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Systematic Review and Comparative Outcomes Analysis of NHP Liver Allotransplants and Xenotransplants

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ABSTRACT

Patients with fulminant liver failure ineligible for transplantation have a high mortality rate. With recent progress in genetic modifications and clinical achievements, using pig livers as a bridge-to-transplant has regained popularity. Preclinical testing has been done in small cohorts of nonhuman primates (NHP), and maximum survival is limited to 1-month. We conducted a systematic review and comparative outcomes analysis of NHP-liver xenotransplantation and gathered 203 pig-to-NHP and NHP-to-NHP transplants reported in 23 studies. Overall, NHP survival after pig-liver xenotransplantation was limited (1, 3, 4 weeks: 18.0%, 5.6%, 1.1%), compared to NHPs after allotransplantation (1, 3, 4 weeks: 60.6%, 47.4%, 45.4%). A focus on pigs with genetic modifications evidenced some short-term survival benefits (1, 3, 4 weeks: 29.1%, 9.1%, 1.8%). The use of the auxiliary transplant technique was also associated with better short-term results (1, 3, 4 weeks: 40.9%, 9.1%, 4.5%). Causes of graft and animal loss were mostly rejection and liver failure in allotransplants, while bleeding, liver, and respiratory failure predominated in xenotransplants. Notably, the 1-month survival rate for NHP-allotransplants was significantly lower than the national > 98% rate for human liver transplants. This data confirms the short-term improvements brought by genetic modifications and auxiliary implantation in the NHP model, which remains imperfect.

1 | Introduction

Liver disease ranks as the 11th most common cause of death worldwide, claiming about two million lives every year, which is roughly 4% of all deaths globally [1]. In the spectrum of liver diseases, acute liver failure is one of the most life-threatening conditions, requiring liver transplantation in up to 60% of the cases depending on the cause [2]. The survival rates following transplantation are high and far exceed those of conservative treatment [3, 4]. However, organ shortage results in not all patients receiving a transplant on time. Of note, approximately 10 000 patients are awaiting a liver transplantation in the United States [5], and many more could benefit from auxiliary xenogeneic liver support following extensive liver resections for otherwise nonresectable tumors (i.e., not leaving enough remnant liver) [6]. In this setting, xenotransplantation has surfaced

Abbreviations: AHLXT, auxiliary/heterotopic liver xenotransplantation; Allo, allotransplantation; ALS, anti-lymphocyte serum; ATG, anti-thymocyte globulin; Aux/hetero, auxiliary/heterotopic; AZA, azathioprine; BMCs, bone marrow cells; CI, confidence interval; Cs, corticosteroid; CsA, cyclosporin; CVF, cobra venom factor; CyP, cyclophosphamide; DKI, double knock-in with human CD46 and human thrombomodulin; DKO1, double knock-out with GGTA1 and CMAH; DKO2, double knock-out with GGTA1 and B4galNT; GM, genetically modified; hPCC, human prothrombin concentrated complex; HR, hazard ratio; LoCD2b, rate anti primate CD2 IgG2b; LXT, liver xenotransplantation; MD-3, chimeric anti-ICAM-1 monoclonal antibody; MMF, mycophenolate mofetil; MST, median survival time; NHP, nonhuman primate; QKO, quadruple knock-out with GGTA1, CMAH, B4galNT2, and iGb3s; RTX, rituximab; sCR1, soluble complement receptor 1; TKO, triple knock-out with GGTA1, CMAH, and B4galNT2; TMA, thrombotic microangiopathy; WBI, whole body irradiation; WT, wild type; Xeno, xenotransplantation.

as a promising solution [7–14] with pigs emerging as optimal donors due to their ease of breeding, organ size match, and the possibility for genetic manipulation [9–11, 15]. Advancements in immunosuppressive strategies and genetic modifications have expanded their feasibility, resulting in longer survival periods, especially for kidneys and hearts [16, 17], allowing initial clinical cases to be performed [18–26]. Despite these advancements, liver xenotransplantation has not achieved comparable success, and the maximum survival in the pig-to-nonhuman primate (NHP) model remains limited to 34 days [27]. Although modest, this graft survival was achieved after decades of work and improvements such as the use of Gal depletion [28], heterotopic surgical implantation [29], human CD55 transgene insertion [30], alpha 1,3-galactosyltransferase gene-knockout pigs [31], and the use of enhanced immunosuppression with co-stimulation blockade [27, 32, 33]. Failures are due to severe coagulopathy, thrombotic microangiopathy, and subsequent graft loss due to uncontrolled activation of the coagulation cascade, and severe thrombocytopenia [9]. Major physiological differences in the levels and function of the numerous proteins produced by the liver explain the greater difficulty in interchanging the liver between species. Historically, several attempts have been made using nonmodified pig livers transplanted in an auxiliary fashion [34] or connected via an ex vivo circuit [35–37]; however, survival was limited to a few days. With now improved pigs and in order to prepare potential future clinical trials, experiments have been conducted in decedents using ex vivo perfusion [38, 39] and heterotopic implantation [40], and one involving auxiliary implantation of a xeno-liver in a living human following an extensive hepatocellular carcinoma resection [6]. Addressing and resolving the challenges posed by liver xenotransplantation holds the potential to successfully develop a bridge to allotransplantation and save numerous lives.

To aggregate what is known so far and better define the current limitations of preclinical models, we conducted a literature review and a comparative outcomes analysis of NHP liver allotransplantation and xenotransplantation. We aim to discuss the achievements of NHP models and advocate for increased clinical translations, particularly among end-stage liver disease patients for whom traditional transplant options are not available.

2 | Materials and Methods

2.1 | Study Design and Search Criteria

We conducted a systematic review and a comparative analysis of individual pig-to-NHP and NHP-to-NHP liver transplantation experiments, encompassing both allotransplantation and xenotransplantation, from database inception until January 2024. Searches were conducted in MEDLINE, PubMed, and Google Scholar. We included relevant original publications published in English. Our search criteria included the following keywords: “Liver” “Xenotransplant”, “Xenotransplantation”, “Orthotopic”, “Auxiliary”, “Non-human primate”, “Monkey”, “Baboon”, “Cynomolgus”, “Rhesus”, “Chimpanzee”, “Pig”, and “Allotransplant”. Reporting followed the recommendations in the preferred reporting items for systematic reviews and meta-analysis (PRISMA) statement standard. Results were first exported as Excel files and then manually evaluated for inclusion and exclusion criteria. This study did not require IACUC or IRB approval as it utilized publicly available data and did not involve any new animal subject.

2.2 | Study Selection

R.P.H.M. and K.S. screened all records. Our inclusion criteria encompassed orthotopic and auxiliary liver xenotransplants and orthotopic allotransplants. We excluded studies performing ex vivo organ perfusion, including other organ transplants besides the liver, performed in different species, studies lacking adequate information for individual animal survival assessment, reviews, case reports already reported elsewhere, duplicates, quotations without published documentation, and cases lacking published records (study selection flowchart in Figure 1). A total of 23 studies were selected and subjected to comprehensive analysis.

2.3 | Data Review

We categorized factors such as donor type, recipient, NHP species and number, transplant type (orthotopic vs. auxiliary), immunosuppression regimen, animal and graft survival, and cause of death.

