A Novel Biomarker for Arrhythmogenic Right Ventricular Cardiomyopathy

A New Paradigm for Disease

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A Novel Serum Biomarker for ARVC

- Lessons from the Paradigm of Skin Desmosomal Disease
- Testing and Validation of Hypothesis
- Biomarker Specificity: Other Cardiomyopathies/Pathologies
- Evidence for a Pathophysiologic Role (Not Just a Biomarker)
- Therapeutic Tractability & Future Directions
Basis of Hypothesis

• ARVC programs occasionally identify isolated cases
• Dermatology collaborations increased our understanding of the etiology of desmosomal disease
• Desmosomal skin disorders may have either genetic or auto-immune etiologies
• Many ARVC cases are gene-elusive: autoimmunity might be an alternate pathophysiology, and may act as a marker for disease
• We chose to test all ARVC subjects, and were amazed to find a consistent auto-antibody in all cases.
Lessons from the Paradigm of Skin Desmosomal Disease

Skin Conditions
- Skin fragility - ectodermal dysplasia syndrome
- Hypotrichosis with scalp vesicles
- Localized recessive hypotrichosis
- Striate palmoplantar keratoderm
- Pemphigus Foliaceus
- Pemphigus Vulgaris

Heart Conditions
- Arrhythmogenic Right Ventricular Cardiomyopathy

Genes:
- PKP1
- DSC3
- DSG4
- DSG1
- DSG3
- JUP
- DSP
- DSC2
- DSG2
- PKP2

 Syndromes:
- Skin fragility - ectodermal dysplasia syndrome
- Hypotrichosis with scalp vesicles
- Localized recessive hypotrichosis
- Striate palmoplantar keratoderm
- Pemphigus Foliaceus
- Pemphigus Vulgaris
- Naxos Disease
- Carvajal Disease

Genetics:
- AD
- AR
Proteins of the Desmosome and Area Composita

Experimental Plan

- Sera: Isolated Cases, Familial Cases and Controls
- Cases defined by ARVC Task Force Criteria (2010)
- Sera assessed against Desmosomal (DSC2, DSG2) & N-Cadherins by Western & ELISA
- Human Validation Cohort, Boxer Dog ARVC and other forms of cardiomyopathy assessed
- Further studies of pathophysiology
Anti-DSG2 Antibody: Primary Cohort and Controls

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Anti-DSG2 Antibody: Validation Cohort

Z1 Z2 Z3 Z4 Z5 Z6 Z7 Z8 Z9 Z10 Z11 Z12 Z13

250 Kd 130 Kd 100 Kd 75 Kd 55 Kd 35 Kd

Anti-DSG2 Antibody: Additional Controls

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Anti-DSG2 Antibody: Other Cardiomyopathies

Anti-DSG2 antibody: Hypertrophic Cardiomyopathy Patients

Anti-DSG2 antibody: Dilated Cardiomyopathy Patients
Human Enzyme-Linked Immuno-Sorbent Assay (ELISA)

ELISA Optical Density of Samples

Diagnostic groups

0 1 2 3 4
Optical Density
Human Enzyme-Linked Immuno-Sorbent Assay (ELISA) ROC

Anti-DSG2 Biomarker ROC Curve
Anti-DSG2 Antibody: Boxer Dogs

Anti-DSG2 antibody: Boxer dogs with ARVC

Anti-DSG2 antibody: Healthy Boxer dogs
Anti-DSG2 Ab & Disease Burden

PVC vs Pixel Count

- PVC count
- Pixel count of Blot

Graph showing correlation between PVC count and pixel count of Blot.
Anti-DSG2 Ab: Effects on Gap Junction Function

Cell Microinjection
Dye Transfer

Mean dye transfer distance in iPSCs

Control | Normal | Anti-DSG2 | Patient 91 | Patient 99
Distance travelled (microns)

Samples

p=0.0025**  p=0.0002***  p=0.0001***
ARVC Biomarker: Next Steps

A. Exon Map

B. Protein Structure

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Anti-DSG2 Epitope (Proximal Extracellular)
Conclusions/Implications

• Anti-DSG2 Ab is Sensitive & Specific for ARVC
• Ab Identifies All ARVC (Genes: PKP2, DSC2, DSP, DSG2, TTN, Gene –ve, Canine ARVC)
• Ab Identifies Severity as Measured by PVC Count
• Ab Induces Gap Junction Dysfunction
• Ab may be Target for Immune Therapies
Further Assessment

• Replicate in additional affected, controls
• Assess possible ARVC and phenotype negative class 1 carriers
• Seeking Collaborators (willing to):
  – Obtain local REB/IRB
  – Provide (anonymized) clinical data/sera
  – No disclosure of status (unless a local return of research results process completed)
Commercialization

• Seeking partners/Several potential models
  – Develop CLIA (UKAS, EA) – approved test
    • Within Hospital for Sick Children
    • Spin-Off
  – Licensing
    • eg: Collection via cardiac genetic testing company; analysis via diagnostic laboratory (?MML or LabCorp)
Other ARVC Therapies in Development
Other ARVC Therapies in Development

- 9 of 80 drugs improved gap junction function in iPSC-CMs
- Assessing dose-response
Anti-DSG2 Ab Based Therapy

• In an age of precision medicine, antibodies involved in the pathophysiology of ARVC could be specifically removed by complexing the antibody or removing the specific clone of antibody-producing cells

• This technology will depend on defining the specific epitope
CAR (& CAAR) T-cell Therapy

5. Cell Infusion

**Autologous approach**
Reengineered T cells are infused back into the patient.

**Allogeneic approach**
The same CAR-T cells can benefit multiple patients and thus save costs.
CAR (& CAAR) T-cell Therapy

CAAR

CAAR T cell

Autoreactive B cell

Pathogenic autoantibodies

Dsg2

CD137

CD3ζ

Kill