Published in final edited form as:

Curr Opin Endocrinol Diabetes Obes. 2014 August ; 21(4): 287–292. doi:10.1097/MED.
0000000000000076.

Biomarkers in Type 1 diabetes: Application to the clinical trial setting

James E. Tooley and Kevan C. Herold, MD

Department of Immunobiology and Internal Medicine, Yale University, New Haven, CT 06520

Abstract

Purpose of Review—Biomarkers of type 1 diabetes are important for assessing risk of developing disease, monitoring disease progression, and determining responses to clinical treatments. Here we review recent advances in the development of biomarkers of type 1 diabetes with a focus on their utility in clinical trials.

Recent Findings—Measurements of auto antibodies and metabolic outcomes have been the foundation of monitoring type 1 diabetes for the past 20 years. Recent advancements have led to improvements in T cell specific assays that have been used in large-scale clinical trials to measure antigen specific T cell responses. Additionally, new tools are being developed for the measurement of β cell mass and death that will allow for more direct measurement of disease activity. Lastly, recent studies have used both immunologic and non-immunologic biomarkers to identify responders to treatments in clinical trials.

Summary—Use of biomarkers in the study of type 1 diabetes have largely not changed over the past 20 years, however recent advancements in the field are establishing new techniques that allow for more precise monitoring of disease progression. These new tools will ultimately lead to an improvement in understanding of disease and will be utilized in clinical trials.

Keywords

Type 1 diabetes; biomarkers; clinical trials

Introduction

Preclinical and clinical evidence suggest that Type 1 diabetes (T1D) is an autoimmune disease resulting from T cell mediated destruction of pancreatic β cells causing blood glucose dysregulation. (1). It occurs over two stages: a clinically silent period characterized by the development of insulinitis, seen in animal models by immune cell infiltration of the pancreatic islets, and an overt diabetes stage, which occurs once a majority of β cells have died and blood glucose levels can no longer be regulated. Because insulinitis occurs when considerable β cell mass remains, the most clinical benefit can be realized by detecting and directing therapies to this stage of disease. Additionally, differentiating those individuals at-

risk who will and will not progress to overt disease is essential for enrollment into preventative clinical trials.

Clinical studies with biologics – anti-CD3 mAbs, CTLA4Ig, and rituximab – have been able to modify the progression of the T1D(2-10). However, not all drug treated individuals respond to therapy. Identifying individuals who are most likely to respond to therapies is key since a personalized therapeutic approach will improve safety and efficacy.

Clinical trials have relied on metabolic measurements as endpoints, most commonly, C-peptide response to mixed-meal tolerance tests or clinical parameters such as HbA1c and insulin usage. However, these metabolic measurements can be affected by environmental factors including glucose control, adherence to clinical management, and physician practices. Optimally, methods that can detect the disease process itself including β cell mass, β cell death or immune dysfunction represent important measures for assessing therapeutic effects. This represents an unmet need in the field necessary not only to select subjects for therapies, but also to understand the reason why disease recurrence has been frequent in clinical trials (Table 1).

Here we review immunologic and metabolic biomarkers in prediction of developing T1D and their utility in clinical trials (Table 2). We also discuss the future of measuring disease specific T cell responses and directly measuring β cell mass, insulinitis and β cell death in T1D.

aAbs in disease prediction and clinical trials

aAb generated towards the pancreas were first describe in 1974 by Bottazzo *et al.* and they remain the only clinically measured sign of insulinitis(11). The initial assays, which involved detection of immunoglobulins that recognize pancreatic islet antigens, are still performed today. There are now at least five biochemically identified β cell targets recognized by auto antibodies. Those most commonly measured are aAbs to glutamic acid decarboxylase (GAD65), insulin associated aAb (IAA), insulinoma associated protein 2 (IA-2, previously known as ICA-512), islet specific glucose-6-phosphatase catalytic subunit related protein (IGRP), and the most recently described zinc transporter 8 (ZnT8)(12, 13). Insulin is the only β cell specific autoantigen.

aAbs are thought to develop as a result of β cell death and subsequent exposure of autoantigens to the immune system. As disease progresses, specificities to additional aAbs appear to develop sequentially, yet this process appears not to follow a specific timeframe or sequence (14). Development of additional aAbs could represent epitope spreading of the autoimmune response or even waxing and waning of antigen specific responses.

