Pilot Evaluation Of A Clinical Xeno Heart Transplant Regimen In A Preclinical Model

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Relevant Disclosures

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- *Gene edited pigs were provided by eGenesis and NSRRC (NIH grant U42OD011140)*
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- *D.K.C. Cooper is a consultant to eGenesis*

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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, Sd)
  - Add human regulatory molecules
    - Complement: CD46, CD55, CD59
    - Coagulation: TBM, EPCR, TFPI
  - Add anti-inflammatory 'transgenes'
    - CD47, HLA-E/β2, HO-1, A20, CD39

Background

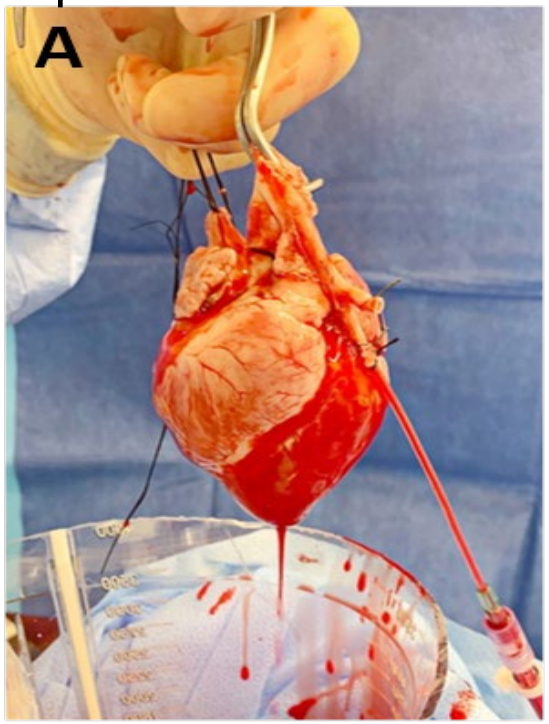
- We evaluated hearts from multi-GE pigs in baboon transplants treated with a novel costimulation-based immunosuppressive regimen and cold-perfused ‘ischemia minimization’.

