The Next Generation of Biomarkers in Type 1 Diabetes:
Taking Lessons from Disease Pathogenesis, Natural History, and Response to Prevention/Intervention Therapies

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Disclosures

The speaker serves, or has served in the last 5 years, as a compensated adviser to, or received research / personal financial support from:

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What is a Biomarker?

“A characteristic that is objectively measured and evaluated as an indicator of...

• normal biological processes,
• pathogenic processes,
• or pharmacologic responses to a therapeutic intervention”

Classic Model of T1D Pathogenesis

- Genetic Predisposition
- (?Precipitating Event)
- Overt Immunologic abnormalities
  - Normal insulin release
  - Progressive loss insulin release
  - Glucose normal
  - Overt diabetes
  - C-peptide present
  - No C-peptide

Age (years+)

Eisenbarth, NEJM 1986
Biomarkers Derived from the Classic Model of T1D Pathogenesis (i.e., Past)

Genes
- Genetic Predisposition

Autoantibodies
- Overt Immunologic abnormalities
- Progressive loss insulin release

Glucose Tolerance
- Normal insulin release
- Glucose normal
- Overt diabetes
- C-peptide present
- No C-peptide

Eisenbarth, NEJM 1986
Current Model for the Pathogenesis and Natural History of Type 1 Diabetes

1. Precipitating events might occur in utero

2. Genetic predisposition probably the key driver or linkage to immune abnormalities

3. Beyond precipitating, environment might influence entire natural history

4. Although overall loss of β cells is potentially linear, it could show a relapsing or remitting pattern

(Precipitating event)

Genetic predisposition

β-cell mass

Age (years)

Normal insulin release

Overt immunological abnormalities

Glucose normal

Progressive loss of insulin release

Overt diabetes

C-peptide present

No C-peptide

5. Presence of two or more islet autoantibodies might represent asymptomatic type 1 diabetes

6. Increasing glucose excursions as individual approaches symptomatic onset

7. Some patients produce low concentrations of C-peptide long after onset

8. β-cell mass not always zero in longstanding patients

Atkinson, M; Eisenbarth, G.S.; Michels, A. Lancet, 2014
Potential “Future” Biomarkers Derived from Current Model for the Pathogenesis and Natural History of Type 1 Diabetes

Atkinson, M; Eisenbarth, G.S.; Michels, A. Lancet, 2014
Practical Evolution of Biomarkers for Type 1 Diabetes

“Something is Wrong”
- Genetic Susceptibility
- Autoantibodies
- Declining Metabolic Status

“This is What is Wrong”
- Mechanistic Insights that Guide:
  - Disease State
  - Pathogenesis

“This is How we will Respond to the Wrong”
- Improved Prediction
- Therapies for Prevention/Cure
- Better Treatment Modalities
Example - The Evolution of Genetics

1980’s to Present – Biomarkers that Define Risk for Type 1 Diabetes

Note: Too many; Too little OR; Notions of GWAS “Bust”

Concannon P, Rich S, Nepom GT
Example - The Evolution of Genetics
Present to Future – Genetics Impacts Biomarkers that Reflect Pathogenesis

- Number of autoreactive cells
- Functional correlates that promote disease
- Regulatory phenotypes that influence disease progression
- Beta cell function/damage

“Beyond GWAS”
Biomarkers in Type 1 Diabetes - Three Areas of Most Need

1. Patient Stratification
   - Distinguish healthy, at-risk from not at-risk

2. Heterogeneity of T1D disease
   - Distinguish healthy, at-risk from pre-diabetes
   - Quantify functional beta cell mass

3. Stratification & Response to Specific Therapy
   - Define “Degree” of Disease
   - Detect on-going autoimmunity and beta-cell regeneration

Disease Staging

At Risk
Pre-Diabetes
Recent Onset
Established Diabetes

Time

Beta Cell Mass

100%

0%
Example of “Stratification & Response to Specific Therapy” – How Outcomes Might, Hypothetically, Influence Care and Entry into Intervention Trials

- **T1D diagnosis**
- **Initial insulin therapy**
  - Reduce acute hyperglycemia
  - Stratify based on biomarkers & demographics
    - Rapid c-peptide loss
      - More “Aggressive” Clinical Trial
    - Slow c-peptide loss
      - Intensive insulin Rx
      - Standard insulin Rx
      - Less “Aggressive” Clinical Trial

