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Designer Organs: Ethical Genetic Modifications in the Era of Machine Perfusion

Irina Filz von Reiterdank,[1,2,3] Raphaela Bento,[1,2,4] Insoo Hyun,[5] Rosario Isasi,[6] Susan M. Wolf,[7] J. Henk Coert,[3] Aebele B. Mink van der Molen,[3] Biju Parekkadan,[4] and Korkut Uygun[1,2]

[1]Center for Engineering for Medicine and Surgery, Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, USA; email: KUygun@mgh.harvard.edu

[2]Shriners Children's Boston, Boston, Massachusetts, USA

[3]Department of Plastic, Reconstructive and Hand Surgery, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands

[4]Department of Biomedical Engineering, Rutgers University, Piscataway, New Jersey, USA

[5]Center for Life Sciences and Public Learning, Boston Museum of Science, Boston, Massachusetts, USA

[6]Dr. John T. Macdonald Foundation Department of Human Genetics and Institute for Human Genomics, University of Miami School of Medicine, Miami, Florida, USA

[7]Law School, Medical School, and Consortium on Law and Values in Health, Environment & the Life Sciences, University of Minnesota, Minneapolis, Minnesota, USA

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Abstract

Gene therapy is a rapidly developing field, finally yielding clinical benefits. Genetic engineering of organs for transplantation may soon be an option, thanks to convergence with another breakthrough technology, ex vivo machine perfusion (EVMP). EVMP allows access to the functioning organ for genetic manipulation prior to transplant. EVMP has the potential to enhance genetic engineering efficiency, improve graft survival, and reduce posttransplant complications. This will enable genetic modifications with a vast variety of applications, while raising questions on the ethics and regulation of this emerging technology. This review provides an in-depth

discussion of current methodologies for delivering genetic vectors to transplantable organs, particularly focusing on the enabling role of EVMP. Organ-by-organ analysis and key characteristics of various vector and treatment options are assessed. We offer a road map for research and clinical translation, arguing that achieving scientific benchmarks while creating anticipatory governance is necessary to secure societal benefit from this technology.

Contents

1. INTRODUCTION

Gene therapy is currently one of the most exciting frontiers in medical science, capturing the imagination of researchers and the public alike due to its potential to fundamentally alter disease treatment paradigms. Among its many applications, transplantation stands out as an area where gene therapy can have an immediate and transformative impact given the persistent challenges, including donor shortages, rejection (1), and complications such as ischemia-reperfusion injury (IRI) (2). These are areas where gene therapy can directly intervene to improve outcomes (**Figure 1**). By enhancing organ preservation, reducing rejection and infection rates, and addressing complications at the organ level, gene therapy could significantly extend organ viability and ultimately extend the lives of transplant organ recipients.

Current efforts largely focus on ex vivo genetic engineering and in vivo gene transfer. Incorporating genetic engineering within ex vivo machine perfusion (EVMP) for transplant surgery offers a compelling example of its potential. EVMP (3, 4) itself has proven to be efficient in enhancing organ preservation and reducing IRI in the clinic, leading to increased use of transplant organs. As a platform, EVMP provides a modality for studying gene therapy dynamics under controlled

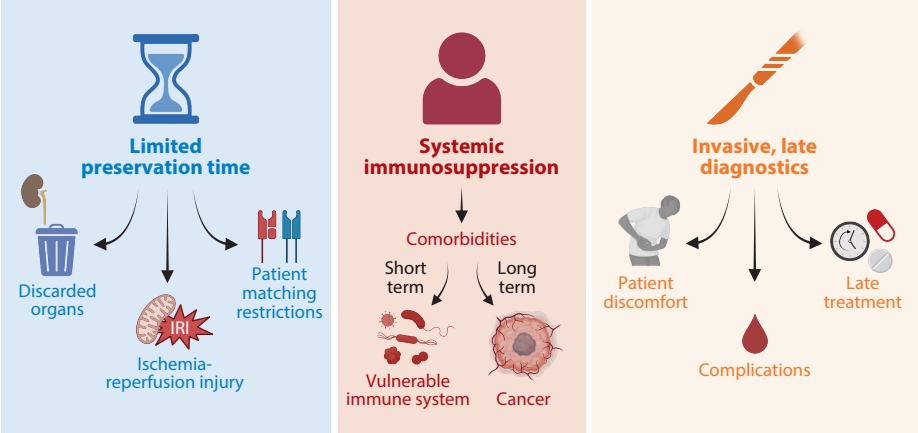

Figure 1

The transplantation field faces critical challenges. Major examples include the limited preservation time for transplant organs; the need for lifelong systemic immunosuppression in organ transplant recipients; and the fact that invasive, late diagnostics lead to loss of transplanted organs and significant patient comorbidities and mortality. Figure adapted from images created in BioRender; Filz von Reiterdank, I. 2025. **https://BioRender.com/x77u607**.

yet physiological conditions using discarded organs (5). Thus, while gene therapy can significantly benefit the field of transplantation, EVMP systems, in turn, offer a robust model to advance our understanding of gene therapy.

Moreover, gene therapy can integrate biomarkers into transplantable organs for real-time monitoring and early rejection detection (6–9), potentially reducing the need for invasive biopsies (10) and lifelong immunosuppression. This would minimize associated comorbidities such as metabolic syndromes, renal toxicity, opportunistic infections, and malignancies (11).

The scope of gene therapy in transplantation could extend far beyond immunological issues and open a gateway to even more revolutionary uses. Transplanted organs could one day be enhanced to outperform their natural counterparts, with livers designed to metabolize new substances or hearts that maintain autonomic rhythm without pacemakers.

Combining genetic engineering and EVMP could yield great benefits as a multi-treatment modality in the field of organ transplantation. Despite these promising advancements, the reality is that clinical translation of these techniques remains nascent. As we elucidate in this review, the current delivery methods are not yet robustly established, and significant scientific and technical challenges persist. We explore the work that has laid the foundation for these promising innovations and address the immense potential of these techniques. Subsequently, we address the ethical, regulatory, societal, and scientific challenges that must be navigated to realize their full potential. Our discussion not only covers the technical and clinical aspects but also considers the expected wider societal implications of such profound changes, including the need for anticipatory governance to ensure these technologies are developed responsibly and equitably.

2. CREATING A SMART GRAFT

2.1. Leveraging Ex Vivo Machine Perfusion for Vector Delivery

Vector delivery modalities can vary by animal or surgical model. Common techniques include local tissue injection or systemic intravascular injection. Generally, smaller animal models are more suited to direct injection of the vectors into the organ of interest (12, 13). In humans, direct

injection is particularly suitable for ophthalmic applications (14–16). Intravascular injection has been successful in both rodents and humans but poses challenges in targeting specific organs (17–20). For whole organs, neither in vivo intravascular injection nor direct tissue injection achieves full graft penetration, and both modalities bear respective risks of off-target side effects and local tissue damage (21).

Current organ preservation methods postprocurement include static cold storage (SCS) and EVMP (22). SCS is unsuitable for cell transduction due to hypothermic conditions inhibiting the necessary aerobic metabolism (23), although some gene delivery with subsequent transgene expression has been reported (21, 24–28). EVMP offers a range of solutions to vector delivery and organ preservation challenges (**Figure 2**). Isolated access to the vascular pedicle of the organ ensures homogeneous perfusion (9), avoids off-target effects, enables higher vector dosages, and allows repeated circulation. It also maintains organ quality and extends preservation (3). EVMP

