JOURNAL OF
EMERGING INVESTIGATORS

Article

The impact of genetic, drug, and procedural factors on cardiac xenograft survival days in non-human primates

Eric Zhang[1], Yongping Hao[1]
[1] Winston Churchill High School, Potomac, Maryland

SUMMARY

Each year, millions with heart failure go untreated due to a rapidly growing organ shortage, prompting researchers to explore cardiac xenotransplantation, animal-to-human heart transplants, as a potential solution. Different gene modifications, drug therapies, and procedures have been tested on primates in preclinical xenotransplant survival studies to mitigate immune activity and organ rejection. However, such studies examine only a few factors in isolated settings. A synthetic study combining individual trials is needed to simultaneously evaluate multiple key factors associated with xenograft survival in a broader context. We hypothesized that more genetic modifications to the donor organ, immunosuppressive regimens with calcineurin regulators, mTOR regulators, αCD154 antibodies, cobra venom factor (CVF), and prophylactic supportive drugs, and transplant procedures involving nonischemic heart preservation and heterotopic surgery all extend xenograft survival time. Preclinical data were compiled from published studies and analyzed using negative binomial mixed models. Overall, triple transgenic hearts significantly outperformed single and double transgenic genotype hearts. Calcineurin/rapamycin regulators increased survival, while αCD154 antibodies, CVF, and supportive drugs had insignificant effects. Heterotopic transplants and non-ischemic heart preservation extend survival compared to orthotopic transplants and static cold storage. By consolidating nearly two decades of research, these findings can serve as a reference for developing an optimal immunosuppression regimen for future human testing. Further research should investigate additional gene combinations, refine drug regimens and dosages, and optimize non-ischemic heart preservation and other transplant procedures. Accelerating the development of a working human xenotransplant model can limit unnecessary animal testing and end the organ shortage.

INTRODUCTION

Heart failure is a rapidly growing healthcare concern. In 2023, the number of heart failure diagnoses for U.S. adults was 6.7 million, a number which is expected to reach 8.5 million by 2030 (1). This has resulted in a continued increase in heart transplants, which remain the best treatment for end-stage heart failure (2). Unfortunately, the demand for heart transplants outpaces the available supply. Each year, over 3,000 patients remain on waitlists for a heart transplant (3). In 2021, a 38-year-old patient with rapidly progressing heart failure had to wait seven months before getting surgery (4). To address growing shortages and waiting times, researchers have been investigating xenotransplantation as an alternative to traditional heart transplantation.

Xenotransplantation is an emerging field studying the transfer of animal cells, tissues, and organs into humans. For decades, scientists have tried devising methods to prolong xenograft survival to match the efficacy of regular transplants (5). Regarding genetics, there is one main consideration: the genotype of the donor organ. By genetically engineering pig embryos, researchers can remove animal epitopes and insert genes that express human membrane proteins to mitigate the immune response (5). For example, the expression of the galactose epitope on porcine cells can be eliminated by using galactosyltransferase gene knockout (GTKO), greatly reducing hyperacute rejection. Similarly, inserting thrombomodulin (*TBM*), a gene encoding a protein responsible for regulating coagulation, and *CD46*, a gene which encodes a human complement regulatory protein, lowers the risk of blood clots and reduces the inflammatory response (6, 7). In this study, we choose to evaluate the effects of single-transgenic GTKO organs, double transgenic GTKO+*CD46* organs, and triple transgenic GTKO+*CD46*+*TBM* organs since these genotypes are the most well-documented and studied (5).

Regarding drugs, four main treatments have been used to modulate the immune response and mitigate external complications: calcineurin inhibitors and mTOR regulators (CNI/mTOR regulators), anti-CD40 (αCD40) or anti-CD154 (αCD154) monoclonal antibodies, cobra venom factor (CVF), and supportive drugs (5). CNI/mTOR regulators form immunophilin complexes with specific FKBP proteins. This disrupts downstream phosphorylation and interferes with the signaling and transcription of *IL2* and other cytokines important for T-cell activation (8). αCD40 and αCD154 interfere with the CD40-CD40L signaling pathway by blocking the interaction between CD40 and CD40L, which is crucial for T-cell activation and inflammatory responses (9). CVF is a C3b protein analog that releases anaphylatoxins to cause complement immune depletion (10). Supportive drugs typically consist of antimicrobials such as ganciclovir and cefazolin, which are administered prophylactically to mitigate the risk of opportunistic infections in post-transplantation (11). Epogen, another support drug, is also administered to combat anemia by stimulating red blood cell production (12). It is frequently administered in preclinical renal xenografts, where anemia is a persistent issue despite porcine erythropoietin production (13). Instead of regulating the immune system

https://doi.org/10.59720/24-299

directly, they serve as prophylactic treatments against post-transplant complications.

Regarding procedures, there are two main considerations: organ preservation and transplantation technique. When transferring the donor organ to the recipient, doctors must decide whether to use static cold storage (SCS) by keeping the organ chilled with ice or do non-ischemic heart preservation (NIHP) by actively perfusing the organ with an oxygenated preservation solution using an external device (5). Doctors must also decide whether to conduct an orthotopic xenotransplant (OXTx) or a heterotopic xenotransplant (HXTx). In OXTx, doctors remove the native heart entirely and attach the new donor heart, but in HXTx, doctors leave the native heart within the recipient and attach the donor organ in parallel (14). For over a decade, HXTx has been the predominant method for preclinical xenotransplantation studies, in part due to reduced costs, complexity, and increased safety if the donor organ is rejected (15).

Several meta-analyses have analyzed the associations between experimental factors like drug or gene combinations and survival time in lung and kidney xenotransplants (16, 17). However, to the best of our knowledge, no similar analysis has been carried out on heart xenografts. Additionally, recent developments in the literature on pig-to-primate heart transplantation mostly describe only a few factors in isolated settings (5). There is a need to conduct a comprehensive study to reflect the progress made, simultaneously examine multiple key factors associated with xenograft survival, and improve our understanding of xenograft rejection in a broader context.

