Extended Survival of 9- and 10-Gene Edited Pig Heart Xenografts with Ischemia Minimization and CD154 Costimulation Blockade –Based Immunosuppression

I. Ileka[1], R. Chaban[1], K. Kinoshita[1], G. McGrath[1], Z. Habibabady[1], A. Calhoun[1], A. Maenaka[1], M. Ma[1], V. Diaz[1] ,M. Dufault[1], L. Burdorf[2], W. Eyestone[2], K. Whitworth[3], D.K.C. Cooper[1] R.N. Pierson III[1]

[1]Center for Transplantation Sciences, Massachusetts General Hospital and Harvard Medical School, Boston, MA
[2]Revivicor, Inc., Blacksburg, VA
[3]National Swine Resource and Research Center (NSRRC), Columbia, MO

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Relevant Disclosures

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- *I. Ileka is supported by T32 5T32 AI007529-24*
- *Gene edited pigs were provided by Revivicor and NSRRC (NIH grant U42OD011140)*
- *Tonix Pharmaceuticals provided TNX-1500*, a humanized, Fc-modified, dimeric anti-CD154 mAb*
- *This work was supported by NIH grants UO1 AI153612 (Pierson), U19 AI090959 (Cooper), and sponsored research agreements with Tonix Pharmaceuticals*

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* TNX-1500 is an investigational new biologics and is not approved for any indication

Background

- Gene-edited (GE) pigs for Xenotransplantation.
  - Remove CHO antigen targets of preformed Ab
    - TKO (Gal-1,3-αGal, Neu5Gc, β4Gal)
  - Add human regulatory molecules
    - Complement: CD46, CD55, CD59
    - Coagulation: TBM, EPCR, TFPI
  - Add human anti-inflammatory ‘transgenes’
    - CD47, HLA-E/β2μg, HO-1, A20, CD39

Background

- We evaluated hearts from multi-GE pigs in baboon transplants treated with a novel costimulation-based immunosuppressive regimen and cold-perfused ‘ischemia minimization’.

Methods

- Current study:
  - 3-, 9-, or 10-GE pig hearts
  - novel costimulation-based immunosuppressive regimen
  - cold-perfused storage technique designed to minimize graft ischemia.
- Eight baboons recipients received heterotopic heart transplants using Steen's cold-perfusion ischemia minimization
  - 3 Reference pig hearts (Ntl. Swine Resource & Research Ctr: NSRRC)
    - 3-GE pigs (n=3): GTKO.β4GALNT2KO.hCD55
  - 5 Multi-GE pig hearts (Revivicor)
    - 9-GE pigs (n=3): GalKO.β4GalNT2KO.GHRKO.hCD46.hCD55.hTBM.hEPCR.hCD47.hHO-1
    - 10-GE pigs (n=2): GalKO.β4GalNT2KO.GHRKO.CD46.CD55.hTBM.hEPCR.hCD47.hHO-1.CMAHKO

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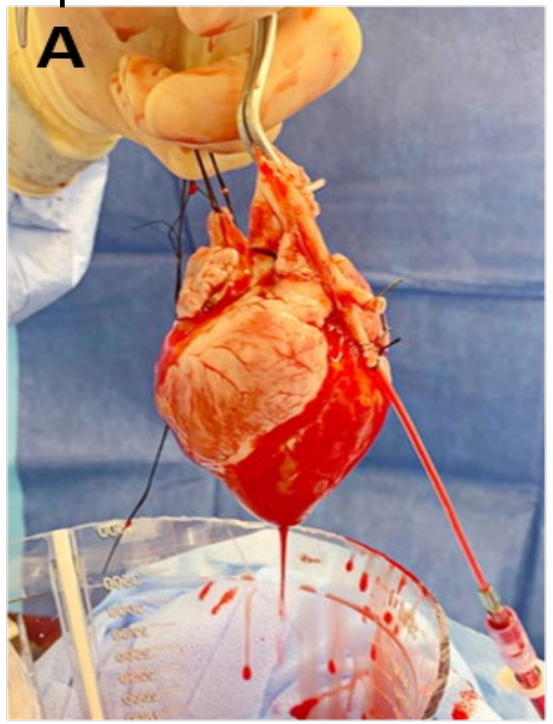
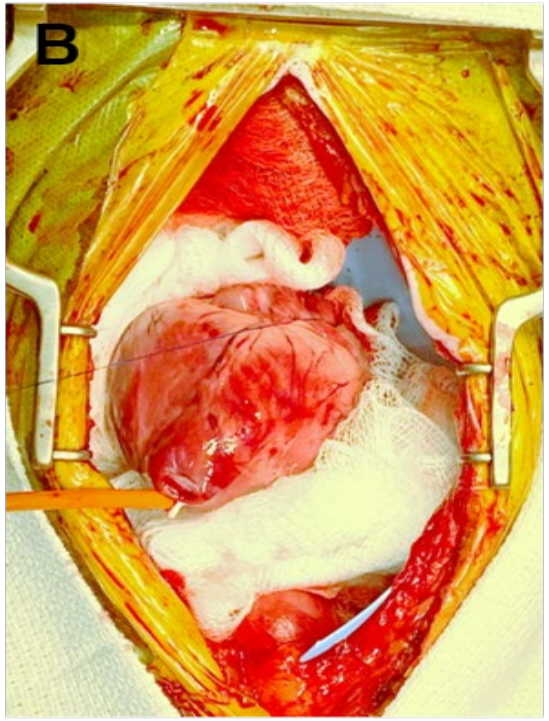
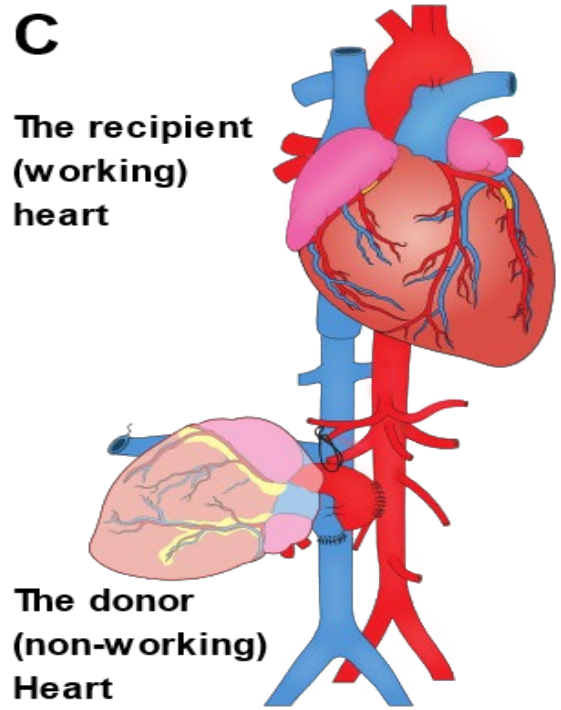
Methods: Heterotopic Heart Transplantation