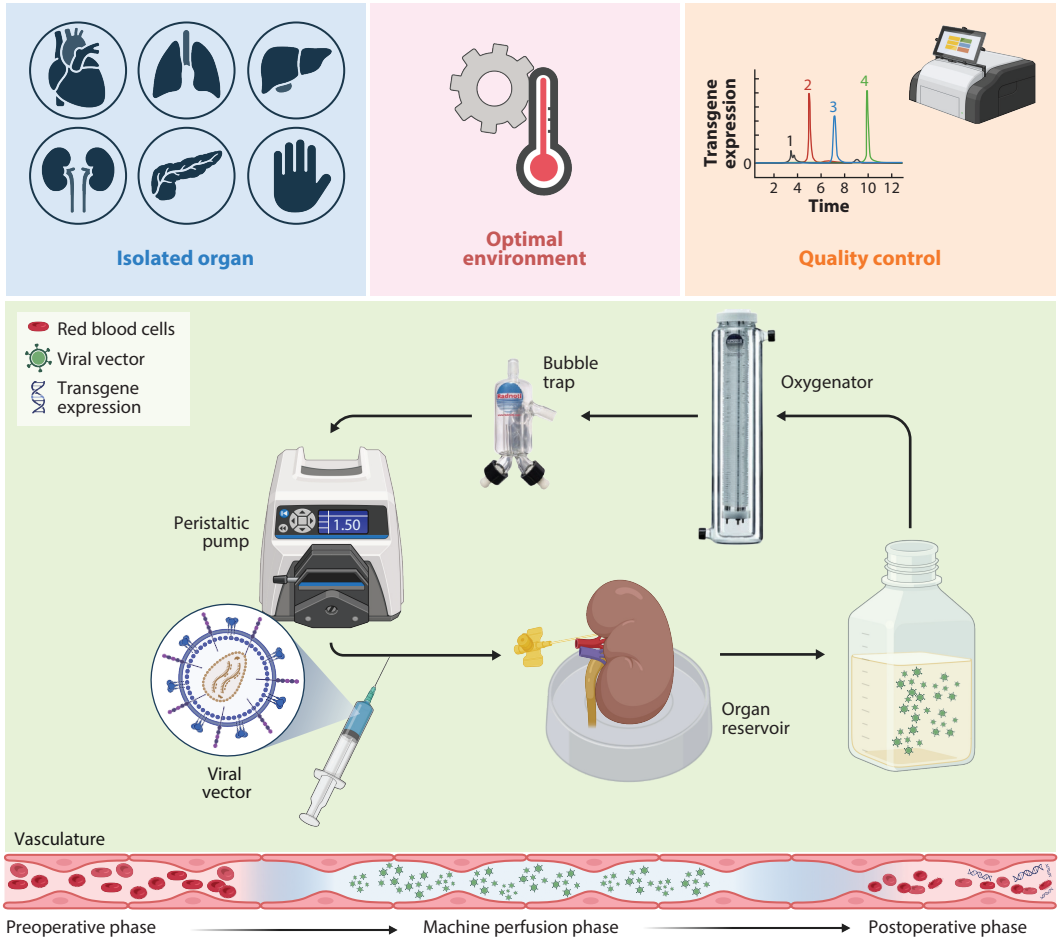

Figure 2

Ex vivo machine perfusion offers a platform to genetically modify whole organs. The technique offers isolated access to organs to expose them to high vector dosages, with the possibilities of preserving them in an environment optimal for both organ preservation and gene therapy, as well as metabolic and genomic monitoring for quality control. Figure adapted from images created in BioRender; Filz von Reiterdank, I. 2025. **https://BioRender.com/u92t189**.

provides a high level of control over key parameters, such as (aerobic) metabolism, temperature, flow, and optimized perfusion solutions, producing ideal environments for transduction. Importantly, ex vivo gene delivery results in decreased vector-associated inflammation pre- and posttransplantation (29).

Besides its therapeutic qualities, EVMP allows real-time assessment of secreted biomarkers to measure transduction efficiency. However, control over where transduction occurs within the organ remains limited, necessitating robust quality control methods. Additionally, EVMP can serve as a preclinical platform with physiological conditions to evaluate gene therapy dynamics in human organs (5).

In summary, EVMP enhances precision and efficacy of gene therapy in transplantation, offering superior control, reducing off-target effects, and providing real-time monitoring, making it a key platform for advancing genetic engineering of transplantable organs.

2.2. Therapeutic Targets for Local Treatment of Grafts

Therapeutic targets in organ engineering focus on improving graft survival. Roughly, therapeutic targets can be divided into four areas of interest: organ rejection (diagnostic and therapeutic), IRI, infection, and genetic disease. Examples of specific targets within these areas are shown in **Figure 3**. For instance, modulating immune responses can drastically reduce organ rejection,

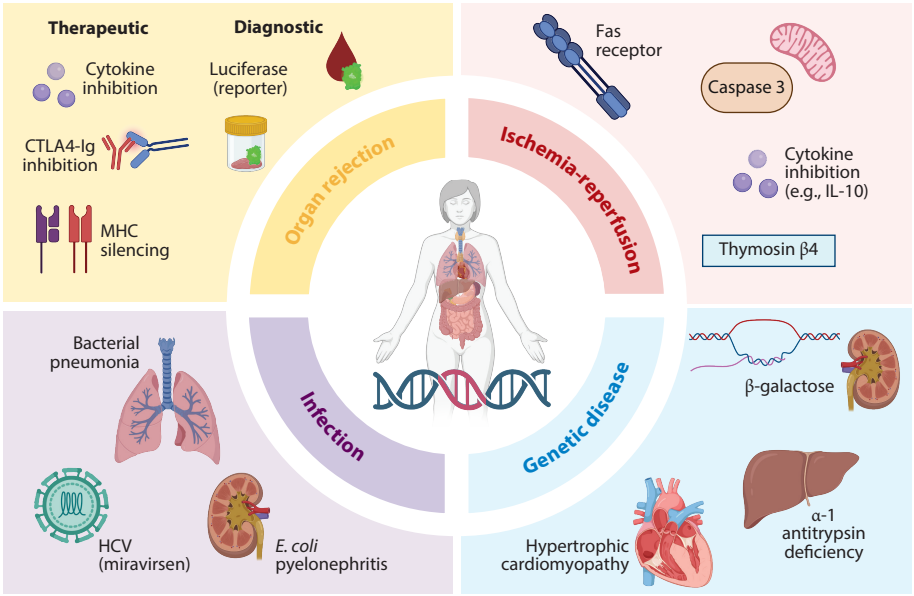

Figure 3

Gene therapy of transplant organs offers a wide range of potential treatment targets. Using EVMP, postoperative outcomes can be improved by inserting diagnostic markers for rejection or infection and therapeutic constructs for treatment into the organ. Genetic manipulation of organs can also inhibit IRI pathways and treat organs when genetic diseases affect organ function. Using gene transfer to optimize organ quality can reduce the need for future organ transplantation. Abbreviations: CTLA4, cytotoxic T lymphocyte associated protein 4; *E. coli*, *Escherichia coli*; EVMP, ex vivo machine perfusion; HCV, hepatitis C virus; Ig, immunoglobulin; IL, interleukin; IRI, ischemia-reperfusion injury; MHC, major histocompatibility complex. Figure adapted from images created in BioRender; Filz von Reiterdank, I. 2025. **https://BioRender.com/n28o558**.

a primary challenge in transplantation. Genes promoting tolerance or suppressing immune responses could achieve unprecedented acceptance. Genetic modifications enhancing IRI resilience could improve survival and extend preservation. By using cryopreservation techniques in tandem, EVMP can be leveraged from a synergistic genetic modulation and cryopreservation perspective to enable organ banking (30, 31).

Biomarkers serve as indirect therapeutic targets, enabling highly specific and sensitive organ health tracking without serial biopsies and guiding immunosuppressive therapy. Ideal biomarkers should be highly sensitive to local inflammation and detectable in the systemic circulation at low concentrations, without being immunogenic (32). Common biomarkers used thus far are nanoluciferase and *Gaussia* luciferase.

Addressing genetic diseases through organ engineering is perhaps the most innovative aspect, offering a cure for inherited monogenic diseases, such as Wilson's disease, and reducing the need for lifelong medical interventions. This form of local treatment could be provided either in situ by creating a closed circuit that perfuses the organ in question (33) or by removing the organ and performing autotransplantation (34) if viral contamination of the systemic circulation is a concern.

Safety measures, such as suicide switches, can be incorporated to turn off the integrated gene sequence, allowing physicians to negate the inserted gene construct by administering agents of choice such as doxycycline. Even genetic modifications envisioned as permanent could be reversed. Accidental administration of these agents should be avoided in these patients.

By targeting the four key therapeutic areas, as well as stimulating the discovery of new ones, we move closer to the vision of creating smart grafts—organs that not only perform their required functions but also possess enhanced resilience, reduced immunogenicity, and the capability to provide real-time health monitoring.

