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REVIEW ARTICLE

The Immunomodulatory Potential of Normothermic Liver Perfusion: Reconditioning the Graft's Immune Profile Before Transplantation

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Abstract

Normothermic machine perfusion (NMP) has emerged as a promising technique in liver transplantation, addressing limitations of static cold storage by preserving organs under near-physiological conditions. Beyond its known benefits in viability assessment and reduction of ischemia-reperfusion injury, recent evidence highlights its immunomodulatory potential. This review explores the evolving role of NMP as a dynamic immunological platform capable of reconditioning donor liver immune profiles before transplantation. The liver harbors a rich network of innate and adaptive immune cells, including Kupffer cells, dendritic cells, T cells, and liver sinusoidal endothelial cells (LSECs), all of which contribute to early allograft injury and long-term rejection. The controlled ex vivo environment of NMP provides a unique opportunity to intervene therapeutically by flushing inflammatory mediators, replenishing anti-inflammatory agents, or delivering gene therapies directly to the graft. By altering the immune milieu prior to implantation, NMP could reduce early allograft dysfunction and chronic rejection, while improving recipient immune tolerance. We detail the mechanistic pathways involved in graft immune modulation during NMP, including cytokine clearance, cellular reprogramming, and targeted immunotherapy. We also evaluate the feasibility of interventions such as mesenchymal stem cell delivery, anti-inflammatory agents, and extracellular vesicle therapy during perfusion. Challenges including graft-specific immune profiling, therapeutic delivery logistics, and clinical translation are critically analyzed.

1. Introduction

Liver transplantation remains the definitive treatment for end-stage liver disease and acute liver failure. However, the growing disparity between organ demand and availability has led to the increased use of marginal or extended criteria donor livers. These grafts are more prone to ischemia-reperfusion injury, which is a major trigger of immune activation, early allograft dysfunction, and long-term rejection (Liu and Man, 2023). Traditionally, static cold storage has been the standard for liver preservation. It offers little scope to assess or modify graft viability or immunogenicity prior to implantation. Normothermic machine perfusion (NMP) has emerged as a transformative technology in organ preservation. By maintaining the liver at body temperature and supplying it with oxygenated perfusate, NMP mimics physiological conditions and sustains metabolic activity (Dengu et al., 2020; Resch et al., 2020). This not only allows real-time assessment of organ function but also opens avenues for therapeutic intervention during perfusion.

The liver is an immunologically complex organ, populated by resident macrophages (Kupffer cells), liver sinusoidal endothelial cells, dendritic cells, and a heterogeneous population of lymphocytes (Kotlyarov, 2025). Under ischemic stress, these cells release damage-associated molecular patterns that activate innate immune pathways and prime adaptive responses. These immunologic cascades are central to graft injury, rejection, and the need for long-term immunosuppression. NMP creates a window of opportunity spanning several hours during which targeted strategies can be employed to detoxify the graft, suppress pro-inflammatory signaling, enhance anti-inflammatory pathways, and even deliver gene-modifying agents. Studies

have demonstrated that NMP can reduce cytokine levels, preserve endothelial integrity, and modify immune cell activation states, all of which are crucial for reducing graft immunogenicity (Vargas et al., 2023) (Figure 1A & B). This review aims to synthesize current understanding and recent advances in the immunomodulatory role of NMP. We explore how NMP affects cellular and molecular immune components of the liver, the therapeutic interventions under investigation, and the challenges to clinical application. As the field moves toward personalized transplantation, leveraging NMP for immune reconditioning may play a critical role in improving outcomes and reducing immunosuppressive burden.

