Preventing type 1 diabetes in childhood

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Abstract

Type 1 diabetes (T1D) is an autoimmune disease in which the insulin-producing beta cells of the pancreas are destroyed by T lymphocytes. Recent studies have demonstrated that monitoring for pancreatic islet autoantibodies combined with genetic risk assessment can identify the majority of children who will develop T1D when they still have sufficient beta-cell function to control glucose concentrations without the need for insulin. Additionally, there has been recent success in secondary prevention by immunotherapy and primary prevention approaches to inhibit the initiating autoimmune process have entered large-scale clinical trials. By changing the focus of T1D management from late diagnosis and insulin replacement to early diagnosis and beta-cell preservation, we can anticipate a future without the need for daily insulin injections for children with T1D.

Type 1 diabetes (T1D, formerly insulin-dependent diabetes mellitus) is an autoimmune disease that irreversibly destroys the insulin-producing beta cells in the pancreatic islets of Langerhans. Although autoantibodies to islet cell components are robust markers of the disease process, evidence indicates that the beta-cell damage is caused by T cell cytotoxicity and cytokine release in concert with disease mechanisms within the beta cell itself. Progressive loss of insulin secretory capacity eventually leads to hyperglycemia that can develop at any age, but has a median age of diagnosis of 12 years. The discovery of insulin in 1921 and its use for the first time as a replacement therapy in 14 yr old Leonard Thompson, on Wednesday Jan 11th 1922, in Toronto, Canada was a landmark in medical science. Prior to this, children with T1D died from insulin deficiency due to uncontrolled fatty acid mobilisation and ketone body production resulting in life-threatening acidosis (diabetic ketoacidosis, DKA). However, the lengthened survival of people with T1D following the discovery of insulin revealed the problem of long-term complications such as blindness from retinopathy due to raised glucose concentrations that occurred despite insulin therapy.

The Diabetes Control and Complications Trial (DCCT) showed in 1993 that improving glycaemic control could delay long-term complications [1]. However, despite major advances in insulin pharmacokinetics and delivery in the last two decades, only a minority of children and adults with T1D can achieve optimal levels of glycaemic control in the long-term [2-4]. One hundred years after the discovery of insulin, an alternative approach has become possible: to avoid the need for insulin by interrupting the autoimmune disease process at an early (pre-clinical) stage (secondary prevention) or by preventing the onset of autoimmunity in the first place (primary prevention) . In this Review, we discuss how this might be achieved, particularly in children, the potential advantages this approach might bring, and the challenges that remain to be overcome.

Delaying the need for insulin therapy

Clinically, T1D typically presents with several weeks of weight loss and polydipsia (extreme thirst) and/or polyuria (excessive urination) due to hyperglycemia. This was

traditionally considered to be the time of disease onset. However, it has since become clear that this represents a late stage of the disease when an estimated 80% or more of beta-cell function has been lost. It is now possible to identify T1D in a preclinical phase in which pancreatic beta-cell function is still sufficient to control blood glucose concentrations without the need for insulin therapy (Figure 1). Slowing the loss of beta-cell function delays the need for insulin and even if insulin is eventually required, this still has multiple benefits.

Firstly, delaying a diagnosis of clinical T1D extends the period of a life free from the daily burden of continuous monitoring, daily dietary and exercise challenges, multiple insulin injections and the risk of hypoglycaemia; all of which are consequences of insulin therapy and so do not exist for as long as insulin therapy is not required. This is particularly beneficial to the many individuals who struggle to achieve glycaemic control with insulin therapy and are at the highest risk of long-term complications and costs to the healthcare system. Secondly by definition, glucose concentrations during this insulin-free period are below the threshold that contribute to the risk of long-term complications, resulting in prolonged benefits by reducing early exposure to elevated glucose concentrations. As demonstrated in the DCCT and its long-term follow-up (Epidemiology of Diabetes Interventions and Complications Study, EDIC), improved metabolic control in the early years of T1D reduces complication rates even 30 years later [5]. Thirdly, if or when patients eventually progress to requiring insulin, preservation of even limited residual beta-cell function has been shown to be associated with less hypoglycaema as well as improved glucose control (indicated by lower amounts of glycosylated haemoglobin, HbA1c) and reduced risk of long-term complications such as retinopathy [6,7]. Lastly, cross-sectional analyses indicate that metabolic control improves after the age of 25 [2], presumably due to greater maturity and a more regularised lifestyle. Hence, increasing the age at which insulin is required is likely to significantly contribute to reduced lifetime glycemic exposure.