2.3. Strategic Selection of Gene Delivery Vectors

Selecting the right vector for the desired therapeutic effect is crucial (35). An ideal vector should be nonimmunogenic and nontoxic, enable precise and efficient transduction, and be cost-effective and easy to produce (17). Achieving robust gene expression in an entire organ can be particularly challenging; the most suitable carrier should be able to target tissues with high efficiency and minimal off-target effects. Each gene therapy modality and delivery system exhibits its own advantages and limitations (**Figure 4**), which should be carefully considered during vector design and selection.

Viral vectors are one of the most commonly used gene delivery tools (36) due to their ease of production and efficacy in infecting numerous cells (17). Nonintegrative viral vectors, such as adenovirus (Ad) and adeno-associated virus (AAV), are ideal for quick and transient transgene expression. AAVs are multi-serotype vectors that can be easily tailored for targeted applications (37) and exhibit the lowest immunogenicity (38), but they have limited payload capacity, restricting their application to smaller cargoes. Lentiviruses (LVs), on the other hand, are integrative retroviruses with high payload capacity that can confer long-term and stable transgene expression (39, 40). Yet, these vectors are also commonly associated with immunogenic responses and insertional mutagenesis due to random host genome integration.

Nonviral modalities, such as lipid nanoparticles (LNPs), polymers, and peptides, are also valuable alternatives as gene delivery vectors (41). Their overall low immunogenicity, high packaging capacity, and low cost of production enable a diverse array of applications (42). These particles can be tailored to carry several types of cargoes, such as messenger RNA (mRNA), small interfering RNA (siRNA), and complementary DNA (cDNA). They can also carry larger genetic engineering tools, such as CRISPR/Cas systems (43), which are not well suited for viral delivery due to their

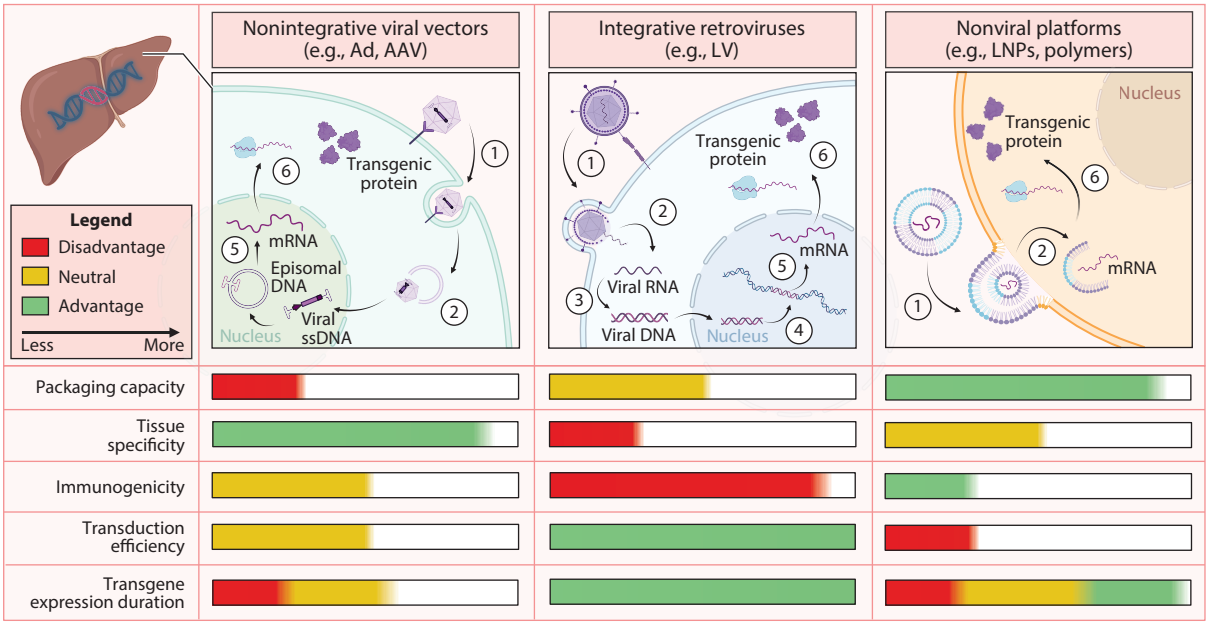

Figure 4

Main gene delivery applications in organ genetic engineering. Different vectors can be used in gene therapy and organ engineering, each exhibiting advantages to be leveraged and drawbacks that must be addressed for proper translation to the clinic. Abbreviations: AAV, adenoviral-associated vector; Ad, adenoviral; LNP, lipid nanoparticle; LV, lentivirus; mRNA, messenger RNA; ssDNA, single-stranded DNA. Figure adapted from images created in BioRender; Imbriani Bento, R. 2025. **https://BioRender.com/f01j511**.

size. The main drawbacks, however, include low efficiency in transducing certain types of cells and low stability depending on the route of administration.

The decision to use either modality must be strategic, considering the intended application and the risk–benefit ratio for each target organ and patient. Some applications may benefit from short-term gene expression, such as minimizing IRI in the early postoperative period, while others will necessitate long-term and stable transgene expression postoperatively. Ongoing research is continuously refining gene-editing technologies, vector design, and delivery systems to develop safer, more precise, and efficient tools with minimal off-target effects, enabling their use across a broader range of applications in organ transplantation.

2.4. Transduction Assessment

In the research phase, several methods can be employed to perform quality control assessment of homogenous transduction during EVMP. For example, different cell types populating the grafts can be isolated after perfusion (44) or after transplantation. Fluorescent or luminescent imaging can show transduction ex vivo and in vivo, depending on the type of reporter genes (45). For real-time assessment of gene construct translation, blood draws can be used to detect secreted biomarker levels, and biopsies can be used to assess tissue-specific gene expression, substantiating translation of the genetic construct rather than presence only. Other recipient organs should be assessed to confirm no off-target transduction, validating delivery specificity and assessing long-term side effects. While EVMP offers additional pathways for quality control and safety prior to transplantation, time to transgene expression is vector-dependent, in some cases extending beyond the time window of EVMP, therefore requiring transplantation to assess transduction. The onset

of gene expression can vary significantly among different viral vectors and can be leveraged depending on the therapeutic target. Ad typically achieves robust transgene expression within hours to days after transduction (46). However, AAVs and LVs exhibit a much later onset of expression, usually 2–7 days in vitro and 3–21 days in vivo (47–49).

Due to their relative novelty, as well as regulatory challenges and ethical concerns, many of these systems have yet to be explored in organ engineering. Nonetheless, genetic engineering has advanced considerably (50). With the increasing approval of gene therapy applications globally, genetically modified organs in transplantation may become a routine practice in the clinic, as long as safety and efficacy are established by the field and ethical standards of regulatory bodies are met.

3. OVERVIEW OF CURRENT LITERATURE CONCERNING ORGAN TRANSPLANTATION

Gene therapy in organ transplantation offers innovative solutions to traditional challenges. This section reviews recent advancements in gene therapy across organs, each presenting unique challenges and opportunities. Key areas for future research include optimizing viral doses, viral circulation times, and perfusion parameters (e.g., perfusate composition, temperature, volume) to balance transduction efficiency with preservation. Current studies often lack clarity on whether viral particles are flushed out before transplantation. While few studies show long-term follow-up, no adverse effects, such as transduction of other organs or vector immunogenicity in the recipient, are reported. Furthermore, doses and transduction temperatures are rarely compared, though high viral titrations (10^{10}–10^{13}) (51) and physiological body temperature (37°C) are commonly used. An overview of studies on genetic engineering of organs using EVMP for vector delivery, arranged by vector used for delivery, is provided in **Table 1**.

Standardizing transduction monitoring will be essential, especially for constructs with constitutive promoters, which may exhibit a baseline of biomarkers arising from retrotransduction contaminants in the viral solution pool (52). These baseline levels should be corrected, for example, by determining not only biomarker levels but also viral DNA levels during perfusion and by assessing biomarker expression at a gene level (translation of transduced genes) rather than their mere activity. Only two studies were found that monitored secreted biomarkers as evidence of transgene expression (35, 53). Current follow-up is limited to 6 weeks in small animals (35) and 7 days in large-animal models, with the latter only including postmortem assessment of transduction (54). The persistence of biomarkers and therapeutic proteins, as well as the long-term effects of virally transduced organs, must be further investigated.

