CELLULAR THERAPY
for
DIABETES PREVENTION

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No conflict of interest
to be disclosed

Practical Ways to Achieve Targets in Diabetes Care.
Keystone, July 17, 2011
Insulin-Dependent Diabetes Mellitus (Type 1 Diabetes)

T1D is a systemic disease, characterized by hyperglycemia, hyperlipidemia and hyperamino-acidemia. It is caused by a decrease in the secretion of insulin, due to the destruction of the β cells of the pancreas. It is frequently associated with specific lesions of the microcirculation, neuropathic disorders and a predisposition to atherosclerosis.

Human islet of Langerhans with insulitis
BONE MARROW → BLOOD → THYMUS → T CELLS → THYMIC SELECTION

Anti-SELF → Anti-NON-SELF
BONE MARROW → BLOOD → THYMIC SELECTION → THYMUS → T CELLS → Anti - SELF

Anti - NON-SELF → 95% → 5%
Thymus-specific deletion of insulin induces autoimmune diabetes

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Insulin expression in the thymus has been implicated in regulating the negative selection of autoreactive T cells and in mediating the central immune tolerance towards pancreatic β-cells. To further explore the function of this ectopic insulin expression, we knocked out the mouse Ins2 gene specifically in the Aire-expressing medullary thymic epithelial cells (mTECs), without affecting its expression in the β-cells. When further crossed to the Ins1 knockout background, both male and female pups (designated as ID-TEC mice for insulin-deleted mTEC) developed diabetes spontaneously around 3 weeks after birth. β-cell-specific autoimmune destruction was observed, as well as islet-major histocompatibility complex (MHC) alleles are major contributors of genetic susceptibility to T1D (Todd et al., 1987; Morel et al., 1988). Autoreactive T cells are released into the circulation because of the faulty presentation of self-antigens by disease-susceptible MHC molecules, which hinder the negative selection process in the thymus (Trucco, 1992; McDevitt, 2001). To date, only two antigens solely expressed in β-cells have been identified: insulin and islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP) (Lieberman et al., 2003; Gianani and Eisenbarth, 2005). Cytotoxic T cells specific for either antigen were isolated from naïve NOD mice, and were able to transfer T1D to NOD–scid mice. However, insulin may have a primary function, because the immune response against IGRP could be prevented by inducing tolerance to [pro]insulin in NOD mice (Krishnamurthy et al., 2006). Indeed, in pre-diabetic patients, insulin-specific autoantibodies are detected frequently, long before the onset of clinical symptoms, and their affinities correlate with the progression of the autoimmune attack (Achenbach et al., 2004). Similarly, insulini-
AUTOIMMUNITY

BONE MARROW

BLOOD

THYMUS

T CELLS

VIRUS

Anti - SELF

Anti - NON-SELF

FAN et al. EMBO J, 28:2812, 2009
Type 1 diabetes onset: the pancreatic environment

Steady-state flux of APC through islets

Microenvironmental anomaly:
Activation and mobilisation of resident and extra-islet APC into islet

1

T-cell and cytokine-dependent
β cell impairment and apoptosis

Migration of activated APC to peripheral lymph nodes

2

β-cell apoptosis:
Uptake of autoantigens by APC

3

CD8+ Tc

Activation of autoreactive CD4+ and CD8+ T-cells in pancreatic lymph nodes

4

CD4+ Tc

Cytokine-induced β-cell impairment/
进一步的凋亡促进

5

6

Type 1 diabetes onset: the pancreatic environment

Steady-state flux of APC through islets

Microenvironmental anomaly: 
Activation and mobilisation of resident and extra-islet APC into islet

T-cell and cytokine-dependent 
\( \beta \) cell impairment and apoptosis

Migration of activated APC lymph nodes

\( \beta \)-cell apoptosis: 
Uptake of autoantigens by APC

pancreatic lymph nodes

Activation of autoreactive CD4\(^+\) and CD8\(^+\) T-cells in pancreatic lymph nodes

Cytokine-induced \( \beta \)-cell impairment/ 
Further apoptosis promotion

Type 1 diabetes onset: the pancreatic environment

Microenvironmental anomaly:
Activation and mobilisation of resident and extra-islet APC into islet

