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Review

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Arresting type 1 diabetes: are we there yet? Obstacles and opportunities

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Abstract

More than 100 years after the discovery of insulin, the exact etiology and pathophysiology of type 1 diabetes (T1D) remains elusive, but our knowledge is growing. This leads to louder calls to initiate a risk screening for T1D in the general population. This risk screening could be based on the genetic risk (in the general population or targeted HLA genotyping in family members of persons with T1D) or on the screening for autoantibodies in blood (e.g., antibodies against insulin, GAD, IA2, or ZnT8). The presence of autoantibodies is known to convey a clearly increased risk of progressing to T1D, particularly when two or more antibody types are present. It remains a point of discussion whether screening efforts are cost-effective. At present, in the absence of interventions capable of delaying the onset of disease, the only benefit of screening is the earlier diagnosis of T1D, thus avoiding life-threatening diabetic ketoacidosis (DKA). Nevertheless, large consortia (e.g., INNODIA and TrialNet) are currently focusing on not only disease biomarkers but also biomarkers of therapeutic effect of interventions. All hope is thus focused on the arrival of intervention strategies that could arrest the ongoing immune destruction of the beta cell and thus delay clinical disease onset. Thus far, attempts have focused on either protecting the beta cell or arresting the immune response, but the future seems to be one of combination therapy. Here, we perform a scoping review on the pathogenesis of T1D, discuss screening strategies, and present promising intervention strategies.

Keywords: Type 1 diabetes, prevention, intervention, cure

TYPE 1 DIABETES: AN AUTOIMMUNE DISEASE WITH A MAJOR ROLE FOR THE BETA CELL ITSELF

Type 1 diabetes (T1D) is an immune-mediated disease in which the insulin-producing beta cells are destroyed by the immune system, resulting in absolute insulin deficiency[1]. The classic hypothesis states that, in a genetically predisposed individual, activation of the immune system by one or multiple environmental triggers results in rapid destruction of the pancreatic beta cells[2]. More recently, attention is shifting towards malfunction of the pancreatic beta cells as a trigger of the immune response, albeit in a genetically at-risk individual[3]. This stresses the importance of genetics in T1D.

With already over 60 loci associated with increased susceptibility to T1D, some genes are linked to beta cell dysfunction and others to immune cell dysfunction, with the HLA region being the major contributor[4,5]. The genetic complexity of T1D is illustrated by the fact that the majority of new T1D diagnoses are made in individuals having no known family history of T1D, despite the 15-fold increased risk for T1D and 2-fold increased risk for coexisting associated autoimmune diseases in individuals having a first-degree relative with T1D[6,7]. In addition, many people carrying the highest risk HLA haplotypes do not develop T1D[8]. However, HLA genes remain the basis of genetic risk prediction models in T1D. The Global Platform for the Prevention of Autoimmune Diabetes (GPPAD) is a European platform that makes use of an enriched genetic risk score (with 47 SNPs) in a general population of newborns to identify those individuals with a 25-fold increased risk of developing T1D (1.3% versus 0.4%, in a Western European background)[9].

The trigger of how T1D occurs in a genetically at-risk individual remains to be elucidated. For years, the immune system was believed to be the only culprit. This issue is from studies in animal models of T1D, such as the NOD mouse that spontaneously develops a disease very similar to T1D, as well as from the detection of autoantibodies against several peptides and proteins of the beta cell in those with recently diagnosed T1D. Moreover, the presence of these autoantibodies in the blood has been shown to have a predictive value for the development of T1D in normoglycemic individuals. These observations were first made in first-degree relatives (and thus genetically predisposed individuals), but they have been extended to the general population. There is now evidence that the presence of two or more autoantibodies almost certainly predicts the evolution to T1D in normoglycemic individuals[10]. Typical autoantibodies are antibodies against glutamic acid decarboxylase (GAD), protein tyrosine phosphatase (IA-2 or ICA512), zinc transporter 8 (ZnT8), and insulin itself[11]. Although these autoantibodies are interesting biomarkers in the prediction of the evolution to T1D, they most likely do not play a pathogenic role. When studying the pancreas of people with T1D who died around the time of diagnosis, it is mainly a cellular infiltrate that is observed (insulitis)[12]. In animal models, the disease is transferred in immune compromised animals by immune cells and not by antibodies[13]. The immune cells responsible for the immune attack remain unknown. However, despite the importance of HLA class II genes in the genetic predisposition, it is not the CD4+ T lymphocyte but rather the HLA class I restricted CD8+ T lymphocyte that is implicated in direct beta cell destruction[14]. However, many other immune cell types could play a role, with an emerging role for innate or non-lymphocyte cells, such as NK cells or neutrophils.