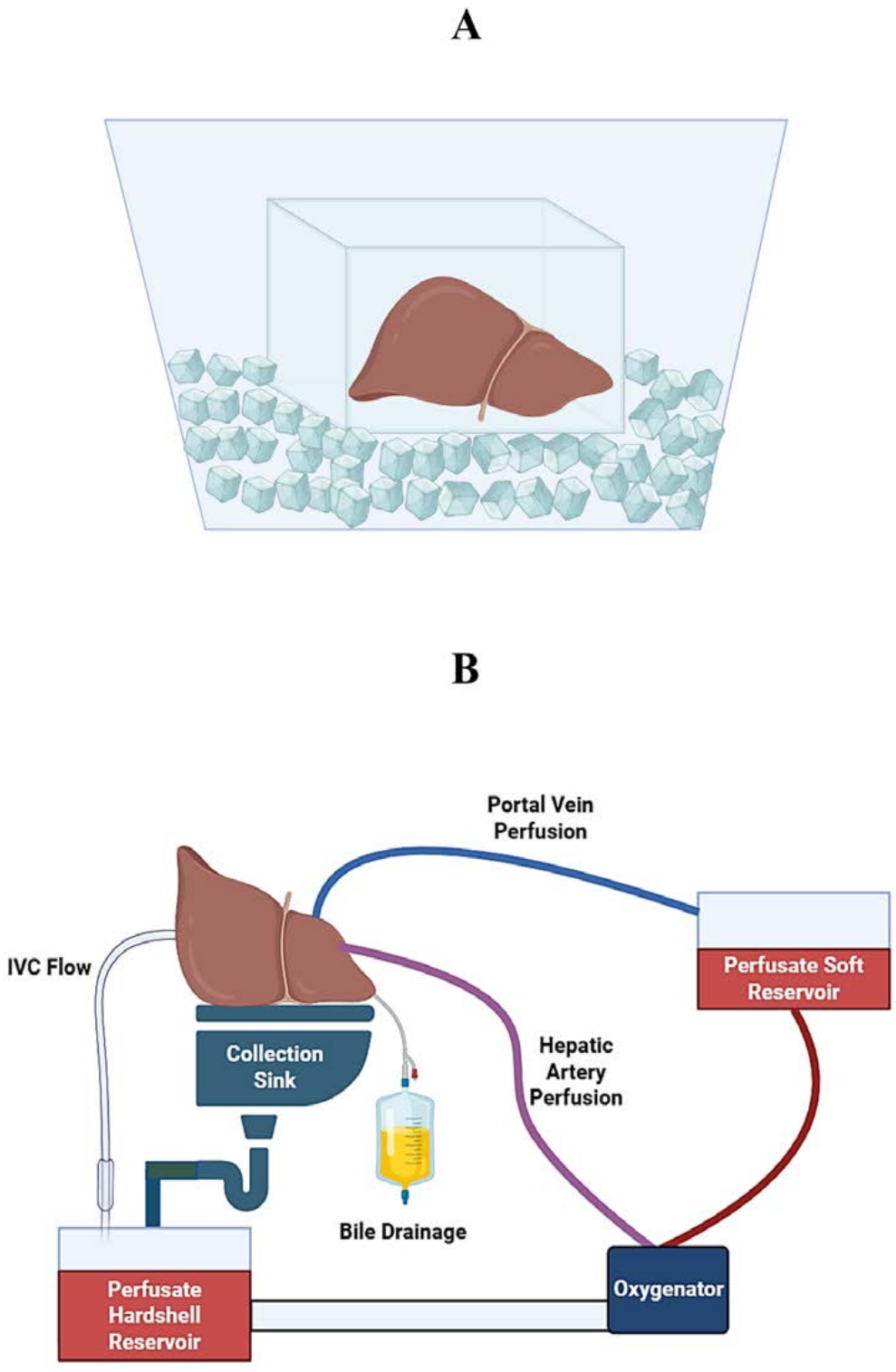

Figure 1: (A) Static Cold Storage, and (B) Normothermic Machine Perfusion device for preservation

