Learning From Past Failures of Oral Insulin Trials

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Very recently one of the largest type 1 diabetes prevention trials using daily administration of oral insulin or placebo was completed. After 9 years of study enrollment and follow-up, the randomized controlled trial failed to delay the onset of clinical type 1 diabetes, which was the primary end point. The unfortunate outcome follows the previous large-scale trial, the Diabetes Prevention Trial–Type 1 (DPT-1), which again failed to delay diabetes onset with oral insulin or low-dose subcutaneous insulin injections in a randomized controlled trial with relatives at risk for type 1 diabetes. These sobering results raise the important question, "Where does the type 1 diabetes prevention field move next?" In this Perspective, we advocate for a paradigm shift in which smaller mechanistic trials are conducted to define immune mechanisms and potentially identify treatment responders. The stage is set for these interventions in individuals at risk for type 1 diabetes as Type 1 Diabetes TrialNet has identified thousands of relatives with islet autoantibodies and general population screening for type 1 diabetes risk is under way. Mechanistic trials will allow for better trial design and patient selection based upon molecular markers prior to large randomized controlled trials, moving toward a personalized medicine approach for the prevention of type 1 diabetes.

TYPE 1 DIABETES IS PREDICTABLE

Before a disease can be prevented, it must be predicted. The ability to assess risk for developing type 1 diabetes (T1D) has been well documented over the last two decades (1). Using genetic markers, human leukocyte antigen (HLA) DQ and DR typing (2), islet autoantibodies (1), and assessments of glucose tolerance (intravenous or oral glucose tolerance tests) has led to accurate prediction models for T1D development (3). Prospective birth cohort studies Diabetes Autoimmunity Study in the Young (DAISY) in Colorado (4), Type 1 Diabetes Prediction and Prevention (DIPP) study in Finland (5), and BABYDIAB studies in Germany have followed genetically at-risk children for the development of islet autoimmunity and T1D disease onset (6). These studies have been instrumental in understanding the natural history of T1D and making T1D a predictable disease with the measurement of antibodies in the peripheral blood directed against insulin and proteins within β-cells (glutamic acid decarboxylase [GAD], islet antigen 2 [IA-2], and zinc transporter [ZnT8]). Having two or more islet autoantibodies confers an ~85% risk of developing T1D within 15 years and nearly 100% over time (7). The American Diabetes Association now recommends screening islet autoantibodies in relatives of T1D patients through available clinical research studies (8), which is predominantly the National Institutes of Health–funded Type 1 Diabetes TrialNet Pathway to Prevention Study in the U.S. (9).

Efforts are also under way to screen children in the general population for islet autoantibodies, as approximately 85% of all diagnosed T1D case subjects lack a family history. In Bavaria, Germany, the Fr1da study is screening children ages 2–5 years for islet autoantibodies and has already screened >25,000 children, with 0.4% having multiple islet autoantibodies (10). The study plans to screen 100,000 children. Another large-scale screening effort is under way in the U.S.; the Autoimmunity Screening for Kids (ASK) program is screening children and adolescents in the Denver, CO, metro area for islet autoantibodies along with tissue transglutaminase autoantibodies in celiac disease (gluten sensitivity) (11). In sum, T1D can be predicted by measuring islet autoantibodies, and thousands of individuals including young children are being identified through screening efforts, necessitating the need for treatments to delay and prevent disease onset.

ORAL INSULIN FOR PREVENTION

Antigen-specific immunotherapies hold the promise of potentially inducing tolerance by inhibiting effector T cells and inducing regulatory T cells, which can act locally at tissue-specific sites of inflammation (12). Additionally, side effects are minimal with these therapies. As such, insulin and GAD

