

Challenges in developing endpoints for type 1 diabetes intervention studies[†]

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Summary

Development of efficient and safe intervention strategies for preserving and/or restoring endogenous insulin production in type 1 diabetes has encountered a wide range of challenges, including lack of standardized trial protocols and of consensus on appropriate efficacy endpoints. For the greatest part, difficulties resided in choosing the most suitable assay(s) and parameter(s) to assess the β -cell function. It is now an accepted approach to evaluate endogenous insulin secretion by measuring C-peptide levels (with highly sensitive and normalized measurement methods) in response to a physiologic stimulus (liquid mixed-meal) under standardized conditions. Preventive interventions mandate the identification of well-defined, reliable and validated mechanistic or immunological markers of efficacy that would correlate with (and predict) the clinical outcome. This has not been consistently achieved to date. However, it has been generally agreed that for preventive studies performed very early in the disease course (in subjects without signs of autoimmunity against β -cells) development of two or more islet related autoantibodies could be employed as biomarkers of disease and thereafter, diagnostic criteria of diabetes serve as suitable endpoints.

This report summarizes the conclusions of the D-Cure workshop of international experts held in Barcelona in April 2007 and the current recommendations and updates in the field. Copyright © 2009 John Wiley & Sons, Ltd.

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The ultimate goal of therapies aiming to reverse type 1 diabetes mellitus (T1DM) is to restore or preserve β -cell mass and function while altering the autoimmune process directed against the pancreatic islet β cells. With emergence of new therapies evaluated in clinical research there is an obvious need for establishment of consensus regarding appropriate outcome measures. In this review article we outline the minutes of a D-Cure workshop which convened a panel of international experts to facilitate this aim (Barcelona, April 2007), with an update of previously published recommendations and an assessment of the current status of endpoints in immune therapy in T1DM.

Summary of previous recommendations in T1DM clinical trials

1. *Primary outcome for T1DM clinical trials to preserve β -cell function.* In 2001 an American Diabetes Association (ADA) workshop evaluated several potential efficacy outcome measures and concluded that the measurement of stimulated C-peptide under standardized conditions (which provides a direct estimate of the endogenous insulin secretion) is the

most suitable primary outcome for clinical trials of therapies aimed at preserving or improving β -cell function in new-onset patients [1]. To facilitate comparison of intervention therapies, the Immunology of Diabetes Society (IDS) has suggested guidelines to standardize protocols [2]. The 2-h mixed-meal tolerance test (MMTT) was recommended for the assessment of β -cell function and the primary outcome was defined as a significant difference in the 2-h area under the curve (AUC) C-peptide levels in response to a MMTT between treated and control groups over time.

2. *Clinical value of β -cell function preservation.* Evidence from the Diabetes Control and Complication Trial (DCCT) indicated that preservation of endogenous insulin production results in better metabolic control and long-term clinical outcome (less hypoglycaemia and less end-organ complications) [3–5]. Whilst this data is not unequivocal and should be substantiated, it is expected that maintenance of even some residual β -cell function could provide clinical benefits.

3. *Limitation of other potential endpoints* (haemoglobin A_{1c} (HbA_{1c}), insulin dose, severity of hypoglycaemia, diabetic complications, immune markers) [1]. Since near-normal glycemic control is the standard-of-care for newly diagnosed T1DM patients, differences in HbA_{1c} between treatment and placebo groups are minimal and thus cannot serve as robust measures of efficacy. The same applies for insulin dose, an indirect and imperfect reflection of β -cell function, highly affected by various factors (e.g. subject compliance, exercise, type of insulin, intra-individual insulin pharmacokinetics and dynamics, insulin sensitivity). In most subjects with new-onset disease ketoacidosis and severe hypoglycaemia are too infrequent to warrant their use as endpoints. In addition, the assessment of hypoglycaemia in clinical trials is difficult unless the precise DCCT criteria are used. Finally, although diabetes-specific autoantibodies are used as markers for the development of T1DM, there is no consistent data that interventions alter their expression. Likewise, T-cell assays to monitor the effects of therapeutic interventions on disease onset or progression still require further development.

4. *Optimum stimulus to estimate C-peptide reserve.* Although the MMTT is commonly used in the United States to assess residual insulin secretion, the Glucagon Stimulation Test (GST) has been employed in Europe. The Type 1 Diabetes TrialNet and The European C-peptide Trial (ECPT) study groups compared these tests in two parallel randomized multicenter studies [6]. Both have indicated that the MMTT is a more sensitive test of residual β -cell function, the peak C-peptide response being significantly higher compared to the GST, conceivably due to the incretin effect of oral glucose stimulus or inherently greater response to combined stimuli of mixed-meal compared with glucagon. Repeat testing also demonstrated that the MMTT was slightly more reproducible. Therefore the standardized MMTT was defined as the preferred test to assess β -cell function in T1DM trials.

Holes in our knowledge regarding C-peptide

There are a number of limitations with C-peptide that should be considered with its use, and pertain to its validation as a surrogate endpoint in T1DM trials.