2.4 | Statistical Analysis

We used Kaplan–Meier analyses to plot survival and assess differences between groups. Cox analyses were used to compute hazard ratios (HRs). Significance was determined based on P-values < 0.05. Median survival, confidence intervals (CI), and HRs were reported.

3 | Results

3.1 | Baseline Animal Characteristics

Our initial search found 1001 articles, and after the removal of duplicates, nonrelevant articles, and manual screening, 23 studies were selected (Figure 1). These studies include 14 studies only on xenotransplantation (Table 1) [27, 28, 30, 32, 33, 41–49], 6 studies only on allotransplantation (Table 2) [50–55], and 3 studies combining both xenotransplants and allotransplantations (Table 3) [29, 31, 56]. Selected articles reported a total of 95 pig-to-NHP liver xenotransplants and 108 monkey-to-monkey liver allotransplants.

Among xenotransplant cases, 34 used wild-type (WT) donor pigs and 61 used genetically modified (GM) donor pigs. Recipients included 57 baboons, 19 cynomolgus monkeys, 9 rhesus monkeys, 9 Tibetan monkeys, and one chimpanzee. Seventy-one underwent orthotopic liver xenotransplantation, while 24 underwent auxiliary/heterotopic procedures. The average donor weight was 7.8 ± 2.9 kg, ranging from 1.2 to 19 kg, and the average recipient weight was 10.1 ± 4.5 kg, ranging from 4.5 to 24.5 kg. Animal survival varied from 0 to 34 days. Details on pig-to-NHP xenotransplants are presented in Tables 1 and 3. A breakdown of NHP species and immunosuppression regimens is shown for WT versus GM pigs and for orthotopic versus

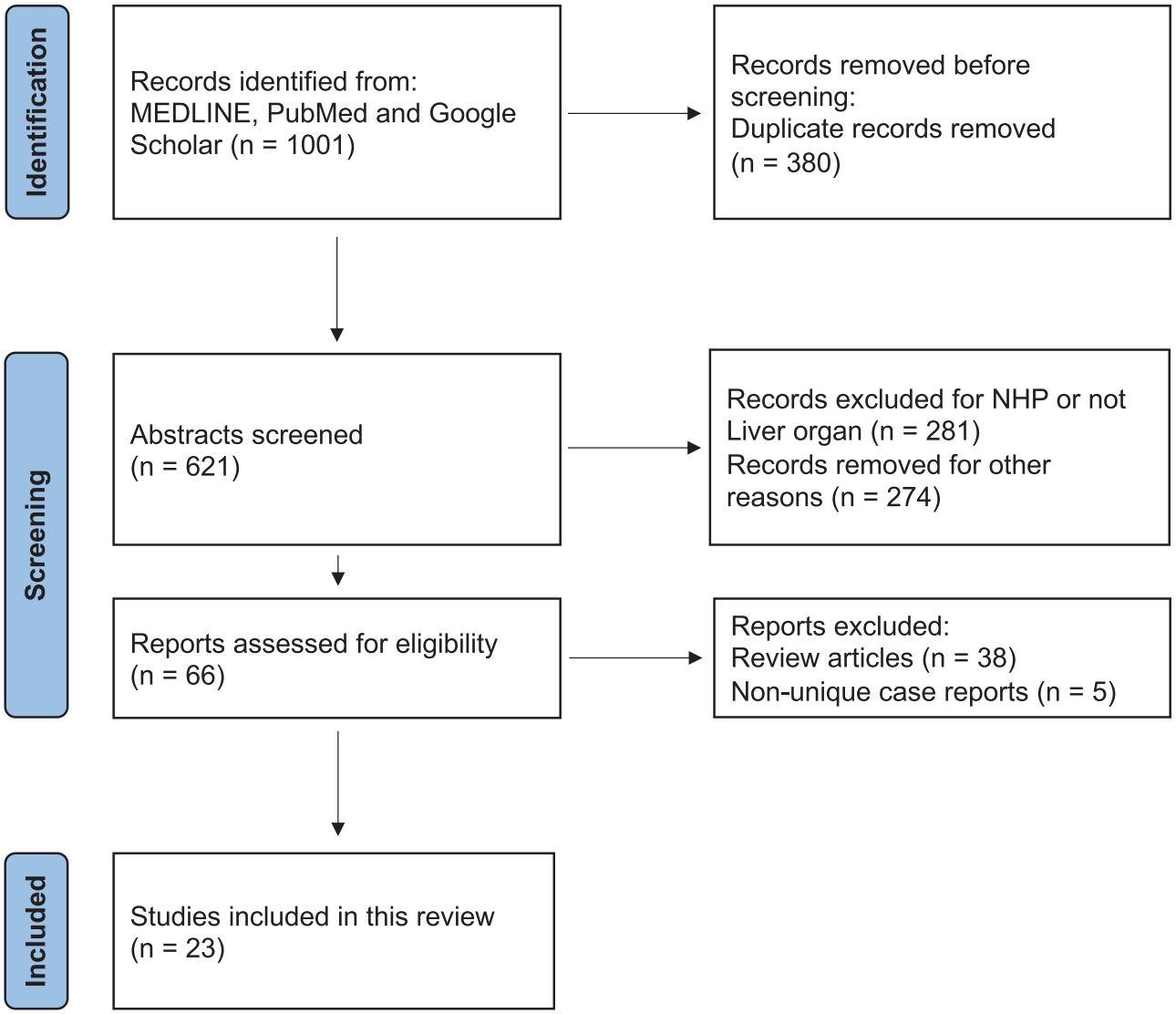

FIGURE 1 | Identification and selection of studies reporting on liver xenotransplantation in NHPs. NHP, nonhuman primates.

auxiliary/heterotopic cases (Table S1). NHP recipients who underwent liver xenotransplantation from WT versus GM pigs had comparable utilization rates of baboons (58.8% vs. 60.6%) and cynomolgus monkeys (11.8% vs. 24.6%), but different utilization rates of rhesus monkeys (26.5% vs. 0%). Orthotopic cases had a higher proportion of baboons (69.0% vs. 33.3%) and Tibetans (0% vs. 37.5%) compared to auxiliary/heterotopic. More intense immunosuppression was more commonly used in the GM pig group and the auxiliary/heterotopic groups.

Among allotransplant cases, recipients included 42 cynomolgus monkeys, 41 baboons, and 25 rhesus monkeys. All underwent orthotopic liver allotransplantation. The average donor weight was 6.2 ± 2.5 kg, ranging from 2.7 to 13 kg, and the average recipient weight was 8.9 ± 5.7 kg, ranging from 2.6 to 23 kg. Animal survival varied from 0 to 1035 days. Details on NHP-to-NHP allotransplants are presented in Tables 2 and 3.

