Chapter 12

Clinical Trials for the Prevention of Type 1 Diabetes
Selected New Onset Trials as of 2012

- **Delay Loss C-peptide**
  - Cyclosporine
  - Anti-CD3
  - Anti-CD20 (B cells)
  - CTLA4-Ig (Co-stim block)
  - Cytoxan-ATG-GCSF

- **No Effect C-Peptide**
  - MMF
  - Daclizumab
  - Canakinumab
  - IL1-RA
  - Nasal Insulin

- **Accelerate Loss C-Peptide**
  - IL2 - Rapamycin
Trialnet/Immune Tolerance Network

NEW ONSET TRIALS

RELATIVES of PATIENTS TYPE 1 DM

1-800-HALT-DM1
Screen, \textit{in silico}, large chemical libraries of drug-like small molecules for their abilities to interact with I-A^g^7 structural pockets by high-throughput molecular docking.

**Molecular Docking**

- **Test high-scoring molecules**
- **Libary of small molecules**
- **Target protein**

**High-Throughput Screening**

- **Test all molecules**
- **Hits**

Rapid and economical

Expensive, false positives

Ostrov

No MHC specific compounds identified to date by conventional high throughput screening.
We can now predict type 1 diabetes and prevent in animal models.

We cannot now prevent type 1 diabetes in man.

PATHWAYS:

Immunomodulation/suppression

Antigen specific Rx

Environmental (e.g. diet, microbiome) intervention
General Paradigm

- Identify Genetic Susceptibility
- Detect Initial Autoantibodies/Other Immunologic
- Monitor Metabolic/Physiologic Decompensation
- Treat Overt Disease Prior to Morbidity/Mortality
- Basic/Clinical Research to Allow Prevention
Thoughts

- Prevention DM and Preservation B cells at onset Important
- Prediction high risk “easy”
  - 1 million in U.S. developing DM(>=2 Abs)
- Multiple Therapies animal models
- Explosion Immunotherapies man
- Outcome – Diabetes/ C-peptide/ continuous glucose monitoring
- International collaboration (ITN/Trialnet)
- NEED CLEARER THERAPEUTIC TARGETS (e.g. Primary Trimolecular Complexes) or Combinations
- NEED IMMUNOLOGIC BIOMARKERS EFFICACY
Prevention of Type 1 diabetes

**Primary:**
1. Autoimmunity
2. $\beta$-cell loss
3. Clinical diabetes

**Genetic susceptibility**

**Complications**

**Secondary**

**Tertiary**

Autoimmunity → $\beta$-cell loss → Clinical diabetes

Genetic susceptibility → No autoimmunity → Clinical remission

Rewers-BDC
Secondary Prevention

X Goal - induction of diabetes remission and preservation of C-peptide

X non-antigen-specific interventions

X antigen specific interventions
IDS Guidelines for Intervention Trials
Greenbaum and Harrison: Diabetes 52:1059, 2003

- Diagnosis ADA criteria
- Document: age, sex, pubertal, family history, glucose, bicarb, ketoacidosis, weight loss, symptoms, HbA1c, islet autoab, insulin Rx, HLA
- Phase I >=18
- GAD, IA-2, IAA(<2 wks), and if DM ICA C-peptide>=.2 nmol/L, early = <12 weeks from diagnosis
- >=2 year trials
- Randomize, blind, mask, safety review, tight control, and continue insulin
- 2 hr. AUC C-Peptide with meal tolerance test, no AM insulin except pump basal, fasting glucose 4-11.1 mmol/l
- Measure islet autoAb other immune with HLA
“Positive” Trials

ANTIGEN SPECIFIC
- Oral Insulin ?
  Skyler: TrialNet

IMMUNE MODULATION
- Anti-CD3
  Herold; Chatenoud: JDRF, ITN, TrialNet
  (Phase 3 not meeting HbA1c endpoints)
- Anti-CD20
  Peskovitz-TrialNet
- Abatacept (CTLA4-Ig)
  Wherret-TrialNet
Potential Timing of Intervention Studies