An interesting alternative hypothesis poses that T1D is the result of a dysfunctional beta cell that is cleared by a correctly functioning immune system. In this perspective, T1D etiology becomes comparable to effective anti-tumor immunity and is not classified as an autoimmune disease. Arguments favoring this hypothesis point to a primary defect in insulin-producing beta cells as the initial trigger. This is supported by recent observations suggesting smaller pancreatic volumes in those affected or at-risk of T1D[15,16]. Furthermore, clear signs of beta cell stress can be detected in those on their way to developing T1D, exemplified by an increased proinsulin-to-insulin ratio[17]. This increased ratio suggests abnormalities in

insulin processing and vesicular trafficking[18]. Moreover, non-specific triggers associated with T1D, such as increased metabolic demand or viral infections, have been shown to induce endoplasmic reticulum (ER) stress and thereby stress beta cells[19]. This results in a vicious cycle, as ER stress may again increase the visibility of the beta cells to the immune system, thereby initiating further destruction of non-affected islets[20]. Indeed, a beta cell under attack results in the release of additional pro-inflammatory cytokines and chemokines by the beta cells and, as such, attracts even more cells of the immune system[21]. Moreover, beta cells overexpress HLA class I molecules, creating an additional homing beacon for cytotoxic T cells[22]. Whereas both the immune-mediated and the beta cell-centric hypothesis hold their ground, T1D is most likely the result of a complex network of dysfunctions in both the beta cells and the immune system[23]. Interestingly, this is demonstrated, among others, in the observation that stressed beta cells not only misfold insulin but also misprocess other proteins and peptides, leading to the formation of neo-antigens generating novel epitopes[24]. These novel epitopes by themselves then result in an aberrant immune response as they are believed to trigger the peripheral activation of CD4+ and CD8+ autoreactive T lymphocytes[25].

All these different hypotheses [Figure 1] are not only scientifically intriguing but also probably the reason for the limited success of interventions pursuing an arrest of beta cell destruction. Indeed, interventions attempting the restoration of immune tolerance using antigen-specific therapies have failed, and pure immune suppression or modulation has only succeeded in temporarily delaying the decline of functional beta cell mass[26]. These failed interventions also triggered the belief that T1D is a heterogeneous disease, where in some, the immune system may be the causal factor (and then still different immune cell types may be prominent in the attack), whereas in others, more beta cell defects are responsible for the final beta cell disappearance.

TYPE 1 DIABETES: SEARCH FOR BIOMARKERS

The presence of islet-specific autoantibodies, not only in genetically predisposed individuals but also in the general population, remains the best predictor of progression towards T1D[27]. Nevertheless, the application of islet-specific autoantibodies as a predictor of T1D is not ready for daily clinical practice. One of the main issues remains the extent of heterogeneity, with the risk of T1D depending on the antibody titer, affinity, immunoglobulin subclasses, and target epitopes on single or multiple islet autoantigens[28]. Insulin autoantibodies (IAA) and GAD autoantibodies (GADA) are most frequently the first appearing autoantibodies, with a higher prevalence of the former in children and the latter in adults[29]. Moreover, the presence of only one islet autoantibody is not sufficient to determine the evolution towards T1D. In a prospective cohort study, only 15% of children with one islet autoantibody developed T1D within 10 years, compared to 70% of those with at least two islet autoantibodies[10]. A recent study showed that autoantibody appearance usually happened before six years of age, with optimal screening ages for initial islet autoantibody screening in children at two and six years of age[30]. Therefore, the consensus states that only the positivity of at least two islet autoantibodies confers a high risk of developing symptomatic T1D[31].

