

Designer organs: The future of personalized transplantation

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Abstract

Organ transplantation is the definitive treatment for end-stage solid organ diseases, yet biological and logistical barriers reduce the rate of successful organ transplants. As such, there is a need for gene therapy and gene modulation strategies in the organ transplantation setting to prevent rejection, expand the donor pool of available organs, and attenuate ischemia-reperfusion damage. As we are entering an era of “precision medicine,” the organ transplant field is becoming equipped with the tools necessary to personalize and optimize organs designed specifically to withstand injurious pathways that occur during transplantation, such that the concept of “designer organs” will be a reality in the near future. In this review, we highlight the recent progress using gene knockout and knock-in strategies used mainly in the context of xenotransplantation. We also discuss advancements in CRISPR-Cas9 gene editing and RNA interference in relation to organ transplantation. Lastly, we discuss the exciting future implications of customized gene therapy in the transplantation setting, and its ability to potentially create a future where organs intended for transplant are personalized to maximize both graft and patient survival.

KEYWORDS

CRISPR-Cas9, designer organs, ex situ perfusion, gene editing, gene modulation, gene therapy, graft treatment, machine perfusion, machine perfusion preservation, organ preservation, personalized medicine, precision medicine, RNA interference

1 | INTRODUCTION

Transplantation is the definitive treatment for end-stage solid organ diseases. However, the ultimate success of this procedure has unfortunately led to its downfall—the donor organ shortage. There are now over 106 000 patients on the solid organ transplant waiting list in the United States while only approximately 39 000 transplants were performed in 2020 (Based on OPTN data as of November 16, 2021). Unfortunately, the overall waitlist mortality is persistently high, with rates ranging from 6.9% (for pancreas) to 28.3% (for heart/lung), with an all organ waitlist mortality of 16.6% (Based on OPTN data as of November

16, 2021). In an effort to combat the organ shortage, transplant surgeons have broadened the acceptance criteria to include “extended criteria donors” (i.e., donors of advanced age, donors after circulatory death, donors with excessive steatosis, and others) of which organ damage is more frequent leading to organ loss.¹

In light of the donor organ shortage, the research and surgical communities have been motivated to discover novel organ protective strategies to solve this crisis. Previous areas of investigation include pharmacologic methods of graft treatment such as preservation solution additives (i.e., antioxidant and anti-inflammatory agents)² as well as more dynamic and therapeutic approaches



including *ex situ* machine perfusion.³ However, manipulation of the graft via gene editing and modulation has recently garnered support as a precise method of altering and protecting the graft from damage as well as conferring favorable qualities to suboptimal organs to ensure that they can be transplanted with acceptable outcomes.

While approaches to genetic modification of a graft may vary, the rationale of graft treatment focuses on three main areas—how to eradicate lifelong systemic immunosuppression and avoid immune rejection, improve the graft shortage dilemma, and attenuate or prevent ischemia-reperfusion damage.^{4–6} The emergence of new technologies within the past decade, such as RNA sequencing and gene editing and modulation strategies, have enhanced our understanding of these pathological mechanisms unique to transplantation from the whole-organ level to single-cell resolution.^{7,8} These genetic advances have driven the organ transplantation field to its current state, such that the concept of “designer organs” is not that far from realization. The future may be one where transplant teams can individualize and genetically modify organs to withstand the harsh demands of transplantation prior to recipient implantation.

In this review, we highlight the overall need for gene therapy and modulation strategies in the context of solid organ transplantation (SOT). We discuss recent advancements using gene knockout and knock-in strategies to facilitate xenotransplantation, focusing mainly on cluster regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein 9 (Cas9). We also highlight the recent progress on gene silencing strategies in SOT including RNA interference (RNAi), such as small interfering RNAs (siRNAs) and antisense oligonucleotides (ASOs). Finally, we discuss the future prospects regarding gene therapy strategies in the organ transplantation field. This review is not meant to be an exhaustive discussion of all gene therapy strategies utilized in the organ transplant setting. Rather, it is intended to highlight some of the major applications of gene therapy and gene modulation strategies, and their future potential in the organ transplantation field.