**GENETICALLY AT-RISK**

- GENETIC PREDISPOSITION
- BETA CELL INJURY
- MULTIPLE ANTIBODY POSITIVE
- LOSS OF FIRST PHASE INSULIN RESPONSE
- DYSGLYCEMIA
- NEWLY DIAGNOSED DIABETES
- C-Peptide
  - β-cell mass
- “PRE”-DIABETES
- DIABETES
- NEUALLY DIAGNOSED DIABETES
Residual β cell function in DCCT participants


855 with T1DM of 1-5 years

552 “non-responders” (65%)
[Sustacal stimulated C peptide < 0.2 pmol/ml]

303 “responders” (35%)
[Sustacal stimulated C peptide 0.2 - 0.5 pmol/ml]

274 intensive
278 conventional
138 intensive
165 conventional

Results
1. Intensively treated responders’ conversion to non-responders was delayed (p<0.001)
2. Within the intensively treated group, responders compared with non-responders had:
   A. Lower Hgb A₁c (p<0.01) for the first 4 years of the study
   B. 50% reduced risk for retinopathy progression
   C. Despite the lower Hgb A₁c, responders risk for severe hypoglycemia (seizure or coma) was 65% less than that of intensively treated non-responders

D. Harlan
ADA Workshop Report: C-Peptide is the Appropriate Outcome Measure for Type 1 Diabetes Clinical Trials to Preserve Beta Cell Function
Diabetes: 53:250-264, 2004

- DCCT: C-peptide stimulated >0.2 nmol/l
  - lower fasting glucose
  - Intensive Rx: less hypoglycemic coma – 6.6 versus 17.3/100 pt years
  - Less progression retinopathy with 9 years f/u 27.6% versus 43.5%

<table>
<thead>
<tr>
<th>C-peptide</th>
<th>Adult 1-5 yrs</th>
<th>Adult &gt;5 yrs</th>
<th>&lt;18 age 1-5 yrs</th>
<th>&lt;18 age &gt;5 yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;.2</td>
<td>48%</td>
<td>8%</td>
<td>33%</td>
<td>3%</td>
</tr>
<tr>
<td>&gt;.5</td>
<td>15%</td>
<td>2%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
C-Peptide Basic Information

- Secreted in 1 to 1 molar ratio with insulin
- Negligible first pass hepatic extraction
- High quality, specific assays that accurately measure the low levels of type 1 diabetes
- $1 \text{ ng/ml} = 0.331 \text{ nmol/l}$
- Detection limit $\approx 0.1 \text{ ng/ml}$ or $0.03 \text{ nmol/l}$
- $T_{1/2}$ for insulin and c-peptide are different
  - Insulin $\approx 3 \text{ min.}$, c-peptide $\approx 35 \text{ min.}$

Palmer 2004
Progressive Loss C-peptide Post Diagnosis (SEARCH Diab Care 2009)

DCCT
Fast >= .23 ng/ml
DESIGN OF STUDIES OF β-CELL PRESERVATION
Distribution of 2 h Peak Value From MMTT
As a Function of Age At Diagnosis

Density

Age < 12 y
Age 12 - 17 y
Age ≥ 18 y

Peak/Stimulated C-peptide nmol/L

Diabetes 53: 250-264, 2004

Palmer 2004
C-Peptide in DCCT

MMTT stimulation of 3736 T1DM patients, age 13-39 y.o., with diabetes of 1-15 years duration.

Palmer 2004
C-PEPTIDE IN DCCT SCREENED SUBJECTS

Diabetes 53: 250-264, 2004

Palmer 2004
C-PEPTIDE IN DCCT SCREENED SUBJECTS

Adolescents (N = 1304)

Duration of T1DM (years)

N = 466

Stimulated C-peptide (nmol/L)

0.0

0.1

0.2

0.3

0.4

0.5

0.6

0.7

0.8

0.9

1.0

11%

22%

67%

N = 838

0.4%

2.6%

97%

Diabetes 53: 250-264, 2004
Effect of Intensive vs Conventional Therapy on β-Cell Function