As the above-mentioned tackles the obstacles in the use of islet-specific autoantibodies as a biomarker, the quest for novel biomarkers is on. Novel biomarkers can contribute to a more precise prediction of disease, a better understanding of the heterogeneity of the disease, and predict or at least measure the response to therapy. In INNODIA (Innovative Medicines Initiative of the European Commission; www.innodia.eu), a public–private partnership, over 50 clinical centers in Europe, both pediatric and adult clinics, are collecting samples from new-onset T1D patients and unaffected family members of people living with T1D. In a natural history study spanning several years, modular interrogation platforms for the analysis of cellular and molecular features of beta cell and immune cell biomarkers have been established. These include proteomes, lipidomes, and metabolomes, as well as a full immunome and RNA analyses. A highly standardized

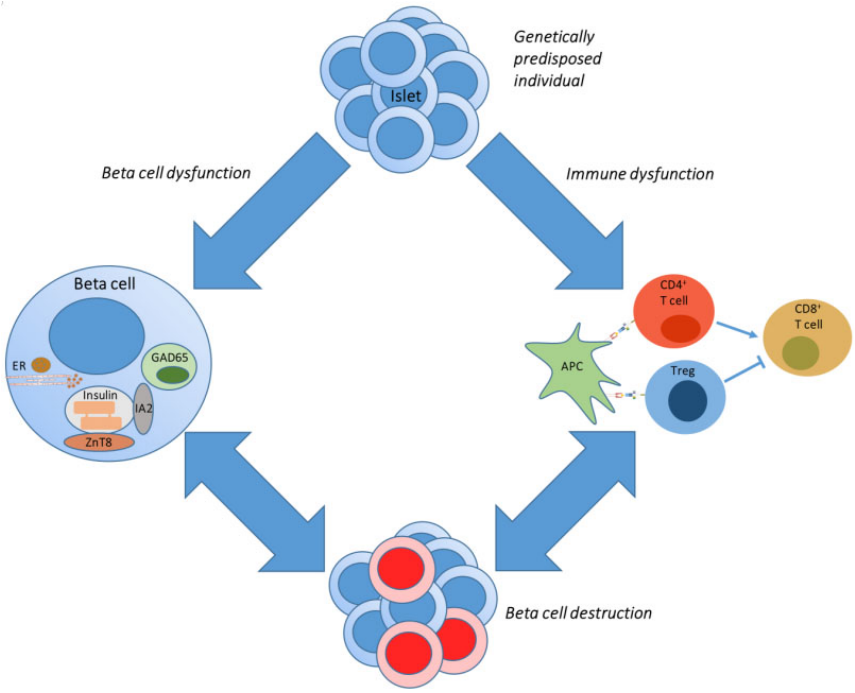

Figure 1. Pathogenesis of type 1 diabetes. In a genetically predisposed individual, a sudden trigger results in beta cell dysfunction or aberrant activation of the immune system, as depicted by the upper blue arrows. This eventually results in the destruction of the beta cells. Beta cell dysfunction can also trigger a (healthy) immune response to clear the dysfunctional beta cells (depicted by double-headed arrows). In a similar way, beta cell destruction by the immune system will cause stress on the remaining beta cells, resulting in further beta cell apoptosis. APC: Antigen-presenting cell; ER: endoplasmic reticulum; GAD: glutamic acid decarboxylase; IA2: islet-antigen 2; Treg: regulatory T cell; ZnT8: zinc-transporter 8.

collection of samples, followed by highly standardized and quality-controlled analyses in accredited laboratories, should allow robust conclusions when performing an integrated multi-omics natural history study on samples of new-onset T1D individuals or antibody-positive first-degree relatives of people with T1D. Of importance, these biomarker analyses are also included in the clinical trials running in the INNODIA network, thus not only opening the path to biomarkers of disease but also raising hope for the discovery of biomarkers of therapeutic effect and success of interventions.