2 | THE NEED FOR GENE THERAPY STRATEGIES IN SOLID ORGAN TRANSPLANTATION

Before the emergence of immunosuppressive drugs, the success rate of SOT was dismal, except in cases where isografts were transplanted between identical twins.⁸ Nowadays, the side effects of long-term immunosuppression and chronic rejection threaten the success of allograft transplantation as a definitive therapy for end-stage organ

diseases.⁴ Immunosuppressive agents currently in use are toxic, non-specific, and potentiate the risk of malignancy and opportunistic infections.⁹ While acute rejection is currently controlled with pharmacologics, chronic rejection remains a threat and is ineffectively addressed by present therapies. Therefore, genetic manipulation of donor organs to reduce the requirement for systemic immunosuppression and thus promote long-term graft survival is promising.

There is also a need to explore gene therapy strategies as a result of the organ shortage, a byproduct of the success of SOT over the past decade. This shortage is forcing the transplantation community to use higher-risk grafts and even explore possibilities beyond human donor organs to keep pace with the outweighing demand. It is predicted that as medicine and public health continue to advance, diseases of the aging, such as heart and kidney failure, will increase in prevalence, creating even more of a strain on limited organ supply.^{6,10} The donor organ shortage is further worsened due to early graft loss as a result of ischemic injury, which increases the risk of acute and chronic rejection leading to organ failure. These scenarios highlight the potential for gene therapy and/or modulation strategies in SOT to prevent rejection, expand the organ pool, and provide graft protection (Figure 1).

3 | GENE THERAPY DELIVERY STRATEGIES

It has been said that in a broader sense, organ transplantation was the first successful application of gene therapy.¹¹ Others have commented that the Achilles heel of gene therapy is gene delivery.¹² That is, the therapeutic utility of gene therapy lies in the efficacy of its delivery strategy, such that optimal delivery is achieved when therapeutic effects are reached in the target organ without significantly triggering the host immune response. There are numerous strategies to deliver gene therapy products including viral vectors (e.g., lentivirus, adenovirus, and adeno-associated virus (AAV)) as well as nonviral vectors (e.g., extracellular vesicles, nanoparticles, cell-penetrating peptides, cationic lipids, conjugates, and polymers). Though viral vectors tend to exhibit greater transduction efficiency compared to nonviral vectors, concerns about viral gene therapy include mutagenesis at the site of gene insertion,¹³ which may cause uncontrolled transgene expression. Other considerations of viral vectors include tissue tropism, gene size intended for delivery, as well as viral infection causing rejection,¹⁴ though this risk is minimal.

The use of AAVs, in particular, has recently emerged as a promising strategy for therapeutic gene delivery in the lab and clinic. For example, AAV was used for delivery of



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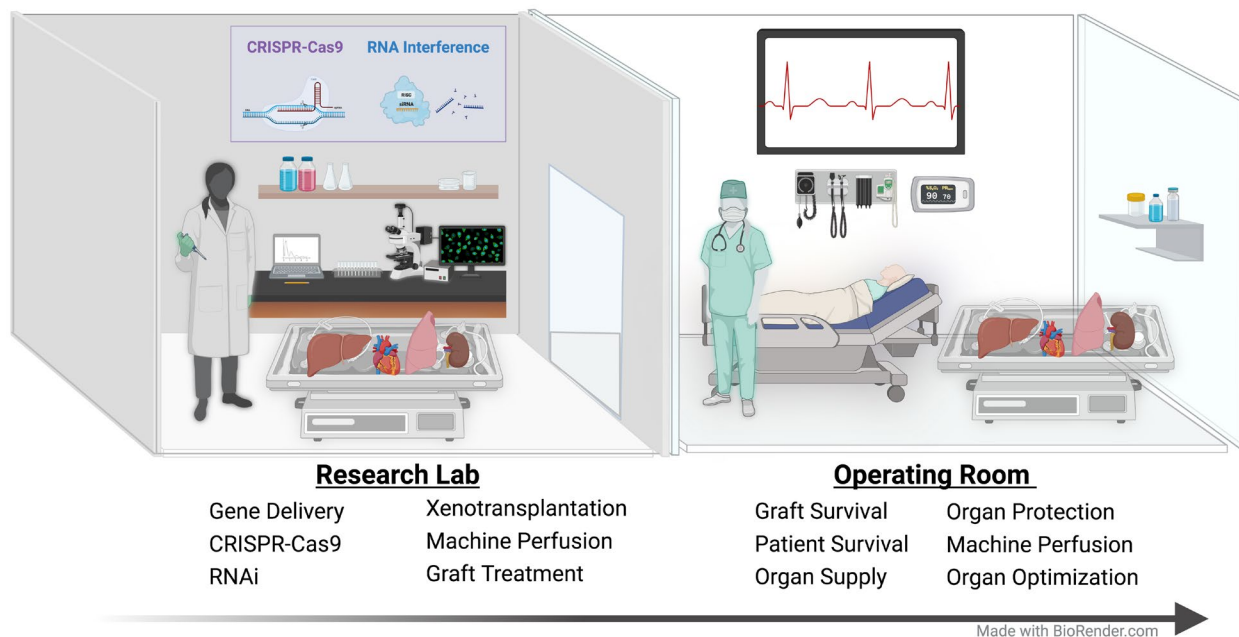