98.2% of patients with recent onset T1D, diagnosed on clinical parameters, are positive for 1 aAb, while 79.4% are positive for 2 aAbs (12). The sensitivity of any single biochemical aAb ranges between 58 and 68%, but the combination of three aAbs has a sensitivity and specificity of 83 and 92% respectively in differentiating patients with recent onset T1D and healthy control subjects (15). Therefore, positive aAbs are used to diagnose T1D in young patients and even older patients thought to have type 2 diabetes. Indeed, in the UKPDS, aAb

+ individuals, who were thought to have Type 2 diabetes at the time of clinical trial enrollment had 5 times greater odds of requiring insulin treatment after 6 years (16), suggesting these adult patients had autoimmune diabetes rather than the more common Type 2 diabetes.

In addition to being used for diagnosis, aAbs are useful in predicting disease development in at-risk relatives of patients with T1D(17, 18). Progression of T1D differs based on which aAb is positive – specifically patients with lower levels of IAA and IA2 (but not GAD65 or ZnT8) seem to progress slower (19). Also, early (by 9 months of age) expression of insulin aAbs identified 4 out of 5 children who progressed to diabetes by age 4 (20).

Being positive for a single aAb can be a transient event (20) and in addition, subjects who are positive for only a single aAb have about a 10% chance of developing disease within 5 years, even if there is a family history of T1D. The risk for diabetes increases greatly as the number of recognized different specificities increases. Individuals who are positive for three aAbs have a risk for T1D that approaches 90% within 8 yrs (13). The prediction of T1D in individuals with positive aAbs depends on the population being studied. In the Diabetes Autoimmunity Study in the Young (DAISY), aAb positivity was predictive in offspring of diabetic parents who were HLA-DR3/4 DQ8. There was a high frequency of false or transiently positive tests in those who did not express these high-risk haplotypes(21).

Collectively, these findings suggest that the number and titer of biochemical aAbs identifies individuals at high risk for disease, but their titers and positivity do not appear to be tightly correlated with disease progression. Nonetheless, although not primary effectors of β cell killing, they may have other pathologic function that may identify active disease (22).

T cell markers in T1D

The importance of T cells in the pathogenesis of T1D is apparent and has been highlighted in multiple clinical and laboratory studies. These studies include the finding that CD8+ T cells make up the majority of cell infiltrates in human insulinitis (23), diabetes antigen specific CD4+ and CD8+ T cells can be found in T1D patients (24-29), and T cells from diabetogenic NOD mice can transfer disease to immune deficient mice(30). Additionally, we recently showed that diabetes antigen reactive CD4+ T cells, isolated from patients with T1D, could cause insulinitis and β cell death when they were transferred into NOD/scid γ c-/- mice that expressed human HLA-DR4 as a transgene (31).

Pathogenic T cells in patients with T1D have been identified by measuring T cell cytokine production in response to diabetic associated antigens (ELISPOT), T cell proliferation assays to diabetes associated antigens or to islet proteins(immunoblot), and identification of diabetes antigen specific T cells by class I or II MHC tetramers (Q-dots). In blinded studies the sensitivity and specificity of the ELISPOT, T cell proliferative, and immunoblot assays for patients with T1D vs healthy control subjects were between 60-74% and 69-88% respectively (15, 32).

The reproducibility of antigen specific T cell assays have been questioned (15). However a recent study showed the reproducibility of measuring antigen specific T cell responses in the

large multicenter TRIGR trial (33). Furthermore, the T-Cell Workshop Committee of the Immunology of Diabetes Society has published recommendations on T cell handling for analysis in clinical trials in an attempt to help improve the reproducibility of these assays(34). The standardization of sample handling and of functional T cell assays has made considerable progress in the last 10 years. The logistics of using fresh samples for studies in clinical trial settings remain problematic, but some of the assays e.g. Class I MHC tetramer studies, can be performed with frozen samples, which is important for their use in trials.

In clinical trials, population measurements of polyclonal T cells have distinguished responders to treatments. After treatment with teplizumab, clinical responders showed an increase in CD8+ central memory cells (4) whereas responders to alefacept (soluble LFA3) showed a reduced frequency of effector T cells and an increase in the ratio of Treg/Teff (35). Surprising results have also emerged from studies of antigen specific T cells. In the rituximab trial of patients with new onset T1D, the T cell proliferation response to diabetes-associated islet specific and neuronal antigens increased over 12 months in responders, but did not change in the non-responders(36).

Class I MHC tetramers have been used to study the frequency of autoantigen specific T cells in patients with reactivation of autoimmunity following islet cell transplant (37) and to track the effects after immune therapy with anti-CD3 mAb (24, 38). In the latter, the frequency of GAD65 and insulin B chain peptide reactive T cells increased following treatment with anti-CD3 mAb.