1. Steady-state flux of APC through islets

2. Delta cell

3. Migration of activated APC to peripheral lymph nodes

4. Beta-cell apoptosis: Uptake of autoantigens by APC

5. Cytokine-induced beta-cell impairment/further apoptosis promotion

6. Activation of autoreactive CD4+ and CD8+ T-cells in pancreatic lymph nodes

- Alpha cell
- Beta cell
- APC
- Delta cell
- T-cell and cytokine-dependent beta-cell impairment and apoptosis
- Cytokines
- CD4+ Tc
- CD8+ Tc

CD4+ T cell activation is regulated by signals derived from the TCR/CD3/CD4 complex and the CD40L and CD28/CTLA-4 co-stimulatory molecules.
Co-stimulation blockade as the means to force DC to become regulatory.

Experimental approach:
Propagate dendritic cells (DC), the most powerful APC, from 4-5 week-old female NOD mouse bone marrow progenitors cultured in GM-CSF (4 ng/mL) and IL-4 (1000 U/mL).

Treat bone-marrow-derived DC with a combination of CD40, CD80 and CD86 phosphorothioate-phosphorothioate-modified oligo-deoxyribonucleotides, targeting the 5’ terminal regions of each primary transcript: AS-ODN. Then re-infuse them.
In vivo trafficking of fluorescently labeled DC

1. DC’s were generated and labeled with 505nm FluoSpheres or 800nm Qdots.

2. Two million DC’s were injected subcutaneously “near“ the pancreas.

3. Mouse organs were harvested on day 2.
In vivo trafficking of fluorescently labeled DC

Organs

- 1 Pancreatic LN (circled)
- 1 Mesenteric LN
- 2 Spleen
- 3 Pancreas
- 4 Large Intestine
- 5 Liver
- 6 Adipose Tissue
- 7 Thymus
- 8 Lung
- 9 Kidney
In vivo trafficking of fluorescently labeled DC

* = Note that the rodent food is auto-fluorescent producing signal in the intestine.
Type 1 diabetes onset: the pancreatic environment

1. Steady-state flux of APC through islets

2. Microenvironmental anomaly: Activation and mobilisation of resident and extra-islet APC into islet

3. β-cell apoptosis: Uptake of autoantigens by APC

4. Cytokine-induced β-cell impairment/further apoptosis promotion

5. Migration of activated APC to peripheral lymph nodes

6. Activation of autoreactive CD4+ and CD8+ T-cells in pancreatic lymph nodes

T-cell and cytokine-dependent β cell impairment and apoptosis
Reversal of hyperglycemia in new onset diabetes NOD Mice with multiple AS-ODN DC administrations

![Graph showing blood glucose levels over time for different mice.](image)

- **Start of Insulin**
- **End of insulin**
- **First DC injection**

- Blood glucose (mg/dL)
- Time (weeks)

- **8 injections, 1/week**

Giannoukakis et al. unpublished results
Whole Monkey Picture 24 hours post injection

- Internal Localization
- Injection Sites

Head

Feet
Unlabeled DC

Spleen

Pancreas Head

Pancreas Tail

Fluorescence-labeled DC

Spleen

Pancreas Head

Pancreas Tail

Monkey 12-09-09

Monkey 3-15-10
To confirm that autologous diabetes suppressive dendritic cells can be engineered and without side-effects.

1. Obtain leukocytes via apheresis
2. Engineer DC towards a “diabetes-suppressive” capacity under GMP/GLP conditions; provide mixture of AS-ODN (CD40/CD80/CD86)
3. Test potency, sterility and divide into individual administration aliquots
4. Administer to volunteer intradermally
5. Immunological, biochemical, physiologic monitoring to establish safety

To confirm that intradermal administration of autologous diabetes suppressive dendritic cells (DC) is safe, non-toxic and without side-effects.