FIGURE 1 The future of personalized transplantation is heading towards a reality where organs are individualized and modified to maximize both graft and patient survival [Color figure can be viewed at wileyonlinelibrary.com]

genetic load during machine perfusion prior to liver implantation in a rodent liver transplant model, with preliminary results demonstrating that AAVs can be used to deliver a variety of gene-editing technologies during ex situ preservation.¹⁵ Clinically, AAVs show potential as a result of their sustained duration of effect, with several clinical trials ongoing to treat a variety of human diseases.¹⁶ Though neutralizing antibodies exist against several AAV serotypes in humans, the prevalence of serum neutralizing antibodies is low, making AAV an appealing delivery method for gene therapy.¹⁷ On the contrary, engineered adenoviruses efficiently transduce human cells in the lab, but wild-type variants can also infect people. Indeed, nearly 60% of some populations are seropositive for recombinant adenoviruses, with some individuals exhibiting adenoviral-deactivating antibodies.¹⁸ While certain delivery vectors are more stable than others, the development of non-immunogenic gene delivery vehicles for durable host genome integration is an area of exciting exploration, especially as it pertains to the organ transplantation field.

4 | THE EMERGENCE OF XENOTRANSPLANTATION

While still in its infancy and is subject to great debate, the use of animal organs, namely from pigs and non-human primates (NHPs), serves as a potential solution for

expanding the donor pool of available organs for transplant, at least serving as a bridge until a human organ becomes available. The interest in xenotransplantation was recently reignited when a kidney from a genetically-edited pig donor (termed GalSafe) was xeno-transplanted into a brain-dead donor at NYU Langone Health, with monitoring of kidney function for 54 h post-transplantation.¹⁹ Although this relative success is questionable in terms of its broad application to humans, it helped to redirect attention toward xenotransplantation as a potential strategy to overcome the shortage of human donor organs.

5 | APPROACHES TO GENETIC ENGINEERING OF ANIMALS FOR XENOTRANSPLANTATION

Several strategies have been used to genetically modify animals for xenotransplantation.²⁰ The discovery of synthetic, programmable nucleases has revolutionized the field of genetic engineering. Among these newly discovered nucleases, CRISPR-Cas9 has been used most widely in the gene editing field as this technology resembles molecular scissors with high precision capable of cleaving DNA at specific locations. Cas9 and its guide RNA can be delivered to target cells using viral or nonviral methods to achieve therapeutic effects.^{21,22} Following DNA cleavage, the double-strand DNA break is repaired by nonhomologous end-joining or homology-directed repair, producing



the desired genome modification. These nuclease-specific genetic engineering strategies have enabled *in vitro* and *in vivo* gene editing in an efficient and specific manner suitable for xenotransplantation applications. CRISPR-Cas9 especially has been used in xenotransplantation studies for its ease of use, low cost, wide *in vivo* applicability, enhanced specificity, ability to target multiple DNA sequences in the same cell using different guide RNAs, and minimal off-target effects with the development of new and improved Cas9 enzymes.²³ The following discussion surrounding gene editing in xenotransplantation primarily focuses on CRISPR-Cas9 technology as it is the method most frequently and recently employed to selectively knockout and knock-in genes, thus surmounting many of the biological barriers encountered in xenotransplantation.