N = 303 With 1 – 5 y duration and C-Peptide 0.2 – 0.5 pmol/mL

Risk Reduction 57% (CI: 39, 71%)

P < 0.0001
<table>
<thead>
<tr>
<th>MMTT Stimulated C-peptide (nmol/l)</th>
<th>≥ 0.05</th>
<th>0.05-0.10</th>
<th>0.1-0.2</th>
<th>&gt;0.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Glucose (mg/dl)</td>
<td>222 ± 6</td>
<td>206 ± 12</td>
<td>217 ± 11</td>
<td>117 ± 6*</td>
</tr>
<tr>
<td>HbA₁C (%)</td>
<td>9.3 ± 0.1</td>
<td>9.8 ± 0.3</td>
<td>9.2 ± 0.2</td>
<td>8.4 ± 0.2*</td>
</tr>
<tr>
<td>Insulin Dose (u/kg)</td>
<td>0.78 ± 0.02</td>
<td>0.75 ± 0.04</td>
<td>0.64 ± 0.02*</td>
<td>0.52 ± 0.02*</td>
</tr>
</tbody>
</table>

* = p < 0.05

Palmer 2004
HbA_1C in Intensive Rx DCCT Patients

- Non-responders
- Responders


BENEFITS OF $\beta$-CELL PRESERVATION

DCCT Intensive Therapy Group

3+ Step Retinopathy Progression

Risk Reduction: 58% (CI: 27, 76)
$p < 0.001$

Year of Follow-up

Cumulative Incidence (%)

Non-responders

Responders

Non-Resp, N: 276
Respond, N: 138

Diabetes 53: 250-264, 2004

Palmer 2004
BENEFITS OF $\beta$-CELL PRESERVATION

DCCT Intensive Therapy Group

Sustained 3+ Step Retinopathy Progression

Risk Reduction: 79% (CI: 9, 95)

$p < 0.012$

Non-responders

Responders

Non-Resp, N: 276
Respond, N: 138

Year of Follow-up

Diabetes 53: 550-264, 2004

Palmer 2004
## Retinopathy and Nephropathy After 6 Years of DCCT Intensive Therapy Based upon Entry C-Peptide

<table>
<thead>
<tr>
<th>Stimulated C-peptide (nmol/l)</th>
<th>Undetectable</th>
<th>Minimal</th>
<th>Baseline only</th>
<th>Sustained</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 0.03</td>
<td>0.04 – 0.2</td>
<td>0.21 – 0.5 and ≤ 0.2 at 1 yr</td>
<td>0.21 – 0.5 at entry and 1 year</td>
<td></td>
</tr>
</tbody>
</table>

- **Retinopathy progression ≥ 3 step**: 4.6 times
- **Albuminuria**: 4.4 times

Diabetes Care 26:832-836, 2003

Palmer 2004
Benefits of β-Cell Preservation in DCCT Hypoglycemia with Coma/Seizure

62% Risk Reduction

Diabetes 53:250-264, 2004
Palmer 2004
Insulin Induced Hypoglycemia

C-Peptide Responders

C-Peptide Non Responders

Diabetes 37:81-88, 1988

Palmer 2004
Insulin Induced Hypoglycemia Counter Regulatory Responses in C-Peptide Responders and Non Responders.

Diabetes 37:81-88, 1988

Palmer 2004
General Immunomodulation/suppression
Trialnet Abetacept (CTLA4-Ig)  
Tihamer et al Trialnet Lancet June 28, 2011

- Patients new onset 6-45 years old
- Randomized placebo controlled trial with 2 years monthly infusions Abetacept (n=77) or Placebo (n=35)

**OUTCOME**
- C-peptide AUC at 2 years 59% higher (.378 nmol/L) versus placebo (.238)
- Delayed loss C-peptide only to 0-6 months, then parallel loss
- Lower HbA1c and lower insulin dose Abetacept group
Prevention and treatment of NOD diabetes with anti-CD20 mAb (Chang-yun et al, 2007)

hCD20 tg mice were treated 4x within 10 days with anti-hCD20 mAb

At 4 or 9 weeks of age:

At diagnosis:
Pescovitz et al. NEJM 2009
Rituximab, B-lymphocyte depletion, and preservation of beta-cell function.