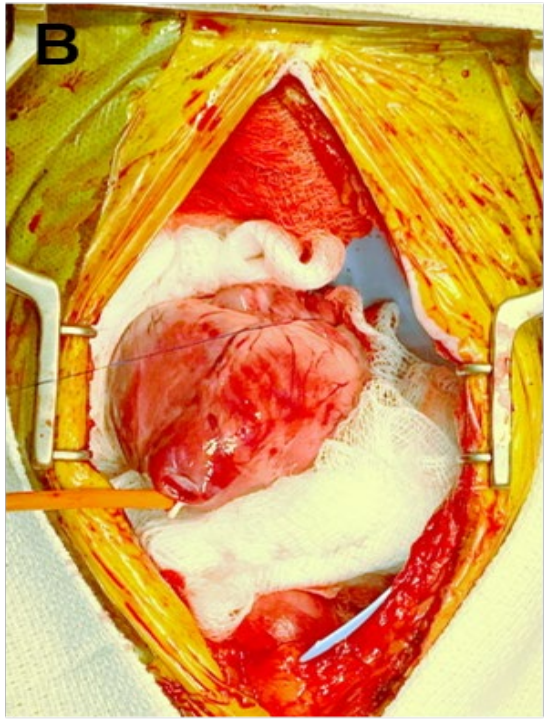
Methods

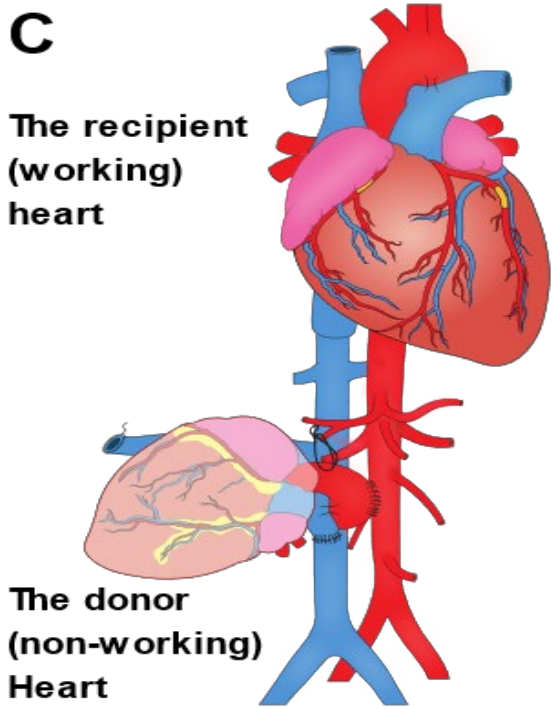
- Pig Donors
  - 3-, 11-, or 15-GE pig hearts
  - novel costimulation-based immunosuppressive regimen
  - cold-perfused storage technique designed to minimize graft ischemia.
- Five baboons' recipients received heterotopic heart transplants using Steen's cold-perfusion ischemia minimization
  - 3 Reference pig hearts (Ntl. Swine Resource & Research Ctr: NSRRC)