Our study aims to evaluate the association between genetic, drug, and procedural factors and xenograft survival based on up-to-date experimental studies involving GTKO pig-to-primate heart transplants. Specifically, we tested three main hypotheses. In preclinical snapshot studies, both double and triple transgenic hearts significantly outperformed standard GTKO hearts, indicating a strong association with patient life expectancy (6, 7, 18). Therefore, our first hypothesis predicted that both triple transgenic GTKO+*CD46*+*TBM* genotype hearts and double transgenic GTKO+*CD46* genotype hearts would be associated with increased survival compared to standard GTKO pig hearts. Next, we observed that researchers frequently incorporated CNI/mTOR regulators, αCD40, and CVF into immunosuppression regimens in order to mitigate the risk of hyperacute and antibody-mediated rejection (19-21). Similarly, the administration of prophylactic support drugs is frequently done to mitigate the risk of post-transplant infections and anemia, thereby increasing patient survival time (12). Thus, for our second hypothesis, we expected CNI/mTOR regulators, αCD154, CVF, and prophylactic supportive drugs to be associated with longer xenograft survival compared to regimens that include αCD40 but lack other specific drug therapies. Finally, the literature suggests that HXTx is used more extensively and outperforms OXTx in preclinical cardiac xenotransplants, and NIHP has seen similarly impressive results despite being a relatively recent medical innovation (22-25). Therefore, we hypothesized that transplant procedures involving NIHP and HXTx would extend xenograft survival compared to procedures using ischemic heart preservation and orthotopic surgery.

Cardiac xenograft data were compiled from published studies and analyzed using a negative binomial mixed model (NBMM). We found that GTKO+*CD46*+*TBM* genotype hearts significantly outperformed GTKO and GTKO+*CD46* genotype hearts. CNI/mTOR regulators significantly increased survival, whereas αCD154 provided no advantage over αCD40. CVF and supportive drugs had negligible effects, and both HXTx and NIHP extended survival compared to OXTx and SCS respectively. Ultimately, understanding the relative efficacy of these major treatments can highlight optimal strategies for further enhancing xenotransplantation outcomes and guide the development of new strategic and scientific approaches.

RESULTS

We conducted a literature search in Google Scholar, PubMed, and ScienceDirect to compile heart xenograft survival data. Our analytical dataset comprised 116 trials and 15 studies from 2005 to 2024 (**Table 1**) (19, 22-24, 26-36). We evaluated seven factors: organ genotypes, CNI/mTOR regulators, monoclonal antibodies, CVF, supportive drugs, surgical techniques, and heart preservation techniques. We provided boxplot statistics for cardiac xenograft survival days for these seven genetic, drug, and procedural factors (**Figure 1**). The cardiac xenograft survival had a mean of 96.8 days, a median of 57.5 days and a range of 0 to 945 days. There was substantial variation in average survival days amongst subgroups within each factor included in this study, ranging from 34.8 to 133.5 days.

We constructed a NBMM for cardiac xenograft survival days to account for significant overdispersion in the data. Additionally, NBMMs are designed for count data and modeled with random effects to mitigate bias induced by drug regimen differences, time-variant variables, and other study-specific factors by accounting for the correlation between trials from the same study. This enabled us to generalize our findings and quantify relative effects on a larger scale.

Author(s), Year	Original Trial Count	Final Trial Count
Mohiuddin et al., 2014 (19)	n = 9	n = 9
Längin et al., 2018 (22)	n = 13	n = 13
Mohiuddin et al., 2014 (23)	n = 22	n = 22
Goerlich et al., 2021 (24)	n = 16	n = 5
Lee et al., 2018 (26)	n = 19	n = 15
Iwase et al., 2015 (27)	n = 7	n = 3
Brenner et al., 2017 (28)	n = 9	n = 9
Mohiuddin et al., 2014 (29)	n = 5	n = 5
Singh et al., 2019 (30)	n = 3	n = 3
Kuwaki et al., 2005 (31)	n = 10	n = 8
Ezzelarab et al., 2015 (32)	n = 15	n = 3
DiChiacchico et al., 2020 (33)	n = 6	n = 1
Singh et al., 2018 (34)	n = 13	n = 11
Park et al., 2021 (35)	n = 1	n = 1
Brenner et al., 2020 (36)	n = 8	n = 8

Table 1: Cardiac xenograft survival study list. 15 studies, ranging from 2005 to 2020, were screened for trial survival data. Trials with missing or inadequate survival data and trials with additional factors that could not be analyzed were removed from the final analytical dataset as outlined in the Materials and Methods section.