There are a number of potential explanations for study results that have shown either no change or even increased frequency and proliferation of antigen reactive T cells with successful immune therapy. One is that the frequency of the cells in the peripheral blood does not reflect what has occurred in the pancreas, but may even reflect egress of the antigen specific cells from the pathologic site or other sites, such as the gut following therapy. We and others have shown that anti-CD3 mAb can cause the migration of T cells to the GI tract where they acquire regulatory function(39, 40).

β cellular biomarkers

Though β cells are the focus of the autoimmune attack in T1D our ability to directly measure β cell mass, death and even insulin secretion are currently limited. The difficult anatomical location of the pancreas and because β cells make up only 2-3% of pancreatic tissue have made it challenging to establish biopsy and imaging techniques to directly study β cells *in vivo*.

Currently, development of new techniques to measure β cell mass and inflammation are underway. In NOD mice, researchers have been able to magnetically label diabetogenic T cells and image their recruitment to the pancreas in real time (41). Additionally in humans, magnetic nanoparticles have been used to visualize pancreatic inflammation in T1D by exploiting vessel leakiness for extravasation of the nanoparticles into inflammatory tissues (42).

Other studies have used MRI, PET, and SPECT to directly image pancreatic β cells. β cells express the vesicular monoamine transporter (VMAT), which can be identified with 18F-fluoropropyl-dihydrotetrabenazine (18F-DTBZ)(43-45). There is some uncertainty about the specificity of the expression of VMAT on pancreatic β cells since a signal in the exocrine pancreas has been noted by some investigators in rodents (46). In humans, ongoing studies are evaluating background staining with 18F-DTBZ to clarify this question. Clearly, the utility of the method will depend on its sensitivity and the variability in measurement since the average loss of C-peptide at the end of the first year of T1D in contemporary studies was modest: -0.017 pmol/mL/month or approximately 29% of the baseline(47).

The endpoints commonly used in clinical trials measure provoked C-peptide responses and do not directly measure β cell death, yet reducing β cell death is the treatment objective of therapeutics. We recently developed an assay to measure β cell death by detection of the levels of β cell derived *INS* DNA(48). *INS* DNA derived from β cells has unmethylated CpG sites that enable gene transcription. The levels of unmethylated *INS* DNA are increased in patients with recent onset T1D and (in unpublished studies) are increased in individuals at risk for the disease who progress to T1D. We found that there was a decrease in the levels of unmethylated *INS* DNA in patients who were treated with teplizumab suggesting that drug treatment decreased the rate of β cell death (49). Additionally, in preclinical models of T1D, a microRNA expressed in large amounts in β cells, has been shown to be elevated in the blood after β cell death: a finding that remains to be validated in humans (50).

Biomarkers for prediction of clinical responses to interventions

The utility of aAb measurements as biomarkers of response to immune therapy is not established. Improvements in metabolic outcomes with anti-CD3 mAbs were not associated with significant changes in aAb titers(3, 5). Conversely, anti-CD20 mAb treatment which targets B cell subsets, but not plasma cells, improved β cell function over the first year of disease and significantly decreased IAA titers in 40% of drug treated patients compared to 0% of placebo(51). In the Oral Insulin DPT-1 trial, subjects with the highest titers of insulin aAbs showed the most significant protection from T1D (52). Conversely, in GAD65/alum trials, immunization resulted in an increase in the titer of anti-GAD65 aAbs, and also altered cytokine responses to GAD65, but there was not a significant effect of the treatment on C-peptide responses (53, 54).

Not all individuals with new onset T1D respond equally to intervention. In a recent teplizumab trial, 45% of drug treated individuals showed robust responses – with an average C-peptide loss of < 10% after 2 years, whereas 55%, the “non-responders” showed a decline in C-peptide that was similar to untreated subjects(2).

In our recent study of teplizumab, there were differences in the T cell phenotypes, before drug treatment in clinical responders and non-responders. Specifically, clinical responders showed an increased frequency of CD8+ effector memory T cells and terminally differentiated CD8+ effector T cells and a lower frequency of IFN γ + CD8+, subpopulations of naïve CD4+ and CD8+ and CCR4+ memory CD4+ T cells(2). Unexpectedly, metabolic features also distinguished responders and non-responders to drug treatment. Clinical responders had lower HbA1c levels and insulin use at the time of study entry compared to

non-responders. These differences were not explained by the levels of stimulated C-peptide, which were not different in the responders and non-responders at the time of study entry. Likewise in the TrialNet abatacept study, HbA1c < 6.0% tended to have better responses compared to those with higher levels(8).