Importantly, the identification of patients at a preclinical stage of T1D in itself has benefits, resulting in a reduction in presentation in DKA by up to 90% in children under age 5 years [8,9]. DKA around the time of diagnosis has been associated with

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neurocognitive deficits [10]. In addition, diagnosis at the preclinical stage allows time for families to adjust to the diagnosis and prepare for insulin therapy and glucose monitoring in a calm outpatient setting in advance of it being required [9].

Staging type 1 diabetes – identifying pre-clinical disease

In the early phases of the autoimmune process when beta-cell loss is limited, blood glucose concentrations are normal and cannot be used to diagnose the disease. Hence a pre-requisite for delaying or preventing the need for insulin therapy is the discovery of biomarkers that can identify early disease in most if not all individuals. As the disease progresses, glucose concentrations begin to rise but the individual may still be asymptomatic. A combination of biomarkers of the autoimmune process and glucose concentrations can then be used to stage progression of disease towards insulin dependence.

Autoantibodies as biomarkers of early disease

The identification of strong familial and genetic associations as well as islet-specific autoantibodies [to insulin (IAA), glutamate decarboxylase (GADA), islet antigen 2 (IA2A), islet specific zinc transporter (ZnT8A) (11,12)] in people with T1D has paved the way for the study of individuals at risk of future T1D. Such natural history studies have included individuals who have a family member with T1D (first degree relative studies) and/or those recruited from the general population without [13] or with [14] high T1D genetic risk. These studies have revealed that over 90% of children with T1D have autoantibodies to at least one islet-specific autoantibody at diagnosis and that these can appear years before clinical diagnosis of T1D. The first autoantibody to appear is against insulin, with a peak incidence around age 12 months [14,15]. Up to 90% of children with a single type of islet-specific autoantibody do not progress to T1D, but seroconversion to the presence of two or more autoantibodies (which occurs at a median age of 2.1 yrs) comes with an 84% risk of clinical T1D by the age of 18 [16]. A disease model of presymptomatic autoimmune beta-cell destruction identified by the presence of islet-specific autoantibodies opens up the possibility of intervening early, before clinical diagnosis of T1D to maximise preservation of beta cells. The very high risk associated with two or more islet-specific autoantibodies has prompted a move to

defining multiple autoantibody positive individuals as having pre-clinical T1D: stage 1 when glucose concentrations are normal, with progression to stage 2 when they start to rise (impaired glucose tolerance) and stage 3 when concentrations reach the standard criteria for clinical diagnosis of T1D [17] (Figure 1).

Advances in islet autoantibody detection are making mass screening more feasible. Reliable testing for 2-3 autoantibodies (typically antibodies to GAD, IA2 and ZnT8) can now be performed on as little as 4 μl of blood using advanced luciferase immunoprecipitation system (LIPS) technology [18] or an agglutination PCR-based detection system [19]. This allows reliable detection of islet autoantibodies from dried blood spots on filter paper or small capillary samples that can be obtained at home or in other community settings and sent by mail to the laboratory. Indeed, PCR-based islet autoantibody testing has recently been made available to the general public in the USA and is being introduced in Europe and Australia. Experience from the TrialNet Pathway to Prevention study, which has screened over 200,000 family members aged 2.5-45 years related to a child with T1D, indicated that 3.8% were single-autoantibody positive and 3% were multiple-autoantibody positive. In the general population, rates are around 1/10th of this (0.3% [8]).

Children who identified as single-autoantibody positive and those who are multiple-autoantibody positive and normoglycaemic will need to join a monitoring programme linked to clinical care providers. Cost-effective and acceptable arrangements for monitoring and monitoring intervals have yet to be defined. However, recent progress has been made in defining those in whom the disease is progressing more rapidly [20], which will allow alternative therapies to be offered to children who continue to progress following initial immunointervention, further prolonging the insulin-free period.