A comparison of baseline characteristics between the xeno and allotransplant groups is provided in Table S2. The xenotransplant group included more recipient baboons (60.0% vs. 38.0%) but fewer cynomolgus (20.0% vs. 38.9%) and rhesus (9.5% vs. 23.2%), respectively ($p < 0.05$). Tibetan monkeys were used as recipients in two studies and chimpanzees in one xeno-group study, but none in the allotransplant group. In xenotransplant cases, antithymocyte globulin (63.2% vs. 20.4%), corticosteroid (70.5% vs. 50.0%), cobra venom factor (46.3% vs. 0.9%), and tacrolimus (41.1% vs. 19.4%) were used significantly more than in allotransplant cases ($p < 0.001$). Similar cyclophosphamide usage was found between xeno and allotransplant groups (20.0% vs. 20.4%).

3.2 | Liver Graft and Animal Survival

Despite a steady increase in the maximum animal survival of pig-to-NHP xenotransplants over the past 50 years (Figure 2), the survival rate remains significantly lower than that of allotransplantation. The median survival of pig-to-NHP liver xenotransplantation was 3 days (ranging from 0 to 34 days), significantly lower than the median survival of NHP-to-NHP liver allotransplantation, which was 20 days (ranging from 0 to 1035 days, $p < 0.001$) (Table 4, Figure 3A). Overall, xenogeneic liver

TABLE 1 | Studies reporting wild-type and genetically modified pig-to-NHP liver xenotransplants.

Author/ year	Donor type (*n*)	Recipient species (*n*)	Number	Transplant type	Immunosuppression and other therapies	Animal survival time (days)	Cause of death	Major findings and insights
Calne et al. (1968) [41]	WT	Baboon	7	Orthotopic	None AZA, Cs Cs	0.25 0.38 1.5 0.25 0.79 1.25 3.5	Hemorrhage Hemorrhage Liver failure Hemorrhage Liver failure Hemorrhage Bronchopneumonia	Using Human fibrinogen to stop bleeding demonstrates the potential of immunosuppression in increasing survival time up to 3.5 days.
Powelson et al. (1994) [28]	WT	Cynomolgus (4) Baboon (2)	6	Orthotopic	Gal depletion, WBI, ATG, Pig BMCs	0.08 0.17 0.21 0.54 3 3.1	Dead during surgery Dead during surgery Dead during surgery Dead during surgery Renal failure Aspiration of Gastric content	Using anti-thymocyte globulin for the first time.
Hayashi et al. (1998) [42]	WT	Baboon	1	Auxiliary	sCR1	0.21	Bleeding	Use of sCR1 in combination with auxiliary LXT.
Ramirez et al. (2000) [30]	WT (3) hCD55 (2)	Baboon	5	Orthotopic	CyP, CsA, Cs	0.13 0.17 0.33 4 8	Bleeding Bleeding Bleeding Arrest Sepsis and coagulopathy	The first use of transgenic pig. (h-DAF) for LXT. Considering the presence of IgM, IgG, C3, C4, C5-9b as a confirmation for hyperacute rejection.
Ramirez et al. (2005) [43]	WT (4) hCD55.CD59HT (5)	Baboon	9	Orthotopic	CyP, CsA, Cs, Daclizumab, RTX, MMF	0.08, 0.13, 0.33, 0.67 0.54 0.75 0.83 0.88 1	Multiorgan failure and DIC Thrombosis Thrombosis Thrombosis Intestinal volvulus Primary xenograft dysfunction	Using livers of poly transgenic pigs, which expressed human complement regulatory proteins CD55, CD59, and H-transferase.

(Continues)

TABLE 1 | (Continued)

Author/ year	Donor type (*n*)	Recipient species (*n*)	Number	Transplant type	Immunosuppression and other therapies	Animal survival time (days)	Cause of death	Major findings and insights
Kim et al. (2012) [44]	GTKO, MGH HS	Baboon	3	Orthotopic	Cs, ATG, Tacrolimus, CVF, AZA, anti CD154, LoCD2b	6 8 9	Bleeding Bleeding and enterococcal infection Bleeding and enterococcal infection	Using aminocaproic acid for platelet count stabilization after profound thrombocytopenia. Persistence of TMA, coagulopathy, and bleeding despite sufficient platelet level.
Yeh et al. (2014) [45]	GTKO, MGH HS	Baboon	3	Auxiliary	Cs, ATG, Tacrolimus, CVF	6 9 15	Liver failure Sepsis Grossly xenograft ischemia and stiff	Unprecedented survival up to 15 days, despite thrombocytopenia and bleeding reduction that could be avoided by supplementation with primate clotting components.
Ji et al. (2015) [46]	GTKO, WZ MS	Tibetan	3	Heterotopic Auxiliary	Cs, MMF, ATG, Tacrolimus, CVF, anti CD154, Dashen	2.2 4.8 13.6	Pulmonary edema Pulmonary infection Pulmonary infection	Early activation of recipient tissue factors played a pivotal role in coagulation dysregulation post-LXT. Identifying the role of Kunitz domain 1 as the most important factor in incompatibility between pig tissue factor pathway inhibitor and human tissue factors.
N-Alvarez et al. (2016) [47]	GTKO, MGH HS	Baboon	7	Orthotopic	Cs, ATG, Tacrolimus, CVF, hPCC (6) Cs, ATG, Tacrolimus, CVF, hPCC, anti CD154, LoCd2b	1 3 5 5 6 7 6	Intrahepatic necrosis Intrahepatic necrosis Renal and respiratory failure Sepsis Intrahepatic necrosis Bleeding, renal and respiratory failure Diffused bleeding	Using Human Prothrombin Complex Concentrate (hPCC) as coagulation factor supplementation in combination with LoCD2b prevents TMA development, reduces transfusion requirements, and improves thrombocytopenia, especially in the form of a continuous dose.

(Continues)

TABLE 1 | (Continued)

Author/ year	Donor type (*n*)	Recipient species (*n*)	Number	Transplant type	Immunosuppression and other therapies	Animal survival time (days)	Cause of death	Major findings and insights
Shah et al. (2016) [32]	GTKO, MGH HS	Baboon	1	Orthotopic	Cs, ATG, tacrolimus, CVF, hPCC, Belatacept	25	Euthanized due to cholestasis and plantar ulcer	Using the combination of hPCC and belatacept as a co-stimulating blockade and considering the importance of CMV-negative donors in increasing recipients' survival time.
Shah et al. (2017) [33]	GTKO, MGH HS	Baboon	4	Orthotopic	Cs, ATG, Tacrolimus, CVF, hPCC, anti-CD40mAb	5 8 25 29	Euthanized due to cholestasis and plantar ulcer Euthanized following a seizure Euthanized because of worsening LFTs Euthanized due to rising LFT and plantar ulcer	Using Anti-CD40mAb instead of belatacept led to **nearly 1-month of survival**. Significant reduction in blood transfusion and platelet counts spontaneous recovery, probably due to posttransplant splenectomy, which overthrew this theory that coagulation factor supplements may be able to be withdrawn over time.
Zhang et al. (2017) [48]	GTKO, WZ MS (3) GTKO (Bama) (2) GTKO-hCD46 (1)	Tibetan	6	Heterotopic Auxiliary	ATG, CVF, Tacrolimus, MMF, Cs	5 11 14 3 6 12	Pulmonary hemorrhage Pulmonary edema Pulmonary infection Renal Failure Airway obstruction Pulmonary infection	Found cytokine levels as predictive markers for xenograft damage, suggested support for native liver recovery. Auxiliary/heterotopic xenotransplantation could be used to provide temporary support for native liver recovery.
N-Alvarez et al. (2018) [49]	GTKO	Baboon	2	Heterotopic Auxiliary	ATG, Tacrolimus, CVF, CS	4 11	Abdominal and respiratory distress, sever pulmonary edema Euthanized due to hypoglycemia and lethargy	Xenogeneic auxiliary/heterotopic liver transplantation could prevent steatosis and inflammation and aid in the native liver segment and hepatocyte cell growth or recovery in acute or posthepatectomy liver failure patients.