PREVENTION OR ARREST OF TYPE 1 DIABETES: ARE WE THERE YET?

Why have we not cured T1D yet? It is not because of a lack of trying. The list of studies already performed in people with new-onset T1D or unaffected high-risk family members is already very long. In an attempt to exploit resources and efforts, the scientific community, together with pharmaceutical companies and patient advisors, have joined globally into large consortia. Examples include the Type 1 Diabetes TrialNet [established in 2001 as a National Institutes of Health (NIH)-funded and Juvenile Diabetes Research Foundation (JDRF)-supported international clinical trial network that emerged from the Diabetes Prevention Trial Type 1 (DPT-1)] and the more recent INNODIA consortium (a European partnership among academic institutions, industrial partners, and patient organizations)[32]. These consortia have the advantage of being multi-centered and often have a master protocol, allowing comparison between different studies.

To date, interventions mainly focused on either targeting an ongoing immune response by general or specific immune suppression or modulation [Table 1] or the induction of tolerance to beta cell-related

Table 1. Overview of clinical trials targeting arrest of new-onset type 1 diabetes

Trial	Mechanism	Location	Effect	Phase	DOP/DOFR	Refs.
Cyclosporin A	Calcineurin inh	Eu	Transient	2	1986	[58]
Rituximab	Anti-CD20 mAb	Au, Eu, NA	Transient	2	2009	[59]
Mycophenolate + Daclizumab	IMDPH inh + anti-CD25 mAb	NA	No	2	2010	[60]
PROTÉGÉ - Teplizumab	Anti-CD3 mAb	NA	No	3	2011	[44]
Abatacept	CTLA-4-Ig	NA	Transient	2	2011	[61]
AbATE - Teplizumab	Anti-CD3 mAb	NA	Transient	2	2013	[62]
T1DAL - Alefacept	LFA-3-Ig	NA	Potential	2	2013	[63]
DEFEND - Otelixizumab	Anti-CD3 mAb	Eu, NA	No	3	2014	[42,43]
START - High-dose ATG	HD anti-thymocyte globulin	NA	No	2	2016	[35]
Low-dose ATG	LD anti-thymocyte globulin	NA	Beneficial	2	2018	[37]
Verapamil	Calcium channel-blocker	NA	Beneficial	2	2019	NCT02372253
T1GER - Golimumab	Anti-TNF-α mAb	NA	Beneficial	2	2020	[50]
Anti-IL-21 + Liraglutide	Anti-IL-21 mAb + GLP-1 RA	NA, Eu	Beneficial	2	2021	[56]
Tocilizumab	Anti-IL-6 receptor mAB	Au, NA	Ongoing	2	2021	NCT02293837
Ladarixin	CXCR1 and CXCR2 Inh	Eu	No	2	2022	[64]
DIABIL-2 - IL-2	Recombinant human IL-2	Eu	Ongoing	2	/	NCT02411253
ITAD - IL-2	Recombinant human IL-2	Eu	Ongoing	2	/	NCT03782636
Iscalimab	Anti-CD40 mAb	Eu	Ongoing	2	/	NCT04129528
I-DIT - Ixekizumab	Anti-IL-17 mAb	Eu	Ongoing	2	/	NCT04589325
UST1D2 - Ustekinumab	Anti-IL-12 / Anti-IL-21 mAb	NA	Ongoing	2-3	/	NCT03941132
MELD-ATG	LD anti-thymocyte globulin	Eu	Ongoing	2	/	NCT04509791
BANDIT - Baricitinib	JAK1 and JAK2 Inh	Au	Ongoing	2	/	NCT04774224