2. Immunological Landscape of the Liver and Transplantation Challenges

2.1. Key Immune Cells in the Liver

The liver contains several key immune cells that play critical roles in both homeostasis and pathological responses, such as ischemia-reperfusion injury and transplant rejection (Tang et al., 2022). Kupffer cells are among the first responders to damage-associated molecular patterns released during organ procurement and cold storage. Upon activation, Kupffer cells produce pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), as well as reactive oxygen species (ROS), which exacerbate ischemia-reperfusion injury and promote the recruitment of circulating leukocytes (Aboelez et al., 2024). Liver sinusoidal endothelial cells are essential for maintaining vascular integrity and immune regulation, functioning as antigen-presenting cells that modulate T cell responses (Shetty et al., 2018). Damage to liver sinusoidal endothelial cells during transplantation enhances graft immunogenicity by upregulating adhesion molecules and promoting leukocyte infiltration. Dendritic cells serve as professional antigen-presenting cells, activating T cells and shaping adaptive immune responses toward either tolerance or rejection (Audiger et al., 2017). Additionally, T cells and natural killer cells contribute significantly to post-transplant immunity; upon activation, they mediate acute cellular rejection through cytotoxic mechanisms and drive chronic allograft vasculopathy via sustained inflammatory and fibrotic pathways (Ravindranath et al., 2021). Together, these immune cells orchestrate complex interactions that determine the balance between graft acceptance and rejection.

2.2 Ischemia-Reperfusion Injury as an Immune Trigger

Ischemia-reperfusion injury is central to early graft inflammation (Lutz et al., 2010). The sudden restoration of oxygen leads to oxidative stress, mitochondrial dysfunction, and cell death, releasing damage-associated molecular patterns that trigger Toll-like receptor (TLR) signaling and inflammasome activation. This cascade fuels innate and adaptive immune responses, enhancing graft immunogenicity.

2.3. Long-term Immune Challenges

Persistent low-grade inflammation can lead to chronic rejection, bile duct loss, and fibrosis. Moreover, conventional immunosuppressants, while reducing rejection, increase the risk of infections, malignancy, and metabolic complications. NMP offers an interventional platform to attenuate these immunologic processes before they translate into clinical graft injury (Land and Linkermann, 2025).

3. Mechanisms of Immune Modulation by Normothermic Perfusion

3.1 Cytokine Clearance and Detoxification

The increase in cytokine release and DAMP accumulation induced by cold ischemia are pivotal in triggering inflammation upon reperfusion (Slegtenhorst et al., 2014). NMP facilitates the continuous circulation and filtration of perfusate, allowing removal or dilution of harmful inflammatory mediators. Key cytokines such as TNF-α, IL-6, IL-1β, and HMGB1 are found in high concentrations in livers subjected to static cold storage (W. Liu et al., 2021). During NMP, these molecules are released into the perfusate but do not accumulate due to ongoing clearance mechanisms. Some perfusion protocols incorporate adsorptive columns or hemoadsorption filters to enhance cytokine removal.

3.2 Reprogramming of Immune Cells

The liver harbors a diverse population of immune cells, including Kupffer cells, Dendritic cells, liver sinusoidal endothelial cells, and monocyte-derived macrophages, all of which exhibit phenotypic plasticity in response to environmental stimuli (Wen et al., 2021). NMP offers a unique opportunity to reprogram these cells toward an anti-inflammatory state. Transcriptomic and proteomic analyses demonstrate that Kupffer cells during NMP downregulate pro-inflammatory markers (e.g., CD86, iNOS, MHC-II) while upregulating anti-inflammatory mediators (e.g., IL-10, TGF-β, Arg1) (Lorenz et al., 2009). Similarly, liver sinusoidal endothelial cells which typically express adhesion molecules like VCAM-1 and ICAM-1 during ischemia return to a quiescent phenotype under perfusion conditions (Guo et al., 2022). Metabolic reprogramming also occurs, with macrophages and endothelial cells shifting from glycolysis to oxidative phosphorylation. This phenotypic modulation is highly dependent on NMP controlled oxygenation, pH stability, nutrient supply, and physiological shear stress, collectively reducing the liver's immunogenicity and priming it for improved post-reperfusion outcomes (Table 1).