https://doi.org/10.59720/24-299

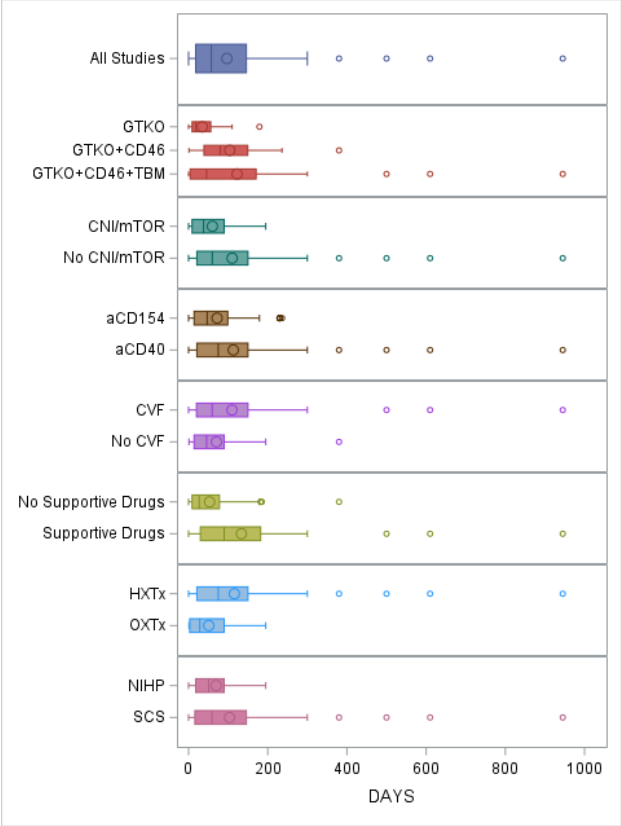

Figure 1: Boxplots for cardiac xenograft survival days by associated factors. Mins, Q1, medians, means, Q3, and maxes were reported graphically for all 116 trials analyzed in this study. Inside the box, the vertical bar represents the median, and the circle represents the mean. GTKO: galactosyltransferase gene knockout; TBM: thrombomodulin insertion; CNI/mTOR: calcineurin/rapamycin regulators; monoclonal antibodies: αCD154 and αCD40; CVF: cobra venom factor; supportive drugs: ganciclovir, cefazolin, epogen; HXTx: heterotopic xenotransplant; OXTx: orthotopic xenotransplant; NIHP: nonischemic heart preservation; SCS: static cold storage (19, 22-24, 26-36).

After running the NBMM, we summarized the statistical associations between seven genetic, drug, and procedural factors and cardiac xenograft survival days. Survival period extensions compared to reference groups were calculated by exponentiating regression coefficients (RC) since NBMMs are logarithmic. In the genetic group, triple-transgenic organs were associated with increased survival compared to single-transgenic GTKO organs (RC = 1.49, p = 0.011). This is equivalent to extending the survival period by a factor of 4.44. Double transgenic organs were only marginally significant (RC = 0.78, p = 0.080). In the drug group, CNI/mTOR regulators were strongly associated with survival (RC = 1.06, p = 0.021), the same as extending the survival period by 2.90 times. Using αCD154 instead of αCD40 provided no major advantage (RC = 0.14, p = 0.648). CVF (RC = 0.05, p = 0.959) and supportive drugs (RC = 0.38, p = 0.614) also did not significantly prolong the days survived. In the procedural group, HXTx significantly outperformed OXTx (RC = 2.20, p = 0.023), equivalent to extending the recipients' survival by 9.03 times. Compared to SCS, NIHP was also associated with survival (RC = 1.18, p = 0.006), equivalent to prolonging xenograft survival by a factor of 3.27.

DISCUSSION

In this study, we evaluated three main hypotheses concerning factors that influence cardiac xenograft survival. First, triple and double transgenic hearts were expected to outperform standard GTKO hearts. Second, the use of CNI/mTOR inhibitors, CVF, and supportive drugs was anticipated to enhance graft survival, with αCD154 predicted to be more effective than an αCD40-based regimen. Third, we hypothesized that NIHP and HXTx preservation strategies would improve survival outcomes compared to SCS and OXTx. Overall, we found that triple transgenic organs, CNI/mTOR regulators, HXTx, and NIHP were the most crucial factors for extending patient survival. Conversely, single and double transgenic organs, CVF, supportive drugs, OXTx, and SCS did not show a significant effect. While all trials used monoclonal antibodies, trials with αCD154 and αCD40 showed similar outcomes.

Triple transgenic organs with GTKO, *CD46*, and *TBM* gene edits significantly extended cardiac xenotransplant survival compared to the single transgenic GTKO organ. However, double transgenic organs with GTKO and *CD46* were only marginally significant. Our findings are generally consistent with literature. Individually, the insertion of human *TBM* and *CD46* have been observed to mitigate organ rejection (6, 7, 18). However, we speculate that GTKO and *CD46* organ gene modifications alone may not sufficiently counteract the immune response. Our reduced analysis, when other factors are not controlled for, showed that double transgenic organs with GTKO and *CD46* are significant predictors of survival. This might explain the preclinical success of *CD46* modified donor organs in a snapshot setting but indicates that even two gene edits of GTKO and *CD46* together are insufficient. Our findings support the transgenic strategy of incorporating multiple gene edits. This matches previously observed trends in lung xenotransplants, where xenotransplants with more gene edits roughly correlated to longer recipient survival times (17). Over the last 30 years, researchers have been experimenting with different gene combinations, including but not limited to *CD46*, *CD55*, *CD59*, *CD37*, GTKO, and *TBM*. Recent studies utilizing sextuple transgenic xenotransplants reported survival past nine months, which is an exponential increase in life expectancy (37). New insights gained from human and *ex vivo* trials can further our understanding of gene therapy in the context of preclinical and future clinical investigations.

For drug therapies, CNI/mTORs significantly increase cardiac xenotransplant survival. Literature on this matter is generally consistent with this observation. By itself, CNIs can induce nephrotoxicity, which is why they are almost always administered together with mTOR regulators (38). In monkey renal allografts studies, CNI/mTOR regulators such as tacrolimus and sirolimus, and corticosteroid-based immunosuppression regimens significantly prolonged survival (20). Furthermore, mTOR regulators have been shown to prevent overgrowth of the donor heart (22, 36). For these reasons, we believe these medications are an indispensable component of an optimized immunosuppression regimen for human trials. Future research should continue to optimize dosages and test the viability of other CNI/mTOR regulator combinations.