Stratification & Response to Specific Therapy:

1. **Rapid c-peptide loss**
   - More “Aggressive” Clinical Trial
2. **Slow c-peptide loss**
   - Intensive insulin Rx
   - Standard insulin Rx
   - Less “Aggressive” Clinical Trial

Example of how outcomes might hypothetically influence care and entry into intervention trials.
Biomarkers in Type 1 Diabetes – Three Predominant Routes for Discovery

Patient Stratification  Heterogeneity of T1D disease  Response to Specific Therapy

Distinguish
• Healthy, not at-risk
• Healthy, at-risk
• Stages, Progression

Define “Established” Disease, Disease Staging

1. Quantify functional beta cell mass, beta cell health, beta cell death, & beta cell regeneration (?!)

2. Detect on-going autoimmunity, immunopathology & pancreatic pathology/status

3. Metabolic control

Beta Cell Mass

At Risk  Pre-Diabetes  Recent Onset  Established Diabetes

Time

0%  //  100%
Five Examples of How Evolution in Knowledge *Might* Influence Future Biomarkers for Type 1 Diabetes
Potential Biomarker #1 – Given the Paucity of Insulitis, More “Direct” Markers of Beta Cell Health will be Used

- At Risk
- Asymptomatic Diabetes
- Symptomatic Diabetes

Functional Beta Cell Mass

Beta Cell Death

Time
Past – Insulitis a “Hallmark Lesion”
Hence, Hope for “CRP” Like Biomarker

Staged at Islet Level

Present/absent; number islets

Edited, courtesy Al Powers
“The lesion should be established in a minimum of three islets, with a threshold level of CD45+ cells/islet before the diagnosis can be made.”
Future - Methylated Insulin as a Marker of Beta Cell Death (i.e., More Beta Cell...Less Intra-Islet Immune)

Detection of β cell death in diabetes using differentially methylated circulating DNA

Eitan M. Akirav\textsuperscript{a,1}, Jasmin Lebestchi\textsuperscript{a}, Eva M. Galvan\textsuperscript{a}, Octavian Henegariu\textsuperscript{a}, Michael Akirav\textsuperscript{b}, Vitaly Ablamunits\textsuperscript{a}, Paul M. Lizardi\textsuperscript{c}, and Kevan C. Herold\textsuperscript{a,2}

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Akirav, E.M. et. al. PNAS, 2012
Potential Biomarker #2 – Features of the Pancreas, including Exocrine Features, may Provide a Biomarker for Type 1 Diabetes
Past – As “Type 1 Diabetes is a Beta Cell Specific Disease”, Pancreatic Contributions are Largely Ignored. However...

- **Gross Anatomy**
  - Smaller pancreas (weight and volume)

- **Immunology**
  - Autoantibodies to exocrine antigens
  - C4D deposition
  - Pancreatic infiltrates

- **Physiology**
  - Exocrine insufficiency

- **Pathology**
  - Exocrine atrophy
  - Fibrosis
  - “Leaky” vasculature
Present – CD4, CD8, and DC Infiltration of Pancreas is More Pronounced in Type 1 Diabetes

Teresa Rodríguez-Calvo,1 Olov Eklöw,1,2,3 Natalie Amirian,1 Jose Zapardiel-Gonzalo,4 and Matthias G. von Herrath1,5

Increased Immune Cell Infiltration of the Exocrine Pancreas: A Possible Contribution to the Pathogenesis of Type 1 Diabetes

DOI: 10.2337/db14-0549

Rodriguez-Calvo, 2014, Diabetes online
Present/Future - Smaller Pancreas in the Natural History of Type 1 Diabetes; MRI?, Ultrasound?