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have both been used as antigen-based approaches in T1D (13). Oral insulin has been evaluated in two large randomized double-blinded placebo-controlled trials over the last two decades. First in the Diabetes Prevention Trial–Type 1 (DPT-1) and then in the TrialNet clinical trials network, which succeeded the DPT-1 study group (14). The DPT-1 enrolled relatives at increased risk for T1D having islet autoantibodies, including insulin autoantibodies, and an intact first-phase insulin response to an intravenous glucose tolerance test to receive 7.5 mg of oral insulin or placebo (n = 372). After 6 years of treatment, there was no delay in T1D onset. However, a post hoc analysis revealed a potential delay in diabetes onset in those relatives with higher titers of insulin autoantibodies (15), thus prompting a repeat trial through TrialNet. The TrialNet study screened, enrolled, and followed 560 at-risk relatives over 9 years from 2007 to 2016, and results have been recently published (16). Unfortunately, this trial failed to meet the primary end point of delaying or preventing diabetes onset. It should be noted that there were four different strata of patients included in this trial according to autoantibodies profiles and first-phase insulin release. In the stratum with a certain autoantibody profile (confirmed insulin autoantibodies in addition to islet cell autoantibodies or GAD/IA-2) and a low first-phase insulin release (n = 54), there was a statistically significant delay in T1D onset (16). Analyzing the other strata and all participants together revealed no delay in T1D development. Similar to the DPT-1 trial, a subset of participants responded.

Although there are subtle differences between the DPT-1 and TrialNet oral insulin trials, when comparing the two there are a number of sobering findings. First, the risk of T1D development was nearly identical in the placebo arms of these two trials even though participants were screened and enrolled over a decade apart. Second, the therapy was identical —7.5mg capsules of human insulin taken orally once a day. Third, the overall results were the same in terms of not meeting the primary end point of delaying T1D onset; however, there were subgroups of responders that were different in both trials. These findings raise the question, "Is oral insulin a viable therapy for T1D prevention?" The answer is close to "no" for the dose and stage of T1D tested in these two trials.

Many factors influence the potency and efficacy of antigen-specific therapy such as dose, frequency of dosing, route of administration, and, importantly, timing in the disease process. Can oral insulin induce a phenotype of antigen-specific tolerance in at-risk relatives with insulin and other islet autoantibodies? This question is being evaluated in a mechanistic trial conducted by TrialNet, Immune Effects of Oral Insulin. This two-arm multicenter open-label study randomized participants to receive 67.5 mg of oral insulin daily or 500 mg every other week for 6 months and then follow-up for 6 months off therapy. The trial rapidly enrolled children and adolescents (n = 92) within 1 year, and final results are anticipated in the next year. This study evaluates two oral insulin dosing regimens at one time in the disease process.

In children without islet autoimmunity but with HLA-DQ-DR haplotypes and family history conferring high T1D risk, the Pre-POINT pilot study evaluated escalating doses of oral insulin up to 67.5 mg daily, which resulted in protective immune responses to insulin (17). Proinflammatory IFN-γ CD4 T-cell responses directed to proinsulin were not observed, while there appeared to be an induction of proinsulin-responsive regulatory T cells. With the ability to induce a protective immune response toward insulin, a larger primary prevention trial (individuals without T1D-associated autoantibodies) to prevent the development of islet autoantibodies is now under way with a higher dose of oral insulin. It should be noted that the oral insulin treatment arms in the Pre-POINT study only included six participants, with only several individuals per group having insulin autoantibody and T-cell responses. This raises the question of trial design and statistical powering for such mechanistic trials.

ADAPTIVE CLINICAL TRIAL DESIGNS

Over the last two decades, most T1D clinical trial designs have randomized participants 1:1 or 2:1, drug to placebo, in a double-blind two-arm design, especially those intervention trials in new-onset T1D (18). Primary end points have been delay in T1D onset for prevention trials or stimulated C-peptide area under the curve at 12 months with new-onset trials. These designs have served the field well and provided reliable human data for efficacy. However, there are limitations including the speed at which these trials can be completed, the number of interventions evaluated, dose optimization, and evaluation of mechanistic hypotheses.