The table is ranked based on the date of publication (DOP) or, if not available, the date of the release of the first results (DOFR). If no results are available yet, the study is marked by a dash. ATG: Anti-thymocyte globulin; Au: Australia; CTLA-4: cytotoxic T lymphocyte-associated protein 4; CXCR: C-X-C chemokine receptor; Eu: Europe; GLP-1: glucagon-like peptide 1; HD: high dose; Ig: immunoglobulin fusion protein; IL: interleukin; IMDPH: inosine monophosphate dehydrogenase; Inh: inhibitor; JAK: Janus kinase; LD: low dose; LFA-3: lymphocyte function-associated antigen; mAb: monoclonal antibody; NA: North America; NCT: national clinical trial; TNF: tumor necrosis factor; RA: receptor agonist; Refs.: references.

antigens to prevent T1D [Table 2].

Interventions arresting an ongoing immune response by general immunosuppression, with cyclosporin A as a prime example, were the first to demonstrate the potential to induce disease remission in new-onset T1D. Despite these promising results, the major obstacles associated with this strategy are disease recurrence and the adverse effects associated with general immunosuppressive drugs (reviewed in[33]). This breakthrough resulted in the quest for an immunosuppressive or immunomodulatory drug that could overcome both obstacles. As such, to date, many immune agents have been tested, with low-dose anti-thymocyte globulin (ATG) and teplizumab (anti-CD3 antibody) being the most promising in people with new-onset T1D[34].

ATG finds its origin in transplantation, and, compared to the relatively higher doses (6.5 mg/kg) used in the randomized controlled Study of Thymoglobulin to ARrest T1D (START) trial, it is found to be more effective in new-onset T1D when used in lower doses (2.5 mg/kg)[35-37]. The protective effect of the lower dose is based on a transient T lymphocyte depletion followed by a T lymphocyte reconstitution in favor of regulatory T lymphocytes, resulting in a shift towards tolerance induction[38]. This is further established by the observation that the addition of G-CSF to a low-dose ATG regimen led to a decrease in the protective effect of ATG[36]. In INNODIA, in phase II, randomized, placebo-controlled, MELD-ATG trial, researchers are testing if even lower doses of ATG would be effective in arresting the decline of functional beta cell mass

Table 2. Overview of clinical trials using antigen-specific therapy in type 1 diabetes

Trial	Mechanism	Location	Effect	Phase	DOP/DOFR	Refs.
DPT-1	Parenteral Insulin	NA	No	3	2002	[65]
DPT-1	Oral Insulin	NA	No	3	2005	[66]
DIPP	Nasal Insulin	Eu	No	3	2008	[67]
Pre-POInT	Oral Insulin	Eu	Potential	1-2	2015	[68]
TrialNet	Oral Insulin	Eu, NA	No	3	2017	[69]
DIAPREV-IT	SC GAD-Alum	Eu	No	2	2018	[70]
Pre-POInT-Early	Oral Insulin	Eu	No	2	2021	[71]
DIAGNODE	ILIT GAD-Alum + Vit D3	Eu	Potential	2	2021	[72]
DIAPREV-IT 2	SC GAD-Alum + Vit D3	Eu	Ongoing	2	2020	NCT02387164
INIT-II	Nasal Insulin	Au, NZ	Ongoing	2	/	NCT00336674
FR1da	Oral Insulin	Eu	Ongoing	2	/	NCT02620072
PINIT	Nasal Insulin	Eu	Ongoing	2	/	NCT03182322
Oral Proinsulin + IL-10	Oral Proinsulin + IL-10	Eu, NA	Ongoing	1-2	/	NCT03751007
IMPACT	SC IMCY-0098	Au, Eu, NA	Ongoing	2	/	NCT04524949

The table is ranked based on the date of publication (DOP) or, if not available, the date of the release of the first results (DOFR). If no results are available yet, the study is marked by a dash. Au: Australia; Eu: Europe; GAD: glutamic acid decarboxylase; IL: interleukin; ILIT: intralymphatic; NA: North America; NZ: New Zealand; Refs.: references; SC: subcutaneous; Vit: vitamin.

in people with newly diagnosed T1D (NCT04509791).