    - 3-GE pigs: GTKO.β4GALNT2KO.CD55
  - 2 Multi-GE pig hearts (eGenesis) (TKO = GTKO.CMAHKO.β4GALNT2KO)
    - TKO.hCD46.CD55.TBM.TFPI.HLAE/β2M.CD47.CD59.CD39.A20.HO1.PD-1 (**EGEN-4737**[15-GE])
    - TKO.hCD46.CD55.TBM.TFPI.HLAE/β2M.CD47.EPCR (**EGEN-4417**[11-GE])

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°C, 40-50 mmHg, immersed in reservoir; LV vent

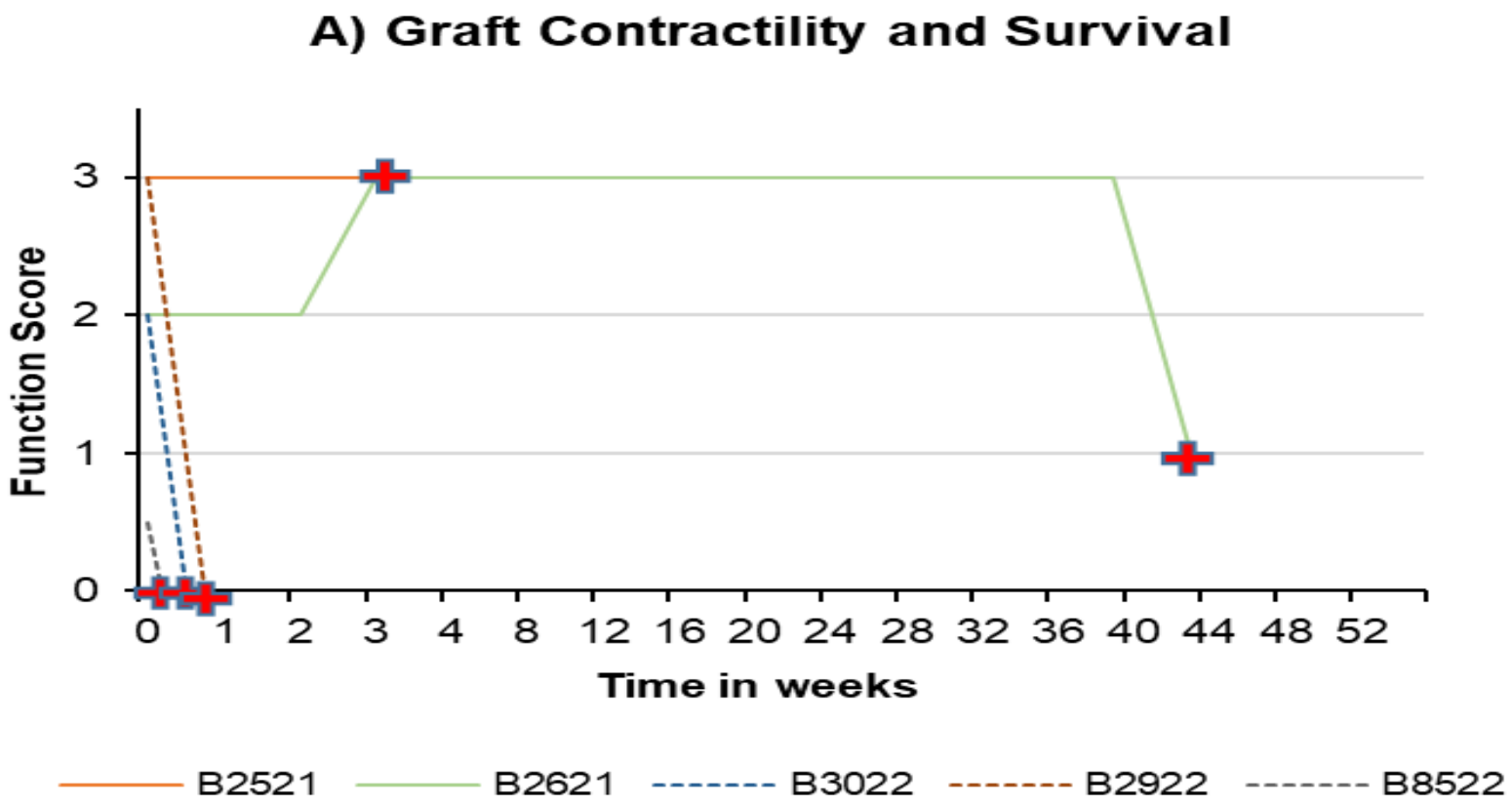
Methods: Recipient Immunosuppresive Treatment Regimen