![Graphs showing changes in C-Peptide, HbA1c, Insulin Dose, and B-Lymphs over 12 months of treatment with Rituximab compared to control groups.](image-url)
Insulin Needs after CD3-Antibody Therapy in New-Onset Type 1 Diabetes

Keymeulen et al, N. Engl J Med
2005; 352:2598-608

-- C-Peptide increase at 6 months compared to controls, decline thereafter but even at 18 months improved C-peptide, less insulin for treated patients.

-- Moderate cytokine release syndrome, reactivation of EBV infection with recovery, no persistent complications.
Anti-CD3 therapy in NOD mice
Effective at Onset

Chatenoud 1997

anti-CD3 only effective in recent-onset diabetic animals, a short therapeutic efficacy window.

splenocytes from treated “cured” mice still transferred disease into irradiated male NODs

cyclophosphamide induced disease relapse in “cured” mice 10-15 weeks after anti-CD3 therapy

anti-CD3 F(ab)'2 fragments were protective

cyclosporine A administration at the time of anti-CD3 therapy prevented therapeutic effect
Attempts to understand the effects of non-mitogenic anti-CD3 antibodies in vitro

**Alegre 1995**
- chimeric 2C11/mouse IgG3 antibody $\rightarrow$ non-mitogenic in vitro, effective in vivo without cytokine release

**Smith 1997**
- 2C11 IgG3 induces unresponsiveness to secondary challenge in T cell clones but not primary T cells, CsA blocks induction of unresponsiveness (anergy)
- 2C11 IgG3 induces partial TCR signaling events

**Smith 1998**
- 2C11 IgG3 selectively anergizes Th1 clones but not Th2 clones
- Proximal TCR signaling events in Th1 and Th2 clones are similar
Updated Data from Phase I/II Trial of Anti-CD3 in New Onset T1DM

Herold et al. Diabetes 54:1763-9 2005
TCR Stimulation with modified anti-CD3 mAb expands CD8+ T cell population and induces CD8+CD25+ Tregs
Bisikirska et al JCI 115:2904, 2005

Correlation CD4/CD8 in vitro versus ex vivo anti-CD3 Rx'd

Non-Responders

Responders

CD4/CD8 ratio in vitro

CD4/CD8 ratio in vivo

0 0.5 1 1.5 2 2.5

0 1 2 3 4
Belghith et al: TGF-b-dependent mechanisms mediate restoration of self-tolerance induced by antibodies to CD3 in overt autoimmune diabetes

Nat Med 9 (2003), 1202-1208
Modern anti-CD3 antibodies in clinical use

- YTH 12.5: Rat IgG2b monoclonal antibody specific for human CD3
- OKT3: Mouse IgG2a monoclonal antibody specific for human CD3
- ChAglyCD3: Molecular engineering - Asn297→Ala297
- hOKT3 Ala-Ala: Leu234→Ala234, Leu235→Ala235

Fathman
Initial hOKT3 Trial, Kevan Herold, Columbia Univ., NY

- <6 weeks off diagnosis; age 8 - 35
- 23 treated patients and 23 control subjects
- post-Sustacal C-peptide for 2 years
- non-FcR anti-CD3 mAb affects activated but not naïve T cells; appears to specifically anergize activated Th1/Tc1 cells
- problems:
  - Activation of T cells \textit{in vivo} following cross linking of the mAb.
  - Development of neutralizing antibodies to the murine mAb.
  - Transient depletion of T cells
Anti-CD3 Monoclonal Antibody in New-Onset Type 1 Diabetes Mellitus

Kevan C. Herold, MD; William Hagopian, MD, PhD; Julie A. Auger, BA; Ena Poumian-Ruiz, BS; Lesley Taylor, BA, David Donaldson, MD; Stephen E. Gitelman, MD, David M. Harlan, MD; Danlin Xu, PhD; Robert A. Zivin, PhD; & Jeffrey A. Bluestone, PhD