Alternative clinical trial designs, such as adaptive trial designs using Bayesian statistics, can overcome some of these issues. Adaptive designs use accumulating data from the trial to modify certain aspects of the study, such as enrollment and treatment group assignments. This "learn as we go" approach relies on biomarkers to drive decisions on planned trial modifications. One such example is an adaptive dose-finding trial for low-dose IL-2 therapy, Adaptive Study of IL-2 Dose on Regulatory T cells in Type 1 Diabetes (DILT1D) (19). This is particularly pertinent, as a trial using IL-2 and rapamycin effectively increased regulatory T cells but resulted in a transient decline in β-cell function (20). The DILT1D study measured the percentage change in regulatory T cells ($CD3^+CD4^+CD25^{hi}CD127^{lo}$ cells) following a single subcutaneous injection of IL-2. There was an initial learning phase from the first five participants, and then subjects were randomized to doses predicted to have a 10% to 20% increase in regulatory T cells (21). Next steps include identifying the optimal repeated dosing regimen to increase regulatory T cells while minimizing effector T-cell activation. One of the significant limitations for adaptive trial designs in the T1D field, at the present time, is the lack of validated biomarkers for short-term readouts to inform trial adaptations. However, large-scale collaborative efforts are ongoing to define biomarkers of T1D-specific immune dysfunction and β-cell stress and death (9,22).

T1D IS A HETEROGENEOUS DISEASE

T1D prevention has proven much more difficult than originally thought, challenging the paradigm that T1D is a single disease. T1D is indeed a heterogeneous disease in terms of age of diagnosis, islet autoantibody profiles, and the rate of loss of residual β-cell function after clinical onset. Children have a much more rapid loss of residual insulin production (measured as C-peptide area under the curve following a mixed-meal tolerance test) after diagnosis than older adolescents and adults (23,24), indicating that childhood and adult-onset T1D are not identical. Further evidence for subtypes of T1D come from studies of human pancreata of T1D organ donors in which children (0–14 years of age) within 1 year of diagnosis had many more inflamed islets compared with older adolescents and adults aged 15–39 years old (25). Additionally, a younger age of T1D onset ($<$7 years) has been associated with higher numbers of $CD20^+$ B cells within islets and fewer insulin-containing islets compared with an age of onset $\geq$13 years associated with fewer $CD20^+$ islet infiltrating cells and more insulin-containing islets (26,27). This suggests a much more aggressive autoimmune process in younger children and distinct endotypes (a subtype of a condition defined by a distinct pathophysiologic mechanism), which has recently been proposed for T1D (27). It is noteworthy that the study of the target organ, inflamed pancreatic islets, from recent-onset organ donors led to these insights, and further work studying antigen-specific immune cells and β-cells within inflamed islets holds promise for defining pathogenic mechanisms, biomarkers, and novel therapeutic targets (28–31).

LESSONS FROM INDUSTRY: DRUG DEVELOPMENT

Safe and specific therapies capable of being used in children are needed for T1D prevention. The vast majority of drug development involves small biotechnology companies, specialty pharmaceutical firms, and large pharmaceutical companies, more so than traditional academia. A large amount of preclinical and clinical research (phase 1, 2, and 3 studies) are needed to advance a drug candidate through the development pipeline to achieve U.S. Food and Drug Administration (FDA) approval for a given disease. A recent analysis of over 4,000 drugs from 835 companies in development during 2003–2011 revealed that only 10.4% of drugs that enter clinical development at phase 1 (safety studies) advance to FDA approval (32). However, the success rate increases 50% for the lead indication of a drug, i.e., a drug specifically developed for one given disease (32). Reasons for this include strong scientific rationale and early efficacy signals such as correlating pharmacokinetic (drug levels) to pharmacodynamic (drug target effects) tests for the lead indication. Lead indications also tend to have smaller, better-defined "homogenous" patient populations than nonlead indications for the same drug. This would imply that the T1D field needs more companies developing drugs specifically for T1D, not type 2 diabetes or other autoimmune diseases with later testing to broaden a drug's indication.

