Islet cells in human T1D: from recent advances to novel therapies - A symposium-based roadmap for future research.

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ABSTRACT

There is a growing understanding that the early phases of type 1 diabetes are characterised by a deleterious dialogue between the pancreatic beta cells and the immune system. This, combined with the urgent need to better translate this growing knowledge into novel therapies, provided the background for the JDRF-DiabetesUK-INNODIA-nPOD symposium entitled “Islet cells in human T1D: from recent advances to novel therapies” which took place in Stockholm, Sweden, in September 2022. We provide below an overview of the main themes addressed in the symposium, pointing to both promising conclusions and key unmet needs that remain to be addressed in order to achieve better approaches to prevent or reverse T1D.

INTRODUCTION

Type 1 diabetes (T1D) is a chronic autoimmune disease with a strong inflammatory component that results from the interaction between predisposing genes and environmental factors (e.g. viral infections and diet). This interaction triggers an autoimmune assault against pancreatic beta cells that provokes local inflammation (insulitis) and progressive beta cell functional impairment as well as loss due to apoptosis. The prevalence of T1D has doubled in 25 years in children, and people living with the disease lose on average ≥11 years of life expectancy (Livingstone et al., 2015, Gregory et al., 2022). Although interventions targeting the immune system have shown efficacy in delaying disease onset (Herold et al., 2019), there are presently no therapies that prevent or cure the disease. The limited success of drugs aiming to arrest/revert autoimmunity and to protect beta cells in T1D is mainly due to the poor understanding of the molecular pathogenesis driving the autoimmune destruction of human beta cells, as well as the heterogeneity of pathogenesis resulting in different patient “endotypes” (Eizirik et al., 2021, Bluestone et al., 2021). Immune-mediated inflammation ("initiation phase") contributes both to the primary induction and secondary amplification of the immune assault, and inflammatory mediators trigger functional suppression and apoptosis of beta cells. This takes place in the context of a “dialogue”
between invading immune cells and the target beta cells, which is modulated by T1D candidate genes acting both on the immune system and on beta cells (Bergholdt et al., 2012, Eizirik et al., 2012, Polychronakos and Li, 2011, Eizirik et al., 2020). This dialogue is mediated by cytokines/chemokines released by beta cells, immune cells and by putative immunogenic signals delivered by dying or “altered” beta cells. Immune cells – particularly CD8+ T cells that recognise antigens presented in the context of conventional HLA class I (HLA-I) – mediate beta cell apoptosis. Beta cells contribute to this process by up-regulating the expression of the HLA-I molecules. The up-regulated HLA-I may present neoantigens generated as a consequence of beta cell endoplasmic reticulum (ER) stress, post-translational modifications, changes in alternative splicing (AS) and/or other mechanisms (Azoury et al., 2020, Roep et al., 2021, Piganelli et al., 2020, Gonzalez-Duque et al., 2018). The pathogenesis of T1D has been proposed to consist of stages 1-3, preceded by a putative stage 0 (prior to seroconversion), as depicted in Fig.1. It is important to state that not all individuals at stage 1 (autoantibody positive) will progress to stage 3 (hyperglycaemia and clinical diagnosis), and there is heterogeneity in disease progression and presentation.

The rationale for the JDRF-DiabetesUK-INNODIA-nPOD symposium entitled “Islet cells in human T1D: from recent advances to novel therapies” which took place in Stockholm, Sweden, between September 23-25, 2022, was provided by the following observations: 1) the key role played by the deleterious dialogue between the immune system and pancreatic beta cells in triggering the autoimmune process in T1D (Fig.1); 2) the urgent need to better translate this growing knowledge into novel (and probably combined) therapies for the disease, including better alignment of preclinical research with these goals. The main points discussed in the symposium are outlined below, culminating in a series of conclusions that should hopefully serve as a roadmap for future research in the field, with the ultimate goal to prevent the progressive beta cell dysfunction and loss characteristic of the disease.

**The progressive beta cell-immune system dialogue in T1D and functional follow up in clinical trials**

The meeting started with an overview lecture provided by Chantal Mathieu, emphasising the importance of the dialogue between beta cells and the immune system and the role for chemokines in this process.
The understanding that beta cells are not simply inert victims of the immune system, but contribute to the natural history of the disease (Martens et al., 2021), is promoting the search for combined therapies to prevent or revert T1D, aiming to both “re-educate” the immune system and boost the beta cell’s resistance to the immune assault. This provides the basis for novel and planned clinical trials implemented by the European consortium INNODIA (https://www.innodia.eu/).

1. Human islets in health and disease: the phenotype of human pancreatic beta cells in T1D

Mark Atkinson presented a lecture titled, “The Network for Pancreatic Organ Donors with Diabetes (nPOD) experience: histology of beta cells in T1D” where he discussed the impact that improvements to existing technologies (e.g., confocal microscopy and light sheet fluorescent microscopy), new image analysis methods, and the development of novel technologies (e.g., Co-Detection by Imaging (CODEX), Cytometry by Time Of Flight (CyTOF)) have made on our mechanistic understanding of T1D pathogenesis. These technologies, combined with the availability of extremely rare and valuable tissues from individuals with or at increased risk of T1D, have led to many discoveries that might be considered ‘transformational’ in terms of their thought. Indeed, these recent advancements have uncovered multiple previously undescribed physiological contributors to T1D, many related to aspects of not only the endocrine but extending to the exocrine pancreas. To be clear, evidence for the autoimmune component in T1D remains strong, including presentation of a moving 3D image of an nPOD donor (20-year-old T1D subject with 7 months disease duration) stained for glucagon and CD3, clearly demonstrating the presence of immune cells within the islet, interspersed amongst endocrine cells (Fig.2).

Klaus Kaestner discussed the “Transcriptomics of beta cells from T1D pancreata”, and the role played by the Human Pancreas Analysis Programme (HPAP), a consortium undertaking deep phenotyping of the human endocrine pancreas and its interaction with the immune system. HPAP generates and distributes high value data sets for the diabetes research community (Kaestner et al., 2019). Pancreatic and immune tissues undergo histology, multi-omics and cellular studies before data are integrated into PancDB (https://hpap.pmacs.upenn.edu/). The programme has made a rapid impact in...
the field (Wang et al., 2019, Fasolino et al., 2022, Japp et al., 2021, Fasolino et al., 2020, Doliba et al.,
2022, Bosi et al., 2022). HPAP single-cell multiomics is enabling new insights into pancreatic
subpopulations in the T1D pancreas. For example, these data enabled the recent identification of MHC-
class II transcripts in a novel subset of pancreatic ductal epithelial cells during T1D, with a tolerogenic
dendritic cell gene expression profile suggesting attempted immune regulation (Fasolino et al., 2022).

Dr. Kaestner went on to discuss alpha cell hypersecretion of glucagon from islets in non-diabetic GAD
autoantibody positive individuals: single cell RNAseq (scRNAseq) has identified upregulation of alpha
cell glycolytic genes, which may underpin the augmented glucagon release in the GADA+ pancreas
(Doliba et al., 2022), consistent with other mechanistic studies by the group (Bahl et al., 2021). The
lecture concluded with recent transcriptional analysis of residual beta cells in the T1D pancreas (Wang
et al., 2019).