3.1. Heart

Heart preservation time is the most limited, requiring transplantation within 4–6 h, which restricts perfusion time and poses a barrier for transduction with vectors requiring extended exposure times such as LVs. Consequently, studies have used Ad and AAV during EVMP, which can achieve transduction with shorter exposure times and have easier high-titration production.

Gene therapy in hearts has been tested in small-animal models via direct injection and catheter-based intra-arterial injections (55). The heart's vulnerability to IRI makes gene therapy using EVMP a critical area of exploration. Shah et al. (56) delivered transgenes during SCS targeting the β-adrenergic receptor-signaling system in rabbits, improving in vivo contractibility, demonstrated by improved systolic ventricular function assessed by Langendorff EVMP at 5 days posttransplantation. By day 14, most transgene expression was absent, typical for the type of AAVs used. Another study delivered thymosin Q4 (TQ4) during 45-min in situ warm Langendorff perfusion

Table 1 Overview of studies on genetic engineering of organs using EVMP for vector delivery, arranged by vector used for delivery

Vector	Concentration of vector	Target	Organ (species)	Temperature	Exposure time	Major findings	Reference and Year
Ad	1×10^{11} (in 20 mL)	β-galactosidase gene	Kidney (pig)	37°C	12 h (ex vivo) versus 2 h (in vivo)	Expression of reporter gene in 85% of glomeruli using EVMP and 75% using in situ perfusion Significant decrease of expression at postoperative days 14 and 21	142 (1996)
Ad	1×10^{10} (pig), 4×10^{10} (human)	hIL-10	Lung (pig, human)	37°C	12 h	Pig: Reduced inflammation and enhanced function at 4 h post-tx, relative to control organs with IL-6 and IL-1b production inhibition; most transduced cells were epithelial cells and alveolar macrophages Human: Improved function and favorable cytokine shift (decreased IL-1b, -8, -6, and -10) at end of EVLP	23 (2009)
Ad	1×10^{10}	GFP versus IL-10 versus empty cassette	Lung (pig)	37°C	12 h	Ex vivo delivery (EVLP) shows higher hIL-10 and GFP trends in plasma and tissue, respectively, compared with in vivo delivery IL-10 transduced lungs show significantly higher lung oxygenation compared with GFP and nontransfected lungs Results persist over 4 h of reperfusion in donor	143 (2012)
Ad	1×10^{10}	hIL-10	Lung (pig)	37°C	11 h	Superior gas exchange and lower histologic inflammation score at 7 days post-tx with no signs of toxicity in blood analysis	54 (2017)
Ad	5×10^{13}	Firefly luciferase	Heart (pig)	37°C	2 h	Transgene expression in tissue at postoperative day 5 (immunostaining and qPCR); no off-target effects	58 (2019)
Ad	1×10^{8}, 1×10^{6}, and 1×10^{4}	GFP	Kidney (canine)	32°C versus 4°C	24 h	Transgene expression increased with dose during NMP No fluorescence found in cold perfused and nontransfected (control) kidneys	63 (2002)

(*Continued*)

Table 1 (*Continued*)

Vector	Concentration of vector	Target	Organ (species)	Temperature	Exposure time	Major findings	Reference and Year
AAV	5×10^{11} loaded in 5×10^{9} microparticles	GFP	Lung (pig)	37°C	6 h	Whole organs were genetically modified using vector carrying microparticles during EVLP intended to avoid viral immunogenicity In vitro, in some cases, optimized gene delivery was seen	79 (2014)
AAV	4×10^{8}	GFP	Liver (rat)	4–6°C	2 h	Small sample size ($n = 2$) showing transgene expression (immunohistochemistry) at 24 h post-tx	68 (2021)
rAAV	2×10^{13}, 8×10^{13}, 8×10^{13}, and 1×10^{14}	Firefly luciferase	Heart (pig)	37°C	2 h	Durable and dose-dependent transgene expression shown by qPCR and staining of tissue biopsies at end of study (30–35 days) in heterotopically transplanted allografts, with no evidence of off-target transgene expression in the liver	59 (2023)
AAV (14 variants)	4.5×10^{10} vg/kg of body mass (total dose), 3.2×109 vg/kg of body weight (per variant)	eGFP	Liver (human)	32–36°C	±7 days	Comparison of 14 AAV vector variants in split human liver model using vector specific barcodes for identification Showed results using NGS with increased transduction efficiency for AAV-SYD12 and AAV-LK03, especially in absence of neutralizing antibodies Suggests Kupffer cells play key role in vector clearance	5 (2024)
LV	1×10^{11}	SLA I and II (shB2m and shCIITA) versus empty cassette versus control	Lung (pig)	37°C	2 h	Silencing of SLA I and II was 67% and 53%, respectively, in cells isolated after EVLP and stimulated with IFN-γ and TNF-α No signs of decreased cell viability or tissue integrity were shown	77 (2019)

(*Continued*)

Table 1 (*Continued*)

Vector	Concentration of vector	Target	Organ (species)	Temperature	Exposure time	Major findings	Reference and Year
LV	1.5×10^{11}	MHC I and II silencing (rat shβ2m and CIITA), NL	Kidney (rat)	31–32°C	2 h	NL in plasma and urine at 1 and 6 weeks post-tx, somewhat decreasing in signal In kidney tissue, ±70% downregulation of targeted genes at EoS, with no off-target effects	35 (2020)
LV	1.7×10^{11}	NL	VCA (rat)	21°C	3.5 h	Up to 12 days stable luciferase levels in supernatant of cells isolated after EVMP	44 (2022)
siRNA	50 nM (in 20 mL)	MMP-2 (renal failure)	Kidney (rat)	3–5°C	22 h	Improved graft function and mitochondrial integrity at the end of EVMP	60 (2016)
siRNA	Not reported	Fas receptor	Liver (rat)	37°C versus 4°C	4 h	Higher transgene expression in HOPE group (fluorescent confocal microscopy)	66 (2019)
siRNA	38 nM (500 μg)	Fas receptor (acute liver failure, post-tx IRI)	Liver (rat)	4°C	1 h (HOPE) versus 2 h (in vivo) + 22 h (SCS)	Fas inhibition decreases IRI severity at 24 h post-tx in SCS protocol No successful inhibition detected in HOPE group	69 (2022)
siRNA	60 nM	MHC II silencing (CIITA)	Vessels (human to mouse)	37°C	6 h	Silencing of MHC II in human vessels shows >80% suppression at 1–2 weeks and ±20% suppression at 6 weeks in an immunodeficient mouse model	85 (2017)
Miravirsen (oligonucleotide)	500 mg/L (±100 μM)	Prevention of HCV reinfection	Liver (pig)	37°C (MP) versus 4°C (SCS)	12 h	Improved miravirsen uptake with significant transgene expression in EVMP group	27 (2017)
Engineered rat fibroblasts	5×10^6	GLuc	Liver (rat)	37°C	3 h	Increasing engraftment over perfusion time (2, 4, and 6 h) No harmful effects in perfusion parameters	45 (2019)
Engineered rat fibroblasts	1×10^6	GLuc	VCA (rat)	21°C	3 h	In vivo plasma luciferase detection up to 3 days post-tx, after which signal returns to background levels	53 (2021)

Abbreviations: AAV, adenoviral-associated vector; Ad, adenoviral; eGFP, enhanced GFP; EoS, end of study; EVLP, ex vivo lung perfusion; EVMP, ex vivo machine perfusion; GFP, green fluorescent protein; GLuc, *Gaussia* luciferase; HCV, hepatitis C virus; hIL, human IL; HOPE, hypothermic oxygenated perfusion; IFN-γ, interferon gamma; IL, interleukin; LV, lentivirus; IRI, ischemia-reperfusion injury; MHC, major histocompatibility complex; MMP, metalloproteinase; MP, machine perfusion; NGS, next-generation sequencing; NL, nanoluciferase; NMP, normothermic machine perfusion; post-tx, posttransplant; qPCR, quantitative polymerase chain reaction; rAAV, recombinant AAV; SCS, static cold storage; siRNA, small interfering RNA; SLA, swine leukocyte antigen; TNF-α, tumor necrosis factor alpha; VCA, vascularized composite allograft; vg, viral genomes.

of mini-pig hearts followed by heterotopic transplantation and showed prolonged graft survival (57). TQ4 reduced inflammation, necrosis, and vascular reaction (acute rejection) and, in parallel, improved capillary density. At day 18, gene expression was found in the hearts, while gene expression in other organs remained within background range. Bishawi et al. (58) performed 2-h normothermic EVMP in pigs and showed gene expression of nonsecreted firefly reporters (Ad) in tissue on day 5 posttransplantation. Recently, the Duke group (59) showed follow-up up to 35 days in pigs that underwent heterotopic heart transplantation after 2 h of Langendorff perfusion with recombinant AAV, which was first optimized in vitro. Although a nonsecreted marker was used, quantitative polymerase chain reaction (qPCR) and antiluciferase immunofluorescent staining of tissue biopsies at day 35 showed significant expression levels compared with native controls. Despite not washing out the hearts at the end of EVMP, qPCR of the liver showed no signs of off-target effects.

