over 14 days post-transplantation in all groups, with no intergroup differences.¹⁴⁰

A meta-analysis of NGAL levels was performed on three studies.^{138,141,221} The study by Lohmann et al., measuring NGAL at day 2 post-transplantation, were analysed separately.¹⁴⁰ Due to large differences in absolute values between studies, only SMD were used. The pooled SMD was -1.68 (95% CI: -5.60 to 2.25), indicating no statistically significant difference between treated and control groups (**Figure 4**). Heterogeneity was substantial ($I^2 = 95.5\%$, $\tau^2 = 11.14$), and Cochran's Q test confirmed significant between-study variability ($p < 0.001$). Post-transplant NGAL results were consistent with perfusion-phase findings. Overall, meta-analysis showed no significant impact of stem cell-based therapies on NGAL levels during or after perfusion.

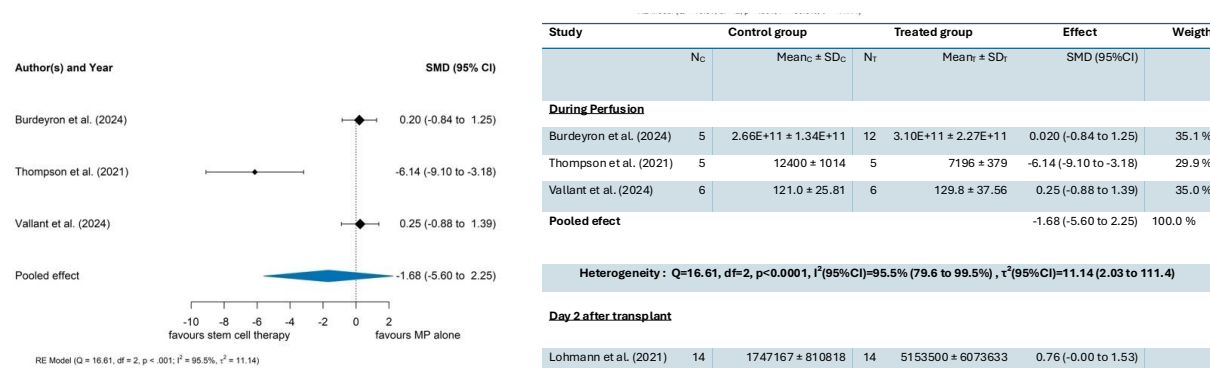


Fig 4. NGAL Levels During or at the End of Perfusion: Meta-Analysis of Standardized Mean Differences. This figure presents a meta-analysis of three studies reporting NGAL levels during or at the end of machine perfusion.^{138,141,221} NGAL levels reported by Lohmann *et al.* (2021) at day 2 post-transplantation are analysed separately.²²⁶ Due to substantial differences in absolute mean values across studies, only standardized mean differences are presented, not mean differences. The pooled effect is not statistically significant, with an SMD [95% CI] of -1.68 (-5.60 to 2.25), indicating no clear impact of stem cell or stem cell-derived product administration on NGAL levels during or at the end of perfusion. Heterogeneity among studies was high ($I^2 = 95.5\%$), supported by Cochran's Q test ($p < 0.001$) and a between-study variance τ^2 of 11.14. Results for NGAL measured two days post-transplantation are consistent with findings during perfusion.

One study reported reduced ischemic injury in kidneys treated with HOPE plus EVs compared to controls. In the same study, renal caspase-3 expression—an apoptosis marker—was significantly higher in controls than in EV-treated kidneys.²²⁴ Another study observed no significant difference in leukocyte infiltration between MAPCs-treated and control kidneys, but found reduced flavin mononucleotide (FMN) release, a mitochondrial injury marker, in the treated group.¹³⁸ In HMP settings, another group reported lower levels of tissue injury markers (lactate dehydrogenase, lactate) and oxidative stress, in MSCs- or EVs-treated kidneys compared to controls.²²² A subsequent study confirmed reduced oxidative stress, as evidenced by decreased glutathione levels, in kidneys treated with both HMP and NMP combined with EVs.²²¹

3.7 — Inflammatory Response

A consistent pattern across studies was the modulation of inflammatory responses following stem cell or EVs administration. Two studies reported a reduction in the pro-inflammatory cytokine interleukin-6 (IL-6) in kidneys subjected to NMP with either nKSPCs or A-MSCs, compared to controls. However, only one of these studies demonstrated a statistically significant decrease.¹³⁹ A separate investigation reported a significant decrease in IL-6 levels two hours post transplantation in the HOPE group relative to controls. However, in the same study, IL-6 secretion increased in all NMP groups to similar levels.¹⁴⁰ The expression of the pro-inflammatory marker interleukin-1 beta (IL-1 β) was significantly decreased in studies using nKSPCs and MAPCs combined with NMP, or HMP/NMP plus EVs, when compared to control groups.^{138,221,223} In contrast, an upregulation of IL-1 β was observed in kidneys treated with MSCs in an HMP model, although this increase did not reach statistical significance.¹⁴¹ The pro-inflammatory chemokine interleukine-8 (IL-8) levels were found to be significantly reduced in nKSPCs-treated kidneys in one study,²²³ whereas another study reported significant higher IL-8 expression in kidneys treated with BM-MSCs and A-MSCs compared to controls.¹³⁹ Furthermore, one study reported a non-significant reduction in interleukin-18 (IL-18), a pro-inflammatory cytokine, in kidneys treated with HMP or NMP combined with EVs, compared to control groups.²²¹ At the mRNA level, IL-10, IL-8, and transforming growth factor-beta (TGF- β)—a cytokine involved in fibrosis and immune regulation—were all significantly reduced in the HOPE group one hour after transplantation, compared to controls.¹⁴⁰

The anti-inflammatory cytokine interleukin-10 (IL-10) was significantly increased in MAPCs-treated kidneys, but not in other models.¹³⁸ Data from another study showed a significant increase in interleukin-1 receptor antagonist (IL-1RA), an anti-inflammatory mediator, suggesting a potential shift toward an anti-inflammatory profile following treatment with HMP or NMP combined with EVs.²²⁴

One study also assessed Hepatocyte Growth Factor (HGF) and Vascular Endothelial Growth Factor (VEGF)—key in regeneration and angiogenesis—reporting significantly increased expression in HOPE plus EVs-treated kidneys compared to controls.²²⁴ Additionally, transcriptomic analyses revealed activation of immunomodulatory genes such as FOXP3, SOCS5, and ISG15, as well as metabolic regulators including Idh2, Ndufs8, and Pdhhb, reinforcing the molecular basis for observed immunologic changes.^{221,222}

3.8 — Histological findings

Histological assessment of tissue injury was performed in six out of nine included studies. Across all experimental groups, and independently from the experimental group, common histopathological features included inflammation, tubular atrophy, and varying degrees of fibrosis. Two studies reported significantly reduced tissue damage in kidneys treated with MSCs, EVs, or HOPE combined with EVs compared to controls. Specifically, treated groups exhibited decreased tubular epithelial cell flattening, tubular necrosis, and luminal obstruction compared to control groups.^{222,224} Another study demonstrated improved structural preservation in kidneys treated with HMP/EVs or NMP/EVs, with enhanced tissue integrity observed after 5 hours of NMP. However, histological lesions such as tubular dilation, epithelial detachment, and inflammatory infiltrates were still evident following NMP in this study.²²¹

3.9 — Cell tracking and Localization

Several studies employed tracking techniques to confirm cell delivery and localization within renal tissues. Among them, three studies used fluorescent labelling methods,^{138,141,223} while one study assessed cell retention by quantifying the presence of the Y chromosome in the renal cortex.¹⁴⁰ MSCs and MAPCs were frequently found in the renal cortex, glomeruli, or peritubular spaces following perfusion.^{138,140,141} One study reported that nKSPCs remained detectable in the renal cortex for up to six hours.²²³ Cell delivery appeared limited in another study.¹²⁶ Only the highest dose (10 million MSCs) led to detectable retention, primarily within glomerular capillaries. However, many of these cells were disintegrated, with fluorescent labelling indicating membrane fragments rather than intact MSCs. Kinetic data further showed that most cells disappeared rapidly from the perfusion circuit, especially when no kidney was connected, suggesting trapping outside the organ. Even with a kidney, only minimal retention occurred after the first passage. No quantification per high-power field was provided, and flow cytometry detected very few MSCs in renal tissue, reinforcing the conclusion that cell engraftment was low and largely ineffective.¹²⁶

4. Discussion

This is the first systematic review and meta-analysis assessing the therapeutic potential of combining stem cell-based therapies with dynamic organ preservation to reduce renal ischemia-reperfusion injury and enhance creatinine clearance. Regardless of stem cell source or perfusion temperature, the studies included in this systematic review and meta-analysis did not provide conclusive evidence of a significant impact of stem cell-based intervention during machine perfusion on renal injury and function, although it should be noted that there was considerable methodological variation between studies. Several studies reported decreased levels of pro-inflammatory cytokines and increased anti-inflammatory mediators, suggesting a potential immunomodulatory effect, although a meta-analysis of these outcomes was not possible. Nevertheless, these changes did not translate into a significant functional improvement during perfusion in preclinical studies. One possible explanation for this apparent discrepancy relates to the temporal dynamics of tissue repair following ischemia–reperfusion injury. Most studies included in this review performed normothermic perfusion for relatively short periods, typically between two and seven hours. While this time-frame may be sufficient to detect early molecular or inflammatory changes, it may be insufficient for these biological effects to translate into measurable functional recovery. Experimental models of renal ischemia–reperfusion injury indicate that tissue repair processes, including tubular regeneration and endothelial recovery, occur over time scales of days to weeks following reperfusion. Tubular cell proliferation begins within 24–48 hours, peaks around 72 hours to day 4, and functional recovery is largely achieved by day 7–14^{227,228}, while endothelial repair involving pro-angiogenic signalling may extend beyond 7 days post-reperfusion.^{229,230} Consequently, reductions in inflammatory mediators observed during *ex vivo* perfusion may represent early biological signals whose functional consequences would only become apparent after transplantation and/or prolonged *in vivo* reperfusion.^{147,212}

The biological mechanisms underlying the potential benefits of stem cell-based therapies during machine perfusion remain incompletely understood. Increasing evidence suggests that the therapeutic activity of stem cells is largely mediated through paracrine signalling rather than durable engraftment within the target tissue. Stem cells secrete a wide range of bioactive molecules—including cytokines, growth factors, and extracellular vesicles—that can modulate inflammatory pathways, reduce oxidative stress, and promote tissue repair processes.^{231,232} Experimental studies in kidney and other solid organ models have shown that stem cell-derived extracellular vesicles can reproduce many of the immunomodulatory and cytoprotective effects attributed to the cells themselves.^{147,216,233} These vesicles contain regulatory proteins, lipids, and microRNAs capable of influencing endothelial integrity, mitochondrial function, and immune cell activation during ischemia-reperfusion injury.

Importantly, several studies included in this review indicate that cell retention within the kidney is limited, with many infused cells entrapped within glomerular capillaries or rapidly cleared from the perfusion circuit.¹²⁶ These findings support the hypothesis that the observed biological effects may primarily reflect transient paracrine interactions rather than sustained cellular integration into renal tissue.

Although machine perfusion has gained clinical relevance, most clinical studies to date have focused on comparing perfusion modalities (e.g., HMP vs. NMP, with or without oxygen), while the integration of regenerative therapies into these platforms remains largely experimental.^{52,55,108,234} Protocols across studies show marked heterogeneity, involving various cell types (e.g., MSCs from bone marrow or adipose tissue, MAPCs, urine-derived progenitors, or EVs) and different ischemic durations, perfusion temperatures, devices, pressures, and perfusate compositions. This variability limits the ability to draw definitive conclusions regarding efficacy or optimal conditions for therapeutic delivery. Moreover, cell dosing remains highly heterogeneous across studies. Few investigations have compared different doses with standardized endpoints, and no consensus has emerged regarding the optimal number of stem cells to use. While higher doses (e.g., 10 million cells) appear necessary for detectable renal retention, even at this level, only a minority of glomeruli show MSCs presence.¹²⁶ In many cases, fluorescent labelling indicated cell debris rather than intact MSCs, suggesting that cell death occurs shortly after infusion. These observations strongly indicate that cell viability and biodistribution are critical challenges. Quantitative data on how many stem cells reach the renal parenchyma remain insufficient. The rapid decline of MSCs counts from the perfusate, particularly when kidneys are not connected to the circuit, further suggests that many cells are trapped or lost outside the graft. This could explain the absence of a dose-response relationship and reinforce the hypothesis that therapeutic effects are likely mediated via paracrine signaling rather than sustained engraftment.^{231–233,235} As for the type of therapeutic agent, both stem cells and EVs have been tested, but direct comparisons are rare. Only a few studies assessed different MSCs sources (e.g., bone marrow vs. adipose-derived), and no one provided conclusive evidence favoring one over another. Similarly, comparisons between stem cells and EVs are limited and inconclusive, making it difficult to recommend one strategy over another. Another source of heterogeneity relates to the experimental models used across studies, which should be considered when interpreting translational relevance. While rodent models are useful for mechanistic investigations, porcine models more closely resemble human renal anatomy and transplantation conditions, which may influence the interpretation and translational relevance of the reported outcomes.

A major gap in the current body of evidence is the lack of post-transplantation data. Notably, only one study included in this review evaluated the impact of stem cell administration during machine perfusion in a transplantation setting with post-operative follow-up.¹⁴⁰

In this porcine autotransplantation model, kidneys treated with MSCs during NMP were subsequently transplanted and monitored for 14 days. Although the intervention was technically feasible and safe, no significant improvement in post-transplant renal function was observed compared with controls. Despite these neutral findings, this study provides important proof-of-concept for integrating cell-based therapies into *ex vivo* perfusion platforms prior to transplantation. The absence of functional improvement may reflect the limited sample size, the complexity of post-transplant injury mechanisms, or insufficient exposure time to the therapeutic intervention. These observations highlight the need for future studies to incorporate transplant models with clinically relevant endpoints, including delayed graft function, graft survival, and long-term renal function.

Finally, the quality of evidence is limited. Risk of bias was often assessed as unclear due to poor reporting of key methodological aspects, such as randomization, blinding, or allocation concealment. Small sample sizes, inconsistent reporting of cell characterization and viability, and variability in perfusion protocols further limit the strength of conclusions that can be drawn. Standardization of delivery protocols, systematic cell tracking, and robust functional assessment in transplant models will be essential to advance the field. Beyond biological efficacy, the clinical translation of stem cell-based therapies during machine perfusion raises important logistical and economic challenges. The production of clinical-grade MSCs requires specialized GMP-compliant facilities, rigorous quality control, and regulatory oversight, which increase both costs and implementation complexity.¹¹⁷ Future translational studies should therefore assess not only biological efficacy but also the scalability and cost-effectiveness of these approaches. To support future study design, **Table 4** summarizes the key parameters that should be considered in experimental protocols investigating stem cell therapies during machine perfusion.

Table 4. Recommended Parameters for Designing Future Experimental Protocols Involving Cell Therapy During Organ Perfusion

Category	Parameter to Consider	Recommendation / Rationale
Injury model	Warm Ischemia Time (WIT) / Cold Ischemia Time (CIT)	Precisely document to allow for meaningful comparisons across studies
Perfusion System	Hypothermic (HMP) vs. Normothermic (NMP)	Clearly specify the mode; cell effects may vary depending on temperature
Perfusate Composition	Type (blood-based, acellular, with supplements)	Standardize as much as possible to reduce biological variability
Cell Type	MSCs, endothelial cells, EV	Report cell source, phenotype, viability, and characterization methods
Delivery Method	Bolus, continuous infusion, intra-arterial administration	Choose a consistent and traceable delivery route; include systematic tracking (e.g., labelling)
Perfusion Duration	Total perfusion time & time of cell delivery	Provide exact timing to ensure reproducibility and interpretation of effects
Cell Tracking	Labelling, imaging, histology, Spatial transcriptomics, AI-based tissues analysis	Include standardized tracking to evaluate cell fate and localization
Functional Assessment	Post-perfusion transplant model	Use robust functional endpoints (e.g., histology, biomarkers, graft survival)
Study Design	Randomization, blinding, allocation concealment	Ensure methodological rigor to reduce risk of bias
Sample Size	A priori calculation if feasible	Optimize statistical power to support valid conclusions

This study has some limitations. Publication bias is possible, as studies with negative or non-significant results are less likely to be published. Outcome heterogeneity may affect the robustness of pooled results. The quality of included studies varied, with some showing methodological limitations. Differences in study design, animal species and donor conditions, and outcome definitions may further limit comparability. In the present study, pooled analyses were therefore intended to complement the qualitative synthesis and to provide an overview of potential effect directions across studies investigating stem cell-based interventions during machine perfusion. Given the limited number of studies and methodological variability, the results of the meta-analysis should be interpreted cautiously and primarily considered as an exploratory summary of the currently available evidence rather than definitive estimates of therapeutic efficacy. Moreover, funnel plots were generated to explore potential publication bias; however, because fewer than ten studies were included in the meta-analyses, interpretation of funnel plot asymmetry should be considered with caution.

In summary, this systematic review and meta-analysis of preclinical studies on stem cell-based therapies during machine perfusion did not demonstrate significant improvement in renal injury or function during perfusion, regardless of stem cell source or perfusion protocol. However, such interventions might be associated with immunomodulation and increased anti-inflammatory cytokines. Clinical translation will likely depend on overcoming challenges in delivery efficiency, mechanistic understanding, and rigorous validation in transplant models. In particular, the relative contribution of viable cells, paracrine signalling, and the release of bioactive molecules following cell apoptosis remains incompletely understood and warrants further investigation.

5. Supplementary File

Supplementary Methods

1. Search Strategy

All experimental studies investigating the effect of stem cell-based therapies combined with machine perfusion vs. (vehicle) control on graft function, urine production, or tissue lesions in animal models or patients undergoing kidney transplantation were considered for inclusion. Duplicate records were removed, and studies were screened for eligibility using Covidence.²²⁰ Initially, titles that were clearly irrelevant to the review's objectives were excluded. The abstracts of the remaining articles were independently evaluated by two reviewers (MN and EDS) to determine their suitability based on the predefined inclusion criteria. Any discrepancies between the two reviewers were resolved through consensus or by contacting a third reviewer (NG) in case of persistent discrepancies. Conference abstracts and other forms of grey literature were not included in this review, as they typically lack the methodological detail required for thorough quality assessment. Furthermore, the examination of reference lists from the included studies did not yield any additional eligible articles beyond those already identified through the systematic search. Subsequently, full-text articles that were retrievable were assessed for final inclusion. The protocol was submitted to PROSPERO on May 13, 2025, prior to commencing the review's drafting, and is currently awaiting publication.

2. Study Selection and Data extraction

Specific outcomes were not pre-defined as inclusion criteria, as we anticipated that the number of studies available on this topic would be limited. All articles meeting the predefined inclusion criteria were considered. No restrictions were applied regarding the publication date to ensure comprehensive coverage of relevant literature. Data extraction was performed in a standardized manner using a prespecified data extraction table in Excel, capturing key study characteristics, including: (1) animal model, (2) sample size, (3) type and duration of perfusion, (4) perfusate composition, temperature, and pressure settings, (5) stem cells/EVs origin and isolation technique, and (6) timing, duration, administration method, and dose of stem cells/EVs. Outcome parameters varied depending on the specific type of dynamic preservation assessed in each study. Comparison of the therapeutic effects of stem cell-based interventions during machine perfusion was based on the following main reported outcomes: (1) perfusion characteristics (flow, intrarenal resistance), (2) functional outcomes (creatinine clearance and urine output during perfusion), (3) biomarkers of kidney injuries, (4) inflammatory markers, (5) histological findings, and (6) cell/EVs localization or tracking.

3. Risk of Bias and Quality Assessment

The Systematic review center for laboratory animal experimentation (SYRCLE's) risk of bias tool for animal studies derives from the Cochrane Risk of Bias framework but has been specifically refined to capture sources of bias unique to animal intervention studies. It incorporates signaling questions designed to guide systematic and reproducible assessments of bias, thereby enhancing the transparency, rigor, and interpretability of the review findings.²¹⁹ For studies on human organs, methodological quality was assessed using the National Institutes of Health (NIH) scoring tools.

4. Statistical Analysis

When only medians and interquartile ranges were reported, means and standard deviations were estimated. When studies reported multiple treatment or control subgroups, these were combined to avoid double-counting. Pooled means and standard deviations were calculated using standard formulas accounting for group sizes and variances. Outcomes were harmonized across studies. To account for different measurement scales, standardized mean differences (SMDs) with 95% confidence intervals (CI) were computed. Forest plots were used to visualize study weights and pooled results. Heterogeneity was assessed using I^2 , τ^2 , and Cochran's Q statistic. A $I^2 < 25\%$ indicated low heterogeneity; $> 75\%$ indicated high heterogeneity. A p-value < 0.05 was considered statistically significant. All analyses were performed using the metafor package in R (v4.3.0; R Core Team, Vienna, Austria).

Supplementary Figures and Tables

Table S1. PubMed, Embase and Scopus Search Strategy

Bibliographic databases	Concept	Search
Medline (PubMed)	#1: Stem cell/MSC	"Mesenchymal Stem Cells"[Mesh] OR "Stem Cell" OR "Stromal Cell" OR "Stem Cells" OR "Stromal Cells" OR MSCs OR "progenitor cells" OR "progenitor cell" OR "Extracellular Vesicles"[Mesh] OR extracellular-vesicle*[tiab] OR exovesicle*[tiab] OR apoptotic-bod*[tiab] OR exosome*[tiab] OR Endosomes[mesh] OR ectosome*[tiab] OR endosome*[tiab] OR microparticle*[tiab] OR microvesicle*[tiab] OR Secretome*[tiab] OR multivesicular-bod*[tiab]
	AND	
	#2: Machine perfusion	"Organ Preservation"[Mesh] OR "Perfusion"[Mesh] OR "Reperfusion"[Mesh] OR "Reperfusion Injury"[Mesh] OR "Transplants"[Mesh] OR "machine perfusion" OR "normothermic machine perfusion" OR "normothermic preservation" OR "normothermic abdominal perfusion" OR "normothermic extracorporeal membrane oxygenation" OR "nmp" OR "hypothermic preservation" OR "hypothermic perfusion" OR "hmp"
	AND	
Embase (Elsevier)	#3: Kidney	"Kidney"[Mesh] OR "Kidney*"[tiab] OR "renal*"[tiab]
	#1: Stem cell/MSC	'mesenchymal stem cell'/syn OR 'stem cell' OR 'stromal cell' OR 'stem cells' OR 'stromal cells' OR mscs OR 'exosome'/exp OR exosome*:ti,ab,kw OR 'extracellular vesicle*':ti,ab,kw OR exovesicle*:ti,ab,kw OR 'apoptotic bod*':ti,ab,kw OR ectosome*:ti,ab,kw OR 'endosome'/exp OR endosome*:ti,ab,kw OR microparticle*:ti,ab,kw OR microvesicle*:ti,ab,kw OR Secretome*:ti,ab,kw OR 'multivesicular bod*':ti,ab,kw
	AND	
	#2: Machine perfusion	'organ preservation'/exp OR 'organ preservation':ti,ab,kw OR 'machine perfusion':ti,ab,kw OR 'dynamic preservation':ti,ab,kw OR 'normothermic machine perfusion':ti,ab,kw OR 'hypothermic machine perfusion':ti,ab,kw OR 'nmp':ti,ab,kw OR 'hmp':ti,ab,kw OR 'hope'/exp OR 'hope':ti,ab,kw OR 'hypothermic oxygenated machine':ti,ab,kw
SCOPUS (Elsevier)	AND	
	#3: Kidney	'kidney'/exp OR kidney*:ti,ab,kw OR renal*:ti,ab,kw
	#1: Stem cell/MSC	"Mesenchymal stem cell" OR Stem cell* OR Stromal cell* OR msc OR "extracellular vesicle*" OR exovesicle* OR "apoptotic bod*" OR exosome* OR ectosome* OR endosome* OR microparticle* OR microvesicle* OR secretome* OR "multivesicular bod"
	AND	
SCOPUS (Elsevier)	#2: Machine perfusion	"Organ preservation" OR "Machine perfusion" OR "Dynamic preservation" OR "Normothermic machine perfusion" OR "hypothermic machine perfusion" OR "NMP" OR "HMP" OR "HOPE" OR "hypothermic oxygenated machine"
	AND	
	#3: Kidney	"Kidney" OR "Renal"

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Table S2: Detailed Risk of Bias Assessment using SYRCLE’s tool for articles reporting on animal studies

	References	Lohmann et al, 2021	Vallant et al, 2024	Pool et al, 2019	Burdeyron et al, 2024	Pool et al, 2020	Gregorini et al, 2017
Selection bias	Was the allocation sequence adequately generated and applied?	?	?	?	?	?	?
	Were the groups similar at baseline or were they adjusted for confounders in the analysis?	+	?	?	?	?	?
	Was the allocation adequately concealed?	?	?	?	?	?	?
Performance bias	Were the animals randomly housed during the experiment?	?	+	+	+	+	+
	Were the caregivers and/or investigators blinded from knowledge which intervention each animal received during the experiment?	+	?	?	?	?	?
Detection bias	Were animals selected at random for outcome assessment?	?	?	?	+	+	?
	Was the outcome assessor blinded?	+	?	?	?	?	?
Attrition bias	Were incomplete outcome data adequately addressed?	+	?	?	?	?	+
Reporting bias	Are reports of the study free of selective outcome reporting?	+	?	+	+	+	+
Other bias	Was the study apparently free of other problems that could result in high risk of bias?	+	+	+	+	+	+

+, yes = low risk; ?, unclear risk; -, no = high risk; / not relevant or applicable

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Table S3: SYRCLE’s signaling questions for bias assessment for articles reporting on animal studies

Reference	Lohmann et al, 2021	Vallant et al, 2024	Pool et al, 2019	Burdeyron et al, 2024	Pool et al, 2020	Gregorini et al, 2017
Did the investigators describe a random component in the sequence generation process ?	?	?	?	?	?	?
Was the distribution of relevant baseline characteristics balanced for the intervention and control groups?	+	?	?	?	?	?
If relevant, did the investigators adequately adjust for unequal distribution of some relevant baseline characteristics in the analysis?	/	/	/	/	/	/
Was the timing of disease induction adequate?	+	+	+	+	+	+
Could the investigator allocating the animals to intervention or control group not foresee assignment due to one of the following or equivalent methods? (*)	?	?	?	?	?	?
Did the authors randomly place the cages or animals within the animal room/facility?	?	?	?	?	?	?
Is it unlikely that the outcome or the outcome measurement was influenced by not randomly housing the animals?	?	+	+	+	+	+
Was blinding of caregivers and investigators ensured, and was it unlikely that their blinding could have been broken?	+	?	?	?	?	?
Did the investigators randomly pick an animal during outcome assessment, or did they use a random component in the sequence generation for outcome assessment?	?	?	?	+	+	?
Was blinding of the outcome assessor ensured, and was it unlikely that blinding could have been broken?	+	?	?	?	?	?
Was the outcome assessor not blinded, but do review authors judge that the outcome is not likely to be influenced by lack of blinding?	/	/	/	/	/	/
Were all animals included in the analysis?	+	+	+	+	+	+

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Reference	Lohmann et al, 2021	Vallant et al, 2024	Pool et al, 2019	Burdeyron et al, 2024	Pool et al, 2020	Gregorini et al, 2017
Were the reasons for missing outcome data unlikely to be related to true outcome? (e.g., technical failure)	+	?	?	?	?	+
Are missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups?	+	?	?	?	?	?
Are missing outcome data imputed using appropriate methods?	?	?	?	?	?	?
Was the study protocol available and were all of the study's pre-specified primary and secondary outcomes reported in the current manuscript?	?	?	?	?	?	?
Was the study protocol not available, but was it clear that the published report included all expected outcomes?	+	?	+	+	+	+
Was the study free of contamination (pooling drugs)?	+	+	+	+	+	+
Was the study free of inappropriate influence of funders?	+	+	+	?	+	+
Was the study free of unit of analysis errors?	+	+	+	+	+	+
Were design-specific risks of bias absent?	+	+	+	+	+	+
Were new animals added to the control and experimental groups to replace drop-outs from the original population?	+	+	+	+	+	+

+, yes = low risk; ?, unclear risk; -, no = high risk; / not relevant or applicable

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Table S4: Quality assessment of studies including human kidneys according to the NIH quality assessment score

	References	Vallant et al, 2024	Rampino et al, 2022	Oliveira Arcolino et al, 2022	Thompson et al, 2020
Described as randomized	Was the study described as randomized, a randomized trial, a randomized clinical trial, or an RCT?	NR	NR	NR	NR
	Adequate randomization: was the method of randomization adequate (i.e., use of randomly generated assignment)?	NR	+	NR	NR
Treatment allocation-two interrelated pieces	Allocation concealment: was the treatment allocation concealed (so that assignments could not be predicted)?	NR	NR	NR	NR
Blinding	Were study participants and providers blinded to treatment group assignment?	NR	NR	NR	NR
	Were the people assessing the outcomes blinded to the participants group assignments?	NR	NR	NR	NR
Similarity of groups at baseline	Were the groups similar at baseline on important characteristics that could affect outcomes (e.g., demographics, risk factors, co-morbid conditions)?	-	+	-	+
Dropout	Was the overall drop-out rate from the study at endpoint 20% or lower of the number allocated to treatment?	+	+	+	+
	Was the differential drop-out rate (between treatment groups) at endpoint 15 percentage points or lower?	+	+	+	+
Adherence	Was there high adherence to the intervention protocols for each treatment group?	-	+	+	+
Avoid other interventions	Were other interventions avoided or similar in the groups (e.g., similar background treatments)?	-	+	+	+
Outcome measures assessment	Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	+	+	+	+
Power calculation	Did the authors report that sample size was sufficiently large to be able to detect a difference in the main outcome between groups with at least 80% power?	NR	NR	NR	NR
Prespecified outcomes	Were outcomes reported or subgroups analyzed prespecified (i.e., identified before analyses were conducted)?	+	+	+	+