Changes from Study Entry to 12 Months in the Total C-Peptide Response to Mixed-Meal Tolerance Testing

Monoclonal-Antibody Group

Control Group

The Ratio of CD4+ and CD8+ T-Cells in the Monoclonal-Antibody Group According to the Presence or Absence of a Response to Treatment

### MAJOR PHASE 3 TRIALS DID NOT MEET PRIMARY ENDPOINT 2011

? Low dose Tolerx/ ?Multinational Population study
Macrogenics

<table>
<thead>
<tr>
<th>Name</th>
<th>Brand Name</th>
<th>Biotech</th>
<th>Pharma</th>
<th>Trial</th>
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<tbody>
<tr>
<td>hOKT3gamma1 (Ala-Ala)</td>
<td>Teplizumab</td>
<td>Macrogenics</td>
<td>Lilly</td>
<td>Protege</td>
</tr>
<tr>
<td>ChAglyCD3</td>
<td>Otelixizumab</td>
<td>Tolerx</td>
<td>GSK</td>
<td>DEFEND</td>
</tr>
</tbody>
</table>
Heat Shock Protein 60 enhances CD4+CD25+ regulatory T cell function via innate TLR2 signaling

Zanin-Zhorov, Cahalon, Tal, Margalit, Lider, Cohen

European Nicotinamide Diabetes Intervention Trial

EASD: No Prevention Progression to Diabetes 9/2002
Mycophenolate Mofetil (MMF) and Zenapax (DZB)

Peter Gottlieb, BDC & VMR

(<8 weeks off diagnosis)

- MMF protects BB rats from developing DM
- MMF effective in a number of autoimmune conditions and in transplantation
- DZB effective as part of transplantation regimens
- IL2-R+ cells increased at dx of DM, harbor autoreactive T cells (mouse and man)
- known toxicities of drugs are low

NO EFFECT NEW ONSET DIABETES TRIALNET STUDY
BCG Vaccination at Onset
Allen et al. 1999

Fasting C-Peptide

Age

< 12

>=12

Stimulated C-Peptide
Environment and progression
- BCG vaccination before age 1 month (n=206)

Islet autoantibody development

Diabetes development in Ab pos

$P = 0.01$

A. Ziegler

Huppmann, Diabetes Care 2005
Antigen Specific Rx
Insulin

- Beta Cell Specific
- Predominant T-cell reactivity islets NOD
- Insulin expressed lymphoid tissue by dendritic and macrophage-like cells
- Thymic messenger RNA for insulin related to “protective” insulin allele
- Proinsulin expression in thymus prevents NOD diabetes
“Pathogenic” Peptide: Insulin B:9-23
Structure of a human insulin peptide (B:9-23)- HLA-DQ8 complex and susceptibility to type 1 diabetes

Prevention of Diabetes with B:9-23 Peptide “Immunization” with adjuvant

D.Daniel, D.Wegmann. PNAS, 1996
Prevention of type 1 diabetes in mice by tolerogenic vaccination with a strong agonist insulin mimotope
Carolin Daniel,1,4 Benno Weigmann,3 Roderick Bronson,4 and Harald von Boehmer1,2
JEM 2011
**Insulin Peptide Induction Anaphylaxis**

**Liu et al. JCI 2002**

- Insulin B:9-23 in saline – 7 injections = death NOD
- Anaphylaxis dependent upon both IgG and IgE antibodies
  Histamine and Platelet Activating Factor
- Anaphylaxis following subcutaneous injection prevented with addition RR to peptide to produce peptide with neutral pl while peptide able to prevent diabetes of NOD mice
• double-blind Phase I/II trial is to test safety and efficacy of an altered insulin peptide ligand
• Patients will be randomized to one of two dosing schedules:
  ☐ Biweekly patients randomized to receive NBI-6024 (0.1mg, 1.0 mg, or 5.0mg) or placebo. Biweekly and monthly patients will be randomized to receive either NBI-6024 (1.0mg) or placebo. Study duration is up to 60 weeks.
  ☐ Eligible patients ages of 12-35 and diagnosed within the past 6 weeks.
Recent and Ongoing Antigen-specific Immunotherapy Trials in New Onset T1 DM