In a similar but separate analysis, selection biomarkers were found to substantially increase the success rate of drug approvals across all phases of drug development. Using a selection biomarker as part of study inclusion criteria increased drug approval threefold from 8.4% to 25.9% when used in phase 1 trials, 28% to 46% when transitioning from a phase 2 to phase 3 efficacy trial, and 55% to 76% for a phase 3 trial to likelihood of approval (33). These striking data support the concept that enrichment of patient enrollment at the molecular level is a more successful strategy than heterogeneous enrollment in clinical intervention trials. Selection biomarkers in drug development are predictive biomarkers used to identify an individual more likely to respond to treatment. A second type of biomarker, a prognostic biomarker, is used to identify the likelihood of a clinical event or disease progression. As mentioned earlier, the risk of T1D development was identical in the placebo arms of the two large oral insulin trials, indicating that the prognostic biomarker of islet autoantibodies with normoglycemia is accurate and reproducible. However, insulin autoantibody titers did not predict treatment response in the follow-up TrialNet oral insulin study despite a post hoc analysis from the DPT-1 trial indicating that this subgroup of patients may have clinical benefit (15).

Taken together, new drugs designed specifically for children at risk for T1D and a biomarker selecting patients for a treatment response may increase the likelihood for a successful prevention trial; however, experimental confirmation in clinical trials is needed.

PERSONALIZED THERAPIES ON THE HORIZON

To eventually delay and prevent the onset of T1D, a rationale strategy is needed moving forward. First, more human clinical trials are needed to test therapies, define drug mechanisms, and ultimately understand human T1D pathology. Second, we advocate testing personalized therapies directed at specific molecular targets implicated in disease pathogenesis with a biomarker-driven and hypothesis testing approach in early-stage clinical trials. To that end, several therapies are being developed and investigated in such a manner. Small molecules, peptide immunotherapy, and autologous peptide-loaded tolerogenic dendritic cells are all targeting major histocompatibility complex (MHC) class II molecules in T1D. HLA class II genes confer significant T1D genetic risk (34) and encode MHC class II proteins found on B cells, macrophages, and dendritic cells. Approximately 50–60% of all patients at risk for and with T1D have the HLA-DR4/DQ8 haplotype, making it an attractive molecular target for therapeutic intervention.

HLA-DQ8 has been targeted with small "drug-like" molecules (35), and it was discovered that methyldopa (Aldomet) blocks the ability of DQ8 to present self-peptides, thus inhibiting CD4 T-cell activation (36). Methyldopa is a well-known oral antihypertensive agent used to treat both children and adults for more than 50 years (37); it is currently indicated for the treatment of pregnancy-induced

hypertension. The repurposing of methyldopa to engage DQ8 and specifically block antigen presentation was evaluated in an open-label dose escalation study with recent-onset T1D patients having the DQ8 allele and residual insulin production (36). A follow-up multicenter randomized double-blinded placebo-controlled crossover trial conducted by TrialNet, Methyldopa for Reduction of DQ8 Antigen Presentation in At-Risk Subjects for Type 1 Diabetes, is set to begin enrollment in 2018 (NCT03396484). This mechanistic trial will evaluate the safety, immunologic efficacy, and mechanism of action of methyldopa in individuals at risk for T1D with the primary outcome assessing the effect of methyldopa versus placebo on the change in insulin-specific DQ8 antigen presentation. Results from such a mechanistic trial design can help inform the design and patient selection for a future trial powered for T1D prevention.