6 | BIOLOGICAL HURDLES OF XENOTRANSPLANTATION

Today, there remain three major barriers inhibiting the clinical success of xenotransplantation in humans for which gene therapy strategies have been explored. These include the elicited immune response and rejection of xenografts in recipients,²⁴ the physiological bioincompatibility between xenografts and humans,²⁵ and the risk of zoonotic infection transmission between graft and recipient.²⁶ While these biological hurdles are often considered independently in the context of xenotransplantation, all three barriers—immunity, incompatibility, and infection—indeed intersect and dictate the future clinical success of xenotransplantation.⁶

7 | IMMUNE REJECTION OF XENOGRAFTS

The most daunting challenge in utilizing porcine xenografts is an inevitable and aggressive immunological rejection of xenografts.²⁷ Early studies attempting to transplant porcine organs in humans failed primarily due to hyperacute rejection occurring within minutes to hours after transplantation.²⁸ The reason for hyperacute rejection was found to be pre-formed natural human antibodies against the porcine carbohydrate xenoantigens, Gal α 1-3Gal, N-glycolylneuraminic acid (Neu5Gc), and non-gal glycan Sda, which lead to rapid complement activation and formation of the membrane attack complex, resulting in graft destruction and organ rejection.^{29,30}

The genes encoding the xenoantigens Gal α 1-3Gal, Neu5G, and Sda, namely α -1,3-galactosyltransferase (GGTA1), cytidine monophosphate-N-acetylneuraminic

acid hydroxylase (CMAH), and β -1,4-N-acetylgalactosaminyltransferase (B4GALNT2), respectively, have been the focus of several gene knockout strategies to reduce xenograft immunogenicity and enhance human compatibility.³¹ Indeed, the first GGTA1 knockout pigs were generated in 2002, and since the discovery of CRISPR-Cas9 in 2012, groups have generated pigs with single, dual, or triple knockout genes encoding the three enzymes necessary for porcine carbohydrate xenoantigen formation.³²

The longest survival of xenografts thus far is 945 days in a cardiac xenograft transplant model using donor pigs with a GGTA1/CD46/thrombomodulin genetic knockout background,³³ 499 days in a kidney xenograft model using donor pigs of a GGTA1 and CD55 genetic knockout background,³⁴ 14 days for lung xenografts from pigs with a GGTA1/CD47/CD55 genetic knockout background,³⁵ and 29 days for liver xenografts in a GGTA1 porcine knockout model.³⁶ All models included an immunosuppressive regimen. Other gene alteration strategies tested *in vivo* include the addition of six human transgenes to protect against inflammation and reduce activation of macrophages and T cells (i.e., CD46, CD55, TBM, EPCR, CD47, and HO1),³⁷ the insertion of human anti-apoptotic and anti-inflammatory genes (i.e., A20³⁸ and HO1³⁹), overexpression of human HLA-E/B2 microglobulin to inhibit natural killer cells,⁴⁰ and knockout of MHC Class 1 molecules by disrupting the 7 alleles of the classic MHC Class 1 swine leukocyte antigen genes.⁴¹ Taken together, these studies suggest that CRISPR-Cas9 genetic deletion and/or manipulation of genes related to complement, innate immunity, and inflammation are viable therapeutic targets for successful xenograft transplantation in preclinical pig-to-NHP models.

Promisingly, recent results demonstrate that when human blood is perfused through pig livers via *ex vivo* perfusion, Neu5Gc deletion results in reduced human anti-pig antibody binding and promotes organ function and survival.⁴² Despite this, it is predicted that triple knockout pigs with the addition of protective human transgenes will be the most optimal organs for xenotransplantation in humans because humans produce minimal to no naturally-existing antibodies to triple knockout porcine cells.^{31,37} Additionally, it has been suggested that a pig with a total of 9 genetic modifications (a donor triple knockout pig expressing human complement-regulatory proteins CD46 and CD55, coagulation-regulatory proteins thrombomodulin and EPCR, and HO1 and CD47) will supply organs (mainly kidneys and heart) that would function for a clinically-relevant period of time (>12 months) following transplant in patients with end-stage organ failure, in addition to an immunosuppressive regimen that adequately controls the adaptive immune response.³¹



To date, CRISPR-Cas9 gene editing has allowed testing of nearly 40 different combinations of porcine gene knockout and human gene knock-in models in less than a decade, with the most sophisticated transgenic pig model containing 3 pig gene deletions (GGTA1, CMAH, and B4GALNT2), insertion of 9 human genes (CD46, CD55, CD59, B2M, HLA-E, CD47, THBD, TFB1, and CD39) as well as the inactivation of 25 loci in porcine cells implicated in endogenous retroviruses.^{43,44} Thus, there are numerous genetically-modified pig models currently available in xenotransplantation research each with their own demonstrated benefits. For a comprehensive review of these models, we direct readers to recently published reviews in the field.^{23,45}