- DPT-1 Parenteral Insulin: No Effect
- DPT-1 Oral Insulin: No Effect/Subgroup?
- DIPP (intranasal): No Effect
- Italy/France Oral Insulin: No Effect
- Joslin/ITN Ins B chain in IFA: Immune Effects
- Altered Insulin B:9-23 peptide: No Effect
- hGAD s.c. in alum (Diamyd): No Effect (follow up)
DPT-1 Staging Scheme

**ICA Positive**
- HLA DQA1*0102/B1*0602 Not Eligible
- **IVGTT**
  - Low FPIR x 2
    - **OGTT Non-Diabetic**
      - Eligible Parenteral
    - IAA Positive
      - **OGTT**
        - IGT or IFG
          - Eligible Oral
        - Normal
          - Eligible Oral
- Intact FPIR
  - Eligible Oral
Parenteral Antigen Protocol

- Randomized, controlled, unmasked
- Experimental Group:
  4 days Continuous IV Insulin Infusion
  - at Baseline and yearly thereafter
  Low Dose Subcutaneous Insulin
  - 0.125 U/kg bid Human Ultralente
- Control Group: Close Observation

NO EFFECT: NEJM 346: 1685, 2002
DPT Parenteral Insulin Trial
NEJM 346:1685-1691, 2002

- 84,228 Relatives Screened
- 3152 ICA Positive
- 372 > 50% Projected 5 year risk
- 339 Randomized to Injection/Observation
- Diabetes: 69 Treated, 70 Observation group
- Insulin at dosage used in high risk no effect
- Multiple anti-islet autoantibodies predict DM

NO EFFECT: NEJM 346: 1685, 2002
Oral Antigen Protocol

- Randomized, controlled, double-masked
- Experimental Group: Oral Insulin
- Control Group: Matched Placebo
- Began Sept 1996

No Overall Effect: ? Major Subgroup
high insulin autoantibodies protection
Skyler Oral Presentation 2004 IDS
Insulin Antibodies in islet antibody-positive subjects given intranasal insulin

Oral Tolerance: Mode of Action

Oral Antigen

Protective Cytokines

Regulatory (Th2) Lymphocytes Producing Protective Cytokines

Inhibition of β-Cell Autoimmunity & Diabetes Prevention

Insulin Producing β-cells

Autoimmune Lymphocytes
Tertiary Prevention (early in clinical disease)

- Beta cell function
- Time

100 %

20%

Preserve Beta cells
STOP complications

Clinical onset of disease
Intensive Insulin Therapy

Continuous Glucose Monitoring Systems

Continuous Subcutaneous Insulin Infusion

Insulins
Effect of Intensive vs Conventional Therapy on β-Cell Function

N = 303 With 1 – 5 y duration and C-Peptide 0.2 – 0.5 pmol/mL
Risk Reduction 57% (CI: 39, 71%)
P < 0.0001

DPT-1 Oral Study – Time to Diabetes by Treatment

Survival Distribution Function

Years Followed

Years Followed

STRATA: Oral Insulin  Oral Placebo

P- Value = 0.176 (Log Rank Test)

Treated

Control

N = 186

N = 186

Survival Distribution Function

Diabetes Care 28:1068-76 2005
Insulin Effect Most Evident in Subjects with Baseline IAA ≥ 300

N=63 (Ins.) and 69 (Plac.)

Log-rank P=0.01
Peto Pr. P=0.01
Hazard Ratio: 0.41 (0.21, 0.80)
DIPPP Protocol

Main Cohort (n=38,000)

• Newborns screened for genetic risk
• High risk babies followed serially for ICA (n=81)
• ICA-positive children randomized to nasal insulin or placebo

No Effect
IMDIAB: Oral insulin cytokine and IgG subclass

- New onset trial, no preservation c-peptide
- Culture TGFbeta increased
  Culture IFNgamma decreased
- IgG1 Insulin antibodies decreased
  IgG3 insulin antibodies decreased
- IMMUNE EFFECT? Rx too late

