A New Perspective in T1D:
Targeting the β Cell

Raghu G. Mirmira, MD, PhD
Professor of Medicine, The University of Chicago
Adjunct Professor of Biomedical Engineering, Purdue University
Adjunct Professor of Pediatrics, Indiana University
Discovery of Insulin, 1921

Banting and Best
Sugar Theory:
“.....the adherents of [this theory] have consistently held that the islets produce a substance which, in one or another way, controls carbohydrate metabolism.”
Whether it is possible that nature will restore the diseased pancreas when the strain is taken off [it] by administering this extract and while the other functions of the body and bodily strength is restored, is merely a hope. It may be possible.”

James Havens, 1922
Advances in T1D Treatments

1922

1970’s

Today
Kinds of Research

Basic Research

- Cell-free systems
- Cell Culture
- Animal Models

Translational Research

- Patient samples
- Pre-clinical drug testing
- Biomarker identification

Clinical Research

- Testing new treatments
- Comparing Treatments
- Defining disease biology
Loss of β Cell Mass in T1D

G. Eisenbarth, J. Skyler
Limited Effects of Immune-based Therapies

**Anti-T Cell agent**

- AUC (pmol/mL/24min) vs Month
- Anti-CD3 vs Comparison

**Anti-B Cell agent**

- pmol/mL vs Time in months
- Rituximab vs Placebo

**Anti-memory T cell agent**

- Change from baseline 4-hr C-peptide AUC (pmol/mL) vs Time in weeks
- Alefacept vs Placebo

References:
- Rigby, et al. 2015
- Herold, et al. 2005
T1D Pathogenesis: A Dialog Between Different Cell Types

- **β cell**
  - Ability to succumb to stress
  - Mass
  - Function

- **B cell**
  - Interacts with Macrophage

- **T cell**
  - Interacts with Dendritic cell

- **Macrophage**
  - Interacts with B cell

- **Dendritic cell**
  - Interacts with T cell
Autoimmunity vs. Something More: Why does it matter?

1. Diagnosis of the disease: Does it change our criteria for diagnosis?

2. Treatment of the disease: Are there targets that we should be considering in addition to the immune system? Personalized medicine

3. Prevention of the disease: When do you begin prevention, and what are you targeting? Personalized medicine

4. Prevalence/Incidence of the disease: how are healthcare resources placed?
Insulin positive islets after 8 years of T1D

6046
18 years old
8 year duration
Caucasian
Female

AutoAb: IA2A+ZnT8+
C peptide: <0.05 ng/ml
BMI: 25.2
Can we do more than we are currently doing?

Data suggest that preserving the β cells that are still present at diagnosis can help persons with T1D:

- Decrease average blood sugars
- Decrease blood sugar variability
- Decrease rates of severe hypoglycemia
- Decrease long-term complications of the disease
Unmet Clinical Needs

• Safe, tolerable drug therapies that will preserve remaining residual β cell insulin production

• Drugs may need to be used in combinations, targeting different parts of the process that results in T1D
β Cell Dysfunction and Neoantigen Production Precede Development of Autoimmunity

Inflammation (Environment)

Inflammatory Signaling (NO, MAPK, Ox Stress)

Biomarkers

Proinsulin
Chromogranin
GAD65
Zn-T8
IA-2

β cell Stress/Death
PTMs
Neoantigen exposure

Autoimmunity

Translation
ER stress

Maganti, et al, Islets 2014
β Cell ER stress in the Development of T1D

- PERK
- IRE1-α
- ATF6
- sXbp1
- Bip
- ATF4
- peIF2a

Pro-inflammaotory Cytokines (TNF-α, IL-1β)

- Protein misfolding
- Proinsulin
- Activation of modifying enzymes (tTG)
- Alternative mRNA splicing
- Dysglycemia

JNK
CHOP

ER Fragmentation
Apoptosis
Hyperglycemia
Elevated proinsulin:C-peptide ratio in humans with new onset T1D

Williams, et al. Transl. Res. 2015

The Mirmira Lab Strategy for Identifying and Engaging Targets for Diabetes

**Model Systems**

- Identify target in rodent β cell lines
- Modulate target in rodent islets:
  - Inhibitors/Activators
  - siRNA/CRISPR
- Modulate target in zebrafish, mouse, pig models *in vivo*:
  - Inhibitors/Activators, morpholino conditional knockouts

**Humans**

- Verify target in human β cell lines (EndoC β H1)
- Modulate target in human islets:
  - Inhibitors/Activators
  - Adenovirus
- Pilot studies in humans:
  - Are there biomarkers suggestive of target activity?
  - Intervention studies
Can reduction of β-cell “stress” modify the course of T1D?