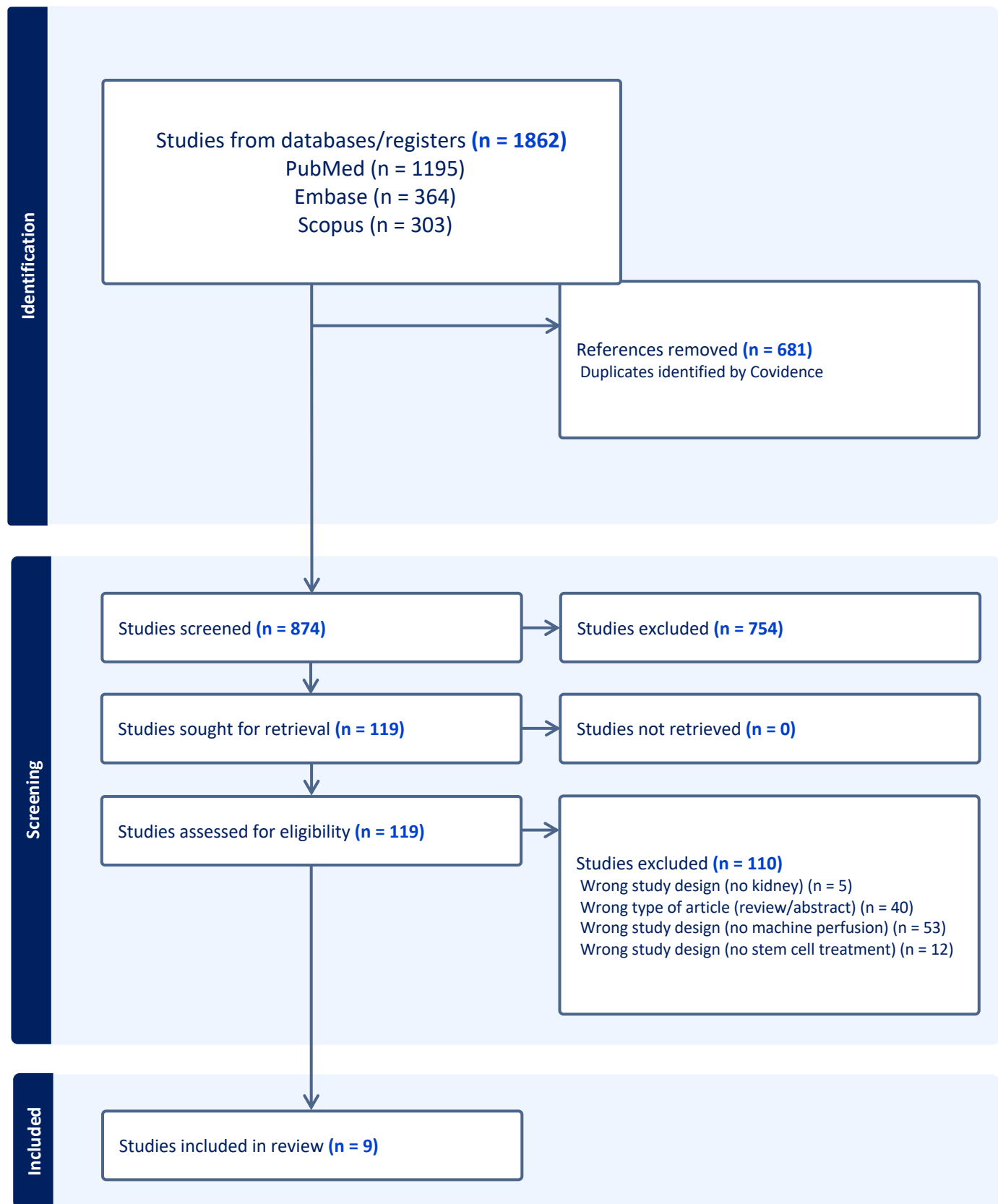
Chapter 3. From Preservation to Regeneration: Stem Cell-Derived Therapies in Machine-Perfused Kidney Transplants – A Systematic Review and Meta-analysis

	References	Vallant et al, 2024	Rampino et al, 2022	Oliveira Arcolino et al, 2022	Thompson et al, 2020
Intention-to-treat analysis	Were all randomized participants analyzed in the group to which they were originally assigned, i.e., did they use an intention-to-treat analysis?	NR	NR	NR	NR

+, yes = good; ?, Other* = fair; -, no=poor

*Other: CD, cannot determine; NR, not reported

Figure S1. PRISMA Flow Diagram



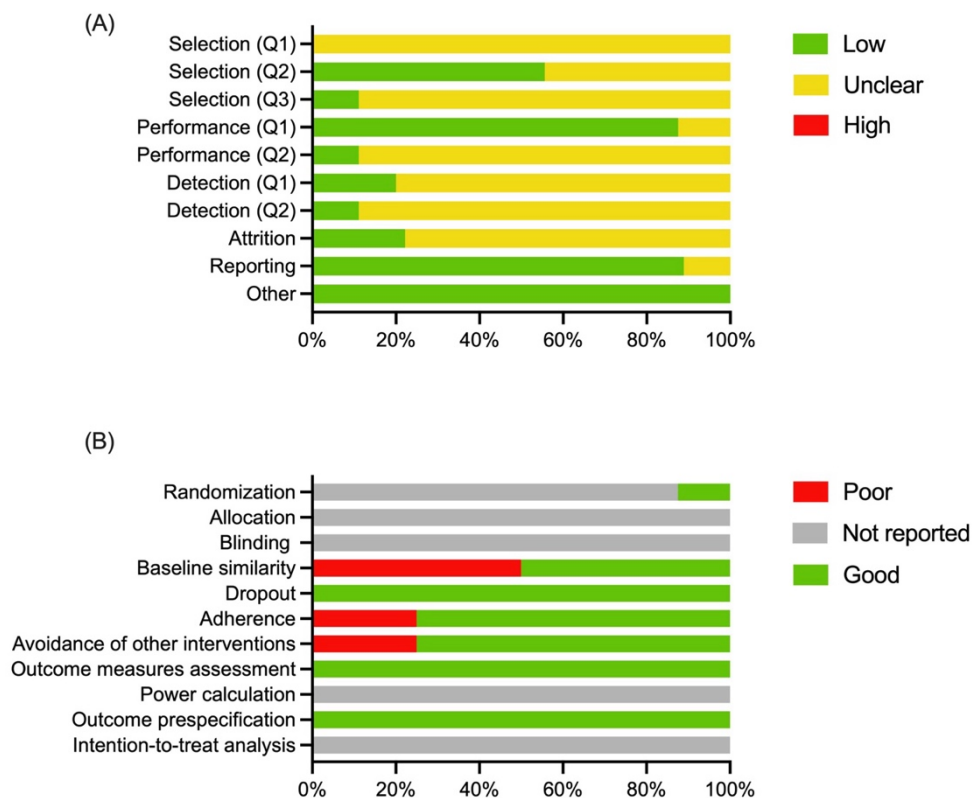
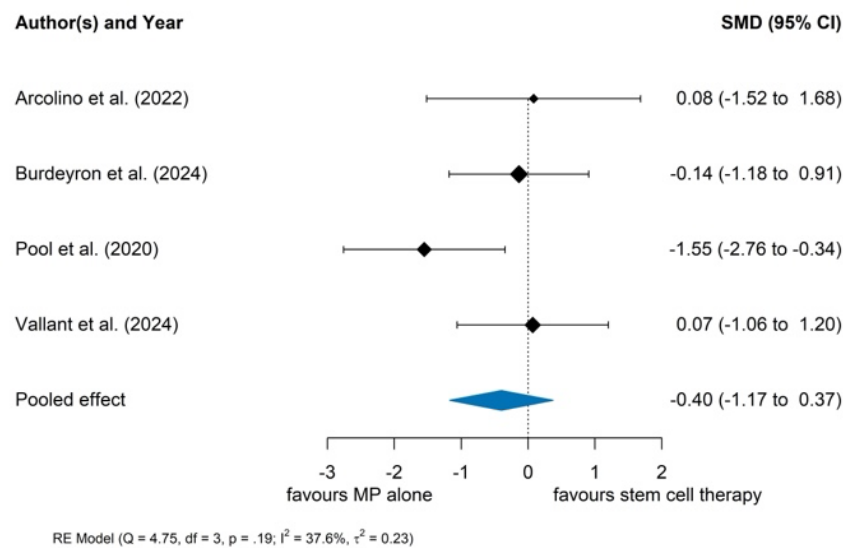


Figure S2. Risk of bias assessment (A) Risk of bias assessment using SYRCLE's tool for animal studies; (B) Risk of bias assessment using NIH tools for human studies.

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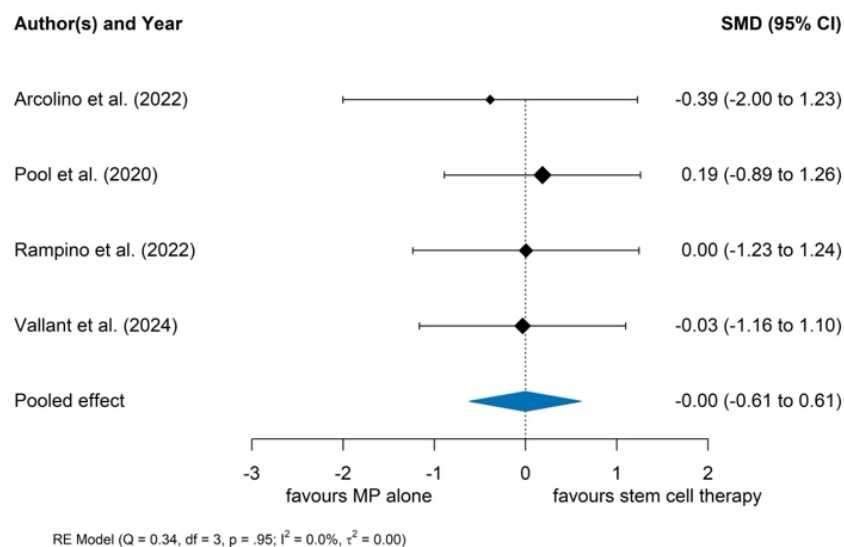


Study	Control group		Treated group		Effect SMD (95%CI)	Weight
	N _C	Mean _C ± SD _C	N _T	Mean _T ± SD _T		
Arcolino et al. (2022)	3	26.40 ± 9.40	3	27.10 ± 1.00	0.08 (-1.52 to 1.68)	17.2 %
Burdeyron et al. (2024)	5	46.75 ± 10.78	12	41.83 ± 39.49	-0.14 (-1.18 to 0.91)	30.0 %
Pool et al. (2020)	5	27.50 ± 5.50	10	15.55 ± 7.90	-1.55 (-2.76 to -0.34)	25.3 %
Vallant et al. (2024) – Mean Fractional excretion of sodium rate	6	0.79 ± 0.21	6	0.81 ± 0.30	0.07 (-1.06 to 1.20)	27.4 %
Pooled effect					-0.40 (-1.17 to 0.37)	100 %

Heterogeneity: Q=4.75, df=3, p=0.19, I²(95%CI)=37.6% (0.00 to 95.49%), τ²(95%CI)=0.23 (0.00 to 8.17)

Figure S3. Fractional excretion of sodium (%) during perfusion: Meta Analysis of Standardized Mean Differences. This figure summarizes the meta-analysis of four studies evaluating fractional excretion of sodium during machine perfusion. Due to differences in units of measurement in Vallant et al. (2024), only standardized mean differences are reported. The pooled effect is not statistically significant, with an SMD [95% CI] of -0.40 (-1.17 to 0.37), indicating no measurable impact of stem cell or stem cell-derived product administration on fractional excretion of sodium during perfusion. Heterogeneity across studies was low to moderate (I² = 37.6%), with no significant heterogeneity detected by Cochran’s Q test (p = 0.19), and between-study variance τ² = 0.23.

Chapter 3. From Preservation to Regeneration: Stem Cell-Derived Therapies in Machine-Perfused Kidney Transplants – A Systematic Review and Meta-analysis



Study	Control group		Treated group		Effect SMD (95%CI)	Weight
	N _C	Mean _C ± SD _C	N _T	Mean _T ± SD _T		
Arcolino et al. (2022) – ml/min	3	277.7 ± 84.42	3	248.4 ± 13.38	-0.39 (-2.00 to 1.23)	14.3 %
Pool et al. (2020) – ml/min	5	165.0 ± 103.0	10	178.5 ± 45.17	0.19 (-0.89 to 1.26)	32.3 %
Rampino et al. (2022) – ml/min	5	183.0 ± 71.30	5	183.3 ± 36.90	0.00 (-1.23 to 1.24)	24.3 %
Vallant et al. (2024) – ml/min/100g	6	51.77 ± 13.79	6	51.33 ± 11.42	-0.03 (-1.16 to 1.10)	29.1 %
Pooled effect					-0.00 (-0.61 to 0.61)	100 %

Heterogeneity : Q=0.34, df=3, p=0.95, I²(95%CI)=0.00% (0.00 to 40.7%) , τ²(95%CI)=0.00 (0.00 to 0.27)

Figure S4. Renal Blood Flow during or at the end of the perfusion: Meta Analysis of Standardized Mean Differences. This figure presents the meta-analysis of four studies assessing renal blood flow during or at the end of machine perfusion. Due to differences in measurement units in the Vallant et al. (2024) study, only standardized mean differences are reported. The pooled effect is not statistically significant, with an SMD [95% CI] of -0.00 (-0.61 to 0.61), indicating no observable effect of stem cell or stem cell-derived product administration on renal blood flow during perfusion. Heterogeneity across studies was minimal (I² = 0.0%), with Cochran's Q test rejecting heterogeneity (p = 0.95), and between-study variance τ² = 0.0.

Chapter 3. From Preservation to Regeneration: Stem Cell-Derived Therapies in Machine-Perfused Kidney Transplants – A Systematic Review and Meta-analysis

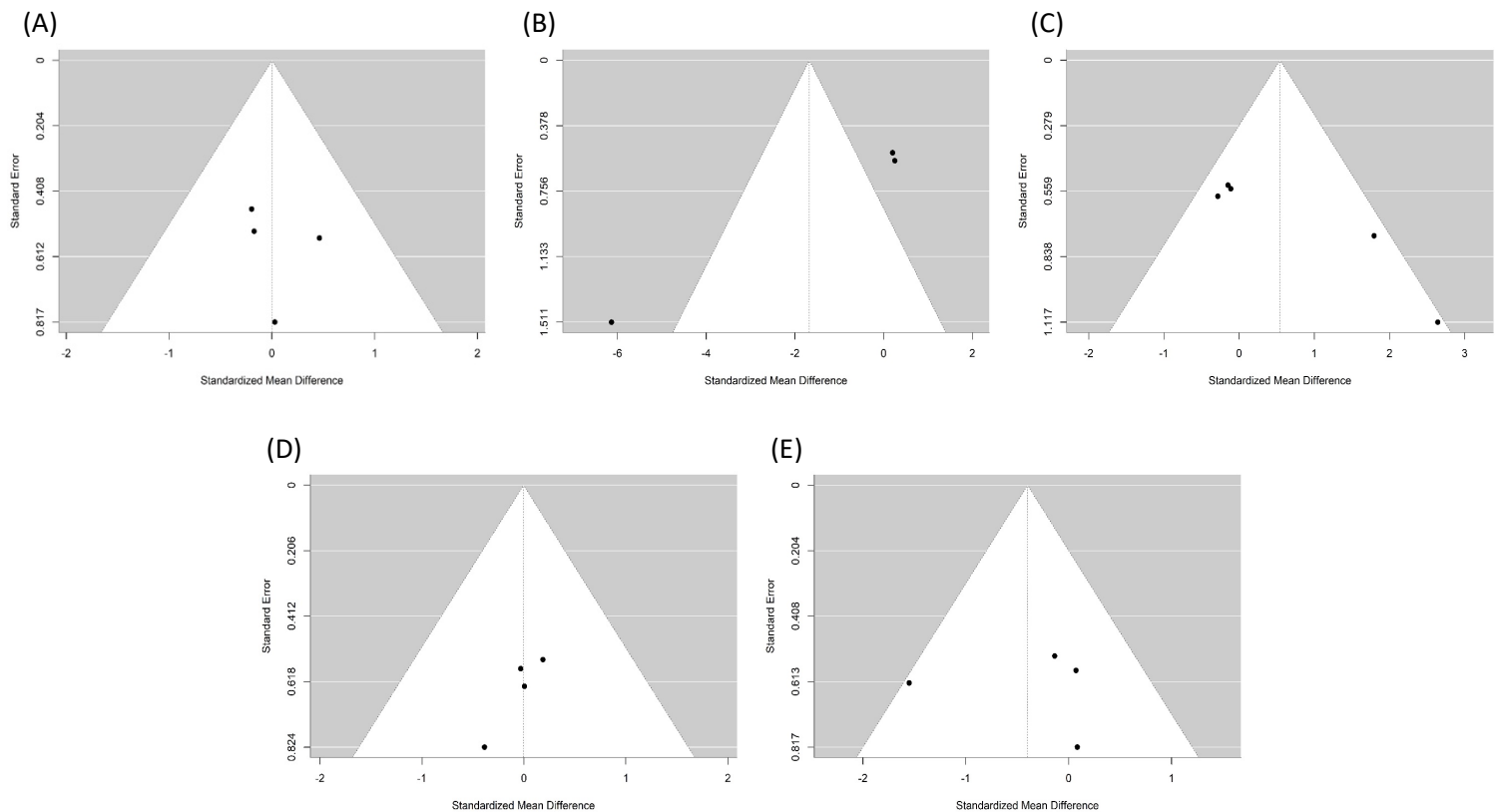


Figure S5. Funnel plots assessing potential publication bias for outcomes included in the meta-analyses: (A) creatinine clearance (Egger's test $p = 0.71$), (B) NGAL levels ($p = 0.071$), (C) mean urine output ($p = 0.026$), (D) renal blood flow ($p = 0.073$), and (E) fractional excretion of sodium ($p = 0.98$). Each point represents an individual study included in the meta-analysis. Egger's regression test was used to evaluate funnel plot asymmetry. Because fewer than ten studies were included in each analysis, the interpretation of funnel plot asymmetry should be considered with caution due to limited statistical power.

Part II. Intra-arterial MSCs
Delivery During NMP of Kidney
Grafts: From Model
Development to Human
Application

Chapter 4. Simultaneous *Ex Situ* Normothermic Perfusion of Paired Kidneys in Pigs

Published as:

Navez M, Gilbo N, Vandermeulen M, Pinto Coelho T, Erpicum P, Lagny MG, Malisoux C, Guarin C, Thierry G, Delanaye P, Cavalier E, Jouret F, Detry O. Simultaneous Ex Situ Normothermic Perfusion of Paired Kidneys in Pigs. *Artif Organs*. 2025;49(9):1405-1416

Abstract

Normothermic Machine Perfusion (NMP) is essential in renal transplantation to improve organ viability before transplantation. This study presents a proof of concept for simultaneous *ex situ* perfusion of paired porcine kidneys using exogenous creatinine and iohexol clearances to assess renal function, with the primary objective of examining intra-individual differences.

Five kidney pairs (n=10) were harvested from pigs, preserved at 4°C for 3 h, and subjected to 6-hour NMP. Each pair was perfused with a solution containing red blood cells and perfusion parameters were monitored continuously. Biochemical parameters were assessed using hourly perfusate and urine samples. Kidney function was evaluated using creatinine, introduced during the priming procedure.

No significant differences were observed between paired kidneys in terms of perfusion and biochemical parameters. Both kidneys maintained stable mean arterial pressures (67.40 ± 12.08 mmHg for right vs. 71.50 ± 4.36 mmHg for left) and flows (68.90 ± 38.61 mL/min vs. 54.00 ± 26.08 mL/min), with consistent electrolyte balance and pH levels. The high inter-individual variability in perfusion and biochemical parameters underscores the importance of paired comparisons.

This pig model of simultaneous NMP of paired kidneys demonstrates that the intra-individual variance is low, which makes it possible to test treatments prior to kidney transplantation using one kidney as a valid comparator of the other.

1. Introduction

Kidney transplantation (KTx) is the best treatment for end-stage renal disease and is associated with lower morbidity and mortality rates than dialysis.²⁵ However, only approximately 20 percent of patients on the waiting list receive a kidney transplant each year, highlighting the fundamental challenges posed by the imbalance between organ availability and clinical demand.^{236,237} Static cold storage (SCS) remains the standard of care for kidney preservation owing to its simplicity, cost-effectiveness, and efficiency. Nevertheless, prolonged cold ischemia can lead to acute tubular necrosis, resulting in delayed graft function and necessitating posttransplant dialysis care.^{238–240} The use of kidneys from donations after circulatory death (DCD) and extended criteria donors (ECD) to expand the donor pool has become increasingly prevalent in transplantation. However, DCD grafts undergo a period of donor warm ischemia, which can severely damage the kidneys and exacerbate ischemia-reperfusion injury (IRI). In addition, DCD kidneys are more vulnerable to damage during cold preservation.^{31,241} High-risk clinical characteristics and extended cold ischemia periods contribute to the frequent discarding of marginal kidneys.^{242,243}

Recent advances in organ preservation and graft evaluation are essential for optimizing the use of fragile kidney grafts. Studies have suggested that *ex situ* kidney perfusion may serve as an effective alternative to SCS.^{57,76,244,245} While there is interest in both normothermic and hypothermic perfusions, there is no consensus on the ideal perfusion strategy for *ex situ* kidney perfusion.^{53,246} Hypothermic Machine Perfusion (4–10°C) allows the use of a simpler perfusion system and a commercially available perfusate, but the reduced metabolic activity of the graft limits the ability to evaluate kidney transplant viability. Normothermic machine perfusion (NMP) is a promising method of organ preservation that uses a warmed, oxygenated, red cell-based, plasma-free solution with extracorporeal membrane oxygenation.^{105,108} By maintaining the organ in a state near physiological conditions, NMP may allow functional testing by restoring its function *ex situ*. Additionally, NMP may permit metabolic recovery by restoring adenosine triphosphate (ATP) levels depleted by warm and cold ischemia.^{247,248} NMP has the potential to optimize the use of DCD kidneys, as their suitability could be evaluated prior to transplantation and treatments could also be administered within this context.^{138,140} Preserving these kidneys under optimal conditions before transplantation ensures immediate functionality and long-term effectiveness.^{75,249,250} Pig kidney NMP models have previously been employed to assess drug efficacy, explore cell therapy, and study metabolism in various injury contexts, with the underlying assumption that both kidneys from the same animal would respond similarly during NMP. However, to the best of our knowledge, this assumption has not been validated experimentally.

This study aimed to develop a model for the simultaneous normothermic *ex vivo* perfusion of porcine kidney grafts obtained from the same donor, with one kidney serving as a paired control.

2. Material and Methods

2.1 — Ethics and Animals

Landrace and Pietrain crossbreed laboratory pigs weighing 30–35 kg were acquired from the Walloon Agricultural Research Centre (CRA-W farm, Gembloux, Belgium). The animals were used for blood collection and kidney procurement. The Animal Ethics Committee of the University of Liege approved the experimental protocols (21-2447). All personnel involved in animal experiments were held by the Federation of European Laboratory Animal Science Associations licenses. All animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institute of Health.

2.2 — Experimental Design

After surgical procurement, 5 kidney pairs underwent simultaneous *ex situ* NMP preservation 3 h after procurement, with creatinine and iohexol injected into the perfusion solution (**Figure 1**). Perfusate and urine samples were collected at 30-minute intervals and hourly, respectively, throughout the 6-hour NMP period. Continuous monitoring of flow and pressure was conducted using hourly data. Kidney biopsies were performed at the end of NMP.

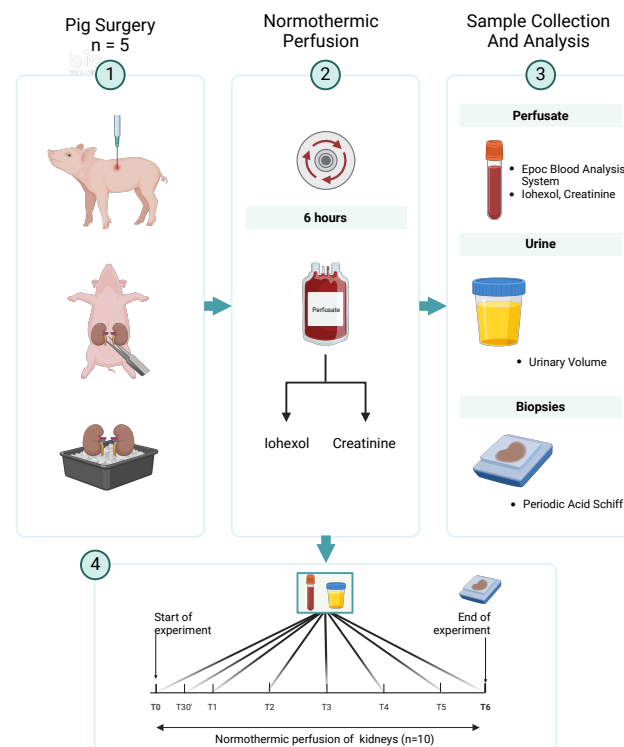


Figure 1. Overview of the experimental design. Ten porcine kidneys underwent concurrent machine perfusion preservation approximately 3 hours post-procurement, with the injection of creatinine and iohexol in the perfusion solution (n=10).

2.3 — Animal Experiments

Animals were first sedated 30 minutes before entering the operating room with an intramuscular injection of tiletamine/zolazepam (4.4 mg.kg⁻¹ equivalent to 2.2 mg.kg⁻¹ of tiletamine and 2.2 mg.kg⁻¹ of zolazepam, Zoletil 100®, Virbac, Leuven, Belgium: containing 250 mg of tiletamine and 250 mg of zolazepam), a peripheral catheter was placed in an auricular vein and intravenous anaesthesia was then induced before orotracheal intubation and maintained during pulmonary ventilation, dissection and euthanasia. Mechanical ventilation was provided by a Datex-Ohmeda ADU S5 care station ventilator, and ventilatory parameters were adapted to allow sufficient oxygenation (PaO₂ > 100 mmHg) and avoid hypercapnic acidosis (PaCO₂ between 35 and 45 mmHg). Anaesthesia was provided using a continuous infusion of propofol (Diprivan®, 20 mg/mL, Aspen Pharma, Dublin, Ireland) at a rate between 2 and 4 mg.kg.h⁻¹ depending on hemodynamic parameters, and analgesia was performed using boluses of 0.2 mcg.kg⁻¹ of sufentanyl (Sufenta®, 5mcg/mL, Viatrix, Canonsburg, Pennsylvanie, USA). Curarization was performed after intubation with boluses of 20 mg cisatracurium (Nimbex® 2 mg · mL⁻¹, Aspen Pharma, Dublin, Ireland) when signs of decurarization were evident. An arterial catheter was placed in the carotid artery to monitor the hemodynamic parameters and blood gas sampling.

After a median laparotomy, the abdominal aorta and inferior vena cava were dissected and isolated. Following the intravenous administration of 25,000 IU of heparin (Leo Pharma B.V., Amsterdam, Netherlands), the abdominal aorta and inferior vena cava were cannulated. After thoracic aorta clamping, the kidneys were flushed through the aortic cannula with 2L of cold (4°C) preservation solution (IGL-1; Institut Georges Lopez, Lissieu, France) and the abdominal cavity was cooled with 4°C saline solution. The pig's blood mass was then drained for salvage, and cardiac arrest was confirmed. Anesthesia and ventilation were stopped once death was confirmed.

Whole blood was collected during renal flush-cooling through the vena cava cannula and processed using a cell-saving system (Xtra™ autotransfusion system; LivaNova, Zaventem, Belgium) to yield concentrated red blood cells without the presence of leukocytes. After bilateral nephrectomy, renal arteries and veins were dissected and cannulated. Kidneys underwent between 3h and 3h30 SCS. Before connecting them to the perfusion circuit, both kidneys were flushed on the back table with 500 mL of IGL-1 at 4°C.

2.4 — Normothermic Machine Perfusion Set-Up

The two kidneys from the same pig underwent simultaneous 6-hour NMP using identical settings on separate NMP circuits (**Figure 2**). Each consisted of one kidney reservoir (Organ Recovery Systems, Inc., Chicago, IL, USA), roller pump, membrane oxygenator (Dideco Perfusion Tubing Systems, LivaNova, Zaventem, Belgium), and heat exchanger (Stockert GmbH, Freiburg, Germany) connected to coated tubing (Soring Group LivaNova, Belgium). The arterial pressure (WPI, Friedberg, Germany) and flow rates (Sonotec, Halle, Germany) were continuously measured. The renal artery was cannulated and connected to the device while the renal vein was left open for drainage. The ureter was then cannulated for urine collection. The perfusion circuits were primed with a perfusion solution composed of homologous red blood cells and an isotonic electrolyte solution (Plasmalyte, Baxter, Lessines, Belgium) to achieve a hematocrit of 20–30% (**Table 1**). Flolan (0.5 mg, GSK, Wavre, Belgium) was continuously infused, and urine was replaced by Plasmalyte in a 1:1 ratio. A parenteral nutrient solution (Aminomix 2 Novum; Fresenius Kabi, Willebroek, Belgium) was administered to maintain a perfusate glucose level above 100 mg/dL and 10mL of sodium bicarbonate (8.4%, B Braun, Melsungen, Deutschland) were added to the perfusate to adjust basal pH. The perfusion temperature was maintained at 38°C, with an airflow rate of 100 mL/min of oxygen and an FiO₂ of 21% administered through the oxygenator. The perfusion solution was prepared and divided into two separate circuits. After reperfusion, the mean arterial perfusion pressure was gradually increased to 60–80 mm Hg in a pressure-controlled system. Intrarenal Resistance (IRR) was calculated by dividing the pressure by the flow and was expressed in mmHg/mL/min/100g of tissue.