3.3 Endothelial Protection and Glycocalyx Preservation

The endothelial glycocalyx, a carbohydrate-rich layer lining liver sinusoidal endothelial cells, plays a crucial role in regulating vascular permeability, leukocyte adhesion, and cytokine signaling (Gultom and Rieben, 2024). Cold ischemic storage disrupts this protective layer, exposing adhesion molecules and facilitating neutrophil infiltration. In contrast, NMP maintains physiological shear stress and oxygen delivery, preserving glycocalyx integrity and endothelial function. Perfused livers exhibit lower levels of glycocalyx degradation products compared to static cold storage. Additionally, NMP enhances endothelial nitric oxide synthase (eNOS) expression, improving vasodilation and reducing leukocyte adhesion, while decreasing markers of endothelial activation such as von Willebrand factor and E-selectin (Campos Pamplona et al., 2023; Dabravolski et al., 2022; Knijff et al., 2022). By mitigating endothelial injury, NMP reduces microvascular dysfunction, platelet aggregation, and the release of damage-associated molecular patterns, ultimately diminishing post-transplant immune activation.

3.4. TLR and NLR Pathway Downregulation

Innate immune activation during liver transplantation is largely driven by the recognition of damage-associated molecular patterns through pattern recognition receptors, including TLRs and NLRs. TLR4, for instance, binds HMGB1 and heat shock proteins, triggering NF-κB activation and pro-inflammatory cytokine release (X. Liu et al., 2021). Similarly, NLRP3 inflammasome activation promotes IL-1β and IL-18 maturation, exacerbating inflammation and pyroptosis (Toldo and Abbate, 2024). However, NMP has been shown to suppress these pathways, with biopsies revealing reduced expression of TLR4, MyD88, and NLRP3, along with diminished NF-κB nuclear translocation. This downregulation limits the activation of liver-resident macrophages, decreases dendritic cell maturation, and attenuates the recruitment of circulating immune cells post-reperfusion, thereby reducing the risk of early graft inflammation.

3.5 Reduced Dendritic Cell Maturation

Dendritic cells are pivotal in initiating adaptive immune responses by presenting antigens to T cells. Ischemic stress promotes Dendritic cells maturation, characterized by upregulated MHC-II and co-stimulatory molecules (CD80/CD86), which primes alloreactive T-cell responses post-transplantation (Gordon et al., 2014). NMP counteracts this process by maintaining Dendritic cells in a semi-mature or tolerogenic state, as evidenced by lower expression of MHC-II, CD80, and CD86 compared to static cold storage preserved livers. These tolerogenic Dendritic cells are more likely to produce immunomodulatory factors such as indoleamine 2,3-dioxygenase and IL-10, fostering regulatory T-cell induction rather than effector T-cell activation. By dampening Dendritic cells -driven alloimmunity, NMP may reduce the need for aggressive immunosuppression and improve long-term graft acceptance.

Table 1: Immunomodulatory Mechanisms of Normothermic Liver Perfusion

Mechanism	Immune Effect	Mechanism of Action	Immune Modulation Observed
Cytokine clearance	Reduces pro-inflammatory mediators (e.g., TNF-α, IL-6, IL-1β)	Kupffer cells, LSECs	Decreased inflammation, lower risk of early graft injury
Macrophage and endothelial reprogramming	Shifts to anti-inflammatory phenotype (↑IL-10, ↓CD86, ↓iNOS)	Kupffer cells, LSECs	Reduced graft immunogenicity
Glycocalyx preservation	Maintains vascular integrity, ↓syndecan-1, ↑eNOS	Liver sinusoidal endothelial cells (LSECs)	Improved microcirculation, ↓neutrophil adhesion
TLR/NLR pathway downregulation	Suppresses NF-κB activation, ↓TLR4, ↓NLRP3	Innate immune cells	Reduced DAMP-mediated signaling and cytokine release
Inhibition of dendritic cell maturation	↓MHC-II, ↓CD80/86 expression, ↑IDO, ↑IL-10	Dendritic cells	Promotes tolerogenic responses, ↓T-cell priming
Metabolic shift in immune cells	Oxidative phosphorylation	Macrophages, LSECs	Enhances cell survival and immune quiescence