(Continues)

TABLE 1 | (Continued)

Author/ year	Donor type (*n*)	Recipient species (*n*)	Number	Transplant type	Immunosuppression and other therapies	Animal survival time (days)	Cause of death	Major findings and insights
Lee et al. (2023) [27]	DKO 2 (1) DKO1 (1) TKO (3) QKO (2) TKO+DKI (1)	Cynomolgus	8	Orthotopic	RTX, ATG, CVF, Etanercept, anti CD154, Cs, Sirolimus	0 0 0 0 7 1 3 5	Bleeding Bleeding Bleeding Bleeding Acute gastric dilatation Bleeding and hemorrhage in liver graft Multi-organ dysfunction syndrome, hepatocyte necrosis Multi-organ dysfunction syndrome, Biliary sepsis, hepatocyte necrosis, primary graft non function	Explored the impact of different surgical methods on recipient survival time after liver xenotransplantation. Left auxiliary LXT led to an optimal possible survival time of **34** days without thrombocytopenia or anemia.
	DKO 2 DKO 1	Cynomolgus	2	Right Auxiliary	RTX, ATG, CVF, Etanercept, Anti CD154, Cs, Sirolimus	0 0	Bleeding Bleeding	
	DKO 1 (3) DKO1 + DKI (2)	Cynomolgus	5	Left Auxiliary	RTX, ATG, CVF, Etanercept, Anti CD154, Cs, Sirolimus	5 7 22 6 34	Asphyxia, hepatocyte vascular necrosis Asphyxia, hepatocyte vascular necrosis Multi-organ dysfunction syndrome, hepatocyte necrosis and primary graft non function Aspiration pneumonia, sepsis, hepatocyte necrosis and intravascular thrombi Anemia, hepatocyte necrosis	

Abbreviations: AHLXT, auxiliary/heterotopic liver xenotransplantation; ALS, anti-lymphocyte serum; ATG, anti-thymocyte globulin; AZA, azathioprine; BMCs, bone marrow cells; Cs, corticosteroid; CsA, cyclosporin; CVF, cobra venom factor; CyP, cyclophosphamide; DKI, double knock-in with human CD46 and human thrombomodulin; DKO1, double knock-out with GGTA1 and CMAH; DKO2, double knock-out with GGTA1 and B4galNT; hPCC, human prothrombin concentrated complex; LoCD2b, rate anti primate CD2 IgG2b; LXT, liver xenotransplantation; MMF, mycophenolate mofetil; QKO, quadruple knock-out with GGTA1, CMAH, B4galNT2, and iGb3s; RTX, rituximab; sCR1, soluble complement receptor 1; TKO, triple knock-out with GGTA1, CMAH, and B4galNT2; TMA, thrombotic microangiopathy; WBI, whole body irradiation; WT, wild type.

TABLE 2 | Studies reporting NHP-to-NHP liver allotransplants.

Author/year	Donor type (*n*)	Recipient species (*n*)	Number	Transplant type	Drugs and immunosuppression regimen	Animal survival time (days)	Cause of death	Study purpose
Gotoh et al. (1991) [50]	Cynomolgus	Cynomolgus	12	Allo	None (5) Tacrolimus (7)	5 6 8 12 63 5 7 7 39 497 789 912	Arterial thrombosis Rejection Rejection Rejection Rejection Dehydration Arterial thrombosis Atrial thrombosis Sepsis Euthanized due to hypoalbuminemia and anemia Cholangitis **Alive**	Undertaken to explore the efficacy and safety profile of FK-506 in the context of liver transplantation. The findings conclusively demonstrated FK-506's potent immunosuppressive properties, establishing it as a formidable agent in liver transplantation protocols.
Gridelli et al. (1993) [51]	Baboon (5) Vervet (13)	Baboon	18	Allo	Cs, AZA, CsA, ATG	0.42 32 48 49 57 0 0 0.25 0.5 1 2 2 2 20 27 40 340 360	Cerebral damage Sacrificed due to Acute rejection Sacrificed due to mild rejection Sacrificed due to moderate rejection Aspergillus Bleeding Bleeding Pulmonary edema Respiratory arrest Cerebral damage Hyperacute rejection Hyperacute rejection Liver F Sepsis Sepsis Hemorrhage VitB12 deficiency Alive	Demonstrated that liver NHP-to-NHP allotransplantation can attain long-term survival with excellent liver function via an immunosuppressive protocol that can be safely used clinically.

(Continues)

TABLE 2 | (Continued)

Author/ year	Donor type (*n*)	Recipient species (*n*)	Number	Transplant type	Drugs and immunosuppression regimen	Animal survival time (days)	Cause of death	Study purpose
Oura et al. (2011) [52]	Cynomolgus	Cynomolgus	24	Allo	Dead under 48 h (10) No (3) ASKP1240 (every 3 days) (4) ASKP1240 (Weekly) (7)	< 2 4,6,7 11 90 188 209 19 31 98 168 272 278 1035	Sever ischemic reperfusion (9), bleeding (1) Acute rejection Biliary Leakage Chronic rejection Chronic rejection Chronic rejection Ischemic reperfusion injury Ischemic reperfusion injury Liver abscess Liver abscess Cholangitis Cholangitis Had been alive until the last follow-up	Undertaken to explore the efficacy and safety profile of ASKP-1240, a fully human anti-CD40 mAb, in the context of liver transplantation. The findings conclusively demonstrated ASKP-1240's immunosuppressive potential without causing a serious side effect, establishing it as a formidable agent in liver transplantation protocols.
Kim et al. (2017) [53]	Cynomolgus (1) Rhesus (5)	Cynomolgus (1) Rhesus (5)	6	Allo	None (2) Tacrolimus, Rapamycin, Cs (4)	5, 6 0 5 52 84	Acute cellular rejection Hepatic vein obstruction Euthanized due to gastrointestinal infection Acute cellular rejection **Had been alive until the last follow-up**	To investigate surgical techniques of liver allotransplantation in nonhuman primates as a powerful method for basic and prehuman research, demonstrating that the procedure for allogeneic orthotopic liver transplantation in a nonhuman primate model closely resembles that of conventional deceased donor liver transplantation in humans.