1. *C-peptide is not a measure of β -cell mass and the C-peptide response reflects function rather than anatomy.* An important limitation of intervention trials in T1DM is the lack of standardized methods to directly measure β -cell mass *in vivo*. While newer imaging techniques (e.g. positron emission tomography, magnetic resonance imaging, scintigraphy, neurofunctional imaging) are undergoing development as non-invasive methods of β -cell mass measurement, metabolic tests have been used as surrogate markers [7]. The peripheral concentration of C-peptide (co-secreted with insulin on an equimolar basis and not cleared by the liver) depends not only on its rate of production but may be affected by factors such as the volume of distribution and renal clearance, mainly under non-steady-state conditions (e.g. physical exercise) [8–10]. Therefore, the C-peptide response to a mixed meal may not accurately reflect actual insulin production, particularly in individuals who change body mass over time. And, since β -cell function can be altered by many variables in addition to mass, conclusions about β -cell mass cannot be inferred based upon measurement of C-peptide alone. Other methods have been developed and validated in animal models to provide a more accurate measurement of insulin secretion [11–15]. These methods would provide a better alternative measure of pancreatic function but still do not provide a quantitative measurement of β -cell mass.

Studies in autologous islet transplants recipients (following pancreatectomy) showed a close relationship between β -cell mass and functional response to a glucose-potentiated arginine stimulation test [16]. These studies suggest an approach that may be useful for prospective evaluation of β -cell mass, but the validity of the method over time (considering complicating factors such as hyperglycemia) has not been determined. Drugs that directly alter insulin secretion can affect performance in these studies independent of β -cell mass [17,18]. Likewise, a hyperglycemic glucose clamp with arginine stimulation at its conclusion has been used to assess maximal β -cell function with the assumption that maximal function correlates directly with mass, but this assumption deserves validation [19].

Finally, even maximal stimulation tests may not identify β cells that are dysfunctional but still present and may potentially become functional. Studies of non-obese diabetic (NOD) mice have suggested that a significant proportion of β cells are degranulated at the time of diagnosis but may recover with treatments such as anti-CD3 monoclonal antibodies (mAb) [20]. These cells may respond in an impaired manner to physiologic stimuli. At times, the recovery of the responses has been misinterpreted as β -cell regeneration (through

replication/neogenesis) since recovery of β -cell function cannot be distinguished from an increase in β -cell mass with functional studies. Similar mechanism of regranulation and improvement of insulin production might occur when metabolic control is achieved in patients with new-onset T1DM (partial remission). Recovery of degranulated and/or dysfunctional cells may also account for the apparent effect of intensive glucose control on improvement of β -cell function seen in the DCCT [3].

Although direct assessment of β -cell mass is essential for studies of agents that are postulated to stimulate β -cell growth, functional, rather than anatomic evaluation may be more significant. For example, studies of the effects of exendin-4 on the remission of diabetes following treatment with anti-CD3 mAb indicate that the drug enhances β -cell function rather than mass [21]. Nonetheless, the rates of reversal of diabetes were improved when the drug was given.

2. *C-peptide levels are lower in children, and clinically significant levels of C-peptide responses have mainly been evaluated in adults.* The DCCT only included subjects as young as 13 years of age. The Diabetes Prevention Trial-1 (DPT-1) and other studies indicate a direct relationship between age and C-peptide responses [22,23]. Previously it was suggested that an increase in C-peptide responses occurs during puberty, but the data from the DPT-1 also shows that C-peptide increases with age in prepubertal children [6,23,24].

Therefore, the identified level of 0.2 pmol/mL as the threshold for clinically significant C-peptide responses may be appropriate for adults, but a lower level may afford similar metabolic advantages in children. Second, the DCCT only enrolled subjects with a stimulated C-peptide level of <0.5 pmol/mL. The natural history and clinical significance of higher, but still reduced levels of C-peptide in this non-obese population have not been studied.

3. *There are no prospective data showing that an intervention that improves C-peptide results in an improvement in the natural history of the disease, such as a decrease in the development of end-organ or even short-term metabolic complications.* The data from the DCCT provides important information to support the premise that improved C-peptide responses are associated with improved clinical parameters including HbA_{1c}. However, glucose control was the primary study variable, and only in a subgroup analysis could the contribution of C-peptide be evaluated.

An analysis of the DCCT data has suggested that retention of even modest residual β -cell function is associated with reduced hypoglycaemia, and from the islet transplant experience, even subjects who require insulin treatment to control glycemia still benefit from some residual insulin production in terms of reduced severe hypoglycaemia [5,25]. There is, however, very little information about what level of β -cell function is required for prevention of hypoglycaemia.