1. Obtain leukocytes via apheresis
2. Engineer DC towards a “diabetes-suppressive” capacity under GMP/GLP conditions; provide mixture of AS-ODN (CD40/CD80/CD86)
3. Test potency, sterility and divide into individual administration aliquots
4. Administer to volunteer intradermally
5. Immunological, biochemical, physiologic monitoring to establish safety

Eligibility criteria:

a) Written informed consent conforming to the institutional guidelines obtained from the patient.
b) Documented evidence of insulin-requiring type 1 diabetes of >5 years duration.
c) Adequate immune competence, as indicated by positive reaction to one or more of the common DTH skin tests that are part of the Multitest CMI™ test system (Pasteur-Merieux Connaught) and as indicated by the manufacturer. Proof of vaccination for tetanus no more than 10 years before.
d) Age 18-35.
da) Adequate hematologic function:
   1) Absolute neutrophil count > 1,000/mm³
   2) Absolute lymphocyte count > 1,000/mm³
db) Hemoglobin > 9 gm/dl
dc) Platelets > 100,000/mm³
dh) Liver function tests: -Bilirubin (total) < 1.7 mg/dl
   -Alkaline phosphatase < 78 u/L (2 x ULN)
   -SGOT < 54 u/L (2 x ULN)
   -Lactic dehydrogenase < 180 u/L (2 x ULN)
di) Kidney profile:-Serum electrolytes: a) Sodium 135-145 mEq/L; b) Potassium 3.5-5.0 mEq/L;
   c) Bicarbonate 21-28 mEq/L; d) Chloride 100-108 mmol/L;
   e) Serum creatinine <4.5 mg/dL (3 x ULN);f)BUN 8-25 mg/dL
dj) No prior history of radiation therapy, immunotherapy, or chemotherapy
dk) Evidence of prior immunization to tetanus
dl) Absence of HIV, HPV, Hepatitis B and C, Cytomegalovirus, and Epstein-Barr virus
dm) At least four weeks since any prior radiation, immunotherapy or chemotherapy
Eligibility criteria:

a) Written informed consent conforming to the institutional guidelines obtained from the patient.

b) **Documented evidence of insulin-requiring type 1 diabetes of >5 years duration.**

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j) No prior history of radiation therapy, immunotherapy, or chemotherapy

k) Evidence of prior immunization to tetanus

l) Absence of HIV, HPV, Hepatitis B and C, Cytomegalovirus, and **Epstein-Barr virus**

m) At least four weeks since any prior radiation, immunotherapy or chemotherapy
Exclusion criteria:

a) One or more of the Eligibility Criteria are not met.
b) A significant history or current evidence of cardiac disease including, but not limited to, congestive heart failure, coronary artery disease, angina pectoris, uncontrolled hypertension, serious arrhythmias; or myocardial infarction within the previous six months.
c) Evidence of active infection requiring antibiotic therapy.
d) History of other concurrent diseases.
e) Pregnant or lactating women.
f) Patients requiring systemic corticosteroids.
g) Any other immune disorder including but not limited to other autoimmune diseases like rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, or ankylosing spondylitis.
h) Pregnancy.
i) History of radiation therapy, immunotherapy, or chemotherapy.
j) Breastfeeding.

The following therapies cannot be administered while patients are undergoing treatment on this protocol:

a) Radiation therapy.
b) Chemotherapy.
c) Corticosteroids (except when administered in life-threatening circumstances), other particle or cell vaccine therapies.
Requirements for DC Isolation: The GMP Facilities

15th Floor BST

= Good Manufacturing Practice
NIH-funded, IRB- and FDA-approved Safety Study

To confirm that intradermal administration of autologous diabetes-suppressive DC is safe, non-toxic and without side-effects.

Major Concerns:

i) Hypersensitivity of different grades
ii) “Cytokine storm” effects
iii) Induction of other autoimmunity
iv) Compromise anti-pathogen immunity
v) Rash
vi) Vitiligo
<table>
<thead>
<tr>
<th>Biochemistry (blood+urine):</th>
<th>Hematology</th>
<th>Immune Profiling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>WBC, RBC, Reticulocytes, Hgb, HCT, MCV, MCH, MCHC, RDW, Mean platelet volume</td>
<td>Autoantibodies: ANA, anti-thyroglobulin IA-2, GAD</td>
</tr>
<tr>
<td>Alkaline Phosphatase</td>
<td></td>
<td>Cytokine panel</td>
</tr>
<tr>
<td>Alanine Aminotransferase</td>
<td></td>
<td>FACS analysis:</td>
</tr>
<tr>
<td>Aspartate Aminotransferase</td>
<td></td>
<td>B220+ CD11c-</td>
</tr>
<tr>
<td>Fasting C-peptide</td>
<td>Automated differential</td>
<td>CD3+ CD4+/CD3+ CD8+</td>
</tr>
<tr>
<td>Creatinine</td>
<td>Poly</td>
<td>CD3+ CD4+ CD44+/CD3+ CD8+</td>
</tr>
<tr>
<td>Glucose</td>
<td>ABS Poly</td>
<td>CD4+ CD4+ CD44+ CD8+ CD8+ CD44+</td>
</tr>
<tr>
<td>HBA1c (glycosylated)</td>
<td>Lymphocytes</td>
<td>CD4+ CD45RA+/CD8+ CD45RA+</td>
</tr>
<tr>
<td>Insulin levels</td>
<td>ABS Lymphocytes</td>
<td>CD4+ CD69+/CD8+ CD69+</td>
</tr>
<tr>
<td>Insulin requirements</td>
<td>Monocytes</td>
<td>CD4+ CD4+ CD45RA+ CD69+/CD8+ CD45RA+ CD69+</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>ABS Monocytes</td>
<td>CD4+ CD83+ HLA-DR+</td>
</tr>
<tr>
<td>Lipids (HDL, VLDL, LDL, cholesterol, TG)</td>
<td>ABS Eosinophils</td>
<td>CD83+ CD11c+</td>
</tr>
<tr>
<td>Electrolytes (Na, K, Cl, CO₂)</td>
<td>Basophils</td>
<td>CD83+ CD11c+ HLA-DR+</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>ABS Basophils</td>
<td>CD4+ CD25^{HIGH} FOXP3+</td>
</tr>
<tr>
<td>Thyroglobulin</td>
<td>Platelets</td>
<td>ELISPOT (IFNg)-MabTech</td>
</tr>
<tr>
<td>Total protein</td>
<td></td>
<td>ELISPOT (CEF peptide pool)-MabTech, MLR, cytokine panel in MLR</td>
</tr>
<tr>
<td>TSH</td>
<td>Prothrombin time</td>
<td></td>
</tr>
<tr>
<td>BUN</td>
<td>Activated PTT</td>
<td></td>
</tr>
</tbody>
</table>

RESULTS IN 10 PATIENTS (3 months after last DC administration) OUT OF THE 16 ENROLLED
<table>
<thead>
<tr>
<th>Patient Roster</th>
<th>Treatment Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>P001 Complete</td>
<td>AS-ODN DC</td>
</tr>
<tr>
<td>P002 Complete</td>
<td>Control DC</td>
</tr>
<tr>
<td>P004 Complete</td>
<td>AS-ODN DC</td>
</tr>
<tr>
<td>P006 Complete</td>
<td>AS-ODN DC</td>
</tr>
<tr>
<td>P008 Complete</td>
<td>AS-ODN DC</td>
</tr>
<tr>
<td>P009 Complete</td>
<td>Control DC</td>
</tr>
<tr>
<td>P010 Complete</td>
<td>AS-ODN DC</td>
</tr>
<tr>
<td>P012 Complete</td>
<td>AS-ODN DC</td>
</tr>
<tr>
<td>P013 Ongoing</td>
<td>AS-ODN DC</td>
</tr>
<tr>
<td>P014 Ongoing</td>
<td>Control DC</td>
</tr>
</tbody>
</table>

Failed Initial Screening:
PO03, PO05, PO07, PO11, PO15, PO16
CLINICAL AND LABORATORY EVALUATIONS IN ESTABLISHED DIABETICS: BASELINE, THERAPY-CONCURRENT AND POST-TREATMENT FOR STUDY DURATION (1 year)

RESULTS IN 10 PATIENTS (3 months after last DC administration) OUT OF THE 16 ENROLLED: NO SIGNIFICANT DIFFERENCES COMPARED TO BASELINE, PRE-TREATMENT VALUES

Dear Drs. Witten and Schneider:

RE: Outcome and reporting of data of phase I study entitled "Autologous Dendritic Cell Therapy for Type 1 Diabetes Suppression: A Safety Study" and approved under FDA IND # BB-12858.