All cardiac xenograft trials contained monoclonal antibodies. However, using αCD154 did not significantly extend cardiac xenograft survival compared to using αCD40. Our

https://doi.org/10.59720/24-299

findings are unexpected as although both antibodies target the same mechanism, preclinical evidence appears to favor αCD154 for longer survival (19, 23, 39). However, previous reports have indicated that αCD154 usage is associated with thrombocytopenia, bleeding, and other complications absent in trials using αCD40 (19, 27). This suggests a tradeoff between overall survival time and health quality during the survival period. Future xenotransplantation studies should thoroughly compare the effects of αCD154 and αCD40 and investigate whether dual administration mitigates such health tradeoffs.

Unexpectedly, CVF did not significantly prolong cardiac xenograft survival. In rat and mouse models, CVF delayed host death by a few days to a week but did not prevent antibody-mediated rejection (21, 40, 41). We suspect that transgenic donor organs make complement depletion via CVF mostly redundant. Specifically, previous literature discussed how embedding human transgenes like *CD46* can disrupt the complement system by preventing C3b deposition, reducing B and T cell activity and preventing the formation of the membrane attack complex (42). This may explain why our results show no significance compared to significant prolongation observed in older animal studies with fewer organ gene modifications (41). Overall, we believe the effect of CVF in drug regimens is minimal when utilizing multigene-edited pig hearts. Researchers should instead focus on refining targeted antibody therapies, CNI/mTOR regulators treatments, and organ gene modifications to substantially improve recipient survival.

Unexpectedly, supportive drugs showed no significant impact on cardiac xenograft survival. Recent literature on these drugs in the context of xenotransplants is scarce. One study highlighted the reduced efficacy of ganciclovir, cefazolin, and similar prophylactic treatments in pig-to-baboon xenotransplants, further noting evidence of *in vitro* toxicity depending on the dosage (12). Epogen is not approved for human heart surgery by the Food and Drug Administration, and its unofficial use as a substitute for traditional blood transfusions has yielded mixed results (43). We suspect that using lab-raised animals and eliminating endogenous retroviruses via CRISPR technology have lowered overall infection risks, which might explain the statistically insignificant effect of prophylactic treatment (44). The effect of epogen on cardiac xenografts is still inconclusive and warrants further investigation. Future research is needed to further our understanding of epogen and antimicrobials in the context of recent xenotransplant advances.

Amongst transplantation procedures, HXTx significantly prolonged xenograft survival compared to OXTx. This finding is generally consistent with literature. OXTx was observed to be associated with early perioperative cardiac xenograft dysfunction in previous studies (15). In our view, currently established HXTx models are safer than OXTx alternatives in preclinical settings where the native recipient heart is still functional. However, this does not mean OXTx models should be discarded. An OXTx approach using multi-gene edited pig hearts has yielded a survival period of up to nine months (37). Additionally, OXTx has been the standard for human procedures, so an optimized orthotopic model would be more transferable from primate to human trials. In contrast, heterotopic heart transplants have only been performed in a handful of human cases worldwide (25). Furthermore, there may be cases where the native heart must be removed, making HXTx impossible. As we move into clinical trials, the HXTx and OXTx procedures should both be considered and refined.

Finally, NIHP significantly improved xenograft survival compared to SCS. Several reasons may explain this. All NIHP-containing studies used an 8°C preservation solution and a portable heart-lung machine to mimic *in vivo* physiological conditions to minimize ischemia (13,16, 18). This has led to substantially less perioperative cardiac xenograft dysfunction compared to SCS trials (13,16, 18). In human-to-human trials, NIHP showed better heart function than SCS based on several metrics such as less mitochondrial dysfunction and reduced loss of cardiomyocytes (45, 46). NIHP might be critical in extending survival, reducing complications, and maintaining stronger organ function. Further research may focus on refining and exploring the effectiveness of certain electrolyte concentrations within preservation solutions and engineering more reliable portable heart-lung machines to optimize a NIHP model.

There are several limitations in this study. First, we did not adjust for variations in humane endpoints. In all trials, subjects were either euthanized during sudden health emergencies and organ rejection or died naturally due to common complications such as hyperacute rejection and infection. To the best of our knowledge, the difference between outcomes with and without researcher intervention was negligible. Second, we could not directly assess the effect of drug dosages due to inconsistent intervals of administration and unreported quantities in literature. However, drug combination and dosage differences were controlled by study-level random effects. Third, only xenograft survival days were analyzed. which may not necessarily indicate normal or improved organ function. Fourth, pig and primate age, gender, weight, and similar characteristics were not considered. Fifth, we did not differentiate between induction, maintenance, or additive regimens when constructing the data set for drug factors. Sixth, differences in preservation solutions among NIHP trials from different studies were not considered. Finally, the associations shown do not guarantee causality between factors and survival.

Cardiac xenotransplantation can be a powerful tool to eventually end the organ shortage. Before clinical trials, further inquiry is needed to fully understand and optimize the genetic, drug-related, and procedural components in pig-to-primate preclinical transplants. This study used secondary data from 15 individual snapshot studies (116 trials) to identify key factors associated with xenograft survival on a larger scale. We found that accounting for other factors, triple transgenic organs, HXTx, NIHP, and CNI/mTOR regulators were essential for prolonging xenograft survival. We suggest researchers continue testing different genotype combinations with single transgenic organs as a baseline. Our findings also indicate that either αCD154 or αCD40 can provide effective immune blockade. Furthermore, we observed that CVF and supportive drugs had a statistically insignificant impact compared to other key genes, drug regimens, and procedural variables. These findings summarize nearly two decades of preclinical cardiac xenotransplantation research and can help optimize future orthotopic and heterotopic xenotransplant approaches in upcoming human survival studies. Further studies should continue testing combinations with additional

https://doi.org/10.59720/24-299

untested factors. Incorporating such trials may offer new insights, specifically into unobserved confounding factors. Additionally, optimal drug dosages can be quantified by testing trials with the same drug regimens but with dosages varying by specific intervals. Ultimately, these findings may accelerate the development of a working human xenotransplantation procedure which could limit unnecessary testing, animal suffering, and end-organ shortage.