Monetini et al. Diabetologia 47:1795, 2004
Prevention of Type 1 diabetes

Primary: 1a autoimmunity
           1b cell loss
           1c clinical diabetes

Autoimmunity → 1b → β-cell loss → 1c → Clinical diabetes

Genetic susceptibility → 1a → No autoimmunity → ? → Clinical remission

Complications → Tertiary

Secondary
Effect of Insulin Injections on Diabetes Frequency

BB/Wor RATS

% Diabetes

Control

Insulin

Gotfredsen
Effect of Insulin Injections on Diabetes & Insulitis

Female NOD Mice

- **% Diabetes**
  - Placebo: 60
  - Insulin: 10

- **Insulitis Score**
  - Placebo: 2.5
  - Insulin: 0.5

Atkinson
Diamy press release: European Phase III study with Diamyd® reported on May 9. The study did not meet the primary efficacy endpoint of preserving beta cell function at 15 months in patients newly diagnosed with type 1 diabetes, although a small positive effect was seen. Diamyd® was well tolerated as demonstrated by a similar number of adverse events in the Diamyd® treated groups as well as in the placebo treated group.

An ongoing parallel US Phase III study, DiaPrevent, was fully enrolled in December 2010, and results are expected in the summer of 2012.

DIAMYD GAD65 FOLLOW UP TRIALS

TRIALNET RANDOMIZED TRIAL NEGATIVE

No effect two or three doses GAD-alum on stimulated C-peptide loss, HbA1c, total insulin dose. Lancet June 27, 2011

PHASE 3 EUROPEAN TRIAL NEGATIVE

Diamy press release: European Phase III study with Diamyd® reported on May 9. The study did not meet the primary efficacy endpoint of preserving beta cell function at 15 months in patients newly diagnosed with type 1 diabetes, although a small positive effect was seen. Diamyd® was well tolerated as demonstrated by a similar number of adverse events in the Diamyd® treated groups as well as in the placebo treated group.

An ongoing parallel US Phase III study, DiaPrevent, was fully enrolled in December 2010, and results are expected in the summer of 2012.
GAD-Alum "Vaccine"

Ludvigsson et al NEJM 359:1909, 2008 GAD Treatment and Insulin Secretion in Recent-Onset Type 1 Diabetes
GAD Treatment and Insulin Secretion in Recent-Onset Type 1 Diabetes

Ludvigsson et al NEJM 2008: 359

Stimulated C-Peptide

Fasting C-Peptide

Figures of absolute values generated from Table 3
Central Nervous System Destruction Mediated by Glutamic Acid Decarboxylase-Specific CD4+ T Cells

Amanda R. Burton, Zachary Baquet, George S. Eisenbarth, Roland Tisch, Richard Smeyne, Greg J. Workman and Dario A. A. Vignali

J. Immunol. published online Mar 26, 2010; doi:10.4049/jimmunol.0903728

Anti-GAD TCR + or – B Lymphocytes

TCR Retrogenic Induction High Levels GAD65 Autoantibodies
Casein Hydrolysate 6-8 months

GAD  P=.74
ZnT8 P=.37

>=1 Antibody p=.03 (n=50)
>=2 antibodies p=.12 (n=25)

Knip et al NEJM Nov 2010
GAD $P = 0.74$

Znt8 $P = 0.37$

Knip et al. NEJM Nov 2010
TNF Blocker-Etanercept New Onset Type 1 Diabetes: 24 week Mastrandrea et al Diabetes Care 32:1244-1248, 2009
Trialnet/ITN

- Trailnet----- Oral Insulin Prevention Trial
- ITN--------- Anti-CD3
- Trialnet--- GAD65 in Alum
- Trialnet---Abetacept (CTLA4-Ig)
- IL2 and Rituximab
1. Non-myeloablative Autologous Bone Marrow (Cytox+ATG)
2. Cord Blood Autotransplant
3. Dendritic Cells
4. Regulatory T Cells
C-peptide levels and insulin independence following autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus.