Rachel Witten, MD  
Division of Clinical Evaluation and Pham/Tox  
Office of Cellular, Tissue and Gene Therapies/CBER  
Food and Drug Administration  
1401 Rockville Pike  
Rockville, MD 20852-1448

Bruce S. Schneider, MD  
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August 19, 2010

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Biochemistry (blood+urine):

- Albumin
- Alkaline Phosphatase
- Alanine Aminotransferase
- Aspartate Aminotransferase
- Fasting C-peptide
- Creatinine
- Glucose
- HBA1c (glycosylated)
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Hematology:

- WBC, RBC, Reticulocytes, Hgb, HCT, MCV, MCH, MCHC, RDW, Mean platelet volume
- Automated differential
- Poly
- ABS Poly
- Lymphocytes
- ABS Lymphocytes
- Monocytes
- ABS Monocytes
- Eosinophils
- ABS Eosinophils
- Basophils
- ABS Basophils
- Platelets

Activated PTT

Immune Profiling (peripheral blood/serum)

Autoantibodies: ANA, anti-thyroglobulin IA-2, GAD

Cytokine panel

FACS analysis:

- B220+ CD11c- CD3+ CD4+ CD8+
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- CD4+ CD45RA+ CD8+ CD45RA+
- CD4+ CD69+ CD8+ CD69+
- CD83+ HLA-DR+
- CD83+ CD11c+
- CD83+ CD11c+ HLA-DR+
- CD4+ CD25 HIGH FOXP3+

ELISPOT (IFNg) - MabTech

ELISPOT (CEF peptide pool) - MabTech, MLR, cytokine panel in MLR
### Insulin Requirements:

- **Lantus**: 30 Units Daily
- **Humalog**: 2-5 Units Daily
- **Total Daily**: 0.5-0.6 units/kg

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**Subject 001**: This patient was a 21-year-old male with established diabetes. He met all inclusion criteria and had no exclusion criteria. I was familiar with this patient from his earlier clinical course. There were no difficulties experienced during his series of intradermal injections. During follow-up, he had no experiences outside routine activities of daily living. He had no acute changes in his diabetes control. He had no other acute illnesses which met the standard of an adverse event. His examinations during the course of the study were unchanged from his initial examination. He successfully completed the study and had no negative comments. He received 30 units Lantus long-acting insulin with multiple daily injections of Humalog. His average insulin dose remained unchanged throughout study. If eligible, he has requested to continue participation for phase 2.
Blood Glucose (mg/dL)
HbA1c Glycosylation
Anti-GAD65 Antibody Titer

Antibody Titer (IU/ml)

Time Post Initial Treatment (Weeks)
C-peptide
Concentration (ng/ml)
Treg Cells

FoxP3+ CD25 High CD4+

Percentage of FoxP3+ CD25 High in CD4+ PBMC (%) vs. Time Post Initial Treatment (Weeks)
Breg Cells?
PHENOTYPES OF Breg IN MICE
(Mauri & Ehrenstein, *Trends in Immunology*, 29: 34, 2007)

1) CD19+ CD43- CD21^{HIGH} CD23+ CD24^{HIGH} IgD+ IgM+
   [produce IL-10]

2) CD19+ CD1d+ CD21+ CD23- IgM+ CD24+ CD62L+
   [produce IL-10 and/or TGF\beta, induce Treg]

3) *B220+ CD19+ CD43- IgM+ [produce IL-10]*

4) CD19+ CD43+ CD5+
   [produce IL-10 and IFN\alpha]

5) CD19+ CD43- CD80+ CD86+ CD40+
   [produce IL-10, induce Treg]

6) *CD19+ CD1d+ CD5+ [produce IL-10]*
Putative Breg content in pancreatic LN in response to AS-ODN DC s.c. administration in vivo (n=3 mice)
Putative B_{reg} content in pancreatic LN in response to AS-ODN DC s.c. administration *in vivo* (n=3 mice)

Column-enriched B220+CD19+ from pancreatic lymph nodes

Putative B_{reg} (CD1d+CD5+B220+CD19+)

dendritic cell treatment in vivo (NOD mice)
**B_{reg} Cell Population 3 Days Post DC Injection**

- Two populations of IL-10+ B220+ CD19+ were found.