Peptide immunotherapy, with a peptide of proinsulin (C19-A3), is also targeting patients with a specific HLA-DR4 molecule, DRB*04:01. The proinsulin peptide was eluted from antigen-presenting cells with DR4 and shown to elicit T-cell responses in T1D patients (38). A phase 1 dose-finding trial assessed intradermal peptide injection in established T1D patients, showing safety and tolerability. A signal of immune efficacy was observed in the low-dose group with the induction of interleukin-10 (IL-10), an anti-inflammatory cytokine associated with regulatory T cells, upon stimulating peripheral blood mononuclear cells with proinsulin peptide (39). A follow-up phase 1b randomized placebo-controlled trial was conducted in new-onset T1D patients with residual insulin production having the DR4 allele, again showing safety and suggestions of an IL-10 response in treatment responders (40). Six DR4-restricted peptides from proinsulin and IA-2 have been combined into a single product that will be administered to new-onset T1D patients in a trial being developed with the Immune Tolerance Network (ITN); additionally, a mechanistic trial in those at risk is being considered in TrialNet.

A third therapeutic approach involves dendritic cells that function to process and present antigens to activate CD4 T cells; however, these cells also have tolerogenic properties. Dendritic cells with a regulatory phenotype can suppress effector T cells, induce regulatory T cells, and provide infectious tolerance (i.e., spreading of tolerance to other antigens than those carried by the dendritic cell) (41). Early-phase clinical trials in a number of autoimmune diseases, including T1D, have isolated a patient's own dendritic cells, cultured with factors (such as vitamin D3 and dexamethasone) to induce a regulatory phenotype and transferred these cells back into the patient, showing safety (42–44). A mechanistic trial is now under way in T1D to pulse tolerogenic dendritic cells with islet peptides known to bind HLA-DR4 prior to injecting them back into a patient in an attempt to induce antigen-specific tolerance (45). The primary outcome of the trial is safety and feasibility, with secondary end points evaluating T-cell–specific immune responses and stimulated C-peptide production.

CONCLUSIONS

Despite being predictable, the delay and prevention of T1D onset has proven a significant challenge. Heterogeneity exists within T1D, and validated biomarkers are needed to define disease subtypes, which can allow for patient selection at a molecular level for intervention trials. A paradigm shift toward hypothesis testing in biomarker-driven mechanistic clinical trials holds promise for defining drug dose and mechanism of action. In this manner, therapy will be personalized and enhance the chance for successful T1D prevention in subsequent randomized controlled trials.

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References

1. Bonifacio E. Predicting type 1 diabetes using biomarkers. Diabetes Care 2015;38:989–996
2. Erlich H, Valdes AM, Noble J, et al.; Type 1 Diabetes Genetics Consortium. HLA DR-DQ haplotypes and genotypes and type 1 diabetes risk: analysis of the type 1 diabetes genetics consortium families. Diabetes 2008;57:1084–1092
3. Ferrannini E, Mari A, Nofrate V, Sosenko JM, Skyler JS; DPT-1 Study Group. Progression to diabetes in relatives of type 1 diabetic patients: mechanisms and mode of onset. Diabetes 2010;59:679–685
4. Barker JM, Barriga KJ, Yu L, et al.; Diabetes Autoimmunity Study in the Young. Prediction of autoantibody positivity and progression to type 1 diabetes: Diabetes Autoimmunity Study in the Young (DAISY). J Clin Endocrinol Metab 2004;89:3896–3902
5. Kupila A, Keskinen P, Simell T, et al. Genetic risk determines the emergence of diabetes-associated autoantibodies in young children. Diabetes 2002;51:646–651
6. Ziegler AG, Hummel M, Schenker M, Bonifacio E. Autoantibody appearance and risk for development of childhood diabetes in offspring of parents with type 1 diabetes: the 2-year analysis of the German BABYDIAB study. Diabetes 1999;48:460–468
7. Ziegler AG, Rewers M, Simell O, et al. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. JAMA 2013;309:2473–2479
8. American Diabetes Association. Sec. 2. Classification and diagnosis of diabetes: *Standards of Medical Care in Diabetes—2018*. Diabetes Care 2018;41(Suppl. 1):S13–S27
9. Battaglia M, Anderson MS, Buckner JH, et al. Understanding and preventing type 1 diabetes through the unique working model of TrialNet. Diabetologia 2017;60:2139–2147
10. Raab J, Haupt F, Scholz M, et al.; Fr1da Study Group. Capillary blood islet autoantibody screening for identifying pre-type 1 diabetes in the general population: design and initial results of the Fr1da study. BMJ Open 2016;6:e011144
11. Gesualdo PD, Bautista KA, Waugh KC, et al. Feasibility of screening for T1D and celiac disease in a pediatric clinic setting. Pediatr Diabetes 2016;17:441–448
12. Michels AW, von Herrath M. 2011 Update: antigen-specific therapy in type 1 diabetes. Curr Opin Endocrinol Diabetes Obes 2011;18:235–240
13. Skyler JS. Prevention and reversal of type 1 diabetes–past challenges and future opportunities. Diabetes Care 2015;38:997–1007
14. Diabetes Prevention Trial–Type 1 Diabetes Study Group. Effects of insulin in relatives of patients with type 1 diabetes mellitus. N Engl J Med 2002;346:1685–1691
15. Skyler JS, Krischer JP, Wolfsdorf J, et al. Effects of oral insulin in relatives of patients with type 1 diabetes: The Diabetes Prevention Trial–Type 1. Diabetes Care 2005;28:1068–1076