4. *The relationship between metabolic control and β -cell function is not well understood in T1DM* (Figure 1). In the DCCT, a reduced rate of C-peptide loss was seen in the intensively controlled group compared to

subjects who received standard therapy, but it is not clear whether this reflects the direct effect of hyperglycemia or other metabolites on β -cell function or alternatively an effect of glucose control on the natural history of the disease [3]. Other studies have supported a direct relationship between glycemic control and improvement in C-peptide although the improvement was of short duration [26]. However, the data from the DCCT do not establish whether improvement in C-peptide is associated with improved natural history of the disease or reduced complications or quality of life. In the subgroup analysis, it is not clear whether improved clinical outcomes are the cause or the effect of higher levels of β -cell function.

Trials of islet transplantation in subjects with T1DM that show acquisition of C-peptide responses and improved complications provide strong support for the use of C-peptide as a surrogate endpoint for clinical benefit. A small study indicated that glycemic lability and hypoglycaemia (incidence and degree) were significantly reduced in C-peptide-positive islet transplant recipients (with or without supplementary exogenous insulin) compared with non-transplanted T1DM subjects, the results being achieved in the setting of a significantly lower HbA_{1c} in all transplanted subjects, but the data need to be substantiated [27].

5. *How does C-peptide and change in C-peptide over time vary in subsets of recently diagnosed patients?* The absolute C-peptide levels reached as an indicator of residual β -cell function as well as the percentages of T1DM patients achieving a certain fasting and stimulated C-peptide endpoint 1 or 2 years after diagnosis vary considerably between studies. This is due to a number of critical factors including, among the most relevant ones, age at disease onset, basal and stimulated C-peptide response at diagnosis, genetics including human leukocyte antigen (HLA) genotype, sample size, severity of metabolic decompensation at diagnosis, presence of insulin resistance, insulin usage and whether near-normal glycemia (with HbA_{1c} consistently <7%) is obtained with intensive therapy, which may strongly influence the outcome of the therapeutic intervention [5,28–33].

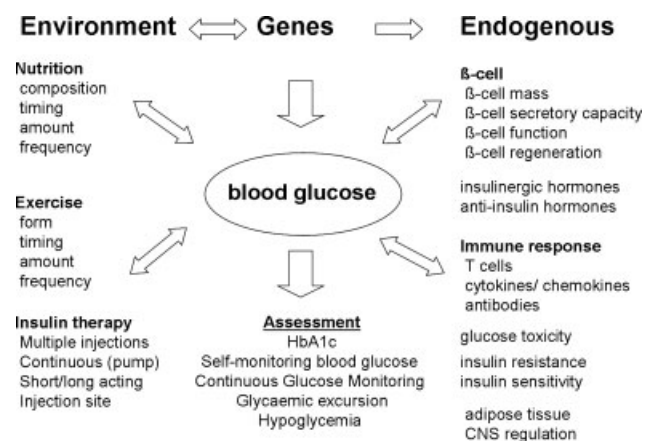


Figure 1. Complex interaction of environmental, genetic and endogenous factors and blood glucose

At the time of diagnosis β -cell secretion in response to a MMTT is about half the normal response [34]. The decrease of stimulated peak C-peptide is rapid in the 6-month pre-diagnosis period and even greater in the first 3 months afterwards, suggesting that there is an acceleration of post-challenge C-peptide loss once glycemic levels are in the diabetic range [35]. Studies suggest that significant β -cell function can be preserved even 4–5 years after onset, but extended analysis of the data is required in order to correct the findings for the parameters mentioned above [3,6]. A small proportion of patients with new-onset T1DM enrolled in a 2-year prospective study had an improvement of the secretory response to a mixed meal at follow-up visits compared to baseline [34]. This increase was not sustained over time, but it indicates that not all patients have a constant decline in β -cell function. Other studies also reported a short-term increase of the secretory response [36]. If this transient improvement is due to an actual increase in insulin secretion by the repair of β -cell mass and/or function or rather just to the correction of precipitating factors is not clear.

At present there are no definite indicators that identify individuals with a slow *versus* a more rapid loss of insulin secretion over time and the factors that may predict these changes remain to be better investigated prospectively. Two recent longitudinal investigations have found that serum interleukin 1 receptor antagonist was associated with preserved β -cell capacity, while chemokines CCL3 and CCL5 with decreased β -cell function, but these findings need further evaluation to define their role in disease progression and the causal relationship [37,38]. Data from the control (placebo) groups enrolled in different intervention trials (who actually represent the 'gold-standard' for the natural history of β -cell failure after diagnosis) seem to indicate that the decline in insulin production is highly variable and influenced by factors such as age at disease onset, residual β -cell function at baseline, genetics, immune status or insulin secretory pattern (early/delayed response to physiologic stimuli) [19,30,33,34,37,39]. Nevertheless, a precise interpretation of the results is difficult due to heterogeneity of studies populations and because the tests assessing the secretory response were different. Thus, a well characterization of the phenotype and genotype of patients included in the analysis should be done.