Marcela Brissova considered the role of “islet cell physiology in T1D”, a disease of dysregulated
glucose homeostasis. Currently, all forms of diabetes are diagnosed by elevated blood glucose. The
failure of clinical diagnosis and treatment to incorporate molecular or genetic features of diabetes is a
limiting factor in building efforts towards precision medicine and disease prevention. To bridge this gap,
several resources in the U.S. are generating multimodal data from human pancreas or islets supported by
robust organ donation mechanisms. These resources include HPAP (Kaestner et al., 2019)
(https://hpap.pmacs.upenn.edu/), nPOD (https://www.jdrfnpod.org), Human Islet Phenotyping Program
(https://hubmapconsortium.org) and the Human Atlas of the Neonatal and Early Life Pancreas
(https://pancreatlas.org/datasets/531/overview). Dr. Brissova and her colleagues at Vanderbilt University
participate on various levels in all five programs, four of which are currently connected via the data-
agnostic Pancreatlas platform (Saunders et al., 2020) (https://pancreatlas.org/), a curated resource to
share imaging datasets developed by the Vanderbilt team. To integrate functional, genomic, and genetic
data with key donor characteristics across age, sex, ancestry, and disease state, a robust islet phenotyping
pipeline was developed (Hart et al., 2018, Brissova et al., 2018, Doliba et al., 2022, Haliyur et al., 2021,
Haliyur et al., 2019) and allowed analyses of human islet preparations from >600 donors thus far.
including adult and pediatric individuals with and without T1D (Brissova et al., 2018, Haliyur et al., 2021). These approaches revealed that endocrine cell composition varies considerably in adult islets and is highly associated with hormone secretion, possibly influencing alpha-beta-delta cell paracrine interactions. In addition, the team found that beta cell composition and insulin secretion are influenced by sex and self-reported ancestry. During postnatal development, islet endocrine cell composition changes considerably and both beta and alpha cells undergo functional maturation, thus further raising questions about gene(s) and environmental factors influencing endocrine cell allocation, beta cell mass, and diabetes predisposition. Studies of isolated islets from individuals diagnosed with T1D (stage 3) showed that residual $\beta$ cells have relatively normal function, whereas alpha cells have an intrinsic defect resulting in dysregulated glucagon secretion. Brissova postulated that several mechanisms could be responsible for alpha cell dysfunction in T1D such as extreme beta cell loss, chronic hyperglycemia, or repeated hypoglycemia leading to changes in alpha cell state mediated by alterations in expression of key islet-enriched transcription factors (Brissova et al., 2018, Wang et al., 2019, Camunas-Soler et al., 2020). Recent work using islets from non-diabetic adult single GAD autoantibody positive individuals led to a surprising discovery that alpha cells appear to have dysregulated secretory output prior to symptomatic T1D (Doliba et al., 2022). Collectively, these insights demonstrate that T1D is a complex disease requiring integrated studies by collaborative interdisciplinary teams to bring molecular insight into heterogeneity across multiple levels to achieve precision medicine in diagnosis and management. Integrating cellular function with molecular signatures and genetics, whilst fostering interactions of basic scientists and clinicians could inform future clinical trials aiming at T1D prevention.

“What do the islets look like in T1D?” To address this question, Sarah Richardson reflected on the value of pancreas biobanks in which T1D islets can be examined in their native environment. Globally there are <700 T1D donor pancreata, with the majority accessible today residing within the nPOD and the Exeter Archival Diabetes Biobanks (EADB; https://pancreatlas.org/datasets/960/explore). Advanced technologies such as scRNAseq and CyToF are providing exciting and informative insights into the pathology of T1D (Wang et al., 2019, Damond et al., 2019)), but are frequently restricted to a small
subset of individuals diagnosed between 10-30 years of age, with a duration of diabetes of >2y. Donors with young-onset (age <10y) and short duration (<2y); those with long duration (>35y); and those with late-onset (age >30y) receive less attention due to limited access. The need to study ‘all’ groups is highlighted by the variation in hallmark T1D-related pathologies, which differ depending on ‘who you look in’ and ‘when you look’.

Collating data from EADB and nPOD to examine the presence of residual beta cells reveals proportionally fewer insulin-containing islets (ICIs) in donors diagnosed age <7y at onset, with residual beta cells declining rapidly beyond this. In contrast, those diagnosed older (age ≥13y), tend to have more residual ICIs at onset which can be preserved for many years post-diagnosis (Carr et al., 2022, Leete et al., 2016). Encouragingly, these data mirror circulating C-peptide levels observed in living individuals with comparable age-at-onset and disease duration (Carr et al., 2022), suggesting that C-peptide will be a valuable window into the pancreas in future intervention trials. The presence of insulitis (accumulation of infiltrating immune cells at the islet), is relatively rare in T1D pancreata (particularly with disease duration >1 year), being described as an ‘elusive lesion’ by Peter In’t Veld (In’t Veld, 2011). In those with a disease duration of <1y, insulitis is more commonly found in those diagnosed young, highlighting again the importance of studying young-onset, short-duration donors to improve our understanding.

Studies of short-duration donors reveal two forms of disease, or endotypes: T1D Endotype 1 (T1DE1) and 2 (T1DE2) (Leete et al., 2016)(Fig.3). These endotypes differ according to insulitis severity, infiltrating immune-cell profiles (particularly B-lymphocytes), the proportion of residual beta-cells and proinsulin localisation within beta cells. T1DE1 is associated with young age-at-onset (age <7y) and a rapid loss of circulating insulin, whereas individuals diagnosed ≥13 years of age (T1DE2) often retain detectable C-peptide long after diagnosis and retain normal proinsulin localisation in most islets. Individuals diagnosed later tend to maintain better blood glucose control and develop fewer complications, which aligns with these pancreatic studies where more beta cells are preserved.

Interestingly, in these T1DE2 donors, there is evidence that beta cells may be defending themselves against the immune attack, through the upregulation of non-classical class I HLA molecules, such as HLA-E, -F and -G and the checkpoint inhibitor PD-L1 (Richardson et al., 2016, Colli et al., 2020a, Wyatt et al., 2019). These can all negatively regulate, or inhibit, immune cells and may protect beta cells.
from bystander effects of infiltrating cells and reduce auto-reactive T cell killing. This could be informative for beta cell replacement strategies in the future.

Carmella Evans-Molina opened her lecture on “in vivo beta cell function in pre-clinical T1D diagnostics” by discussing the analysis of individuals with autoantibody positivity from cohorts such as the TrialNet Pathway to Prevention study and Diabetes Prevention Trial of T1D (DPT-1) (Ferrannini et al., 2010, Evans-Molina et al., 2018), which suggests there are several predictable metabolic checkpoints that are observed during the progression of T1D. Initially, there is a loss of early C-peptide responses, as well as reduced C-peptide area under the curve responses during oral glucose tolerance tests (OGTT) that can be detected up to six years prior to the onset of Stage 3 T1D (Evans-Molina et al., 2018). An important caveat is that individuals from these cohorts are identified as antibody positive in cross-sectional screening, so their exact timing of seroconversion is unknown. Nonetheless, during a second phase that continues until approximately two years before diagnosis, there is relative stability in OGTT C-peptide measures (Evans-Molina et al., 2018). Beginning about two years before diabetes onset, there is evidence of rising glycemia and declining C-peptide secretion. Metabolic deterioration accelerates one year to six months prior to Stage 3 T1D onset, with marked declines in beta-cell glucose sensitivity, rate sensitivity, and potentiation coupled with decreased insulin sensitivity, and markedly rising blood glucose levels (Ferrannini et al., 2010, Evans-Molina et al., 2018). Furthermore, data published following the symposium revealed elevations in blood glucose prior to the appearance of islet autoantibodies (prior to stage 1-2) in children with a high genetic risk score for T1D (Warncke et al., 2022).

Dr. Evans-Molina went on to consider age as an important modifier of T1D risk, as it is well-accepted that age is inversely associated with the risk of developing T1D in those with autoantibodies (Anand et al., 2021). Whether age influences patterns of C-peptide decline before and after the onset of Stage 3 disease is a critical question, especially as it relates to the selection of individuals for disease-modifying interventions and the timing of these interventions during periods of active, accelerated disease. In a recent analysis of DPT-1 data, Evans-Molina showed that younger and older progressors to T1D had a
similar rate of decline of both beta cell glucose sensitivity and insulin sensitivity prior to the onset of Stage 3 T1D. This stands in contrast to patterns observed after the onset of Stage 3 T1D where children manifest a more rapid loss of C-peptide following clinical diagnosis (Greenbaum et al., 2012, Hao et al., 2016). Consistent with these findings, measurable serum C-peptide and histologic evidence of residual insulin-containing islets in long-duration disease are less likely to be observed in individuals with younger age of onset (McKeigue et al., 2019, Davis et al., 2015, Lam et al., 2017, Oram et al., 2019).