Other non-immunologic parameters have also been found to predict clinical responses. In the Protégé and Delay trials of teplizumab, clinical responders were younger and had a shorter disease durations than non-responders (4, 10). The responses to rituximab also tended to be better in younger subjects (9). Thus, in addition to immunologic differences, it is important to consider differences in other disease relevant measures.

Even among subjects who were treated with agents that did not show efficacy overall, there may be subgroups for whom interventions are effective. These observations are not surprising since there are genetic polymorphisms and acquired differences between individuals that may modify drug response, and not all forms of the disease may have the same cause or respond to therapy in the same manner. Identification of individuals likely to respond to a specific therapy represents a valuable tool for improving the efficiency and equipoise of clinical trials.

Conclusions

While there have been advances in the development of immunologic measurements, the relationships between these parameters and metabolic progression of disease and therapy responses are still under investigation, and a single test cannot substitute for the measurement of C-peptide responses. Detection of biochemical aAbs remains the most reproducible and predictive measurement to identify individuals at high risk for the disease, but does not provide information about the process in real time. Cellular biomarkers have been widely used, but there is limited information on how changes in cell subsets can predict immune therapy responses.

The heterogeneity in responses to immune interventions highlights the importance in identifying biomarkers that can identify individuals most likely to respond to a given intervention. To date, the most reliable parameters have been metabolic and demographic, but further immune studies may refine our ability to match interventions with patients.

Acknowledgments

Conflicts of interest: Dr. Herold has received grant funding from MacroGenics Inc.

Funding organizations: Supported by grants DK045735, RR024139, AI102011, JDRF 2012-546, DK095639, State of Connecticut Biomedical Research Award 2012-0222, DK057846, DP3DK101122 James E. Tooley is a Howard Hughes Medical Institute Medical Research Fellow.

References

1. Herold K, Vignali DA, Cooke A, Bluestone J. Type 1 diabetes: Translating mechanistic observations into effective clinical outcomes. *Nat Rev Immunol*. 2013; 13
- 2**. Herold KC, Gitelman SE, Ehlers MR, Gottlieb PA, Greenbaum CJ, Hagopian W, et al. Teplizumab (anti-CD3 mAb) treatment preserves C-peptide responses in patients with new-onset

type 1 diabetes in a randomized controlled trial: Metabolic and immunologic features at baseline identify a subgroup of responders. *Diabetes*. 2013 Jul 8. Patients treated with teplizumab in the AbATE trial had a 75% improvement in C-peptide response compared to controls at 2 years. Responders to treatment were distinguished from non-responders by HbA1c, insulin usage and immunologic parameters.