6. *What are the optimal ways to express C-peptide from the MMTT?* There are several ways to express the results of a MMTT (e.g. amount of change, rate of change, absolute levels, change past a certain threshold, duration or percentage of patients achieving a certain endpoint), but there is no clear evidence indicating which of them is the optimal parameter, nor what degree of change would correlate with clinical outcomes. Ideally, longitudinal studies investigating this correlation would provide the best knowledge that answers the question.

Data from the TrialNet-EPCT study indicates a high correlation of basal C-peptide with peak and AUC-mean response after MMTT ($r = 0.93$ and 0.95 , respectively)

[6]. However, there is no direct demonstration that basal C-peptide measurement is clinically meaningful, while data from the DCCT strongly emphasize the relevance of stimulated response.

In the DCCT the residual endogenous insulin secretion was determined by measuring the C-peptide levels at 90 min after boost ingestion: a value of ≥ 0.20 pmol/mL was defined as 'clinically relevant' [4]. The maximum post-stimulus response also occurred at ~ 90 min in the TrialNet-EPCT study [6]. However, previous studies using the same test have shown that some individuals with T1DM might have a delayed peak meal-induced insulin secretory response and the pattern of response was predictive of the progression of disease over the 2-year follow-up: the decline of the insulin secretory response was significantly less in subjects with delayed peak [34]. Thus the time-to-peak C-peptide levels may be used as an additional parameter, acknowledging that some patients might have a peak response at slightly different time points.

For an overall assessment of the secretory response to the mixed meal stimulus the C-peptide AUC calculation is useful. Some investigators have used 4-h, while most prefer the 2-h MMTT [40–43]. The 4-h test provides information of the entire response and might be useful in the case of patients with impaired β -cell function that do not reach their maximum values in the first 2 h. However, taken into account that the duration of the test is a significant issue posing burden to patients and investigators in a clinical setting, and because during a long test some impediments (hypo- or hyperglycemia) might occur, mainly in subjects with minimal residual function, it is probably reasonable to advise a 2-h test [2].

Standardization of C-peptide measurements

The C-peptide standardization programme was initiated subsequent to the 2001 ADA workshop in order to assess whether C-peptide results could be normalized to enable data combination and/or comparisons from different laboratories and studies. Initial comparisons of three different assays demonstrated that C-peptide stability even at -20°C was in part assay dependent, not improved by aprotinin and that several assays could reliably measure C-peptide on both plasma and serum (plasma being slightly more stable) [44]. It was also shown that the C-peptide results generated by different methods used by laboratories worldwide (and in some cases even by using the same method) were not always comparable, especially at higher concentrations. This finding has important implications for studies in which C-peptide results from different laboratories involved in multicenter trials are compared or combined for data analyses as well as for clinicians following patients over time, if samples are tested at different laboratories.

Normalization of C-peptide results from clinical samples achieved a significant improvement in comparability,

while using the World Health Organization standards did not. This indicated that matrix-compatible calibrator materials are necessary to attain effective normalization of C-peptide results [45]. Subsequent studies evaluating the use of a liquid chromatography–mass spectrometry method to harmonize C-peptide results indicated significant differences in unadjusted data between laboratory means, but not after normalization using serum specimens. Effective normalization of C-peptide values to a definitive reference method is feasible, but it may not necessarily be consistent for methods that demonstrate high imprecision [46]. Preliminary results of studies evaluating calibrators for C-peptide indicate that pooled serum calibrators provide the same reduction in variability as single-donor serum calibrators. Meetings with manufacturers are being conducted to initiate the development of a more generalized standardization programme and a backup reference laboratory is being established.

Intervention trials in recently diagnosed T1DM

Primary endpoint

One accepted approach is to consider significant difference in the 2-h C-peptide AUC response to a standardized mixed-meal challenge between treated groups and control groups over time as primary endpoint.

Recent intervention studies have used various inclusion criteria: fasting C-peptide >0.1 nmol/L or >0.2 nmol/L, random C-peptide >0.2 nmol/L or were independent of initial C-peptide concentrations and it appears that there is no specific threshold advisable, although patients with higher baseline stimulated C-peptide benefited more from immunotherapy than those with lower levels in some studies [19,39–43,47–49]. It might be worth mentioning that subjects diagnosed with T1DM in clinical settings have significantly lower C-peptide levels at diagnosis compared with individuals diagnosed in prevention trials [29,35,50]. Moreover, T1DM diagnosed through a screening and regular follow-up programme has a less severe onset and a milder clinical course in the first year post-diagnosis [51]. This provides a strong rationale for an early intervention for preserving the residual β -cell function. Additional support comes from the DPT-1 report indicating an increase of the rate of C-peptide decline mainly in the first 3 months post-diagnosis [35]. Thus it is critically important that a therapeutic intervention is implemented as early as possible, probably more so in the young in whom the rate of loss of β -cell function is greater (due to a more aggressive autoimmune process). Equally important is a good metabolic control through intensive insulin therapy implemented before immune intervention (ideally at diagnosis), since this by itself is bringing obvious benefits.