MATERIALS AND METHODS

Study and Data Selection

The initial selection consisted of studies utilizing GTKO donor hearts and primate recipients. The selection was limited to English literature published before June 2nd, 2024. Google Scholar, PubMed, and Science Direct were used as the primary search engines. We used variations of keywords such as "GTKO", "GT-KO", "heart", "cardiac xenotransplantation", "survival", "xenograft survival" and "pig to primate" and screened all abstracts and full texts if available. Individual trials were excluded in this step if they did not use GTKO organs, did not reference cardiac or heart grafts, or encountered surgical errors. Survival time was recorded as the number of days after initial xenograft implantation before organ failure, rejection, removal, or primate death. In cases where only the mean survival time was reported for repeated experiments, multiple trials with the mean survival time were logged. This resulted in the identification of 193 trials (24 studies) (**Figure 2**).

The data were then rearranged in Excel for the factors of interest: gene modifications (GTKO, GTKO+*CD46*, GTKO+*CD46*+*TBM*), CNI/mTOR, monoclonal antibodies (αCD40, αCD154), CVF, supportive drugs, surgical procedures (HXTx, OXTx), and heart preservation techniques (SCS, NIHP). Trials using CNI and mTOR regulators were grouped because of frequent dual administration and similar mechanisms (5,31). Other factors were also grouped during this step but could not be analyzed due to limited data, namely having fewer than 15 trials across at least 2 studies. Factors present in all trials from one study were controlled as described in the statistical analysis portion. All studies and trials that contained factors with limited counts, showed collinearity, contained incomplete drug regimens, or did not have adequate survival data were removed. The final analytical dataset comprised 116 trials from 15 studies (**Figure 2, Table 1**).

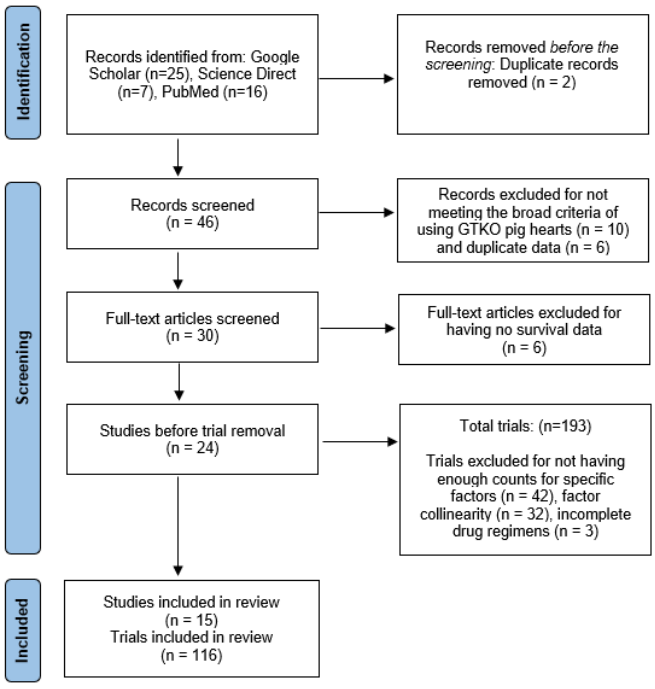

Figure 2: Modified PRISMA flow diagram of literature search on cardiac xenograft survival. The diagram outlines all procedures to construct the analytical dataset consisting of 116 trials from 15 studies. Trials were excluded if there were fewer than 15 trials across at least two studies for a specific factor.

Statistical Analysis

Poisson regression and other generalized linear models are useful and computationally efficient methods for individual patient survival analysis (47). A Poisson regression was initially used to evaluate the association between primate survival days after xenotransplant and genetic, drug, and procedural factors. Descriptive analysis showed that survival days data exhibited significant overdispersion, meaning the variance far exceeded the mean. To account for this, an NBMM was used to fit the data (48). NBMMs are generalized linear models that have previously been used to model over-dispersed count data with additional random effects (48). In our model, study-level random effects were implemented to mitigate external noise from drug regimen differences, time-variant variables, and other study-specific factors by accounting for the correlation between trials from the same study. This was an appropriate assumption given that the same authors oversaw such trials, used the same procedures, and had the same laboratory conditions.

The Pearson chi-square statistic test for overdispersion was used to confirm that the NBMM was more appropriate than a Poisson mixed model. The subgroup with the largest number of trials was used as the reference group for each factor. Additional subset analyses were done to interrogate explanatory factors individually. $p < 0.05$ indicated statistical significance, and $p < 0.10$ indicated marginal significance. All statistical analyses were done in R software version 4.3.2 with MASS and lme4 packages (49, 50).

Received: September 2, 2024
Accepted: December 6, 2024
Published: June 13, 2025

REFERENCES

1. Bozkurt, Biykem, et al. "Heart Failure Epidemiology and Outcomes Statistics: A Report of the Heart Failure Society of America." *Journal of Cardiac Failure*, vol. 29, no. 10, Oct. 2023, pp. 1412-51. https://doi.org/10.1016/j.cardfail.2023.07.006.
2. Westerdahl, Daniel E and Jon A. Kobashigawa. "Heart Transplantation for Advanced Heart Failure." *Cardiac Intensive Care,* 2019, pp. 504–24. https://doi.org/10.1016/B978-0-323-52993-8.00048-5.
3. "Organ Donation Statistics." *Health Resources and Services Administration*. https://www.organdonor.gov/learn/organ-donation-statistics. Accessed 13 Sept. 2024.
4. Maltes, Sérgio, et al. "Challenges of Organ Shortage