2. The role of T1D candidate genes on beta cells – from genetics to new therapies

John Todd, in his lecture entitled “Genes to clinic in type 1 diabetes”, showed that the candidate gene GLIS3 is clearly a beta cell fragility gene acting particularly in early life, predisposing to T1D in individuals diagnosed under the age of 7. Of note, the gut microbiome encodes functional insulin cross-reactive mimotopes of insulin that may promote tolerance to insulin and tolerance to similar bacterial proteins e.g. transketolase. Particularly in DR3/4-DQ8trans dimer-positive individuals anti-insulin T cells escape from the thymus in the first months of life, especially during weaning (6 months onwards, 3 months before IAAs appear). Translation of genetic information to the clinics should consider the following points: a. Interval dosing of low-dose IL-2 in children is safe and provides a steady-state increase in Tregs. If the Oxford IL-2 trial (ongoing) shows positive results, efforts to bring IL-2 into T1D prevention trials, and production of a non-invasive form of IL-2 should be commenced, including using IL-2 as a maintenance therapy; b. Healthy weaning should be promoted (especially in families with T1D and families in which anti-islet autoantibody screening has identified an autoantibody-positive child); c. Healthy living during pregnancy could reduce the incidence of T1D; d. Reduction of childhood obesity could reduce T1D incidence by ~30%; e. Promotion of gut microbiota and gut epithelial health could prevent T1D.

Lorenzo Pasquali addressed the theme “Inflammation and regulatory landscape of beta cells provides new insights into the genetics of type 1 diabetes”. He explained that exposure of human islets to a pro-inflammatory environment causes profound changes in beta cell gene expression and
regulatory landscape. This process results in the activation of distal cis-regulatory elements bound by immune responsive and tissue-specific transcription factors (TFs). Activation of these noncoding regulatory elements is coupled with 3D chromatin conformation changes enabling the contact with their target gene promoters. Beta cell cytokine responsive enhancers harbour T1D risk variants, suggesting a new mechanism whereby the variants act at a beta cell level but are functional only upon islet cell perturbation, for instance as mediated by pro-inflammatory cytokines (Ramos-Rodríguez et al., 2019, Eizirik et al., 2020). Exploring these relationships could open an avenue to the identification of new T1D candidate genes acting at the beta cell level.

Single cell approaches allow detection of a heterogeneous beta cell response to the proinflammatory environment which could in turn inform on a specific, targetable, beta cell sub-population. Moreover, single cell analyses may uncover a cell type specific noncoding regulatory response to cytokines; indeed, cellular responses may be islet beta cell type specific, which might capture cues of the molecular basis of intrinsic beta cell fragility, as compared to other islet cell populations.

Closing the session, Decio L. Eizirik discussed “From candidate genes to new therapies for T1D – the TYK2 story”, a project developed in close collaboration with Carmella Evans-Molina. He described that a challenge for studies on risk polymorphisms that may contribute to T1D is how to translate this knowledge into new therapies for the disease. Eizirik has previously described that 80% of the candidate genes for T1D are expressed in human islets (Eizirik et al., 2012), a finding also observed in the target tissues of other autoimmune diseases (Szymczak et al., 2021). He next compared the gene signatures induced by the cytokine IFNα (an “early cytokine” that may contribute to three key features of the histology of human islets in T1D, namely up-regulation of HLA class I, induction of ER stress and apoptosis) against the signatures observed in FACS-purified human beta cells obtained from patients affected by T1D and then “mined” the common genes detected against gene signatures induced by a panoply of drugs in other cell systems (Colli et al., 2020b). This indicated that inhibition of TYK2, a kinase that transduces the early signals of IFNα and is a candidate gene for T1D (polymorphisms that decrease TYK2 signaling decrease disease risk), could revert the IFNα-induced signature. Additional
testing of TYK2 inhibitors, done in collaboration with the Evans-Molina group, including agents already in advanced state of evaluation for the treatment of other autoimmune diseases, indicates that TYK2 inhibition protects human beta cells \textit{in vitro} against IFN\(\alpha\), alone or in combination with IL-1\(\beta\) (Coomans de Brachène et al., 2020), and has clear beneficial effects in two models of autoimmune diabetes in mice (unpublished data). Importantly, TYK2 deletion in stem cell-derived \(\beta\)-cells protects these cells against both IFN and CD8\(^+\) T-cell-induced damage (Chandra et al., 2022). As a whole, these data suggests that TYK2 inhibition is potentially an interesting approach to protect pancreatic beta cells in the early stages of T1D.

3. Beta cell stress in T1D

Heiko Lickert discussed \textit{“New insights into beta cell failure and recovery”}. He described that beta cells are heterogeneous in health and disease and understanding the underlying mechanisms may allow to protect or even regenerate a functional beta cell mass (Bader et al., 2016, Roscioni et al., 2016, Yoshihara et al., 2020). Using a scRNA-seq-based evolutionary comparison of healthy mouse, pig and human islets revealed that alpha and beta cell heterogeneity is conserved among species (Tritschler et al., 2022). Interestingly, isolated human islets across several healthy donors showed mature, immature, ER stressed and MHC class I and II / autoantigen high expressing clusters of beta cells, suggesting that the isolation procedure induces stress and unleashes beta cell plasticity. Streptozotocin (STZ)-induced diabetes and sustained hyperglycaemia for 100 days combined with scRNA-seq of mouse islets identified markers and pathways of beta cell dedifferentiation (Sachs et al., 2020). Single and combinatorial pharmacology revealed that exogenous insulin directly acts on beta cells, reducing glucotoxicity and contributing to restore beta cell identity and function. Interestingly, glucagon-like peptide 1 receptor (GLP-1R) can be targeted to deliver a GLP-1-estrogen conjugate to beta cells to induce a nuclear response and protect beta cells from ER stress-mediated cell death, avoiding the side effects of estrogen on female tissues (Sachs et al., 2020). It is well known that insulin/IGF signaling is essential for beta cell survival, proliferation and function and that beta cell insulin/IGF resistance causes overt diabetes (Goldfine and Kulkarni, 2012, Jain et al., 2022). The discovery of the insulin inhibitory
receptor in beta cells may allow specific sensitisation of insulin/IGF signaling in islet beta cells to trigger proliferation and survival (Ansarullah et al., 2021). In conclusion, understanding paths and mechanisms of beta cell stress, loss of identity and dedifferentiation and identifying and validating druggable molecular targets may allow to protect and/or regenerate beta cells in type 1 (and 2) diabetes.