None of the studies measured secreted biomarkers in the systemic circulation, and all used Ad and AAV, leveraging their relatively short transduction time. Long-term studies on rejection attenuation in heart transplant have not yet been performed.

3.2. Kidney

The kidney's ischemic resistance allows up to 24 h of EVMP, enabling long exposure time with lower vector doses, reducing toxicity risks. The kidney's small size, relatively low flow rates, and availability of urine for noninvasive assessments are additional advantages.

A key target is reducing chronic injury. Moser et al. (60) used hypothermic EVMP in rodent kidneys to silence metalloproteinase 2 (MMP-2) expression, involved in severe acute and chronic renal injury. After 22 h of hypothermic machine perfusion, graft injury improved. However, using MMP-2 and MMP-9 inhibitors showed even greater decreased levels, suggesting that other MMPs are also involved. In an SCS model, Yang et al. (61) injected naked caspase-3 siRNA into murine kidneys undergoing 24-h SCS to decrease apoptosis and reduce IRI. Although reduction of apoptosis was shown, the effect was unclear posttransplantation. Similarly, Zheng et al. (62) silenced complement 3, RelB, and Fas in a murine transplant model with 2-week follow-up. In all SCS groups (4, 6, and 12 h), reduced apoptosis and inflammation [proinflammatory cytokines, interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF-α)] were shown posttransplantation. Conversely, using EVMP, Brasile et al. (63) transduced canine kidneys with a recombinant Ad containing a green fluorescent protein (GFP) reporter gene. Although group sizes were small ($n = 2$), transduction of the intimal layer of blood vessels was achieved in a dose- and temperature-dependent manner. The dose of 10^8 IU/g of tissue at 32°C was most effective compared with lower dosages and 4°C (63). More recently, Yuzefovych et al. (35) used EVMP at 21°C to attenuate rejection in a rodent transplant model, silencing major histocompatibility complex (MHC) I and II through LVs encoding β2-microglobulin and the rat class II transactivator, and showing stable gene expression until 6 weeks posttransplantation. Additionally, the vector contained a nanoluciferase reporter, which was detectable in plasma and urine throughout the postoperative period. Remarkably, transcript levels of β2-microglobulin and the class II transactivator were decreased by 70% at 6 weeks posttransplantation (35).

Research suggests that high vector doses, long exposure times, and near-physiological temperatures are beneficial to transduction efficiency. Furthermore, one of the few studies using a secreted biomarker detectable in plasma and urine showed stable transduction levels of the therapeutic element against rejection during a follow-up of several weeks. Understanding the extent of rejection attenuation in more clinically relevant models in combination with longer follow-up times will be of major interest to the field.

3.3. Liver

Similar to kidneys, livers have relatively high ischemia resistance, allowing for EVMP times of up to 12–18 h. Biomarkers could potentially be secreted in bile, and detected in feces, although this is yet to be shown in EVMP studies, let alone in vivo. A challenge with the liver is its large size, requiring high flow rates and thereby increasing the total volume in which the vectors circulate and increasing the number of cells to be transduced. However, one great advantage is the ease with which liver tissue is generally transduced compared with other tissue types (64, 65).

Instead of using a vector, the Massachusetts General Hospital (MGH) group (45) used engineered fibroblasts to express a secreted luciferase reporter and GFP during rat liver EVMP at 37°C. After 3 h of EVMP, accumulated biomarkers were washed out, and stable, linearly increasing biomarker secretion was shown over the next 3 h. Distribution throughout the organ was visualized using near-infrared fluorescence imaging. Other studies demonstrated successful siRNA uptake under normo- and hypothermic conditions, targeting the Fas receptor in rat livers (66). The same siRNA technique was later used to target p53 to modulate apoptosis (67). In subsequent studies, Ad-mediated delivery of a GFP-containing sequence during hypothermic liver perfusion was demonstrated through immunohistochemistry analysis at 24 h posttransplantation (68, 69). Comparing FAS siRNA administration methods, systemic administration before procurement showed better results at 24 h posttransplantation than administration during 1 h of hypothermic EVMP, with significantly decreased transaminase levels and lower proinflammatory cytokines. Confocal microscopy showed absorption of the FAS siRNA, but no statistically significant differences in the apoptotic index, necrosis levels, and FAS protein transcription between treated and untreated groups were observed. Differences might be explained by the longer exposure time (2 h) at body temperature (37°C) versus 1 h of hypothermic conditions (4°C) during EVMP.

The potential of antisense oligonucleotide therapy was first shown by Goldaracena et al. (27) by silencing hepatitis C virus (HCV) virulence in a porcine model and comparing delivery methods, showing improved effect using the EVMP model (37°C) versus no significant effect in the SCS group (4°C). Although current antiviral agents against HCV are highly effective, diminishing the relevance of this specific target for gene therapy in a clinical setting, this approach offers a potential strategy to prevent (re)infection (70). As a method for preclinical evaluation of vectors in a clinically relevant model, Cabanes-Creus et al. (5) perfused split human livers with 14 AAV variants, identifying AAV-SYD12 and AAV-LK03 as superior, especially in the absence of neutralizing antibodies. This study provides more extensive and detailed analysis of genetic engineering and is one of the few studies conducted directly in human organs, providing more robust and clinically relevant evidence. However, interactions between the AAV variants and the study's limited sample size indicate the need for broader validation to ensure replicability.

3.4. Lung

Lungs can be perfused for up to 12 h, offering ample time for genetic modification. Unique to lungs is the intrabronchial delivery of vectors, which favors minimal toxicity, avoids transduction of other organs, and does not require flushing, as intrabronchial delivery is naturally limited to the lungs, potentially allowing for repeated gene therapy after transplantation (24). Nonetheless, in most cases, ex vivo lung perfusion (EVLP) is still necessary to accommodate this technique.

In rats, AAV transduction with human IL-10 (hIL-10) improved IRI and postoperative graft function (71–73). De Perrot et al. (73) showed significantly improved posttransplant graft function if lungs were retrieved at least 12 h after donor transtracheal administration of AdhIL-10. The group also observed that a dose of methylprednisolone (30 mg/kg) injected 3 h before starting transduction prevented the release of TNF-α and improved lung oxygenation. However, the

expression of hIL-10 was not enhanced within 12 h, in contrast to earlier studies with later evaluation times at 24–72 h that showed increased gene expression (24, 74). Another study in a humanized mouse model was not able to show a benefit from prednisolone treatment on AAV transduction (5).