(Continues)

TABLE 2 | (Continued)

Author/ year	Donor type (*n*)	Recipient species (*n*)	Number	Transplant type	Drugs and immunosuppression regimen	Animal survival time (days)	Cause of death	Study purpose
Kato et al. (2017) [54]	Cynomolgus	Cynomolgus	5	Allo	CsA, MMF, Cs	0 2 11 22 23	Euthanized due to hypotension during surgery Euthanized due to anuria Hemodynamic collapse from IVC thrombosis Euthanized at the end point of the study Euthanized at the end point of the study	To investigate surgical H-shunt venovenous bypass techniques for liver allotransplantation in nonhuman primates (cynomolgus) as one of the most widely used animals in prehuman studies, demonstrating success with long-term posttransplant survival rate.
Hong et al. (2021) [55]	Rhesus	Rhesus	11	Allo	None (2) Cs, Tacrolimus, Sirolimus (4) MD-3, Cs, Tacrolimus, Sirolimus (5)	5, 6 36 52 62 66 5 96 216 412 730	Graft Dysfunction du to acute T cell mediated rejection Diarrhea [a] Graft Dysfunction due to T cell mediated rejection Graft Dysfunction due to T cell mediated rejection Cholestasis Sepsis with diarrhea[a] Diarrhea[a] Cholestasis Graft dysfunction with ascites Euthanized at the end point of the study	Undertaken to investigate the efficacy and safety profile of a chimeric anti-ICAM-1 monoclonal antibody (MD-3) in the context of liver transplantation. The findings conclusively demonstrated that short therapy with MD-3 improved liver allograft survival to 2 years without the maintenance of an immunosuppressant.

Abbreviations: Allo, allotransplantation; ALS, anti-lymphocyte serum; ATG, anti-thymocyte globulin; AZA, azathioprine; Cs, corticosteroid; CsA, cyclosporin; CVF, cobra venom factor; CyP, cyclophosphamide; MD-3, chimeric anti-ICAM-1 monoclonal antibody; MMF, mycophenolate mofetil; NHP, nonhuman primate; RTX, rituximab.

[a]Diarrhea happened as an adverse drug reaction. ASKP1240: a fully human anti-CD40 monoclonal antibody.

TABLE 3 | Studies reporting liver Xeno and allotransplantation at the same time.

Author/ year	Transplant type	Donor type (*n*)	Recipient species (*n*)	Number	Immunosuppression and other therapies	Animal survival time (days)	Cause of death	Major findings and insights
Calne et al. (1970) [56]	Orthotopic-Xeno	WT	Rhesus (3) Chimpanzee (1)	4	ALS None	0.5 0.5 0.5 0.33	Bleeding Bleeding Bleeding Bleeding	Using anti-Lymphocyte serum in combination with human fibrinogen and aminocaproic acid. Demonstrating coagulation dysregulation as the predominant barrier.
	Allo	Rhesus (6) Cynomolgus (3)	Rhesus	9	None ALS	1.5 2.5 7 8 15 248 0.79 3.5 20	Peritonitis Hemorrhage Killed, Moribund due to cellular rejection Hemorrhage Pneumonia Cholangitis with jaundice Bleeding Bleeding Portal phlebitis with multi liver abscess	Using liver allotransplantations as control groups for xenotransplantation.
Y Luo et al. (1998) [29]	Orthotopic-Xeno Heterotopic-Xeno	WT	Rhesus (6) Baboon (2)	8	Non (3) CsA, Cs, Dashen (3) CsA, CyP, Cs	0.08, 0.08, 0.08 0.08,0.08,0.23 0.08, 0.08	All dead due to acute cellular and early vascular rejection and fibrosis	Comparing orthotopic and heterotopic LXT demonstrated that recipients who underwent heterotopic LXT had longer survival time.

(Continues)

TABLE 3 | (Continued)

Author/ year	Transplant type	Donor type (*n*)	Recipient species (*n*)	Number	Immunosuppression and other therapies	Animal survival time (days)	Cause of death	Major findings and insights
	Allo	Baboon (8) Vervet Monkey (14)	Baboon	22	CsA, CyP, Cs	0 0 6 16 35 35 42 62 2 6 10 20 35 60 60 123 0 0 0 28 38 45	Postoperative hypovolemic shock Hemorrhage Hypovolemic shock and multi organ failure **Euthanized electively** Euthanized due to cholangitis **Euthanized electively** Euthanized due to acute rejection **Euthanized electively** Cerebral embolus Hemorrhage Hemorrhage Sepsis **Euthanized electively** **Euthanized electively** **Euthanized electively** **Euthanized electively** Hemorrhage Hemorrhage Hemorrhage Euthanized due to weight loss and weakness Euthanized due to weight loss and weakness Euthanized due to weight loss and weakness	Liver concordant and discordant NHP-allotransplantations were used as control groups for xenotransplantation groups to investigate histopathology or rejection.

(Continues)

TABLE 3 | (Continued)

Author/ year	Transplant type	Donor type (*n*)	Recipient species (*n*)	Number	Immunosuppression and other therapies	Animal survival time (days)	Cause of death	Major findings and insights
Ekser et al. (2010) [31]	Orthotopic-Xeno	WT (1) GTKO (2) GTKO/hCD46 (8)	Baboon	11	Non Cs, MMF, ATG, Tacrolimus Cs, MMF, ATG, Tacrolimus CyP, Cs, MMF, Tacrolimus, CVF CyP, Cs, MMF, Tacrolimus	0.21 0.13 6 0.13 0.83 1 4 5 6 6 7	Euthanized due to bleeding Size mismatch Sever bleeding in Abdomen and lung Size mismatch Primary non function of the liver Primary non function of the liver Sepsis Sever bleeding in abdomen and lung Sever bleeding in abdomen Sever bleeding in abdomen, small intestine, pericardial and myocardium Sever bleeding in abdomen and lung	Using GTKO pigs for the **first** time. Considering profound thrombocytopenia developed within an hour after liver reperfusion, diffuse spontaneous hemorrhage, and TMA as the main limiting survival factors. Suggesting LXT as a bridge to allotransplantation.
	Allo	Baboon	Baboon	1	Cs, MMF, ATG, Tacrolimus, CVF	30	Euthanized due to weakness or weight loss	Using liver allotransplantations as control groups for xenotransplantation.

Abbreviations: Allo, allotransplantation; AHLXT, auxiliary/heterotopic liver xenotransplantation, ALS, anti-lymphocyte serum; ATG, anti-thymocyte globulin; AZA, azathioprine; Cs, corticosteroid; CsA, cyclosporin; CVF, cobra venom factor; CyP, cyclophosphamide; LXT, liver xenotransplantation; MMF, mycophenolate mofetil; NHP, nonhuman primate; TMA, thrombotic microangiopathy; WT, wild type, Xeno, xenotransplantation.