3. Herold KC, Gitelman SE, Masharani U, Hagopian W, Bisikirska B, Donaldson D, et al. A Single Course of Anti-CD3 Monoclonal Antibody hOKT3 γ 1(Ala-Ala) Results in Improvement in C-Peptide Responses and Clinical Parameters for at Least 2 Years after Onset of Type 1 Diabetes. *Diabetes*. 2005 Jun; 54(6):1763–9. [PubMed: 15919798]
- 4**. Herold KC, Gitelman SE, Willi SM, Gottlieb PA, Waldron-Lynch F, Devine L, et al. Teplizumab treatment may improve C-peptide responses in participants with type 1 diabetes after the new-onset period: a randomised controlled trial. *Diabetologia*. 2012 Oct 21. Patients treated with teplizumab after the new onset period may still receive benefits in preservation of C-peptide response. Responders to treatment had an increase in CD8⁺ central memory cells at 3 months after treatment and tended to be younger and have decreased HbA1c at study entry.
5. Herold KC, Hagopian W, Auger JA, Poumian-Ruiz E, Taylor L, Donaldson D, et al. Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. *N Engl J Med*. 2002; 346(22):1692–8. [PubMed: 12037148]
6. Keymeulen B, Vandemeulebroucke E, Ziegler AG, Mathieu C, Kaufman L, Hale G, et al. Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. *N Engl J Med*. 2005 Jun 23; 352(25):2598–608. [PubMed: 15972866]
7. Keymeulen B, Walter M, Mathieu C, Kaufman L, Gorus F, Hilbrands R, et al. Four-year metabolic outcome of a randomised controlled CD3-antibody trial in recent-onset type 1 diabetic patients depends on their age and baseline residual beta cell mass. *Diabetologia*. 2010 Apr; 53(4):614–23. [PubMed: 20225393]
8. Orban T, Bundy B, Becker DJ, Dimeglio LA, Gitelman SE, Goland R, et al. Co-stimulation modulation with abatacept in patients with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled trial. *Lancet*. 2011 Jun 28.
9. Pescovitz MD, Greenbaum CJ, Krause-Steinrauf H, Becker DJ, Gitelman SE, Goland R, et al. Rituximab, B-lymphocyte depletion, and preservation of beta-cell function. *N Engl J Med*. 2009 Nov 26; 361(22):2143–52. [PubMed: 19940299]
10. Sherry N, Hagopian W, Ludvigsson J, Jain SM, Wahlen J, Ferry RJ Jr, et al. Teplizumab for treatment of type 1 diabetes (Protege study): 1-year results from a randomised, placebo-controlled trial. *Lancet*. 2011 Jun 28.
11. Bottazzo GF, Florin-Christensen A, Doniach D. Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. *Lancet*. 1974 Nov 30; 2(7892):1279–83. [PubMed: 4139522]
12. Wenzlau JM, Juhl K, Yu L, Moua O, Sarkar SA, Gottlieb P, et al. The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. *Proc Natl Acad Sci U S A*. 2007 Oct 23; 104(43):17040–5. [PubMed: 17942684]
13. Verge CF, Gianani R, Kawasaki E, Yu L, Pietropaolo M, Jackson RA, et al. Prediction of type I diabetes in first-degree relatives using a combination of insulin, GAD, and ICA512bdc/IA-2 auto antibodies. *Diabetes*. 1996 Jul; 45(7):926–33. [PubMed: 8666144]
14. Yu M, Johnson JM, Tuohy VK. A predictable sequential determinant spreading cascade invariably accompanies progression of experimental autoimmune encephalomyelitis: a basis for peptide-specific therapy after onset of clinical disease. *J Exp Med*. 1996 Apr 1; 183(4):1777–88. [PubMed: 8666934]
15. Herold KC, Brooks-Worrell B, Palmer J, Dosch HM, Peakman M, Gottlieb P, et al. Validity and reproducibility of measurement of islet auto reactivity by T-cell assays in subjects with early type 1 diabetes. *Diabetes*. 2009 Nov; 58(11):2588–95. [PubMed: 19675135]
16. Turner R, Stratton I, Horton V, Manley S, Zimmet P, Mackay IR, et al. UKPDS 25: auto antibodies to islet-cell cytoplasm and glutamic acid decarboxylase for prediction of insulin requirement in type 2 diabetes. UK Prospective Diabetes Study Group. *Lancet*. 1997 Nov 1; 350(9087):1288–93. [PubMed: 9357409]