Raghu Mirmira addressed the search for “Circulating biomarkers of beta cell stress and death”. In early T1D, beta cells are subjected to stresses such as inflammation, viral infections, hypoxia/ischemia, and impaired nutrient handling as a result of insulin deficiency (Chen et al., 2017, Eizirik et al., 2009, Wilcox et al., 2016). Under these conditions, beta cells trigger emergency responses, including the “integrated stress response (ISR),” which is highlighted by the activation of one or more of 4 kinases (PERK, HRI, PKR, GCN2) that lead to the phosphorylation of the translation factor eIF2α (Taniuchi et al., 2016). Phosphorylated eIF2α blocks the translational initiation of many mRNA transcripts in an attempt to divert energy expenditure toward cellular recovery processes (Muaddi et al., 2010). If the block is not alleviated, then the cell is bound for an apoptotic fate. Thus, like many emergency responses in the cell, the ISR balances cellular fate. During the ISR, translationally-inhibited mRNAs are compartmentalised into discrete processing bodies (PBs) and non-membranous inclusions known as stress granules (SGs) (Wheeler et al., 2016, Kedersha et al., 2013, Namkoong et al., 2018). PBs function in normal RNA decay, though this idea remains somewhat controversial (Luo et al., 2018). SGs, by contrast, are induced by cellular stressors (e.g. inflammation, viral infections) (Decker and Parker, 2012) and function as cellular signaling “nodes,” whereby their predominant fate (recycling of their contents to translating ribosomes, diversion to lysosomes for degradation, or fusion into endosomal cycling cascades) may ultimately determine cell survival. Pancreatic tissue sections from autoantibody-positive non-diabetic individuals showed an increase in general stress granule formation in beta cells, whereas in longstanding T1D stress granules were significantly reduced. A perspective has been emerging that SGs may intercept other intracellular pathways (Kedersha et al., 2013, Buchan et al., 2013, Buchan and Parker, 2009), including endosomes, which are eventually released as extracellular vesicles (EVs) (Baixauli et al., 2014, Jimenez et al., 2019). All EVs carry cell/stress-specific cargo that includes proteins, nucleic acids and lipids. The inclusion of SGs into forming endosomes and EVs (Jimenez et
al., 2019) may augment a mechanism by which a cell’s “state of emergency” can be communicated extracellularly. Dr Mirmira’s data demonstrate that the triggering of the ISR results in the inclusion of insulin mRNA within SGs. In humans with new onset T1D, insulin mRNA is detectable in circulation, whereas in longstanding T1D (where beta cells are few or dysfunctional), insulin mRNA is undetectable. These findings hold the promise that interrogation of EVs from individuals at risk for T1D might uncover mRNA species that reflect ongoing stress within beta cells. Such knowledge has the potential to identify high-risk individuals for whom intervention might prevent subsequent T1D.

Anath Shalev discussed the approaches her group is developing regarding “Reducing beta cell stress – clinical potential”. Recent clinical trial data (Xu et al., 2022, Ovalle et al., 2018) demonstrate that continuous oral use of the approved blood pressure medication, verapamil, in subjects with T1D delays loss of beta cell function and lowers insulin requirements for at least 2 years post-diagnosis. Verapamil downregulates the detrimental protein thioredoxin interacting protein (TXNIP) and promotes an anti-oxidative, anti-apoptotic and immunomodulatory gene expression profile in human islets. Oral verapamil treatment reverses T1D-associated increases in serum chromogranin A (a T1D autoantigen) and in proinflammatory factors (interleukin-21 levels and T-follicular-helper cells). This is the first indication that verapamil may also affect the autoimmunity of T1D in addition to its effects on beta cell survival. As such this could also help explain why verapamil is successful even without additional bona fide immunomodulation. Importantly, since verapamil is very well tolerated and its effects are not dependent on the presence of insulitis, testing it for the prevention of T1D in at-risk subjects seems warranted and would provide an attractive risk-benefit ratio. In addition, global longitudinal serum proteomics analysis revealed that chromogranin A, which can also be detected by a simple clinical blood lab test, provides a promising novel marker to help monitor successful therapy or disease progression in T1D. These studies provide an example on how findings in human islets and diabetes mouse models other than NOD can be successfully translated into clinical trials.
In the last lecture of the session, Alberto Pugliese discussed “Exploring circulating miRNAs as biomarkers of disease progression, beta cell loss and stress”. miRNAs are critical regulators of gene expression and modulate pathways relevant to T1D. During the past decade, studies have explored associations of circulating miRNAs with T1D, and to improve prediction of T1D risk and progression, including the decline of insulin secretion. Studies differed in design, cohorts, assays employed to measure miRNA levels, number of miRNAs measured, sample types tested (serum, plasma, rarely vesicles), and data analysis approaches. Despite the above differences, a review of published literature shows that among miRNAs whose circulating levels were associated with T1D/islet autoimmunity or disease progression, 35 were reported by more than 2 independent studies (range 2-8 studies reporting associations), including 12 miRNAs reported by at least 3 independent studies. These observations suggest that circulating miRNAs can be exploited as disease biomarkers with further advances and validation. This will require access to replication cohorts, and ideally future studies should examine longitudinal samples. Ongoing studies are now relying on RNAseq based platforms to examine levels of higher numbers of miRNAs (extending to the 2,000 range), and the sample volume requirement is down to microliters, with minimal impact on existing repositories. It is possible that several of the earlier associations, based on RT-PCR assays that assessed a more limited number of miRNAs, may not be replicated due to differences in assay format and yet there is potential for discovery of more and stronger associations. Reconciling RT-PCR and RNAseq data may be challenging, but should be attempted, and standardised assays should be developed for the clinical setting. Studies of miRNA responses in the context of disease relevant metabolic/immunological stimulations would be helpful to better understand the biological impact of miRNAs, unveil dysregulation of miRNAs that may not be evident in the steady state, and to improve our understanding of the cellular origin of miRNAs in the circulation. To this end, initial studies showed feasibility of these approaches for example by measuring changes in miRNA levels in patients, in vivo, during a mixed meal tolerance test, and in parallel during glucose stimulated insulin secretion from isolated pancreatic tissue.

4. Beta cell replacement and protecting beta cells from the immune system
Timo Otonkoski addressed the question “**Beta cells from ES- and iPS- cells: how close are we to endogenous beta cells?**” by presenting a comprehensive characterisation of human pluripotent stem cell-derived islets (SC-islets) and primary human islets, focusing on their structure, function (both in vitro and in vivo following murine implantation), gene expression and metabolism as described in recent reports (Balboa et al., 2022, Barsby et al., 2022). Using a modified 7-stage differentiation protocol, he showed that when SC-islets are given sufficient time to mature *in vitro*, they develop into islet-like aggregates that consist of nearly 100% endocrine cells (40-50% of both alpha- and beta cells, 5% delta cells and 5-10% enterochromaffin-like cells, which may in fact represent endocrine progenitors) (Fig.4). The SC-islets present dynamic glucose-stimulated insulin secretion that closely matches adult islet controls, except for a somewhat lower extended second phase. When transplanted into immunodeficient mice, the SC-islets become highly vascularised and innervated. However, as compared with implanted adult islets, there is a longer lag time before SC-islets fully control the glycaemia of diabetic mice. The SC-islets demonstrate signs of metabolic immaturity, for example strong responsiveness to exogenous pyruvate, and lower coupling of mitochondrial respiration to glucose-induced insulin secretion. However, these metabolic differences appear to quickly decrease in vivo, after implantation in mice, in agreement with transcriptomic data of maturation associated genes and pathways. Overall, SC-islets represent a valuable source of human pancreatic endocrine cells that can be effectively used both for the modelling of pathogenic mechanisms behind various types of diabetes (Balboa et al., 2021), and for the development of cell replacement therapies for insulin deficiency.

In the lecture entitled **“Stem cell to beta cell: state of the art and therapeutic potential”** Louise Winkel provided an industrial perspective on stem cell to beta cell technology, including discussion of regulatory, manufacturing and logistical challenges that remain to bring a stem-cell based therapy to the clinic. Novo Nordisk has been active in stem cell research for over 20 years. In late 2018, Cell Therapy R&D was established as an independent unit and has since grown to cover the full pharmaceutical value chain, from research to commercial, including a GMP manufacturing site in Fremont. In addition to
several exploratory projects, 3 projects are moving towards the clinic: Parkinson’s disease, Chronic heart failure and T1D, all but the latter in close collaboration with external partners.