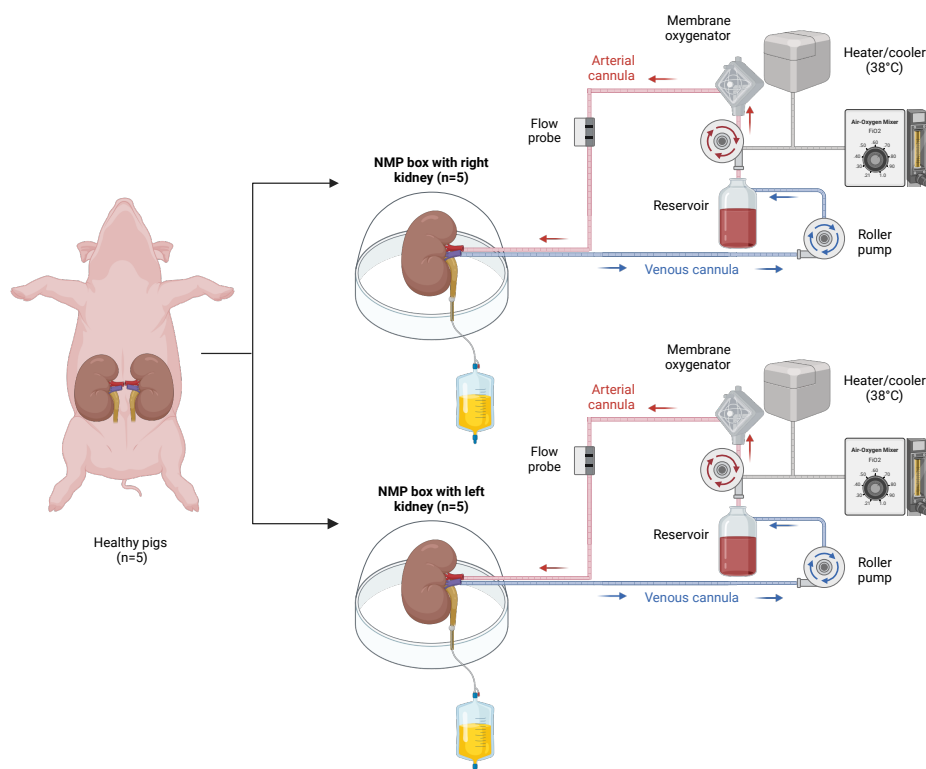


Figure 2. Overview of the simultaneous perfusion of two kidney grafts from the same pig.

Table 1: Composition of the perfusate used for the simultaneous NMP of kidneys.

NMP Perfusate Solution	
Priming solution	
Autologous concentrated red cells	500 mL
Plasmalyte	500 mL
Additives in bolus	
Albumin 20%	100 mL
Cefuroxime	1500 mg
Sodium bicarbonate (8,4%)	10 mL
Mannitol (15%)	13.4 mL
Creatinine	75 mg
Iohexol	518 mg
Infusions during perfusion	
Epoprostenol	8.3 µg/h
Plasmalyte	10 mL/h

2.5 — Sample Collection and Analyses

The sampling line was attached to the arterial inflow cannula. Perfusate samples were collected using SST™ BD Microtainer® (New Jersey, USA) blood collection tubes and subsequently centrifuged at 2,000g for 5 min at 4°C.

The resulting supernatant was aliquoted and preserved at -80°C until further analysis. All samples were analysed, and all data points were included in the analysis. One pig was excluded because its pH level fell below the threshold of 7. All other pigs used in this study met the inclusion criteria. Biochemical parameters were assessed in the perfusate obtained from the arterial line using an Epoc Blood Analysis System (Siemens Healthcare NV, Dilbeek, Belgium). Kidney function was evaluated using a prespecified dose of creatinine (GE Healthcare, Diegem, Belgium) and iohexol (Omnipaque 240 mg I/mln, GE Healthcare, Diegem, Belgium), which were introduced into the perfusion solution during the priming phase. These compounds were then cleared from the perfusate by the kidneys over time. Kidney biopsies were taken at the end of perfusion, and histological samples were harvested, fixed in 4% formalin, embedded in paraffin, and stained with hematoxylin-eosin and Periodic Acid-Schiff (PAS). Histopathological analysis was performed by an expert nephropathologist in a blinded manner.

2.6 — Statistical Analyses

Given the exploratory nature of this study, which aimed to establish a simultaneous perfusion model, formal sample size calculation was not feasible. A sample size of $n=5$ animals was chosen to account for the biological variation typically observed between experimental units in large-animal models. Results are presented per kidney or expressed as the mean $\pm$ standard deviation. Data were visualized using the GraphPad Prism software (GraphPad Software, San Diego, CA, USA, version 10). A generalized linear mixed model was employed to evaluate the effects of time, the kidney, and the interaction between time and the kidney. A compound symmetry covariance matrix was used to account for the repeated measures. Residual normality and homoscedasticity assumptions were checked, and the data were log-transformed when necessary. For the two parameters, beta regression was applied owing to the specific data characteristics. Model quality was assessed using Akaike's Information Criterion and Bayesian Information Criterion, with smaller values indicating a better model fit. Statistical significance was defined at the 5% level ($p<0.05$) using SAS (version 9.4) and R (version 4.2.2), with the Inormimp package for censored data imputation.

3. Results

3.1 — Animal Characteristics

The characteristics and preoperative blood parameters of the five pigs are presented in **Table 2**. No significant difference was observed between the right (119.0 ± 19.1 g) and left kidney weights (118.4 ± 23.2 g), $p=0.62$. The mean hemoglobin level was 9.38 ± 0.81 g/dL, while the creatinine level averaged 0.84 ± 0.21 mg/dL. The pH levels had a mean of 7.42 ± 0.03 , and the mean pO₂ level was 194.8 ± 54.9 mmHg. The mean glucose level was 129.8 ± 15.6 mg/dL.

The minor inter-individual variations in the cold ischemic period showed no correlation with the perfusion parameters.

Table 2: Overview of animals and perfusion characteristics of basal pig.

	Pig 1	Pig 2	Pig 3	Pig 4	Pig 5	Mean $\pm$ SD
Animal Weight (Kg)	33	32	33	32	35	33.0 $\pm$ 1.2
Right Kidney Weight (g)	110	153	113	110	109	119.0 $\pm$ 19.1
Left Kidney Weight (g)	111	157	95	118	111	118.4 $\pm$ 23.2
Hemoglobin (g/dL)	8.4	9.6	9.1	10.6	9.2	9.38 $\pm$ 0.81
Creatinine (mg/dL)	0.67	0.90	1.15	0.63	0.85	0.84 $\pm$ 0.21
pH	7.459	7.389	7.411	7.416	7.432	7.42 $\pm$ 0.03
pO ₂ (mmHg)	187.5	281.9	160.4	205.3	138.9	194.8 $\pm$ 54.9
Glucose (mg/dL)	120	135	108	147	139	129.8 $\pm$ 15.6
Cold Ischemic Time (min)	178	210	207	190	183	193.6 $\pm$ 14.3

3.2 — Hemodynamic and Metabolic Function Parameters

Five kidney pairs were perfused; the perfusion parameters are listed in **Table 3**. Hemodynamic parameters, including arterial flow and intrarenal resistance, are illustrated in **Figure 3A** for arterial flow and **Figure 3B** for IRR. Their comparison during the 6-hour perfusion in five right kidneys *versus* five left kidneys is presented in **Table 4**.

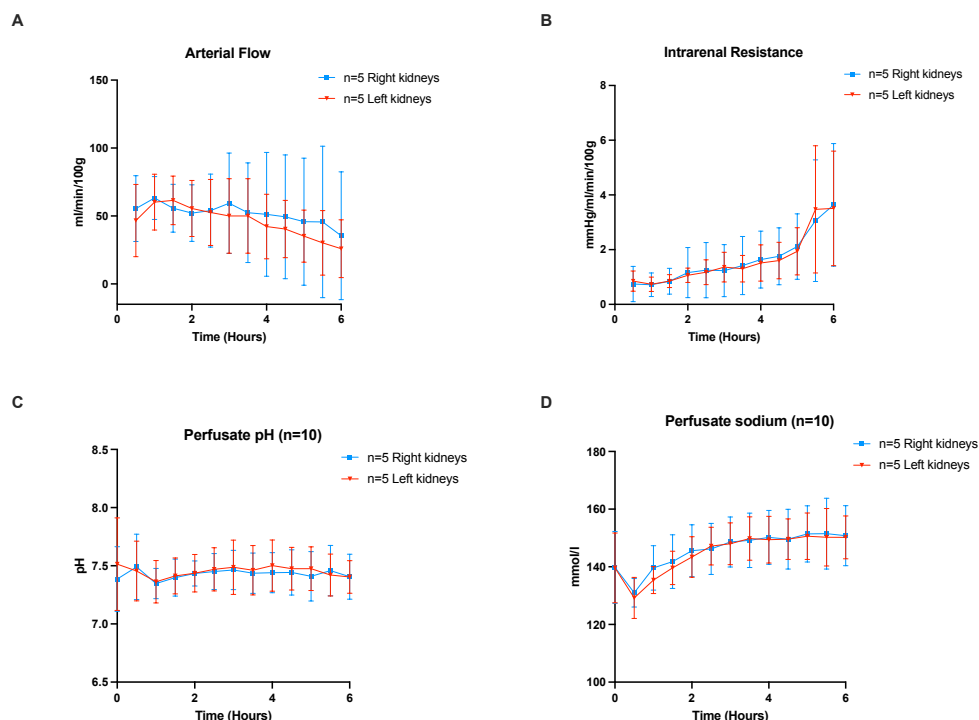


Figure 3. A, Arterial flow (n = 10) in mL/min/100g in right and left kidneys perfusions. B, Intrarenal Resistance (n = 10) in mmHg/mL/min/100g in right and left kidneys perfusions. C, Perfusate pH (n = 10) in right and left kidneys perfusions. D, Perfusate sodium (n = 10) in mmol/L in right and left kidneys perfusions.

Chapter 4. Simultaneous Ex Situ Normothermic Perfusion of paired Kidneys in Pigs

Table 3: Hemodynamic and metabolic function parameters per pair of kidneys¹.

Kidney pair	Pair 1		Pair 2		Pair 3		Pair 4		Pair 5		<i>p-value</i>
	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	
Arterial Pressure (mm Hg)	76.8 ± 9.1	76.5 ± 10.1	79.6 ± 12.9	77.6 ± 14.2	71.4 ± 8.7	67.8 ± 8.6	64.8 ± 11.9	63.7 ± 10.0	49.9 ± 25.0	67.2 ± 18.7	0.51
Arterial Flow (mL/min/100g)	53.8 ± 18.0	66.8 ± 25.9	41.6 ± 22.9	30.1 ± 14.3	42.0 ± 16.1	44.7 ± 14.3	18.9 ± 7.2	24.0 ± 8.7	101.6 ± 28.8	63.8 ± 12.4	0.56
Resistance index (mmHg/mL/min/100g)	1.5 ± 1.0	1.4 ± 1.3	1.2 ± 0.8	1.2 ± 0.5	1.6 ± 0.9	1.9 ± 0.8	3.3 ± 1.6	2.5 ± 1.9	0.4 ± 0.1	0.8 ± 0.2	0.92
pH	7.67 ± 0.07	7.74 ± 0.10	7.36 ± 0.13	7.39 ± 0.18	7.42 ± 0.16	7.37 ± 0.04	7.26 ± 0.08	7.27 ± 0.07	7.44 ± 0.12	7.49 ± 0.16	0.15
Sodium (mmol/L)	155.8 ± 10.02	150.7 ± 9.3	141.0 ± 10.9	142.5 ± 13.3	150.1 ± 5.1	147.4 ± 5.5	134.2 ± 3.8	135.3 ± 3.8	147.0 ± 4.9	147.5 ± 6.4	0.90
Chloride (mmol/L)	97.4 ± 7.9	94.9 ± 9.0	101.6 ± 4.6	104.3 ± 5.7	103.2 ± 2.5	101.0 ± 3.5	97.1 ± 5.2	97.4 ± 5.2	100.2 ± 4.6	101.5 ± 4.2	0.69
Calcium (mmol/L)	0.63 ± 0.35	0.76 ± 0.30	0.75 ± 0.17	0.80 ± 0.15	1.12 ± 0.14	1.08 ± 0.13	1.00 ± 0.12	1.01 ± 0.12	0.50 ± 0.08	0.54 ± 0.06	0.20
Potassium (mmol/L)	2.8 ± 0.8	2.8 ± 1.1	2.4 ± 0.9	2.3 ± 1.3	2.5 ± 1.0	2.4 ± 0.8	5.8 ± 0.6	5.3 ± 0.7	2.9 ± 0.7	2.8 ± 1.0	0.04
Hemoglobin (g/dL)	10.6 ± 0.8	11.1 ± 1.2	11.1 ± 1.0	12.0 ± 1.1	6.9 ± 0.4	7.1 ± 0.4	6.7 ± 0.7	6.8 ± 0.4	6.4 ± 0.4	6.1 ± 0.7	0.35
Hematocrit (%)	31 ± 2.2	32.7 ± 3.4	32.6 ± 3.1	35.2 ± 3.2	20.3 ± 1.4	20.9 ± 1.4	19.5 ± 1.9	19.9 ± 1.1	18.9 ± 1.1	17.9 ± 2.0	0.41
Glucose (mg/dL)	84.4 ± 22.8	91.7 ± 28.1	173.3 ± 34.4	178.1 ± 34.9	120.0 ± 25.9	134.4 ± 28.6	113.5 ± 26.9	110.6 ± 23.9	126.4 ± 14.7	134.2 ± 17.1	0.12
Arterial PO ₂	89.1 ± 20.1	100.0 ± 7.0	120.0 ± 21.6	110.3 ± 6.1	107.7 ± 38.8	107.8 ± 40.9	115.5 ± 13.7	110.3 ± 26.0	91.9 ± 26.3	103.0 ± 29.1	0.27
Arterial PCO ₂	39.9 ± 9.1	33.7 ± 11.0	37.7 ± 16.3	32.5 ± 5.2	40.6 ± 7.2	38.2 ± 0.8	34.2 ± 11.1	35.9 ± 11.4	39.6 ± 6.4	44.1 ± 15.0	0.96
Temperature (°C)	35.9 ± 1.8	37.4 ± 2.0	33.1 ± 5.0	35.3 ± 1.9	35.5 ± 2.1	35.1 ± 1.5	33.4 ± 1.2	34.1 ± 1.6	36.5 ± 2.3	34.9 ± 1.8	0.60

¹ Values represented mean ± SD. Time-averaged longitudinal mean value compiled from 30'- measurements over the course of the perfusion

Table 4: Comparison of perfusion characteristics between right and left kidneys from the same pig.

	Right kidneys (n=5)	Left Kidneys (n=5)	Paired t-test p
Arterial pressure in mm Hg	67.40 ± 12.08	71.50 ± 4.36	0.51 (ns)
Arterial flow in mL/min/100g	51.59 ± 30.67	45.90 ± 19.29	0.56 (ns)
IRR in mm Hg/mL/min/100g	1.60 ± 1.06	1.58 ± 0.64	0.92 (ns)
pH	7.43 ± 0.17	7.46 ± 0.18	0.15 (ns)
Sodium in mmol/L	147.70 ± 8.53	147.50 ± 6.63	0.90 (ns)
Potassium in mmol/L	3.04 ± 1.46	2.80 ± 1.38	0.04 (*)
Calcium in mmol/L	0.76 ± 0.28	0.82 ± 0.21	0.20 (ns)
Hematocrit in %	24.60 ± 6.35	25.40 ± 8.05	0.41 (ns)
Hemoglobin in g/dL	8.36 ± 2.11	8.66 ± 2.70	0.35 (ns)
Glucose in mg/dL	123.30 ± 33.46	130.10 ± 30.34	0.12 (ns)
Cumulative urine (mL)	75.60 ± 44.17	90.40 ± 47.55	0.18 (ns)

3.3 — Arterial Pressure, Flow and Intrarenal Resistance

Arterial pressure increased significantly over time ($p < 0.0001$). The arterial flows showed no significant difference, with right kidneys exhibiting a mean flow of 51.59 ± 30.67 mL/min/100g and left kidneys 45.90 ± 19.29 mL/min/100g ($p = 0.56$). The flow rate decreased significantly over time ($p < 0.0001$) regardless of the kidney side (left or right). There was no difference in IRR of right (1.60 ± 1.06 mmHg/mL/min/100g) vs. left kidneys (1.58 ± 0.64 mmHg/mL/min/100g, $p = 0.92$). Resistance increased significantly over time ($p < 0.0001$), regardless of the kidney side (left or right). The paired t-test results for arterial pressure, arterial flow, and IRR indicated that there was no difference between the right and left kidneys during the 6-hour NMP ($p > 0.05$). Of note, for further experiments testing conditioning strategies, 47% of data variability was explained by inter-individual differences, which underlines the importance of intra-individual simultaneous paired comparisons. Generalized Linear Mixed Model (GLMM) is represented in **Suppl. File Table 1**.

3.4 — pH Levels

The pH course of each perfused kidney is shown in **Figure 3C**. The average pH values for the right and left kidneys were 7.43 ± 0.17 and 7.46 ± 0.18 ($p = 0.15$), respectively, and no significant changes detected during the 6-hour NMP period. The variability in the pH levels was largely attributed to inter-animal differences, accounting for 59% of the total variability. No statistically significant differences were observed between the left and right kidneys ($p > 0.05$).

Figure 4A presents paired data that enable visual assessment of inter-organ differences between the right and left kidneys of each animal. The individual variations were minor, as the mean difference was close to zero, suggesting no significant difference between the right and left kidneys.

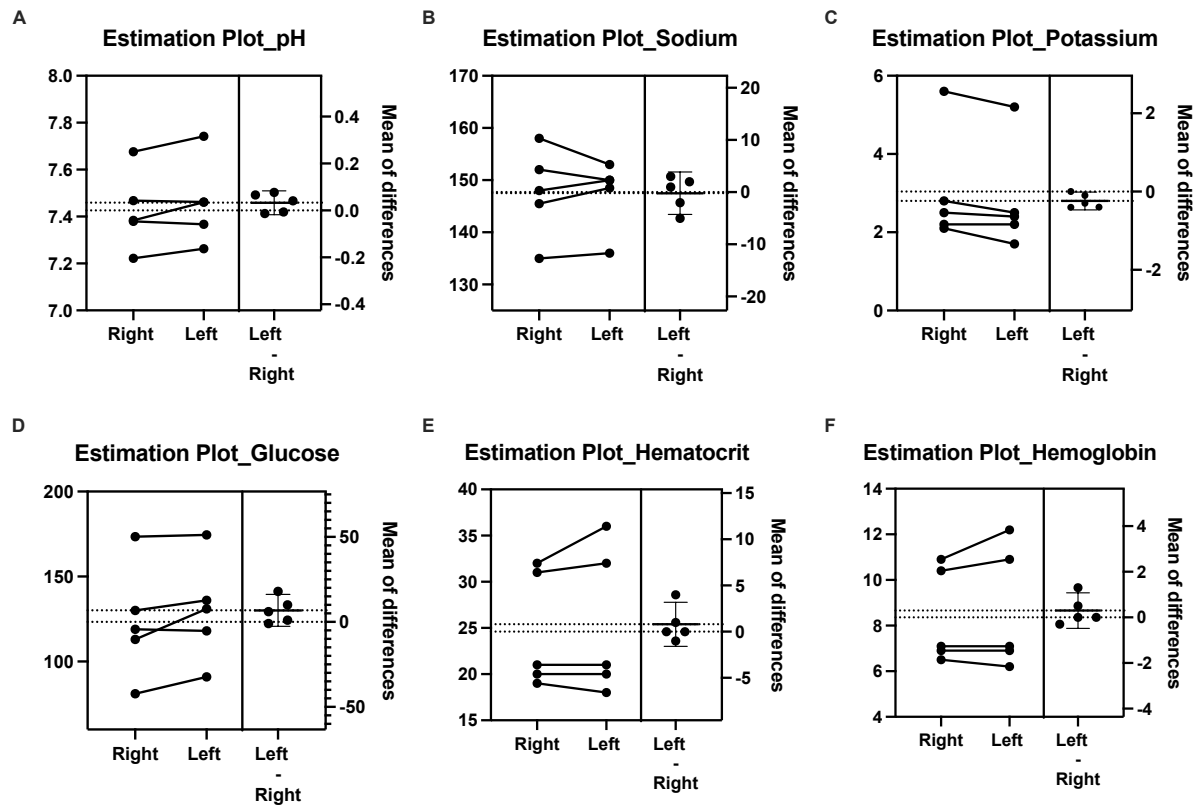


Figure 4. A–F, Estimation plots comparing physiological measurements taken from the right and left kidneys of the same pig during a 6-hour normothermic perfusion. The six panels illustrate differences between sides for pH, Sodium, Potassium, Hemoglobin, Hematocrit, and Glucose levels.

3.5 — Electrolytes Measurements: Sodium and Potassium

As illustrated in **Figure 3D**, the sodium levels in both kidneys started at approximately 140 mmol/L and showed minor fluctuations during perfusion. Sodium concentrations measured 147.70 ± 8.53 mmol/L for right kidneys and 147.50 ± 6.63 mmol/L for left kidneys, showing no significant difference ($p=0.90$). However, the sodium levels increased over time ($p<0.0001$), with 38% of the variability explained by individual pig differences. As shown in **Figure 4B**, the mean difference was slightly positive, indicating a slight tendency toward higher sodium levels on the left side. While some animals exhibited larger discrepancies, the average suggests that these differences are not systematic. Potassium levels (**Table 3**) remained stable but were significantly higher in both kidneys of pair 4 than in the other kidneys ($p<0.0001$). No significant differences were observed between the left and right kidneys ($p>0.05$).

In **Figure 4C**, potassium levels indicate that variations exist between individuals, but are mostly balanced, indicating a lack of significant preference.

3.6 — Glucose Measurements

Glucose concentrations declined significantly and progressively over the perfusion period, reflecting consumption despite the administration of a parenteral solution to regulate glucose levels. This decline was similar in both the kidneys. Glucose concentrations were 123.30 ± 33.46 mg/dL in the right kidneys and 130.10 ± 30.34 mg/dL in left kidneys ($p=0.12$). In **Figure 4D**, the glucose measurements show considerable individual variability, but the mean difference suggests a balanced distribution.

3.7 — Haematological Parameters: Haemoglobin and Haematocrit Levels

Both parameters exhibited no significant differences between the kidneys, indicating stable perfusion. No significant time or kidney effect was observed for hematocrit and hemoglobin levels, with 21% and 19% variability attributed to pig differences, respectively. Hematocrit values ranged from 20% to 35% with a slightly positive mean difference, suggesting a small tendency for higher levels on the left side, although these individual differences were not consistent across all animals in **Figure 4E**. **Figure 4F** show haemoglobin levels ranging from approximately 6 to 13 g/dL, with a mean difference near zero, indicating no significant side differences, despite some individual variations.

3.8 — Urine Production

The functional assessment was further supported by hourly urine output measurements. Cumulative urine output did not differ significantly between the right and left kidneys, with both maintaining steady production over the 6-hour period. Cumulative urine volume increased significantly over time ($p<0.0001$), with a significant kidney effect and no interaction effect, and 15% of the variability was explained by pig differences.

3.9 — Assessment Of Renal Function: Creatinine and iohexol clearances

Creatinine clearance remained consistent across all kidneys, reflecting stable glomerular filtration rates throughout perfusion. Creatinine concentration significantly decreased over time ($p<0.0001$), with 21% variability attributed to inter-pig differences (**Figure 5A**). Paired t-test analyses comparing the right and left kidneys revealed no statistically significant differences in the elimination of creatinine ($p=0.43$) from the perfusate.

Similarly, iohexol levels decreased significantly over time during NMP ($p < 0.0001$), with no intra-individual right *versus* left difference. The inter-individual difference between pigs was responsible for 6.4% of the variability. A similar pattern of creatinine and iohexol elimination during NMP was observed across all samples ($n=10$). The elimination of both tracers mainly occurred during the first two hours *post* infusion (**Figure 5B**). The GLMM indicated a significant association between iohexol and creatinine clearances ($p < 0.0001$), which was independent of time ($p=0.14$). Note that a time-dependent reduction in clearance was observed at later time points, particularly at 180 minutes compared to baseline ($p=0.0134$). Iohexol clearance was characterized by a coefficient of 0.0118 ± 0.0017 ($p < 0.0001$). Of note, the AIC (-7.0) and BIC (-7.8) criteria confirmed the robustness of the model fit (**Suppl. File Table 2**).

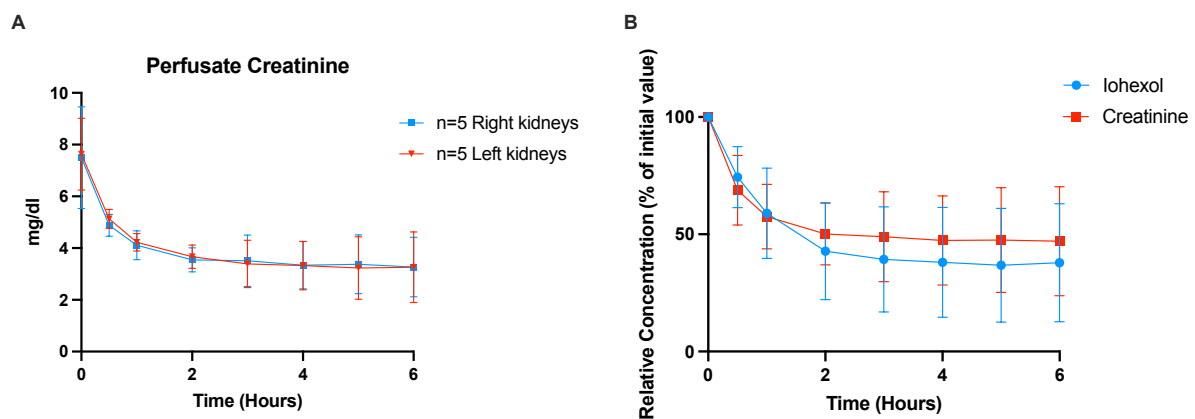


Figure 5. A, Perfusate creatinine in mg/dl: Mean and SD of 5 right and 5 left kidneys perfusions ($n=10$). B, Relative concentration of creatinine and iohexol during perfusion ($n=10$).

3.10 — Histological Results

Histological analysis of the kidney biopsies was performed exclusively at the end of perfusion. PAS staining revealed features suggesting acute tubular necrosis with marked loss of the brush border and thinning of the tubular epithelium lining the proximal tubule, with no evidence of cellular desquamation into the lumen. Peritubular capillaritis was observed in all the samples to the same extent (**Figure 6**).

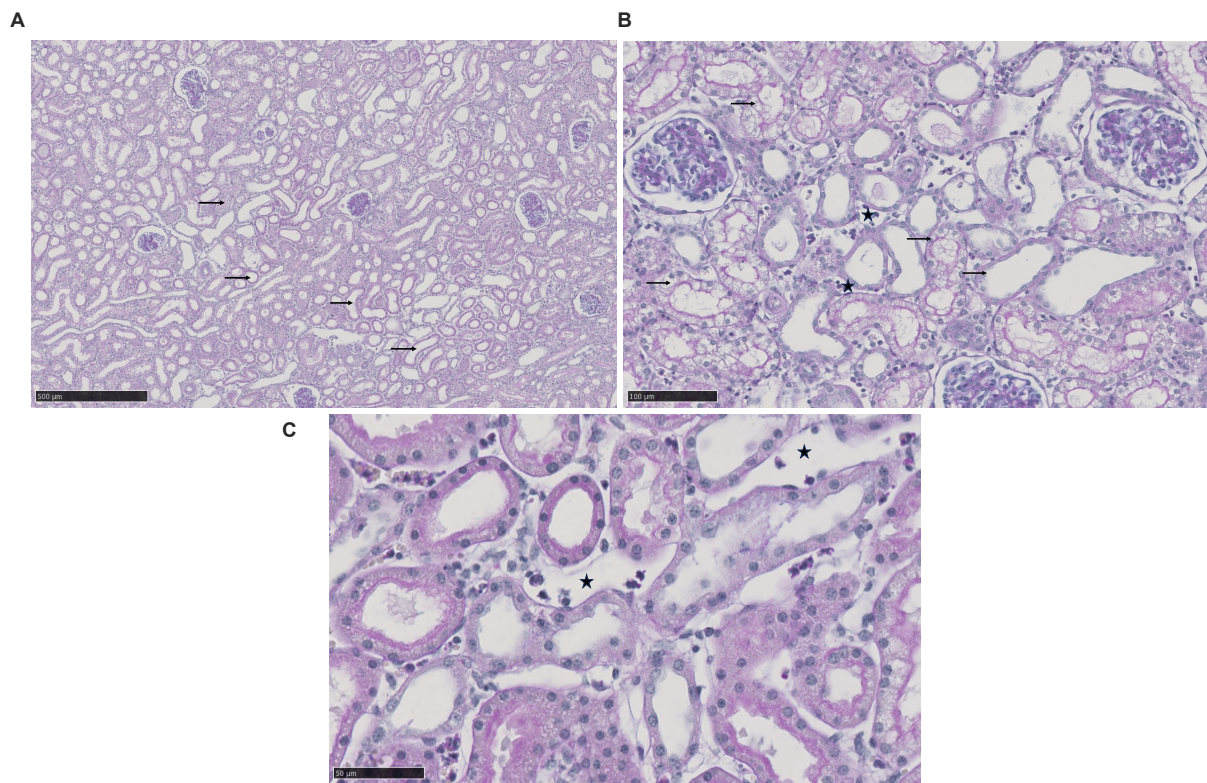


Figure 6. A and B, Acute tubular necrosis with thinning of the tubular epithelium and cytoplasmic vacuolization of proximal tubular epithelial cells. Original Magnification x5 (A); x20 (B), PAS staining. C, Peritubular capillaritis. Original magnification x 40, PAS staining. Scale bars = 500 μm (A), 100 μm (B), 50 μm (C)

4. Discussion

The primary aim of this study was to develop a pig model for the simultaneous *ex vivo* normothermic perfusion of two kidneys from the same animal to demonstrate the potential use of one kidney as a control for the other. This model opens the possibility of studying conditioning strategies to prevent or limit IRI. Furthermore, our findings demonstrate several critical aspects of renal physiology and function during NMP, offering valuable contributions to the knowledge on KTx and preservation.