4. Therapeutic Interventions During NMP for Immune Reconditioning

NMP has emerged not just as a preservation tool, but as a therapeutic window during which a range of targeted interventions can be delivered directly to the liver. This ex vivo platform enables real-time, organ-specific strategies to reduce immunogenicity and improve transplant outcomes. Several therapeutic modalities are being explored, including cell-based therapies, extracellular vesicles, pharmacological agents, gene therapy, and immune tolerance inducing approaches. Each intervention exploits the unique features of the NMP environment physiological temperature, sustained metabolism, and controlled perfusion to recondition the immune landscape of the liver graft before implantation.

4.1 Mesenchymal Stem Cells

Mesenchymal stem cells are widely recognized for their immunomodulatory, anti-inflammatory, and regenerative capabilities, making them attractive candidates for liver graft reconditioning during NMP. When administered into the perfusate, mesenchymal stem cells have been shown to injured areas within the liver parenchyma, particularly sites of endothelial and macrophage activation. Once engrafted, mesenchymal stem cells secrete a milieu of bioactive factors including IL-10, TGF-β, prostaglandin E2, and indoleamine 2,3-dioxygenase (IDO), which collectively suppress innate immune responses and promote an anti-inflammatory microenvironment (Volarevic et al., 2017). They also actively shift hepatic macrophage polarization from an M1 (pro-inflammatory) to an M2 (anti-inflammatory, pro-reparative) phenotype, while attenuating neutrophil infiltration and oxidative stress. Preclinical studies in rat and porcine liver perfusion models have consistently demonstrated improved histological appearance, reduced levels of TNF-α and IL-6, and enhanced endothelial integrity following mesenchymal stem cells therapy (Jiao et al., 2021, 2019). Importantly, mesenchymal stem cells do not require engraftment into the recipient after transplantation, making them a safe, transient modulator of the donor liver's immune environment.

4.2 Extracellular Vesicles

Extracellular vesicles, particularly exosomes derived from mesenchymal stem cells or endothelial progenitor cells, offer a cell-free alternative for immunomodulation during NMP. These nano-sized vesicles carry a anti-inflammatory microRNAs (e.g., miR-21, miR-146a), proteins, and lipids capable of influencing immune cell signaling, suppressing inflammation, and promoting tissue repair (Li et al., 2021). During perfusion, extracellular vesicle can be selectively delivered into the hepatic vasculature, where they are taken up by Kupffer cells, liver sinusoidal endothelial cells, and hepatic stellate cells, altering their gene expression profiles. Notably, extracellular vesicle has been shown to decrease NF-κB signaling and upregulate protective pathways such as PI3K-Akt and STAT3, thereby preserving endothelial glycocalyx and reducing leukocyte adhesion molecule expression (C. Zhang et al., 2019). Extracellular vesicle therapy during NMP has also been associated with reduced necrosis, lower transaminase release, and enhanced mitochondrial function in experimental models. Because extracellular vesicle are non-immunogenic and can be lyophilized or synthesized at scale, they represent a promising, controllable, and regulatory-friendly approach for liver graft preconditioning.

4.3 Pharmacologic Agents

The normothermic perfusion environment allows for the controlled administration of pharmacological agents directly into the liver, providing high concentrations with minimal systemic exposure. Corticosteroids such as methylprednisolone are commonly used to weak inflammatory cascades by suppressing cytokine production (e.g., TNF-α, IL-1β), stabilizing lysosomal membranes, and reducing endothelial permeability. Anti-cytokine biologics like anti-TNF agents (e.g., infliximab) or IL-6 receptor antagonists (e.g., tocilizumab) can be infused into the perfusate to neutralize specific inflammatory mediators, offering precision immunosuppression (Chang et al., 2021). Furthermore, blockade of innate immune receptors through TLR4 antagonists (e.g., eritoran, TAK-242) can mitigate the activation of Kupffer cells and reduce downstream pro-inflammatory signaling triggered by damage-associated molecular patterns. These agents, when delivered during NMP, can prevent the escalation of sterile inflammation and reduce the priming of adaptive immune responses. The ability to monitor drug kinetics, cytokine profiles, and metabolic markers in real-time makes NMP an ideal platform for pharmacological modulation of the liver immune environment.

