

Perspectives in Diabetes

Guidelines for Intervention Trials in Subjects With Newly Diagnosed Type 1 Diabetes

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Type 1, or insulin-dependent diabetes, is an autoimmune disease that culminates in the destruction of insulin-producing β -cells in the islets of the pancreas. Studies in the nonobese diabetic (NOD) mouse model of spontaneous type 1 diabetes provide “proof-of-concept” that the disease is preventable (1). People with type 1 diabetes and their relatives, researchers, government, and industry are eager to move forward and test candidate intervention/prevention therapies in humans. Such therapies may entail risks, including accelerated loss of β -cell function, malignancy, and infection. Scientifically and ethically, investigators are obliged to maximize the information gained from intervention trials and minimize risks. One way of achieving this is by standardizing trial protocols. Standardization of islet autoantibody assays (2–13) and of the intravenous glucose tolerance test for measuring first-phase insulin response (14–18) has been a major advance, allowing stratification for disease risk among relatives. The literature on intervention trials in newly diagnosed type 1 diabetic patients (19–44) reveals that entry criteria, trial design and duration, and outcome measures differ considerably. Adoption of standardized protocols would permit comparative and pooled data analysis and facilitate evaluation of potential therapies.

Our purpose here is to highlight issues pertaining to trial variables and suggest ways of standardizing protocols for phase I and II intervention trials in newly diagnosed patients. These issues will be discussed under three major headings: trial subjects, trial design, and trial outcome measures.

SUBJECTS: INCLUSION CRITERIA

Diagnosis of diabetes

Background. Type 1 diabetes can have different clinical presentations that presumably reflect the nature of the underlying disease pathology, to which we have no direct

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AUC, area under curve; MMTT, mixed-meal tolerance test.

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access. Some patients present acutely with dehydration and ketoacidosis, whereas others have minimal or no symptoms (45,46). Natural history studies have indicated that these differences may correlate with the rate of loss of β -cell function and residual β -cell function, determined by genetic (47–49) and other (50–66) factors that modify disease pathology. However, the relationship between the nature of the clinical presentation and the effectiveness of intervention therapy is not known (Table 1).

Proposal

- Define onset of diabetes from time of diagnosis by a physician, based on recognized, e.g., American Diabetes Association, criteria.
- Document the following at clinical presentation: age, sex, pubertal status, family history of diabetes, blood glucose, bicarbonate, presence or absence of ketoacidosis, weight loss, polyuria, polydipsia, HbA_{1c}, islet autoantibodies, insulin requirement, and HLA typing.

Age

Background. The natural history of pre- and postclinical type 1 diabetes varies with age. Specifically, the rate of β -cell destruction is inversely related to age (50–53,58). This age effect is directly associated with the number of susceptibility HLA class II (e.g., DR 3,4; DQ 2,8) (47,48,67) and class I (e.g., A24) (49) alleles. The more susceptibility alleles there are, the younger the age of onset and diagnosis, with a more autoaggressive immune response reflected by the number of islet antibodies (68–70). Therefore, the requirement for effective intervention treatment is likely to be more demanding in younger subjects. On the other hand, a slower rate of β -cell destruction in older subjects may indicate a wider window of opportunity for intervention; although, if the process was “regulated,” it would be important that intervention treatment did not jeopardize this.

Although an upper age limit may delineate classic type 1 diabetes from slowly progressive type 1 diabetes or latent autoimmune diabetes of adults (71–73), the combination of clinical type 2 diabetes and autoantibodies may still occur in children and younger adults (74). Age is also an issue with respect to consent and recruitment.

Proposal

- Match subjects in treatment and control groups as closely as possible for age.
- In phase I trials, enroll only subjects ≥ 18 years of age.