Figure. Time course of total area under the curve (AUC) of C-peptide levels during mixed-meal tolerance test in 12 patients continuously insulin free and in 8 patients transiently insulin free.

- 22 No Ketoacidosis
- 20 Insulin Free
- 8 Transient

Couri et al JAMA 15:1573, 2009
Immunotherapy Trials in New Onset Type 1 DM

- MMF and DZB - Peter Gottlieb, TrialNet
- HSP 65 p277 s.c. - (Peptor) – Jerry Palmer, Seattle
- Multi-dose DZB - Henry Rodriguez, Indiana
- Exanitide and DZB – David Harlan, NIH
- Oral hIFN-alpha - Kristina Rother, NIH
- Anti-CD20 – Mark Peskovitz, Indiana, TrialNet
- Anti-CD3 – Protege Macrogenics
- Multidose anti-CD3 hOKT3 - Kevan Herold, ITN
- Rapamycin and IL-2, Greenbaum-ITN
- CTLA4Ig – Tihamer Orban, TrialNet
- GAD 65 in Alum – Diamyd
- Proinsulin DNA Vaccine – BayHill
- ATG (Sandostat) – Steve Gitelman, UCSF, ITN, TrialNet
- Gastrin and EGF – Phase I Trial
- Alpha 1 Anti-trypsin trials: Peter Gottlieb-BDC and ITN
Subset of Trials in New Onset Type 1 DM

- Horse anti-Thymocyte - no lasting effect
- Cyclosporine A - no lasting effect
- Imuran - no lasting effect
- Corticosteroids - no lasting effect
- Plasmapheresis - no lasting effect
- BCG (Denver) - no effect
- Nicotinamide (DENIS/ENDIT) - no effect (At risk)
- Gluten-free diet (Italy) - no effect
- Q fever vaccine s.c. - no effect
- HSP Peptide p277 - ?delay adults/no effect children
- hOKT3 gamma1 (Ala-Ala) - > 1 yr effect
- Anti-CD20 - => 0.5 yr effect
- Abetacept - = 0.5 yr effect
- Anti-CD3 Macrogenics - endpoint HbA1c/ins dose missed
Examples Non-antigen Specific Immunotherapy Trials in Type 1 DM

• MMF and DZB – Trialnet (Gottlieb) No effect
• Multidose anti-CD3 ITN X1 > 1yr effect (Herold and Chatenoud)
• HSP 65 p277 s.c. - (Peptor) – LADA ?
• Multi-dose DZB - Henry Rodriguez, Indiana ?
• Oral hIFN-alpha - Staley Brod, Texas ?
• NIP study “fish oil” - Trialnet (Chase) ?
• Nicotinamide Endit No Effect
• Rituximab (anti-B Cell) Trialnet X1 1 yr effect
Primary Prevention

- Xautoantibodies or diabetes as the endpoint
- Xavoidance of environmental agents?
- Xinduction of autoantigen tolerance?
Primary Prevention Trials

- DPT-1 - Parenteral/Oral Insulin
- DIPP - Nasal Insulin
- INIT - IntraNasal Insulin Trial
- ENDIT - Nicotinamide
- TRIGR - Casein Hydrolysate (Cow’s Milk Elimination)

Gleevec (Imatinib) reverses NOD diabetes
- Tyrosine kinase inhibitor (Abl, PDGFR, cKit, c-Fms)
Sunitinib reverses NOD diabetes
- Tyrosine kinase inhibitor (c-Kit, PDGFR, c-Fms)
PDGFR immunoglobulin inhibitor results in transient reversal NOD diabetes

Conclusion: Mechanism action inhibition inflammation by blocking PDGFR (Platelet derived Growth Factor Receptor) rather than T cell targeting of islets, leading to long-term remission diabetes of NOD
Role of the intestinal tight junction modulator zonulin in the pathogenesis of type 1 diabetes in BB diabetic-prone rats

Watts et al PNAS 102:2916, 2005

% Diabetic

- Zonulin Inhibitor FZI/0
- Control
What are we missing?

Assay for Pathogenic T cells.

? TETRAMER

? ELISPOT
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