17. Krischer JP, Cuthbertson DD, Yu L, Orban T, Maclaren N, Jackson R, et al. Screening strategies for the identification of multiple antibody-positive relatives of individuals with type 1 diabetes. *J Clin Endocrinol Metab*. 2003 Jan; 88(1):103–8. [PubMed: 12519837]
18. Krischer JP, Schatz D, Riley WJ, Spillar RP, Silverstein JH, Schwartz S, et al. Insulin and islet cell auto antibodies as time-dependent covariates in the development of insulin-dependent diabetes: a prospective study in relatives. *J Clin Endocrinol Metab*. 1993; 77(3):743–9. [PubMed: 8370696]
19. Achenbach P, Warncke K, Reiter J, Naserke HE, Williams AJ, Bingley PJ, et al. Stratification of type 1 diabetes risk on the basis of islet autoantibody characteristics. *Diabetes*. 2004 Feb; 53(2): 384–92. [PubMed: 14747289]
20. Yu L, Robles DT, Abiru N, Kaur P, Rewers M, Kelemen K, et al. Early expression of anti insulin auto antibodies of humans and the NOD mouse: evidence for early determination of subsequent diabetes. *Proc Natl Acad Sci U S A*. 2000 Feb 15; 97(4):1701–6. [PubMed: 10677521]
21. Barker JM, Barriga KJ, Yu L, Miao D, Erlich HA, Norris JM, et al. Prediction of autoantibody positivity and progression to type 1 diabetes: Diabetes Autoimmunity Study in the Young (DAISY). *J Clin Endocrinol Metab*. 2004 Aug; 89(8):3896–902. [PubMed: 15292324]
22. Reijonen H, Daniels TL, Lernmark A, Nepom GT. GAD65-specific auto antibodies enhance the presentation of an immunodominant T-cell epitope from GAD65. *Diabetes*. 2000 Oct; 49(10): 1621–6. [PubMed: 11016444]
23. Coppieters KT, Dotta F, Amirian N, Campbell PD, Kay TW, Atkinson MA, et al. Demonstration of islet-auto reactive CD8 T cells in insulinitic lesions from recent onset and long-term type 1 diabetes patients. *J Exp Med*. 2012 Jan 16; 209(1):51–60. [PubMed: 22213807]
24. Pinkse GG, Tysma OH, Bergen CA, Kester MG, Ossendorp F, van Veelen PA, et al. Autoreactive CD8 T cells associated with beta cell destruction in type 1 diabetes. *Proc Natl Acad Sci U S A*. 2005 Dec 20; 102(51):18425–30. [PubMed: 16339897]
25. Roep BO, Stobbe I, Duinkerken G, van Rood JJ, Lernmark A, Keymeulen B, et al. Auto- and alloimmune reactivity to human islet allografts transplanted into type 1 diabetic patients. *Diabetes*. 1999; 48(3):484–90. [PubMed: 10078547]
26. Skowera A, Ellis RJ, Varela-Calvino R, Arif S, Huang GC, Van-Krinks C, et al. CTLs are targeted to kill beta cells in patients with type 1 diabetes through recognition of a glucose-regulated preproinsulin epitope. *J Clin Invest*. 2008 Oct; 118(10):3390–402. [PubMed: 18802479]
- 27*. Unger WW, Pearson T, Abreu JR, Laban S, van der Slik AR, der Kracht SM, et al. Islet-specific CTL cloned from a type 1 diabetes patient cause beta-cell destruction after engraftment into HLA-A2 transgenic NOD/scid/IL2RG null mice. *PLoS One*. 2012; 7(11):e49213. HLA-A2 restricted IGRP specific CD8+ cells are increased in the peripheral blood of patients with T1D. These cells are capable of lysing HLA-A2 expressing murine islets *in vitro* as well as the destruction of murine islets *in vivo* in HLA-A2 transgenic immune deficient mice. [PubMed: 23155466]
28. Arif S, Moore F, Marks K, Bouckennooghe T, Dayan CM, Planas R, et al. Peripheral and islet interleukin-17 pathway activation characterizes human autoimmune diabetes and promotes cytokine-mediated beta-cell death. *Diabetes*. 2011 Aug; 60(8):2112–9. [PubMed: 21659501]
29. Arif S, Tree TI, Astill TP, Tremble JM, Bishop AJ, Dayan CM, et al. Autoreactive T cell responses show proinflammatory polarization in diabetes but a regulatory phenotype in health. *J Clin Invest*. 2004 Feb; 113(3):451–63. [PubMed: 14755342]
30. Miller BJ, Appel MC, O'Neil JJ, Wicker LS. Both the Lyt-2+ and L3T4+ T cell subsets are required for the transfer of diabetes in nonobese diabetic mice. *J Immunol*. 1988 Jan 1; 140(1):52–8. [PubMed: 3275717]
- 31*. Viehmann Milam A, Maher SE, Gibson J, Lebastchi J, Ruddle NH, Herold KC, et al. A humanized mouse model of autoimmune insulinitis. *Diabetes*. 2014 Injection of human CD4+ T cells pulsed with autoantigen-derived peptides into HLA-DR4 transgenic immune deficient mice resulted in insulinitis and β cell death.
32. Seyfert-Margolis V, Gisler TD, Asare AL, Wang RS, Dosch HM, Brooks-Worrell B, et al. Analysis of T-cell assays to measure autoimmune responses in subjects with type 1 diabetes: results of a blinded controlled study. *Diabetes*. 2006 Sep; 55(9):2588–94. [PubMed: 16936208]