Inflammation

↑ 12-LOX

↓

Reactive Oxygen Species
ER Stress

Blockade of 12-LOX early in T1D pathogenesis may halt autoimmunity

12-LOX Signaling (Ox Stress) → β cell Stress/Death

Inflammation (Environment) → Autoimmunity

Proinsulin, Chromogranin, GAD65, Zn-T8, IA-2

ER stress → Neoantigen exposure

From: Maganti, et al, Islets 2014
12-LOX staining increased in T1D islets

Grzesik et al., JCEM 2015
Generation of Islet-specific 12-LOX Knockout Mice on a T1D prone strain of mice

- NOD-Alox15-fl/fl
- NOD-PdxPB-Cre
- NOD Islet-specific Knockout Mice

Piñeros,, et al. Unpublished
Islet 12-LOX knockout protects animals from type 1 diabetes with reduced insulitis

Males

Females

Piñeros, et al. Unpublished
12-LOX Inhibitors—Team Science

Screen Library (NIH/NCGC)
David Maloney, NIH/NCATS

Biochemistry in vitro
Ted Holman, UCSC

Zebrafish validation
IUSM

Human Islet validation
Jerry Nadler, EVMS

Preclinical validation in mouse models
IUSM
12-LOX Inhibitors exhibit species specificity

ML355
Human specificity

ML351
Mouse specificity

ML127
Human and Mouse
Zebrafish alox orthologs are inhibitable with ML compounds

### Zebrafish 12LOX IC₅₀ data

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ (uM)</th>
<th>Max Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML355</td>
<td>5.5+/−2</td>
<td>65%</td>
</tr>
<tr>
<td>ML127</td>
<td>0.34+/−0.3</td>
<td>33%</td>
</tr>
<tr>
<td>ML351</td>
<td>&gt;100</td>
<td></td>
</tr>
</tbody>
</table>

### Human 12LOX IC₅₀ data

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ (uM)</th>
<th>Max Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML355</td>
<td>0.34+/−0.1</td>
<td>95%</td>
</tr>
<tr>
<td>ML127</td>
<td>1.0+/−0.2</td>
<td>95%</td>
</tr>
<tr>
<td>ML351</td>
<td>&gt;50</td>
<td></td>
</tr>
</tbody>
</table>

Hernandez-Perez, et al. Unpublished
Mammals and fish have similar pancreata

Abhi Kulkarni

Ryan Anderson, PhD
A T1D Model in Zebrafish—no, really!

*Tg*(insulin:NTR)*s950* fish → Metronidazole (MTZ) → β-Cell Death

0 hr MTZ  1 hr MTZ  3 hr MTZ  6 hr MTZ  9 hr MTZ  12 hr MTZ

Kulkarni, et al. Ox Med Cell Longev 2018
Immune Cell Infiltration in the Zebrafish T1D Model

*Tg(mpeg:GFP)\textsuperscript{gl22} x Tg(insulin:NTR)\textsuperscript{s950} fish*

<table>
<thead>
<tr>
<th>Control</th>
<th>9 hrs ablation</th>
<th>12 hrs ablation</th>
</tr>
</thead>
<tbody>
<tr>
<td>mΦ:GFP Ins Gcg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mΦ:GFP Ins Gcg DNA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Kulkarni, et al. Unpublished
The Effect OF ML-355 on Immune Cell Homing in the Zebrafish T1D Model

Control (6hrs)  ML-355 (6hrs)

Kulkarni, et al. Unpublished
“Humanized” mice to test human 12-LOX inhibitors

Tersey, et al. Unpublished
ML355 protects against diabetes in humanized *ALOX12* mice

Tersey, et al. *Unpublished*
Where do we go from here? Building a 12-LOX Company (Veralox Therapeutics)

12-LOX - Chemical equity for previously undruggable target

Product of multiyear, multi-institution collaboration leading to a first-in-class, potent and selective 12-LOX inhibitor

Lead molecule in IND-enabling, next-gen work underway

On track to deliver IND at YE 2020, pipeline expansion through 2nd generation drug products in development

Initial work in HITT and T1D with future pipeline in a target

Attracted top tier strategic investors and key opinion leaders for both HITT and T1D, with future expansion into additional indications

HITT: Heparin-induced Thrombocytopenia and Thrombosis
T1D: Type 1 Diabetes
Lab members, collaborators, funding

Mirmira Lab
Sarah Tersey
Marimar Hernandez
Farooq Syed
Marisa Fisher
Ryan Anderson
Kara Benninger
Jennifer Nelson
Karishma Randhave
Esther Levasseur
Chris Reissaus
Annie Pineros-Alvarez
Abhishek Kulkarni
Cody Sorrell

IUSM
Emily Sims
Carmella Evans-Molina
Linda DiMeglio
Amelia Linnemann
Kieren Mather

ULB
Decio Eizirik

IBRI
Teresa Mastracci

EVMS
Jerry Nadler
Maggie Morris-Fears

PNNL
Tom Metz
Ernesto Nakayasu
BobbieJo Webb-Robertson

Mt. Sinai
Adolfo Garcia-Ocana
Donald Scott

Weill-Cornell
Laura Alonso

NIH National Institute of Diabetes and Digestive and Kidney Diseases
UC4 DK104166
R01 DK060581
R01 DK105588
P30 DK097512

JDRF Improving Lives. Curing Type 1 Diabetes.

IIDP Integrated Islet Distribution Program

nPOD Network for Pancreatic Organ Donors with Diabetes