Throughout the 6-hour normothermic perfusion period, we observed stable hemodynamic and metabolic parameters, indicating effective perfusion and organ functionality. Arterial pressure, flow, and intrarenal resistance were maintained, and no significant differences were observed between the right and left kidneys of each animal. The stability of these parameters suggests that our perfusion model successfully preserved renal hemodynamic, which is crucial for maintaining organ viability during preservation.^{206,250,251}

Electrolyte levels, including sodium and potassium levels, remained stable throughout the perfusion period, indicating the effective regulation of ionic balance. The absence of significant differences between the right and left kidneys further supports the reliability of our perfusion model for maintaining electrolyte homeostasis. These results are consistent with previous reports highlighting the ability of NMP to preserve electrolyte balance and support renal metabolic functions.^{88,252,253} Glucose concentrations decreased progressively over the perfusion period, reflecting metabolic consumption despite the administration of a parenteral nutrient solution. This decline in glucose levels suggests active metabolic processes during NMP, as confirmed by previous studies demonstrating the restoration of ATP levels and metabolic activity in kidneys subjected to NMP.^{72,254} The comparable metabolic activity observed in both kidneys indicated that this model effectively supported glucose metabolism, an essential aspect of organ preservation. Consistent urine production observed during the 6-hour perfusion period serves as a functional indicator of renal activity and viability. The cumulative urine output did not differ significantly between the right and left kidneys. This study highlights the potential of NMP as a transformative approach for KTx. By maintaining physiological conditions and enabling real-time functional assessment, NMP can optimize organ utilization, particularly for kidneys, from DCD and ECD sources.

There is no clear consensus on perfusion protocols, including the use of red blood cells or hemoglobin-based oxygen carrier-based perfusion fluid.^{72,255} Strategies for circuit structure, perfusate composition, and assessment of perfused organ viability vary across published studies, necessitating ongoing research to determine the optimal method for organ perfusion.⁷¹ Studies have shown that using whole blood in liver perfusion can provoke a heightened inflammatory response due to the presence of immune cells, resulting in elevated cytokine levels and greater tissue damage.²⁵⁶ However, researchers indicated that during kidney perfusion, there was no significant difference in cytokine levels or renal function between whole blood and RBC-based perfusates, highlighting the benefits of leukocyte-depleted blood in reducing excessive inflammation.⁹¹ Considering this evidence, we selected an RBC-based perfusate to maintain sufficient oxygenation while minimizing immune activation. The red blood cells used for perfusion in this study were sourced from the same pig from which the kidneys were harvested, further enhancing the relevance and interest of this study.

Our pilot study pioneers the application of iohexol clearance as a reproducible method for assessing glomerular filtration in porcine kidneys during NMP. Thus far, most studies about NMP have evaluated the renal function using creatinine as the conventional biomarker.^{75,76} However, creatinine levels are subject to significant well-known non-GFR-related variability, which may similarly impact measurements in pig kidneys. Furthermore, the endogenous circulating porcine creatinine may bias the clearance of human creatinine used as GFR tracer during the NMP.

In order to circumvent these creatinine-related limitations in GFR assessment, iohexol clearance has been developed and validated in research and clinical routine.¹³ Note that iohexol clearance has not been tested in human kidneys under dynamic NMP. Still, we demonstrate here an intra-individual right *versus* left concordance of iohexol clearance across kidney pairs. The inter-pig variability in clearance rates, likely due to differences in vascular resistance or NMP settings, did not affect the intra-pair reproducibility. Future research will explore iohexol clearance in larger cohorts and assess the effects of extended perfusion times and ischemia on GFR to validate our pilot study across diverse preclinical and clinical settings.

Our study has certain limitations. The use of a well-controlled pig kidney model with short cold ischemia and a limited sample size makes it difficult to establish the precise reference ranges for normal kidney function during NMP. Rather than defining strict thresholds, this study aimed to assess the feasibility of the model. To further investigate the sensitivity of NMP in detecting kidney dysfunction, it would be valuable to develop a model incorporating a defined period of warm ischemia to induce ischemic injury. Additionally, while the dual kidney perfusion approach ensures standardized conditions and minimizes variability in experimental comparisons, it does not replace clinical single-organ perfusion. Instead, it provides a controlled framework for evaluating therapeutic interventions to mitigate ischemia-reperfusion injury.

In conclusion, this study establishes the use of a paired kidney NMP model, in which one kidney serves as a control for the other. This experimental design provides a robust framework for evaluating therapeutic interventions in the context of NMP^{125,133,148,257}. This allows for the assessment of the true impact of these treatments on the kidneys under identical conditions and from the same donor. These findings contribute to the growing evidence supporting NMP as a valuable tool in KTx, with the potential to improve organ utilization and patient outcomes. Further research and clinical trials are necessary to validate these findings and establish NMP as a standard practice for KTx.

5. Supplementary File

Table 1: Results of the generalized linear mixed models on perfusion parameters.

	Art.pressure (mmHg)	Flow (mL/min)* ²	IRR*	pH	Na+ (mmol/L)	K+(mmol/L)*
Temps	13.7 ± 1.27** (p < 0.0001)	-0.0026 ± 0.00033 (p < 0.0001)	0.0043 ± 0.0003 (p < 0.0001)	/	8.48 ± 0.48** (p < 0.0001)	-0.0058 ± 0.00064 (p < 0.0001)
Temps (log)	/	/	/	/	/	0.000015 ± 1.74x10-6 (p < 0.0001)
AIC	886.4	134.1	115.0	-154.8	669.8	24.8
BIC	885.6	133.4	114.2	-156.0	669.0	24.0
ICC	0.19	0.47	0.43	0.59	0.38	0.59
Covarian ce	70.3 ± 52.6 (p = 0.18)	0.24 ± 0.17 (p = 0.17)	-0.47 ± 0.26 (p = 0.14)	0.021 ± 0.013 (p = 0.12)	52.9 ± 37.8 (p = 0.16)	0.15 ± 0.11 (p = 0.16)
Residual	98.8 ± 13.2 (p < 0.0001)	0.13 ± 0.018 (p < 0.0001)	0.0043 ± 0.0003 (p < 0.0001)	0.014 ± 0.0018 (p < 0.0001)	14.5 ± 1.93 (p < 0.0001)	0.039 ± 0.0058 (p < 0.0001)
	Glu (mg/dL)	Hct(%)**	Hb (g/dL)**	Cum. urine (mL)**	Creat (mg/dL)*	Iohexol (mg/dL)
Temps	0.47 ± 0.55 (p < 0.0001)	/	/	0.0051 ± 0.00057 (p < 0.0001)	-0.0018 ± 0.0002 (p < 0.0001)	-0.11 ± 0.013 (p < 0.0001)
Temps (log)	-0.0015 ± 0.00015 (p < 0.0001)	/	/	-0.67 ± 0.12 (p < 0.0001)	/	/
AIC	1151.6	-178.89	-188.54	-203.92	27.9	663.1
BIC	1150.8	-180.45	-190.10	-206.26	27.1	662.3
ICC	0.54	0.14	0.13	0.15	0.21	0.064
Covarian ce	966 ± 692.49 (p = 0.17)	0.21	0.19	0.32	0.037 ± 0.027 (p = 0.18)	48.7 ± 43.3 (p = 0.26)
Residual	352.7 ± 45.3 (p < 0.0001)	1.30 ± 0.97 (p = 0.090)	1.22 ± 0.90 (p = 0.088)	1.71 ± 1.30 (p = 0.094)	0.056 ± 0.0071 (p < 0.0001)	199.2 ± 32.7 (p < 0.0001)

² *Logarithmic transformation of the outcome

**beta regression

Table 2: Generalized Linear Mixed Model Analysis Assessing the Effects of Time, Iohexol, and Their Interaction (Time × Iohexol).

Effect		Coefficient	SE	DF	t Value	p-valeur
Intercept		1.1966	0.1315	4	9.10	0.0008
Temps	30	0.05923	0.1827	16	0.32	0.7500
p = 0.054	60	-0.2429	0.2161	16	-1.12	0.2776
	120	-0.1307	0.1765	16	-0.74	0.4697
	180	-0.4476	0.1611	16	-2.78	0.0134
	0	0	.	.	.	.
Iohexol (mg/dL)		0.01180	0.001701	36	6.94	<.0001
Iohexol*Temps	30	-0.00458	0.003329	36	-1.38	0.1770
P = 0.14	60	0.000800	0.005081	36	0.16	0.8758
	120	-0.00374	0.004915	36	-0.76	0.4522
	180	0.007563	0.004403	36	1.72	0.0945
	0	0	.	.	.	.

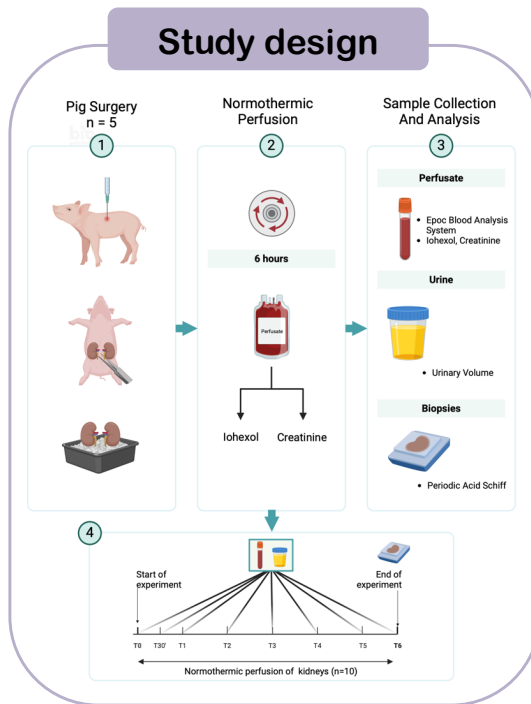
AIC = -7.0

BIC = -7.8

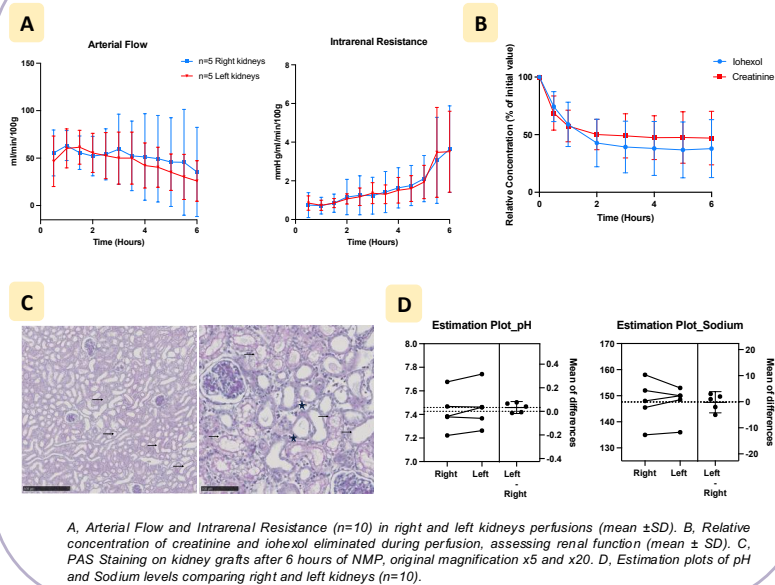
Simultaneous *ex situ* normothermic perfusion of paired kidneys in pigs

Margaux Navez *, Nicholas Gilbo, Morgan Vandermeulen, Tiago Pinto Coelho, Pauline Erpicum, Marc Gilbert Lagny, Clarisse Malisoux, Claire Guarin, Gabriel Thierry, Pierre Delanaye, Etienne Cavalier, François Jouret and Olivier Detry

Study design



Results



This model of **parallel and simultaneous normothermic perfusion** of pig kidneys could be used for testing innovative therapies, both in swine kidneys and human discarded grafts, using the **contralateral kidney as a paired control**



Chapter 5. Infusion of hMSCs during NMP of porcine kidneys: a randomized, paired, blinded, preclinical study

Manuscript submitted and in review in *Scientific Reports*:

Navez M., Gilbo N., Vandermeulen M., Erpicum P., Lagny M.G., Malisoux C., Guarin C., Pinto Coelho T., Thierry G., Lechanteur C., Briquet A., Baudoux E., Le Goff C., Cavalier E., Caers J., Jouret F., Detry O. *Infusion of human mesenchymal stromal cells during normothermic machine perfusion of porcine kidneys: a randomized, blinded, preclinical study.*

Abstract

Kidney transplantation from donation after circulatory death (DCD) donors is increasingly used but remains associated with warm ischemic injury. Normothermic machine perfusion (NMP) enables functional assessment and therapeutic interventions. Mesenchymal stromal cells (MSCs) display immunomodulatory and regenerative properties, yet their translational efficacy during NMP remains unclear.

In a porcine model, six pairs of kidneys subjected to 30 minutes of warm ischemia followed by 3 hours of static cold storage underwent 6 hours of NMP. In each pair, one kidney randomly received an intra-arterial injection of placebo, while the contralateral kidney received 10 million clinical-grade human bone marrow–derived MSCs (hMSCs). Perfusion characteristics, glomerular filtration, tissue injury, and inflammatory markers were assessed.

hMSC infusion during NMP was technically feasible and hemodynamically well tolerated, with no adverse effects on perfusion stability. Perfusion parameters, urine output, and creatinine/iohexol clearance showed no significant differences between groups. NGAL and cytokines levels increased during perfusion, but hMSCs did not alter their dynamics.

Intra-arterial delivery of clinical grade hMSCs during NMP was safe but did not improve renal function or reduce histological injury. These results highlight the challenges of achieving MSC engraftment during *ex situ* perfusion and highlight the need for refined strategies such as repeated dosing, prolonged perfusion, or extracellular vesicle therapy. Despite the negative findings, this study presents a highly translational large-animal model, supporting further investigation of MSC-based therapies in human kidneys discarded for transplantation.

1. Introduction

Kidney transplantation remains the optimal treatment for end-stage renal disease; however, its potential is constrained by a significant global shortfall of suitable organ donors.²⁵⁸ This shortage contributes to a prolonged transplantation waiting lists, and has driven a growing reliance on grafts obtained from donation after circulatory death (DCD) donors.^{25,31,237} These DCD donors may increase organ availability for transplantation.²⁵⁹ Evidence suggests that kidneys from DCD donors can achieve short- and mid-term survival rates comparable to those obtained from donation after brain death (DBD) donors. However, the higher incidence of primary graft dysfunction, coupled with the detrimental effects of the warm ischemia time inherent to DCD procurement, remains a significant limitation.^{34,260}

Machine perfusion is emerging as a valuable approach to kidney graft preservation, offering advantages over static cold storage (SCS), the current gold standard, by enabling both improvement and assessment of graft quality prior to transplantation.^{234,244,261–263} In particular, normothermic machine perfusion (NMP) offers significant advantages to overcome current limitations in organ quality and preservation time. This technology provides enhanced preservation conditions, extends viable storage duration, enables kidney quality assessment, facilitates organ reconditioning, and allows for targeted therapeutic interventions.^{53,85,245} NMP holds promise for clinical benefits, potentially improving early graft function, reducing ischemia–reperfusion injury, and enabling viability assessment prior to transplantation.^{73,75}

Recently, regenerative medicine has emerged within the field of organ transplantation, particularly in conjunction with machine perfusion techniques.^{138,212} A potentially therapeutic intervention during NMP is the administration of mesenchymal stromal cells (MSCs), known for their immunomodulatory, anti-inflammatory, and regenerative properties.^{162,264,265} We and others have previously investigated the use of MSCs in the setting of liver and kidney transplantation in early-phase clinical studies, demonstrating their favorable tolerability profile.^{133,266–268}

Building on this background, the present study investigates the potential therapeutic effects of clinical-grade human mesenchymal stem cells (hMSCs) in a large animal model. Specifically, we examine hMSCs perfused into renal grafts during NMP with the goal of mitigating ischemia–reperfusion injury.¹¹⁶ The primary objective was to assess the feasibility of delivering hMSCs to ischemic porcine kidneys during NMP. Secondary objectives included evaluating their impact on perfusion quality, severity of renal tissue injury, modulation of key inflammatory markers, and overall kidney function.

2. Material and Methods

2.1 — Ethics and Animals

This study was designed and reported in accordance with the ARRIVE guidelines 2.0 for reporting animal research. Female laboratory pigs (30–60 kg), resulting from a Landrace and Pietrain cross, were sourced from the Walloon Agricultural Research Centre (CRA-W farm, Gembloux, Belgium). These animals were used for the collection of washed red blood cells and the procurement of kidneys. The study protocol was reviewed and approved by the Animal Ethics Committee of the University of Liege (approval number 21-2447). No formal sample size calculation was performed; six animals were deemed sufficient to assess feasibility and biological effects in this randomized, blinded, preclinical large-animal model, in accordance with the principles of animal reduction and refinement. Animal care and handling complied with the guidelines set out in the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health and the National Academy of Sciences, ensuring ethical and humane treatment throughout the experimental process.

2.2— Study Design

Six pairs of kidneys were perfused simultaneously using NMP, following the protocol recently published by our group.¹⁶³ Following a 30-minute period of warm ischemia, kidneys underwent 3 hours of SCS, after which they were subjected to 6 hours of NMP. At 45 minutes of NMP, an intra-arterial injection was administered: kidneys were randomly assigned to receive either placebo (NMP group) or 10 million clinical-grade bone marrow-derived hMSCs (NMP + hMSC group). Perfusate and urine samples were collected every 30 minutes and every hour, respectively, during the 6-hour perfusion period. Renal tissue biopsies were obtained at the end of the NMP period (**Figure 1**).

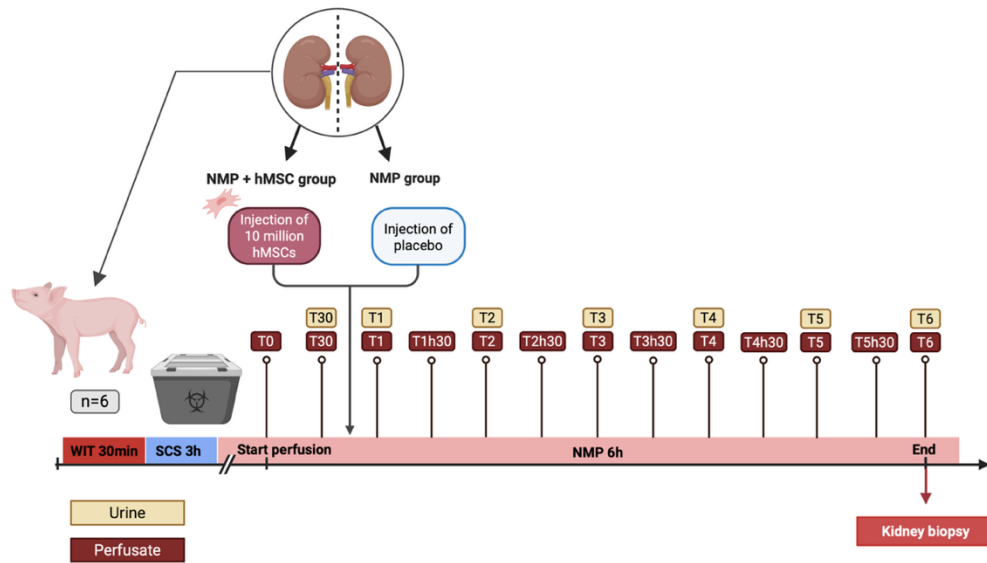


Figure 1. Experimental design and samplings at different timings. Samples of urine and perfusate were collected during NMP. Biopsies were performed at the end of NMP. NMP, normothermic machine perfusion; T, timing; WIT, warm ischemia time; SCS, static cold storage; hMSCs, human mesenchymal stromal cells.

2.3— MSCs and Placebo Preparation

Detailed methodologies for clinical-grade hMSC culture have been reported previously.¹¹⁶ In summary, hMSCs used in this study were produced under clinical-grade conditions by the Laboratory of Cell and Gene Therapy (LTCG), a GMP-licensed of accredited facility at the University Hospital of Liege. hMSCs were isolated from bone marrow aspirates of healthy allogeneic donors and processed via automated Ficoll-based mononuclear cell isolation closed-system. Cells were expanded in culture up to passage 2 using serum-containing media supplemented with gamma-irradiated foetal bovine serum. At passage 2, cells were harvested, formulated in a cryopreservation medium containing saline, human albumin, and 10% dimethyl sulfoxide, then frozen using a controlled-rate protocol before storage in the vapor phase of liquid nitrogen. For this study, hMSCs were thawed and directly diluted with a saline–albumin solution prior to intra-arterial administration during NMP. The final cell product was perfused within one hour post-thawing to ensure stability and viability.¹¹⁶ The placebo consisted of a 20 mL syringe containing saline, human albumin and DMSO, identical in appearance and volume to the syringe containing 10 million thawed hMSCs, prepared as described above.

2.4— Randomization

For each kidney pair, one kidney was randomly assigned to receive a placebo treatment (NMP group), while the contralateral kidney received the hMSC-based therapy (NMP + hMSC group). Randomization was performed by the LTCG, which was responsible for preparing and supplying both the placebo and hMSC products. On the day of the experiment, two identically prepared opaque syringes, labelled 'right' and 'left', were provided and administered to the corresponding kidneys in a blinded manner under standardized experimental conditions. To ensure blinded and unbiased evaluation, the surgical team, as well as all personnel involved in kidney graft procurement, NMP, sample collection, and data analysis, remained blinded to group allocation throughout the entire study.

2.5— Surgical Procedure

Operative procedures have been described previously.¹⁶³ Briefly, animals were sedated thirty minutes prior to surgery via intramuscular injection of tiletamine/zolazepam (4.4 mg/kg; Zoletil 100, Virbac, Leuven, Belgium). An auricular vein catheter was placed, followed by intravenous induction of anaesthesia and orotracheal intubation. Anaesthesia was maintained using continuous propofol infusion (2–4 mg/kg/h; Aspen Pharma, Dublin, Ireland), boluses of sufentanyl (0.2 µg/kg Viatrix; Canonsburg, Pennsylvania, USA). Animals were mechanically ventilated to maintain PaO₂ >100 mmHg and PaCO₂ between 35–45 mmHg. A carotid arterial catheter was inserted for hemodynamic monitoring and blood sampling. After intravenous administration of 25,000 IU of heparin, the abdominal vessels were cannulated. Warm ischemia was induced by clamping the thoracic aorta and maintained for 30 minutes. Subsequently, the kidneys were flushed in situ via the aortic cannula with 2 L of cold (4°C) IGL-1 preservation solution (Institut Georges Lopez, Lissieu, France), and the abdominal cavity was cooled with 4°C saline and topical slush ice. Whole blood was collected via the vena cava cannula during the flush and processed using a cell-saving system (Xtra autotransfusion system; LivaNova, Zaventem, Belgium) to obtain leukocyte-depleted, concentrated red blood cells. Animals underwent euthanasia by exsanguination, resulting in cardiac arrest under general anaesthesia. Following bilateral nephrectomy, the renal artery and the ureter were dissected and cannulated. Kidneys were then preserved by SCS in IGL-1 solution at 4°C for 3 hours. Prior to connection to the NMP circuit, each kidney was weighed and flushed on the back table with 500 mL of cold IGL-1 solution.

2.6— Normothermic Machine Perfusion

As described in a previous study by our group, both kidneys from the same pig underwent simultaneous 6-hour NMP using two identical and independent, pressure-controlled, pulsatile perfusion systems. These were custom-made devices adapted from two discontinued clinical cardiopulmonary extracorporeal circulation systems (Stockert GmbH, Freiburg, Germany).¹⁶³ Each NMP circuit consisted of a kidney receptacle, roller pump, membrane oxygenator (Dideco Perfusion Tubing Systems; LivaNova, Zaventem, Belgium), and heat exchanger (Stockert GmbH, Freiburg, Germany) connected to coated tubing (LivaNova; Zaventem, Belgium). The arterial pressure (WPI, Friedberg, Germany) and flow rates (Sonotec, Halle, Germany) were continuously measured. The renal artery was cannulated and connected to the NMP circuit, while the renal vein was left open to drain into the circuit for reperfusion. The ureter was cannulated for urine collection. The NMP circuits were primed with a solution of autologous red blood cells and isotonic electrolyte solution (Plasmalyte; Baxter, Lessines, Belgium) to achieve a haematocrit of 20–30%. Flolan (Epoprostenol sodium, 7.5 µg/h; GSK, Wavre, Belgium) was continuously infused. Urine output was replaced 1:1 with Plasmalyte, and a parenteral nutrition solution (Aminomix 2 Novum; Fresenius Kabi, Willebroek, Belgium) was added to maintain glucose levels > 100 mg/dL. Sodium bicarbonate (10 mL, 8.4%; B Braun Melsungen, Germany) was used to maintain a pH in the range of 7.2 to 7.5. Perfusion temperature was maintained at 38 °C with an oxygen flow of 100 mL/min at FiO₂ 21%. During perfusion, arterial pressure was gradually increased to a target range of 60–80 mmHg. Intrarenal resistance (IRR) was calculated as the ratio of arterial pressure to flow and expressed in mmHg/mL/min/100 g of tissue.

2.7— Perfusate and Tissue Collection

A sampling line was connected to the arterial inflow cannula for perfusate collection. Samples of 2 mL were drawn into SST BD Microtainer tubes (BD, New Jersey, USA), centrifuged at 2000 × g for 5 minutes at 4 °C. Supernatant was aliquoted and stored at –80 °C for subsequent analysis. Biochemical parameters (pH, sodium, potassium) were measured in the arterial line perfusate using the Epoc Blood Analysis System (Siemens Healthcare NV, Dilbeek, Belgium). Renal function was assessed via clearance of predefined concentrations of creatinine (75mg/L) and iohexol (518 mg/L) added to the perfusate during circuit priming (GE Healthcare, Diegem, Belgium). Clearance of these markers over time was used to evaluate excretory performance. The glomerular filtration rate was measured based on creatinine clearance and expressed as mL/6h/100g of kidney tissue. Perfusate and urine concentration of creatinine was determined at each sampling time point and cumulatively over the 6-hour NMP period to establish the filtration rate. All values were normalized to the kidney weight. At the end of perfusion, kidney biopsies were obtained for histological analysis.

Tissue samples were fixed in 4% formalin, paraffin-embedded, and stained with haematoxylin-eosin and Periodic Acid–Schiff. Histopathological assessment was conducted by an experienced nephropathologist blinded to group allocation. All collected samples were analyzed, and no data points were excluded from the study.

2.8— ELISA

Levels of neutrophil gelatinase-associated lipocalin (NGAL) were measured using commercially available enzyme-linked immunosorbent assay kits [human Lipocalin-2/NGAL Quantikine ELISA kits (R&D Systems, Minneapolis, MN, USA)]. A custom electrochemiluminescent Milliplex® assay was performed on perfusate samples to quantify cytokine expression. The 8-plex panel included IFN- γ , IL-2, IL-10, IL-1 α , IL-6, TNF- α , IL-1 β and IL-8 (PCYTMAg-23K; Millipore, Darmstadt, Germany). All assays were carried out according to the manufacturer's instructions.

2.9— Statistical Analysis

A total of six animals ($n = 6$) were selected to account for expected biological variability in large-animal models. Results are reported as mean $\pm$ standard deviation. Data visualization was performed using GraphPad Prism (version 10). For comparative analyses, the area under the curve (AUC) was determined, followed by a non-parametric Wilcoxon signed-rank test. A generalized linear mixed model was used to assess the effects of time, treatment group (kidney), and their interaction, using a compound symmetry covariance structure to account for repeated measures. Assumptions of normality and homoscedasticity were verified, and log transformation was applied where appropriate. For two specific parameters, beta regression was used due to the distribution of the data. Model fit was evaluated using Akaike and Bayesian Information Criteria (AIC/BIC), with lower values indicating better fit. Statistical significance was defined as $p < 0.05$. All statistical analyses were conducted using SAS (version 9.4) and R (version 4.2.2), with the *Inormimp* package used for censored data imputation.