16. Krischer JP, Schatz DA, Bundy B, Skyler JS, Greenbaum CJ; Writing Committee for the Type 1 Diabetes TrialNet Oral Insulin Study Group. Effect of oral insulin on prevention of diabetes in relatives of patients with type 1 diabetes: a randomized clinical trial. JAMA 2017;318:1891–1902
17. Bonifacio E, Ziegler AG, Klingensmith G, et al.; Pre-POINT Study Group. Effects of high-dose oral insulin on immune responses in children at high risk for type 1 diabetes: the Pre-POINT randomized clinical trial. JAMA 2015;313:1541–1549
18. Ehlers MR. Strategies for clinical trials in type 1 diabetes. J Autoimmun 2016;71:88–96
19. Waldron-Lynch F, Kareclas P, Irons K, et al. Rationale and study design of the Adaptive study of IL-2 dose on regulatory T cells in type 1 diabetes (DILT1D): a non-randomised, open label, adaptive dose finding trial. BMJ Open 2014;4:e005559
20. Long SA, Rieck M, Sanda S, et al.; Diabetes TrialNet and the Immune Tolerance Network. Rapamycin/IL-2 combination therapy in patients with type 1 diabetes augments Tregs yet transiently impairs β-cell function. Diabetes 2012;61:2340–2348
21. Todd JA, Evangelou M, Cutler AJ, et al. Regulatory T cell responses in participants with type 1 diabetes after a single dose of interleukin-2: a non-randomised, open label, adaptive dose-finding trial. PLoS Med 2016;13:e1002139
22. Mirmira RG, Sims EK, Syed F, Evans-Molina C. Biomarkers of β-cell stress and death in type 1 diabetes. Curr Diab Rep 2016;16:95
23. Hao W, Gitelman S, DiMeglio LA, Boulware D, Greenbaum CJ; Type 1 Diabetes TrialNet Study Group. Fall in C-peptide during first 4 years from diagnosis of type 1 diabetes: variable relation to age, HbA_{1c}, and insulin dose. Diabetes Care 2016;39:1664–1670
24. Greenbaum CJ, Beam CA, Boulware D, et al.; Type 1 Diabetes TrialNet Study Group. Fall in C-peptide during first 2 years from diagnosis: evidence of at least two distinct phases from composite Type 1 Diabetes TrialNet data. Diabetes 2012;61:2066–2073
25. In't Veld P. Insulitis in human type 1 diabetes: a comparison between patients and animal models. Semin Immunopathol 2014;36:569–579
26. Leete P, Willcox A, Krogvold L, et al. differential insulitic profiles determine the extent of β-cell destruction and the age at onset of type 1 diabetes. Diabetes 2016;65:1362–1369
27. Arif S, Leete P, Nguyen V, et al. Blood and islet phenotypes indicate immunological heterogeneity in type 1 diabetes. Diabetes 2014;63:3835–3845
28. Michels AW, Landry LG, McDaniel KA, et al. Islet-derived CD4 T cells targeting proinsulin in human autoimmune diabetes. Diabetes 2017;66:722–734
29. Kent SC, Mannering SI, Michels AW, Babon JAB. Deciphering the pathogenesis of human type 1 diabetes (T1D) by interrogating T cells from the "scene of the crime". Curr Diab Rep 2017;17:95