8 | BIOLOGICAL INCOMPATIBILITY OF XENOGRAFTS

Graft loss due to clotting following the hyperacute rejection phase of xenotransplantation is the second biological hurdle and is due to the incompatibility of coagulation between humans and pigs. The dysregulation of coagulation regulators between species is governed mainly by thrombomodulin present in the blood vessels of the transplanted xenografts.⁴⁶ Porcine thrombomodulin is incompatible with human protein C,⁴⁷ leading to thrombin generation, coagulation, and inflammation. To prevent the coagulation incompatibility in xenotransplantation, groups have generated transgenic pigs expressing human thrombomodulin, which has demonstrated favorable results in pig-to-NHP cardiac xenotransplant studies.^{48,49} Thrombomodulin has also been shown to have anti-inflammatory as well as anticoagulant properties, further demonstrating its protective role when incorporated into xenograft models. While several studies have highlighted the importance of incorporating multiple human transgenes into xenotransplantation models, it is unclear at this time the specific genetic alterations needed to completely prevent coagulation complications following xenotransplantation. However, it is certain that the expression of human thrombomodulin in transgenic animal models is more efficient and less toxic than the continued administration of systemic anticoagulant agents.⁶

9 | INFECTION TRANSMISSION RISK WITH XENOGRAFTS

The third and final hurdle preventing successful clinical translation of xenotransplantation using pigs is due to

zoonotic infection concern, primarily for porcine endogenous retroviruses (PERVs). It is known that under stress, porcine cells release PERVs, which can infect human cells *in vitro*.⁵⁰ There is a concern for the pathogenicity of PERVs in immunosuppressed xenograft recipients as well as the potential for PERVs to become further virulent through mutations or recombination with other human viruses.²⁷ There have been no cases of human PERV infection since its discovery in 1995.⁵¹ Nevertheless, caution has been taken with research efforts directed toward preventing zoonotic infection as a result of porcine xenotransplantation. Using CRISPR-Cas9, groups have been able to inactivate more than 60 copies of PERV insertions to reduce the infectious risk threefold by specifically targeting the retroviral gene polymerase, *pol*, a reverse transcriptase universal to all PERVs needed for viral production.⁵²

Despite the attention toward using gene editing technology to reduce the risk of porcine zoonotic infections in xenotransplantations, many have argued that SOTs of any type pose an infectious risk to the recipient, such that the concern for infectious transmission in xenotransplantation is becoming less of a public health threat.⁵³ The reasons for a reduced infectious risk from pigs are numerous, however, some of the most frequently cited reasons include robust screening protocols implemented over multiple generations of breeding, the ability to decrease potential infections with isolation and vaccination measures, and recent advancements in genetic engineering, thus lowering the risk for infection to a greater degree than in allotransplantation.^{6,54} Undoubtedly, public concerns with zoonosis raised by the COVID-19 pandemic will be a major obstacle to overcome.

10 | THE FUTURE OF XENOTRANSPLANTATION

While the future of xenotransplantation is indeed exciting as a result of gene editing technologies, it is important to note that the potential for organ replacement would still vary between the type of transplantable organ.⁵⁵ Recent progress in artificial pancreas and cardiac device development such as partial and total artificial hearts, as well as cellular therapies that enhance function, are re-shaping the pancreas and heart transplantation landscape.⁵⁶⁻⁶⁰ However, in the case of liver, lungs, and kidneys, transplantation remains the definitive treatment for end-stage diseases as no fully implantable devices or cellular therapies exist due to organ complexity. For the liver, the use of allogeneic and xenogeneic hepatocyte transplants is gaining traction in the field when treating inherited genetic disorders, though their efficacy in systemic diseases is unlikely given the limited potential of stem cells to



proliferate and replace large areas of damaged or diseased tissue.⁵⁵

Though the concept of designer organs in cases of xenotransplantation is appealing, the rapid and recent progress within the field has encouraged regulatory authorities to critically examine xenotransplantation guidelines, particularly in cases of proposed clinical trials. In particular, clinical trials may not involve patients in whom allotransplantation is contraindicated due to conditions such as chronic infection or malignancy, as this would likely confirm a poor prognosis beyond xenotransplant consideration.⁶¹ Additionally, initial xenograft clinical trials must demonstrate sustained life-supporting xenograft function and survival in preclinical models.^{61,62} Thus, the organ transplant field awaits clinical implementation of xenotransplantation with cautious optimism, one where there is hope for a future devoid of organ shortages with the realization that careful steps must be taken to ensure such a reality.