Different efficacy tests and endpoint assessments have been employed in intervention trials so far and changes

in fasting, peak and C-peptide AUC over time have been reported [19,39–43,47–49]. There is general agreement that measurement of fasting C-peptide alone (although easier to perform) is not sufficient to detect subtle effects of therapeutic interventions. IDS currently advocates the difference in 2-h C-peptide AUC response to a standard MMTT between treatment and control groups over time as the primary outcome [2]. Because effective glucose control through implementation of an intensive insulin regimen at diagnosis can lead to slower decline of stimulated C-peptide, it would be reasonable that in the intervention trials the changes from baseline are followed beyond the first year (possibly 2 years) for both groups [4,52,53].

There is not enough evidence to recommend a certain absolute/relative change of C-peptide levels after an immune therapy that would correlate with long-term clinical outcomes. However, an effective treatment should demonstrate maintenance/increase in C-peptide response or at least an attenuation of the decline rate compared with the control group. A recent report indicated that a single course of immune therapy in subjects with new-onset T1DM resulted in preservation of C-peptide for up to 5 years and this was accompanied by reduced insulin use [54]. This is the first long-term follow-up after an immune intervention demonstrating benefits in terms of prolonging β -cell function for longer than previously expected.

The difference in the natural disease progression associated with the factors discussed above (e.g. age at diagnosis, genetics, immune or metabolic status) might influence the response to immune therapy. Therefore this might need to be tailored to different subgroups accordingly (age, HLA genotype, titer/number of autoantibodies [aAb]), but there is no data so far that would support this differentiation in terms of dosing, duration of therapy or selecting a certain drug.

In terms of compliance, it is probably worth mentioning that immune interventions trials conducted so far had low withdrawal rates (four studies did not report any drop-out and other four reported that 77–98% of enrolled patients finished the study) [19,39–42,47–49].

Secondary endpoints

For the reasons discussed in previous sections and because it remains unclear which of the parameters resulting from the MMTT is more useful, peak and incremental C-peptide responses, as well as time-to-peak C-peptide levels should be included in the analysis as complementary endpoints. Their addition would provide a more comprehensive evaluation of the changes in C-peptide response induced by a therapeutic intervention.

Other possibly indicative parameters should be evaluated as secondary (supportive) efficacy outcomes. For an intervention to demonstrate efficacy on preservation of β -cell function, it would be expected to also result in a meaningful decrease in mean daily insulin requirements

compared to the control arm at similar glycemic control [55]. However no generally accepted magnitude of effect on exogenous insulin dose that could be considered predictive of clinical benefits has been established. HbA_{1c} will be rather seen as a measure of trial integrity since it is not expected to substantially differ between treatment groups. In fact a trial that results in substantial differences would be problematic from the standpoint of interpreting the C-peptide results. An index that combines the HbA_{1c} level and the number of insulin units used/day may give additional meaningful information. Consistent with its relationship with β -cell function, there seems to be an inverse relationship between C-peptide AUC and insulin dose (Figure 2). However, the metabolic status is influenced both by the insulin secretion and insulin sensitivity, so probably an assessment of insulin resistance should also be included.

Glycemic excursions are expected to be reduced in individuals with preserved insulin production since they are less dependent on exogenous insulin [56]. This measure has clinical importance particularly since observations from the DCCT (and its follow-up study) suggest that intensively managed individuals (with presumably reduced glycemic excursions) had a reduced rate of secondary end-organ complications even at the same HbA_{1c} levels as individuals who receive conventional management [57,58]. A limitation of this approach is that the glycemic excursions are dependent on many factors other than residual β -cell function but an ability to modulate them represents a clear immediate benefit in terms of avoidance of hypoglycaemia and potentially reduced long-term complications. An evaluation of frequency and severity of hypoglycaemic events should also be done.

An alternative index that would satisfy the need for a clinical endpoint and a measurement of β -cell

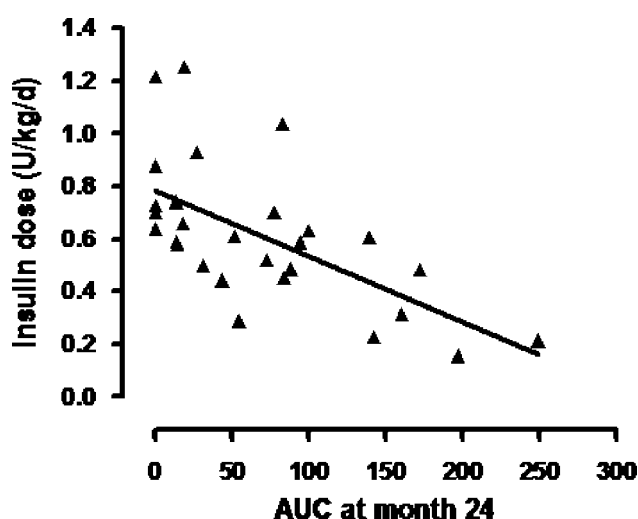


Figure 2. C-peptide AUC and insulin dose in subjects in a Phase I/II trial of anti-CD3 mAb. Control and drug treated subjects underwent a MMTT 24 months after diagnosis of T1DM. For each individual, the C-peptide AUC during a 4-h MMTT and the insulin dose at the time of the study are plotted. $R^2 = 0.403$, $p = 0.0002$, slope = -0.002509 ± 0.0005769

function at the same time could be used. A study in T1DM islet grafts recipients has indicated that C-peptide corrected to glucose values correlates well with the mass of transplanted islets and is more indicative of clinical outcome than C-peptide alone, but it is not known whether in newly onset T1DM this index is more reliable [59].