- 33**. Hadley D, Cheung RK, Becker DJ, Girgis R, Palmer JP, Cuthbertson D, et al. Large-scale prospective T cell function assays in shipped, unfrozen blood samples: experiences from the multicenter TRIGR trial. *Clin Vaccine Immunol*. 2014 Feb; 21(2):203–11. Polyclonal and post vaccination T cell responses were analyzed in 4,919 samples of fresh heparinized blood shipped by courier within North America in the TRIGR trial. The majority of samples were viable up to 3 days after blood draw. The reproducibility of T cell function assays were comparable to other established blood biochemical tests. [PubMed: 24334687]
34. Mallone R, Mannering SI, Brooks-Worrell BM, Durinovic-Bello I, Cilio CM, Wong FS, et al. Isolation and preservation of peripheral blood mononuclear cells for analysis of islet antigen-reactive T cell responses: position statement of the T-Cell Workshop Committee of the Immunology of Diabetes Society. *Clin Exp Immunol*. 2011 Jan; 163(1):33–49. [PubMed: 20939860]
- 35**. Rigby M, DiMeglio L, Rendell M, Felner E, Dostou J, Gitelman S, et al. Targeting effector memory T cells with alefacept in new onset type 1 diabetes: 12 month results from the TIDAL study. *Lancet Endocrinology and Metabolism*. 2013 A recently reported phase II clinical trial of alefacept did not meet its primary endpoint, but showed improvements by a number of secondary endpoints suggesting efficacy. Responders to treatment were found to have an increased ratio of Treg/Teff compared to non-responders.
36. Herold KC, Pescovitz MD, McGee P, Krause-Steinrauf H, Spain LM, Bourcier K, et al. Increased T Cell Proliferative Responses to Islet Antigens Identify Clinical Responders to Anti-CD20 Monoclonal Antibody (Rituximab) Therapy in Type 1 Diabetes. *J Immunol*. 2011 Aug 15; 187(4): 1998–2005. [PubMed: 21775681]
37. Vendrame F, Pileggi A, Laughlin E, Allende G, Martin-Pagola A, Molano RD, et al. Recurrence of type 1 diabetes after simultaneous pancreas-kidney transplantation, despite immunosuppression, is associated with auto antibodies and pathogenic autoreactive CD4 T-cells. *Diabetes*. 2010 Apr; 59(4):947–57. [PubMed: 20086230]
38. Cernea S, Herold KC. Monitoring of antigen-specific CD8 T cells in patients with type 1 diabetes treated with antiCD3 monoclonal antibodies. *Clin Immunol*. 2010 Feb; 134(2):121–9. [PubMed: 19837003]
39. Esplugues E, Huber S, Gagliani N, Hauser AE, Town T, Wan YY, et al. Control of TH17 cells occurs in the small intestine. *Nature*. 2011 Jul 28; 475(7357):514–8. [PubMed: 21765430]
40. Waldron-Lynch F, Henegariu O, Deng S, Preston-Hurlburt P, Tooley J, Flavell R, et al. Teplizumab induces human gut-tropic regulatory cells in humanized mice and patients. *Sci Transl Med*. 2012 Jan 25; 4(118):118ra12.
41. Moore A, Grimm J, Han B, Santamaria P. Tracking the recruitment of diabetogenic CD8+ T-cells to the pancreas in real time. *Diabetes*. 2004 Jun; 53(6):1459–66. [PubMed: 15161749]
42. Gaglia JL, Guimaraes AR, Harisinghani M, Turvey SE, Jackson R, Benoist C, et al. Noninvasive imaging of pancreatic islet inflammation in type 1A diabetes patients. *J Clin Invest*. 2011 Jan; 121(1):442–5. [PubMed: 21123946]
43. Freeby M, Goland R, Ichise M, Maffei A, Leibel R, Harris P. VMAT2 quantitation by PET as a biomarker for beta-cell mass in health and disease. *Diabetes Obes Metab*. 2008 Nov; 10(Suppl 4): 98–108. [PubMed: 18834437]
44. Goland R, Freeby M, Parsey R, Saisho Y, Kumar D, Simpson N, et al. ¹¹C-dihydrotetrabenazine PET of the pancreas in subjects with long-standing type 1 diabetes and in healthy controls. *J Nucl Med*. 2009 Mar; 50(3):382–9. [PubMed: 19223416]
45. Souza F, Simpson N, Raffo A, Saxena C, Maffei A, Hardy M, et al. Longitudinal noninvasive PET-based beta cell mass estimates in a spontaneous diabetes rat model. *J Clin Invest*. 2006 Jun; 116(6):1506–13. [PubMed: 16710474]
46. Fagerholm V, Mikkola KK, Ishizu T, Arponen E, Kauhanen S, Nagren K, et al. Assessment of islet specificity of dihydrotetrabenazine radiotracer binding in rat pancreas and human pancreas. *J Nucl Med*. 2010 Sep; 51(9):1439–46. [PubMed: 20720057]
- 47*. Greenbaum CJ, Beam CA, Boulware D, Gitelman SE, Gottlieb PA, Herold KC, et al. Fall in C-peptide During First 2 Years From Diagnosis: Evidence of at Least Two Distinct Phases From Composite Trial Net Data. *Diabetes*. 2012 Jun 11. C-peptide response declines in a biphasic pattern in the first 24 months of disease after diagnosis. Using data from three clinical trials

TrialNet found C-peptide slope was -0.0245 pmol/mL/mo during the first 12 months and -0.0079 pmol/mL/mo during the second 12 months after diagnosis of T1D.

