Phenotyping of transgenic pigs to determine the suitability of xenografts in the treatment of aortic diseases

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Disclosure of Interest

Speaker name: Ewa Strauss

• I do not have any potential conflict of interest

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BACKGROUND

• The synthetic materials used in the surgical treatment of aortic diseases can cause graft infection, which requires the replacement aortic prostesis with biological material, such as auto- or allografts.

• Due to the shortage of multiorgan donors, many patients with the prosthesis infection cannot be treated successfully.

• The domestic pig may be the perfect donor of easily accessible blood vessels for transplantation.
AIM

• Under the MEDPIG project a series of „humanized” pigs were generated to omit species incompatibility

• We focused on the ex vivo studies of the aorta & skin grafts from these transgenic pigs, to determine the suitability of transgenic xenografts in the treatment of aortic diseases.

• In a pilot in vivo study functionality of the aortic grafts were tested through cross transplantation (betwee transgenic pigs).
POLITRANSGENIC:
GAL, FUT, HLA, ZNF

Human genes that regulate the complement system are inserted by the method of DNA microinjection. The introduction of the human genes may mask the porcine epitopes.
1. COMPARATIVE MORPHOLOGY of ANATOMICAL FRAGMENTS

PORCINE AORTA (N=100)  

Photographic documentation  
Angio CT-scans  
Vector graphics

HUMAN AORTA (N=100)  

Angio CT-scans

Osirix system
RESULTS

### THORACIC AORTA [mm]

<table>
<thead>
<tr>
<th></th>
<th>LENGTH</th>
<th>PROXIMAL DIAMETER</th>
<th>WALL THICKNESS</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIG</td>
<td>303.8 ±35.0</td>
<td>19.2 ±1.6</td>
<td>1.65 ±0.8</td>
</tr>
<tr>
<td>HUMAN</td>
<td>249.5 ±46.6</td>
<td>27.8 ±3.6</td>
<td>2.02 ±1.1</td>
</tr>
<tr>
<td>Difference</td>
<td>+ 122%</td>
<td>69%</td>
<td>82%</td>
</tr>
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### ABDOMINAL AORTA [mm]

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<th>LENGTH</th>
<th>PROXIMAL DIAMETER</th>
<th>WALL THICKNESS</th>
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<tbody>
<tr>
<td>PIG</td>
<td>180.7 ±17.0</td>
<td>11.7 ±1.6</td>
<td>1.15 ±0.30</td>
</tr>
<tr>
<td>HUMAN</td>
<td>125.1 ±14.9</td>
<td>20.4 ±4.3</td>
<td>1.18 ±0.52</td>
</tr>
<tr>
<td>Difference</td>
<td>144%</td>
<td>57%</td>
<td>97%</td>
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</table>

Other possibilities of xenografts application
- Combining fragments of 2 blood vessels
- Vascular patches
- Non-anatomical prosteis (by-pass)
- Acellular matrices

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<thead>
<tr>
<th>T H O R A C I C</th>
<th>A B D O M I N A L</th>
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<tbody>
<tr>
<td>P/H=122% P&lt;.001</td>
<td>144% P&lt;.0001</td>
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<table>
<thead>
<tr>
<th>WALL THICKNESS</th>
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<tr>
<td>82%, P=.009</td>
</tr>
<tr>
<td>97%, P=.76 NS</td>
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2. ULTRASTRUCTURE OF THE AORTA

2.1. RESULTS: LIGHT MICROSCOPY (H+E)

Thoracic aorta
Difference in **tunica media** histology:
• shortened elastic fibers
• an increased number of collagen fibers
• numerous clusters of smooth muscle cells
• unusual presence of blood vessels

Abdominal aorta
Difference in **tunica media** histology:
• increased number of smooth muscle cells, indicating rather the muscle type of arteries

There were no histological markers indicating the presence of **atherosclerotic plaques** or **inflammation**
2.2. RESULTS: SCANNING ELECTRON MICROSCOPY

Morphology of the endothelial cell layer of the porcine aorta

A. Aorta of transgenic pig (this study)
Normal porcine endothelia show a continuity and a slight, physiological nuclear bulging and alignment in the direction of flow.