11 | GENE THERAPY AND GENE SILENCING STRATEGIES IN ORGAN TRANSPLANTATION

Several gene therapy strategies have been explored in cases of allotransplantation as well. For example, studies have used adenoviral vectors encoding human interleukin-10 (AdhIL-10) during ex situ machine perfusion of donor lungs in both discarded human and porcine lung models to inhibit pro-inflammatory cytokine secretion and promote improvement in lung function prior to transplantation.^{63,64} The use of mesenchymal stem cells (MSCs) has also been explored in SOTs as a method of modulating the inflammatory response and attenuating tissue damage, and their addition to machine perfusate in discarded human and animal models has been tested in lung, kidney, and liver preclinical and clinical trials and is thoroughly reviewed elsewhere.⁶⁵ Gene therapy strategies in allotransplantation can also be used to correct genetic deficiencies, inborn errors of metabolism, or clotting disorders in donor organs that are associated with an increased risk of graft loss.⁶⁶

Gene silencing strategies have also been implemented in organ transplantation to modulate gene expression at the messenger RNA (mRNA) and protein level. Specifically, RNA interference (RNAi) is a powerful, clinically-established therapeutic technology that enables the repression of disease-associated or overexpressed genes by knocking down the level of target mRNA and thus subsequent protein, and its use in the clinical setting is established. The first-ever RNAi drug received FDA approval in 2018 to treat polyneuropathy caused by

hereditary transthyretin amyloidosis, and several clinical trials using RNAi drugs to treat a variety of human diseases are ongoing.⁶⁷

RNAi therapies, specifically in the form of small interfering RNAs (siRNAs) and antisense oligonucleotides (ASOs), can be chemically-modified for enhanced stability, specificity, and potency, with a robust duration of effect for 6–12 months following a single systemic injection.^{68,69} Delivering RNAi therapeutics during the transplantation process serves as an attractive method of organ protection for their ability to directly treat a procured graft during the ex situ preservation period without the need for systemic therapy, their high specificity with minimal off-target effects, and their ability to be administered without the need for viral transfection agents.⁷⁰ Formulation of RNAi therapies without the inclusion of viral transfection agents eliminates concerns of immunogenicity associated with the transfection agent itself and is an important consideration in the context of SOTs.

The application of RNAi-based therapies has recently been investigated as a method of modulating alloimmune responses before and after transplant to reduce graft injury and induce donor-specific tolerance. Specifically, injury-sensitive endothelial cells have been targeted during the pre-transplant period as donor organs express MHC molecules, and it is the endothelial cell barrier that the recipient lymphocytes first encounter upon reperfusion.⁷¹ Endothelial cells are also primary targets of ischemia-reperfusion injury (IRI) and preformed donor antibody damage.⁷² Thus, reduction of endothelial cell injury may reduce the host alloimmune response. Endothelial cells also express adhesion molecules, such as intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), that recruit leukocytes during IRI.⁷³ Strategies used to target ICAM-1 include anti-ICAM-1 conjugated poly (lactic-co-glycolic acid) (PLGA) nanoparticles (NPs).⁷⁴ Approaches using similar methods have also targeted MHC II on allograft endothelial cells. It was found that NPs (poly(amine-co-ester)) loaded with siRNA targeting MHC II and delivered via ex vivo perfusion decreased endothelial cell MHC II expression for up to 6 weeks, accompanied by decreased graft T cell infiltration and activation.⁷⁵ Other groups have demonstrated the feasibility of conjugating anti-CD31 antibodies to NPs to facilitate endothelial cell uptake and vascular retention in human kidneys during normothermic machine perfusion.⁷⁶ Thus, NPs may serve as a platform for endothelial cell targeting during ex vivo normothermic machine preservation to reduce allograft transplant injury and promote organ function and survival, at least in the short-term post-transplant period.