- The IL-10+ B220^{H+} CD19^{H+} B cell population (P30) increased with AS-ODN DC administration compared to control DC.

- n = 3 mice p = 0.0544
Cell Proliferation in a Mixed Lymphocyte Reaction (MLR)

with

**Breg cells from AS-ODN Treated Mice**

\[ T = \text{CD4}^+ \text{T-cells} \quad (n = 200,000) \]

\[ S = \text{Allo-Splenocytes} \quad (\text{irradiated}; \ n = 200,000) \]

\[ B = \text{B-cells from AS-ODN P30} \quad (n = 20,000) \]

\[ B-\text{high} = \text{B-cells from AS-ODN P30} \quad (n = 200,000) \]

*** where \( p \leq 0.0001 \)
DC-activated B_{regs} in Humans

<table>
<thead>
<tr>
<th>Condition</th>
<th>PBMC</th>
<th>T-cells</th>
<th>DC-Bregs 1:10</th>
<th>Anti-IL-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent BrdU Positive (%)</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Phase I (Safety) Study of Autologous Tolerogenic Dendritic Cells in Type 1 Diabetic Patients

NICK GIANNOUKAKIS, PHD1,2 BRET PHILLIPS, PHD1 DAVID FINEGOLD, MD3 JO HARNABA, MS1 MASSIMO TRUCCO, MD1

OBJECTIVE—The safety of dendritic cells to selectively suppress autoimmunity, especially in type 1 diabetes, has never been ascertained. We investigated the safety of autologous dendritic cells, stabilized into an immunosuppressive state, in established adult type 1 diabetic patients.

RESEARCH DESIGN AND METHODS—A randomized, double-blind, phase I study was conducted. A total of 10, otherwise generally healthy, insulin-requiring type 1 diabetic patients between 18 and 60 years of age, without any other known or suspected health conditions, received autologous dendritic cells, unmanipulated or engineered ex vivo toward an immunosuppressive state. Ten million cells were administered intradermally in the abdomen once every 2 weeks for a total of four administrations. The primary end point determined the proportion of patients with adverse events on the basis of the physician’s global assessment, hematology, biochemistry, and immune monitoring for a period of 12 months.

RESULTS—The dendritic cells were safely tolerated. There were no discernible adverse events in any patient throughout the study. Other than a significant increase in the frequency of peripheral B220+ CD11c− B cells, mainly seen in the recipients of engineered dendritic cells during the dendritic cell administration period, there were no statistically relevant differences in other immune populations or biochemical, hematological, and immune biomarkers compared with baseline.

CONCLUSIONS—Treatment with autologous dendritic cells, in a native state or directed ex vivo toward a tolerogenic immunosuppressive state, is safe and well tolerated. Dendritic cells upregulated the frequency of a potentially beneficial B220+ CD11c− B-cell population, at least in type 1 diabetes autoimmunity.

Our preclinical data in the NOD mouse strain demonstrating prevention and reversal of type 1 diabetes with costimulation-impaired, immunosuppressive dendritic cells (bone marrow-derived dendritic cells treated ex vivo with a mixture of antisense oligonucleotides targeting the primary transcripts of CD40, CD80, and CD86) compelled us to determine the safety of, and possible immune reactions against, such dendritic cells in humans. We therefore generated human dendritic cells analogous to the ones successfully used in those NOD studies (6), concurrently targeting the expression of the same costimulatory molecules ex vivo, envisaging type 1 diabetes cell therapy. We hypothesized that immunosuppressive dendritic cells would primarily be safe and well tolerated and, secondarily, could alter the frequency of immune cell populations potentially beneficial in type 1 diabetes.

RESEARCH DESIGN AND METHODS—This phase I study...
Dear Dr. Trucco,
We have reviewed the Phase 1 safety data you have submitted, and we did not detect any overt safety signals. We feel that the Phase 1 safety data provide an acceptable safety justification for progression to a Phase 2 study.

Sincerely,
Rachel.

Rachel Witten, MD
Division of Clinical Evaluation and Pham/Tox
Office of Cellular, Tissue and Gene Therapies/CBER
Food and Drug Administration
1401 Rockville Pike
Rockville, MD 20852-1448

Phone: 301-827-9134

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Once “safety” is confirmed, “efficacy” will be determined.