Campbell-Thompson, JAMA. 2012
Potential Biomarker #3 - Improved Staging through Biomarker “Combination”

100%  0%

Functional Beta Cell Mass

At Risk  Asymptomatic Diabetes  Symptomatic Diabetes

Beta Cell & Genetics & Immunology & Pancreas

Time
PAST – Multiple Autoantibodies are also Associated with Faster Progression to Symptomatic T1D in T1D Relatives

Numbers 1–4 are number of autoantibodies at screening. Curves indicate occurrence of type 1 diabetes over follow-up (n = 29,035). DPT-1 = Diabetes Prevention Trial–Type 1

Present – 3 Year Risk of Progression to Symptomatic Disease with 10% Increase in HbA1c is 84%
Use of the Diabetes Prevention Trial-Type 1 Risk Score (DPTRS) for Improving the Accuracy of the Risk Classification of Type 1 Diabetes

Jay M. Sosenko, Jay S. Skyler, Jeffrey Mahon, Jeffrey P. Krischer, Carla J. Greenbaum, Lisa E. Rafkin, Craig A. Beam, David C. Boulware, Della Matheson, David Cuthbertson, Kevan C. Herold, George Eisenbarth, Jerry P. Palmer, the Type 1 Diabetes TrialNet and Diabetes Prevention Trial-Type 1 Study Groups*
These results indicate that BHT-3021 induced antigen-specific reductions in CD8 cells reactive to proinsulin, but not to other antigens, and that the magnitude of the reduction was inversely correlated with the improvement in C-peptide.
Future – Genotype Driven Analysis of Immune and Metabolic Functions

The autoimmune disease-associated SNP rs917997 of *IL18RAP* controls IFNγ production by PBMC

Courtney B. Myhr*, Maigan A. Hulme*, Clive H. Wasserfall, Peter J. Hong, Priya Saikumar Lakshmi, Desmond A. Schatz, Michael J. Haller, Todd M. Brusko, Mark A. Atkinson.

![Graph A](image1)

![Graph B](image2)
Potential Biomarker #4 – Heterogeneity in “Normal” Beta Cell Mass Will be Accounted for

- At Risk
- Asymptomatic Diabetes
- Symptomatic Diabetes

Beta Cell Mass
Past – 100% is 100%

Diagram showing the decline in functional beta cell mass over time from At Risk, to Asymptomatic Diabetes, to Symptomatic Diabetes.
Present – Not All Humans are “Created Equal”, in terms of Beta Cell Mass

\[ r = 0.02 \]
\[ p = 0.9 \]
Present – Not All Humans are “Created Equal”, in terms of Beta Cell Mass nor in Their Ability to Replicate Beta Cells

Formation of a Human β-Cell Population within Pancreatic Islets Is Set Early in Life

Brigid E. Gregg, Patrick C. Moore, Damien Demozay, Ben A. Hall, Mei Li, Aliya Husain, Amy J. Wright, Mark A. Atkinson, and Christopher J. Rhodes
Hypothetical Idea #1 – Stress Resulting from a Reduced Beta Cell Mass/Small Pancreas MAY Determine How Quickly T1D Develops

100% Genetic Susceptibility

10% Beta Cells

Triggering Event (?)

Autoimmune Process

Disease Onset

Time (months or years)

Credit: David Harlan
AI Powers

Edited, Courtesy R. Insel
Hypothetical Idea #2 – Stress Resulting from a Reduced Beta Cell Mass/Small Pancreas MAY Represent a Trigger

- Triggering Event (?)
- Autoimmune Process

100% Beta Cells

? 10% Disease Onset

Time (months or years)

- Age?
- BMI?
- Genetics?
Future – Quantification of Beta Cell Mass

FINALLY

• Imaging?
• MicroRNA?
• Omics?
Potential Biomarker #5 - Improved Assays for C-peptide may Stimulate Additional Efforts to Staging and Trials Beyond 100 Days

### Graph

- **Y-axis**: Functional Beta Cell Mass
- **X-axis**: Time
- **Legend**:
  - At Risk
  - Asymptomatic Diabetes
  - Symptomatic Diabetes

### Chart Details

- **Beta Cell Function**
- **Values**:
  - 100% at Risk
  - 0% Symptomatic Diabetes
Past – “Few Patients” Retain Ability to Produce C-Peptide

Peak C-peptide during MMTT (2-h) in patients 18 years of age at onset of diabetes and with type 1 diabetes (T1DM) of 1-15 years’ duration when screened for entry into the DCCT.

Adults (N = 2432)

0.03 min
Present - Functional Beta Cells After Diagnosis of Type 1 Diabetes...Beneficial?

Where Go From Here – Overcome the Biomarker Implementation Gap

- Imbalance between biomarker discovery, validation and application
- Many more biomarkers discovered than available as diagnostic test
Thank You!