TABLE 1
Inclusion criteria

Diagnosis of diabetes	● According to American Diabetes Association criteria
Age	● Phase I trials \geq age 18 years ● Phase II, and III trials \leq age 35 years
Autoantibodies	● One of four to GAD65, insulin (if on insulin <2 weeks), IA2, or ICA
Start of therapy in relationship to diabetes diagnosis	● Baseline MMTT peak C-peptide ≥ 0.2 pmol/l ● If early-onset trial, subjects to be enrolled between 2 and 12 weeks from diagnosis

- Limit entry to subjects aged <35 years.

Autoantibodies

Background. Type 1A diabetes is an immune-mediated disease resulting in loss of β -cells. During the past several decades, islet autoantibodies to the GAD65 isoform (GADAb), tyrosine phosphatase-like insulinoma antigen IA2 (IA2Ab), and insulin (IAA) have been identified in individuals at risk for and presenting with clinical disease. Although up to 10% of patients presenting with clinical type 1 diabetes are islet autoantibody-negative (64) and ~ 10 –15% of patients with clinical type 2 diabetes are autoantibody-positive (71–74), autoantibody measurements remain the best indication that diabetes is immune mediated. Most would agree that the presence of one or more islet autoantibodies (GADAb, IA2Ab, or IAA, measured within 2 weeks of diabetes diagnosis) indicates immune-mediated disease and is a sufficient criterion for entry. More controversial is whether the presence of ICA alone is also a sufficient criterion for entry. Measurement of ICA by immunofluorescence requires a larger sample and is more difficult to perform than newer radioimmunoassays developed for GADAb, IA2Ab, and IAA. In subjects at risk for diabetes, the presence of ICA or any one autoantibody alone may not confer sufficient risk for entry to prevention trials; however, in subjects *with* diabetes, ICA is a marker of immune-mediated disease. Therefore, a patient with diabetes confirmed positive for ICA in the absence of the other three autoantibodies should also be eligible for study enrollment.

Proposal

- Subjects should have at least one of four islet autoantibodies: to GAD65, insulin (if on insulin treatment <2 weeks), IA2, or ICA.

Time from diagnosis

Background. In general, time from diagnosis is inversely related to C-peptide secretion. However, data from the Diabetes Control and Complications Trial and other studies indicate that some subjects with type 1 diabetes continue to have residual C-peptide, even 5 years after diagnosis (50,53,75–78). Time from diagnosis is therefore

not necessarily an accurate index of residual β -cell function. Additionally, measurement of C-peptide secretion when diabetes is poorly controlled is unreliable (see below).

Two models of the disease have been proposed: in one, clinical onset occurs on a continuum of the immune assault, with β -cell function finally being inadequate to maintain normoglycemia; in the other, the process of β -cell injury becomes abruptly destructive, heralding clinical diagnosis (65,66,79,80). In the latter, initiation of treatment within a short timeframe would be essential. In addition, data from cyclosporin trials suggest that early treatment is beneficial. Thus, investigators may wish to enroll subjects relatively soon after diagnosis in “early-onset trials.”

Such early-onset trials should be distinguished from those in which the only entry criterion is residual C-peptide secretion. In the latter, matching for time from diagnosis where there is a small number of subjects or randomizing where there is a larger number of subjects would be particularly important to obviate the potential problem of enrolling “survivors” with persisting C-peptide secretion.

Proposal

- Document peak C-peptide of ≥ 0.2 pmol/l after a liquid mixed-meal tolerance test (MMTT) (Sustacal/Boost). This baseline test should only be done after the subject is metabolically stable (at least 2 weeks after diagnosis).
- Studies defined as early-onset trials should include only subjects <12 weeks from diagnosis. Otherwise, no specific time from diagnosis is recommended.

TRIAL DESIGN

Number of subjects

Background. Phase I and II studies are often not large enough to stratify subjects according to important variables (Table 2).