The Toronto group (75) later investigated intrabronchial in vivo delivery of AAVs in a porcine model during 12-h anesthesia. Only in the ischemia-induced group, hIL-10 transfection showed prevention of the deleterious effect of the AAV itself and association with better lung function compared with the empty vector group. In a similar model, pig lungs that underwent 12-h SCS were transduced during 12-h EVLP followed by transplantation and 4 h of reperfusion in vivo (76). Similarly, significant inhibition of swine IL-6 and IL-1b release was shown. In discarded human lungs, Cypel et al. (23) performed 12-h normothermic EVLP with or without intrabronchial delivery of AdhIL-10 gene therapy, showing significant improvement in lung function (arterial oxygen pressure and pulmonary vascular resistance), a favorable shift from proinflammatory to anti-inflammatory cytokine expression, and recovery of alveolar–blood barrier integrity. Interestingly, using the same construct, EVLP was shown to result in excellent lung function and lower signs of inflammation in porcine lungs compared with in vivo vector delivery into the organ donor. A longer follow-up was performed by Machuca et al. (54), who investigated intrabronchial delivery of AAVs with hIL-10 sequences with a constitutive CMV-promoter during 12h EVLP before single lung transplantation. Cytokine assessment showed presence of the anti-inflammatory hIL-10 until postoperative day 7, with peak levels at day 5. Immediately posttransplantation, lung function was better in the transduced and EVLP control group compared with the SCS group. At day 7, the transduced group showed significantly better gas exchange than the control groups, and histologically lower inflammatory scores were seen. Similar to the immunoengineering of rodent kidneys by Yuzefovych et al. (35), Figueiredo et al. (77) showed homogenous, permanent silencing of swine leukocyte antigen I and II of porcine lung endothelium using LV. After EVLP, lung endothelial cells were isolated, and, after 96 h of in vitro cell culture, 67% and 52% targeted silencing of SLA I and II, respectively, were demonstrated. Notably, this study had a significantly shorter viral circulation time than previous studies, namely, 2 h. More recently, Nykänen et al. (78) targeted IL-10 by perfusing human lungs with engineered mesenchymal stromal cells with augmented anti-inflammatory IL-10 production. Perfusate levels showed rapidly and markedly elevated IL-10 levels after injection. Interestingly, poor metabolic conditions such as an acidic lung microenvironment negatively affected IL-10 levels ex vivo as well as in vitro.

Using a Trojan horse design, McConnell et al. (79) delivered porous silicone microparticles carrying AAV nanoparticles arterially during porcine EVLP. These particles were developed to combat the inflammatory cascade caused by viral vectors. While expression was limited after 6 h of EVLP, levels increased after overnight incubation of tissue samples, suggesting that assessment of transduction immediately postexposure may cause underestimation of transduction efficiency, as seen when performing in vitro transduction. This would align with the time needed to assess transduction during in vitro cell culture.

In summary, genetic modification studies in lungs were first performed using in vivo gene delivery prior to moving to EVLP-mediated transduction, with one study showing a promising beneficial effect of using EVLP in a pig model. Most studies made use of Ad or AAV, with intrabronchial viral exposure times varying from 2 to 12 h, and, accordingly, postoperative follow-up time was up to 7 days. Therapeutic targets were mostly IRI related. One study made use of intravascular delivery of LVs in human lungs aimed at attenuating rejection, showing promise for translation to human organs as well as a potential for more permanent transduction. Interestingly, vector doses varied from 10^{10}–10^{11} independent of the delivery route. Future studies aiming

to achieve long-term transduction, for example, by using AAVs or LVs in a clinically relevant transplantation model, would be of great interest to the field.

3.5. Vascularized Composite Allografts

Vascularized composite allografts (VCAs) consist of multiple tissue components such as skin, muscle, and bone. The most recent additions to this group of organs, as defined in the 2014 Organ Procurement and Transplantation Network Final Rule (42 C.F.R. part 121) (**https://www.ecfr.gov/current/title-42/chapter-I/subchapter-K/part-121**), are urogenital organs and the uterus. Unlike life-saving organs, VCAs aim to improve the quality of life. Therefore, the risks of large surgeries and lifelong immunosuppression must be weighed more carefully than in life-saving transplantation of solid organs. In addition, VCAs have high rejection rates (80). Methods to detect rejection at an early stage or to suppress rejection and reduce the need for immunosuppression could accelerate the field of VCA transplantation. Challenges for transduction include the limited preservation time of 6 h for grafts containing muscle tissue and the presence of tissue types that are relatively hard to transduce (81, 82).

Genetic engineering using EVMP in VCAs is thus far limited to rat models. Two studies used Ad-mediated gene transfers through intra-arterial injections under ischemic conditions (83). Michaels et al. (21) showed transduction of the *lacZ* reporter gene in groin and muscle flaps with high regional expression of ß-galactosidase in all the cell types within the flap. Transduction efficiency was significantly higher with higher doses of viral particles (1×10^6 versus 4×10^{11} plaque-forming units) and with intravascular delivery compared to groups with direct intramuscular injections. Similarly, Xiao et al. (26) performed an Ad-mediated *CTLA4IG* gene transfer, which showed significantly prolonged graft survival, especially in combination with rapamycin treatment.

More recently, the MGH group (53) demonstrated in vivo activity of genetically modified rat fibroblasts in VCAs. Similar to the liver study performed by the same group, engineered rat fibroblasts were perfused, and transplanted VCAs showed detectability of the *Gaussia* luciferase reporter in the plasma until postoperative days 4–5, after which the signal diminished. The Hannover group (84), similarly to their studies on rodent kidneys and porcine lungs, perfused VCAs with LVs encoding nanoluciferase or NeonGreen as reporter genes at 33°C. Here, protamine sulfate was added to enhance transduction efficiency; however, its effect was not investigated. After 3.5 h of viral exposure, keratinocytes, fibroblasts, and microvascular endothelial cells were isolated and showed significantly higher levels of bioluminescence of supernatant in vitro compared with nontransduced controls from day 1 until day 12 of cell culture. Viral flush-out during EVMP prior to cell isolation was not reported.

3.6. Vessels

As a model for organ EVMP, Cui et al. (85) performed 6-h perfusions of human umbilical vessels with siRNA-loaded polyamine-coester (PACE) nanoparticles targeting class II transactivator. Posttransplantation in immunodeficient mouse hosts, these vessels were shown to attenuate MHC II expression on endothelial cells for at least 4–6 weeks, although the MHC II suppression levels decreased from >80% in weeks 1–2 to ±20% in week 8.

3.7. Xenotransplantation

Recent advances in genetic engineering have revitalized xenotransplantation, offering the potential to alleviate the chronic human organ shortage. Genetically modified pigs are promising due to their physiological similarities to humans, although they require a significant number of

genetic modifications to be compatible with humans. While xenotransplantation relies on genetic modification of the donor animal, EVMP could be applied to add further genetic modifications to the organ before transplantation. While EVMP of human organs is more closely aligned with current clinical practice, aspects of xenotransplantation, such as off-target and long-term effects of the genetic modifications as well as the regulatory process of moving to first-in-human (FIH) trials, may be informative to genetically modified human organs (86). Ethical and societal considerations are expected to overlap, such as the importance of considering patient perspectives and their quality of life with genetically modified organs.

As of the date of this writing, four xenotransplantations have been performed in living patients and four in brain-dead patients (87). The first two living heart recipients of 10-gene-edited pig hearts expired within 2 months (88). One of these deaths was linked to the well-known latent porcine cytomegalovirus and roseolovirus (PCMV/PRV) (89). To derisk the high stakes of clinical trials (90), a decedent model was used for pig kidney transplants, which were followed up for three days without complications. Following this, the first pig kidney transplant to a living recipient with 69 genetic modifications was performed with US Food and Drug Administration (FDA) approval for compassionate care (91), but the recipient expired after approximately 2 months for currently unknown reasons. Another patient receiving a pig kidney and thymus gland underwent graft failure after 1 month and expired within approximately 3 months (92). These pioneering cases are critical for refining patient care, improving survival, and ensuring the safety and efficacy of future xenotransplants. As the field progresses, the integration of EVMP for additional genetic engineering and the rigorous evaluation of attendant ethical and regulatory issues will be essential for progress toward clinical application.

4. FUTURE PERSPECTIVES AND CONSIDERATION OF ETHICAL, LEGAL, AND SOCIETAL IMPLICATIONS

4.1. Moving Toward Clinical Trials

Developments in recent years, such as the discovery of CRISPR/Cas (93), have paved the way for clinical genetic modulation, with the first treatment using CRISPR/Cas technology being approved for in vivo use in Europe and the United States as a treatment for sickle cell disease (50). Application of gene therapy in transplant surgery still has many hurdles to overcome before clinical implementation (**Figure 5**), although, in the adjacent field of xenotransplantation, organs from genetically modified pigs have been transplanted to humans in isolated cases (88, 91, 92). EVMP leverages isolated treatment through the vascular pedicle, reducing toxicity and systemic off-target effects, with precise metabolic control. Since EVMP is already used clinically in some parts of the world (94), future work will focus on ensuring the efficacy and safety of gene therapy for transplant organs. Knowledge can be drawn from long-term effects in patients being treated systemically with therapeutic targets similar to those intended for genetic modification using EVMP, with the expectation that local treatment of the transplanted organ should have milder side effects. Recently approved gene therapies for FIH trials are an important step in gathering this knowledge (50).