- 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: 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
B2521	EGEN-4737 (15G)	None	Abdominal dehiscence	20	Antibody mediated rejection
B2621	EGEN-4417 (11G)	None	None	300	Acute cellular rejection (3R) with severe antibody mediated rejection

Results : Graft Survival and Function



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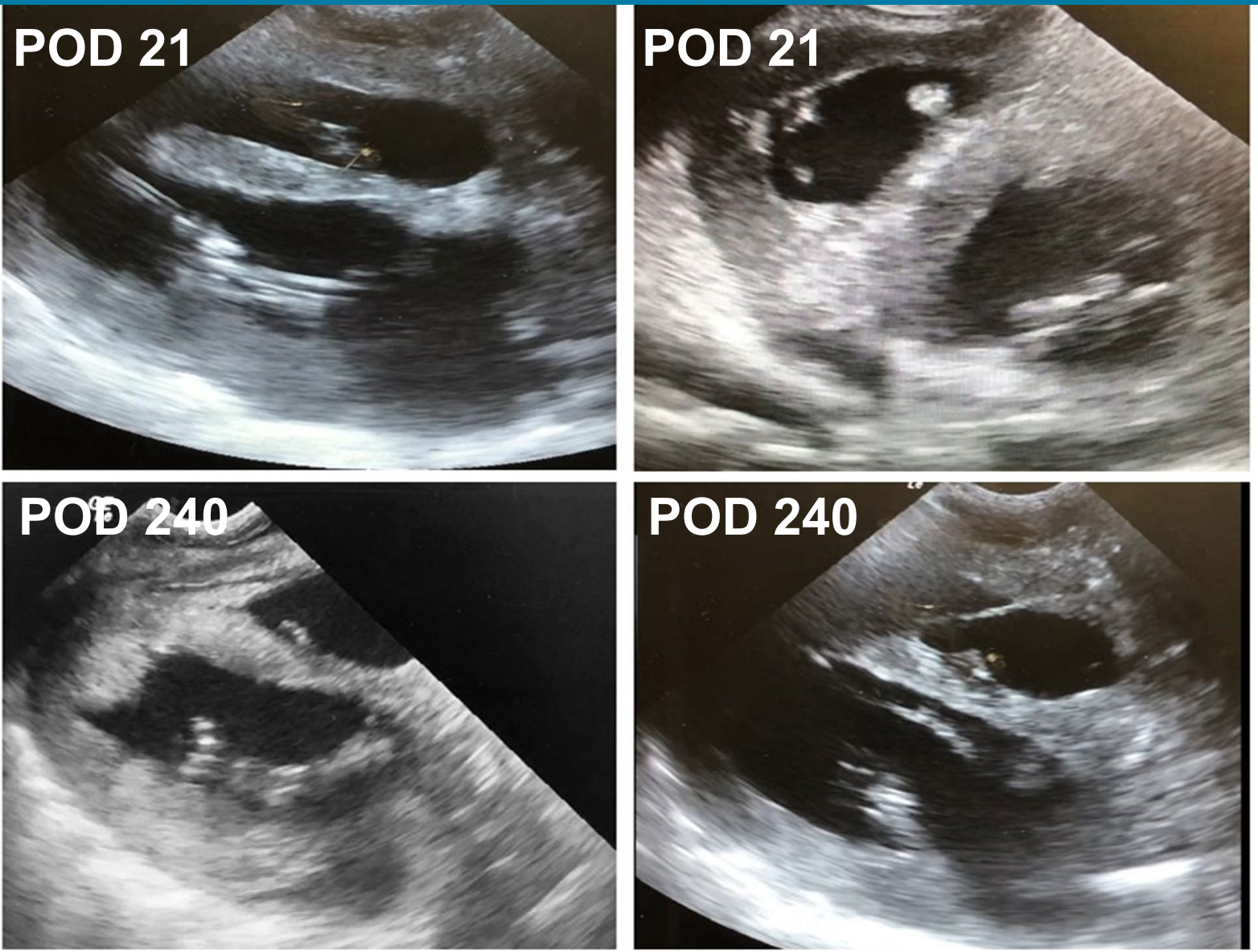
Results: Cardiac Echo, 11-GE Pig