In the search for more specific immunomodulatory agents to arrest a T cell-mediated autoimmune disease, attention has shifted to the use of anti-CD3 monoclonal antibodies. The initial clinical pilot trials using humanized anti-CD3 monoclonal antibodies (i.e., teplizumab or the aglycosylated otelixizumab) were hopeful as they showed preservation of beta cell function[39,40]. However, no one could have predicted what followed. Hereafter, large multicenter, randomized, placebo-controlled phase III trials for both otelixizumab and teplizumab failed. Unfortunately, the main reason for this is an alternation of the study protocol in the former and an incorrect choice of the primary endpoint in the latter. Later, this story of anti-CD3 monoclonal antibodies became a prime example of the importance of choosing the correct study protocol and endpoints (reviewed in[41]) and one of the main reasons large consortia often work with a master protocol. For otelixizumab, this was the DEFEND trial, which was probably unsuccessful due to a 15-fold dose reduction in the effective dose[42,43]. For teplizumab, its large phase III trial was the PROTÉGÉ trial. Here, the study population (patients diagnosed with T1D within the past 12 weeks) and the choice of the endpoint (insulin requirement) were the suspected reasons for therapy failure[44]. Later, the randomized, open-label, AbATE trial narrowed the timeframe of new-onset T1D to enroll patients only with a new diagnosis in the past eight weeks and corrected the primary endpoint to a change in C-peptide. In this way, they demonstrated that teplizumab was able to preserve C-peptide in people with new-onset T1D, with a decline in C-peptide up to seven years after diagnosis in responders[45,46].

The AbATE trial demonstrated that an earlier start of therapy resulted in the rescue of more residual beta cells. Based on this, teplizumab was given even earlier in the disease process of T1D, namely in people with stage 2 T1D (defined as the presence of two or more diabetes-related autoantibodies and dysglycemia). Here, teplizumab was able to delay progression to clinical T1D for up to three years[47,48]. These encouraging results have led to the submission of teplizumab to the regulatory authorities for the delay of T1D in prediabetes.

Another lesson learned from the anti-CD3 trials is that an intervention should not be written off too soon. Indeed, for a long time, it was believed that anti-inflammatory interventions targeting single cytokines (e.g., TNF-α or IL-1) are not successful in T1D[49]. However, the recent phase II, multicenter, placebo-controlled, double-blind, T1GER study, where anti-TNF-α receptor antibodies were administered continuously, showed that the decline of functional beta cell mass, as measured by stimulated C-peptide, could be arrested by continued administration of the antibody[50].

Therapies targeting the restoration of tolerance of the immune system towards beta cell-related antigens, such as insulin or GAD, have failed to be successful[51]. However, this approach remains interesting, as it holds the potential to induce longer-lasting beta cell protection if one could restore tolerance to the beta cell. Hypothesizing that a combination of immune modulation ("clearing the autoimmune attack at the time of T1D diagnosis") and antigen administration ("restoring tolerance") could offer the perspective of long-term effect, several approaches have been designed, and some tested. One of these is the administration of proinsulin (as an autoantigen) in combination with the cytokine IL-10 through a genetically engineered *Lactococcus lactis* as the carrier. This approach allows administering this antigen via an oral route (a pathway known for tolerance induction), and interim results of the phase Ib (open-label) and IIa (randomized, double-blind), multicenter, study with this approach (AG019 Actobiotics™) demonstrate both safety and potentially interesting immune effects[52] (NCT03751007). The investigators even brought in an additional immune modulator in the form of teplizumab, based on successful animal studies[53,54], which again showed good safety and interesting immune effects. Another promising combination of immune modulation and antigen was tested in the DIAGNODE-1 pilot trial, where GAD dissolved in alum was administered into lymph nodes (a more targeted approach), as well as in combination with vitamin D for low-grade immunomodulation[55]. Based on relatively promising results, GAD in alum is now being tested in combination with ibuprofen (DIABGAD, an interventional pilot trial; NCT01785108), etanercept (EDCR, an interventional open-label trial; NCT02464033), or GABA (GABA/Diamyd, a randomized placebo-controlled trial; NCT02002130) as an anti-inflammatory component.