4.4 Gene Therapy

Gene therapy during NMP represents a novel frontier in immunomodulation, allowing for the temporary or permanent alteration of gene expression in donor organs before implantation. The ex vivo setting circumvents many ethical and safety concerns associated with in vivo gene therapy, enabling direct delivery of adenoviral, lentiviral, or non-viral vectors to hepatocytes, Kupffer cells, and liver sinusoidal endothelial cells. One strategy involves the upregulation of cytoprotective genes such as heme oxygenase-1 (HO-1) or IL-10, both of which suppress inflammation, promote oxidative stress resistance, and modulate immune activation (Origassa and Câmara, 2013). Alternatively, silencing or editing of MHC class I/II molecules, co-stimulatory markers (CD80/CD86), or adhesion molecules (e.g., ICAM-1) can reduce the graft's antigen presentation capabilities and minimize alloimmune recognition (Obregon et al., 2017). In one experimental model, adenoviral delivery of IL-10 during NMP led to prolonged allograft survival in rats and reduced recipient T-cell infiltration (Chen et al., 2007). Though still in early stages, ex vivo gene therapy could eventually be used to program the graft toward a state of immune tolerance or hypoimmunogenicity, offering lasting benefits post-transplant.

4.5 Tolerance-Inducing Agents

Beyond generalized immunosuppression, NMP provides a vehicle for delivering targeted tolerance-inducing agents aimed at promoting donor-specific hypo-responsiveness in the recipient. One key approach involves co-stimulatory blockade, where agents such as CTLA4-Ig are infused during perfusion to inhibit CD28-mediated T cell activation. This intervention downregulates T-cell proliferation and promotes the expansion of regulatory T cells (Tregs). Other agents, like rapamycin or TGF-β mimetics, can also foster Treg development and reduce effector T-cell responses (Strauss et al., 2009). Importantly, timing the delivery of these agents during NMP ensures localized exposure to donor antigen-presenting cells, enhancing their tolerogenic potential. In future protocols, immune tolerance induction may be combined with personalized immunogenetic matching and recipient immune profiling to fine-tune therapy. These strategies could reduce or even eliminate the need for chronic immunosuppression, transforming post-transplant care paradigms.

4.6 Immune Monitoring During NMP

Effective immune reconditioning requires robust, real-time monitoring to assess the evolving immunologic status of the graft during perfusion. Cytokine profiling using multiplex assays allows for the quantification of pro- and anti-inflammatory mediators (e.g., IL-6, TNF-α, IL-10) in the perfusate, offering insights into inflammatory resolution or escalation. Flow cytometry and immunohistochemistry of liver biopsies can track the activation status of immune cell populations, such as Kupffer cells, DCs, and T cells (Di Blasi et al., 2020). Advanced techniques like single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics provide detailed molecular fingerprints of the graft's immune microenvironment (Mou et al., 2025), including cellular cross-talk and regulatory network shifts. Such analyses can guide therapeutic adjustments during NMP, stratify risk, and predict post-transplant rejection or tolerance. Ultimately, the integration of immune monitoring into perfusion protocols will be essential for precision-guided immunotherapy and the safe clinical translation of advanced immune interventions (Table 2).