- Standard UW cold perfusate.
- Ischemia minimization: Steen method
  - battery-powered portable perfusion circuit
  - 4°C Steen Solution
  - Protocol 2-hr perfusion
- Heterotopic abdominal xenograft transplant







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Methods: Steen Ischemia Minimization

- Steen: buffered extracellular solution (laboratory-made)
- 240ml of washed human RBCs; 760 ml Steen solution
- Aortic perfusion at 4^{o}C, 40-50 mmHg, immersed in reservoir; LV vent

Methods: Recipient Immunosuppresive Treatment Regimen

- Induction Therapy
  - Antithymocyte Globulin (ATG)
  - αCD20
- Day of Surgery Therapy
  - Thromboxane inhibitor (BIA)
  - TNF Inhibitor (Etanercept)
  - Interleukin 6 inhibitor receptor blocker (Tocilizumab)
- Maintenance Therapy
  - αCD154 (TNX-1500), Mycophenolate Mofetil(MMF), Corticosteroids
  - Interleukin 6 inhibitor receptor blocker (Tocilizumab)

Results: Xenograft survival

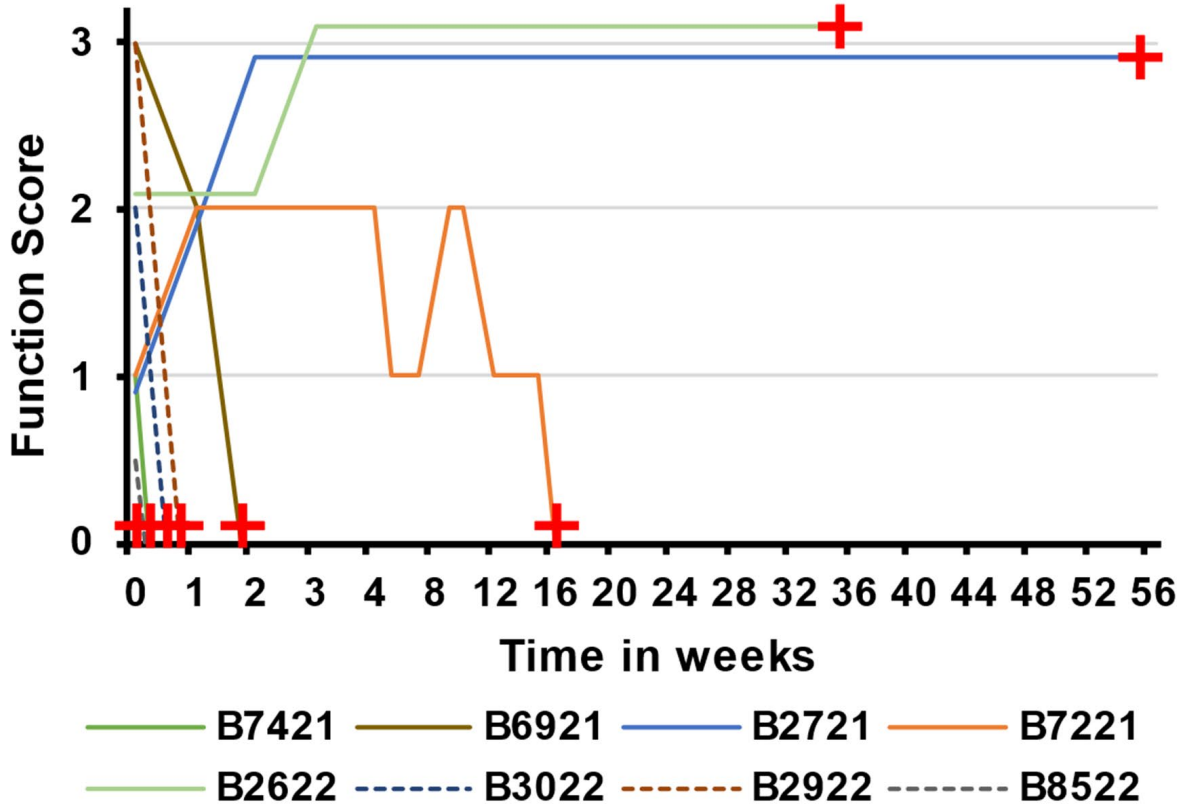
- 3-GE grafts functioned well initially.
  - POD 0: Refractory ventricular fibrillation. No AMR
  - POD 3: Graft necrosis, rupture: AMR
  - POD 5: Graft necrosis, rupture: AMR
- 9-GE
  - POD 0: Refractory Ventricular fibrillation
  - POD 13: AMR (strongly positive preop crossmatch)
  - POD 393: Preserved function, normal myocardium (protocol biopsies): slowly progressive graft hypertrophy
- 10-GE
  - POD 113: Combined CMR, AMR; progressive hypertrophy
  - POD 243: Preserved function, normal myocardium (protocol biopsies); slowly progressive hypertrophy

