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

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Relevant Disclosures

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- Gene edited pigs were provided by Revivicor and NSRRC (NIH grant U42OD011140)
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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-\(\alpha\)Gal, Neu5Gc, \(\beta\)4Gal)
  – Add human regulatory molecules
    • Complement: CD46, CD55, CD59
    • Coagulation: TBM, EPCR, TFPI
  – Add human anti-inflammatory ‘transgenes’
    • CD47, HLA-E/\(\beta\)2\(\mu\)g, 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
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
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 Immunosuppressive 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
<table>
<thead>
<tr>
<th>Animal ID</th>
<th>GE</th>
<th>Anticoagulation</th>
<th>Major complication</th>
<th>Graft Survival (Days)</th>
<th>Final Biopsy</th>
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<td>B3022</td>
<td>3G</td>
<td>Heparin IV</td>
<td>Graft rupture</td>
<td>3</td>
<td>Antibody mediated rejection</td>
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<td>3G</td>
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<td>9G</td>
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<td>0</td>
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<td>9G</td>
<td>Heparin IV for 10 days</td>
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<td>B6921</td>
<td>9G</td>
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<td>10G</td>
<td>Heparin IV for 8 days, then heparin sc for 2 weeks, ASA po daily</td>
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<td>Antibody mediated rejection (Grade 3), Acute cellular rejection (3R)</td>
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<td>10G</td>
<td>Heparin IV for 8 days, then heparin sc continue, ASA po daily</td>
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<td>No evidence of rejection</td>
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</table>
Results: Graft Survival, Function, and Morphology

A) Graft Contractility and Survival

B) Interventricular Septum (IVS)

[Graphs showing time in weeks on the x-axis and function score or IVS thickness in mm on the y-axis, with different lines representing different grafts (e.g., B7421, B6921, B2721, B7221, B2622, B3022, B2922, B8522).]
Results: CRP, IL-6

*Dashed lined is the upper limit of the CRP assay

CRP

IL-6

*Dashed lined is the upper limit of the CRP assay
Results: Clinical condition, cardiac injury

Animal weight

Time in weeks

Troponin I level

Time in weeks
Results: Histology

A B2721 (9GE) D#50
B B2721 (9GE) D#50
C B2721 (9GE) D#275
D B2721 (9GE) D#275
B7221 (10GE) D#50
B7221 (10GE) D#50
Results: Histology
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!

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