Combined genetic and autoantibody screening
The possibility of identifying pre-clinical T1D has been greatly enhanced with information derived from natural history studies. However, how this information could be applied in a whole population to reliably and cost-effectively detect the majority of

individuals before they progress to insulin dependence remains a challenge. Cross-sectional screening for islet autoantibodies in first degree relatives only identifies 10-15% of all T1D cases because the majority of incident cases have no affected first-degree relatives [21]. Recent efforts to perform cross sectional screening in 90,576 children between the ages of 1.75 and 5.99 years has highlighted that a pre-symptomatic diagnosis of asymptomatic T1D defined by multiple islet autoantibodies is possible [8], and was 96.4% sensitive (proportion of true cases identified as multiple autoantibody positive) and >99% specific (proportion of non-T1D identified by lack of multiple autoantibodies) for T1D presenting in the three years after screening. However, it is important to note that this will miss cases presenting before screening, cases that seroconvert after screening, and will identify some children who are "at risk" by being single autoantibody positive but probably will not progress. Recent data from the The Environmental Determinants of Diabetes in the Young (TEDDY) study suggest a single screen for multiple islet autoantibodies between the ages of 3 and 4 years has a near 40% sensitivity (with >90% specificity) for T1D presenting before the age of 12, with a risk of T1D within the next 5 years of 50-60% [22]. A solution to increase the sensitivity of screening is to include a second autoantibody screen at a later stage. However, this may still only achieve a sensitivity approaching 50% for childhood T1D presenting before the age of 12.

Another option is to consider whether improving characterisation of genetic information from birth can make screening more efficient, and enable prediction of T1D in very early life. Identification of newborn babies at high risk ~~according to human leukocyte antigen (HLA) profiling~~ based on known susceptible and protective HLA class II haplotypes (the region of the genome with the strongest effect of risk) has allowed natural history studies to follow a subset of a population accounting for approximately 50% of childhood T1D. Recently, T1D genetic risk scores (GRS), aggregating genetic risk into a continuous risk variable, have increased the sensitivity and specificity of genetic screening. One study focused on recruiting infants with a greater than 10% risk of islet autoimmunity in the first few years of life (~1/1000 infants) for an early life intervention trial, but this misses most childhood cases of T1D [23]. A recently improved GRS, incorporating more information on HLA class II ~~gene~~ haplotype interactions, was able to

stratify nearly 80% of childhood T1D within the top 10% in a population [24]. Combining this risk score with family history information and repeated autoantibody testing in this 10% could allow identification of pre-clinical T1D in the majority of children [25] (Figure 2).

We propose that screening and prevention of insulin requiring T1D in childhood be developed and implemented in two broad phases (Figure 3). Phase 1 comprises serological screening for islet autoantibodies only. This would first be conducted in pre-school children (age 2-5) in whom less than 15% of children with T1D have become insulin requiring. To detect late seroconverters, this would need to be repeated at a later age. Phase 2 would commence with GRS estimation at birth using this to detect early-onset cases (<3 years) and guide the need for and frequency of islet antibody testing (Figure 3). **Once preclinical T1D is identified, secondary prevention (to delay T1D diagnosis) can be undertaken with immunointerventions to reduce autoimmunity, while separate efforts proceed for primary prevention to stop the development of autoimmunity in the first place.**

Recent advances in secondary prevention of type 1 diabetes

Evidence that it is possible to delay the diagnosis of T1D through immunointervention has recently been presented in a landmark study. First degree relatives aged between 8 and 50 years (median age 14 years) from families with T1D received transient T cell modulation with the monoclonal antibody, teplizumab (anti-CD3) in the dysglycaemic phase prior to disease diagnosis (Stage 2, Figure 1). This resulted in a delay in the need for insulin treatment for a median of at least 3 years [20,26]. The importance of this observation has been highlighted by teplizumab being granted "Breakthrough" status by the US Food and Drug Administration (FDA) in 2019 and "Prime" status by the European Medicines Agency (EMA) in 2020. Teplizumab therapy entails 12-14 days of intravenous infusions and causes transient T cell depletion. At least part of the mechanism of action involves engagement of the CD3-epsilon chain on the surface of cytotoxic CD8^{+} T cells delivering a partially agonstic signal that leads to their non-responsiveness and conversion to a partial exhaustion phenotype [indicated by expression of the surface markers eomesodermin (EOMES), T-cell immunoreceptor with Ig and ITIM domains

(TIGIT) and killer cell lectin-like receptor subfamily G member 1 (KLRG-1) (27)]. Other studies also suggest the expansion of regulatory T cell (Treg) populations to explain the longer-term persistence of benefit [28, 29]. Safety monitoring to date has shown no adverse effects beyond the dosing period.