for Heart Transplant: Surviving Amidst the Chaos of Long Waiting Times." *Transplantation Direct*, vol. 7, no. 3, March 2021, p. e671. https://doi.org/10.1097/TXD.0000000000001122.
5. Cooper, David K.C. and Richard N. Pierson, 3rd. "Milestones on the Path to Clinical Pig Organ Xenotransplantation." *American Journal of Transplantation*, vol. 23, no. 3, March 2023, pp. 326-35. https://doi.org/10.1016/j.ajt.2022.12.023.
6. Cowan, Peter J. and Simon C. Robson. "Progress Towards Overcoming Coagulopathy and Hemostatic Dysfunction Associated with Xenotransplantation." *International Journal of Surgery*, vol. 23, no. Pt B, Nov. 2015, pp. 296-300. https://doi.org/10.1016/j.ijsu.2015.07.682.
7. Loveland, Bruce E., et al. "Characterization of a Cd46 Transgenic Pig and Protection of Transgenic Kidneys against Hyperacute Rejection in Non-Immunosuppressed Baboons." *Xenotransplantation*, vol. 11, no. 2, 12 Feb. 2004, pp. 171-83. https://doi.org/10.1046/j.1399-3089.2003.00103.x
8. Jewett, Anahid and Han-Ching Tseng. "Immunotherapy." *Pharmacology and Therapeutics for Dentistry*, Elsevier, 2017, pp. 504-29. https://doi.org/10.1016/b978-0-323-39307-2.00035-7.
9. Zhang, Tianshu, et al. "Update on CD40 and CD154 Blockade in Transplant Models" *Immunotherapy*, vol. 7, no. 8, Aug. 2015, pp. 899-991, https://doi.org/10.2217/IMT.15.54.
10. Vogel, Carl-Wilhelm and David Fritzinger. "Cobra Venom Factor: The Unique Component of Cobra Venom That Activates the Complement System." *Snake Venoms*, Springer, 29 Jan. 2017, pp. 345-404. https://doi.org/10.1007/978-94-007-6410-1_4.
11. Mueller, Nicolas J., et al. "Reduced Efficacy of Ganciclovir against Porcine and Baboon Cytomegalovirus in Pig-to-Baboon Xenotransplantation." *American Journal of Transplantation*, vol. 3, no. 9, Sept. 2003, pp. 1057-64. https://doi.org/10.1034/j.1600-6143.2003.00192.x.
12. Perico, Norberto, et al. "Xenotransplantation in the 21st Century." *Blood Purification*, vol. 20, no. 1, 17 Jan. 2002, pp. 45-54. https://doi.org/10.1159/000046985.
13. D'Ambra, Michael N. " Commentary: We Need to Know More About Erythropoietin." *JTCVS Techniques*, vol. 4, Dec. 2020, pp. 158-59. https://doi.org/10.1016/j.xjtc.2020.07.024.
14. Goerlich, Corbin E., et al. "Heterotopic Porcine Cardiac Xenotransplantation in the Intra-Abdominal Position in a Non-Human Primate Model." *Nature Scientific Reports*, vol. 10, no. 1, 01 July 2020, p. 10709. https://doi.org/10.1038/s41598-020-66430-x.
15. Byrne, Guerard W., et al. "Changes in Cardiac Gene Expression after Pig-to-Primate Orthotopic Xenotransplantation." *Xenotransplantation*, vol. 18, no. 1, 22 Feb 2011, pp. 14-27. https://doi.org/10.1111/j.1399-3089.2010.00620.x.
16. Firl, Daniel J. and James F. Markmann. "Measuring Success in Pig to Non-Human-Primate Renal Xenotransplantation: Systematic Review and Comparative Outcomes Analysis of 1051 Life-Sustaining Nhp Renal Allo- and Xeno-Transplants." *American Journal of Transplantation*, vol. 22, no. 6, June 2022, pp. 1527-36. https://doi.org/10.1111/ajt.16994.
17. Harris, Donald G., et al. "Meta-Analysis of the Independent and Cumulative Effects of Multiple Genetic Modifications on Pig Lung Xenograft Performance During Ex Vivo Perfusion with Human Blood." *Xenotransplantation*, vol. 22, no. 2, 02 Dec. 2014, pp. 102-11. https://doi.org/10.1111/xen.12149.
18. Janeway, Charles A., et al. "Immunobiology: The Immune System in Health and Disease: Appendix Ii. Cd Antigens." 5th ed., Garland Science, 2019.
19. Mohiuddin, Muhammad. M., et al. "Role of Anti-Cd40 Antibody-Mediated Costimulation Blockade on Non-Gal Antibody Production and Heterotopic Cardiac Xenograft Survival in a Gtko.Hcd46tg Pig-to-Baboon Model." *Xenotransplantation*, vol. 21, no. 1, 29 Oct. 2013, pp. 35-45. https://doi.org/10.1111/xen.12066.
20. Qi, Shijie, et al. "Effect of Tacrolimus (Fk506) and Sirolimus (Rapamycin) Mono- and Combination Therapy in Prolongation of Renal Allograft Survival in the Monkey." *Transplantation*, vol. 69, no. 7, 15 April 2000, pp. 1275-83. https://doi.org/10.1097/00007890-200004150-00012.
21. Barker, Roger A., et al. "A Role for Complement in the Rejection of Porcine Ventral Mesencephalic Xenografts in a Rat Model of Parkinson's Disease." *Journal of Neuroscience*, vol. 20, no. 9, 01 May 2000, pp. 3415-24. https://doi.org/10.1523/JNEUROSCI.20-09-03415.2000.
22. Langin, Matthias, et al. "Consistent Success in Life-Supporting Porcine Cardiac Xenotransplantation." *Nature*, vol. 564, no. 7736, 05 Dec. 2018, pp. 430-33. https://doi.org/10.1038/s41586-018-0765-z.
23. Mohiuddin, Muhammad M., et al. "Genetically Engineered Pigs and Target-Specific Immunomodulation Provide Significant Graft Survival and Hope for Clinical Cardiac Xenotransplantation." *Journal of Thoracic Cardiovascular Surgery*, vol. 148, no. 3, Sept. 2014, pp. 1106-13; discussion 13-4. https://doi.org/10.1016/j.jtcvs.2014.06.002.
24. Goerlich, Corbin E., et al. "Blood Cardioplegia Induction, Perfusion Storage and Graft Dysfunction in Cardiac Xenotransplantation." *Frontiers in Immunology*, vol. 12, 08 June 2021, p. 667093. https://doi.org/10.3389/fimmu.2021.667093.
25. Ahmed, Talha and Ankin Jain. "Heart Transplantation." *StatPearls Publishing LLC*. www.ncbi.nlm.nih.gov/books/NBK557571/. Accessed 13 Sept. 2024.
26. Lee, Sung-Jun, et al. "Seven Years of Experiences of Preclinical Experiments of Xeno-Heart Transplantation of Pig to Non-Human Primate (Cynomolgus Monkey)." *Transplant Proceedings*, vol. 50, no. 4, May 2018, pp. 1167-71. https://doi.org/10.1016/j.transproceed.2018.01.041.
27. Iwase, Hayato, et al. "Pig-to-Baboon Heterotopic Heart Transplantation--Exploratory Preliminary Experience with Pigs Transgenic for Human Thrombomodulin and Comparison of Three Costimulation Blockade-Based Regimens." *Xenotransplantation*, vol. 22, no. 3, 03 April 2015, pp. 211-20. https://doi.org/10.1111/xen.12167.
28. Brenner, Poalo, et al. "40 Days Survival after Orthotopic Cardiac Xenotransplantation of Multi-Transgenic Pig Hearts in a Pig-to-Baboon Model with Cd40mab or Cd40l Costimulation Blockade and Xenograft Preservation Using "Steens" Cold Blood Cardioplegia Perfusion." *Transplantation*, vol. 101, no. (5S-3), May 2017, p. S65. https://doi.org/10.1097/01.tp.0000520401.22958.4c.