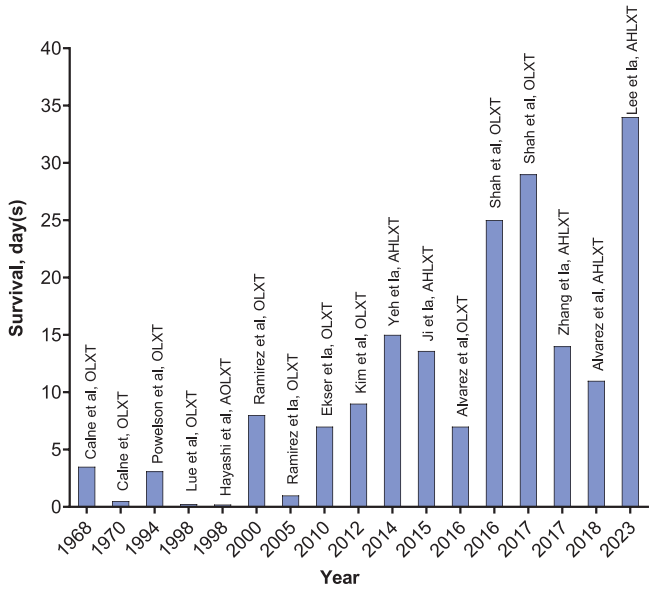

FIGURE 2 | Evolution of survival rates of pig-to-NHP liver xenotransplant recipients. AHLXT, auxiliary/heterotopic liver xenotransplantation; NHP, nonhuman primates; OLXT, orthotopic liver xenotransplantation.

transplants had an approximately five times higher chance of failing compared to allogeneic ones. The median survival time exhibited an expected difference between WT pigs (0.2 days) and GM pigs (6 days; $p < 0.001$) (Table 4, Figure 3B). Using WT pigs also thirtyfold the risk of failure in the xenogeneic group. When comparing implantation techniques, orthotopic implantations remained more challenging with inferior survival compared to auxiliary implantations (median survival: 1 day vs. 6 days; $p < 0.001$) (Table 4, Figure 3C). The orthotopic transplant had doubled the chance of failure compared to the use of an auxiliary technique transplant. Additionally, transplants without co-stimulation or complement blockade, or without B and/or T-cell depletion, had 2.5, 3.8, and 7.2 times higher chances of failure, respectively (Table 4, Figure 4).

Survival analysis of allotransplants stratified for species types revealed some disparities in survival rates (Figure S1).

We further stratified NHP causes of death with a subsequent differentiation based on donor/recipient types and implantation types (Table S3). Across all cases, the main causes of death were bleeding (25.1%), liver failure (16.2%), and rejection (15.8%). In the NHP-to-NHP group, the predominant causes of death included rejection (22.3%), liver failure (17.7%), bleeding (14.9%), infection (12.9%), thrombosis (4.6%), respiratory problems (2.7%), multi-organ failure (1.8%), and other reasons (12.0%), including weakness, cerebral embolus, and adverse drug effects. Notably, 12.0% of all NHP-to-NHP cases were alive until the end of the experiment or euthanized electively. In the pig-to-NHP groups, causes of death were attributed to bleeding (36.8%), liver failure (14.7%), respiratory issues (14.7%), rejection (8.4%), multiple organ failure (7.4%), thrombosis (6.3%), and infection (4.3%), and other reasons (7.4%). As anticipated, animals receiving WT pig livers exhibited a significantly higher mortality rate due to rejection (23.5% vs. 0%) and bleeding (50.0% vs. 29.5%), albeit with a lower incidence of mortality attributed to thrombosis (0% vs. 9.8%). Furthermore, the auxiliary/heterotopic surgical approach was associated with a heightened risk of mortality from respiratory complications compared to the orthotopic implantation (45.8% vs. 4.2%). However, bleeding was substantially lower with the auxiliary/heterotopic implantation (16.7% vs. 43.7%). None of the monkeys in the xenotransplant group were electively euthanized.

4 | Discussion

The advent of gene-editing technology [16, 17] and the advancement in immunosuppression therapies [57] have allowed several teams to reach extended xenograft survival duration. For instance, heterotopic pig-to-NHP heart transplants have shown survival periods of up to 945 days, life-supporting heart transplants have reached 264 days [17], and kidney xenotransplantation has extended survival to 758 days [16]. This progress has pushed some groups to move forward with clinical xenotransplantation [18–21, 23–26]. GM pig hearts have been transplanted in two patients at the University of Maryland [18, 19], and GM pig kidneys have been transplanted in patients with end-stage kidney disease at MGH [58] and New York University (NYU) [59]. Prior to that, pig organs were transplanted in decedents at NYU (hearts and kidneys) [20–22] and the University of Alabama Birmingham

TABLE 4 | NHP median survival time and hazard ratio compared among different organ type and immunosuppression groups.

Groups	Median survival time (days)	Hazard ratio	95% CI	*p*-value
Overall comparison				
Xenotransplants versus allotransplants	3 versus 20	5.1	3.5–7.5	< 0.001
Xenotransplants				
Wild-type versus genetically modified pig donors	0.2 versus 6	30.5	14.6–63.8	< 0.001
Orthotopic versus auxiliary implantation	1 versus 6	2.1	1.3–3.3	< 0.001
Without versus with co-stimulation blockade	0.8 versus 7	2.5	1.6–4.0	< 0.001
Without versus with B/T Cell depletion	0.3 versus 5	3.8	2.0–7.1	< 0.001
Without versus with complement blockade	0.5 versus 6	7.2	4.2–12.3	< 0.001

Abbreviation: CI, confidence interval; NHP, nonhuman primates.

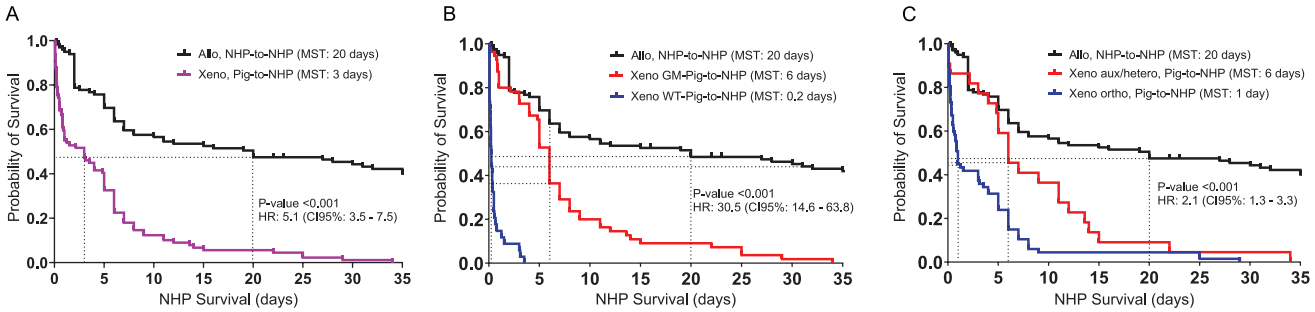

FIGURE 3 | Survival following liver transplantation in NHPs stratified for (A) allo- versus xenotransplantation, (B) wild-type pigs versus genetically modified pigs versus allotransplantation, and (C) surgical method (orthotopic versus auxiliary/heterotopic) versus allotransplantation. Allo, allotransplantation; Aux/hetero, auxiliary/heterotopic; GM, genetically modified; NHP, nonhuman primate; Xeno, xenotransplantation.