Results of a prevention study with a fusion protein that combines cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and immunoglobulin (Ig) to inhibit full T cell activation (abatacept) in first degree relatives at an earlier stage - stage 1 - enrolling subjects age 1-45 years with two or more islet autoantibodies and normal glucose tolerance are expected to be reported in the next year [NCT01773707]. Additional secondary prevention studies with anti-thymocyte globulin (ATG) in children from age 6 years (NCT04291703) at a dose that depletes T effector cells rather than Tregs, and B cell depletion with the CD20 monoclonal antibody, rituximab (to reduce antigen presentation) in combination with abatacept to reduce T cell activation (NCT03929601) in children from age 8 years are also planned.

To date, seven different selective but non-antigen specific immunointerventions have shown evidence of slowing the autoimmune process resulting in beta-cell preservation (improved insulin secretary capacity) in newly-diagnosed (stage 3) T1D in at least one phase 2 study (Table 1). Many others are currently being tested, providing additional candidate therapies for use in prevention studies [30]. Most recently, results of the tumor necrosis factor (TNF) monoclonal antibody (golimumab) have been reported (NCT03298542). When given every 2 weeks as a subcutaneous injection to 84 individuals aged 6-21 years with newly diagnosed T1D, it showed impressive preservation of beta-cell function and was well tolerated [31]. Anti-IL-21 to inhibit trafficking of CD8+ T cells to pancreatic islets in combination with a glucagon like peptide (GLP-1) agonist to promote beta cell survival (NCT02443155) has also shown potential for benefit [32]. Ultra-low dose interleukin-2 to selectively expand Tregs is also being tested in clinical trials in newly-diagnosed children with T1D (Stage 3, e.g. NCT03782636), given the high degree of safety and known genetically-validated mechanisms, and because of reports of clinical efficacy in several other autoimmune diseases.

An alternative to non-antigen specific immunotherapies are immunointerventions that restore tolerance in an antigen-specific manner. No such therapies have proven efficacy at this time, but many approaches based on administering beta-cell derived antigens or epitopes derived from them (e.g. from proinsulin or GAD) are being explored [33]. In general, they have proved safe [33-35] and if an effective platform approach can be established, they might be preferred to non-specific immunotherapies or used in combination with other therapies (Figure 3).

Approaches to primary prevention of type 1 diabetes

The degree of risk of T1D to a child is likely to begin in utero (Figure 1). Given that T1D mothers provide about half the risk of disease in children and that the fathers' contribution to the risk increases with paternal age, it can be assumed that many of the T1D risk alleles inherited by the child begin their effects from birth onwards in a complex interplay with the equally numerous environmental factors [11-12]. Many researchers still seek the disease "trigger", but it is very likely that part of the environmental contribution is a loss of protective factors, related to the hygiene hypothesis and industrialization, underpinning disruption of symbiotic health-promoting intestinal microbiota (dysbiosis) [12]. If we could fully understand and reintroduce these protective factors across the general population we can potentially halt the increasing incidence of T1D [36].

Since the earliest autoantibody to appear is IAA, oral administration of insulin to promote immune tolerance as early as possible after birth with high genetic risk of T1D could be a preventative strategy. Using this platform, a randomized placebo-controlled trial (RCT) of daily oral insulin versus placebo, the Primary Oral Insulin Trial (POInT, NCT03364868), is underway. Treatment is for three years with the primary endpoint being a 50% reduction of the frequency of two or more anti-islet autoantibodies or progression to T1D followed up for up to 7 years, with results expected in 2025 [37]. Pregnant mothers are recruited and DNA is obtained from the blood spots from newborn baby's Guthrie filter card (used in the official neonatal screening program for conditions such as phenylketonuria) or a trial-specific custom filter card. This DNA is genotyped with a GRS of 47 single nucleotide polymorphisms

associated with T1D risk. If the baby is in the top 25% of the GRS and therefore at 10% risk of developing two or more autoantibodies or T1D by age 6 years, then the infant is randomized into the trial, starting treatment between 4 and 7 months of age. The 1,040 children required have been randomized by March 2021, having genotyped nearly 250,000 babies across the five European countries. This infrastructure and networks for initial recruitment and conduct of such large-scale primary prevention trials in T1D, including central biobanking of biological samples and databases is referred to as the Global Platform for the Prevention of Autoimmune Diabetes (GPPAD). GPPAD is currently unique but demonstrates the feasibility of such ambitious efforts across multiple countries in their general populations, rather than relying on first-degree relatives of T1D patients.