As a general rule for any intervention, favourable effects on the above-mentioned endpoints should be balanced against the risks of the particular intervention being tested.

Clinical trials for prevention of T1DM

Pre-autoimmunity (prior to autoantibody detection) primary prevention endpoint

The consensus reached here was that *development of two or more islet related aAb* (glutamate decarboxylase, protein tyrosine phosphatase IA2, islet cytoplasmic aAb and antibodies to insulin, and possibly antibodies to the cationic efflux zinc transporter) serve as suitable endpoint in primary preventive studies when the studies are performed very early in the disease course, i.e. in subjects without signs of autoimmunity against β cells. Several studies have shown that individuals having two or more aAb in their sera have at least a 50% risk of developing diabetes over 5 years and an even greater risk over extended time [60]. Data from the Diabetes Autoimmunity Study in the Young (DAISY) have shown that in first-degree relatives with two or more aAb, the 3-year risk of developing T1DM was 39% and the 5-year risk 68%; those with three aAb had a 100% 5-year risk [61]. Several factors including younger age, higher titers, antibody affinity, the presence of specific combinations of aAb, HLA genotypes, loss of first-phase insulin and glucose intolerance can substantially increase the risk further [62].

HLA-screening of newborn babies can identify a major group of general population newborns with a 20% risk of developing islet aAb and a group of newborns relatives (children with a parent or sibling with T1DM) with a risk of ~55% [63]. However, we should bear in mind that only a fraction of the children developing T1DM are identified on the basis of HLA [64]. Newborn screening programmes, including the Finish Type 1 Diabetes Prediction and Prevention (DIPP), German BABYDIAB, Colorado DAISY and Prospective Assessment in Newborns for Diabetes Autoimmunity (PANDA) have demonstrated the presence of confirmed aAb in the first 2 years of life [65–67]. These children have high rates of progression to clinical disease. Importantly, none of the children that developed diabetes in these prospective studies reverted to negative aAb, but were persistent for at least one at the time of diagnosis [65–67].

Since there is general belief that T1DM is the result of a T-cell-mediated disease, it is hoped that T-cell

markers that correlate immunological efficacy of the intervention and clinical outcome will be developed. Current efforts are mostly directed at the identification of potential T-cell markers at the time of diagnosis, which distinguish patients from non-diabetic controls [68]. While it remains to be determined whether such markers will exist with sufficient accuracy, precision, specificity, linearity, ruggedness and robustness, additional efforts are needed to define T-cell responses that correlate with remission, disease progression and mechanistic effects of the drugs as well as safety. In the case of immune modulation, it is critical to have reliable immune correlates, but many gaps remain to be overcome (e.g. applicability of cryopreserved rather than fresh leukocytes for the retrospective identification of immune changes).

Primary interventional approaches aimed at halting the autoimmune attack before the appearance of two or more aAb must be both efficacious and very safe if this surrogate is to be employed, given the uncertainties related to underlying autoimmune processes. Follow-up of subjects converting to two aAb would be important if the therapy prevents or enhances the seroconversion, and also important if the therapy has no effect on aAb since it still might alter subsequent clinical T1DM. Ideally, immune correlates of efficacy, based on the rationale of any given immune intervention, should be employed. This has not been achieved to date, since reliable measures of immunological efficacy have not been consistently identified, reproduced or validated nor have any been shown to correlate with clinical efficacy. There is a need to correlate mechanistic or immunological efficacy (immune modulation) with clinical efficacy (disease modification) and this is particularly important in the case of pre-diabetes, where demonstration of efficacy mandates the presence of well-defined surrogate markers that occur long before the disease becomes clinically manifest. It would be very important to determine what a favourable immunological profile should look like, and how this might differ between immune intervention strategies.

Post-autoimmunity (after autoantibody detection) primary prevention endpoint

During this stage, prevention strategies could be contemplated both before and after the occurrence metabolic abnormalities (usually detected by oral/intravenous glucose tolerance test). The risk for diabetes in aAb positive children who have indeterminate or impaired glucose tolerance is higher than 90% in 6 years [69]. Evidence from the DPT-1 suggests that subjects at risk for T1DM maintain important β -cell function until about 6 months pre-diagnosis and thereafter the decrease of stimulated peak C-peptide is more rapid (while the decline of fasting C-peptide seems to be much less) [35,70]. A reduced first-phase insulin response (FPIR) (progressively lost during the prodromal phase) is indicative of β -cell damage

and has been shown to be predictive of progression to T1DM in aAb positive first-degree relatives [71,72]. The combination of reduced FPIR and positive aAb facilitates estimation of the T1DM risk in unaffected siblings and accounts for more than 75% of the variation in time to diabetes over a 6-year interval [73,74].