https://doi.org/10.59720/24-299

29. Mohiuddin, Muhammad. M., "One-Year Heterotopic Cardiac Xenograft Survival in a Pig to Baboon Model." *American Journal of Transplantation*, vol. 14, no. 2, Feb. 2014, pp. 488-9. https://doi.org/10.1111/ajt.12562.
30. Singh, Avneesh K., et al. "Cardiac Xenografts Show Reduced Survival in the Absence of Transgenic Human Thrombomodulin Expression in Donor Pigs." *Xenotransplantation*, vol. 26, no. 2, 05 Oct. 2018, p. e12465. https://doi.org/10.1111/xen.12465.
31. Kuwaki, Kenji, et al. "Heart Transplantation in Baboons Using Alpha1,3-Galactosyltransferase Gene-Knockout Pigs as Donors: Initial Experience." *Nature Medicine*, vol. 11, no. 1, 26 Dec. 2004, pp. 29-31. https://doi.org/10.1038/nm1171.
32. Ezzelarab, Mohamed B., et al. "Systemic Inflammation in Xenograft Recipients Precedes Activation of Coagulation." *Xenotransplantation*, vol. 22, no. 1, 11 Sept. 2014, pp. 32-47. https://doi.org/10.1111/xen.12133.
33. DiChiacchio, Laura, et al. "Early Experience with Preclinical Perioperative Cardiac Xenograft Dysfunction in a Single Program." *Annals of Thoracic Surgery*, vol. 109, no. 5, May 2020, pp. 1357-61. https://doi.org/10.1016/j.athoracsur.2019.08.090.
34. Singh, Avneesh K., et al. "Cd4+Cd25(Hi)Foxp3+Regulatory T Cells in Long-Term Cardiac Xenotransplantation." *Xenotransplantation*, vol. 25, no. 2, 17 Dec. 2017, p. e12379. https://doi.org/10.1111/xen.12379.
35. Park, Min Y., et al. "Comprehensive Analysis of Cardiac Xeno-Graft Unveils Rejection Mechanisms." *International Journal of Molecular Sciences*, vol. 22, no. 2, 13 Jan. 2021, https://doi.org/10.3390/ijms22020751.
36. Brenner, Poalo, et al. "Worldwide First Successful and Reproducible Long-Term Survival up to Half a Year: Completed Preclinical Study with Life-Supporting Orthotopic Pig-to-Baboon Cardiac Xenotransplantation (Oxhtx) Fulfilling the Ishlt Prerequisite for Clinical Cardiac Xenotransplantation." *The Journal of Heart and Lung Transplantation*, vol. 39, no. 4, April 2020. https://doi.org/10.1016/j.healun.2020.01.1129.
37. Mohiuddin, Muhammad M., et al. "Progressive Genetic Modifications of Porcine Cardiac Xenografts Extend Survival to 9 Months." *Xenotransplantation*, vol. 29, no. 3, 31 March 2022, p. e12744, Medline, https://doi.org/10.1111/xen.12744.
38. Park, Jeong-Ik and Bo Hyun Jung. "Future-Directed Studies on Immunosuppressive Treatments and Xenografts for Organ Transplantation." *Annals of Liver Transplantation*, vol. 2, no. 1, 31 May 2022, pp. 1-7, https://doi.org/10.52604/alt.22.0003.
39. Perrin, Steven and Marianne Magill. "The Inhibition of Cd40/Cd154 Costimulatory Signaling in the Prevention of Renal Transplant Rejection in Nonhuman Primates: A Systematic Review and Meta Analysis." *Frontiers in Immunology*, vol. 13, 06 April 2022, pp. 861471. https://doi.org/10.3389/fimmu.2022.861471.
40. Mohiuddin, Muhammad M., et al. "Experiments in Cardiac Xenotransplantation. Response to Intrathymic Xenogeneic Cells and Intravenous Cobra Venom Factor." *Journal of Thoracic Cardiovascular Surg*ery, vol. 106, no. 4, October 1993, pp. 632-635.
41. Oh, Joo Y., et al. "Complement Depletion with Cobra Venom Factor Delays Acute Cell-Mediated Rejection in Pig-to-Mouse Corneal Xenotransplantation." *Xenotransplantation*, vol. 17, no. 2, 13 April 2010, pp. 140-6. https://doi.org/10.1111/j.1399-3089.2010.00574.x.
42. Sun, Qin, et al. "Cutting Edge of Genetically Modified Pigs Targeting Complement Activation for Xenotransplantation." Frontiers in Immunology, vol. 15, April 2024, https://doi.org/10.3389/fimmu.2024.1383936.
43. Fishman, James A. "Infection in Xenotransplantation: Opportunities and Challenges." *Current Opinion in Organ Transplantation*, vol. 24, no. 5, Oct. 2019, pp. 527-34. https://doi.org/10.1097/MOT.0000000000000682.
44. Flécher, Erwan, et al. "Heterotopic Heart Transplantation: Where Do We Stand?" European *Journal of Cardio-Thoracic Surgery*, vol. 44, no. 2, 13 March 2013, pp. 201-06. https://doi.org/10.1093/ejcts/ezt136.
45. Pradegan, Nicola, et al. "Nonischemic Donor Heart Preservation: New Milestone in Heart Transplantation History." *ASAIO Journal*, vol. 69, no. 8, Aug. 2023, pp. 725-33. https://doi.org/10.1097/MAT.0000000000002001.
46. Wang, Lan, et al. "(189) Non-Ischaemic Heart Preservation to Improve Donor Heart Quality." *The Journal of Heart and Lung Transplantation*, vol. 42, no. 4, April 2023, pp. S93-S94. https://doi.org/10.1016/j.healun.2023.02.205.
47. Crowther, Michael J., et al. "Individual Patient Data Meta-Analysis of Survival Data Using Poisson Regression Models." *BMC Medical Research Methodology*, vol. 12, 23 March 2012, p. 34. https://doi.org/10.1186/1471-2288-12-34.
48. Yirga, Ashenafi A., et al. "Negative Binomial Mixed Models for Analyzing Longitudinal Cd4 Count Data." *Nature Scientific Reports*, vol. 10, no. 1, 07 Oct. 2020, p. 16742. https://doi.org/10.1038/s41598-020-73883-7.
49. Bates, Douglas, et al. "Fitting Linear Mixed-Effects Models Using Lme4." *Journal of Statistical Software*, vol. 67, no. 1, 7 Oct. 2015, pp. 1-48. https://doi.org/10.18637/jss.v067.i01.
50. "R: A Language and Environment for Statistical Computing." *R Foundation for Statistical Computing*. https://www.R-project.org/. Accessed 13 Sept. 2024.

