Epigenetic biomarkers of destruction of residual beta cells after diagnosis of type 1 diabetes – links to diabetic ketoacidosis and partial clinical remission.

Beta cells of pancreas are some of the most specialized factories in the organism, having a monopoly on the production of insulin, the key hormone for glucose metabolism. The death of beta cells, then, results in absolute insulin deficiency and demands lifelong treatment with subcutaneous insulin. Beta cells can die in many ways – but the most common is being "shot in the back" by an individual`s own immune system which commonly leads to type 1 diabetes (T1D), the most prevalent type of diabetes in children. However, this autoimmune coup does not happen overnight – it is a process, caused by unidentified factors, in which the beta cell number slowly declines over many years. Only when about 80% of cells are gone, then symptoms appear and T1D diagnosis is made. The remaining residual beta cell mass is very important for the first few months after T1D diagnosis – and its well-being can possibly affect long-term prognosis. Most patients after T1D onset enter so-called partial clinical remission (PR), when the remaining cells catch a second breath and increase production of endogenous insulin. In this period – maintaining good glucose control is easier, and it has proven benefits for future risk of developing diabetes complications. PR, however, ends usually within 6-8 months as the remaining cells are destroyed by continued autoimmune reaction. Unfortunately, there are very few available methods to directly observe the process of beta cell death. One of proposed tools tries to use the fact that whenever cells of the body die, their contents spill into the vicinity and end up in the bloodstream. This also applies to each cell production manual – its DNA (deoxyribonucleic acid) sequence, which encodes all proteins produced by the cell. Fortunately, while each cell`s DNA is the same in terms of sequence (each human is made up according to a single manual), none of the cells in the body use all DNA information. They rather read and execute the parts relevant for their function – the remaining pages are glued together and unavailable for transcription. Such gene silencing is often done by methylation (adding a chemical –CH3 group) of specific sites within or around each gene. In effect, each cell carries a unique pattern of methylation over its DNA, allowing us to identify the cell among many others – and, as mentioned before, the beta cells are one of the most standing out individuals. Their genes for insulin are largely unmethylated and can serve as a fingerprint of beta cell. Thus, if we browse cell-free circulating DNA (cfDNA) for beta cell-specific parts (B-cfDNA), we can assess the amount of recently-deceased beta cells.

We intend to use this measurement in the clinical setting. We will recruit children with new onset T1D. In this group, we aim to compare levels of B-cfDNA between those who presented with diabetic ketoacidosis (DKA) at T1D onset or not. DKA is a life-threatening complication of T1D resulting from prolonged lack of any insulin in circulation, and usually indicates that T1D symptoms were missed and contact with healthcare was delayed. While it is known that DKA predisposes to more difficulties in future diabetes control, it is not known if the condition itself corresponds with intensity of beta cell destruction. Subsequently, we will determine if B-cfDNA amounts present in circulation at T1D onset can predict whether the patient will enter PR and if so – how long it will last. To that end, we will invite willing patients into a 12-onth follow-up, during which every 3 months we will be collecting blood (for measurement of B-cfDNA and endogenous insulin secretion by proxy of c-peptide, a by-product of insulin) and clinical data to determine the presence of PR.

Furthermore, we also plan to measure B-cfDNA in two control groups. The first one will be diabetes-free autoantibody-negative siblings of children with T1D. Islet-specific autoantibodies are hallmarks of preclinical T1D development and are used to measure the long-term risk of developing T1D. Being positive for at least 2 different autoantibodies means that T1D development is certain, while displaying none makes T1D diagnosis very unlikely. The other group will consist of adult patients with T1D undergoing islet transplants. During this procedures the patients receive pancreatic islets containing beta cells with the aim of improving their endogenous insulin secretion and diabetes control. Unfortunately, not all islets are transplanted successfully and their remnants are known to appear in circulation ~1h after transplant. Such B-cfDNA peak is used in the field as a positive control, and we will utilize it similarly.