48. Akirav EM, Lebastchi J, Galvan EM, Henegariu O, Akirav M, Ablamunits V, et al. Detection of beta cell death in diabetes using differentially methylated circulating DNA. *Proc Natl Acad Sci U S A*. 2011 Nov 22; 108(47):19018–23. [PubMed: 22074781]
- 49*. Lebastchi J, Deng S, Lebastchi AH, Beshar I, Gitelman S, Willi S, et al. Immune Therapy and beta-Cell Death in Type 1 Diabetes. *Diabetes*. 2013 Feb 27. A recently developed technique to detect β cell death by measuring relative amounts of β cell derived INS DNA in serum. This assay was used to show an increase in β cell death in T1D patients and a decrease in rate of death after treatment with teplizumab.
- 50*. Erener S, Mojibian M, Fox JK, Denroche HC, Kieffer TJ. Circulating miR-375 as a biomarker of beta-cell death and diabetes in mice. *Endocrinology*. 2013 Feb; 154(2):603–8. miR-375, an islet enriched miRNA, was found to be increased in two different mouse models of T1D before progression to overt diabetes suggesting its utility as a predictive biomarker of T1D. These studies have yet to be validated in humans. [PubMed: 23321698]
51. Yu L, Herold K, Krause-Steinrauf H, McGee PL, Bundy B, Pugliese A, et al. Rituximab selectively suppresses specific islet antibodies. *Diabetes*. 2011 Oct; 60(10):2560–5. [PubMed: 21831969]
52. Skyler JS, Krischer JP, Wolfsdorf J, Cowie C, Palmer JP, Greenbaum C, et al. Effects of oral insulin in relatives of patients with type 1 diabetes: The Diabetes Prevention Trial--Type 1. *Diabetes Care*. 2005 May; 28(5):1068–76. [PubMed: 15855569]
53. Wherrett DK, Bundy B, Becker DJ, Dimeglio LA, Gitelman SE, Goland R, et al. Antigen-based therapy with glutamic acid decarboxylase (GAD) vaccine in patients with recent-onset type 1 diabetes: a randomised double-blind trial. *Lancet*. 2011 Jun 27.
- 54*. Axelsson S, Cheramy M, Akerman L, Pihl M, Ludvigsson J, Casas R. Cellular and humoral immune responses in type 1 diabetic patients participating in a phase III GAD-alum intervention trial. *Diabetes Care*. 2013 Nov; 36(11):3418–24. T1D patients treated with GAD-alum had an increase in GAD aAbs and Th2 skewing of their cytokine profiles, however no biomarkers were associated with treatment efficacy. [PubMed: 23863909]

Key Points

- Currently clinicians and researches rely heavily on surrogates of the autoimmune process and β cell mass – auto antibodies and metabolic biomarkers – as measures of type 1 diabetes, however advancements are leading to more direct measurements of these variables
- Recent advancements are making it possible to measure diabetes antigen specific T cells – the cells thought to be responsible for the pathogenesis of disease – in clinical trials
- New imaging techniques and biochemical assays currently being developed create the possibility to directly measure β cell mass and β cell death
- Both immune and metabolic biomarkers have been used to identify responders in recently completed clinical trials

Table 1
Goals of biomarkers in Type 1 diabetes clinical trials

• Early detection of insulinitis
• Prediction of development of overt diabetes in at risk subjects
• Identification of responders to treatment
• Direct measurement of β cell mass and death

Table 2
Biomarkers of autoimmunity in Type 1 diabetes

Measurement	Description
Auto antibodies (aAbs)	Detection of immunoglobulins that recognize one of five commonly measured diabetogenic antigens – GAD65, IAA, IA-2, IGRP and ZnT8 – by fluid-phase radioassay.
T cell proliferation	Measurement of PBMC proliferation in response to culture with diabetogenic antigens.
Immunoblot	Measurement of PBMC proliferation in response to to culture with human pancreatic islets cell antigens. Pancreatic islets are separated by electrophoresis and electroblotted onto nitrocellulose membranes for solubilization and addition to culture.
ELISPOT	Measurement of IFN γ secretion by PBMCs in response to culture with synthetic peptides that represent naturally processed diabetogenic antigens.
Tetramer Assay	Detection by flow cytometry of diabetes antigen specific T cells by staining with fluorescently labeled diabetogenic peptide-MHC class I or II complexes.
β cell death	Measured by detecting relative amounts of β cell derived <i>INS</i> DNA in serum. β cell <i>INS</i> DNA contains unmethylated CpG sites that allows for its discrimination from <i>INS</i> DNA derived from other sources.