3. Results

3.1— Animal Characteristics

Six pigs (mean weight: 40.50 ± 9.77 kg) underwent the experimental protocol enabling the procurement of six pairs of kidneys (**Table 1**). In total, six kidneys were allocated to each group: the NMP group (1 right/5 left) and the NMP + hMSC group (5 right/1 left) (**Suppl. Table 1**). Baseline kidney weights were comparable between the control group (138.17 ± 32.46 g) and the treated group (134.83 ± 25.52 g; $p = 0.56$). At the end of perfusion, kidney weights had increased in both groups, with no significant difference observed in kidney weight at the end of perfusion (176.50 ± 45.92 g for NMP group vs. 184.33 ± 55.74 g for NMP + hMSC group; $p = 0.31$). Cold and warm ischemia times were identical between groups (CIT: 183.67 ± 7.61 min; WIT: 30 min). Baseline perfusate creatinine levels were also the same (1.04 ± 0.05 mg/dl in both groups). All kidneys were included in the final analysis.

Table 1. Statistics of characteristics of paired kidneys included in the study (mean, SD and p-value)

	NMP group		NMP + hMSCs group		p-value
	Mean	SD	Mean	SD	
Pig weight (kg)	40.50	9.77	40.50	9.77	-
Pig creatinine, baseline (mg/dl)	1.04	0.05	1.04	0,05	-
Kidney side (R/L)	1/5	-	5/1	-	-
Kidney weight, baseline (g)	138.17	32.46	134.83	25.52	0,56
Kidney weight, end perfusion (g)	176.50	45.92	184.33	55.74	0,31
CIT (min)	183.67	7,61	183.67	761	-
WIT (min)	30.00	-	30.00	-	-

Note : CIT, cold ischemia time ; WIT, warm ischemia time

3.2— Perfusion Characteristics

The administration of hMSCs did not significantly affect the majority of perfusion or biochemical parameters during the 6-hour perfusion period compared with controls (**Suppl. Table 2**). Electrolyte and acid–base homeostasis did not differ between groups, with comparable values for pH, sodium, potassium, $p\text{CO}_2$, and $p\text{O}_2$ (all $p > 0.1$) (**Fig. 2**). Similarly, no significant intergroup differences were detected in glucose, lactate, creatinine, or iohexol clearance. In contrast, the NMP + hMSC group demonstrated significantly higher hematocrit (18.29 ± 2.73 vs. 17.40 ± 2.92 ; $p = 0.018$) and hemoglobin concentrations (6.21 ± 0.91 g/dl vs. 5.92 ± 0.98 g/dl; $p = 0.017$). In addition, mean perfusion pressure was greater in the NMP + hMSC group (53.65 ± 27.58 mmHg vs. 46.43 ± 16.51 mmHg; $p = 0.035$), while cumulative urine output was slightly lower (20.49 ± 15.68 ml vs. 21.70 ± 24.49 ml; $p = 0.033$). No significant difference in vascular resistance was observed between groups (3.28 ± 4.32 vs. 1.96 ± 2.24 ; $p = 0.20$).

Chapter 5. Infusion of hMSCs during NMP of porcine kidneys: a randomized, blinded, preclinical study

Although hMSC-treated kidneys exhibited a tendency towards greater weight gain ($38.33 \text{ g} \pm 17.77 \text{ g}$ for NMP group vs. $49.50 \text{ g} \pm 33.22 \text{ g}$ for NMP + hMSC group), this difference was not statistically significant ($p = 0.44$) (Fig 3).

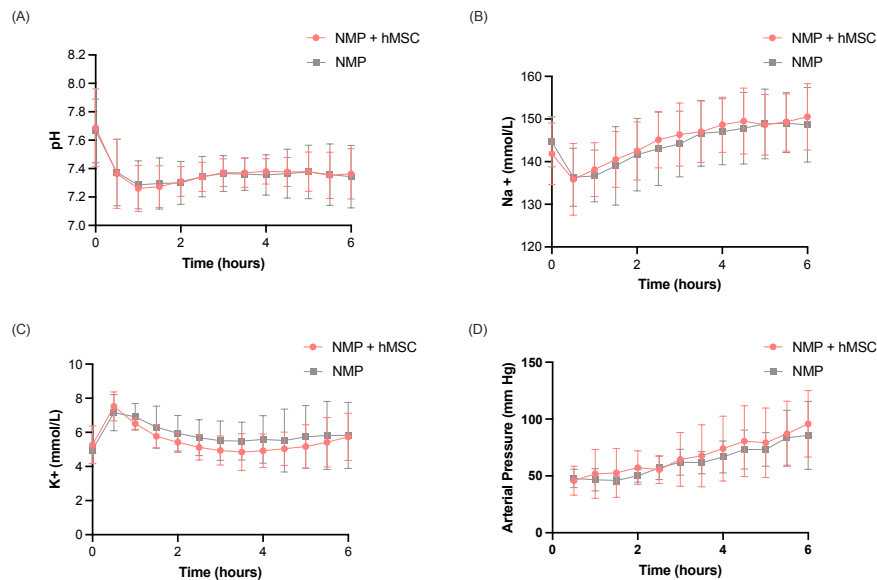


Figure 2. Perfusion characteristics during 6 hours of NMP with administration of placebo or 10 million hMSCs. (A) pH ;(B) sodium levels (mmol/l) ;(C) potassium levels (mmol/l) ;(D) arterial pressure (mmHg). Data presented as mean $\pm$ SD.

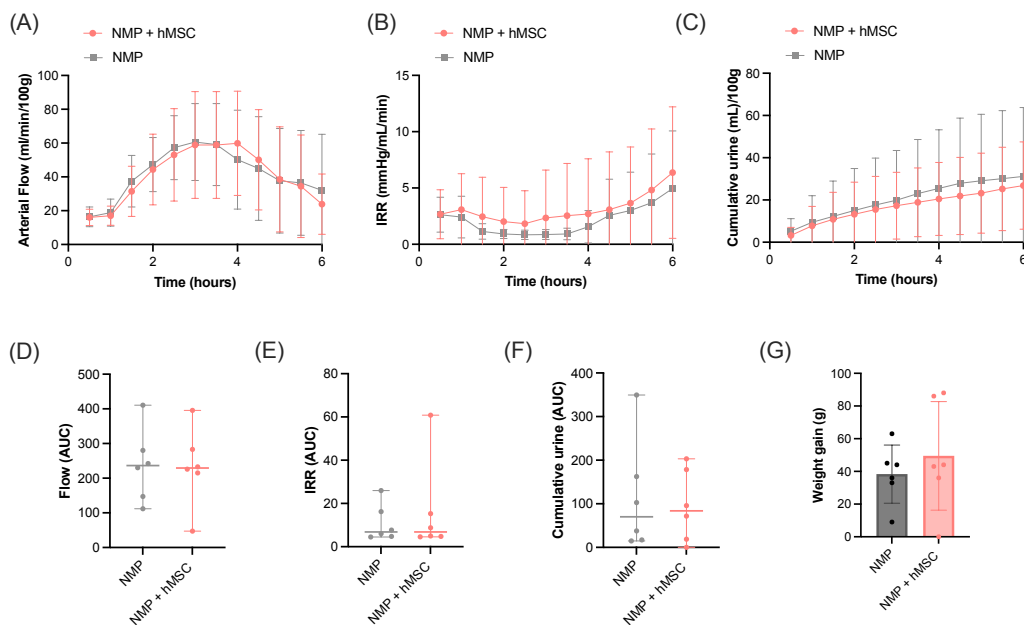


Figure 3. Perfusion characteristics during 6 hours of NMP with administration of placebo or 10 million hMSCs. (A) Arterial flow (ml/min/100g) ;(B) Intrarenal Resistance (mmHg/ml/min) ;(C) Cumulative urine (ml/100g) ;(D) AUC analysis of flow; (E) AUC analysis of intrarenal resistance; (F) AUC analysis of cumulative urine and (G) weight gain (g). Data presented as mean $\pm$ SD.

3.3— Renal Function and Histology

Clearance of creatinine and iohexol from the perfusate was used to evaluate glomerular filtration. Both analytes exhibited similar elimination profiles in the NMP + hMSC and NMP groups, with no significant differences observed in the calculated AUC. Only five kidneys ($n = 5$) were included in the GFR (Glomerular Filtration Rate) analysis, as one kidney pair from the same pig (pair 3) did not produce urine and was therefore excluded from this measurement. GFR followed a similar pattern, with slightly higher mean values in the NMP + hMSC group (108.30 ± 89.14 mL/6h/100g for NMP vs. 131.10 ± 74.12 mL/6h/100g for NMP + hMSC), though without statistical significance ($p > 0.1$). Histological evaluation of the kidney biopsies was conducted solely at the end of perfusion using PAS staining. Findings demonstrated features consistent with acute tubular injury, including loss of the proximal tubular brush border, thinning of the tubular epithelium and vacuolization of tubular cells. Mild peritubular capillaropathy was consistently observed across all samples (**Fig 4**).

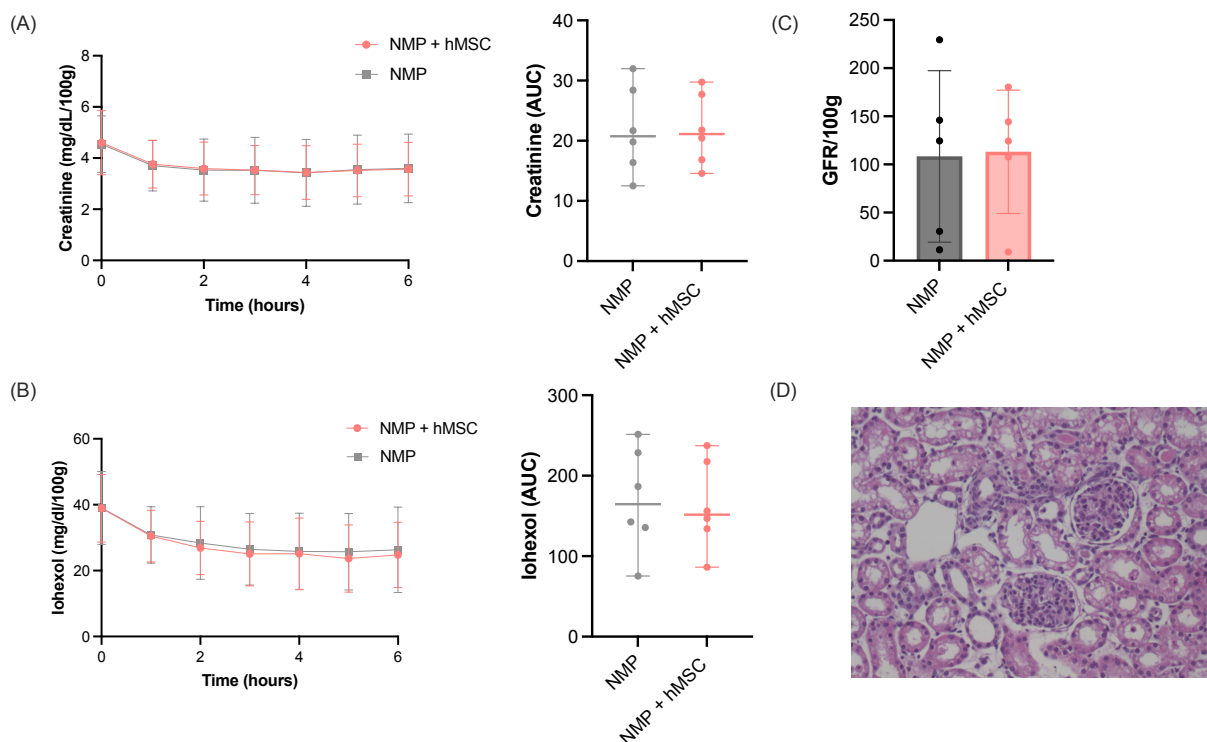


Figure 4. Assessment of renal function during 6 hours of NMP with administration of placebo or 10 million hMSCs. (A) Elimination of creatinine in perfusate (mg/dl/100g); (B) Elimination of iohexol in perfusate (mg/dl/100g). Left panels: kinetics and right panels: AUC analyses. (C) Glomerular filtration rate (ml/6h/100g). (D) Histological representation of kidney biopsies, original magnification x20, PAS staining. Data presented as mean $\pm$ SD.

3.4— NGAL Dynamics

Perfusate concentrations of NGALs increased significantly from baseline to 6 hours in both groups, consistent with progressive injury or stress during NMP. However, no significant intergroup differences were detected at any time point, indicating that hMSC treatment did not alter the temporal pattern of NGAL release (**Fig 5**).

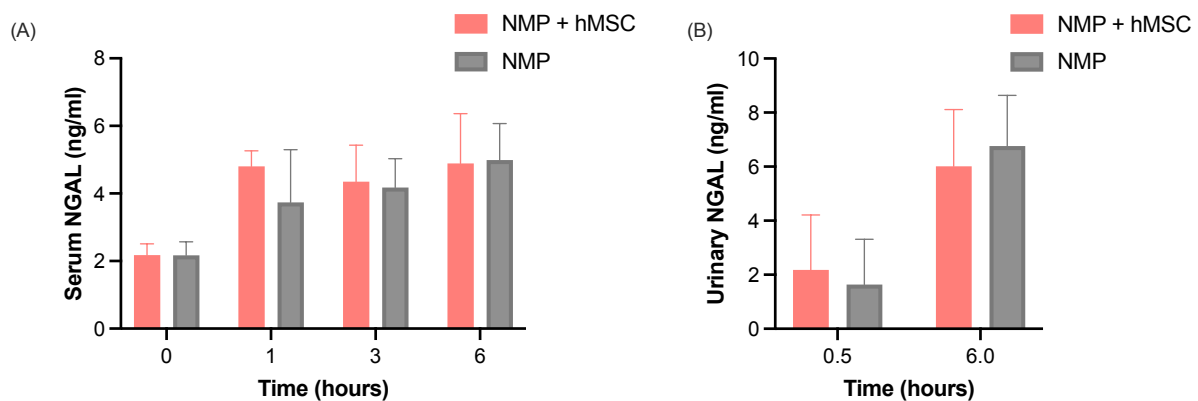


Figure 5. Assessment of kidney injuries during 6 hours of NMP with administration of placebo or 10 million hMSCs. (A) Concentration of NGAL in serum (ng/ml) ;(B) Concentration of NGAL in urine (ng/ml). Data presented as mean $\pm$ SD.

3.5— Cytokine Measurement

No significant differences were observed between the NMP + hMSC group and NMP group for any of the measured cytokines. Nevertheless, a characteristic temporal pattern was noted across both groups: TNF- α concentrations peaked at 3 hours before declining towards baseline, while IL-6 and IL-10, initially nearly undetectable at the onset of perfusion, began to rise after 3-hours, suggesting a delayed cytokine response during NMP. IL-1 α and IL-1 β showed increased levels only at the 6-hour mark of perfusion. IL-2 remained below the detection threshold throughout the perfusion period (**Fig 6**).

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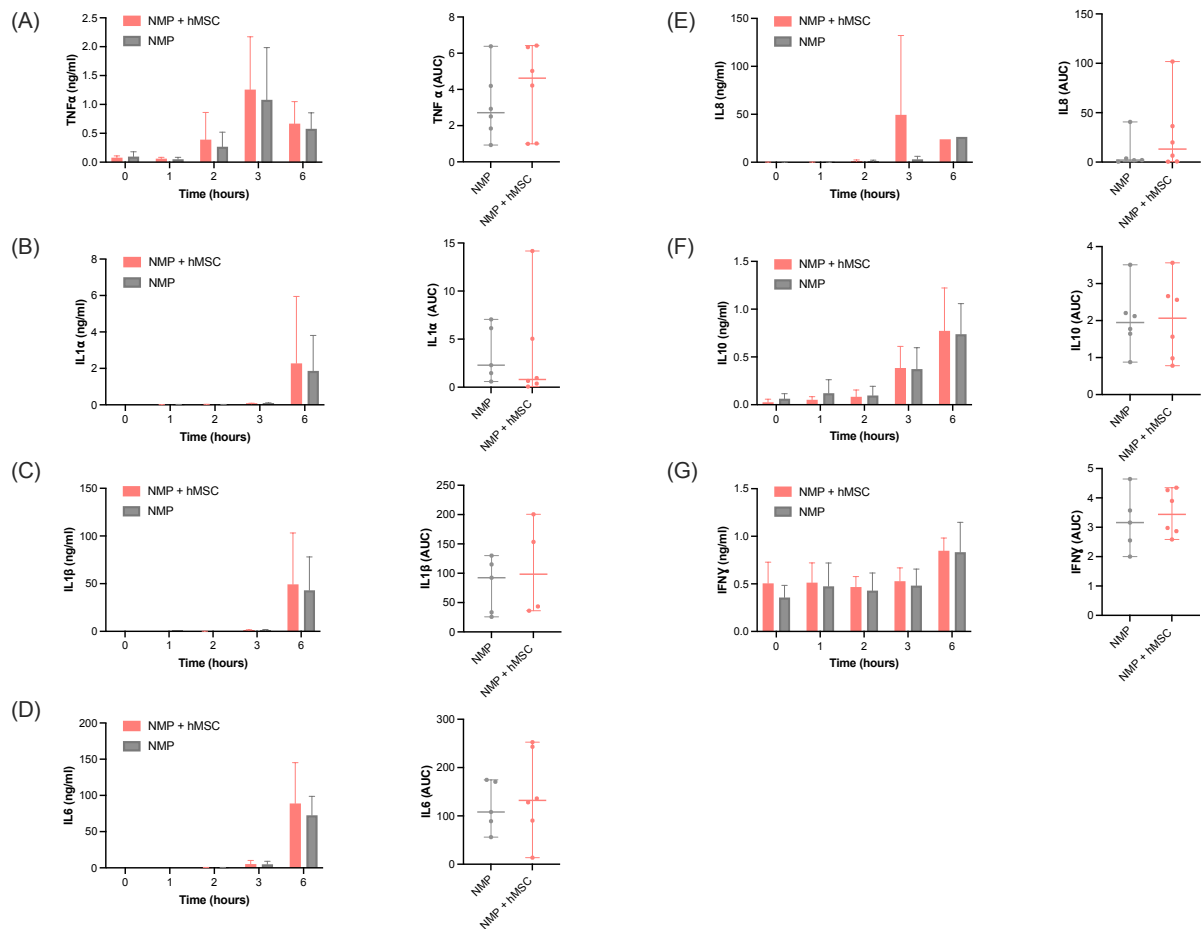


Figure 6. Concentration of cytokines in NMP perfusates: IFN γ , IL-10, IL-1 α , IL-6, TNF α , IL-1 β and IL-8. Left panels: kinetics and right panels: AUC analyses. Data presented as mean $\pm$ SD.

4. Discussion

In this porcine model of NMP, intra-arterial infusion of 10 million clinical-grade human bone marrow–derived MSCs was technically feasible and hemodynamically well tolerated, with no disruption of perfusion hemodynamic or adverse events. Despite seemingly efficient delivery, hMSC treatment failed to improve renal function (as assessed by creatinine clearance and urine output) nor did it reduce ischemia/reperfusion–induced histological injury when compared with controls.

These findings are broadly consistent with previous reports from other groups. Lohmann *et al.* administered a comparable dose of MSCs into porcine kidneys during 4h of NMP and observed no functional or histological improvement after transplantation, despite confirming the presence of MSCs in renal tissue.¹⁴⁰

Pool *et al.* conducted two relevant studies: in 2020, they perfused ischemic pig kidneys with hMSCs for seven hours, resulting in reduced injury markers and increased growth factor release, yet without improvements in perfusion flow or excretory function.¹³⁹ In a 2019 study, the same group demonstrated that most MSCs rapidly disappeared from circulation, with only a limited number of cells retained in glomerular capillaries.¹²⁶ Our inability to detect MSCs post-NMP mirrors these findings, reinforcing the conclusion that cell engraftment during *ex situ* perfusion is minimal. A key methodological distinction between our study and that of Lohmann *et al.* lies in the preservation strategy: while their protocol incorporated a phase of cold oxygenated hypothermic perfusion (oxHMP) prior to NMP, our model utilized SCS followed immediately by NMP. Importantly, the warm and cold ischaemia times used in our study closely mirror those encountered in human DCD donor kidneys, suggesting that this may represent one of the more translationally relevant large-animal models described to date.

The translational potential of MSC therapy in kidney transplantation has also been explored by our group in both preclinical and clinical settings, evaluating the anti-inflammatory, immunoregulatory, and reparative properties of MSCs in models of ischemia–reperfusion and transplantation.^{266,267} In a pilot clinical trial, Erpicum *et al.* administered allogeneic bone marrow-derived MSCs ($\sim 2 \times 10^6$ cells/kg) into kidney transplant recipients and observed improved early graft function and increased regulatory T cell populations, without any adverse events.¹³³ These findings confirmed the safety of hMSC administration in humans and highlighted the potential of MSC-based therapies to enhance post-transplant recovery, although their long-term effects remain to be defined. Notably, donor-specific anti-HLA antibodies (DSAs) were detected in these studies, highlighting the risk of recipient sensitization following direct MSC injection.^{133,152} In this context, NMP offers a strategy to deliver MSCs directly to the graft while theoretically minimizing the risk of recipient immunization.

A key strength of our study lies in the use of clinical grade, hMSCs, manufactured under validated and standardized conditions in accordance with International Society for Cellular Therapy (ISCT) criteria. Unlike other preclinical studies that have employed research-grade, we deliberately selected the same hMSCs that have already been administered to patients in phase I/II clinical trials.¹³³ This strategy maximizes the reproducibility and translational relevance of our findings. Furthermore, with an experimental design that replicates the most recently developed preservation pathways (static cold storage followed by NMP), our study provides a unique and robust model for evaluating MSC-based interventions in a setting that most closely mirrors clinical reality.

Nevertheless, several limitations must be acknowledged. Although limited by a small sample size inherent to large-animal models, our design using paired kidneys minimized inter-individual variability.

Moreover, the consistent absence of effects across functional, histological, and biomarker endpoints strongly suggests that a clinically meaningful benefit of a single MSCs dose during NMP is unlikely. Finally, the lack of standardized protocols in the literature—regarding MSC source, dose, timing, and administration route—remains a major obstacle to inter-study comparisons and reproducibility. This variability underscores the urgent need for harmonized protocols to generate robust and comparable results.

In conclusion, while the safety and feasibility of hMSC delivery during NMP were confirmed, the lack of functional benefit provides critical information for refining future strategies and preventing unnecessary clinical translation of ineffective protocols. Our findings, together with prior reports^{139,140}, indicate that refinements such as repeated dosing, prolonged perfusion, or the use of MSC-derived extracellular vesicles may be necessary to achieve consistent benefits.^{147,224} Most importantly, by employing clinical-grade hMSCs under ischemia and preservation conditions comparable to current clinical practice, this study provides the most translationally relevant large-animal model to date. It thereby contributes valuable insights to guide further studies exploring MSC-based therapies in discarded human kidneys and to inform subsequent steps toward potential clinical translation. Future studies should prioritize strategies to enhance MSC persistence and functional activity, including repeated or prolonged dosing, pre-conditioning of cells, or the use of MSC-derived extracellular vesicles. Harmonization of experimental protocols across centers will be essential to enable direct comparability and accelerate clinical translation.

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5. Supplementary File

Table S1. Characteristics of paired kidneys included in the study.

	NMP #1	NMP #2	NMP #3	NMP #4	NMP #5	NMP #6	NMP/hMSCs #1	NMP/hMSCs #2	NMP/hMSCs #3	NMP/hMSCs #4	NMP/hMSCs #5	NMP/hMSCs #6
Pig Weight (kg)	31	33	37	40	58	44	31	33	37	40	58	44
Pig creatinine, baseline (mg/dl)	0.99	1.06	1.12	1.04	1.02	0.99	0.99	1.06	1.12	1.04	1.02	0.99
Kidney side	Left	Left	Right	Left	Left	Left	Right	Right	Left	Right	Right	Right
Kidney weight, baseline (g)	102	137	107	170	182	131	116	134	100	150	173	136
Kidney Weight, end perfusion (g)	111	170	151	206	245	176	116	177	136	238	259	180
CIT (min)	170	190	180	189	185	188	170	190	180	189	185	188
WIT (min)	30	30	30	30	30	30	30	30	30	30	30	30

Note : CIT, cold ischemia time ; WIT, warm ischemia time

Table S2. Comparison of biochemical and hemodynamic parameters between the NMP group and the NMP + hMSCs Group during 6 hours of NMP (mean, SD and p-value).

	NMP group		NMP + hMSCs group		p-value
	Mean	SD	Mean	SD	
pH	7.37	0.19	7.37	0.17	0.96
pCO₂ (mmHg)	42.84	11.61	43.30	11.59	0.79
pO₂ (mmHg)	106.63	39.93	105.36	40.80	0.81
Na⁺ (mmol/l)	144.14	8.44	144.89	7.96	0.37
K⁺ (mmol/l)	5.86	1.39	5.54	1.18	0.18
Hct (%)	17.40	2.92	18.29	2.73	0.018
Hb (g/dl)	5.92	0.98	6.21	0.91	0.017
Glucose (mg/dl)	129.22	33.88	121.13	27.70	0.14
Lactate (mmol/l)	13.77	4.77	15.48	3.94	0.17
Creatinine (mg/dl)	3.65	1.17	3.69	0.99	0.34
Iohexol (mg/dl)	28.71	11.30	28.09	10.21	0.39
Art. Press. (mmHg)	46.43	16.51	53.65	27.58	0.035
Flow (ml/min)	41.98	26.00	40.12	27.80	0.38
IRR mmHg/ml/min)	1.96	2.24	3.28	4.32	0.20
Temperature (°C)	34.60	3.38	33.87	3.48	0.26
Cum. urine vol. (ml)	21.70	24.49	20.49	15.68	0.033

Chapter 6. Clinical-Grade hMSC Delivery During NMP of Discarded Human Kidneys: A Translational Study

Manuscript in preparation

Abstract

Normothermic machine perfusion (NMP) has emerged as a dynamic platform for pre-transplant organ assessment and therapeutic delivery. Mesenchymal stromal cells (MSCs) hold potential for limiting ischemia–reperfusion injury, but their *ex vivo* delivery to human kidneys under clinically relevant NMP conditions has never been evaluated in a controlled setting.

Seven pairs of discarded human kidneys underwent 6 hours of NMP. Within each pair, one kidney received an intra-arterial injection of 10 million clinical-grade bone marrow-derived human MSCs (hMSCs) and the other received placebo after 45 minutes. Primary outcome was feasibility and safety; secondary outcomes included functional parameters, tubular injury markers, cytokine profiles, and histological assessment. All analyses were conducted in a blinded manner.

Renal blood flow and intrarenal resistance showed significant time effects ($p < 0.0001$ and $p = 0.04$, respectively), with no significant between-group differences, although trends toward higher renal blood flow and lower intrarenal resistance were observed in MSC-treated kidneys during the early perfusion phase. Urine output increased over time in both groups ($p = 0.009$), with numerically higher values in MSC-treated kidneys at multiple time points without reaching statistical significance ($p = 0.61$). Creatinine clearance increased significantly over time ($p = 0.01$) and was consistently higher in MSC-treated kidneys throughout perfusion, without reaching statistical significance ($p = 0.40$). NGAL levels were numerically lower in MSC-treated kidneys at later time points, and cytokine profiles and histological parameters were comparable between groups.

This first blinded, randomized, paired, controlled study demonstrates that clinical grade hMSC delivery during human kidney NMP is safe and directly applicable to a clinical trial setting. Consistent functional trends suggest a potential biological signal, supporting the design of an adequately powered confirmatory study using the paired discarded kidney model described in this study.

1. Introduction

Kidney transplantation is the treatment of choice for patients with end-stage kidney disease, offering superior survival and quality of life compared to dialysis.^{258,269,270} However, the persistent shortage of donor organs remains a major limitation, leading to prolonged waiting times and increased mortality on the transplant waiting list.⁵⁵ To mitigate this shortage, organs from extended criteria donors, particularly elderly donors, are increasingly considered for transplantation.^{241,271} Although this strategy might expand the deceased donor pool, extended criteria kidneys are more vulnerable to ischemia–reperfusion injury and are associated with higher rates of delayed graft function and poorer long-term outcomes.^{272–274} Strategies aimed at improving the evaluation and preservation of marginal donor kidneys are therefore of considerable interest.^{53,275,276}

Normothermic machine perfusion (NMP) has recently emerged as a promising approach for organ preservation and viability assessment.^{277–281} By restoring near-physiological conditions through oxygenated perfusion at body temperature, NMP allows metabolic activity to resume and enables real-time evaluation of graft function.^{282–284} Beyond its role in preservation, NMP also provides an attractive platform for delivering therapeutic interventions directly to the organ before transplantation.^{138,285,286} Such strategies may help limit ischemia–reperfusion injury and potentially improve graft quality prior to transplantation.^{221,223,224}

Mesenchymal stromal cells (MSCs) have attracted significant attention as a potential therapeutic tool because of their immunomodulatory and tissue-protective properties. In preclinical models of renal ischemia–reperfusion injury, MSC administration has been associated with reduced tubular damage, decreased pro-inflammatory cytokine release, and improved renal function recovery.^{124,148,287,288} These effects are thought to be largely mediated through paracrine mechanisms, including the release of anti-inflammatory and trophic factors, rather than direct cell engraftment.^{133,289} While systemic intravenous delivery has been the most used route of administration, it is limited by pulmonary sequestration and poor renal targeting, highlighting the need for more direct delivery strategies.^{290–292} The pivotal kidney transplantation study reported by our group in 2019 concern a phase I–II, open-label, single-centre clinical study in which ten deceased-donor kidney transplant recipients received a single intravenous infusion of third-party allogeneic BM-MSC on day 3 post-transplantation. The intervention was safe and well-tolerated. Compared to historical controls, MSC-treated patients showed improved eGFR trajectories in the first year post-transplant alongside an increased proportion of circulating regulatory T cells.¹³³

The use of NMP as a platform for MSC delivery offers several potential advantages. Compared to intravenous injection, direct intra-arterial administration during NMP allows targeted delivery to the kidney vasculature, bypassing pulmonary sequestration and maximizing local cell concentration, with a reduced risk of immunization. Furthermore, the controlled *ex vivo* environment of NMP enables real-time monitoring of immediate functional and biological responses, providing a unique opportunity to assess the early effects of MSC therapy before transplantation.^{37,38}

In this study, we aimed to evaluate the feasibility and biological effects of administering allogeneic human MSCs (hMSCs) during NMP of discarded human kidneys as a preclinical model. To our knowledge, this represents the first report of intra-arterial delivery of clinical-grade, bone marrow-derived hMSCs during NMP in a paired kidney model in humans, enabling direct intra-donor comparisons under controlled conditions. Leveraging this design, we performed an integrated assessment of renal function and inflammatory responses over a 6-hour perfusion period.