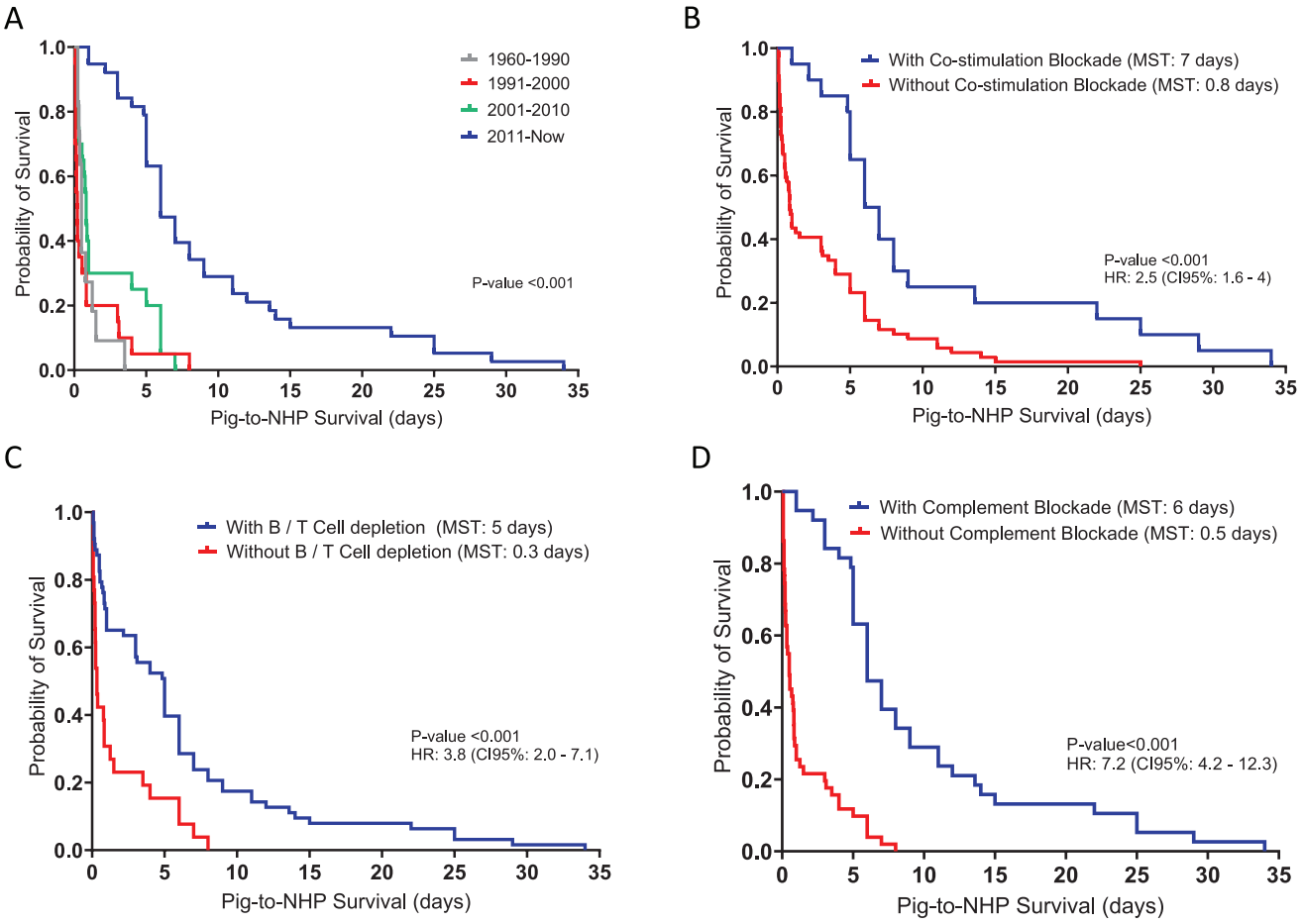

FIGURE 4 | Survival following pig-to-NHP liver xenotransplantation stratified for (A) different era, (B) with versus without using co-stimulation blockade (anti-CD40 mAb, and anti-CD154 mAb), (C) with versus without B/T cell depletion, and (D) with versus without complement blockade (e.g., cobra venom factor). CI, confidence interval; HR, hazard ratio, MST, median survival time; NHP, nonhuman primate.

(kidneys) [23–26]. With regards to the liver, a historical initial attempt was reported in a patient in the 90s, with an auxiliary pig liver graft implanted in a patient with fulminant hepatic failure. The graft function for 36 h, bile production, and lactate clearance were observed, but no neurological improvements were noted [34]. Despite preemptive plasmapheresis and ex vivo perfusion of pig kidneys that removed 90% of the xeno-antibodies, the graft sustained profound antibody-mediated rejection. Ex vivo WT pig liver perfusion via extracorporeal machine perfusion devices was attempted at Duke [36] and Nebraska [35] in 1994, involving 4 and 8 patients, respectively. Some were successfully bridged to transplantation. In 2000, Levy et al. successfully bridged two patients using CD55/CD59 modified livers [37]. However, it became evident at that time that more preclinical work needed to be done, and various groups continued NHP experiments.

Since then, and over the last few decades, a significant amount of preclinical data and achievements have been accumulated (Tables 1 and 3). Although improvement has been observed in the recent decade (Figure 4A), allowed by a more comprehensive

immunosuppression regimen (Figures 4B,C and D) and advanced gene editing in donors, the maximum survival is limited to about 1-month.

We summarize here "all" pig-to-NPH liver xenotransplants performed to date and provide a comparative analysis of the results to guide further experiments and potential further clinical attempts. We report aggregated survival rates, causes of death, and immunosuppressive protocols, exploring variations across donor types and implantation approaches. We highlight the advances provided by (i) genetic modifications (30 times less NHP death), (ii) enhanced immunosuppression (e.g., 2.5-fold less NHP death with co-stimulation blockade), and (iii) auxiliary/heterotopic liver xenotransplantation implantation (2 times less NHP death). The profound impact of genetic modifications was achieved by simple, double, and triple alpha 1,3-galactosyltransferase gene-knockouts and key addition of human transgenes, resulting in a near complete disappearance of hyperacute rejections [27, 31–33, 43–49]. Co-stimulation and complement blockade further prolonged the survival of xenografts [27, 31–33, 44–47, 49]. The improved survival allowed by auxiliary and heterotopic surgical implantations is attributed to the persistence of the native liver, which remains functional alongside the transplanted xenograft, providing relief in performing essential detoxification and synthetic tasks and potentially playing a role in modulating the immune response [60, 61].

The auxiliary/heterotopic approach reduces bleeding risk by preserving native liver hemostatic functions and mitigating coagulopathy and thrombocytopenia. However, increased abdominal pressure from the additional graft may impair diaphragmatic excursion, exacerbating respiratory complications, especially under inflammatory and fluid-shift conditions.

We report median and maximal survival times for the different groups and note that only a small proportion of liver xenotransplants reach 1-month of survival. We also emphasize the inherent limitations of NHP models, as exemplified by the results obtained in the NHP-to-NHP allotransplant model, which we used as a comparative group. The survival of this latter group remains largely inferior to allotransplantation in humans (i.e., > 98% graft survival at 1-month), further highlighting that experimental liver allo and xenotransplantation in NHPs is only an approximate surrogate for clinical studies, prompting cautious evaluation of results and conclusions. Despite significant strides in donor gene editing and immunosuppressive regimens, liver xenotransplant survival rates still lag far behind those of allotransplants. Notably, we highlighted that bleeding remains the predominant cause of failure, underscoring coagulation dysregulation as a critical barrier.