Relative to POD 21, interventricular septal thickness remained stable on weekly measurements until POD 240.

Immunosuppression intensity was reduced on POD 200; hypertrophy progressed and contractility diminished after POD 240, until graft failure and euthanasia on POD 300.

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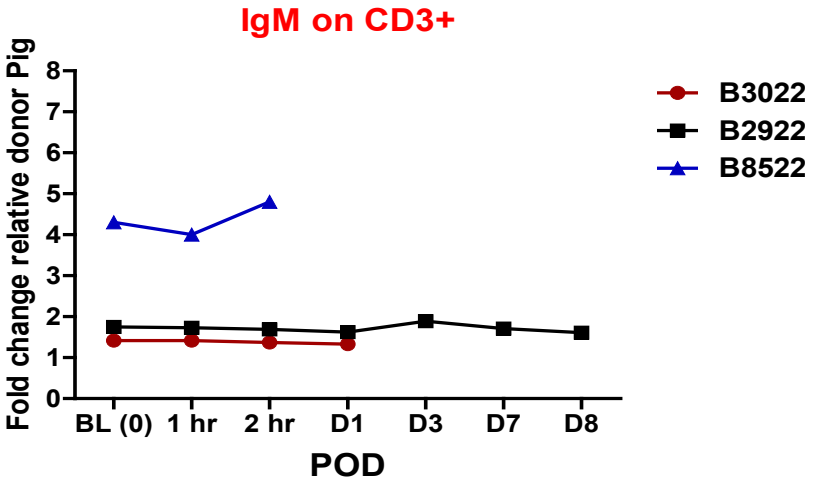
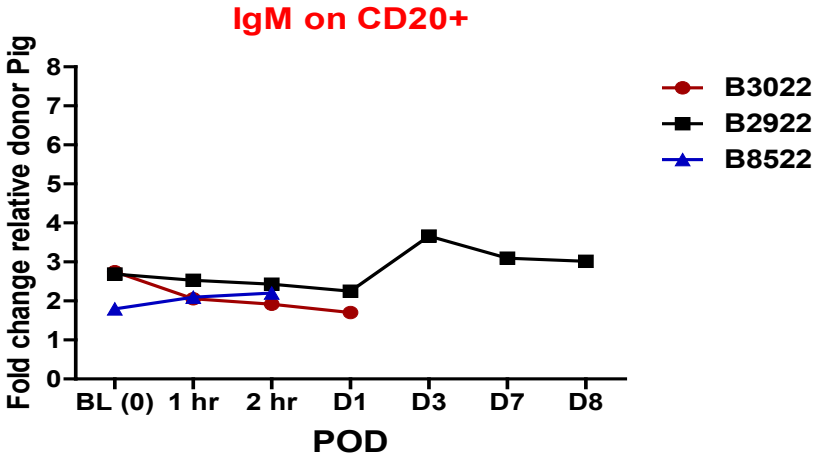
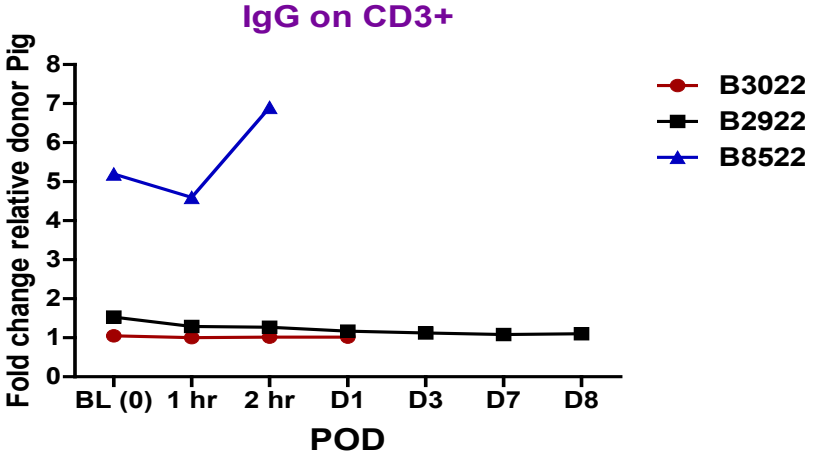
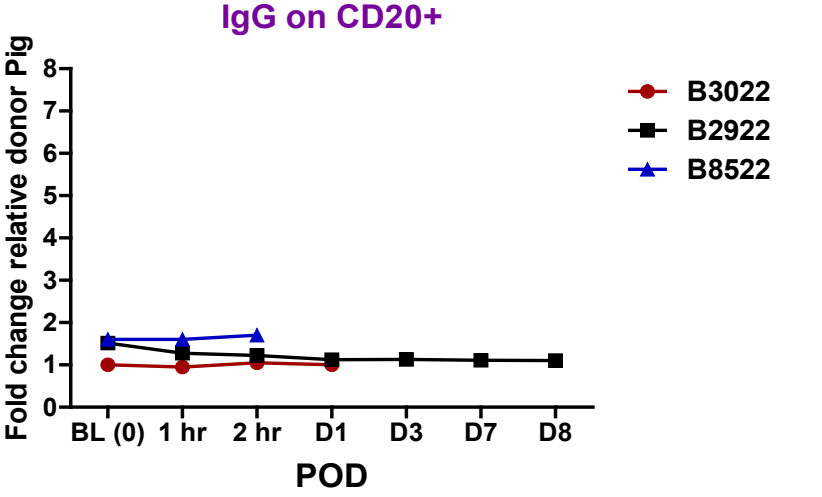
Results: Anti-pig Antibody in 3-GE pigs