Proposal

- Aim to include sufficient numbers of subjects to enable stratification in phase III trials. For smaller trials, collect

TABLE 2
Trial design

Number of subjects	● Aim to include sufficient numbers of subjects to enable stratification
Duration of trial	● Efficacy should be evaluated at a minimum of 2 years
Factors that influence outcome measures	● Aim for tight control (e.g., as close to normal HbA _{1c} as possible without causing hypoglycemia) ● Randomize, placebo-control, and double-mask subjects in phase II and III trials

standardized raw data on all subjects for later combined analysis. Document age, sex, pubertal status, family history of diabetes, time from diagnosis, nature of clinical presentation (see above), HLA, baseline immune marker, and C-peptide status.

Duration of trial

Background. It is assumed that mixed meal- or glucagon-stimulated C-peptide falls after diagnosis, and power calculations may be predicated on intervention reducing the rate of fall. However, data from control arms of trials in recently diagnosed adults indicate there may be little or no fall in C-peptide over the first year (42,43). Therefore, evaluation out to 1 year after either diagnosis or treatment initiation may fail to accurately reflect outcome, particularly in adults in whom there may only be a minimal fall in C-peptide over this period. Evaluation at this time may, however, provide short-term safety data.

Proposal

- Evaluate treatment for at least 2 years, particularly in adults; 1 year may be appropriate for safety.

Factors that influence outcome measures

Background. Diabetes treatment (24,36,78,81,82), physical activity, diet, time of testing, and other variables influence diabetes control and outcome measures.

The standard of care for people with diabetes is “tight” control (i.e., $HbA_{1c} < 7\%$) (83). In some intervention trials, subjects have been taken off insulin when euglycemia was achieved (84–86). It remains unknown whether continuing insulin therapy even during the honeymoon phase is beneficial, but indirect evidence suggests it is (78,81,82). The failure of parenteral insulin to prevent diabetes does not indicate that insulin treatment is without benefit in subjects with diabetes. Thus, unless a subject has reached an “insulin-free” end point (see below), insulin treatment should be continued.

Proposal

- Aim to standardize variables that could influence diabetes control and/or outcome measures.
- Randomize subjects in phase II and III trials.
- Aim to placebo control and double mask.
- Mask blood samples before analysis.
- Review safety and other data by external committee (e.g., a data safety monitoring board), with code broken to investigators and subjects if necessary for reasons of safety. Otherwise, do not break codes for data analysis until termination of the trial.
- Aim for tight control (e.g., as close to normal HbA_{1c} as possible without causing hypoglycemia).
- Continue insulin treatment whenever possible (avoiding hypoglycemia) unless subject has reached an insulin-free end point (see below).

TRIAL OUTCOME MEASURES

Metabolic tests

Background. Several tests can be used to evaluate β -cell function. C-peptide in healthy subjects can be stimulated by intravenous, intramuscular, or subcutaneous glucagon; intravenous sulfonylurea; intravenous glucagon-like pep-

TABLE 3
Outcome measures

Metabolic tests	• 2-h MMTT every 3 months
Immune tests	• Standardized autoantibodies
Primary outcome	• Difference in 2-h AUC C-peptide between treated and control groups
Secondary outcomes	• Insulin dose per kilogram, HbA_{1c} level

ptide 1; intravenous or oral amino acids; intravenous or oral glucose; or a mixed meal (87–92). During intervention with cyclosporin, subjects with type 1 diabetes had C-peptide responses to a MMTT at a time when intravenous glucose and glucagon responses were absent (93). Most studies have only evaluated the C-peptide response to an oral mixed meal over 2 h, although it has been suggested that a 4-h test may provide additional useful information, because many subjects with impaired β -cell function do not reach a peak C-peptide value during 2 h. Unfortunately, a 4-h MMTT can be difficult to perform, particularly in subjects with minimal residual function due to hypo- and hyperglycemia occurring during the test. Alternatively, intravenous glucagon-stimulated C-peptide has been used in new-onset trials. However, there is limited information regarding the relationship between MMTT and glucagon test results (92,94,95), and there are no data indicating that one test is preferable to the other. Nonetheless, for the purpose of having standardized end points, the MMTT is the recommended test. If investigators choose to perform intravenous glucagon stimulation of C-peptide, a MMTT should be performed in addition at least at baseline and annually to obtain comparative data (Table 3).