B and C. massive cell bulging, widened irregular intercellular spaces

Examples of endothelial injury caused by iodinated contrast media (data from the literature)*

3. BIOMECHANICAL STUDIES

3.1. MACRO-SCALE

Flexibility
Relaxation
The curve of the aortic wall tension
Texture analyzer

3.2. NANO-SCALE

7 tests performed at different locations
Nanoidenter Agilent G200
Effect of the cryopreservation of pig aortic tissue (period 30-180 days)

H+E staining of fresh and cryopreserved aortas grafts
3.2. RESULTS

The nano-scale mechanical properties of the porcine aorta (intima & internal elastic membrane)

**FRESH**

**CRYOPRESERVED**

The Young’s modulus [E]

- Rubber
  - Soft tissues
  - 0.01–0.1 GPa
- Glass
- Diamond Engineering materials
  - 1050 - 1210 GPa
4. PRELIMINARY IN VIVO FUNCTIONAL STUDY (4 TG animals)

Cross-transplantation procedure

Two months after surgery

Genetic analysis of imbeding 24 markers

Angio-CT scans

Before transplantation

After transplantation
5. IN VITRO STUDIES OF CYTOTOXICITY

Molecular and functional validation (20 TG + 20 Control)

**CD31 expression**

![Fluorescent microscopy image and cytometric analysis of CD31 expression](image1)

**acLDL uptake**

![Fluorescent microscopy image and cytometric analysis of acLDL uptake](image2)

**THORACIC AORTA**

Aortic Endothelial Cell *in vitro* culture

Brigt field microscopic image of *in vitro* primary porcine aortic endothelial cells
RESULTS: CYTOMETRIC ANALYSIS

Level of cleavage products of the C3, C4 and C5 components of the complement system in porcine endothelial cells.

Relative level of complement-dependent cytotoxicity, measured by LDH release in porcine endothelial cells.
RESULTS: WHOLE-GENOME EXPRESSION ANALYSIS

AFFYMETRIX PORCINE MICROARRAYS
PRIMARY PORCINE AORTIC ENDOTHELIAL CELLS

TRANSGENIC vs CONTROL

CONTROL SERUM-TREATED vs CONTROL UNTREATED

TRANSGENIC SERUM- TREATED vs CONTROL SERUM- TREATED
ACELLULAR BIOMATERIALS – ACELLULAR SKIN GRAFTS

Treatment of ischemic wounds

- Skin derived from transgenic pigs, submitted to chemical and enzymatical acellularization
- Free from Porcine Endogenous Retroviruses
- Radiation sterilization
- Temporary dressing

Currently used in this project in the treatment of burn wounds (accidents in the mining industry) and for donor fields.
SUMMARY

• Morphological, histological, mechanical and functional features of the porcine aorta, indicate the potential usefulness of aortic transgenic xenografts for the treatment of aortic diseases

• Tissue of poly-transgenic pigs are less immunogenic to human, as compared to those from non-transgenic animals

• Acellularization and cryopreservation may extend the applicability of tissue of transgenic pigs in medicine
NEXT STEPS:

• Generation of new poly-transgenic animals using the CRISPR/CAS9 genome editing method
SCIENTIFIC COOPERATION

UNIVERSITY OF LIFE SCIENCES IN POZNAN

- **Department of anatomy of animals**
  Prof. Hieronim Frąckowiak
  Vet Maciej Zdun

- **Department of Biotechnology & Microbiology**
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  PhD Wojciech Białas
  Students (1)

TECHNOLOGY PARK

Wielkopolska Center for Advanced Technologies
PhD Marek Nowicki
Students (1)

NATIONAL RESEARCH INSTITUTE OF ANIMAL PRODUCTION IN BALICE/KRAKOW

PhD Vet, Jarosław Wieczorek
Prof. Jerzy Smorag
PhD Anna Radko

CENTRE FOR EXPERIMENTAL AND INNOVATIVE MEDICINE IN KRAKOW

PhD Vet, Agnieszka Pietsch-Fulbiszewska
PhD Michał Nowakowski

POZNAŃ UNIVERSITY OF MEDICAL SCIENCES

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  Prof. MD, Fryderyk Pukacki
  PhD MD, Łukasz Kruszyna
  PhD MD, Maciej Zieleński
  MD, Hubert Stępak
  MD, Paweł Zawadzki

- **Laboratory of Translational Medicine**
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  PhD med. Joanna Wróblewska
  Msc Jolanta Tomczak
  Msc Lidia Wawrzyńska

- **Department of Histology and Embriology**
  Prof. Maciej Zabel
  PhD Agnieszka Malińska
  PhD Marcin Ruciński

- **Wielkopolska Cancer Centre**
  Prof. MD, Andrzej Mackiewicz

INSTITUTE OF HUMAN GENETICS PAS

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POLISH ACADEMY OF SCIENCES

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Thank you!