Results: Summary

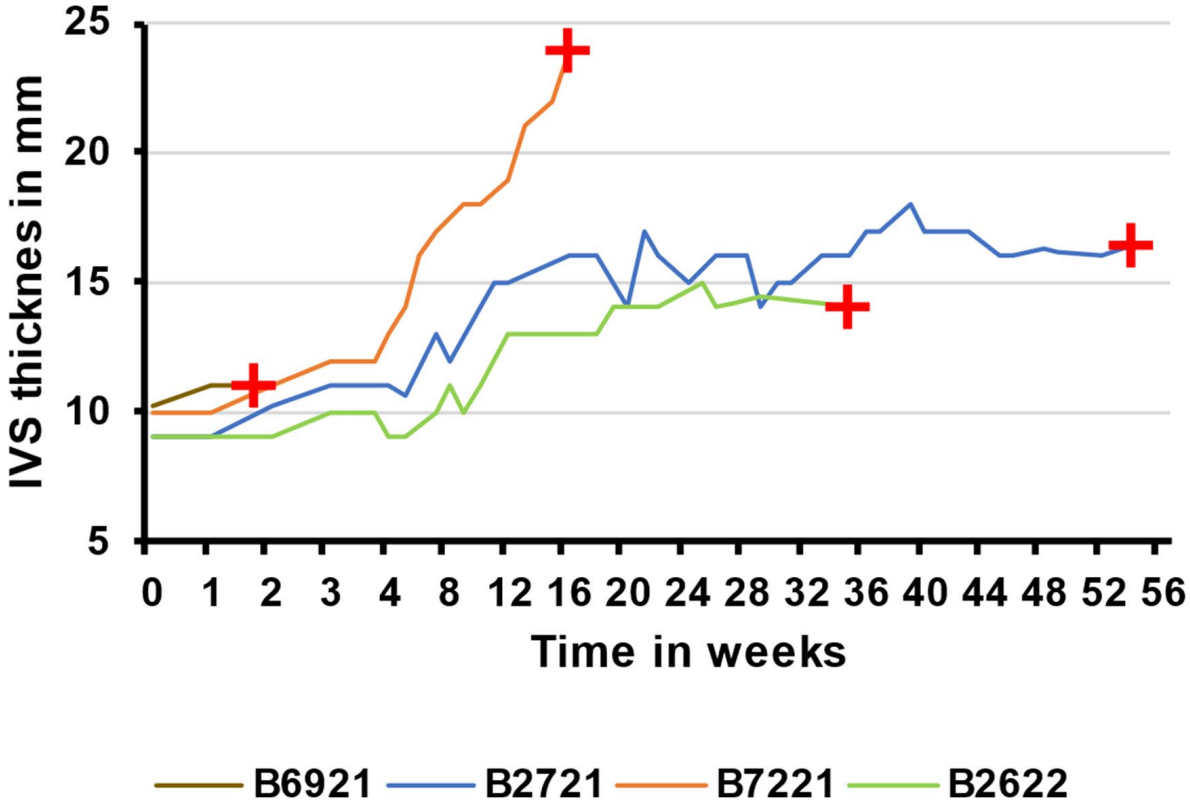
Animal ID	GE	Anticoagulation	Major complication	Graft Survival (Days)	Final Biopsy
B3022	3G	Heparin IV	Graft rupture	3	Antibody mediated rejection
B2922	3G	Heparin IV	Graft rupture	5	Antibody mediated rejection
B8522	3G	Heparin IV	Refractory Ventricular fibrillation	0	No evidence of rejection
B7421	9G	No	Refractory Ventricular fibrillation	0	Antibody mediated rejection
B2721	9G	Heparin IV for 10 days	None	393	No evidence of rejection
B6921	9G	Heparin IV for 10 days	None	13	Antibody mediated rejection
B7221	10G	Heparin IV for 8 days, then heparin sc for 2 weeks, ASA po daily	None	113	Antibody mediated rejection (Grade 3), Acute cellular rejection (3R)
B2622	10G	Heparin IV for 8 days, then heparin sc continue, ASA po daily	None	243	No evidence of rejection

Results: Graft Survival, Function, and Morphology

A) Graft Contractility and Survival



B) Interventricular Septum (IVS)