The protocol for generating functional islet-like clusters from pluripotent stem cells has been developed internally over the past 14 years and is now matured to generate thoroughly characterised, functional cells that reproducibly reverse diabetes in pre-clinical models. This has been a major achievement, not only accomplished by Novo Nordisk but also by several other groups in this field. However, it is “only” the beginning of even bigger challenges to solve, from an industrial perspective. Setting aside the major and complex challenges of route of administration and protection from the immune system in people with T1D, upscaling and development of processes and assays that support large scale good manufacturing practice (GMP) production, quality control and distribution beyond investigational new drug (IND)-enabling studies and Phase 1/2 trials is pivotal to the success of any cell-based therapy.

The development, delivery and commercialisation of stem cell-based therapies differ significantly from traditional drug development. Unlike protein- and peptide-based therapies, few standardised procedures are currently established across the industry or at the regulatory level. Each company must develop and mature both capabilities and processes as projects evolve, which requires very close collaboration across the research and development value chain. In addition, each cell type has different requirements and reliable delivery of cells into the body is extremely complex.

In summary, the field needs to focus on quality, scalability and cell delivery to accomplish our shared goal of helping people living with serious chronic diseases with safe and efficacious cell-based therapies.

Shareen Forbes presented work on “islet transplantation and mesenchymal stem cells”. Islet transplantation is of proven efficacy in T1D, reducing hypoglycaemia, stabilising blood glucose levels and improving quality of life. However, there is a limited supply of donor pancreata and each recipient requires islets from 2-3 donors to substantially impact on glycaemic control. Rates of insulin independence are only 17% in the UK, there is attrition in graft function over time and the scarcity of donor pancreases for transplantation further compounds the problem. Islets are avascular when transplanted into the liver, rendering them vulnerable to hypoxia and immune mediated apoptosis and
reliant on angiogenesis and vasculogenesis to engraft. As such, >60% of transplanted islets die in the first 48 hours post-transplant. Treatments to ameliorate this loss are urgently required.

Mesenchymal stromal cells (MSCs) are found in a number of organs and support cells within tissues. They are defined by the presence and absence of specific cell surface markers, are adherent to plastic and are able to differentiate into fat, bone and cartilage. MSCs from different sources are heterogeneous but can home to sites of inflammation and secrete immunomodulatory and anti-inflammatory cytokines as well as growth factors that are pro-angiogenic. MSCs are currently in Phase 3 clinical trials and have shown efficacy in a number of settings including acute graft versus host disease when given at a high dose intravenously (>1x10⁶ MSCs). They are therefore potential candidates to improve islet engraftment and long-term graft function. Dr. Forbes described their recent efforts to manufacture umbilical cord derived human MSCs to GMP grade, with gene expression studies demonstrating anti-inflammatory, immunomodulatory and pro-regenerative properties (Forbes et al., 2020). In a diabetic immunodeficient mouse model followed for 16 weeks, the Forbes team has shown superior glycaemic control, greater stimulated C-peptide concentrations, increased graft vascularity and delayed rejection with umbilical cord derived MSCs plus islets versus islets alone (Forbes et al., 2020).

A current barrier for the translation of this therapy into humans is the demonstration of a homogeneous cryopreserved banked MSC product, which is efficacious and safe at this proposed dose in islet transplantation. This would enable this allogeneic product to be thawed on demand and infused with islets into humans. Such a strategy would allow islet grafts to function better for longer and allow pancreases to be distributed to more people.

Kevan Herold provided an up-to-date review of “Immunotherapy and interventions in pre-diabetes and diabetes”. A number of immune interventions have been successfully trialed for the treatment of T1D in those with recent onset disease. (Herold et al., 2013, Herold et al., 2005, Herold et al., 2002). Most recently, T1D clinical onset has been delayed in some individuals at risk for the diagnosis but who are free of symptoms for extended periods of time (Herold et al., 2019). However, even with the successes, the end of the disease has not yet been achieved, and further work is needed. The following
observations and conclusions, based on the experience from clinical and preclinical studies may help
guide this work: First, there appears to be a window for successful interventions with the agents that
have been able to modulate the natural disease course. This appears to be in the peri-diagnosis period
(pre and immediately post diagnosis) which is thought to be characterised by active immune killing of
beta cells. The reasons why this period is opportune have not been defined but it may relate to the
susceptibility of immune cells to modulation. Second, the effects of immune therapies have not been
permanent – in most cases tolerance has not been achieved. With B cell depletion with anti-CD20 mAb
(Pescovitz et al., 2009), which can modulate the decline in beta cell function when it is given to patients
with new onset disease, the recovery of B cells in the peripheral blood includes the recovery of
autoreactive B cells (Chamberlain et al., 2016). Likewise with anti-CD3 mAb autoreactive T cells are
not permanently depleted. Recent preclinical and translational studies have highlighted the stem-cell like
features of autoreactive CD8+ T cells in T1D suggesting that these cells are resistant to elimination and
may persist even after immune therapy. It suggests that rather than depleting the autoreactive repertoire,
achieving tolerance will require an environment or ligands that prevent these autoreactive cells from
reactivation. Finally, recent studies have described the contribution of beta cells themselves to their own
demise (Piñeros et al., 2022, Rui et al., 2021, Mallone et al., 2022). They are an important source of
inflammatory mediators that may recruit and activate autoreactive cells. Blocking these mechanisms in
beta cells may solve both the problem of immune cell activation and beta cell killing.

With the progress of immune therapies, a new frontier is opened in which the goals for treatment are
directed towards maintenance of the efficacy seen with single agents, timing of therapies, combinations
of agents that may target immune and beta cells, and selection of patients who are most likely to respond
to interventions. Much like the first descriptions of success with cancer immune therapy, progress in the
field is likely to expand rapidly.

*Coda: Since the symposium the US Food and Drug Administration have approved the use of Teplizumab
(anti-CD3) injection to delay the onset of stage 3 T1D in patients >8 years of age with stage 2 T1D.*

5. Measuring success in replacement/regeneration in pre-clinical and clinical models
Peter Senior opened this session by discussing “Clinical Assessment of Islet Cell Function” from the standpoint of human islet transplantation. The University of Alberta has performed 729 islet transplants on 312 individuals since 1999 with a goal of improving both “metabolic health” and “mental health”. Factors such as reducing glucose variability as well as reducing or abolishing hypoglycaemia and the fear of hypoglycaemia are important in improving “mental health” beyond average glucose or HbA1c levels. Frequent assessments of islet cell function are required following islet transplantation to identify the need for additional islet loading (second or third islet transplants) and/or evidence of rejection. More invasive/extensive assessments of islet function provide more information, but a balance has to be struck with the patient burden of repeated assessments. In addition, stimulated C-peptide levels are very dependent on the stimulus such that glucose-potentiated arginine stimulation (with glucose = 360mg/dl) could release 3-4 times as much C-peptide in islet transplants recipients than arginine alone (Rickels et al., 2005). Formulae to assess insulin sensitivity (e.g. HOMA) and to relate C-peptide to glucose (e.g. Suito Index) improve clinical correlation but are generally developed in the non-insulin treated healthy population and may have limitations in the clinic population. More complex composite scores such as the BETA2 score which includes insulin dose and HbA1c as well as glucose and fasting C-peptide (Ryan et al., 2005, Forbes et al., 2016) are more convenient to use in clinical practice and correlate well with clinical outcome (insulin independence) and invasive measures ($r^2= 0.69$)(Senior et al., 2020b, Lam et al., 2022). Continuous glucose monitoring (CGM) is required to capture variability and can show improvement following islet transplantation compared to hybrid closed loop insulin therapy (Senior et al., 2020a). Further studies are required to indicate if this correlates well with “mental health” in addition to “metabolic health”.