An interventional trial in preclinical T1DM has shown that the non-progressors (subjects who did not develop the disease) had an increase in peak and AUC C-peptide response to three stimuli (mixed meal, oral glucose and intravenous glucose) over time, as opposed to progressors [75]. The preservation of C-peptide response in the pre-diabetic phase seems to indicate non-progression status and may serve as an additional marker to support efficacy in prevention trials.

While measurements of aAb are available and can thus be used as biomarker endpoints, outcomes of preventive interventions in T1DM would obviously include development of clinical diabetes in aAb positive subjects. Of note, the diagnostic criteria for diabetes mellitus, as first established in 1979 (and then revised by the ADA in 2003 and 2007) are based on the findings of prospective studies that evaluated glycemic values associated with increased risk of microvascular complications [76]. Therefore, the diagnostic threshold is not a value indicative of the onset of disease pathogenesis, but rather of a β -cell (residual) function insufficient for maintenance of glycemic control. Nevertheless, these criteria should be used as endpoints to evaluate efficacy of preventive interventions in T1DM clinical trials, until more appropriate thresholds that better reflect the disease development are eventually obtained.

Regulatory perspectives of preventive and therapeutic interventions

Therapies directed at the underlying autoimmune process of T1DM have presented challenges to the regulatory authorities like Food and Drug Administration (FDA) or European Medicines Agency (EMA). The DCCT data presenting the relationship between residual endogenous insulin secretion at study entry and metabolic control or microvascular complications at long-term follow-up has been persuasive to FDA. Although the rather large range of starting C-peptide values in the cohort was not the result of therapeutic intervention and the analysis was not pre-specified, the results were pivotal in changing FDA's view about efficacy endpoints. In February 2008 it reaffirmed the clinical relevance of preservation of β -cell function and recognized the measurement of C-peptide compared to control at 1 year as an appropriate primary efficacy endpoint for therapeutic trials intended to preserve β -cell function in early T1DM [55]. FDA and EMA currently recognize the unmet clinical need for a means of preserving remaining β -cell function in people with new-onset T1DM as well as for effective intervention that prevents T1DM. The following considerations reflect

an understanding of regulatory agencies' perspectives and its convergence with the expert community's view [55].

1. *Target populations – therapeutic indications.* Initial pivotal trials are generally confined to adults and older children. As safety and efficacy is demonstrated, additional trials, which include progressively younger children are allowed and eventually required. In the case of new therapeutic products with novel mechanisms of action, the early studies should reserve pediatric exposure until the metabolism, pharmacodynamics and safety of the new agent are well defined. Until actual clinical benefits are demonstrated, the therapeutic indication should specify 'for the preservation of remaining endogenous insulin secretion in people with recently diagnosed T1DM'. The indicated population would be defined by the parameters used in the pivotal trials.

2. *Study design.* All T1DM trials will be expected to reflect standard-of-care for subjects with recently diagnosed T1DM, aiming for near-normal glycemia (treat-to-glycemic target). For T1DM therapies, FDA would require at least 12 months of controlled trial treatment with indefinite follow-up of the study cohorts, and the durability of effect will be evaluated during the course of the controlled trial and during the unblinded follow-up. Various factors may drive trials of longer (than 1 year) duration. Subjects should be monitored for an extended period to investigate whether they experience a lower frequency of hypoglycaemia, or of chronic complications. Durability (or lack thereof) of the effect of a therapy will be taken into the overall benefit to risk assessment made in the initial drug approval process.

3. *Efficacy endpoints.* The primary and secondary endpoints and their consideration have been largely discussed before and are summarized in Table 1.

4. *Statistical approaches.* Standard statistical approaches would be used to size the pivotal trials and provide pre-specified analysis plans. The key unresolved question is what would be regarded by the regulatory authorities as the minimally acceptable treatment effect size. Given the lack of availability of an approved therapy, it is conceivable that FDA would accept an approximately 20–30% placebo-adjusted difference. A categorical approach might also be accepted. This might consist of the difference in proportions of subjects maintaining a C-peptide response above a pre-specified level. If statistical significance is achieved on the primary endpoint, secondary assessments of efficacy can be considered.

5. *Basis for regulatory approval.* As is the case of any therapeutic product, the regulator weighs the observed or likely benefits against the risks suggested by clinical and nonclinical data to decide about licensing approval and the appropriate product label. FDA recognizes that even some partial preservation of endogenous insulin secretion should result in clinical benefits. The advice of the expert community will be sought on what is a minimally acceptable treatment effect. While such a threshold may become established, the magnitude of benefit that would be required for a particular therapy would depend on the safety profile of the therapy.