Finally, based on the observations described above, suggesting a role in the pathogenesis of T1D for both the beta cells and the immune system, other trials have been conducted in an attempt to combine immune modulation with beta cell protective agents. A recent randomized, placebo-controlled, phase II trial combined liraglutide, a GLP-1 receptor agonist, and an antibody targeting the cytokine IL-21. This combination therapy showed a clear delay in the decline of functional beta cell mass[56].

FUTURE PERSPECTIVES

Why have we not succeeded in arresting T1D? In the most popular animal model, the NOD mouse, the list of successful interventions is long, but a careful reading of the literature shows that these interventions are particularly successful when administered early in the life of the mouse before any autoimmune attack has started[57]. In humans, however, this would be the equivalent of treating newborns. As long as our predictive power is low (genetic risk scores allow enrichment in the general population of up to 25-fold, but they still only provide a risk attribution of around 1%), only very safe interventions will be tolerated. As such, on the GPPAD platform, the cross-sectional cohort Freder1k trial is evaluating the effect of administration of oral insulin on the onset of autoimmunity (autoantibodies against the beta cell) (NCT03316261), and the multicenter, randomized, placebo-controlled, SINT1A study is evaluating the impact of probiotics on islet autoimmunity development (NCT04769037). A major issue with these early interventions is the number of people to be screened, as well as the amount of time these people need to be followed up. Even when individuals at later stages of T1D (such as those with autoantibodies with or without dysglycemia stage 1/2), and thus higher risk to progress towards clinical T1D (stage 3), are studied, these trials are logistically

challenging due to the large cohorts and long follow-up needed. In the future, the combined use of immune interventions together with beta cell augmenting and/or replacement therapy, such as stem cell-based therapy, might offer a solution to compensate for the lost beta cell function at the time of clinical diagnosis.

CONCLUSIONS

Type 1 diabetes remains one of the most common and severe chronic diseases in children, adolescents, and young adults. However, despite its high prevalence, we keep on using a standard therapy that is already more than a century old. Technological advances have helped make giant strides in regulating optimal glucose homeostasis, but the chronic burden of long-term hyperglycemia-related complications remains troublesome. Despite all efforts, the road to a cure for T1D remains long and full of obstacles. The first is the need for biomarkers allowing early screening. The second is the need for superior treatment options, as this pursuit for early biomarkers should not turn into a Sword of Damocles. Whereas interventions thus far have mainly focused on either targeting the ongoing immune response or the induction of tolerance to the beta cell, we believe future research should focus on targeting both. A limitation to this review is that the field of T1D research is extensive and ever ongoing (as the long list of currently ongoing trials shows). Nevertheless, we hope this review shows that the pursuit of early biomarkers can go hand in hand with superior treatment options, as early screening can result in an earlier treatment initiation at a time of a higher residual functional beta cell mass. Thus, when asking the question, "Are we there yet?", we have to say, "Not yet". However, the future does look bright as we have high hopes for the trend towards global consortia as joined effort and pooled resources hopefully have a synergistic effect.

DECLARATIONS

Authors' contributions

Conceptualized the review goals and wrote the manuscript: Mathieu C, Martens PJ
All authors contributed to the article, consent to participate and approved the submitted version.

Availability of data and materials

The data that support the findings of this study are openly available in PubMed at https://pubmed.ncbi.nlm.nih.gov/.

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Conflicts of interest

The authors declared that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

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