2. Material and Methods

2.1 — Ethical Statement

This study was approved by the University Hospital–Faculty Ethics Committee of Liege (reference ID: 2023/20) and conducted in accordance with the Belgian law of 19 December 2008 governing the procurement and use of residual human body material.

2.2 — Study design

Seven pairs of discarded human kidneys procured from the same deceased organ donors underwent 6 hours of simultaneous NMP, allowing paired comparisons between treated and untreated kidneys. Kidneys declined for clinical transplantation were considered for inclusion in the study if they exhibited sufficient residual function (calculated GFR of the deceased organ donor > 40 mL/min), a comparable size at donor computed tomography and a vascular anatomy suitable for arterial cannulation. Following deceased donor aortic cold flush with IGL-1 solution and classical organ procurement, the kidneys were transported to the surgical laboratory on static cold storage (SCS) and connected to the NMP circuits using an autologous red blood cell–based perfusion solution. In donation after circulatory death (DCD) donors, procurement warm ischemia time was defined as the interval between circulatory arrest and initiation of cold perfusion via aortic flush with preservation solution. Cold ischemia time (CIT) was defined as the interval between aortic cold flush and the onset of NMP. After 45 minutes of NMP, kidneys within each donor pair were randomized in a blinded manner to receive either placebo (non-treated kidneys) or an intra-arterial injection of 10×10^6 clinical-grade bone marrow–derived human MSCs (hMSC) (MSC treated kidneys) (**Fig 1**).

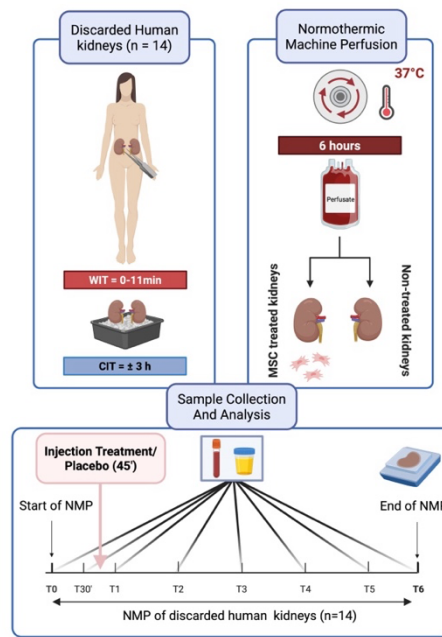


Fig 1. Study design overview. Fourteen discarded human kidneys (7 donor pairs) underwent 6 hours of NMP at 37°C. Within each pair, one kidney received an intra-arterial MSC injection at 45 minutes after NMP initiation, while the contralateral kidney received placebo. Perfusate and urine samples were collected at serial time points (T0–T6), and a renal biopsy was obtained at the end of perfusion. CIT, cold ischemia time; MSC, mesenchymal stromal cell; NMP, normothermic machine perfusion; WIT, warm ischemia time.

2.3 — Human kidney NMP setup

The complete NMP setup and protocol have been previously described.¹⁶³ Upon arrival at the laboratory, renal arteries of both kidneys were cannulated with 14 Fr cannulas (Medtronic, Brussels, Belgium) and ureters were cannulated to allow urine collection for volume measurement and sampling. The kidney pairs were perfused simultaneously for 6 hours using an oxygenated, autologous red blood cell–based perfusion solution and two–clinical cardiopulmonary extracorporeal circulation systems (Stockert GmbH, Freiburg, Germany). Red blood cells were recovered from the deceased organ donor during surgical procurement using a cell-saver device (Xtra autotransfusion system; LivaNova, Zaventem, Belgium). Pressure-controlled NMP was performed at 37 °C with a target arterial pressure of 70–75 mmHg. The volume of perfusate in the circuit was maintained constant by replacing urine output with an equivalent volume of crystalloid solution administered hourly. Perfusate and urine samples were collected hourly throughout the perfusion period. Renal function was assessed by measuring the clearance of an exogenous bolus of creatinine (75 mg/L, Sigma-Aldrich, St. Louis, MO, USA) and iohexol (518 mg/L, GE Healthcare, Diegem, Belgium) added to the perfusate during circuit priming. Creatinine clearance (CrCl) was calculated using the standard formula: $\text{CrCl} = (\text{urinary creatinine concentration} \times \text{urine flow rate}) / \text{perfusate creatinine concentration}$.

Additionally, the fractional excretion of sodium (FENa) was calculated as: $FENa (\%) = (\text{urinary sodium concentration} \times \text{perfusate creatinine concentration}) / (\text{perfusate sodium concentration} \times \text{urinary creatinine concentration}) \times 100$, to assess tubular sodium reabsorption capacity. Physiological parameters were continuously recorded throughout the 6-hour perfusion period, including perfusate blood gas parameters, urine output, and renal arterial pressure and renal blood flow (RBF). Intrarenal Resistance (IRR) was calculated by dividing the pressure by the flow and was expressed in mmHg/mL/min/100g of tissue. Chronic histological lesions were assessed on post-perfusion biopsies by a single nephropathologist (Pauline Erpicum) in a blinded fashion, using a modified Banff scoring system adapted to the pre-transplant setting, as previously described.^{295,296} Parameters included arteriolar hyalinosis (mm), arterial intimal thickening (ah), vascular changes (cv), interstitial fibrosis (ci), tubular atrophy (ct), and the percentage of cortical fibrosis and tubular atrophy (%FIAT). Acute rejection-related Banff parameters (e.g., tubulitis, intimal arteritis, glomerulitis) were not assessed, as they are not applicable in the pre-transplant setting and cannot be distinguished from ischemia-reperfusion injury under NMP conditions.

2.4 — Human Mesenchymal Stromal Cells treatment

The allogeneic, off the shelf, clinical-grade, hMSCs used in this study were isolated, cultured, banked and delivered by the Cell and Gene Therapy Laboratory (LTCG) of the Department of Haematology at the University Hospital of Liege, a Good Manufacturing Practice (GMP)-accredited facility. The use of these cells in clinic was approved by the institutional ethics committee (reference 2025/470). The hMSCs were isolated from the bone marrow of healthy unrelated donors. The methodology for hMSC isolation, expansion, characterization and banking has been previously described.^{116,117,133} The hMSCs were thawed immediately prior to use and diluted in a saline–human albumin solution. The final hMSCs product was infused within one hour after thawing to preserve cell stability and viability. The placebo consisted of a 20 mL syringe containing saline, human albumin, and dimethyl sulfoxide (DMSO), identical in appearance and volume to the syringe containing 10×10^6 thawed hMSCs. Syringes containing either hMSCs or placebo were administered intra-arterially, as close as possible to the graft, by a 5-minutes injection. Treatment allocation was randomized and performed in a blinded manner. All investigators involved in the study remained blinded to group allocation until completion of the study.

2.5 — MSC tracking in kidney tissue

In a separate experiment, one donor kidney pair was exclusively used for MSC tracking, to avoid any confounding effect on the primary experimental outcomes. Prior to infusion, hMSCs were prelabelled with CellTracker CMPTX Red fluorescent dye (C34552, Thermo Fisher Scientific, [Massachusetts, United States](#)) to enable subsequent cell tracking within the renal tissue. Renal biopsies were collected at 30 minutes, 1, 2, 3, 4, 5, and 6 hours post-infusion and immediately embedded in Tissue-Tek OCT compound for cryopreservation. Cryosections of 5 μm thickness were prepared and counterstained with DAPI (4',6-diamidino-2-phenylindole) to visualize cell nuclei. Fluorescence imaging was performed using a Zeiss Axioscan slide scanner, and image analysis was carried out with the Zeiss ZEN image analysis software.

2.6 — Cytokines measurement (ELISA)

Levels of neutrophil gelatinase-associated lipocalin (NGAL), interleukin-10 (IL-10), and tumor necrosis factor- α (TNF- α) were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA). Cytokine concentrations in perfusate samples were further assessed using a custom multiplex assay (Human Luminex Discovery Assay, LXSAHM-09, R&D Systems, Minneapolis, MN, USA). The 9-plex panel included hepatocyte growth factor (HGF), interferon- γ (IFN- γ), interleukin-6 (IL-6), interleukin-18 (IL-18), vascular endothelial growth factor-A (VEGF-A), kidney injury molecule-1 (KIM-1), transforming growth factor- α (TGF- α), interleukin-1 β (IL-1 β), and interleukin-8 (IL-8). All assays were performed according to the manufacturer's instructions and the assessor of these endpoints remained blinded to the treatment allocation throughout the experiments.

2.7 — Statistical Analysis

Statistical analyses were performed using GraphPad Prism (version 10, GraphPad Software, San Diego, CA, USA). Data are presented as mean $\pm$ standard error of the mean (SEM), unless otherwise specified. Longitudinal perfusion parameters were analysed using a mixed-effects model (restricted maximum likelihood, REML) to account for repeated measurements over time and pairing between MSC-treated and non-treated kidneys. The Geisser–Greenhouse correction was applied where appropriate, and multiple comparisons between groups at individual time points were performed using Šídák's correction. Cytokine levels were normalized to baseline (T0) and expressed as log₂ fold change. Given the small sample size and the non-normal distribution of cytokine data, paired comparisons between MSC-treated and non-treated kidneys were performed at each time point using the Wilcoxon matched-pairs signed-rank test. To control for multiple testing across time points and cytokines, false discovery rate (FDR) correction was applied. A two-sided p-value <0.05 was considered statistically significant.

3. Results

3.1 — Organ donors and kidney grafts characteristic

Seven pairs of human kidneys ($n = 14$) were included in the study (Table 1). Donors included both donation after circulatory death (DCD, $n=5$) and donation after brain death (DBD, $n=2$) cases, comprising both male ($n=3$) and female individuals aged between 59 and 84 years. Causes of death were predominantly cerebrovascular accidents or cardiac arrest. The primary reason for discard was advanced donor age, frequently in combination with DCD status or additional comorbidities such as arterial hypertension or type 2 diabetes mellitus. In one case, calcification of the renal artery was also reported. Last serum creatinine levels ranged from 0.83 to 1.18 mg/dL. Cold ischemia times ranged from 2h10min to 3h46min, with one exception in which perfusion was performed the following day, resulting in a prolonged cold ischemia time of 18h04min. In DCD donors, the mean procurement warm ischemia time was 9.2 minutes.

Table 1. Clinical characteristics of donor kidney pairs included in this study

Kidney pair	Donor age (years)	Gender	Donor type	Cause of death	Reason for decline	WIT (time)	CIT (time)	Weight (g)	Terminal Cr (mg/dL)
Pair 1	84	F	DCD	Stroke	Age + DCD	8min	2h39	L – 185g R – 180g	1.11
Pair 2	73	M	DCD	Stroke	Age + DCD	8min	3h16	L – 220g R – 190g	0.86
Pair 3	81	F	DCD	Trauma	Age + DCD + calcification of renal artery	8min	2h10	L – 210g R – 240g	0.86
Pair 4	77	M	DBD	Stroke	Age + ADK history	0	18h04	L – 319g R – 370g	0.83
Pair 5	73	F	DBD	Stroke	Age + HTA	0	3h46	L – 240g R – 238g	1.18
Pair 6	76	F	DCD	CVA	Age + DCD + AHT	11min	3h31	L – 310g R – 290g	0.9
Pair 7	59	M	DCD	Cardiac arrest	DCD + AHT + T2DM	11min	3h26	L – 360g R – 340g	1.02

Note: F, Female; M, Male; DBD, Donation after brain death; DCD, Donation after circulatory death; CVA, Cerebrovascular accident; T2DM, Diabetes mellitus type 2; ADK, Adenocarcinoma; AHT, Arterial Hypertension; WIT, Warm Ischemic Time; CIT, Cold Ischemic Time, L, Left kidney; R, Right kidney; Cr, Creatinine

3.2 — Physiological and functional parameters during NMP

Comparison within kidney pairs showed no major differences in physiological parameters between MSC-treated and non-treated kidneys (**Fig 2**). All kidneys produced urine and demonstrated homogeneous perfusion throughout NMP. Although renal blood flow (RBF) varied significantly over time ($p < 0.0001$), no statistically significant difference was detected between groups (treatment effect: $p=0.66$; time $\times$ treatment interaction: $p=0.83$; Fig. 2A).

However, a trend toward higher RBF was apparent in the treated group throughout the perfusion period. Similarly, while intrarenal resistance (IRR) showed a significant time effect ($p=0.04$), no significant between-group differences were found (treatment: $p=0.33$; interaction: $p=0.71$; Fig. 2B), although a tendency toward lower resistance was observed in the treated group during the two first hours of perfusion. Perfusate sodium concentrations increased significantly over time ($p< 0.0001$), while potassium concentrations declined ($p< 0.001$), with no differences detected between MSC-treated and non-treated kidneys for sodium (treatment: $p=0.97$; interaction: $p=0.30$; Fig 2C). Although no significant treatment effect was observed for potassium ($p=0.50$), a borderline significant interaction between time and treatment was detected ($p=0.048$), suggesting a subtle divergence in the temporal evolution of potassium concentrations between groups (Fig 2D).

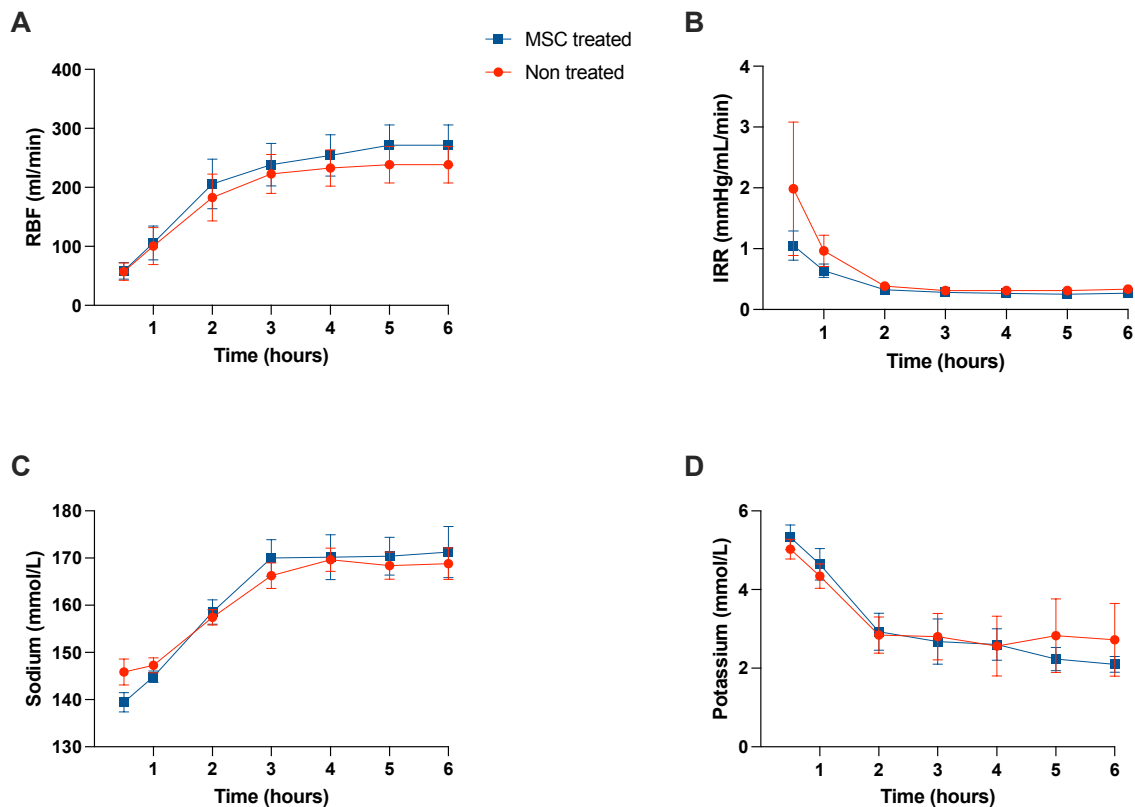


Fig 2. Hemodynamic and electrolyte parameters during normothermic machine perfusion. Longitudinal assessment of perfusion dynamics in paired human kidneys treated or not with MSCs. (A) Renal blood flow (RBF), showing a trend toward higher flow in the MSC-treated group throughout perfusion. (B) Intrarenal resistance (IRR), with a tendency toward lower resistance in the treated group during the early phase of perfusion. (C) Sodium concentration in the perfusate over time. (D) Potassium concentration in the perfusate over time. Data are presented as mean $\pm$ SEM ($n = 7$ pairs). Statistical analysis was performed using a linear mixed-effects model accounting for repeated measures within kidney pairs. No significant treatment effect or time $\times$ treatment interaction was observed for any parameter.

Urine output, expressed as mL/h/100 g of kidney tissue, increased over time in both groups ($p=0.009$), with no significant differences between MSC-treated and non-treated kidneys ($p=0.61$; interaction $p=0.93$; Fig. 3A). Higher urine output values were observed in MSC-treated kidneys at 2, 3, 5, and 6 hours of perfusion, although these differences did not reach statistical significance. To further evaluate renal function during perfusion, filtration capacity was assessed by creatinine clearance (Fig. 3B). Creatinine clearance increased significantly over time ($p=0.01$), reflecting dynamic changes in filtration throughout NMP. No significant differences were observed between groups ($p=0.40$; interaction $p=0.33$), although creatinine clearance values were consistently higher in MSC-treated kidneys throughout perfusion. Tubular function was assessed by the fractional excretion of sodium (Fig. 3C), which showed lower values in MSC-treated kidneys, without reaching statistical significance ($p=0.47$; interaction $p=0.96$). NGAL concentrations increased markedly over time in both groups, consistent with progressive tubular stress during perfusion. Although NGAL levels were lower in MSC-treated kidneys at later time points, these differences did not reach statistical significance (treatment: $p=0.81$; interaction: $p=0.96$; Fig. 3D). Perfusate creatinine concentrations declined progressively over time in both groups ($p<0.001$), with no significant difference between MSC-treated and non-treated kidneys ($p=0.55$; interaction $p=0.99$; Fig. 3E), reflecting effective creatinine elimination during perfusion. Similarly, iohexol concentrations decreased markedly over time in both groups ($p<0.001$), with no significant difference between groups ($p=0.69$; interaction $p=0.95$; Fig. 3F). Across all parameters analysed, post hoc comparisons at individual time points confirmed the absence of significant differences between MSC-treated and non-treated kidneys.

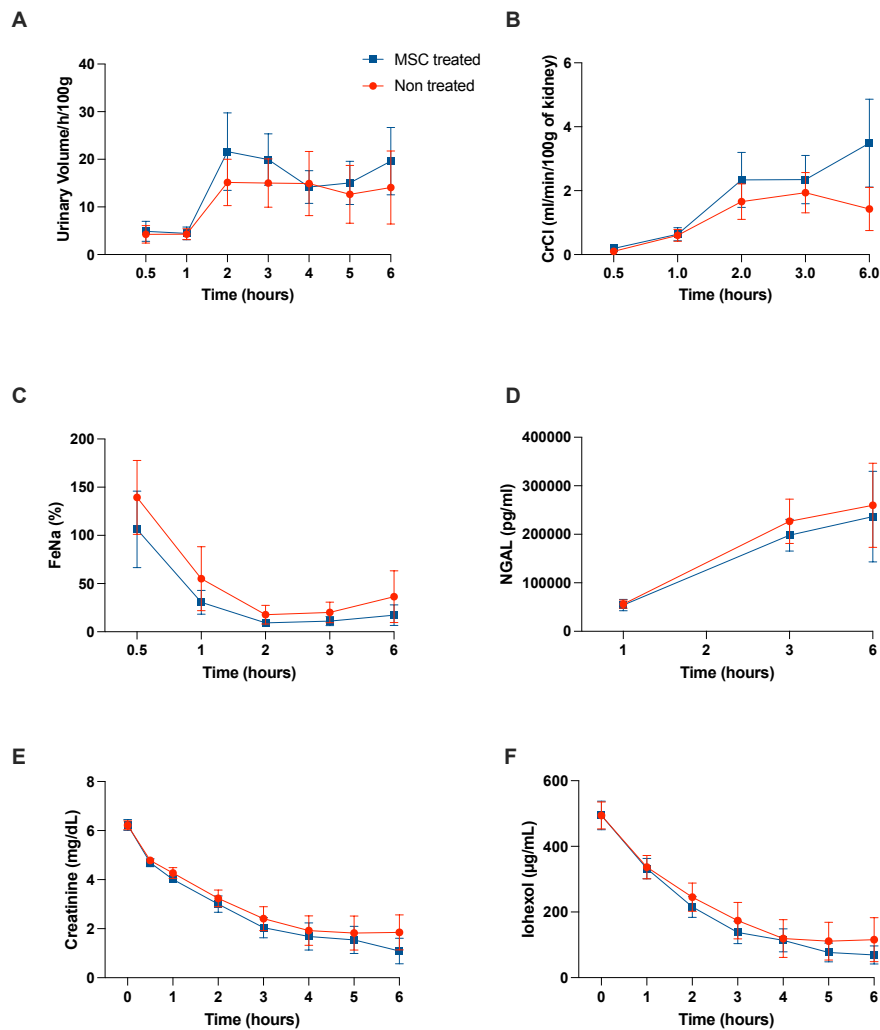


Fig 3. Functional parameters during normothermic machine perfusion. Longitudinal assessment of renal function in paired human kidneys treated or not with MSCs. (A) Urinary volume normalized to kidney weight, with a trend toward higher diuresis in the MSC-treated group. (B) Creatinine clearance (CrCl) per 100g of kidney, as an estimate of glomerular filtration rate, showing a tendency toward higher values in the treated group at later time points. (C) Fractional excretion of sodium (FeNa), reflecting tubular function over time. (D) Neutrophil gelatinase-associated lipocalin (NGAL) levels in the perfusate, as a marker of renal tubular injury. (E) Perfusate creatinine concentrations over time, reflecting progressive creatinine elimination during perfusion. (F) Perfusate iohexol concentrations over time, used as an exogenous marker of glomerular filtration rate. Data are presented as mean $\pm$ SEM (n = 7 pairs). Statistical analysis was performed using a linear mixed-effects model accounting for repeated measures within kidney pairs. No significant treatment effect or time $\times$ treatment interaction was observed for any parameter.

3.3 — Perfusate cytokine assessment

To assess whether MSC administration during NMP modulates the inflammatory milieu of human kidney grafts, we quantified, in the perfusate, a panel of pro- and anti-inflammatory cytokines as well as injury-associated markers (**Fig 4**). Across all conditions, cytokine levels exhibited a time-dependent increase during perfusion. Despite observable variability and differences in magnitude between MSC-treated and non-treated kidneys for selected analytes, no statistically significant differences were identified at any time point after correction for multiple comparisons. Although non-significant trends were noted for certain mediators, including lower levels of IL-6 and TNF- α in MSC-treated kidneys at later time points, these findings were not consistent across the cytokine panel and did not reach statistical significance. Similarly, anti-inflammatory cytokines and injury-related markers, including IL-10, KIM-1, and VEGF-A, followed comparable temporal trajectories in both groups. Collectively, these data indicate that, under the present experimental conditions, MSC delivery does not result in a measurable modulation of the cytokine profile during *ex vivo* perfusion.

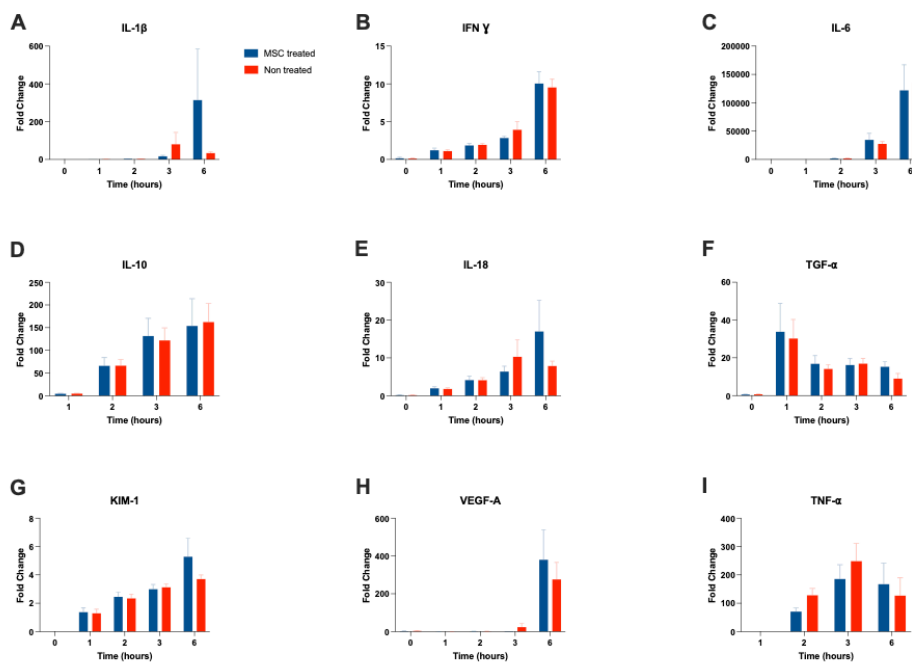


Fig 4. Temporal profile of inflammatory and injury-related biomarkers during NMP expressed as fold change from baseline. Time-dependent changes in perfusate cytokines and injury markers during normothermic machine perfusion (NMP) in MSC-treated and non-treated kidneys, expressed as fold change relative to baseline (T0). (A), IL-1 β ; (B), IFN- γ ; (C), IL-6 ; (D), IL-10 ; (E), IL-18 ; (F), TGF- α ; (G), KIM-1 ; (H), VEGF-A ; (I), TNF- α . Data are presented as mean $\pm$ SD. Fold change was calculated relative to baseline values for each kidney. Due to the wide dynamic range across analytes, y-axis scales differ between panels. Comparisons between MSC-treated and control kidneys at each time point were performed using paired Wilcoxon signed-rank tests on log₂-transformed fold change values, with false discovery rate (FDR) correction for multiple testing. No statistically significant differences were observed between groups.

3.4 — Histological assessment of kidney injury

Biopsies from all 7 donor pairs were sampled after 6 hours of NMP and scored according to the modified Banff classification (**Figure S1, Table S1**). Both non-treated and MSC-treated kidneys displayed mild pre-existing chronic changes, including low-grade mesangial matrix expansion, arteriolar hyalinosis, and interstitial fibrosis with tubular atrophy. The extent of fibrosis and interstitial atrophy (%FIAT) remained below 10% in most samples, except for Pair 6, which showed 15–20% in the non-treated kidney. Within each donor pair, the histological profiles of the two kidneys were comparable, confirming that pre-existing lesions did not confound the assessment of MSC treatment effect.

3.5 — Renal distribution of hMSCs

Fluorescence microscopy of cryosections revealed the presence of CellTracker CMPTX Red-labelled hMSCs (red puncta, indicated by white arrows) dispersed among renal tubular cells counterstained with DAPI (blue) (**Fig 5**). Red-labelled cells were detectable as early as 30 minutes post-infusion. At 1-hour, fluorescent cells appeared more numerous, although their distribution was heterogeneous across the tissue sections, with some areas showing higher cell density than others. From 2 hours onwards, the number of visible labelled cells appeared to gradually decrease, though isolated, red-labelled cells remained detectable up to 6 hours post-infusion. These qualitative observations suggest that infused hMSCs reach the renal parenchyma rapidly and can be detected over a 6-hour period, although the heterogeneity of the biopsies precludes any definitive quantitative conclusion regarding their spatiotemporal distribution.