Table 2. Therapeutic Interventions During Normothermic Liver Perfusion

Intervention	Modality	Mechanism of Action	Key Effects
Mesenchymal stem cells (MSCs)	Cell-based therapy	Secretion of IL-10, TGF-β, IDO; polarization of M2 macrophages	Anti-inflammatory, endothelial repair
Extracellular vesicles (EVs)	Cell-free nano-therapy	Delivery of anti-inflammatory miRNAs and proteins; suppression of NF-κB	↓Cytokine release, preserved glycocalyx, enhanced tissue repair
Pharmacologic agents	Drug infusion during NMP	Anti-cytokines (e.g., anti-TNF), corticosteroids, TLR4 inhibitors	↓Inflammation, precision immunosuppression
Gene therapy	Viral/non-viral vector delivery	Overexpression of IL-10, HO-1; silencing of MHC, ICAM-1, CD80/86	Reduced antigen presentation, ↓alloimmune activation
Tolerance-inducing agents	Co-stimulation blockade (e.g., CTLA4-Ig)	Induces Tregs, inhibits T-effector activation	Graft-specific tolerance, ↓need for post-transplant immunosuppression
Immune monitoring	Real-time cytokine & cellular assays	Flow cytometry, multiplex cytokine profiling, scRNA-seq	Guides therapy, predicts rejection/tolerance

5. Preclinical and Clinical Evidence of Immune Reconditioning

Preclinical studies utilizing rat and porcine liver models have demonstrated that NMP significantly attenuates ischemia-reperfusion injury (Cheng et al., 2021; Zhang et al., 2019), as evidenced by reduced inflammatory markers and diminished neutrophil infiltration, particularly when combined with MSC delivery, which enhances anti-inflammatory IL-10 production. Clinical data, including findings from the VITTAL trial, indicate that NMP-rescued extended criteria donor livers exhibit rejection rates comparable to standard grafts despite higher baseline risk profiles, while subset analyses from the OrganOx consortium reveal that decreased perfusate levels of TNF-α and IL-1β correlate with a reduced incidence of early allograft dysfunction. Ongoing clinical trials are further investigating the safety and immunomodulatory potential of MSC and extracellular vesicle administration during NMP, as well as exploring immune transcriptomic signatures as predictive biomarkers for post-transplant outcomes, aiming to refine personalized immunosuppression strategies and improve graft longevity.

6. Future Perspectives

NLP is evolving beyond organ preservation into a dynamic bioreactor platform for immunological optimization, with future applications potentially including precision immune editing protocols tailored to donor-recipient immunoprofiles through integration of real-time immune monitoring and AI-driven therapeutic modulation during perfusion. Advancements in stem cell and extracellular vesicle therapies, particularly off-the-shelf formulations, along with CRISPR-based ex vivo gene silencing of immunogenic pathways, may enable targeted manipulation of graft immunogenicity, while selective depletion or expansion of immune cell populations during perfusion could become standard practice. This paradigm shifts toward proactive ex vivo immunomodulation may transform transplant immunosuppression from lifelong systemic therapy to the delivery of pre-tolerized grafts, ultimately improving long-term outcomes though realizing this vision will require global collaboration, standardized protocols, and continued innovation in perfusion technology and immunobiology.

7. Conclusion

Normothermic liver perfusion represents a paradigm shift in transplantation, transforming organ preservation into an opportunity for immune reconditioning. By attenuating innate immune activation, enabling targeted therapies, and preserving tissue integrity, NMP can reduce graft immunogenicity and improve long-term outcomes. Though challenges remain in clinical translation, early evidence supports its immunomodulatory potential. As technologies advance, NMP may become central to precision transplant medicine, offering hope for reduced rejection, lower immunosuppressive burden, and enhanced graft survival.

Declarations

Ethics approval statement

Not applicable

Consent to participate

Not applicable

Consent to publish

Not applicable

Data Availability Statement

The data are available from the corresponding author upon reasonable request

Competing Interests

The authors declare that they have no conflict of interest

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Author contribution

Conceptualization, Data curation, Investigation: A.M. Formal analysis, Writing, review, and editing: N.R; I.N. All authors have read and agreed to the published version of the manuscript

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Disclosure use of AI-assisted technologies

During the preparation of this work the authors used AI-assisted technology QuillBot, in order to check the grammar and spell in some sentences

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