Aims of Phase II:

- To improve the physiological indices of functional beta cell mass in new-onset diabetics (MMTT; Glucose AUC, C-peptide, HbA1c, Insulin administrations).

- To reduce the titer of, or eliminate the presence of diabetes-associated auto-antibodies in new-onset diabetics.

- To increase the prevalence of putative regulatory T- and B-cells in new-onset diabetics.
Working principle:
“Rescue and/or regeneration of functional residual beta cell mass can reverse type 1 diabetes”

A number of studies in the NOD mouse model have proven that residual beta cell mass can be rescued by interfering with autoimmunity; prevention of further loss of functional mass facilitates resumption of euglycemia.

Rescue of residual beta cell mass may concurrently facilitate endocrine cell regeneration replenishing lost/dysfunctional beta cell mass to variable degrees; this too can participate in “reversal” of new-onset disease.

Adapted from:
β Cells Can Be Generated from Endogenous Progenitors in Injured Adult Mouse Pancreas

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DOI 10.1016/j.cell.2007.12.015

SUMMARY

Novel strategies in diabetes therapy would obviously benefit from the use of beta (β) cell stem/progenitor cells. However, whether or not adult β cell progeni-
Conversion of adult pancreatic α-cells to β-cells after extreme β-cell loss

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Pancreatic insulin-producing β-cells have a long lifespan, such that in healthy conditions they replicate little during a lifetime. Nevertheless, they show increased self-duplication after increased metabolic demand or after injury (that is, β-cell loss). It is not known whether adult mammals can differentiate (regenerate) new β-cells after extreme, total β-cell loss, as in diabetes. This would indicate differentiation from precursors or another heterologous (non-β-cell) source. Here we show β-cell regeneration in a transgenic model of diphtheria-toxin-induced acute selective near-total β-cell ablation. If given insulin, the mice survived and showed β-cell mass augmentation with time. Lineage-tracing to label the glucagon-producing α-cells before β-cell ablation tracked large fractions of regenerated β-cells as deriving from α-cells, revealing a previously disregarded degree of pancreatic cell plasticity. Such inter-endocrine spontaneous adult cell conversion could be harnessed towards methods of producing β-cells for diabetes therapies, either in differentiation settings in vitro or in induced regeneration.

In vivo adult lineage reprogramming (transdifferentiation)—the notion that adult differentiated cells can change fates from one cell type to another—has had little experimental support from mouse models1,2. In the pancreas, nevertheless, there is evidence of induced exocrine acinar cell reprogramming: ectopic expression of pro-endocrine factors resulted in conversion of acinar cells into insulin-producing β-cells3, allowing us to explore whether new insulin-producing cells can emerge from other sources than pre-existing β-cells, as these were almost totally depleted. For this purpose, we used two in vivo genetic approaches: cell ablation combined with cell lineage tracing21,22. We created a model of inducible, rapid β-cell removal (>99%) by administration of diphtheria toxin (DT)22,23. In mice, the transgenic expres-
Once “safety” is confirmed, “efficacy” will be determined.

Aims of Phase II:

- To improve the physiological indices of functional beta cell mass in new-onset diabetics (MMTT; Glucose AUC, C-peptide, HbA1c, Insulin administrations).

- To reduce the titer of, or eliminate the presence of diabetes-associated auto-antibodies in new-onset diabetics.

- To increase the prevalence of putative regulatory T- and B-cells in new-onset diabetics.
These endpoints should, at the stage of the disease being investigated, be measures of residual endogenous insulin secretion, preferably through dynamic testing because measuring C-peptide during fasting or at random times will not show the small increments in function or mass that might be achieved. In our trial with anti-CD3, we used the hyperglycaemic clamp—the gold standard for measurement of functional β-cell mass—but this test is cumbersome and cannot be done in all centres. The mixed meal tolerance test used by Wherrett and colleagues is well established and standardised to allow reliable conclusions about stimulated C-peptide secretion in large multicentre trials (table). Readouts, such as HbA$_{1c}$ or rate of hypoglycaemia, that agencies might like (owing to their experience in pharma-driven trials on new agents) are not helpful in patients with recent-onset, C-peptide-positive type 1 diabetes.
Study title: Autologous co-stimulation-impaired dendritic cells for T1DM mellitus therapy: A randomised, double-blind phase II efficacy trial to maintain and improve functional beta cell mass in new onset disease