There is a vast literature on the role of the intestinal microbiota in the development of islet autoimmunity and T1D. Overall, microbial dysbiosis and the consequences for immune tolerance, intestinal inflammation and gut epithelial functions are likely causal factors in T1D. Additionally, T1D risk variants alter gut microbial composition and antibodies to commensal bacterial antigens [38]. Both breastfeeding (exclusively), and probiotic use provides some protection, but only if probiotics in commercially available formulas are used in the first 27 days after birth [39]. A randomised controlled trial within GPPAD will test the ability of a daily probiotic supplementation versus placebo starting as early as possible, which is around 42 days owing to the time required to obtain the GRS and consent families with babies with the highest GRS (as in POInT). Treatment will be for 12 months to reduce the frequency of two or more autoantibodies or T1D up to age 6.5 years in 1,144 randomised children. The trial is using a single strain probiotic, *Bifidobacterium longum* subsp. *infantis*, because it is an efficient metaboliser of the human milk oligosaccharides (HMOs) in breast milk. Consequently, the babies should be breast fed as long as possible, in order to optimise beneficial effects of HMOs. HMOs are natural prebiotics, and these and synthetic versions have also been reported to have widespread beneficial effects on gut epithelial functions and the infant immune system [38, 40], but much more research is needed to confirm these effects on autoimmunity and inflammation.

Coxsackievirus is strongly implicated as one of the risk co-factors in early T1D development [40]. A multi-strain vaccine has been developed and needs to be trialled in children at risk of T1D [40]. Administration of tolerogenic peptides or DNA or RNA vectors that encode T1D autoantigens are also probably part of the future for primary prevention [32], along with other antigen-specific approaches. Childhood obesity appears also to be a contributory factor in T1D [41], possibly by influencing the development of the microbiota dysbiosis. Perhaps early dietary and behavioural prevention studies can be designed to reduce T1D incidence, but again within an RCT approach to ensure robust findings.

Conclusions

With sufficient investment and commitment, it should be possible in the current state of knowledge to prevent almost all cases of DKA and the requirement for hospital admission at diagnosis and to move the modal age of diagnosis of T1D from age 12 to around age 15. Further developments, including repeated and sequential interventions, should push the age of diagnosis out further until beta-cell deficiency that requires insulin therapy becomes a rare occurrence under the age of 18. Beyond this, GPPAD and other trials in T1D and trials in atopic or allergic diseases ~~autoimmune~~ diseases such as dermatitis and peanut allergy are expected to yield insights that will lead to successful primary prevention of T1D and ultimately its removal from society.

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Acknowledgments

Figure Legends

Figure 1: Staging of type 1 diabetes

Factors prior to birth and early life exposure combine with genetic risk resulting in autoimmunity. Once autoimmunity is clearly established (stage 1, multiple types of islet-specific autoantibody detected), this represents disease onset with inevitable progression to beta-cell loss, ultimately impacting on the ability to control glucose (stage 2, dysglycaemia) and finally levels of glycaemia diagnostic of type 1 diabetes (T1D) and the need for insulin (stage 3).

Figure 2: Use of genetic risk score at birth to identify the ~10% of neonates at highest T1D risk in childhood

Combining genetic risk score (GRS) and autoantibodies can be used to identify preclinical type 1 diabetes (T1D). a | Neonates can be selected with the top 10-15% of genetic risk as scored by GRS. This identifies 85-93% of the children diagnosed with T1D under the age of 5 years. b | Reducing the risk with age allows the population followed prospectively to be reduced further from 10% to 1% at age 8-10 years by successive rounds of antibody screening and recalculation of combined risk including GRS. Information derived from (24, 25).