The first use of an ASO as a gene modulatory agent in organ transplantation was in 2017 where an ASO targeting



miRNA-122 (Miravirsin) was delivered in a porcine model of ex situ liver machine perfusion.⁷⁷ miRNA-122 was selected as a target for ASO-mediated knockdown for its high expression in hepatocytes and because its presence allows for hepatitis C virus (HCV) replication.⁷⁷ In vitro data confirm ASO-mediated repression of HCV replication during machine perfusion as a proof-of-concept, although it is unlikely that Miravirsin will be implemented in the clinic as a method to prevent HCV reinfections given the high efficacy of current HCV antiviral regimens. Several other groups have since investigated the use of gene modulation strategies during the liver transplantation process to target components necessary for viral replication and genes implicated in IRI such as those involved in inflammation, oxidative stress, and cell death. Numerous RNAi strategies have been tested experimentally in several transplantable organ animal models involving the liver, kidneys, heart, and lungs, and thoroughly reviewed elsewhere.^{2,78,79} However, we highlight that Gillooly et al first demonstrated the feasibility of delivering siRNA during ex situ machine perfusion.⁸⁰ This group delivered unmodified siRNA targeting the apoptotic Fas receptor during ex situ liver perfusion under both hypothermic and normothermic conditions.

In addition to administering RNAi therapeutics during ex situ machine perfusion, groups have demonstrated the feasibility of delivering siRNA in the preservation solution itself. In one such study, a cocktail of unmodified siRNA targeting TNFalpha, Fas, and complement C3 was administered to the heart in a syngeneic model of mouse heart transplantation as part of the preservation solution. After 48 h, siRNA-treated hearts were transplanted into syngeneic recipients and demonstrated sustained beating for >100 days (whereas control grafts lost function within 8 days), improved histology, and diminished neutrophil and lymphocyte accumulation.⁸¹ This was one of the first studies to demonstrate that delivery of siRNA in the preservation solution is feasible and can effectively repress target mRNA expression to protect cardiac function and prolong graft survival against IRI.⁸¹ Other groups have since tested the delivery of a siRNA cocktail solution (targeting complement C3, RelB, and Fas) in a similar mouse model of syngeneic kidney transplantation, highlighting the feasibility and clinical potential of delivering siRNA-based therapies during the preservation period of donor organs.⁸²

12 | POTENTIAL FUTURE PROSPECTS OF GENE MODULATION IN TRANSPLANTATION

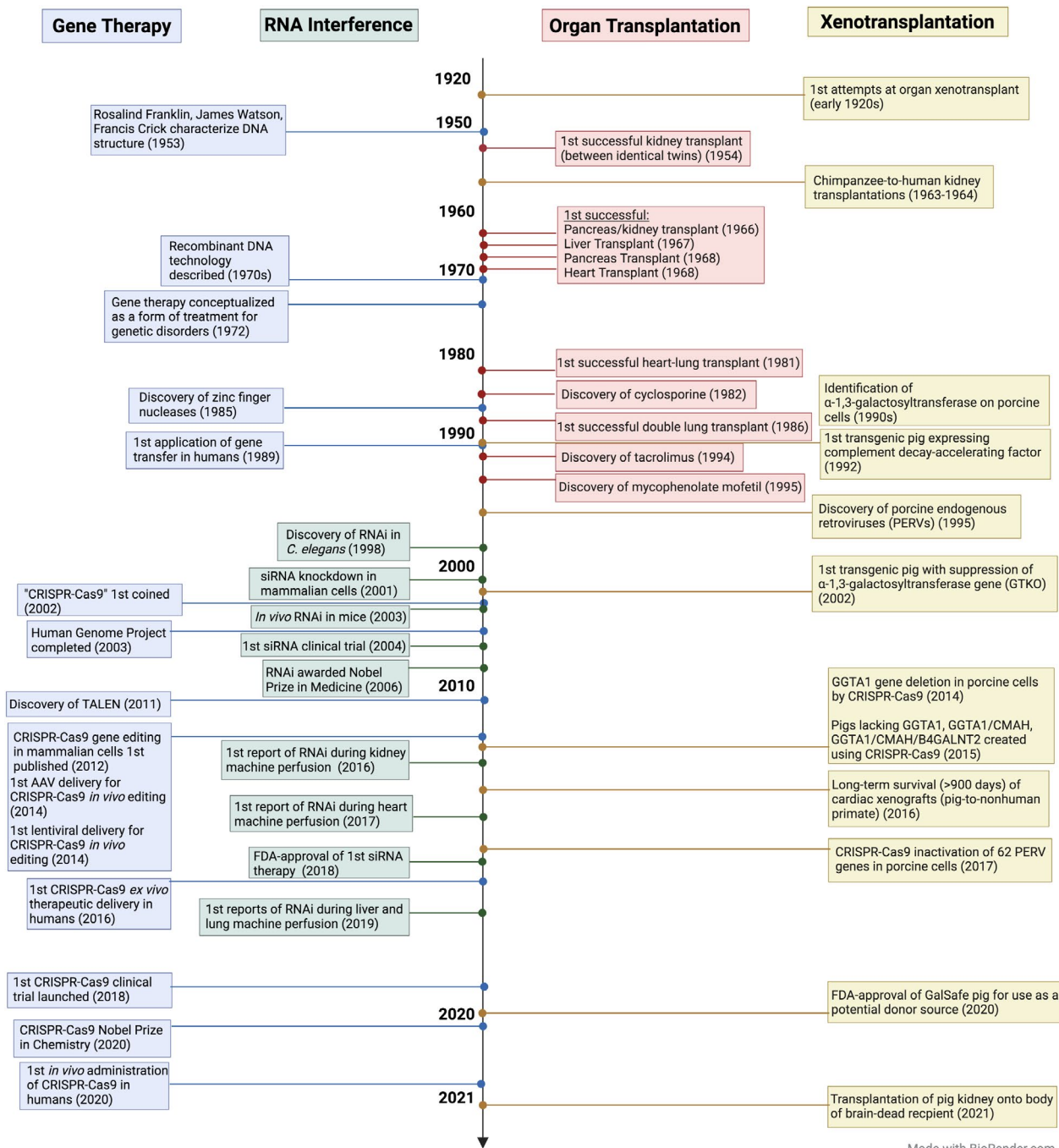
Though RNAi therapeutics have been investigated experimentally as a way to protect grafts against virus