Current research primarily involves ex vivo rodent and porcine models, with some human organ or animal transplantation models for in vivo assessment. Prior to clinical translation, future work will need to optimize four phases: (*a*) vector production, (*b*) gene delivery, (*c*) posttransduction follow-up, and (*d*) identifying ideal targets for optimal posttransplantation outcomes. Regulatory bodies such as the FDA and European Medicines Agency (EMA) will require rigorous data from preclinical trials on larger animals and human cadavers before proceeding to FIH trials.

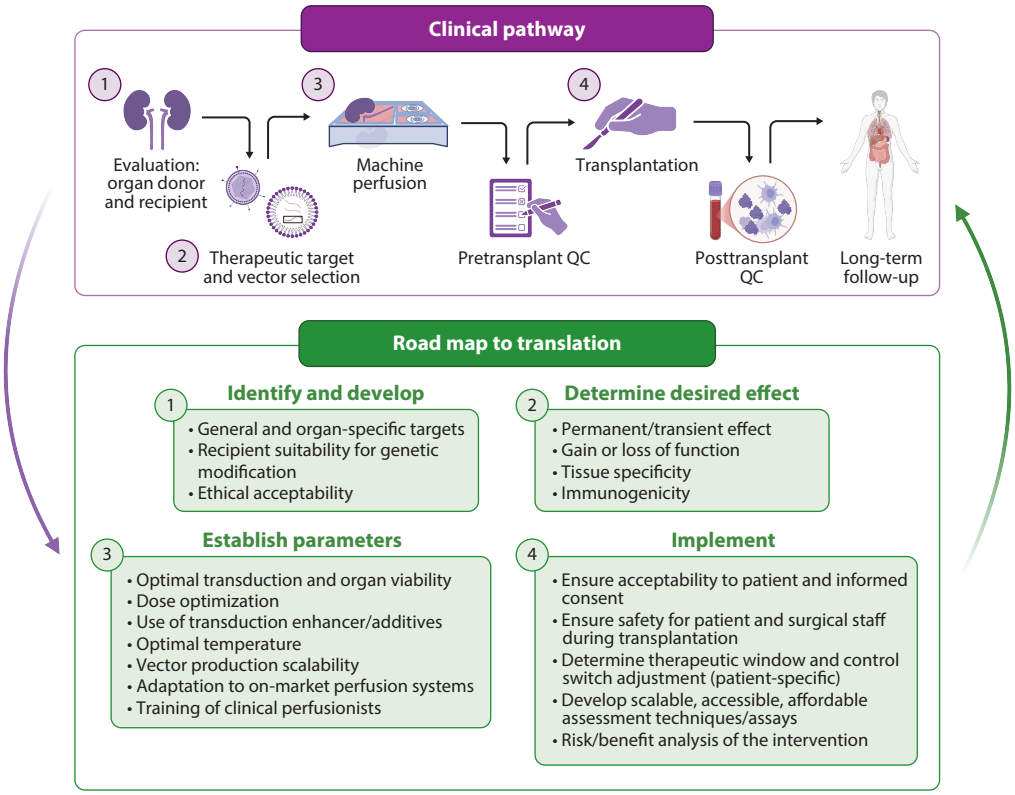

Figure 5

(*Top*) Schematic of future clinical protocols for genetically modified organs for transplantation. (*Bottom*) Road map to translation showing key challenges that need to be addressed to allow for translation to the clinic. Abbreviation: QC, quality control. Figure adapted from images created in BioRender; Imbriani Bento, R. 2025. **https://BioRender.com/o58o878**.

Scientifically, optimizing transduction while minimizing toxicity is key to clinical translation. Robust assessments of the translation of inserted gene constructs need to be established, defining success in a reliable manner throughout the postoperative period. Treatment targets are currently focused on reducing IRI and attenuating rejection. Additional applications could address transient complications in the acute postoperative phase, such as reduced ionotropic function in heart transplants or edema in VCAs, improving patient recovery by reducing intensive care unit and hospital length of stay as well as severe complications such as graft failure. Moreover, EVMP could salvage otherwise discarded organs through local treatment, expanding the donor pool.

Enhancing organ function beyond physiological ability is another goal. For instance, livers could be designed to metabolize otherwise untreatable substances, or hearts could maintain autonomic rhythm, reducing the need for electronic pacemakers. Treating organs with genetic disorders prior to end-stage organ damage may avoid allotransplantation and lifelong immunosuppression (95). Patients could be placed on bypass either in situ or while the organ is removed and treated outside of the body before being reimplanted. Autotransplantation could also be explored for chronic medication delivery, in which VCAs would be procured and perfused with a constitutive or inducible promoter that would treat the chronic disease and then be transplanted back into the patient. This approach would target local effects (21), but systemic targets could also be explored.

Dual or triple gene therapy could allow multiple vectors to work in tandem, circumventing limitations set by the number of genes in the genetic constructs (currently limited to two) (45, 96, 97). Vectors selection could be targeted to application needs, with some requiring high transduction efficiency and others achieving results with lower rates.

Scaling up genetic engineering for organ transplantation could involve organ reconditioning hubs to make this technique available to all centers or equipping existing clinical sites (29). Centralized hubs could offer cutting-edge technologies and catalyze innovation, driving research in gene therapies. Challenges include maintaining organ viability during transport, navigating regulatory requirements, and ensuring equitable access. Furthermore, substantial investments in infrastructure and training would be necessary to support these hubs. Addressing these logistical, oversight, and financial barriers is crucial for integrating genetically engineered organs into mainstream medical practice and extending their benefits globally.

Clear scientific and regulatory goals must be set by the scientists, physicians, and regulatory bodies, to assess the progress of gene therapy in organ transplantation. High-quality assessment methods, minimal transduction efficiency, and maximum acceptable off-target effects should be defined, with safety benchmarks guided by the Common Terminology Criteria for Adverse Events. The translational path from animal models to phased human clinical trials involves testing in human organs, cadavers, and FIH trials. Early engagement with regulatory bodies such as the FDA and EMA, as well as patient groups, should be strategically planned to help address concerns and streamline approvals, reducing the likelihood of costly delays (98). Early patient engagement is crucial, as it ensures that the perspectives and needs of those directly affected are considered, fostering trust and acceptance of new therapies. Ethical considerations in the transplantation field itself, such as those surrounding normothermic regional perfusion (99, 100), should also be integrated to maintain societal trust.

4.2. Ethical, Legal, and Societal Implications and Regulatory Governance

Gene therapy, more properly called gene transfer research when the intervention remains in the research stage as here, has a multi-decade history of oversight. In the United States, that oversight has involved both federal and institutional bodies. However, that oversight has evolved from review by the Recombinant DNA Advisory Committee at the National Institutes of Health (NIH) and local oversight by Institutional Biosafety Committees (IBCs) pursuant to the NIH Guidelines (101) plus FDA review to a more streamlined process (102). Gene transfer protocols now undergo FDA and IBC review, plus any additional review needed at NIH if the research is NIH funded. If the research involves a live human subject (a living donor, a living recipient, or both), that would trigger application of the relevant regulations governing human subjects research, specifically the Common Rule (103) and FDA regulations (104, 105), on such research. If the research involves animal models, then federal statutes, regulations, and guidelines on the ethical use of animals in research would apply (106), which provide for institutional review by Institutional Animal Care and Use Committees.

Conducting research on genetically engineered organs and translating that research to the clinic raises several ethical, legal, and societal implications (ELSI). Here, we focus on (*a*) issues raised by gene transfer research involving ex vivo manipulation of donated organs followed by potential transplant of the allograft into the recipient (excluding the issues posed by manipulation of organs while still in the donor's body) (107); (*b*) issues in genetic engineering of autologous organs for diagnosis, treatment, and, potentially, enhancement; (*c*) issues in genetic engineering of xenografts once they are outside the donor animal's body and being prepared for transplantation into the human recipient; and (*d*) the needed translational pathway for these interventions including bench, animal, ex vivo human, cadaver, FIH, and clinical trials research.