Michael Brehm discussed advancements in “Humanized models to understand the crosstalk between beta cells and the immune system”. Generating such models is a challenging task with several components. First, it is necessary to delete elements of the mouse immune system to ensure that any response to islet grafts is “human” in origin. Deletion of the common gamma chain in NOD scid gamma (NSG) and NOD-Rag1null (NRG) mice impairs signalling through multiple key cytokines (IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21) resulting in lack of T, B and natural killer (NK) cells (Shultz et al., 2005,
Ishikawa et al., 2005, Pearson et al., 2008). Secondly, deletion of murine insulin secretion allows detection of the function of any transplanted islets via monitoring glucose levels. Such “hyperglycaemic” mice can be generated either using the beta cell toxin streptozotocin, or introducing the Akita insulin2 gene mutation which has a dominant negative effect on insulin production by impairing proinsulin folding in all forms of insulin (Pagliuca et al., 2014). Inducible beta cell depletion can be introduced using the rat insulin promotor-diphtheria toxin responsive element (RIP-DTR).

Thirdly, the human immune system needs to be engrafted. This has been done by transferring either human peripheral blood mononuclear cells (PBMC), or haematopoetic stem cells (HSC). Unfortunately, such mice do not have a long lifespan due to Xeno-graft versus host disease, but this can be reduced by deletion of mouse MHC class I and II without irradiation. NSG RIP-DTR MHC-DKO mice reconstituted with 50 million human PBMCs given intraperitoneally that receive diphtheria toxin, reject a human islet allograft as shown by rising glucose levels (Brehm et al., 2019). Islet organoids derived from human pluripotent stem cells can be used in place of islet tissue and these systems have been used to demonstrate that overexpression of PD-L1 protected the organoids from rejection (Yoshihara et al., 2020) . This platform has also been used recently by Doug Melton’s group to show that interferon-gamma signalling plays a major role in rejection of stem cell-derived islets and this can be ameliorated to some degree by deletion of CXCL10 in the stem cells (Sintov et al., 2022). Currently, work is underway to enhance the development of the engrafted human innate immune cells by expression of cytokines such as human IL-15 or human FLT3-ligand. In addition, expression of mouse thymic stromal lymphopoetin (TSLP) has been shown to restore lymph node development in these composite mice, which is otherwise lacking.

James Cantley discussed “Preclinical models and assessment of islet function”, beginning by reviewing rodent models for T1D, including streptozotocin (STZ)-induced insulin-deficient diabetes, dosed to induce acute (Cantley et al., 2013) or progressive (Cantley et al., 2011) beta cell decline, along with the NOD/ShiLtJ (NOD) mouse model of T1D. NOD mice are widely used in preclinical T1D studies with examples of successful translation to human trials including anti-CD3 immunotherapies.
(Herold et al., 1992, Chatenoud et al., 1994, Herold et al., 2019) and stem cell derived pancreatic cell
grafts (Agulnick et al., 2015, Ramzy et al., 2021, Shapiro et al., 2021), and some failures to translate.
Despite steady improvements in pre-clinical study design, adopting many of the principles of clinical
trials (Atkinson, 2011, Grant et al., 2013, Gill et al., 2016), current pre-clinical endpoints are typically
static fed blood glucose measurements with a diabetes diagnostic threshold of >250mg/dl. Continuous
blood glucose monitoring (CGM) has revolutionised the clinical management of diabetes, whilst providing
new insights into glycaemic variability and its relationship with beta cell function (Carr et al., 2021,
Yeşiltepe-Mutlu et al., 2021, Rickels et al., 2020, Gibb et al., 2020), risk of disease progression (Steck et
al., 2022) and diabetes complications (Wilmot et al., 2021). Recent advances in telemetry have enabled
remote CGM in rodents via surgically-implanted carotid glucose sensors, providing new insights into
glycaemic variability (Lo Martire et al., 2018), revealing marked daily glucose fluctuations during the
prediabetic phase in NOD mice (Korstanje et al., 2017) and following minimal-mass islet transplantation
in STZ-induced diabetic rats (King et al., 2017). This has important implications for binary pre-clinical
study endpoints utilising the 250mg/dl threshold, with a risk of false negatives and positives in
prevention or reversal studies. Strategies for quantifying glycaemic variability during the pre-diabetic
and diabetic phases were considered, such as rodent-adapted time-in-range, % coefficient of variation or
interquartile range, providing a graded assessment of disease progression and pre-clinical efficacy,
whilst mirroring human glycaemic metrics. Barriers to wider implementation of preclinical CGM
include expensive and invasive telemetry implants, with further innovation in rodent CGM, application
of human patch systems to larger model species, or better use of static blood glucose measurements
needed to circumvent this.

In the second part of the lecture, Dr. Cantley discussed the challenges of assessing beta cell mass and 3D
structure of the rodent pancreas using conventional histology. A new Insulin1-tdTomato knock-in
reporter mouse generated by the lab was presented which, combined with tissue clearing approaches and
Mesolens imaging (Battistella et al., 2022), has enabled high-resolution imaging of total beta cell mass
in the intact pancreas, whilst providing a proportional readout of insulin1 promoter activity.

6. What is missing for translation?
Steven Parks is a member of the Diabetes UK administrative team and was diagnosed with T1D aged 3 years. He shared key reflections on the **translational gaps that need to be filled to address the needs of patients and their families**. In particular, he emphasised that the needs differ at different ages – for example between young children, teenagers and older adults. The sudden diagnosis of T1D after a very short (weeks) prodrome was a huge shock to his family and better markers able to monitor the decline in beta cell function in pre-diabetes and along with immune and genetic markers to predict who will develop it, is important to improve the preparation and reduce the impact of diagnosis for families. At all stages, the idea of being immunosuppressed long-term as part of therapy raises concerns and risks need to be minimised for example by being islet cell targeted. Following diagnosis, Steve confirmed that variability and unpredictability in glucose control is a major stress for people living with T1D. Reducing the complexity and burden of dealing with the different effects of food, exercise and psychological stress on glucose levels is essential. Although the technology of insulin replacement has advanced considerably, many people do not find it sufficiently discrete, reliable or low burden to engage with it in its current form, especially amongst teenagers and young adults, emphasising the continued need for interventions in prevention and stem cell replacement. At all stages of research from planning to late stages, Steven emphasized the importance of including people living with diabetes and their families to ensure that what appears a good idea to research teams does really meet their needs and comprises an acceptable balance of benefits and risks.

In his lecture entitled **“Clinical endpoints in beta cell preservation – Competing or complementary therapies?”**, Colin Dayan built on Peter Senior’s comments, emphasising the need for improved C-peptide and clinical endpoint assessments in studies of islet cell function. Measurement of the area under the curve (AUC) of insulin/C-peptide over 2 or 4 hrs following a mixed meal tolerance test has been the standard measure of beta cell function for 20 years. However, it is well known that beta cells secrete insulin in 2 phases – a rapid first phase and slower second phase. The first phase is critical to regulate post-prandial peaks of glucose and reduce variability but occurs within 10-15 mins, so it is not well
captured by integration of the C-peptide and glucose response over 2-4 hrs. Consistent with this, there is only modest correlation between AUC C-peptide or the detected peak C-peptide level (typically at around 90 mins) and measures of glycaemic control e.g. HbA1c or CGM parameters (r values typically around 0.4) (Buckingham et al., 2015). Analysis of pooled data from 18 intervention trials of immunotherapy in new-onset T1D, 6 of which met their primary end point, conducted within the Trial Outcome Markers Initiative (TOMI) hosted by the C-Path Consortium has shown that immunotherapy (vs placebo) has significant impact on AUC C-peptide at 6 months but not after 3 months. The same is true for insulin doses. By contrast, differences in HbA1c levels seen at 3 months, suggesting that immunotherapy has an important early impact on beta cell function that is not detected by the AUC metric. Several alternative measures take into account the relationship between glucose and C-peptide levels, as Peter Senior indicated in his presentation, e.g. Insulin dose adjusted A1C (IDAA1c), the Suito Index, the B2 score, and glucose and C-peptide response curves (Sims et al., 2021), however these do not reflect biphasic secretion. The Oral Minimal Model (OMM) developed by Cobelli and colleagues (Cobelli et al., 2014) (Galderisi et al., 2021) can estimate biphasic insulin secretion from the OGTT. The utility of hypoglycaemia as an outcome in clinical trials of beta cell preservation in new-onset T1D is also being explored within TOMI. Dr Dayan went on to describe the use of adaptive platform trials to study multiple combinations of agents (and agents at different doses) for beta cell preservation in a more rapid and efficient manner than with standard randomised controlled trial designs, as illustrated by the T1D Plus platform being developed in the INNODIA network. Work on the most sensitive and rapidly responsive measure of beta preservation will further accelerate these efforts and progress in drug development in this area.