Figure 3. A roadmap to prevent type 1 diabetes in childhood

Diagnosis and intervention prior to requiring insulin can be sequentially improved as newer approaches (e.g./ GRS calculation, combined interventions, more specific interventions to slow beta cell loss) are introduced. The result is a progressively later age of diabetes onset (secondary prevention) leading to the need for insulin to become rarer in childhood. Ultimately, primary prevention approaches are preferable to avoid the need for ongoing immune interventions.

Table 1: Non-antigen-specific immune interventions in T1D

The agents listed in this table have published evidence of preservation of beta cell function from clinical trials in new-onset (stage 3) type 1 diabetes (T1D). See text for mechanisms of action. Clinical trial numbers (clinical trials.gov) or references provided.

Table 1: Non-antigen-specific immune interventions in T1D

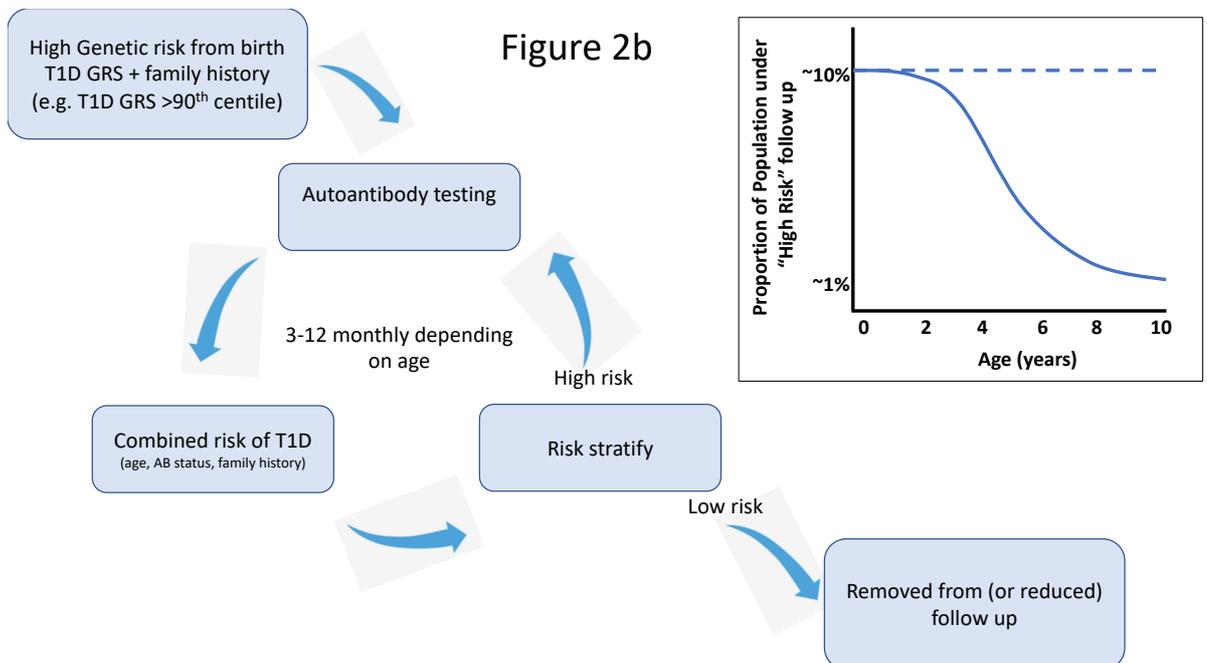| Agent	Target	Age group studied (yrs)	Reference/ Trial
Teplizumab	T cells (CD3)	8-35	[29]
ATG	T cells	12-45	NCT02215200
Rituximab	B cells (CD20)	8-40	NCT00279305
Abatacept (CTLA4-Ig)	T cell activation: CD80, CD86	6-45	NCT00505375
Alefacept	T cells (CD2)	12-35	NCT00965458
Anti-IL21 (NNC0114-0006) (+ liraglutide)	IL-21 (T cells, B cells, NK cells)	18-45	NCT02443155
Golimumab	TNF	6-21	NCT03298542