replication, rejection, and IRI, their implementation in the clinical setting, particularly during organ transplantation, has not yet occurred. RNAi-based therapeutics are dosed based on weight. Thus, large quantities are likely required to reach therapeutic effects in both ex situ and in vivo models. The specificity and duration of effect of RNAi-based therapeutics, as determined by chemical conjugate, backbone, and delivery strategy is nevertheless exciting as it eliminates concerns for major off-target effects and permanent gene modulation, especially when the targets of IRI, for example, are involved in the maintenance of homeostasis with numerous overlapping cellular signaling pathways. Transient repression of gene and protein expression, therefore, may sufficiently regulate immune responses while preventing potential toxicities and adverse effects of prolonged homeostatic signaling repression.

It must be acknowledged, however, that in cases where gene therapy is applied during ex vivo cold preservation, the metabolic function of an organ may limit uptake. The use of machine perfusion at physiologic conditions may therefore serve as a more effective platform for both gene therapy and RNAi-based drug delivery. The transient nature of mRNA silencing seen with RNAi therapeutics on the order of weeks to months is appealing during the transplantation process, where graft function within the first year following transplantation dictates a transplant's long-term success.⁸³ Additional randomized human studies using RNAi therapeutics are necessary, and discarded human organs declined by transplant centers may serve as a strategy to investigate gene modulation therapies in human organ models.

13 | CONCLUSION

The future of organ transplantation is indeed exciting, as numerous gene editing and modulation strategies are currently being explored with promising results. Though no agents have yet to be tested in organ transplantation clinical trials, the potential for such gene-targeting strategies to revolutionize the organ transplantation field is tangible (Figure 2). Unlike other genetic disorders where stable and durable genetic modification may be needed, organ transplantation may require only temporary genetic modulation after organ transplantation. Furthermore, the preservation period offers a uniquely appropriate and clinically necessary period during which to administer gene therapy, and this can be done ex situ preventing risks of off-target effects. *The concept of "designer organs" will enable a future with uniquely improved graft and patient survival outcomes and a downwards trending waitlist,*



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FIGURE 2 Timeline of major advancements in the fields of gene therapy, RNA interference, organ transplantation, and xenotransplantation [Color figure can be viewed at wileyonlinelibrary.com]

where the supply of organs will hopefully one day outweigh the demand.

CONFLICT OF INTEREST

There are no conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS

All authors contributed to the literature search, original draft preparation, and editing and review of the final manuscript.

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