The transformative potential of genetically altered organs requires careful attention to all of these issues. In the United States, that work will necessitate anticipatory engagement with regulators such as the FDA and key stakeholder communities such as patient advocacy groups, transplant clinicians and their professional societies, payers such as the Centers for Medicare & Medicaid Services, and the entities involved in the transplant system including the Department of Health and Human Services (DHHS), Organ Procurement and Transplantation Network, Health Resources and Services Administration, Centers for Disease Control and Prevention, and organ procurement organizations (108, 109). Given the furor that has long surrounded public debate over genetically modified organisms in the food supply (110, 111), ensuring the kind of rigorous testing and oversight that will secure public trust in genetically modified organs will be essential. Surveys and focus group studies can help gauge both clinician and patient receptivity toward genetically modified organs (111, 112). This research, coupled with collaboration with patient advocacy groups, can inform the creation of educational materials that transparently convey both the benefits and the potential risks of gene transfer in transplantable organs, aiding informed consent in research and ultimately in clinical application.

4.2.1. Issues raised by ex vivo gene transfer research in allografts. Research involving genetic alteration of a donated organ while on EVMP for transplantation into a human recipient falls under three major oversight regimes in the United States. The first is the system governing organ donation for transplant. In a detailed analysis, a committee of the National Academies analyzed the need for authorization from the donor while alive or from the donor's surrogate to conduct the research on the organ and advocated for legal and policy change to support this process (102). The second relevant oversight system governs research with living human participants; federal regulations from the DHHS (the Common Rule) (103) and the FDA (104, 105), requiring review by an Institutional Review Board, provide the primary guidance, although some states have additional rules. The third oversight regime focuses on gene transfer research and is guided by the NIH Guidelines (101), requiring IBC oversight at the institutional level, and the FDA rules (113). While this US system for overseeing gene transfer research has evolved over decades, divergent international approaches to the oversight of human gene transfer pose recognized problems. In 2024, the FDA announced the Collaboration on Gene Therapies Global Pilot, an effort to encourage greater harmonization across international regulatory bodies (114).

An extensive and longstanding ELSI literature on gene transfer research and, more recently, gene editing, emphasizes the importance of considering safety, toxicity, off-target effects, persistence (or failure to wash out), participant consent, and inability to withdraw after the genetic intervention (and, here, the transplant) has been performed (115–118). Historically, central to ELSI discussion has been the distinction between somatic genetic alteration without risk of heritability versus the far greater controversy surrounding germline or heritable genetic alteration (119), although recent challenges to the distinction include mitochondrial manipulation (120, 121). In the case of genetically altered organs for transplant, additional issues would be equitable access and avoiding a two-tier regime disadvantaging those patients who need transplants but might lack the resources to access transplants incorporating more expensive interventions (122). Finally, a distinct ELSI literature has considered the issues raised by VCA allografts (e.g., face, hand, penis), which are not lifesaving but can improve quality of life and yet have shown high risk of rejection and complications (123, 124). Here, core issues include ethical selection of patients/participants, ensuring robust informed consent and mechanisms for additional protection in light of participant vulnerability (125), and policy reform to address VCA donation and transplant (126, 127).

4.2.2. Issues raised by gene transfer research in autologous organs. Besides the above issues with allografts, genetic engineering of organs by in situ bypass perfusion or autologous transplantation could add the further complication of reopening long-standing ELSI debates about the difference between treatment and human enhancement, and the ethical permissibility of the latter (128–130). EVMP and gene therapy could be used for the well-accepted goal of treating monogenic disease. However, genetic changes could also be made to patients' own organs to "improve" them beyond their typical species functioning, such as enabling livers to break down harmful new toxins rather than simply restoring the organs to a normal healthy state. Objections to genetic enhancement have included concerns about diverting scarce medical resources from the traditional goals of diagnosing and treating disease, imposing the risks of genetic engineering on patients without the justification of restoring health, redesigning the human body without societal consensus and the ability to predict where multiple efforts to redesign may lead, and reviving human eugenics (128, 131). What may seem reasonable from the standpoint of an individual patient's interest in protection from new toxins (for instance) may be problematic from a societal point of view, especially if multiple different enhancement efforts proceed leading to significant "improvements" and modified autologous organs are not equitably accessible. Although enhancement concerns also apply to allografts, the prospect of using a patient's organs for their own enhancement brings these concerns into sharper relief, since the scarcity of donor organs would not be a limiting factor in the latter case.

4.2.3. Issues raised by ex vivo genetic engineering of xenografts. While an extensive ELSI literature already addresses xenotransplantation, including recent research involving the transplantation of genetically modified porcine organs into human beings (132, 133), the use of EVMP to change animal organs—potentially to very high degrees of genetic modification—raises novel considerations. To the extent that animal organs can be made much more humanlike than not with the aid of EVMP, questions may arise as to whether such pretransplant organs should still be categorized as xenoproducts or whether they would be better viewed simply as highly bioengineered biological constructs. The concept of xenotransplantation relies on a sharp species distinction between the animal donor and the human recipient. For many people, this distinction matters for the purposes of ethics, policy, and personal acceptance. This distinction, however, could be undermined in the future, and the ELSI effects of blurring this line are unknown at this time. Blurring this line may also challenge the regulatory approaches that have been developed in different jurisdictions for animal-derived clinical products (134).

4.2.4. Ensuring a sound translational pathway. Finally, there are ELSI issues involved in ensuring a sound translational pathway. This is likely to involve bench, animal, ex vivo human, cadaver, FIH, and clinical trials research. Especially controversial are cadaver and FIH research. Research in human cadavers has prompted substantial ELSI literature over decades (135). Although research on human cadavers does not constitute human subjects research under US federal regulations from the DHHS and the FDA because the human beings are not living, commentators have nonetheless suggested safeguards to ensure respectful, ethical, and socially acceptable conduct (136, 137). Considerations such as scientific necessity, lack of alternative models, consent consistent with donor intent, and appropriate oversight are central.

FIH clinical trials have also elicited a robust ELSI literature (138). FIH research requires rigorous processes to evaluate the strength of the preclinical evidence and adequacy of research in nonhuman animals, the potential for the intervention to fulfill its clinical promise, and welfare-related considerations such as a favorable risk–benefit ratio, careful participant/patient selection, and robust informed consent processes (139). Determining the acceptability of FIH trials will involve collaboration with regulators such as the FDA. As product classification dictates the

evidence of safety and efficacy necessary for regulatory approval, regulatory frameworks will need to address the unique characteristics of novel gene therapy applications in organs for transplantation.

More broadly, ensuring a sound translational pathway requires analysis of societal value including equity considerations. Translational research is expected to provide tangible societal benefits, which must entail not only improving health outcomes—where genetically altered organs hold great promise—but also reducing health disparities. Historically disadvantaged individuals and communities have often been excluded from participating in scientific research and from receiving the benefits of medical discoveries, which is an especially acute problem in the context of organ allocation (140, 141). An ethical translational pathway should promote equitable access and progress toward fair distribution of transplantable engineered organs.

Finally, stakeholder engagement is vital for a socially responsible clinical translation process (141). Incorporating the values and perceptions of affected individuals and communities during the innovation process—including in the development, conduct, and dissemination of research—provides valuable insights into issues of concern that need to be addressed. This kind of engagement also contributes to public acceptance and uptake of the technology, which is particularly important for research and clinical applications deemed controversial.

5. CONCLUSION

Genetic modification of organs for transplantation is an exciting new avenue in personalized medicine. Significant improvements are necessary to make organ gene therapy a reality for transplant patients in the clinic, especially in three scientific solution areas: early, noninvasive routine detection of rejection episodes; improved immunosuppression; and reduction of the immunogenicity of grafts. Genetic modification using EVMP has the potential to address all three of these issues and more. While current work is mostly limited to ex vivo animal models, early in vivo data suggests that EVMP can make genetic modification a widely applicable method with relatively easy translation to the clinic. An integrated approach, combining rigorous scientific benchmarks, regulatory compliance, attention to ethics, patient engagement, and establishing comparative effectiveness, will support the clinical translation of gene therapy in organ transplantation.

The field of genetic engineering in organ transplantation is now at a critical juncture. While foundational work has laid the groundwork for these innovations, new work is required to bridge the gap between research and clinical application. This review aims to provide a comprehensive overview of the current state of research, identify the existing gaps and challenges, and highlight the immense potential of this approach. By doing so, we seek to stimulate the scientific community, attract essential investment, and engage ethicists and policymakers to ensure these groundbreaking advancements are realized in a manner that maximizes societal benefit and upholds the highest ethical standards.

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