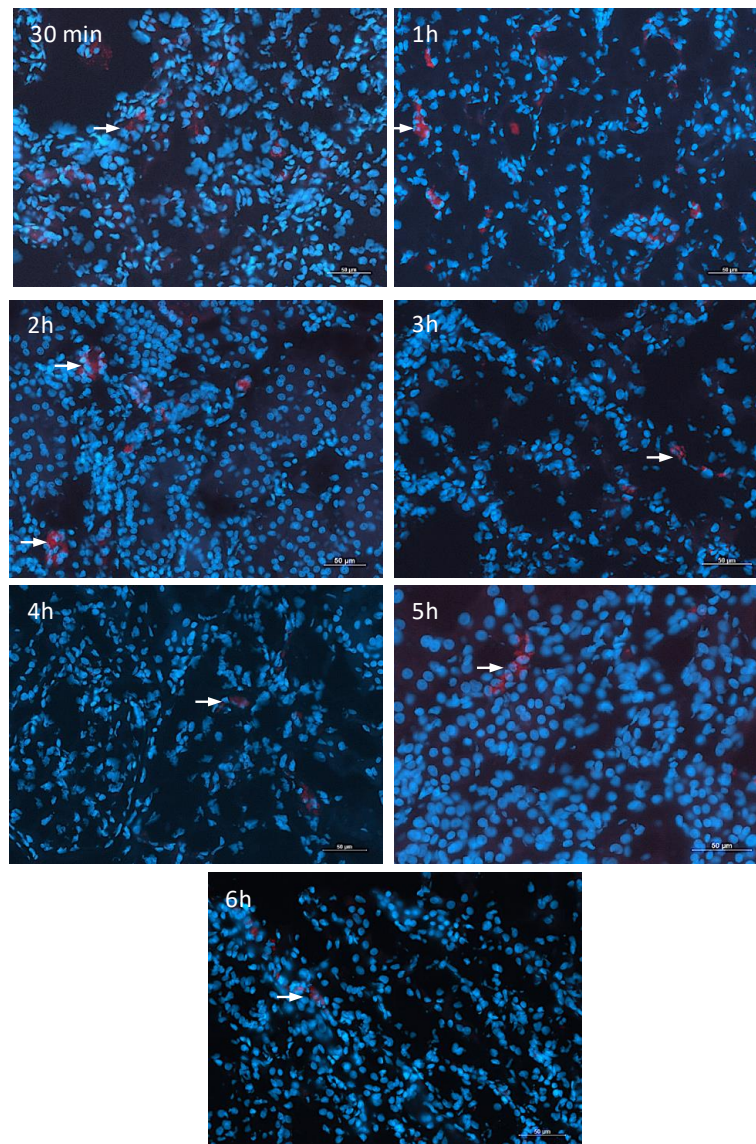


Figure 5. Fluorescence microscopy tracking of hMSCs. Cryosections (5 μm) of renal biopsies collected at 30 minutes, 1, 2, 3, 4, 5 and 6 hours post-infusion were examined by fluorescence microscopy using a Zeiss Axioscan slide scanner. hMSCs prelabelled with CellTracker CMPTX Red (red, white arrows) are visible within the renal tissue counterstained with DAPI (blue). The heterogeneous distribution of labelled cells across tissue sections reflects the inherent variability of the biopsy sampling. Scale bar = 50 μm .

4. Discussion

While MSC-based therapies have shown promising results in preclinical transplantation models, their translation to human grafts under clinically relevant perfusion conditions remains largely unexplored. To our knowledge, the present study is the first to evaluate the effect of clinical-grade, bone marrow-derived, allogeneic hMSC treatment during NMP in a preclinical model in humans using discarded kidneys and a paired design, thereby providing an internal control for inter-donor variability.

Our study demonstrates that intra-arterial hMSC delivery during NMP is technically feasible and safe: functional parameters — including renal blood flow, intrarenal resistance, creatinine clearance, and NGAL levels — evolved comparably between MSC-treated and non-treated kidneys throughout the perfusion period, with no detectable adverse effects on NMP dynamics. Notably, consistently higher creatinine clearance values and greater urine output were observed in MSC-treated kidneys across multiple time points throughout perfusion, alongside a trend toward higher renal blood flow and lower intrarenal resistance in the early perfusion phase. While these differences did not reach statistical significance, their consistency across independent functional parameters is biologically coherent and may reflect a real, though modest, paracrine reparative signal. The sample size of the present study — inherent to the use of discarded organ pairs — was not powered to detect differences of such magnitude, and the possibility that a larger cohort would reveal statistically significant improvements in creatinine clearance or urine output cannot be excluded.

The clinical grade hMSCs used in our study were produced under GMP conditions and administered immediately after thawing, without prior *in vitro* culture or expansion. This approach differs from some earlier studies in which MSCs were cultured prior to administration, which, while potentially beneficial for viability and function, introduces significant practical barriers to clinical implementation and represents a departure from standard GMP release practice.^{126,127,141,297} This ready-to-use, off-the-shelf approach is closely aligned with the logistical constraints of solid organ transplantation *and reflects standard GMP release practice*. Importantly, the laboratory's GMP manufacturing protocol relies on a highly standardized culture process with rigorous batch qualification, which substantially reduces inter-batch variability compared to non-GMP cultured cells used in other studies. These hMSCs have already demonstrated clinical safety in two phase I-II, controlled trials conducted by the same group, in which a single infusion of third-party hMSCs were infused after transplantation in 10 liver and 10 kidney recipients, respectively.^{133,267} In the kidney recipient cohort, hMSC infusion was associated with improved early allograft function and increased regulatory T cell, but also with a risk of immunization against the unrelated hMSC donor Human Leucocyte Antigens (HLA).¹³³ This prior clinical experience provides a strong rationale for the hMSC use during NMP to decrease the risk of immunization and supports the biological plausibility of an effect in the present study.

The time-dependent increase in cytokine levels observed in both groups reflected the expected pattern of ischemia–reperfusion injury during *ex vivo* perfusion, without significant modulation by MSC treatment after correction for multiple comparisons, although non-significant trends were noted in selected cytokines. Histological assessment revealed comparable levels of tubular injury, inflammation, and vascular changes across groups.

These findings are in line with those reported by Lohmann *et al.* in a porcine auto transplantation model, in which MSC infusion during NMP was equally well tolerated and similarly did not yield measurable improvements in renal function or histological injury parameters within a 14-day post-transplant follow-up, despite confirmed MSC retention in the renal cortex.²²⁶

The interpretation of these findings warrants consideration of several contributing factors. First, intra-arterial MSC delivery during machine perfusion is inherently constrained by cell loss within the perfusion circuit: Sierra-Parraga *et al.* showed that circulating MSC numbers decline to approximately 10% of the infused dose within hours of NMP onset,²⁹³ and Pool *et al.* demonstrated that the few intact cells remaining were predominantly trapped in glomerular capillaries rather than distributing to the tubular compartments most affected by ischemic injury.¹²⁶ Consistent with these observations, fluorescence microscopy tracking of CMPTX Red-labelled hMSCs in our study revealed a sparse and heterogeneous distribution of MSCs within the renal parenchyma, with an apparent reduction in detectable MSCs over the 6-hour observation period, suggesting limited and transient engraftment following intra-arterial infusion. The dose of 10×10^6 MSCs was selected based on the work of Pool *et al.*, who identified this as the minimum effective dose for MSC detection and retention in the kidney during NMP, and subsequently used it to demonstrate reduced injury markers and immunomodulatory effects in an *ex vivo* porcine model.^{126,298} Second, the NMP duration may have been insufficient to allow MSC-derived paracrine mediators to exert detectable reparative effects. The growing feasibility of prolonged NMP protocols extending to 24–48 hours opens a broader therapeutic window that future studies combining extended perfusion with biological interventions should explore.^{77,79–81} Third, inter-donor variability — even within a paired design — may have obscured signals of modest magnitude, a limitation inherent to all discarded organ studies. Finally, the optimal MSC dose, infusion timing, and perfusion composition for human kidney NMP remain poorly defined, and as Lohmann *et al.* acknowledged, the absence of detectable benefit should not be equated with biological inactivity.²²⁶ The present findings, combined with converging mechanistic evidence, suggest that intact MSC delivery may not be the optimal strategy for harnessing MSC biological potential during machine perfusion. As argued by Gilbo *et al.*, the rapid loss of viable cells in the perfusion circuit and their predominantly glomerular trapping — rather than distribution to injured tubular compartments — represent fundamental barriers unlikely to be overcome by dose optimization alone, making extracellular vesicles (EV)-based or secretome-based strategies inherently better suited to this context.²³³

In conclusion, this study demonstrates that clinical-grade, allogeneic, hMSC delivery during human kidney NMP is technically feasible, safe, and directly translatable to a clinical setting — establishing a rigorous methodological framework and an ethically grounded experimental platform that future interventional studies can build upon. These findings contribute valuable groundwork for the field, clarifying what remains to be optimized and in which direction future efforts should be directed. The maturation of NMP as an active therapeutic platform, combined with the expanding availability of prolonged perfusion protocols and the growing mechanistic evidence supporting EV-based approaches, identifies realistic and well-supported directions for pre-transplant organ conditioning. The paired discarded kidney model validated here — spanning both DBD and DCD donors under clinically representative conditions — offers an ideal infrastructure to pursue these strategies, with the objective of improving graft quality and outcomes for transplant recipients.

5. Supplementary File

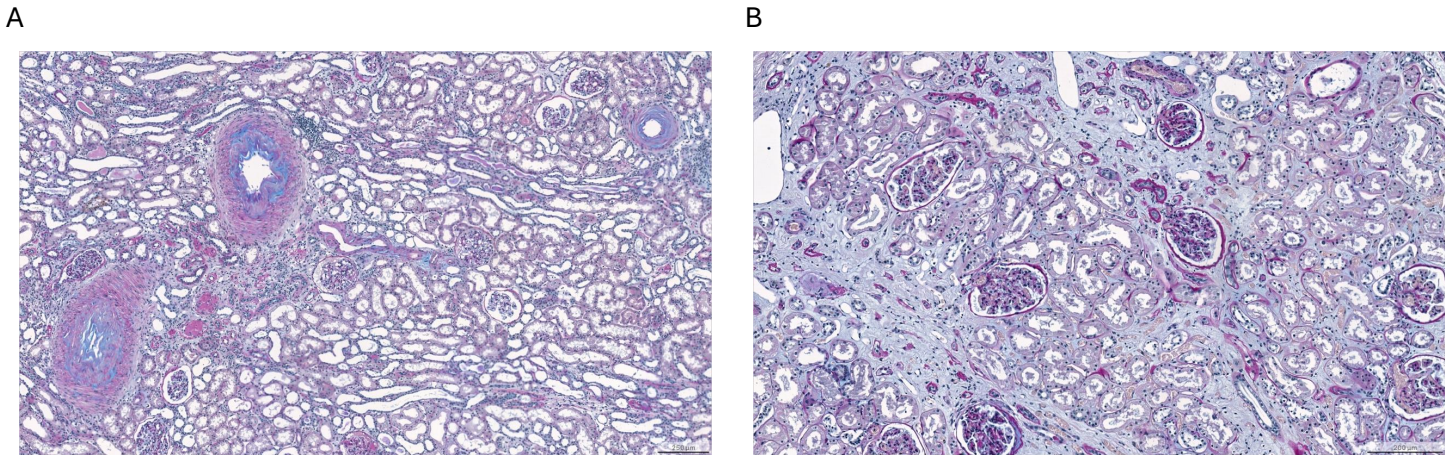


Figure S1. Representative histological images of chronic lesions in discarded human kidneys (Periodic Acid Schiff staining). (A) Low-magnification view showing multiple arteries with concentric fibrous intimal thickening ($cv \geq 1$) leading to vascular luminal narrowing. (B) Intermediate-magnification view showing areas of low-inflammatory fibrosis and tubular atrophy, along with glomeruli exhibiting mild focal and segmental mesangial expansion. Lesions were graded according to the Banff classification.

Table S1— Histological scoring of paired kidney biopsies in non-treated and MSC-treated conditions after 6 hours of NMP

KIDNEY PAIR	NON-TREATED KIDNEY						MSC-TREATED KIDNEY					
	mm	ah	cv	ci	ct	%FIAT	mm	ah	cv	ci	ct	%FIAT
PAIR 1	1	0	1	1	1	5–10%	1	0	1	1	1	5–15%
PAIR 2	1	0	1	0	1	< 5%	1	1	1	1	1	10–20%
PAIR 3	0	0	1	1	1	5–10%	1	1	0	1	1	5–10%
PAIR 4	1	0	1	1	1	5–10%	1	0	1	0	1	5%
PAIR 5	2	2	1	0	1	5%	1	1	2	0	1	<5%
PAIR 6	0	0	0	1	1	15–20%	0	0	1	1	1	5–10%
PAIR 7	3	1	0	1	1	5–10%	3	1	0	0	1	5%

Note: Renal biopsies from seven donor-recipient pairs were assessed according to the Banff classification criteria. For each kidney, the following lesion scores were recorded: mesangial matrix increase (mm), arteriolar hyalinosis (ah), interstitial fibrosis by vascular score (cv), interstitial inflammation (ci), and tubular atrophy (ct). The percentage of fibrosis and tubular atrophy (%FIAT) was estimated semi quantitatively. Scores range from 0 to 3, where 0 indicates absence of lesion and higher values reflect increasing severity. Results are presented for each pair as individual Banff scores.

Part III. General Discussion and Future Perspectives

1. General Discussion

1.1— Overview

Kidney transplantation remains the best available treatment for ESRD, yet its transformative potential continues to be constrained by the persistent gap between organ supply and clinical demand. The progressive expansion of the donor pool using grafts obtained from DCD and ECD has partially alleviated this imbalance, but it has simultaneously introduced new challenges: these organs are inherently more susceptible to IRI, carry a higher risk of DGF, and are disproportionately represented among those discarded prior to transplantation. NMP has emerged in this context as a technological platform — one that not only enables functional assessment of marginal organs under near-physiological conditions but also opens a therapeutic window during which active biological interventions can be delivered directly to the graft before transplantation. MSCs represent one of the most compelling candidates for such *ex vivo* therapeutic delivery. Yet, at the outset of this thesis, the translational evidence base for combining MSC therapy with machine perfusion was fragmented, methodologically heterogeneous, and largely derived from rodent or poorly controlled large-animal experiments. The core scientific question motivating this thesis was therefore whether intra-arterial delivery of clinical-grade MSCs during NMP could attenuate IRI-associated damage and modulate the immunological profile of the renal graft.

This thesis addressed that question through a stepwise translational programme comprising four complementary studies. Together, these studies form a coherent arc — from evidence synthesis, through methodological validation, to large-animal and human organ proof-of-concept — designed to establish the scientific and technical foundations upon which future interventional strategies can be rationally built. The following sections move through the principal findings of this thesis — from the methodological foundations established by the systematic review and the paired NMP model, through the convergent safety and feasibility data, to the interpretation of the absence of functional benefit — before turning to the general limitations of this work and the translational perspectives it opens.

1.2— Lessons from the systematic review

The systematic review and meta-analysis presented in this thesis was, to our knowledge, the first to formally synthesise the totality of published preclinical evidence on stem cell-based therapies during machine perfusion of kidney grafts. Its findings were instructive in two aspects. First, pooled analyses did not demonstrate a statistically significant improvement in renal function during perfusion, regardless of stem cell type or perfusion temperature. This result contrasted with the general optimism in the field and provided a sober empirical baseline for the subsequent experimental work.

Second, more importantly for the design of this thesis, the review exposed the profound methodological heterogeneity characterising the existing literature: studies differed markedly in terms of cell type (bone marrow- or adipose-derived MSCs, multipotent adult progenitor cells, urine-derived progenitors, or extracellular vesicles), cell dose (ranging from 1 to 30 million cells), perfusion temperature and duration, animal species, ischemia conditions, and outcome definitions. This variability made cross-study comparison essentially impossible and, critically, prevented any identification of the conditions under which therapeutic benefit might be achievable.

This diagnosis of heterogeneity was not only descriptive: it directly shaped the experimental strategy of this thesis. If the existing evidence was uninterpretable because studies differed in too many dimensions simultaneously, then the necessary first step was not to add yet another heterogeneous experiment to the pool, but rather to build a rigorously standardised model. A model in which a single, well-defined treatment — MSC administration in this case— could be evaluated against an internal, paired control. This gap led directly to the design of the paired porcine NMP model described in this thesis.

1.3— Building the paired porcine NMP model

The primary objective of the study presented in Chapter 4 was to establish whether the two kidneys harvested from the same donor pig behaved equivalently during simultaneous NMP — a prerequisite for the use of one kidney as a valid biological control for the other. Indeed, when this project was initiated, porcine NMP models were increasingly used to test experimental interventions, but a fundamental methodological problem was rarely acknowledged. Most groups operated a single perfusion device, which meant that kidneys from the same donor — or from two different donors — must be perfused consecutively rather than simultaneously. The consequence was directly acknowledged by Weissenbacher *et al.*: in their porcine study comparing urine recirculation strategies, both kidneys from the same pig were perfused sequentially, with the second organ kept on ice until the first perfusion was complete — resulting in CITs of 2 and 27 hours respectively, simply because only one perfusion system was available. The authors themselves noted that the duration of CIT had no significant impact on arterial flow in their cohort, but a 25-hour difference in CIT between paired organs is a potentially major confounding variable when the aim is to isolate the effect of a specific intervention.²⁹⁹ Pool *et al.*, such as many others, similarly worked with kidneys obtained individually from a slaughterhouse, without any form of paired intra-individual design, making the attribution of differences to a treatment effect rather than baseline organ variability difficult to justify.¹³⁹

This problem is not limited to animal models. Studies using discarded human kidneys face additional, largely unavoidable constraints: organ availability depends on procurement logistics, transport times, and donor characteristics that cannot be standardised or controlled by the research team. As illustrated by studies working with discarded human kidneys, including that of Oliveira Arcolino *et al.*, CITs can vary between paired organs depending on the order of surgical retrieval, the distance from the procurement centre, and the timing of organ arrival at the laboratory.²²³ Inter-donor biological heterogeneity — in age, cause of death, terminal creatinine, and comorbidities — adds a further layer of variability that no statistical adjustment can fully account for. These are inherent limitations of human organ research, and they are entirely legitimate. In a porcine model, however, all these sources of variability are avoidable. Ischemia times, procurement conditions, blood source, and perfusion start can all be standardised and controlled by design.

A central — and technically demanding — part of this thesis was therefore the development, from the ground up, of two simultaneous NMP perfusion systems. Commercially available devices were either cost-prohibitive for paired simultaneous use or insufficiently configurable to our experimental requirements. In consequence, we designed and assembled two home-made NMP circuits based on cardiopulmonary bypass technology, with custom-designed tubing sets, membrane oxygenators, roller pumps, heat exchangers, and dedicated kidney reservoirs. Each circuit was equipped with an electronic gas delivery system supplying oxygen, compressed air, and CO₂ to the oxygenator inlet, enabling precise and independent control of the gas mixture administered to the perfusate — including oxygen fraction, air flow rate, and the optional addition of CO₂ to regulate perfusate pH. Building and optimising these systems occupied a considerable part of the early experimental phase of this thesis: each circuit had to maintain stable arterial pressure, flow, temperature, and oxygenation independently and simultaneously over six hours. The simultaneous paired perfusion model developed in this thesis was built precisely on this principle: by perfusing both kidneys from the same donor animal at exactly the same time, using the same perfusate and identical circuit settings, it eliminates the inter-individual noise that dominates between-group comparisons in less controlled designs, and ensures that any observed difference between the two organs can only reflect the effect of the intervention under evaluation.

The results of our study confirmed this assumption experimentally for the first time: across all haemodynamic, metabolic, and functional parameters assessed during six hours of NMP, no significant difference was observed between paired right and left kidneys. Arterial pressure, flow, intrarenal resistance, electrolyte balance, urine output, and creatinine clearance were all statistically indistinguishable within pairs, despite considerable inter-animal variability. Critically, the generalised linear mixed model revealed that 47% of the total variability in perfusion parameters was attributable to inter-individual differences between animals, rather than to left–right asymmetry within the same donor.

This finding provided the quantitative justification for the paired design: by using contralateral kidneys from the same pig, this inter-individual noise is removed entirely from the comparison, greatly increasing the statistical sensitivity for detecting treatment effects in subsequent experiments.

This study pioneered also the use of iohexol clearance as a functional endpoint during kidney NMP, complementing the more conventional creatinine-based assessment. As discussed previously, endogenous porcine creatinine could introduce a systematic bias with the use of exogenous creatinine as a GFR tracer during NMP, since it competes for tubular handling and measurement. Iohexol, a freely filtered, non-secreted, non-reabsorbed contrast agent, circumvents this limitation entirely. The significant correlation between iohexol and creatinine clearances observed across all ten perfused kidneys — together with the high intra-pair concordance of both tracers — validated this approach and informed its subsequent application in the human kidney study of **Chapter 6**.

An important limitation of the model as initially described was the absence of a warm ischemia period: kidneys were harvested from healthy pigs under general anaesthesia and subjected only to short cold ischemia, conditions that do not reflect the injury profile of DCD donor organs. The importance of warm ischemia in establishing a clinically representative porcine NMP model has been well demonstrated by Kathis *et al.*, whose series of porcine autotransplantation studies consistently used 30 minutes of warm ischemia as a standardised DCD-like injury. This duration induced reproducible tubular injury, elevated perfusate lactate, raised serum creatinine post-transplant, and delayed functional recovery. This profile is simply absent in uninjured kidneys which constitutes the very substrate against which any therapeutic intervention must be evaluated.^{300,301} This limitation was explicitly addressed in the design of **Chapter 5**, which introduced a standardised 30-minute period of donor warm ischemia followed by three hours of static cold storage before NMP. This modification resulted in a model that closely replicates the ischemia–reperfusion conditions encountered in human DCD kidney transplantation, both in terms of injury severity and preservation pathway.

1.4— Safety and feasibility: from porcine model to human kidneys

Across the two experimental studies of this thesis — one in a porcine DCD model and one in discarded human kidneys — the intra-arterial delivery of clinical-grade bone marrow-derived hMSCs during NMP was consistently demonstrated to be technically feasible and haemodynamically safe. In neither study did MSC administration result in adverse perfusion events, circuit instability, or deterioration of macroscopic organ appearance compared to paired control. Arterial pressure, flow rates, intrarenal resistance, and urine output evolved comparably between MSC-treated and control kidneys throughout the six-hour perfusion period in both models.

These observations are not trivial: the intra-arterial route carries an inherent risk of microvascular obstruction in injured organs, and the potential for MSC clumping within the perfusion circuit was a legitimate concern before these experiments were conducted. As discussed in the Introduction, prior work had already established that intra-arterially delivered MSCs are predominantly trapped in glomerular capillaries and rapidly cleared from the perfusion circuit, with circulating cell numbers falling to approximately 10% of the infused dose within hours.^{126,265} The absence of any negative effect — in both porcine and human kidneys — was therefore not to be taken for granted, and should be read in the context of a real prior concern about circuit obstruction and organ damage.

The safety profile documented in this thesis is broadly consistent with data from adjacent fields. In the clinical setting, Erpicum *et al.* from our group had previously demonstrated that intravenous infusion of allogeneic bone marrow-derived MSCs ($\sim 2 \times 10^6$ cells/kg) in kidney transplant recipients was safe and well tolerated, with no acute infusion-related adverse events.¹³³ In the preclinical transplantation literature, Lohmann *et al.* similarly reported no safety concerns following intra-arterial MSC delivery during NMP in a porcine autotransplantation model.¹⁴⁰ What the present data add is the confirmation of this safety profile in discarded human kidneys — under clinically representative ischemia conditions and in a controlled, paired, blinded design — a step that had not previously been achieved in a human organ setting. The reproducibility of this safety signal across two species and two distinct ischemia profiles strengthens confidence in its generalisability.

1.5— Consistency effects across studies and species

The most scientifically important finding of this thesis is the consistent absence of any detectable improvement in renal function or reduction in histological injury following MSC administration during NMP, observed independently across the systematic review, the porcine experimental study, and the human kidney study. This convergence is all the more meaningful given the methodological diversity of the three contexts: the systematic review pooled data across multiple research groups, cell types, and animal models; the porcine study used a controlled DCD-like injury model with standardised ischemia times; and the human study introduced a level of inter-donor biological heterogeneity that cannot be replicated in animal models. That the same absence of functional benefit emerged across all three, despite these differences in context and rigour, substantially strengthens the conclusion.

Across all three bodies of evidence, functional parameters including creatinine clearance, iohexol clearance, urine output, and intrarenal resistance were statistically indistinguishable between MSC-treated and control kidneys throughout the six-hour perfusion period.

Perfusate NGAL concentrations — a sensitive marker of acute tubular injury — increased progressively in both groups but were not significantly modified by MSC treatment after correction for multiple comparisons, although numerically lower values were noted in the MSC group at selected time points in Chapter 6. Cytokine profiles evolved comparably in both groups, reflecting the expected pattern of IRI-associated inflammation during *ex vivo* perfusion. This trajectory was well characterised in human kidney NMP by Weissenbacher *et al.*, who showed that perfusate cytokine and biomarker dynamics during the first six hours of NMP are primarily driven by donor organ quality and ischemia severity rather than perfusion conditions *per se*.⁸⁸ Histological assessment at the end of perfusion revealed comparable levels of tubular injury, peritubular capillaritis, and inflammatory infiltration between groups in both species. This convergence across three independent lines of evidence — a meta-analysis of published preclinical data, a blinded randomised large-animal experiment, and a translational human organ study — provides a level of internal consistency that substantially exceeds what any single study could achieve. It strongly suggests that the absence of benefit is a robust biological signal rather than an error attributable to insufficient statistical power in any individual experiment.

1.6— *The mismatch between perfusion duration and biological repair*

A central interpretive framework for understanding these results is the mismatch between the biological timescale of MSC-mediated repair and the observational window afforded by standard NMP protocols. Experimental models of renal IRI have consistently demonstrated that tubular regeneration is a days-to-weeks process. Surviving tubular epithelial cells undergo partial dedifferentiation before repopulating injured nephron segments — a process that begins within 24–48 hours of reperfusion, peaks between 72 hours and day 4, and results in functional recovery largely complete by day 7–14.^{143,302} Even if MSC-derived paracrine mediators successfully initiated early pro-regenerative signalling during the six-hour NMP window — a biologically plausible scenario given the anti-inflammatory and growth-factor-rich secretome of these cells — the functional consequences of such signalling would, by definition, remain undetectable within the timeframe of *ex vivo* perfusion. As discussed in Chapter 3, reductions in inflammatory mediators during perfusion may represent early biological signals whose functional implications would only become apparent after transplantation and prolonged *in vivo* reperfusion. This temporal limitation is not unique to MSC therapy: it reflects a fundamental constraint of the standard NMP paradigm as currently practised. Six-hour perfusion protocols, while clinically convenient and sufficient for viability assessment, are inherently too short to serve as a readout window for reparative biological processes.

1.7— *Tracking the cells: a technical and translational challenge*

A second explanation for the absence of measurable benefit relates to the fate of the administered cells. Using clinical-grade, cryopreserved MSCs administered immediately post-thawing — rather than freshly cultured or research-grade cells — was not an oversight but a decision driven by two complementary objectives. The first was translational fidelity: the same cell product already administered to patients in clinical trials at our centre was used in both experimental studies, ensuring that efficacy data generated here would be directly applicable to clinical implementation. The second was methodological rigour. The cell product and the matching placebo were prepared and labelled by the LTCG independently of our research team, and delivered to the experimental site in identically appearing, anonymised syringes. As a result, no member of our team involved in organ procurement, NMP monitoring, sample collection, or data analysis knew which kidney had received MSCs and which had received placebo — at any point during the experimental phase or during the initial analysis of results. This external preparation of both the active and control products by a separate GMP-certified laboratory constitutes a strength of this thesis: it eliminated any possibility of performance or detection bias at the experimental level. It is a methodological standard that is rarely achieved in large-animal or *ex vivo* organ research, where the preparation of cell products and the conduct of experiments typically occur within the same team.

However, this organisational choice may come at a biological cost. Cryopreserved cells administered immediately post-thawing may exhibit reduced viability, altered surface marker expression, and impaired paracrine secretion compared with cells that have been allowed to recover *in vitro* for 24–48 hours — effects that Sierra Parraga *et al.* demonstrated experimentally under NMP perfusion conditions.²⁶⁵ Whether this contributed to the absence of detectable effects cannot be determined from our data alone, but it represents a variable that future studies should explicitly address.

1.8— *Learning from the absence of effect*

It is important to emphasise that the convergent absence of benefit documented across Chapters 3, 5, and 6 is not a scientific failure: it is a scientifically valuable and actionable result. The field of organ transplantation has, historically, suffered from the premature clinical translation of interventions supported by heterogeneous and insufficiently controlled preclinical data. The results of this thesis are clear on one point: a single dose of clinical-grade MSCs administered during standard six-hour NMP does not yield measurable functional benefit, whether in ischemic porcine kidneys or in discarded human organs, under rigorous blinded and internally controlled conditions. This finding provides a solid empirical rationale for redirecting future research efforts. The field can now focus its efforts not on repeating what has already been shown not to work, but on understanding why — and on designing the next generation of studies accordingly.

2. General Limitations

Before turning to future perspectives, several overarching limitations of this research deserve mention.

First, all three original studies were limited in sample size — a constraint inherent to large-animal experimental models and to studies on discarded human organs, where logistical, ethical, and financial considerations limit throughput. While the paired design of these studies substantially mitigates the statistical consequences of small sample sizes by eliminating inter-individual variability, the absolute number of experimental units remains potentially insufficient to detect effect sizes. This is particularly relevant for the human kidney study, which included only seven donor pairs, five DCD and two DBD. This cohort, while clinically representative in terms of donor profiles and ischemia conditions, does not provide sufficient statistical power to draw definitive conclusions about subgroup differences between donor types or to detect effects.

Second, the absence of post-transplantation data is perhaps the most consequential limitation of this research. All functional and histological readouts were obtained at the end of *ex vivo* perfusion. This *ex vivo* endpoint, while informative for safety and feasibility assessment, cannot capture the outcomes that matter most clinically: DGF, PNF, and long-term graft survival. MSC-mediated repair processes operate over days to weeks, and their effects — if any — would only become apparent after transplantation and sustained *in vivo* reperfusion. This limits our ability to draw conclusions about the potential clinical impact of MSC delivery during NMP, which can only be properly evaluated in a transplantation model with adequate post-operative follow-up.

Third, histological assessment was performed exclusively on biopsies obtained at the end of perfusion. This cross-sectional approach does not capture the temporal evolution of tissue injury and early repair during the six-hour perfusion period. Changes that may be transient, spatially heterogeneous, or concentrated in compartments not represented by a single cortical core biopsy would remain undetected. Serial biopsies at intermediate time points would provide a more complete picture of the biological trajectory of the graft during MSC exposure, though this approach carries its own practical constraints in *ex vivo* perfusion models.

Fourth, this research evaluated a single MSC product at a single dose and a single time point of administration. Whether alternative MSC sources, higher or repeated doses, or different administration schedules would yield different results cannot be determined from the present data. The generalisation of these findings to other cell products or delivery protocols should therefore be made with appropriate caution.