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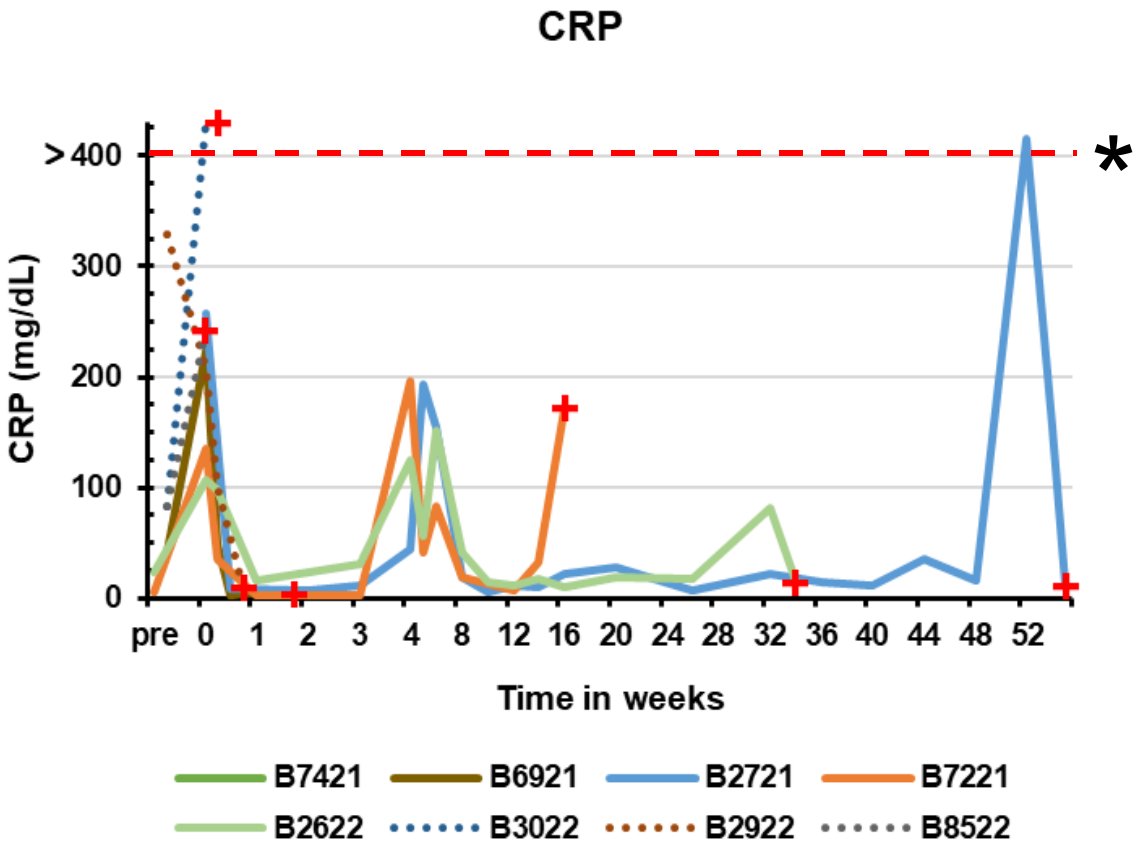
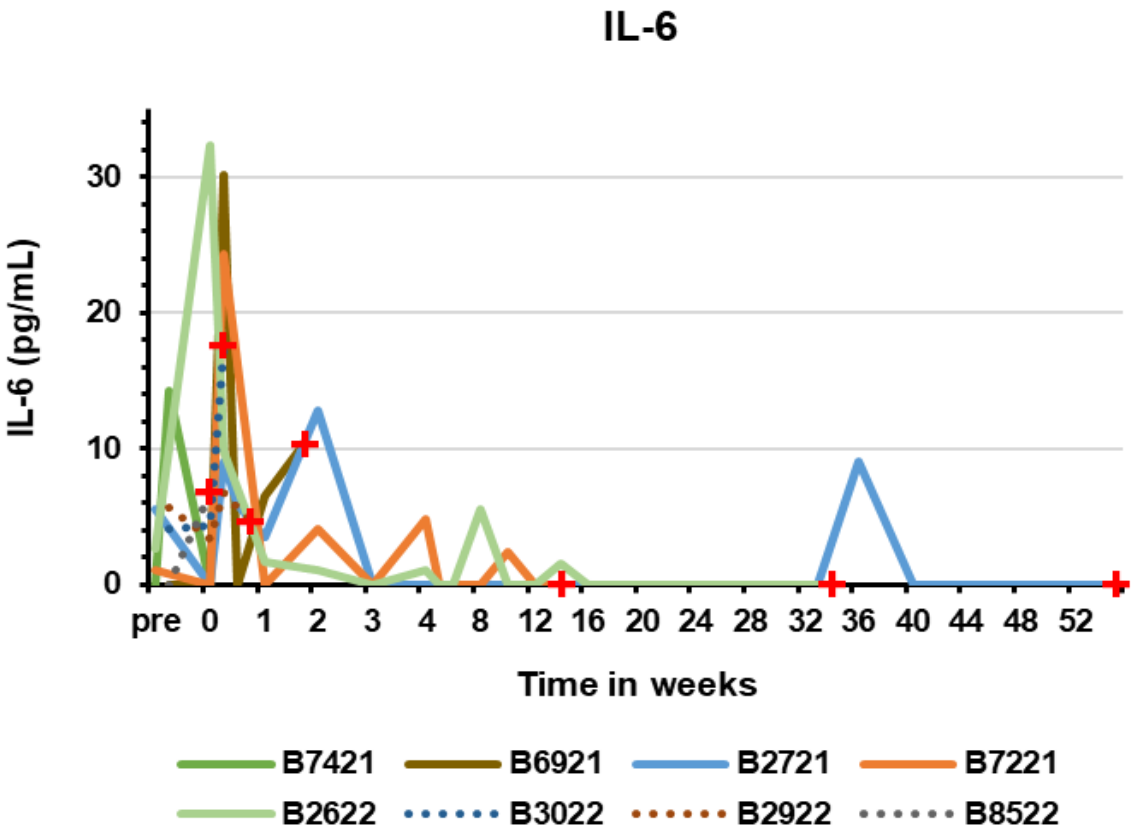
Results: CRP, IL-6