“The closed loop story – advantages and limitations” was the theme addressed by Charlotte Boughton, including recent advances in this field and the recently published CLOuD study applying closed loop therapy from diagnosis of T1D in children (Boughton et al., 2020). Closed loop insulin pump therapy, in which insulin delivery is linked to CGM via an algorithm aims to mimic islet function
as closely as possible. She emphasised the challenge of glucose variability and unpredictability in insulin requirements that Steven Parks had highlighted, especially in very young children. Data from observation of over 2000 days in 114 different individuals showed that children aged 1-6 have a %CV in daily insulin requirements as high as 46%, as compared to values of around 36% in teenagers and young adults (Dovc et al., 2019). Closed loop therapy reduces rather than increases the burden for patients, with users spending on average 36 minutes per day on diabetes management. Hyperglycaemia is potentially detrimental to islet cells (“glucotoxicity”) including impairing beta cell function. It has therefore been hypothesised that improved glycemic control immediately after diagnosis may improve islet cell function and survival. However, reports to date have produced conflicting results (Shah et al., 1989, Buckingham et al., 2013). The Closed Loop from Onset of T1D study (CLOuD) aimed to address this issue in young people aged 10-17 by comparing the use of hybrid closed loop therapy with standard management (43% using pump therapy and 68% using continuous glucose monitoring by 24 months) starting within 21 days of diagnosis (Ware et al., 2022). HbA1c levels were 0.4 percentage points lower at 12 months (6.9 vs 7.3%) and 1 percentage point lower at 24 months (6.9 vs 8.0%) in the hybrid closed loop group, but no difference was seen in AUC C-peptide levels in mixed meal tolerance testing.

Participants in the intensive arm used the closed loop system for 76% of the time, which suggests that it had good levels of acceptability. However, 5 cases of severe hypoglycaemia and one case of diabetic ketoacidosis still occurred in the closed loop group emphasising the remaining unmet need for replacement/preservation of islet function.

7. Conclusions and roadmap for future research.

A suggested roadmap for future T1D research is summarised in Fig.5. New imaging technologies applied to pancreatic biobank tissues and islets are transforming our view of the natural history of T1D. They demonstrate that beta and alpha cell changes occur early during pathogenesis, promote new hypotheses on the role of exocrine cells, and suggest that a stage 0 T1D may exist (beta cell dysfunction and impaired glycaemic control, in the absence of autoimmunity). Further investigation of the role
played by alpha cells in the pathophysiology of T1D, and on why beta but not alpha cells are killed in long-term T1D, is clearly warranted.

Histopathological differences between putative T1D endotypes continue to be explored. Invasive insulitis observed in childhood T1D E1 could be associated with alterations in islet basement membrane components and integrity. In contrast, the islet basement membrane appears structurally more intact in older onset T1D E2 with mild insulitis. Alterations in its integrity could account for different rates of disease progression and possibly for the sudden acceleration close to diagnosis after many years of “status quo preservation”. In addition, the lack of direct immune-beta cell contact in situations where the islet basement membrane is intact supports soluble factors mediating the dialogue between the two cell types, and warrants further investigation. Insulin sensitivity may also play a role in shaping age-related endotypes. Research into the role of gut microbiota in T1D susceptibility, especially during weaning, may provide new insights and potential therapeutic strategies.

The hypothesis of the existence of T1D heterogeneity is starting to move from a phenomenological concept to one supported by novel mechanistic hypotheses. On one side, this invites enhanced efforts in the study of long-term non-progressors to identify disease-protective factors that may point to novel therapeutic targets. On the other, the often advocated immunomodulatory and beta cell-protective combination therapies may also be achieved by single agents targeting both immune and beta cells, e.g. JAK or TYK2 inhibitors and verapamil. In addition, studying young organ donors with multiple autoantibodies but without overt hyperglycaemia (stage 1-2) will provide insights into disease pathogenesis and heterogeneity, interactions between the beta cell and immune system, and potentially revealing new therapeutic targets.

It is likely that combination therapies with investigational products (reducing insulin dependence) and insulin delivery via closed loop systems (refining insulin administration) will become standard therapeutic approaches in the near term. The ability of anti-CD3 administration to delay the onset of T1D provides a great source of optimism for the future of immunotherapies to preserve beta cell function. Improved screening and biomarkers to determine T1D risk will support targeted implementation of these therapies. Although closed loop systems did not improve preservation of residual C-peptide in the recent CLouD trial, it may act as a useful adjuvant for other
immunomodulatory and beta cell-protective therapies. New strategies to improve the success of islet transplant therapies, such as encapsulation or co-transplantation with mesenchymal stromal cells, also have the potential to reduce insulin dependence in the near term. Recent progress in the advancement of stem cell to beta cell replacement therapy has clinical potential in the medium term, although regulatory, manufacturing and cost challenges remain. Finally, development of strategies to drive regeneration of endogenous beta cells remains a long-term research goal which, in combination with recent advances in immunotherapy, could provide stable restoration of a functional pancreatic beta cell mass. Successful and timely development of these therapeutic approaches will require continued improvements in the assessment of glycaemic control/variability and beta cell function metrics as robust clinical/preclinical markers and endpoints. Improved understanding of T1D risk, heterogeneity and identification of prognostic markers will facilitate stratified treatment of the disease.

In summary, this symposium has captured a cross-section of current T1D research, including the aetiology and heterogeneity of T1D along with the current state-of-the-art and clinical potential of treatment strategies, both established, emerging and in development. This research landscape inspires optimism for the future management, treatment and even prevention of T1D.
JDRF-DiabetesUK-INNODIA-nPOD Symposium: Full list of chairs and speakers.

Emily K Sims, Indiana University, IN, USA (chair)
Chantal Mathieu, Katholieke Universiteit Leuven, Belgium (speaker)
Decio L. Eizirik, Universite Libre de Bruxelles, Belgium (co-organiser, chair and speaker)
James Cantley, University of Dundee, UK (co-organiser, chair and speaker).
Colin M Dayan, Cardiff University, UK (co-organiser, chair and speaker).
Esther Latres, JDRF Research, NY, USA (co-organiser and chair).
Mark A Atkinson, University of Florida, FL, USA (chair and speaker).
Sarah J Richardson, University of Exeter, UK (chair and speaker).
Klaus H Kaestner, University of Pennsylvania, PA, USA (speaker).
Marcela Brissova, Vanderbilt University, TN, USA (speaker).
Carmella Evans-Molina, Indiana University, IN, USA (chair and speaker).
Teresa Rodriguez-Calvo, Helmholtz Center Munich, Germany (chair).
Francesco Dotta, University of Siena, Italy (chair).
John A Todd, University of Oxford, UK (speaker).
Lorenzo Pasquali, Universitat Pompeu Fabra Barcelona, Spain (speaker).
Roberto Mallone, Université Paris Cité, Institut Cochin, CNRS, INSERM, Paris, France (chair).
Anath Shalev, The University of Alabama at Birmingham, AL, USA (chair and speaker).
Heiko Lickert, Helmholtz Center Munich, Germany (speaker).
Raghavendra G Mirmira, University of Chicago, IL, USA (speaker).
Alberto Pugliese, Arthur Riggs Diabetes & Metabolism Research Institute, City of Hope, CA, USA (speaker).
Shareen Forbes, University of Edinburgh, UK (chair and speaker).
Timo Otonkoski, University of Helsinki, Finland (speaker).
Louise Winkel, Novo Nordisk, Denmark (speaker).
Kevan C Herold, Yale University, CT, USA (speaker).