30. Babon JA, DeNicola ME, Blodgett DM, et al. Analysis of self-antigen specificity of islet-infiltrating T cells from human donors with type 1 diabetes. Nat Med 2016;22:1482–1487
31. Pathiraja V, Kuehlich JP, Campbell PD, et al. Proinsulin-specific, HLA-DQ8, and HLA-DQ8-transdimer-restricted $CD4^+$ T cells infiltrate islets in type 1 diabetes. Diabetes 2015;64:172–182
32. Hay M, Thomas DW, Craighead JL, Economides C, Rosenthal J. Clinical development success rates for investigational drugs. Nat Biotechnol 2014;32:40–51
33. Thomas DW, Burns J, Audette J, Carroll C, Dow-Hygelund C, Hay C. *Clinical Development Success Rates 2006-2015*. BIO, Biomedtracker, Amplion, 2016
34. Hu X, Deutsch AJ, Lenz TL, et al. Additive and interaction effects at three amino acid positions in HLA-DQ and HLA-DR molecules drive type 1 diabetes risk. Nat Genet 2015;47:898–905
35. Michels AW, Ostrov DA, Zhang L, et al. Structure-based selection of small molecules to alter allele-specific MHC class II antigen presentation. J Immunol 2011;187:5921–5930
36. Ostrov DA, Alkanani A, McDaniel KA, et al. Methyldopa blocks MHC class II binding to disease-specific antigens in autoimmune diabetes. J Clin Invest 2018;128:1888–1902
37. Mah GT, Tejani AM, Musini VM. Methyldopa for primary hypertension. Cochrane Database Syst Rev 2009 (4):CD003893
38. Nagata M, Kotani R, Moriyama H, Yokono K, Roep BO, Peakman M. Detection of autoreactive T cells in type 1 diabetes using coded autoantigens and an immunoglobulin-free cytokine ELISPOT assay: report from the fourth immunology of diabetes society T cell workshop. Ann N Y Acad Sci 2004;1037:10–15
39. Thrower SL, James L, Hall W, et al. Proinsulin peptide immunotherapy in type 1 diabetes: report of a first-in-man phase I safety study. Clin Exp Immunol 2009;155:156–165
40. Alhadj Ali M, Liu YF, Arif S, et al. Metabolic and immune effects of immunotherapy with proinsulin peptide in human new-onset type 1 diabetes. Sci Transl Med 2017;9:eaaf7779
41. Nikolic T, Roep BO. Regulatory multitasking of tolerogenic dendritic cells - lessons taken from vitamin d3-treated tolerogenic dendritic cells. Front Immunol 2013;4:113
42. Jauregui-Amezaga A, Cabezón R, Ramírez-Morros A, et al. Intraperitoneal administration of autologous tolerogenic dendritic cells for refractory Crohn's disease: a phase i study. J Crohn's Colitis 2015;9:1071–1078
43. Benham H, Nel HJ, Law SC, et al. Citrullinated peptide dendritic cell immunotherapy in HLA risk genotype-positive rheumatoid arthritis patients. Sci Transl Med 2015;7:290ra87
44. Giannoukakis N, Phillips B, Finegold D, Harnaha J, Trucco M. Phase I (safety) study of autologous tolerogenic dendritic cells in type 1 diabetic patients. Diabetes Care 2011;34:2026–2032
45. Suwandi JS, Nikolic T, Roep BO. Translating mechanism of regulatory action of tolerogenic dendritic cells to monitoring endpoints in clinical trials. Front Immunol 2017;8:1598