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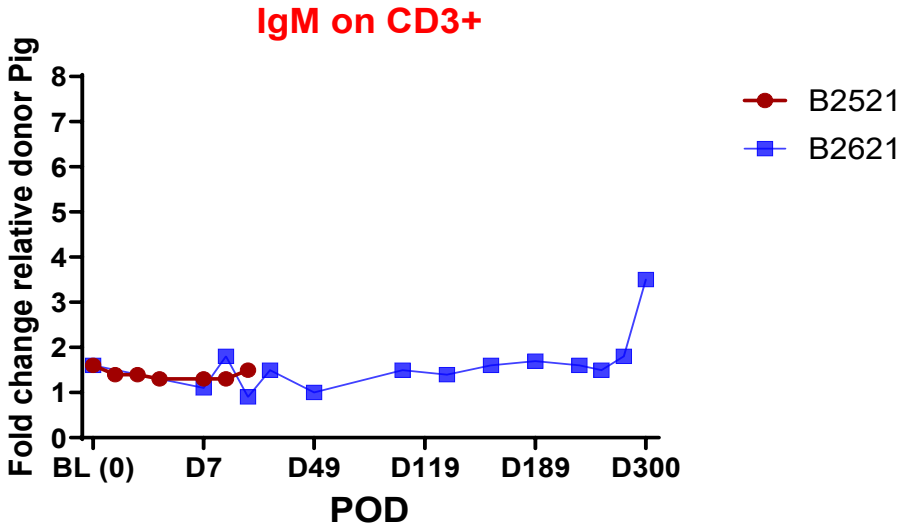
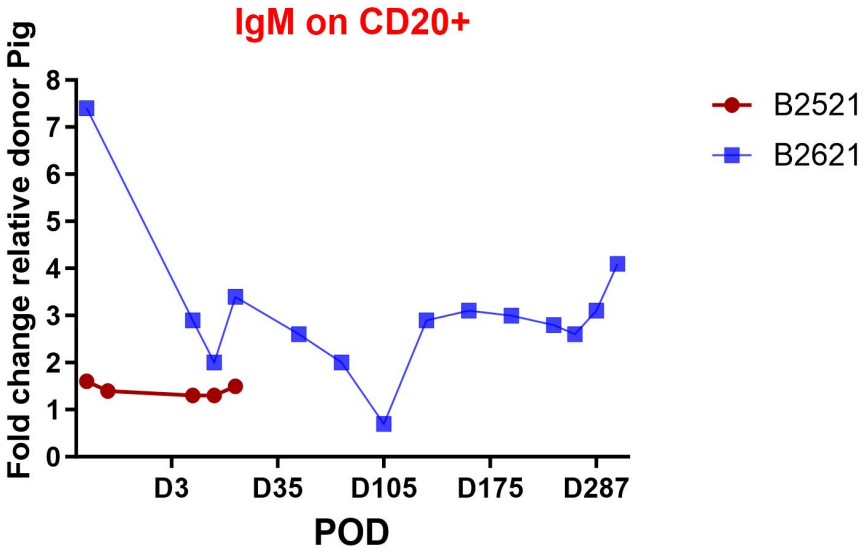
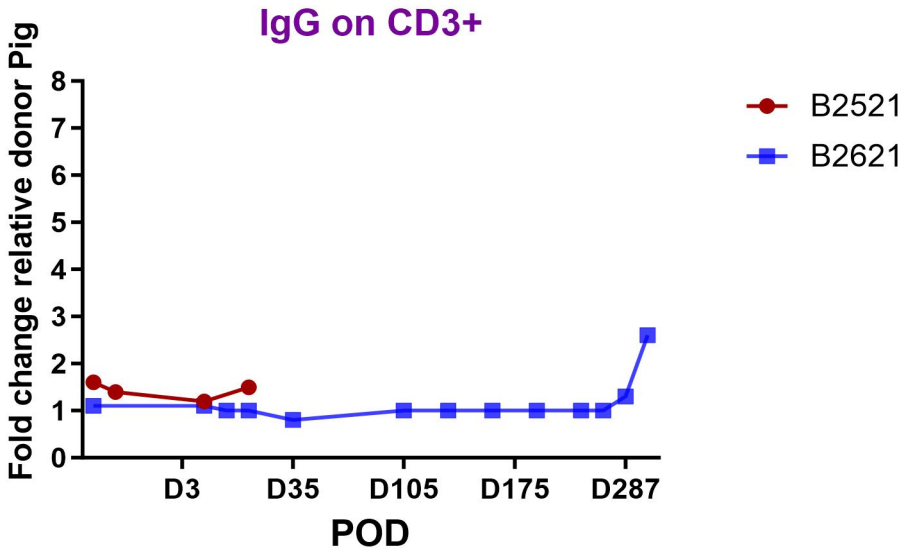
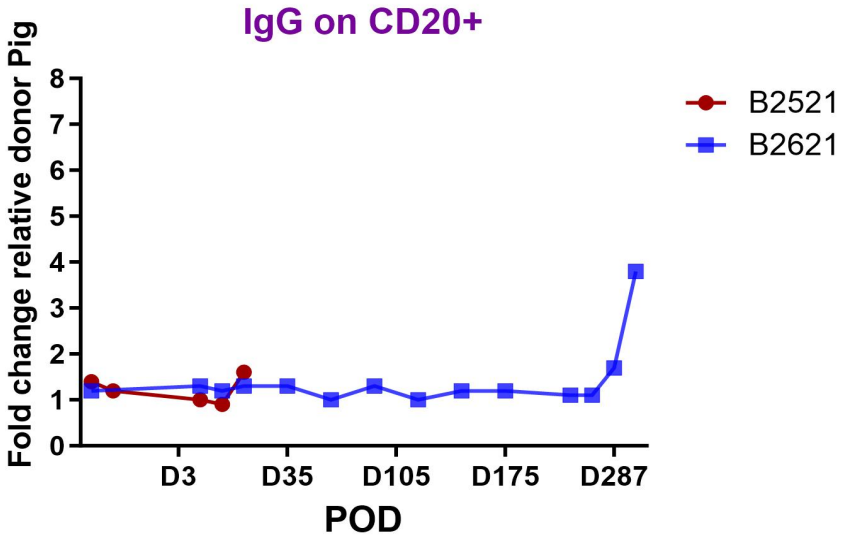
Results: Anti-pig Antibody in multi-GE pigs