Figure 1. Stages of T1D
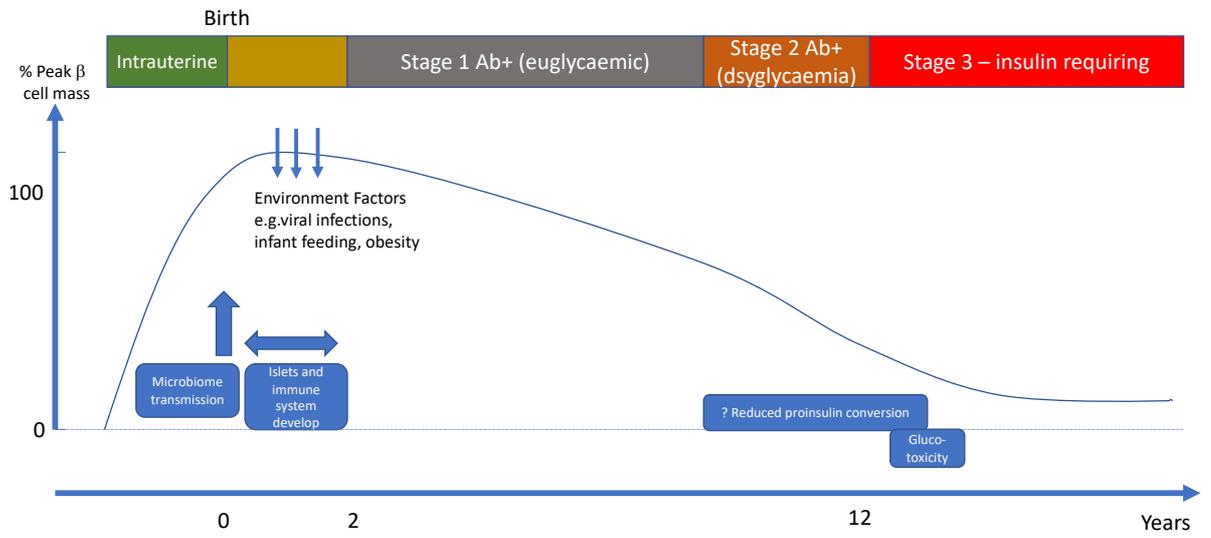
Figure 2a: A genetic risk score at birth can identify the ~10% of neonates at highest type 1 diabetes risk in childhood

90th population centile of T1D genetic risk

Follow up high risk neonates
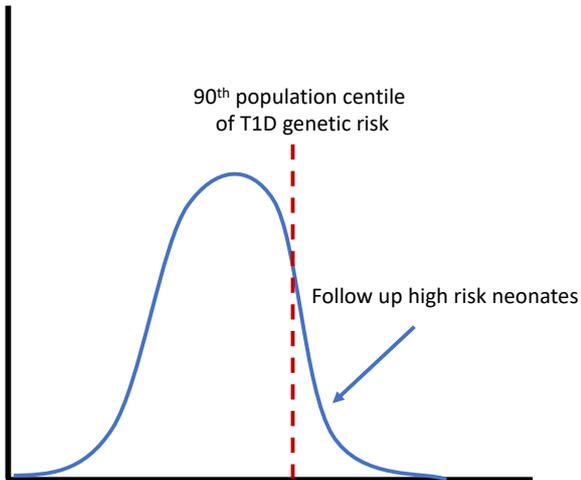
	Sensitivity to detect T1D Cases in early life*	
Population Centile of T1D Genetic Risk	T1D Diagnosed under 3 years	T1D Diagnosed under 5 years
85%	93%	90%
90%	89%	85%

Figure 2b

High Genetic risk from birth T1D GRS + family history (e.g. T1D GRS >90th centile)

Autoantibody testing

3-12 monthly depending on age

Combined risk of T1D (age, AB status, family history)

Risk stratify

High risk

Low risk

Removed from (or reduced) follow up

Figure 3: Preventing diabetes in childhood – a roadmap

Time:

Screening

Phase 1:
Serological screening of all childhood relatives and general population age 3-5 and age 11-13

Phase 2:
Genetic screening of all children at birth followed by risk driven targeted serological screening

2⁰ prevention

Non-antigen specific immunotherapy (NAS)

Sequential/combined NAS immunotherapy

Antigen specific immunotherapy

1⁰ prevention

Antigen specific immunotherapy

Probiotics