*Dashed lined is the upper limit of the CRP assay

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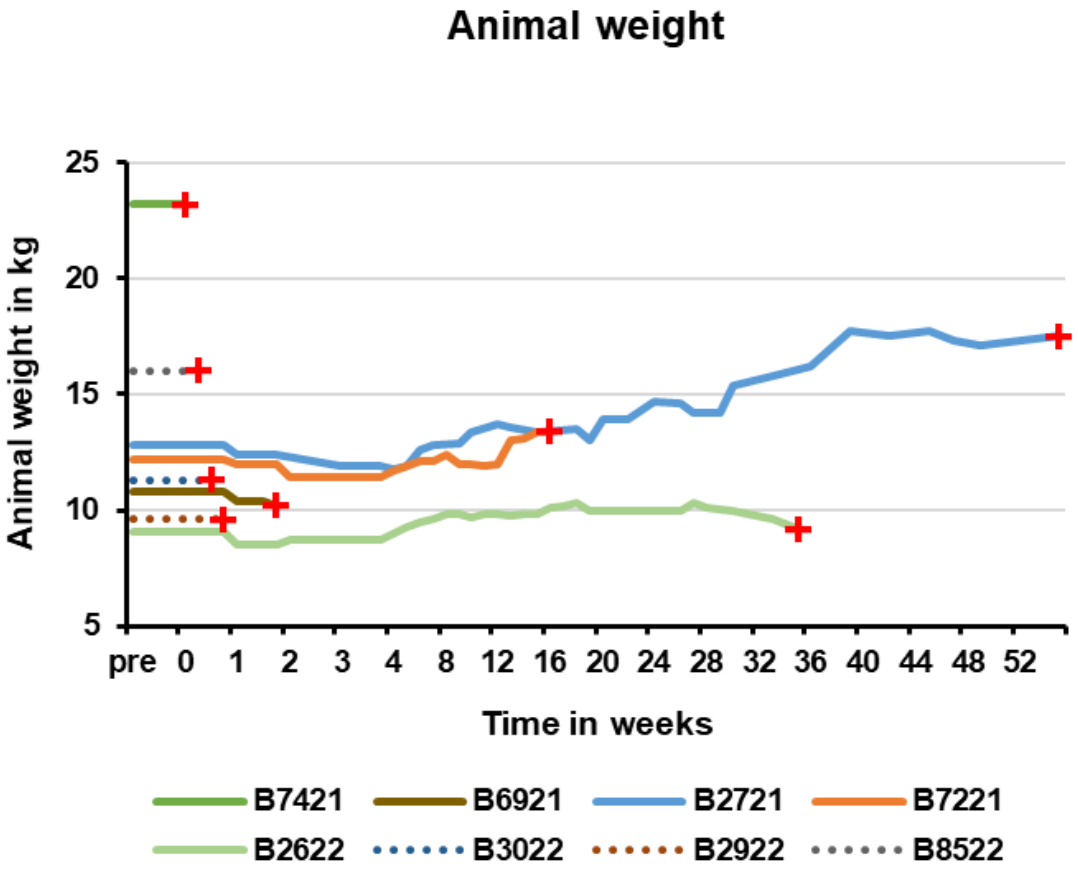
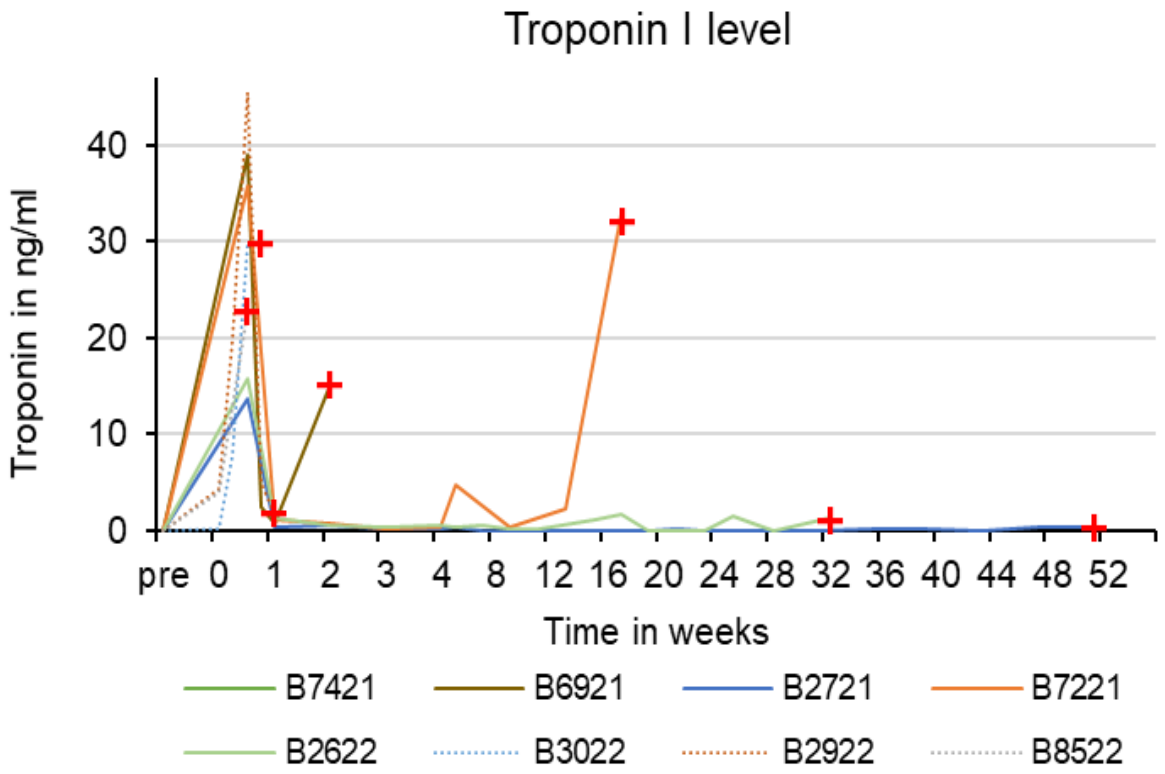
Results: Clinical condition, cardiac injury