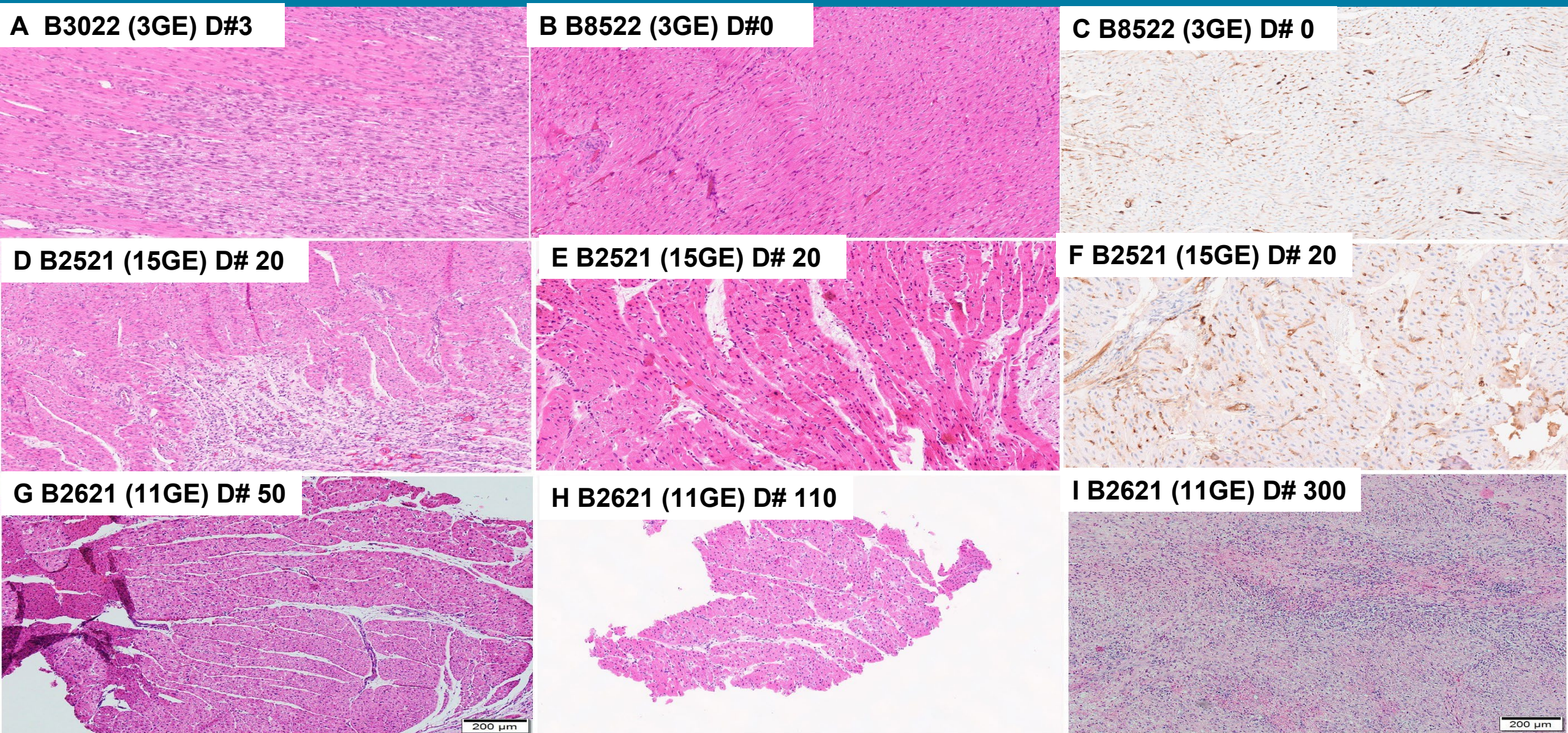






Results: Histology

H&E H&E C4d/C4d/H&E



Conclusion

- This pilot experience with multi-GE porcine hearts in the context of a clinically applicable ischemia minimization and immunosuppressive regimen supports our working hypothesis that is effective to prolong heart xenograft survival.

- We plan to further test this hypothesis in an orthotopic heart xenograft model.

Thank you!

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