Peter Senior, University of Alberta, Canada (speaker).

Michael A Brehm, University of Massachusetts, MA, USA (speaker).

Richard A Oram, University of Exeter, UK (chair).

Steven Parks, DiabetesUK, UK (speaker).

Charlotte K Boughton, University of Cambridge (speaker).

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**Figures and Legends:**

**Figure 1: Progression to Type 1 Diabetes.** Risk factors for type 1 diabetes (T1D) include genetic predisposition and environmental factors, such as viral infections and diet. Although clinical diagnosis of T1D usually occurs at stage 3 when beta cell mass is depleted and hyperglycaemia established, several stages precede this. There is increasing recognition of stage 0, in which beta cell dysfunction and impaired glycaemic control may occur in the absence of autoantibodies. Stage 1 represents seroconversion with engagement of the adaptive immune system and appearance of autoantibodies. During stage 2 beta cell dysfunction and apoptosis is increased impairing glycaemic control, leading to clinical diagnosis at stage 3. An important concept discussed is the reciprocal interaction between the beta cell and immune system throughout disease progression, along with interperson heterogeneity influenced by factors such as age of onset. Figure created with BioRender.com.

**Figure 2: Visualising insulitis in 3 dimensions.** Image of a 3D reconstruction of an nPOD donor (20-year-old T1D subject with 7 months disease duration) stained for glucagon (blue) and CD3 (yellow), revealing presence of infiltrating immune cells within the islet of Langerhans, interspersed with endocrine cells. 3D reconstruction was performed using Arivis Vision4D.
Figure 3: Summary of pancreas Type 1 diabetes endotypes (T1DE). Images of pancreatic sections from a T1DE1 (left panel) or T1DE2 (right panel) donor. Low magnification images of pancreas sections (left inset panels, 1cm scale bars) with each islet annotated as being an uninflamed Insulin-containing islet (ICI) – red; inflamed, defined as >15 CD45+ immune cells/islet, ICI – green; inflamed insulin-deficient islet (IDI) – blue; or uninflamed IDI – pink. T1DE1 donors have evidence of more inflammation, (green and blue islet annotations) and fewer residual insulin-containing islets (red/green islet annotations) than individuals with T1DE2. The higher magnification images (right inset panels, 500µm and 100µm scale bars) demonstrate the presence of peri-insulitis and invasive insulitis in ICIs of a T1DE1 donor, and an example of low level peri-insulitis observed in a T1DE2 donor. Further immune and beta cell characteristics of the endotypes are described in the boxes below.

Figure 4: Human pluripotent stem cell-derived islets (SC-islets). Double immunostaining for insulin and glucagon in the immature SC-islets (Stage 7, week 0) shows many double positive cells. In the mature stage, the hormone positive cells are clearly defined as separate populations in an architecture that resembles the human islet. Dynamic insulin secretion was studied with perifusion. The immature SC-islets do not respond to high glucose, although they are able to respond to exendin-4 and membrane depolarisation induced by potassium chloride (KCl). In contrast, the mature-stage SC-islets show a high response to glucose. (Data from Balboa et al. Nature Biotech 2022. Figure prepared by Väinö Lithovius).

Figure 5: A suggested roadmap for future Type 1 Diabetes Research. Summary of priorities for type 1 diabetes (T1D) research as described in detail in the main text. Pathophysiology (upper section). Risk factors for T1D involve a combination of genetic and environmental factors, including gut microbiota and viral infection. There is an urgent need for more research into the early stages of disease onset, including environmental triggers and the interaction between beta cells and the immune system. Progress in recent years has revealed marked heterogeneity in the age of diagnosis, disease progression and residual beta cell function in people with T1D. Further research into the factors underlying this heterogeneity is needed to generate deeper insights into disease aetiology, identify prognostic markers...
and enable personalised treatment strategies. With increased global obesity, including in childhood, more research into the role of obesity, sedentary lifestyle and insulin sensitivity on T1D progression is warranted. A recurring theme in the meeting was the dysregulation of glucagon secretion from pancreatic alpha cells (in spite of the fact that beta cells but not alpha cells are killed in long-term diabetes), and the imbalance of glucagon and insulin in the T1D pancreas. Finally, alterations in the exocrine and ductal system of the pancreas have been observed in T1D. **Lower section, interventions.**

Summary of established and emerging approaches to treat T1D is provided, with estimated timescales to clinical impact; colour coded: pink, near-term 0-5 years; orange, mid-term 0-8 years; blue, long-term 5-10+ years). Improved pre-diabetes screening will support targeted immunotherapy, beta cell preservation and ultimately T1D prevention. Optimised combination therapies, reduced insulin requirements and smarter insulin delivery have the potential to improve clinical care in the near term. Stem-cell derived cell transplant therapies are in clinical trials with positive early reports and have the potential to improve regulated insulin release in T1D in the mid-term. Likewise, strategies to improve the success of islet transplant therapy have the potential to reduce insulin dependence in the short term. Strategies to regenerate endogenous beta cells have long-term potential to restore regulated insulin secretion in the pancreas which, if coupled to beta cell protective therapies, may reverse T1D. Improvements in preclinical models and protocols can accelerate many of these therapeutic avenues. Figure created with BioRender.com.
Stage 0

Functional beta cell mass
Islet inflammation
Innate
Pancreatic Islet

Stage 1

Adaptive
Cytokines/IFNγ
Autoantibodies

Stage 2

Immune system

Stage 3

Blood glucose

Risk factors

Genetic
Environmental

Pancreatic Islet

Cytokines/IFNα
Autoantigens
Cytokines/chemokines

Immune system

HLA-I

Apoptosis
Islet inflammation
Adaptive
Innate

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### Older-onset (≥13y)
- Low numbers of immune cells (peri-insulitis)
- Infiltrate contains few B cells
- Retain beta cells

### Younger-onset (<7y)
- Many immune cells
- Infiltrate contains many B cells and CD8+ T cells (peri-insulitis and invasive insulitis)
- Fewer residual beta cells at diagnosis that are lost rapidly
- Abnormal insulin processing

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<th>T1DE2</th>
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<tr>
<td>Younger-onset (&lt;7y)</td>
<td>Older-onset (≥13y)</td>
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<td>Retain beta cells</td>
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Stage7, week 0 SC-islet

Stage7, week 6 SC-islet

Insulin secretion

INS GCG DNA
T1D prevention

Stage 0

Stage 1 Stage 2 Stage 3

Environmental triggers

Gut microbiota

Interaction between β-cells and the immune system

Heterogeneity in disease progression and residual β-cells

Role of insulin sensitivity and adiposity

α-cell dysfunction and paracrine regulation

Non-endocrine cell behaviour

Pathophysiology

Interventions

Improved combination therapies; reduced demand for insulin; smarter insulin delivery.

Improved pre-diabetes screening

β-cell protective therapies

Stem cell derived β-cell cell replacement

Improved survival and function of islet transplants

Endogenous β-cell regenerative therapies

Improved preclinical evaluation

T1D reversal

Near term 0-5 years

Mid term 0-8 years

Long term 5-10+ years

Heterogeneity in disease progression and residual β-cells

Role of insulin sensitivity and adiposity

α-cell dysfunction and paracrine regulation

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