Study PI: Massimo Trucco; Primary Institution: University of Pittsburgh School of Medicine; Children's Hospital of Pittsburgh, Pittsburgh, PA; Potential collaborating sites: University of Florida at Gainesville (Gainesville, FL) and Stanford University (Palo Alto, CA), USA

We have discovered, in a phase I study (clinicaltrials.gov identifier NCT00445913) in established adult insulin-requiring T1DM patient volunteers, that autologous unmanipulated (control DC) and co-stimulation-impaired dendritic cells (co-treated ex vivo with a mixture of antisense DNA oligonucleotides targeting the 5' end of the CD40, CD80 and CD86 primary transcripts; immunoregulatory DC - iDC) are safe and well-tolerated. In this trial, we discovered that C-peptide made a transient but real re-appearance in 2/7 iDC recipients, that the same 2/7 iDC recipients maintained the lowest HbA1c among all volunteers, that the same 2/7 individuals exhibited an increase in putative "B-regulatory cells" during the duration of the iDC administration, and that one of these two individuals exhibited a dramatic decrease in anti-GAD antibody levels post-treatment. Although these outcomes were not the goal of in the phase I study and are not statistically-significant, they do portend to a possible benefit in new-onset disease where residual beta cell mass is expected to be considerably higher than in already established diabetics.

Study design: To achieve an any-pair power of 0.90 using the Tukey-Kramer multiple comparison test with and error rate of 0.05 and a standard deviation of 1, 66 patients with new-onset T1DM, between 16-35 years of age who meet all inclusion criteria are eligible for enrollment in the study. They will be randomised into three treatment arms: i) placebo recipients (n=8); ii) control DC (n=8); iii) iDC (n=8). The patients assigned to the DC arms will receive 1 x 10^7 cells (control DC or iDC) delivered as four independent intradermal injections of 2.5 x 10^6 cells at a pancreas-proximal anatomic site (abdominal quadrant subserved by the viscera overlying the pancreas in the anterior abdomen). A total of four vaccines at 2-week intervals will be delivered. If there is no grade 3 or 4 toxicity observed, successive vaccinations will be delivered as planned. For the study, dose-limiting toxicities will be defined as grade 3 or greater, with the following exceptions: chills, malaise and fever are not considered dose limiting. All grade 4 toxicities will be dose limiting, with the exception of lymphocytopenia. To protect patients against serious adverse effects of therapy the stopping rule will be observed, as described further below.
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**Study design:** To achieve an any-pair power of 0.90 using the Tukey-Kramer multiple comparison test with and error rate of 0.05 and a standard deviation of 1, 66 patients with new-onset T1D, between 8-35 years of age who meet all inclusion criteria are eligible for enrollment in the study. They will be randomized into three treatment arms: i) placebo recipients (n=22); ii) control DC (n=22); iii) iDC (n=22). The patients assigned to the DC arms will receive 1 x 10^7 cells (control DC or iDC) delivered as four independent intra-dermal injections of 2.5 x 10^6 cells at a pancreas-proximal anatomic site (abdominal quadrant sub-served by the viscera overlying the pancreas in the anterior abdomen). A total of four vaccines at 2-week intervals will be delivered. If there is no grade 3 or 4 toxicity observed, successive vaccinations will be delivered as planned. For the study, dose-limiting toxicities will be defined as grade 3 or greater, with the following exceptions: chills, malaise and fever are not considered dose limiting. All grade 4 toxicities will be dose limiting, with the exception of lymphocytopenia. To protect patients against serious adverse effects of therapy the stopping rule will be observed, as described further below.
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offering hope for patients. Yet hope is an unsafe friend. In view of some disappointing results from phase 3 immune intervention trials in type 1 diabetes that failed to meet their primary endpoints, particular care seems warranted to avoid raising false expectations among patients and physicians.
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Trucco, J. Clin. Invest. 115: 5-12, 2005