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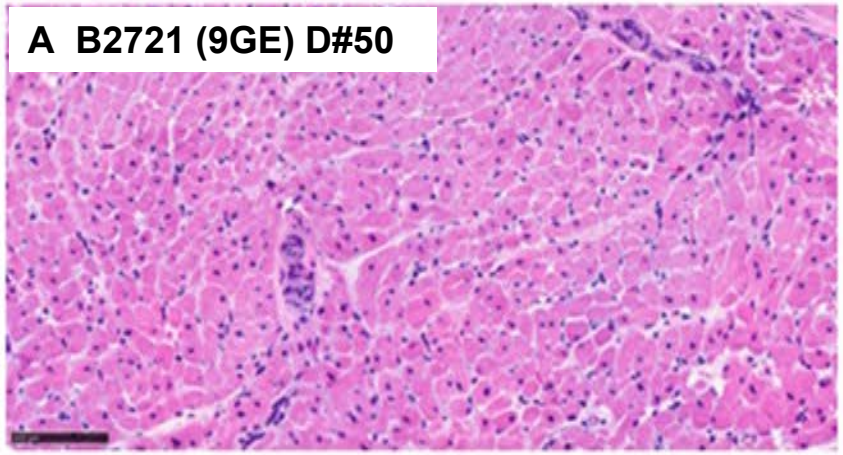
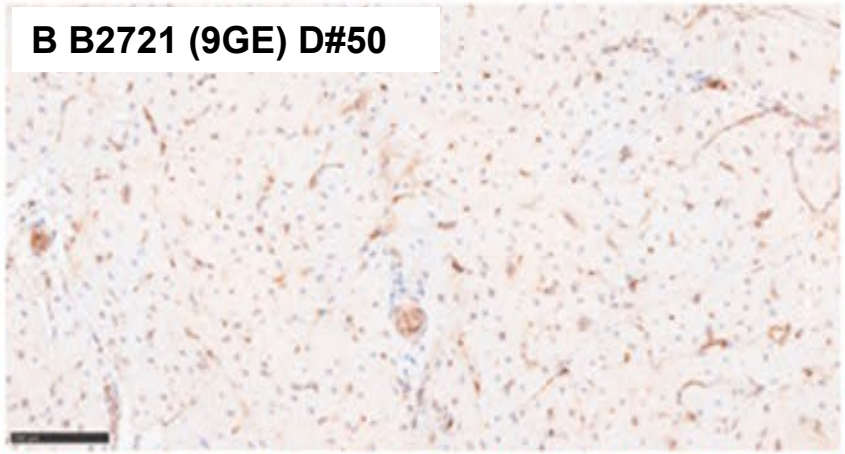
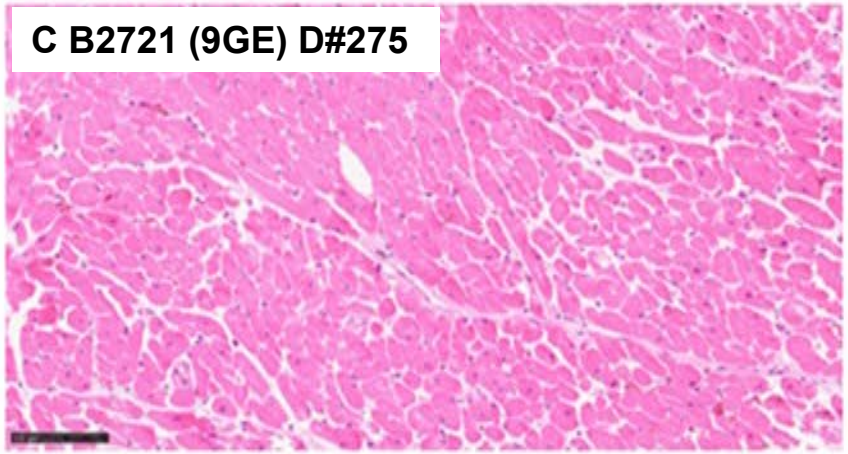
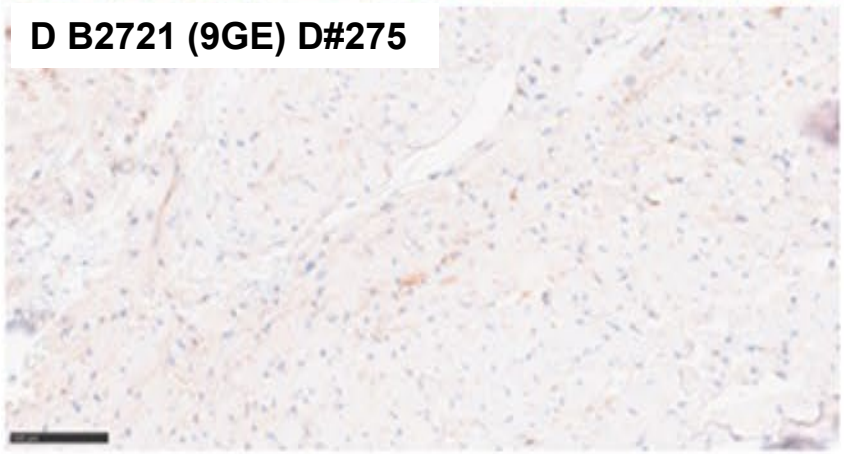
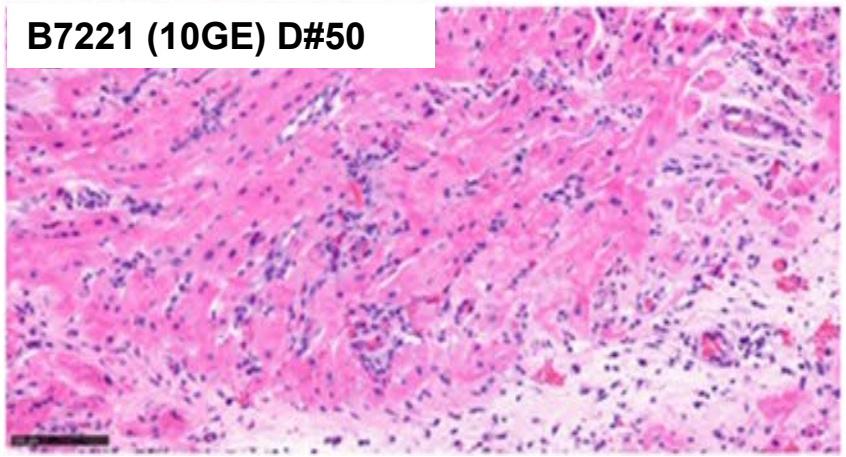
Results: Histology