Table 1. Suggested efficacy endpoints for therapeutic and preventive T1DM intervention trials

Therapeutic intervention trials for T1DM

1. *Primary endpoint:* Δ 2-h C-peptide AUC response to a standardized MMTT^a between treated and control groups over time (baseline versus study end; intermediate and post-study follow-up time points may be considered)

2. *Secondary endpoints:* between treated and control groups over time (baseline versus study end; intermediate and post-study follow-up time points may be considered):

- Δ peak C-peptide
- Δ incremental C-peptide
- Δ fasting C-peptide
- Δ HbA_{1c}
- Δ Exogenous insulin dose (IU/kg body weight/day)
- Δ incidence (number of events and % of subjects affected) of hypoglycaemic events

Preventive intervention trials for T1DM

1. *Pre-autoimmunity primary prevention endpoint* (in subjects negative for aAb): development of two or more islet autoantibodies

2. *Post-autoimmunity primary prevention endpoint* (in subjects positive for aAb): ADA criteria of diabetes

^aStandardized MMTT is the preferred approach for assessment of stimulated C-peptide; diabetes control during peri-test period is important: test should be conducted only if fasting glycemia is in the 70–200 mg/dL range; evening insulin should be administered as usual, morning insulin withheld, if on pump only basal insulin should be continued; during test, measurements should be done (at least) at time –10, 0, 15, 30, 60, 90 and 120 min; liquid meal (boost) should be given at a dose of 6 mL/kg body weight and ingested within 5 min; it should be performed every 3 months [6].

6. *Other considerations.* FDA can be expected to consider a vaccine perspective in evaluating immunomodulatory approaches for both efficacy and safety. Because T1DM therapies will typically not be amenable to exploration of multiple doses and regimens in pivotal clinical trials, FDA may require some assessment of how long a chronic or recurrent therapy should be continued or repeated. Informative data would be provided by randomizing trial subjects to various regimens including no therapy following the completion of the initial treatment period.

Box 1. Current revised recommendations for T1DM clinical trials

1. To assure investments by governmental agencies, insurance and pharmaceutical companies and receive regulatory approval, clinical trials have to show a meaningful and statistically significant effect on appropriate primary outcome(s) and prove a low risk/benefit ratio.
2. Separate recommendations should be made for trials designed to prevent T1DM in high-risk individuals and for intervention trials in newly diagnosed subjects.
3. Interventions should be performed as close as feasible to the diagnosis of diabetes.

4. Primary outcome measure for intervention trials will be C-peptide response to a standardized 2h-MMTT; HbA_{1c}, exogenous insulin dose or hypoglycaemia are rather secondary/supportive endpoints.
5. Measurement of C-peptide levels should be done using highly sensitive and normalized measurement methods.
6. For pre-autoimmunity prevention trials the endpoint should be development of two or more autoantibodies; for post-autoimmunity prevention trials the endpoint should be criteria for diagnosis of diabetes.

Conclusions

Challenges regarding development of means to preserve endogenous insulin production and eventually cure T1DM as well as of efficient interventions to prevent T1DM included lack of consensus regarding appropriate efficacy outcome measures and of standardized recommendations/guidelines to be used in clinical trials. Enormous efforts by the scientific community and governmental or nongovernmental agencies/companies have been made in recent years to facilitate reaching these aims and comparing different therapeutic interventions. This report summarizes the conclusions of the D-Cure workshop held in Barcelona (April 2007) that followed-up the 2001 ADA workshop as well as the current perspectives and updates in the field (Box 1).

Until now there are no efficient, practical and safe methods that reverse the autoimmune process and fully restore the β -cell mass/function. Convincing data from clinical trials indicates though that preservation of residual endogenous insulin production provides significant long-term clinical benefits and therefore this should be the minimum aim of intervention trials in subjects with recently onset T1DM. In spite of all limitations, the assessment of endogenous insulin secretion by measuring C-peptide levels (with highly sensitive and normalized measurement methods) in response to a physiologic stimulus (liquid mixed-meal) under standardized condition is the best approach. MMTT has proved to be a sensitive, reproducible and well tolerated test. The intervention should be performed as early as possible in order to preclude (if successful) further β -cell loss.

Several ways to approach the unresolved issues might be envisioned: (1) performing new trials on natural history of T1DM with an emphasis on C-peptide and other surrogate markers of disease progression, (2) attempting to combine already available data from intervention studies or (3) performing future intervention studies according to a common protocol for disease monitoring. For this,

one would need the involvement of regulatory authorities, trial designers, companies and clinicians performing the studies, as well as the contribution of basic science researchers. Better understanding of the natural history of T1DM and development and validation of new technologies/assays will improve ways to intervene efficiently for prevention and/or cure of T1DM. Certainly, continued update of current recommendations will be required.

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Conflict of Interest

The authors declare no conflict of interest.

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