Finally, the *home-made* nature of the NMP circuits used in this thesis represents an additional limitation that deserves acknowledgement. As described in Chapter 4, both perfusion systems were assembled in-house at a time when no validated, commercially available device dedicated to renal NMP existed in our institution. This approach was a necessity at the outset of this project and constitutes a methodological contribution. However, the field has evolved rapidly over the course of this thesis. Several devices for *ex vivo* kidney machine perfusion are now commercially available in Europe, including the **Kidney Assist** (XVIVO, Sweden) — which supports both HMP and NMP (12–37°C) — the **PerLife® system** (Aferetica, Italy), the **ARK Kidney®** (Ebers Medical Technology, Spain), and the Metra K (OrganOx, UK) (**Figure 1**).^{71,85} The **Kidney Assist Transport**, a portable variant designed primarily for HOPE, received FDA clearance in 2022 but remains available only in select markets. While these devices have CE marking and are used in research and select clinical centres, NMP of the kidney has yet to achieve widespread clinical adoption, in contrast to its more established use for liver, heart, and lung preservation. These systems offer standardised hardware, integrated pressure and flow monitoring, automated regulation of perfusion parameters, and validated software interfaces that reduce operator-dependent variability — advantages that our home-made circuits, however functional, could not fully guarantee over extended perfusion durations. Lantinga *et al.* demonstrated that the choice of device and protocol significantly influences kidney function and perfusion parameters during NMP — an observation that underscores the importance of device standardisation for the reproducibility and cross-centre comparability of results.⁷¹

Kidney Assist XVIVO - Sweden	ARK Kidney® Ebers - Spain	PerLife® Aferetica - Italy	Metra K OrganOx - UK
			
Stationary	Portable	Stationary	Portable
CE Certificate	CE Certificate	CE Certificate	In development
Up to 6 hours	Up to 73 hours	Up to 6 hours	At least 24 hours
10 - 37°C	15 - 37°C	4 - 37°C	25°C or 37°C
0 - 90 mmHg	80 ± 0.4 mmHg		N/A
0 - 1000 ml/min	N/A		N/A

Figure 1. Overview of ex vivo NMP devices currently available or in development for kidney transplantation.

Four perfusion platforms are depicted with their main technical specifications. The Kidney Assist (XVIVO, Sweden) and PerLife® (Aferetica, Italy) are stationary, CE-marked devices primarily used in a back-to-base approach. The ARK Kidney® (Ebers, Spain) is the only CE-marked portable device, enabling NMP during transport with a maximum studied duration of 73 hours *ex vivo*. The Metra K (OrganOx, UK) is a portable automated system designed for prolonged perfusion (≥ 24 hours) with urine recirculation and integrated gas-exchange monitoring; it is currently undergoing clinical evaluation. N/A: parameter not publicly disclosed. CE: “Conformité Européenne” mark under EU Medical Device Regulation (MDR 2017/745). Adapted from Rabelink *et al.* (*Nat. Com.*, 2025),⁸⁵ modified using Biorender.com.

3. Future Perspectives

3.1— Cell tracking: a prerequisite for any future intervention study

Before any new therapeutic strategy is evaluated in our experimental system, one methodological gap must be addressed as a priority: the need for a reliable method to confirm that the administered product reaches the renal parenchyma and distributes to the compartments affected by ischemic injury. As discussed in the limitations section, MSC detection in kidney tissue at the end of NMP remained challenging in both the porcine and human studies. Without robust biodistribution data, interpreting any biological result — positive or negative — remains fundamentally ambiguous, and this limitation would apply equally to any future cell-based or cell-free intervention.

A first important step forward has been achieved through a preliminary experiment using CellTracker™ Red CMPTX, a cell-permeant fluorescent dye that is retained within living cells and does not transfer to neighbouring cells. This experiment demonstrated that intact MSCs could be detected within the renal tissue up to six hours after intra-arterial injection during NMP, confirming that the cells do reach the parenchyma and persist within the organ throughout the perfusion period. This finding is consistent with the results reported by Pool *et al.* in a porcine kidney NMP model, where fluorescently pre-labelled MSCs were localised within glomerular capillaries by immunofluorescence, further validating the feasibility of this tracking approach in the *ex vivo* perfusion setting.¹²⁶ However, while qualitative detection has now been established, a critical next step remains: the quantification of MSCs present within the renal tissue. Knowing that cells reach the organ is necessary but insufficient — understanding how many cells are retained, in which compartments, and at what density is essential to interpret any downstream functional or biological effect. Without this quantitative dimension, the relationship between the administered dose and the observed biological response cannot be established, and dose-optimisation for future efficacy studies will remain empirical.

A more robust long-term solution would be the development of a GFP-transduced MSC research-grade preparation, providing stable and constitutive fluorescence independent of cell viability and enabling discrimination between intact cells and membrane debris. Such a preparation would be used exclusively for biodistribution and quantification studies, never as a substitute for the clinical-grade product in efficacy evaluations. Together, these tracking strategies — qualitative localisation and rigorous quantification — should be considered a prerequisite before any intervention study is initiated in our experimental system.

3.2— From whole cells to extracellular vesicles: refining the therapeutic strategy

Under the conditions tested, no measurable functional signal emerged following MSC administration. This does not prove the absence of any biological effect but raises the question of whether the current strategy reaches the right compartment by the right route. Part of the answer may lie in the cell product itself, and two aspects deserve consideration.

The first concerns cellular potency. MSCs are not constitutively immunosuppressive: their therapeutic activity is conditional upon exposure to an inflammatory microenvironment; whereby pro-inflammatory cytokines activate intracellular signalling cascades that upregulate the secretion of immunosuppressive mediators.³⁰³ Cryopreserved cells administered immediately post-thawing may not have undergone sufficient exposure before injection into the graft — particularly if they are cleared from the circuit before full activation can occur. Pre-conditioning strategies offer one approach to circumvent this limitation.

Hypoxic pre-conditioning, reviewed by Yang *et al.*, promotes MSC survival, enhances HIF-1 α -dependent paracrine secretion, and increases the production of pro-angiogenic and anti-inflammatory mediators — effectively pre-activating the cells before delivery.³⁰⁴ Whether such pre-conditioning strategies can be incorporated into a GMP-compliant release protocol without compromising clinical-grade product specifications warrants prospective regulatory assessment.

The second concerns dosing strategy. Throughout this thesis, MSCs were administered as a single intra-arterial bolus at 45 minutes after NMP initiation — a timing chosen pragmatically but not optimised for biological effect. Given the rapid clearance documented in the literature, the biological exposure of the graft to live MSCs may have been limited to a very short window, far shorter than the six-hour perfusion period. Whether repeated injections at multiple time points — for example at 45, 120, and 240 minutes — could maintain a therapeutically effective cellular dose throughout the perfusion period is an entirely open question. To our knowledge, no published study has tested repeated intra-arterial MSC dosing during kidney NMP, and this represents a gap in the evidence base. Such a strategy would, however, require a longer perfusion window than the six-hour protocol used in this thesis — which links directly to the perspective discussed in the following section — and would necessitate the cell tracking infrastructure discussed previously.

Finally, and arguably more promising, direction is to abandon whole-cell delivery altogether in favour of MSC-derived EVs. Unlike intact cells, EVs are nanoscale membrane-bound particles that distribute passively through vascular and interstitial compartments, carrying regulatory microRNAs, proteins, and bioactive lipids. They are not subject to size-dependent glomerular entrapment, do not require *in vivo* licensing to exert paracrine effects, and can be produced, characterised, and stored under GMP-compliant conditions with a degree of batch-to-batch consistency that is difficult to achieve for living cell products.^{147,305} Rampino *et al.* demonstrated that MSC-derived EVs delivered during HOPE of extended-criteria human kidneys reduced ischemic injury scores, preserved mitochondrial function, and stimulated pro-angiogenic signalling, without adverse haemodynamic effects.²²⁴ More recently, Burdeyron *et al.* showed in a porcine HMP/NMP model that EV delivery from urine-derived progenitor cells was feasible and safe, reduced cell damage markers, and induced a distinct transcriptomic signature in kidney biopsies. Creatinine clearance and NGAL levels during NMP were not significantly different, consistent with the temporal limitation discussed previously.²²¹ Grange *et al.* reviewed the potential applications of EVs across solid organ transplantation, including their delivery during machine perfusion for kidney, lung, and liver grafts, and positioned MSC-derived EVs as a promising tool to reduce IRI and improve graft viability in transplantation.³⁰⁶

Crucially, EVs could be produced from the same bone marrow-derived MSC line already used in our studies, ensuring continuity with the existing translational framework — and could be evaluated using the paired discarded human kidney platform, which represents exactly the experimental infrastructure needed for a rigorous first human organ study.

3.3— A cell-free horizon: pharmacological conditioning strategies as alternatives to MSC-based therapy

Beyond the MSC/EVs paradigm, it is important to recognise, however, that the value of machine perfusion as a therapeutic platform is not contingent on the success of cell-based strategies: the circuit itself represents a delivery vehicle whose utility extends well beyond any single biological approach. Several non-cellular strategies have demonstrated preclinical proof of concept and deserve consideration as alternatives, or complements, should the cellular hypothesis ultimately prove insufficient.

Pharmacological modulation of IRI encompasses several mechanistically distinct approaches. Alkaline phosphatase supplementation during machine perfusion exploits the conversion of extracellular ATP into adenosine to attenuate purinergic pro-inflammatory signalling and has shown favourable metabolic profiles in a porcine HMP/NMP model.³⁰⁷ Mitochondria-targeted antioxidants — in particular MitoQ, a ubiquinone derivative with preferential mitochondrial uptake — have shown significant improvements in *ex vivo* renal blood flow and urine output in both porcine and discarded human kidneys when added to the preservation solution, providing one of the few non-cellular interventions with functional proof-of-concept in human tissue.³⁰⁸ Hydrogen sulphide donors, through induction of a reversible hypometabolic state and mitochondrial protection, have improved DCD graft survival and early post-transplant function in rodent and porcine models.³⁰⁹ Complement inhibition during NMP — targeting either the terminal pathway (C5) or the lectin pathway — represents another tractable approach, given that NMP itself induces measurable complement activation in human kidneys that correlates with pro-inflammatory cytokine release and impaired creatinine clearance.^{92,310,311} Finally, gene therapy approaches, including viral vector-mediated delivery of cytoprotective transgenes such as HO-1 or IL-10 during *ex vivo* perfusion, remain at an early preclinical stage for the kidney, though more advanced for liver and lung grafts.²⁵¹ Franzin *et al.* provide a comprehensive overview of these pharmacological strategies, including senolytic agents, in the context of machine perfusion-based organ reconditioning.³¹² Collectively, these approaches highlight that the therapeutic potential of machine perfusion extends well beyond the cellular biologics currently under investigation, and that the platform retains clinical relevance independently of the fate of the MSC hypothesis.

3.4— *Optimizing the perfusion platform: duration and device*

Whole-cell, EV-based and cell-free strategies would benefit from a longer perfusion window. The six-hour protocol used throughout this thesis is clinically convenient and sufficient for viability assessment but is inherently too short to serve as a readout window for reparative biological processes. The emergence of prolonged NMP protocols extending to 24 hours, demonstrated feasible in both porcine and human kidneys without adverse effects on organ quality, opens a broader therapeutic opportunity.^{77,80,81} An extended window would allow more time for paracrine mediators to initiate and propagate reparative signalling, enable repeated dosing rather than a single bolus, and potentially allow early biological effects to translate into detectable functional changes during perfusion itself.

The transition to a validated commercial platform is also a prerequisite for several of the directions outlined above. Prolonged NMP beyond six hours, repeated therapeutic dosing, and multi-site reproducibility all require a level of circuit stability, automated parameter regulation, and standardised monitoring that our home-made systems were not designed to guarantee over extended durations. The Kidney Assist and comparable commercially validated devices now offer precisely these features, together with regulatory documentation and CE marking that would facilitate ethics submissions for future clinical studies. Moving to such a platform would not simply be a technical upgrade: it would materially expand what is experimentally possible, reduce operator-dependent variability, and make the results generated in our laboratory directly comparable with those produced by other groups. It represents a prerequisite for the kind of collaborative multi-centre validation that will ultimately be necessary before any intervention tested here can be considered for clinical translation.

3.5— *The autotransplantation model: the essential missing step*

As argued throughout this discussion, the most consequential gap in this thesis is the complete absence of post-transplantation data. A dedicated porcine autotransplantation study, incorporating the standardised 30-minute warm ischemia and paired perfusion design, applying EV-based conditioning with confirmed biodistribution, and monitoring grafts for 14 days post-transplant, represents the critical next experimental step before any consideration of clinical implementation. The model of Lohmann *et al.*, which followed MSC-treated kidneys for 14 days after autotransplantation, provides the methodological template — but used intact MSCs under conditions that may not have been optimal.¹⁴⁰ Conducting such a study within the framework established by this thesis — using the paired DCD porcine model and the tracking infrastructure described previously — would represent a direct and well-supported continuation of this work.

3.6— Towards clinical translation

If the autotransplantation model yields encouraging results, the question of clinical translation will arise naturally — but it is important to be realistic about the steps that remain. The human discarded kidney platform developed in this thesis constitutes a valuable methodological foundation, demonstrating the feasibility of conducting blinded, randomised, paired interventional studies on human organs under clinically representative conditions. However, the leap from there to a first-in-human study in transplant recipients is substantial and should not be underestimated.

What makes this perspective nonetheless credible is how rapidly the field is moving. The emerging consensus — well reflected at ESOT 2025 and in the recent literature — is that the primary value of NMP in kidney transplantation may lie not in preservation *per se* but in two applications that are directly relevant to this work. The first is organ rescue: a substantial proportion of kidneys currently discarded are not truly unsuitable for transplantation but simply lack an adequate evaluation framework. 34 Lives, a public benefit company, established the first independent kidney Assessment and Repair Center in the United States in 2025. Their results bear this out: of 158 kidneys initially declined and referred to their centre between April 2024 and May 2025, 142 were ultimately transplanted following sub-normothermic acellular perfusion — a 90% rescue rate.³¹³

The second application is therapeutic delivery — and it is here that the imagination of the field is most active. The same perfusion window that enables functional assessment also allows active interventions to be administered to the isolated organ. MacMillan and Hosgood *et al.* provided perhaps the most striking recent example: by infusing two bacterial enzymes into the NMP circuit, they achieved approximately 80% conversion of blood group A antigens on the renal vasculature to the universal O type within two hours, without adverse haemodynamic effects.³¹⁴ This finding illustrates the range of interventions that the NMP platform can accommodate, and contextualises the MSC and EV strategies explored in this thesis within a much broader landscape of organ engineering during *ex vivo* perfusion.

Before a Phase I study in transplanted kidneys can be contemplated, however, several prerequisites remain. GMP-compliant EV production at therapeutic scale, with validated release criteria and cold-chain logistics compatible with organ procurement timelines, does not yet exist for this specific application. Pharmacokinetic characterisation of EV biodistribution within the human kidney during NMP is needed to establish that vesicles reach the intended compartments. A complete preclinical safety and efficacy dossier, including the autotransplantation data discussed previously, will be required by regulatory authorities. Each of these steps represents a substantial programme of work.

The health-economic case will also need to be made: EV-based therapies are inherently complex and costly to manufacture, and prospective modelling comparing the cost of EV-enhanced NMP against the downstream costs of DGF, dialysis dependency, and graft loss in DCD and ECD recipients will be an essential component of any regulatory and funding submission. **Figure 2** summarises the sequential steps and prerequisites that together define the translational roadmap emerging from this thesis, from methodological groundwork to a potential first-in-human study.

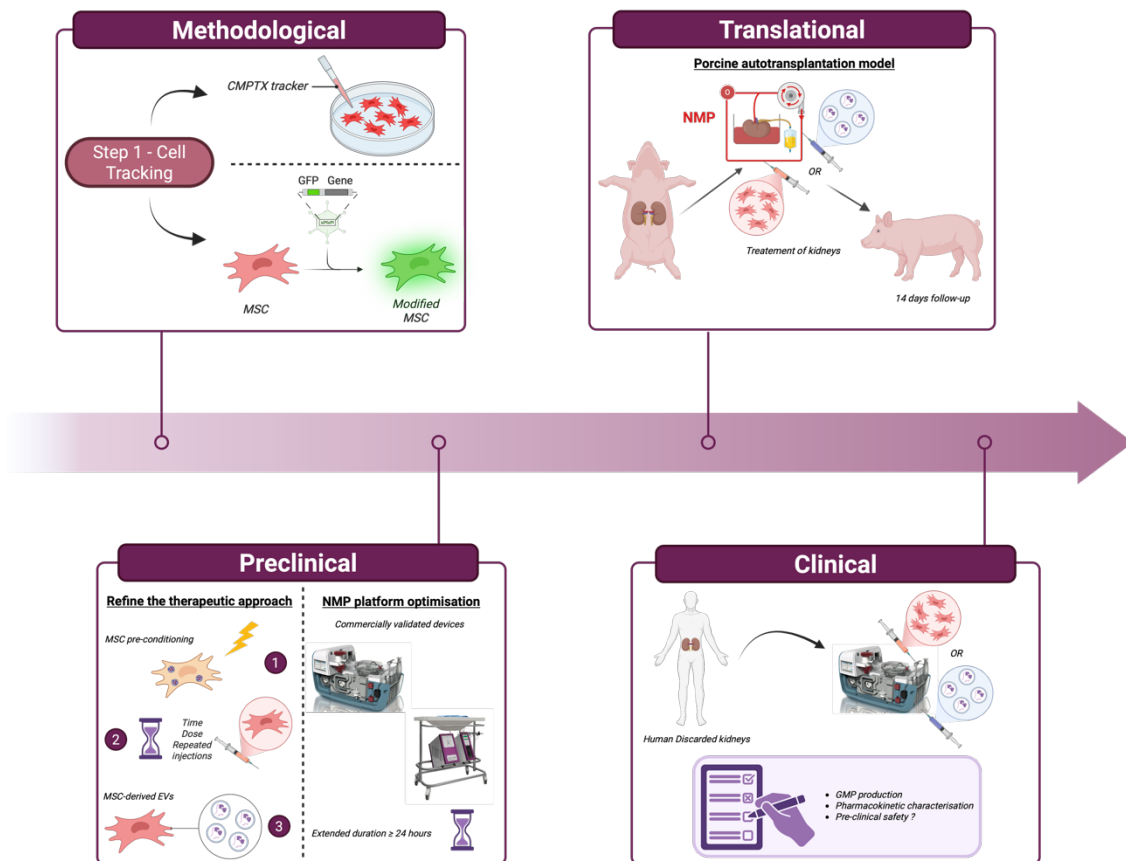


Figure 2. Future perspectives: a translational roadmap toward EV-enhanced NMP in kidney transplantation. Sequential steps from methodological groundwork to clinical implementation, each conditional on completion of the previous stage. MSC: mesenchymal stromal cell; EV: extracellular vesicle; NMP: normothermic machine perfusion; GMP: good manufacturing practice. Figure created with BioRender.com.

4. Conclusion

This thesis set out to address a concrete scientific question: can clinical-grade MSCs delivered intra-arterially during NMP reduce IRI in kidney grafts before transplantation? The answer, based on four complementary studies, is nuanced. The approach is safe and technically feasible. It does not, however, produce measurable functional benefit under the conditions tested — a single dose, six hours of perfusion, in both a porcine DCD model and discarded human kidneys.

The four studies that led to this conclusion each carry their own weight. The systematic review established the first formal evidence synthesis in the field and revealed that the absence of benefit in our experimental work was not an isolated finding but part of a consistent pattern across species, cell types, and protocols. The development of the simultaneous paired porcine NMP model — built from scratch using home-made circuits — was a technically demanding contribution, complete with iohexol clearance as a novel functional marker and a statistically validated rationale for the paired intra-individual design. The porcine intervention study, conducted in a blinded and randomised manner using clinical-grade cells prepared by an independent GMP laboratory, demonstrated that a single dose of hMSCs during six-hour NMP is safe and does not improve renal function. The human discarded kidney study brought this work closer to clinical reality, establishing the platform and the approach on solid enough ground to support future interventional research.

What these results leave open is perhaps as important as what they close. The mechanistic barriers they point toward — the temporal mismatch between perfusion duration and tissue repair kinetics — are specific and addressable. MSC-derived EVs, cell tracking infrastructure, prolonged NMP protocols, and a porcine autotransplantation model with post-operative follow-up are the most clearly supported next steps. In parallel, the field itself is moving: dedicated organ rescue centres are already demonstrating that *ex vivo* perfusion can retrieve organs that would otherwise be discarded, and the NMP platform is increasingly recognised not as a preservation technique competing with HMP, but as an active therapeutic vehicle. The work in this thesis is part of that broader movement.

The paired human kidney platform validated here — spanning DBD and DCD donors, under clinically representative conditions, with rigorous blinding — offers exactly the infrastructure needed to pursue the next generation of interventions. Whether those interventions involve EVs, enzymatic organ modification, gene therapy, or strategies not yet conceived, the methodological groundwork is in place. What this thesis contributes is a rigorous and honest foundation — one from which the field can move forward with greater confidence.

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List of Publications

First-author publications

- **Navez M.**, Gilbo N., Vandermeulen M., Pinto Coelho T., Erpicum P., Lagny M.G., Malisoux C., Guarin C., Thierry G., Delanaye P., Cavalier E., Jouret F., Detry O. *Simultaneous ex situ normothermic perfusion of paired kidneys in pigs*. **Artificial Organs**. 2025;49(9):1405–1416. <https://doi.org/10.1111/aor.15016>
- **Navez, M.**, Detry, O., & Jouret, F. (2026). Mesenchymal/stromal cell therapy in kidney diseases: an update. **Current opinion in nephrology and hypertension**, 35(4), 465–472. <https://doi.org/10.1097/MNH.0000000000001184>
- **Navez, M.**, Dos Santos Barata, E., Maes, N., Levchenko, E., Oliveira Arcolino, F., Burdeyron, P., Steichen, C., Detry, O., Gilbo, N., & Jouret, F. (2026). From Preservation to Regeneration: Stem Cell-Derived Therapies in Machine-Perfused Kidney Transplants - A systematic Review and Meta-analysis. **Kidney & blood pressure research**, 1–25. Advance online publication. <https://doi.org/10.1159/000552604>

Co-authored publications

- Erpicum P., Pinto Coelho T., Bonvoisin C., Bouquegneau A., Grosch S., **Navez M.**, Vandermeulen M., Beguin Y., Detry O., Jouret F., Weekers L. *Delayed graft function: an ongoing clinical challenge*. **Revue Médicale de Liège**. 2023;78(2):107–113.
- Pinto Coelho T., Erpicum P., **Navez M.**, Vandermeulen M., Detry O., Jouret F. *Kidney transplant injury associated with brain death is mediated by TNF α , independently of renal innervation*. **American Journal of Physiology – Renal Physiology**. 2025;329(1):F128–F142.
- Thierry, G., Piette, B., **Navez, M.**, Koch, J. N., Mangon, A., Lagny, M. G., Piette, N., Vandermeulen, M., Gilbo, N., Meurisse, N., Hans, G., Bonhomme, V., & Detry, O. (2026). Oncologic Safety of Irradiated-only Autologous Blood Transfusion in Liver Transplantation for Hepatocellular Carcinoma: A Single-Center Cohort Study. **Transplantation**, 10.1097/TP.0000000000005760. Advance online publication. <https://doi.org/10.1097/TP.0000000000005760>

Under review (first author)

- **Navez M.**, Gilbo N., Vandermeulen M., Erpicum P., Lagny M.G., Malisoux C., Guarin C., Pinto Coelho T., Thierry G., Lechanteur C., Briquet A., Baudoux E., Le Goff C., Cavalier E., Caers J., Jouret F., Detry O. *Infusion of human mesenchymal stromal cells during normothermic machine perfusion of porcine kidneys: a randomized, blinded, preclinical study.* **Scientific reports.**

In preparation

- **Navez M.**, Gilbo N., Vandermeulen M., Erpicum P., Lagny M.G., Malisoux C., Guarin C., Pinto Coelho T., Thierry G., Lechanteur C., Briquet A., Baudoux E., Le Goff C., Cavalier E., Caers J., Jouret F., Detry O. *Mesenchymal Stromal Cell Delivery During Normothermic Machine Perfusion of Discarded Human Kidneys: A Translational Study of Feasibility, Inflammation, and Intrarenal Cell Distribution.*

Scientific Communications

Competitive and invited oral presentations at international and national conferences.

- **Navez, M.** and Steichen, C. (24 April 2026). *Normothermic Machine Perfusion as a platform for stem cell-based treatment.* **RISE 2026**, Poitiers, France.

- **Navez, M.** and Delnooz, E. (16 April 2026). *Modèles de perfusion de greffons rénaux au laboratoire de chirurgie expérimentale.* **WILD 2026**, Liège, Belgium.

- **Navez, M.**, Pinto Coelho, T., Erpicum, P., Vandermeulen, M., Lagny, M.-G., Gilbo, N., Thierry, G., Lechanteur, C., Beguin, Y., Le Goff, C., Cavalier, E., Jouret, F., & Detry, O. (25 October 2025). *Mesenchymal Stromal Cell Therapy under Normothermic Machine Perfusion : a placebo-controlled study in a pig model* [Paper presentation]. **Europerfusion 2025**, Athènes, Greece.

- **Navez, M.**, Gilbo, N., Pinto Coelho, T., Erpicum, P., Vandermeulen, M., Lagny, M.-G., Malisoux, C., Guarin, C., Thierry, G., Briquet, A., Lechanteur, C., Baudoux, E., Beguin, Y., Jouret, F., & Detry, O. (29 June 2025). *Mesenchymal Stromal Cell Therapy under Normothermic Machine Perfusion : a placebo- controlled study in a pig model* [Paper presentation]. **European Society for Organ Transplantation (ESOT) congress**, London, United Kingdom.

- **Navez, M.**, Gilbo, N., Pinto Coelho, T., Erpicum, P., Vandermeulen, M., Lagny, M.-G., Thierry, G., Briquet, A., Lechanteur, C., Beguin, Y., Jouret, F., & Detry, O. (25 April 2025). *Mesenchymal Stromal Cell Therapy during Kidney Preservation under Normothermic Machine Perfusion: a Placebo-controlled Study in a Pig Model* [Paper presentation]. **Belgian Surgical Week 2025**, Ostende, Belgium.

- **Navez, M.**, Gilbo, N., Pinto Coelho, T., Erpicum, P., Vandermeulen, M., Lagny, M.-G., Malisoux, C., Guarin, C., Thierry, G., Briquet, A., Lechanteur, C., Baudoux, E., Beguin, Y., Jouret,

F., & Detry, O. (28 March 2025). *Mesenchymal Stromal Cell Therapy during Kidney Preservation under Normothermic dMACHINE Perfusion: a Placebo-controlled Study in a Pig Model* [Poster presentation]. **Congrès Société Belge de Transplantation (BTS) 2025**, Bruxelles, Belgium.

- **Navez, M.**, Pinto Coelho, T., El Karouni, W., Erpicum, P., Vandermeulen, M., Lagny, M.-G., Gilbo, N., Thierry, G., Jouret, F., & Detry, O. (18 October 2024). *Development of a Model for Simultaneous Normothermic Perfusion in Porcine Kidney Grafts* [Paper presentation]. **Europfusion 2024**, Valencia, Spain.

- **Navez, M.**, Pinto Coelho, T., El Karouni, Erpicum, P., Vandermeulen, M., Lagny, M.-G., Gilbo, N., Thierry, G., Delanaye, P., Cavalier, E., Jouret, F., & Detry, O. (24 May 2024). *Development of a Model for Simultaneous Normothermic Perfusion in Porcine Kidney Grafts* [Paper presentation]. **61st ERA Congress**, Stockholm, Sweden.

- **Navez, M.**, Pinto Coelho, T., El Karouni Walid, Erpicum, P., Vandermeulen, M., Lagny, M.-G., Cheramy-Bien, J.-P., Gilbo, N., Malisoux, C., Guarin, C., Thierry, G., Delanaye, P., Cavalier, E., Jouret, F., & Detry, O. (25 April 2024). *Development of a model of simultaneous normothermic perfusion of kidney grafts in the pig* [Paper presentation]. **BVN-SBN Annual Scientific Meeting 2024**, Vilvoorde, Belgium.

- **Navez, M.**, Pinto Coelho, T., El Karouni Walid, Erpicum, P., Vandermeulen, M., Lagny, M.-G., Cheramy-Bien, J.-P., Malisoux, C., Guarin, C., Thierry, G., Delanaye, P., Cavalier, E., Gilbo, N., Jouret, F., & Detry, O. (15 March 2024). *Development of a model of simultaneous normothermic perfusion of kidney grafts in the pig* [Paper presentation]. **Congrès Société Belge de Transplantation**, Bruxelles, Belgium.

- **Navez, M.**, & Detry, O. (December 2023). *Development of a porcine model of kidney Normothermic Machine Perfusion System for the preservation of kidney grafts* [Poster presentation]. **Société Francophone de Transplantation (SFT)**, Brest, France.

- **Navez, M.**, & Detry, O. (September 2023). *Normothermic ex vivo kidney perfusion associated with mesenchymal stromal cells prior to transplantation – development of a porcine model* [Poster presentation]. **Giga Day**, Liège, Belgium.

Grants & Fellowships

- **FRIA Doctoral Fellowship** – Fonds National de la Recherche Scientifique (FNRS), Belgium
- **Operating Research Grants** – Fondation Léon Fredericq, University of Liège
 - Three competitive operating grants (2024, 2025, 2026)
- **Research Fellowship in Renal Diseases** – Fondation Léon Fredericq, 2026
- **Best Presentation Award**, RISE Congress, 2026