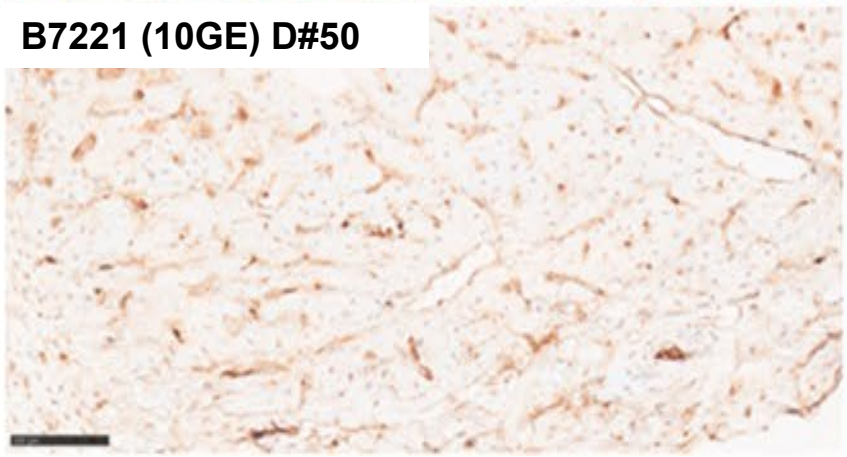












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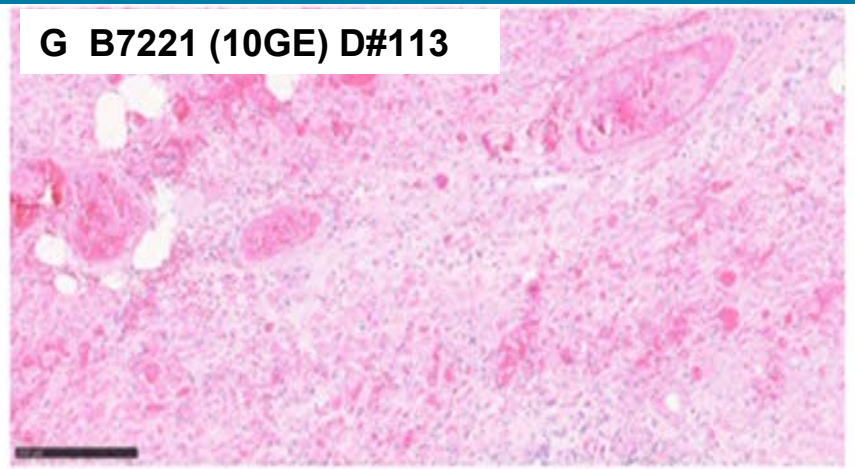
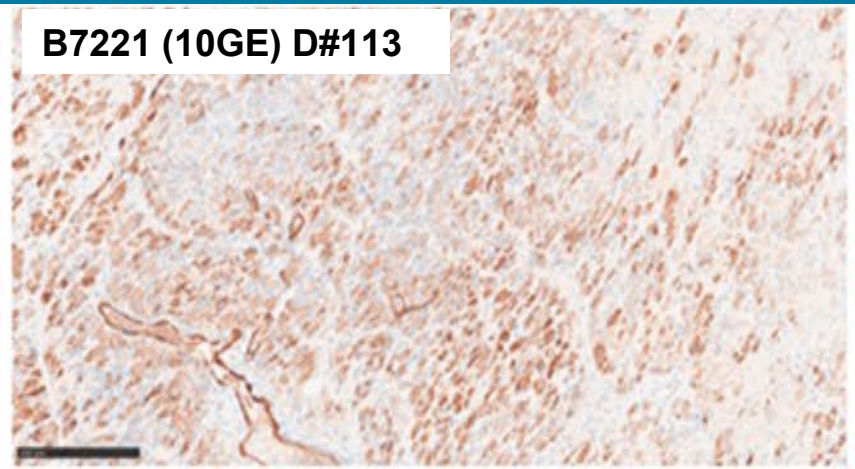
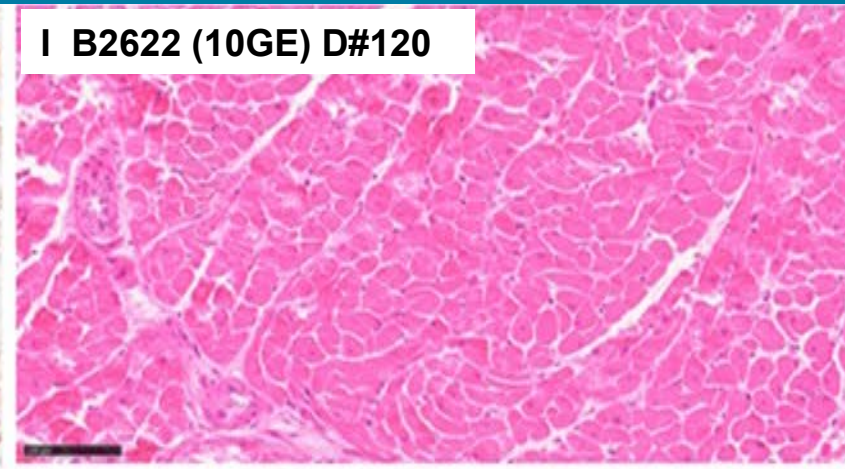
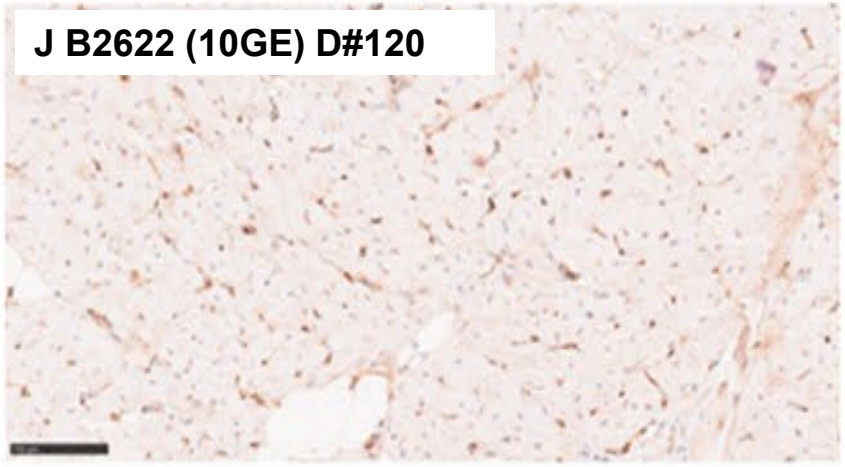
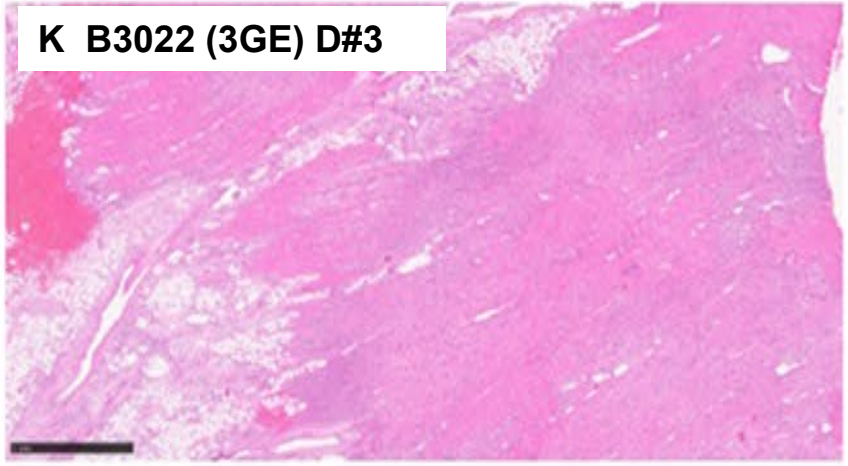
Results: Histology

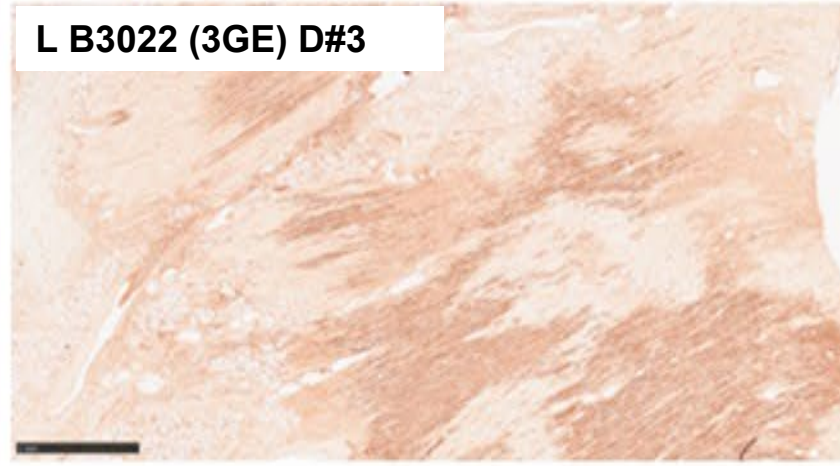












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Conclusions

- Relative to reference genetics without thrombo-regulatory and anti-inflammatory gene expression, 9- or 10-GE pig hearts exhibit promising performance in the context of a clinically applicable regimen including ischemia minimization and αCD154-based immunosuppression.

- Despite GE modifications and use of ischemia minimization, peri-transplant myocardial injury (Troponin leak) and systemic inflammation (CRP, IL-6 elaboration) occur consistently, but do not prevent graft recovery and long-term survival.

- These encouraging results justify further evaluation in an orthotopic heart xenotransplant model.

Thank you!

Key Contributors:
R Chaban
A Calhoun
V Diaz
I Ileka
RN Pierson III

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