The fact that the liver is the only organ for which the physiology cannot be artificially replaced to sustain life makes it both essential and difficult to replace [62]. Xenotransplantation seems to be the most promising approach to support deficient liver function in patients [33]. However, the physiological differences between human and pig liver make this undertaking difficult to achieve compared to other organs, such as the heart or the kidneys. The liver produces most of the circulating proteins (e.g., albumin, cholesterol transport proteins), the complement (e.g., C3), and coagulation proteins (prothrombin), and it is not yet fully understood if those function correctly in humans. Detoxification of drugs, conversion of ammonia, and metabolic functions (e.g., conversion of T4) are also essential functions for which it is still not clear if those adequately translate between species. Given all these differences, it is not surprising to see that the survival of liver xenografts is limited to 1-month [63, 64]. In our analysis, the primary barrier restricting liver xenotransplant survival was uncontrolled bleeding provoked by severe thrombocytopenia and coagulation dysregulation in more than one-third of the NHPs, as initially suggested by Calne et al. [41, 56]. Postxenotransplantation thrombocytopenia has multifactorial causes. It could occur due to excessive platelet activation and aggregation and/or aberrant platelet sequestration and phagocytosis. Porcine von Willebrand factors (vWF), liver sinusoidal endothelial cells (LSECs), and porcine Kupffer cells play key roles in that process. Porcine vWF binds tighter to the human platelet's GpIb receptor because of increased O-linked glycosylation, which leads to platelet degranulation, formatting platelet plugs, and platelet consumption, respectively [65]. A solution could be the interruption of the platelet activation and aggregation pathway by using either an anti-GpIb antibody in combination with desmopressin [66], an anti-GpIIb/IIIa antibody [67], or expression of human vWF in the porcine liver [68]. Another target could be LSECs scavenger receptors, such as ASGR-1. These receptors mediate phagocytosis by binding to platelet B1-4-N-acetylglucosamine glycoprotein on human platelets [69]. Eliminating this glycoprotein by using asialofetuin could reduce species-discordant platelet consumption [70]. Similarly, blocking actions (e.g., siRNA, antibodies, genetic modifications) could target CD18, CD40, and SIRP-α/CD47 receptors on Kupffer cells, thereby reducing platelet phagocytosis and sequestration [71–73]. The other critical barrier is coagulation dysregulation, which results in the constitutive activation of the extrinsic station cascade, even in the absence of immunological evidence of hyperacute rejection. This coagulation dysregulation originates from incompatibilities between the donor's tissue factor pathway inhibitor (TFPI) and the recipient's tissue factor (TF) [46] and/or thrombin-thrombomodulin complex [74]. To address this issue, GM pigs expressing human TFPI and thrombomodulin have been designed, and supplementation with human concentrate can be done [63]. Overall, it appears that GM pig livers are appropriate to support at least part of the function of a human native liver, such as bile detoxification and part of protein production. Some critical functions linked to coagulation seem to be difficult to replace yet. Considering the latter, some patients with extensive liver resection (leaving less than 20% remnant liver) could benefit from partial auxiliary support with a GM pig liver and adequate immunosuppression. This approach was taken in the 71-year-old patient who received an auxiliary liver graft in China and who, to our knowledge, > 1-month posttransplant [6]. To enhance survival outcomes and improve the translational relevance of liver xenotransplantation studies, several steps can be taken. Future studies should integrate further genetic modifications in donor pigs, such as modifications involving Gal or non-Gal deletions, complement regulation, cellular immune response, anticoagulation, anti-inflammatory/anti-apoptotic response, infection control, and organ growth. Advanced immunosuppressive protocols will need to be tested, including those utilizing new generations of anti-CD154 mAb and anti-CD40 mAb, new anti-complement therapies, and other multimodal approaches to block the antibody response.

Addressing key challenges, such as coagulation dysregulation and platelet function, through incorporating human-compatible coagulation factors may further enhance graft survival and better approximate clinical applications in human liver transplant recipients. Our analysis highlights the significant survival benefits of auxiliary/heterotopic liver transplant techniques compared to orthotopic approaches, likely due to the retained functionality of the native liver. These techniques could serve as an effective interim solution for bridging patients to allotransplantation when immediate donor organs are unavailable, reducing the risk of immediate graft failure. Additionally, survival data suggest that cynomolgus monkeys exhibit better outcomes than rhesus monkeys, potentially due to minor MHC compatibility or procedural differences. Consequently, future liver xenotransplant studies may benefit from prioritizing cynomolgus monkeys to improve consistency and translatability, particularly when evaluating GM donor models.

Our study had limitations. The first limitation is the inevitable heterogeneity present across the different studies and the long inclusion period. Investigators used different species/genetic backgrounds for both donors and recipients, different immunosuppression protocols, and different implantation techniques. We accounted for the main differences and performed subgroup analyses; however, we acknowledge the impossibility of reconciling all variables. Nevertheless, the effect of major differences, such as the absence or presence of genetic modification, implantation techniques, allo versus xenotransplant models, and co-stimulation/complement blockade, as well as B and/or T-cell depletion, showed differences as expected. Second, our comparison group with NHP allotransplant is suboptimal because those studies were performed with various interventions and not with the intent to serve as 'optimal' allotransplant controls. Again, it is still reassuring to see that the differences between the xenotransplant group are as expected. Also, it confirms that the NHP allotransplant model is not perfect, with only 12% of the animals (13 out of 108 cases) reaching the study termination time point, suggesting that enhanced donor modifications and improved immunosuppressive strategies are necessary to increase survival rates and relevance in liver xenograft studies. Another potential issue is animal duplication, which is not infrequent; even though we took great care to avoid duplicated reports on the same animal in different publications by the same group, the occurrence of this possibility cannot be completely ruled out. Another point to consider, which is valid for nearly all large animal preclinical studies, is that animal recipients are healthy to start with, which does not fully mimic the situation of a chronically or acutely ill patient. Only studies performed in a clinical setting will be able to adequately address these questions. Again, the rational and risk/benefit ratio of performing those seems justified for dying patients with no other options.

In conclusion, while challenges such as liver protein compatibility with the host, thrombocytopenia, and coagulopathy persist, our study highlights the progressive enhancement of preclinical liver xenotransplantation outcomes. Genetic modifications, such as the incorporation of human-compatible coagulation regulators like thrombomodulin and TFPI, have been pivotal in addressing coagulopathy and improving graft function. Advances in immunosuppressive strategies, including next-generation co-stimulation blockade agents like nonthrombogenic anti-CD40L monoclonal antibodies, and procedural refinements in auxiliary implantation techniques have significantly extended survival, with animal models achieving survival beyond 1-month. Machine perfusion systems have become valuable platforms for testing genetic modifications and immune compatibility, facilitating rapid translational advancements [75, 76,]. Initial clinical compassionate studies are underway and will face unique challenges. However, they are expected to address critical unanswered scientific questions and pave the way for xenotransplantation as a viable bridge to allotransplantation or recovery.

Author Contributions

Raphael Meier designed the study. Kasra Shirini and Raphael Meier collected the data. Kasra Shirini and Raphael Meier analyzed the data. Kasra Shirini and Raphael Meier interpreted the data and wrote the manuscript. Kasra Shirini and Raphael Meier have full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request, though it was all collected from publicly available sources.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.