There are little published data on conditions that affect C-peptide stimulation tests in patients with established type 1 diabetes. An important consideration is the control of diabetes in the peri-test period. Although one study reported no effect of exogenous insulin on the MMTT (96), most protocols advise withholding insulin before the test. Should this only apply to short-acting insulin? What about insulin via the pump? The importance of the prevailing blood glucose level on stimulated C-peptide remains controversial. Some studies suggest no effect (87), whereas others indicate that the test is only valid in the absence of hypoglycemia (94,97) or hyperglycemia (98–100).

Proposal

- Evaluate stimulated C-peptide with the liquid MMTT on a quarterly basis.
- Administer evening insulin as usual but withhold morning insulin of any type. If on the pump, continue the basal rate but withhold the bolus. Conduct the test only if fasting blood glucose is 4–11.1 mmol/l (72–200 mg/dl).

Immune tests

Background. Antibodies (titer, isotypes, IgG subclasses, and epitope specificity) and T-cell responses (proliferation, activation markers, and cytokine production) may change in response to intervention therapy and therefore provide important mechanistic “surrogate marker” information. However, autoantibody changes cannot be used as an outcome measure because the relationship between changes in these markers and therapeutic benefit is un-

known. For example, in the cyclosporin trials, islet antibody levels did not correlate with benefit (101), whereas remission of Graves' hyperthyroidism (an autoantibody-mediated disease) has been associated with a decrease in autoantibody levels (102,103).

The place of markers such as IgG autoantibody subclasses (104,105) and islet antigen-reactive T-cell responses (106–108) is not yet clear. Assays for these cells are being evaluated by Immunology of Diabetes Society Workshops (109). T-cell assays require substantial improvement so that reproducible, quantitative, and qualitative responses can be measured.

Proposal

- Measure islet autoantibodies and freeze sera/plasma for future studies. Consider freezing blood mononuclear cells for future analysis.
- Evaluate immune markers in regard to HLA types.

Primary and secondary outcomes

Background. Studies have reported changes in fasting, peak, and area under curve (AUC) C-peptide values over time. It remains unclear which is most useful. In addition, it is not known whether C-peptide expressed as a function of blood glucose is more reliable. There are prepubertal versus postpubertal/age differences in C-peptide that are often not taken into account.

Withdrawal of insulin should be done only in the context of preventing hypoglycemia, not as a primary goal of treatment. However, in some subjects, therapy may result in restoration of a euglycemic insulin-free state.

Proposal

- Define the primary outcome as a significant difference in the 2-h AUC C-peptide response between treated and control groups over time. In addition, analyze incremental and peak C-peptide responses. Additional analysis, such as time to peak C-peptide response or 4-h AUC for C-peptide, may be an appropriate exploratory outcome.
- Define secondary outcomes as insulin dose per kilogram and HbA_{1c} level.
- Subjects at least 1 year from diagnosis on limited amounts of insulin with normal HbA_{1c} levels on two occasions 3 months apart are potentially "insulin-free." However, before withdrawal of chronic insulin therapy, documentation of normal glucose response is needed. These subjects should undergo a standard oral glucose tolerance test after not receiving insulin for 3 days. The presence of normal glucose tolerance under these conditions indicates an insulin-free state, and chronic insulin administration can be discontinued. Close follow-up with repeated HbA_{1c} and glucose tolerance tests are recommended, with reinstatement of insulin if abnormalities are present.

CONCLUSION

These Immunology of Diabetes Society guidelines have been developed to facilitate comparison of intervention therapies. Development and validation of novel assay technologies as well as new data on alternative outcome measures will undoubtedly require modifications to these recommendations in the future, but the principle that

standardization of clinical intervention trials benefits patients, families, and investigators will continue to underlie these efforts.

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