https://doi.org/10.59720/24-299

APPENDIX

R Code Repository

```
#Set a working file directory to import Excel data into R.
getwd()
setwd("C:/Users/ericz/OneDrive/Desktop/R4DataAnalysis")
getwd()

#Import the dataset for analysis.
xenodata <- read.csv("Xeno2.csv", header=T)
summary(xenodata)

# Install required packages for running a standard Negative Binomial regression model and a negative binomial mixed model.
require(MASS)
require(lme4)

# Define and run the Pearson chi-square test for the overdispersion parameter
overdisp_fun <- function(model) {
    rdf <- df.residual(model)
    rp <- residuals(model,type="pearson")
    Pearson.chisq <- sum(rp^2)
    prat <- Pearson.chisq/rdf
    pval <- pchisq(Pearson.chisq, df=rdf, lower.tail=FALSE)
    c(chisq=Pearson.chisq,ratio=prat,rdf=rdf,p=pval)
}

#This is the code for constructing the standard Poisson regression model.
m_poi0<-glm(formula=DAYS~factor(CYK2)+factor(GN4)+factor(ISCH2)+factor(PCD2)+factor(MCSR)+factor(CVF2)+factor(CD
3),family="poisson", data=xenodata)
overdisp_fun(m_poi0)
summary(m_poi0)

#This is the code for testing a Poisson mixed model with study-level random effects.
m_poi1<-glmer(formula=DAYS~factor(CYK2)+factor(GN4)+factor(ISCH2)+factor(PCD2)+factor(MCSR)+factor(CVF2)+factor(C
D3)+(1|STUDY1),family="poisson",data=xenodata)
overdisp_fun(m_poi1)
summary(m_poi1)

#This is the code for a negative binomial mixed model with study-level random effects.
m_nb1<-glmer.nb(formula=DAYS~factor(CYK2)+factor(GN4)+factor(ISCH2)+factor(PCD2)+factor(MCSR)+factor(CVF2)+facto
r(CD3)+(1|STUDY1),data=xenodata)
overdisp_fun(m_nb1)
summary(m_nb1)

#This outputs regression coefficients, standard errors, and ps into Excel.
co<-summary(m_nb1)$coefficients[,1]
se<-summary(m_nb1)$coefficients[,2]
pvalue<-summary(m_nb1)$coefficients[,4]
est <- cbind(Estimate = round(co,3), round(se,3), round(pvalue,3))
write.csv(est, "m_nb1.csv")
exp_co<-exp(co)
exp_se<-exp(se)
est_exp <- cbind(Estimate = round(exp_co,3), round(exp_se,3), round(pvalue,3))
write.csv(est_exp, "m_nb1exp.csv")
```