Incretin response in immune checkpoint inhibitor-induced diabetes: an observational study

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Immune checkpoint inhibitors (ICIs) have greatly improved cancer treatment, by abolishing the T-cell inhibition induced by neoplastic tissues. However, ICI administration may be associated with various immune-related adverse events (irAEs). ICI-induced diabetes mellitus (DM), is a rare but life-threatening irAE, which may present as a fulminant diabetes (FD, defined by ketosis, plasma glucose concentration ≥ 16 mmol/l, HbA1c level < 8.7% and

fasting C-peptide concentration < 0.1 ng/ml) or a progressive increase in glucose concentration (non-fulminant diabetes, NFD) [1]. The pathophysiological mechanisms underlying ICI-related DM are complex and remain unclear. Pancreatic islet infiltration and beta-cell destruction by T lymphocytes has been highlighted, mostly in cases of FD with ketoacidosis and absolute insulin deficiency [2]. By contrast, insulin resistance related to systemic inflammation is a hallmark of cases in which a progressive increase in glucose concentration is induced by iCIs.

The incretin hormones, GIP (glucose-dependent insulinotropic polypeptide) and GLP-1 (glucagon-like peptide 1), are gut peptides that increase insulin secretion during meals, playing a key role in glucose homeostasis. Changes in their secretion are associated with the development of dysglycaemic states, such as type 2 diabetes (T2D). The mechanisms underlying the impairment of insulin secretion in response to incretin hormones in these diseases have yet to be established. It has recently been shown that changes in gut flora diversity or imbalances between important bacterial species present in the microbiota can affect enteroendocrine cell function, and, thus, the incretin effect [3]. Human gut microbial species modulate host immunity and may, therefore, have a positive or negative effect on cancer prognosis and ICI efficiency. Indeed, some cases of ICI resistance have been attributed to an imbalance of the gut microbiome, as demonstrated by Routy B et al. in a large cohort of patients with non-small cell lung cancer, renal cell carcinoma or urothelial cancer [4]. Conversely, the same team demonstrated that patients responding to PD-1 blockade had faeces enriched in particular bacterial genera (*Akkermansia* and *Alistipes*) [4]. Based on these findings, we hypothesize that interactions between ICIs and the gut microbiome may modify incretin hormone availability, thereby contributing to the pathophysiology of ICI-induced DM.

We tested this hypothesis by comparing the secretion of GIP and GLP-1 during an oral glucose tolerance test (75 g of glucose, OGTT) in three patients with FD and five patients with NFD. The FD patients consisted of one woman and two men, with a median age of 63 years, and a median time to diabetes onset of four months. The ICIs used were nivolumab (*n* = 1), pembrolizumab (*n* = 1) and a combination of durvalumab-tremelimumab (*n* = 1), for lung cancer (*n* = 2) or breast cancer (*n* = 1). None of and the patients was overweight, and median HbA1c level was 7.8% (62 mmol/mol). The NFD subjects consisted of one woman and four men, with a median age of 67 years, and a median BMI of 25.1 kg/m^2. Median time to diabetes onset was six months, and median HbA1c level was 6.6% (49 mmol/mol). The ICIs administered were nivolumab (*n* = 3), pembrolizumab (*n* = 1) and a combination of nivolumab and ipilimumab (*n* = 1), for lung cancer (*n* = 3), breast cancer (*n* = 1) or kidney cancer (*n* = 1). Incretin hormone secretion during the OGTT was compared between the diabetic patients on ICIs, nine T2D patients (7 women and 2 men, median age: 62 years, BMI: 33 kg/m^2, HbA1c: 8.2% or 66 mmol/mol) and 11 healthy controls (9 women,2 men, median age: 46 years, BMI: 23 kg/m^2, HbA1c: 5.2% or 33 mmol/mol). HbA1c and glucose levels during the OGTT were determined in routine biological assays. GIP and GLP-1 levels were assessed by sandwich ELISA (Merck-Millipore protocol). All patients gave informed consent for participation in this study.

As expected, circulating GLP-1 concentrations were lower in all diabetic groups (T2D, FD and NFD) (Figure 1A) than in controls, and the GLP-1 concentrations of the groups of patients treated with ICIs were intermediate between those of T2D and control subjects. Among ICI-treated patients, GLP-1 concentrations were lowest in the FD group. GIP is an important hormone for the control of glucose homeostasis, and GIP concentrations have been reported to be higher in dysmetabolic populations than in controls. Consistent with this

finding, GIP secretion during the OGTT was similar in T2D patients and the NFD group (Figure 1B). Unexpectedly, GIP concentrations, in fasting and during OGTT, were found to be lower in the FD group. This defect, associated with the limited GLP-1 secretion described above, suggests a large change in the functionality of enteroendocrine cells in FD patients.

This observational study highlights changes in incretin hormone secretion in patients treated with ICIs. NFD patients had an incretin hormone secretion profile similar to that of patients with T2D (low GLP-1 and high GIP concentrations), but a discordant profile was observed in FD patients, who displayed a lack of secretion of both incretin hormones. Certain mechanisms known to reduce the incretin effect, such as obesity and severe dysglycaemia, can be excluded in the FD group (because HbA1c levels were in the same range in the FD and T2D groups). Insulin deficiency cannot be considered the origin of the combined changes to incretin hormone secretion either, because it has been shown that patients with other diseases involving insulin deficiency (e.g. type 1 diabetes) have similar levels of meal-stimulated incretin secretion to healthy subjects. It remains unknown whether ICIs affect the functionality of L or K cells, and tests are required to determine whether these compounds have potential direct toxic effects on these cells. Finally, as indicated above, recent data have suggested that some gut microbiota species affect the efficacy of immunotherapy for cancer. In addition to the association between microbiota 16S data and clinical outcomes in populations with cancer, convincing evidence for a direct effect of the microbiota on response to cancer therapy has been provided by the observation that the administration of *Akkermansia muciniphilia* to germ-free mice results in better intra-tumoural T-cell recruitment in response to PD1 blockade [4]. In addition, some metabolites produced by the anaerobic intestinal microbiota may also affect local immunity and enteroendocrine cell function through the production of short-chain fatty acids (SCFAs). Supporting this assertion,

high faecal SCFA concentrations are associated with more effective PD-1 blockade in human cancer treatment [5]. These findings should be seen in the context of current knowledge of the promotion of T2D by gut microbiota imbalance in the general population. Low proportions of *Akkermansia muciniphilia* and SCFA in the faeces are associated with a higher prevalence of metabolic disorders, such as obesity and T2D, and lower levels of incretin hormone availability. These data suggest that changes in the gut microbiota and in the metabolites produced by the gut microbiome may affect the efficacy of cancer therapy, the secretion of incretin hormones and glucose homeostasis.

It therefore appears likely that multiple interactions between the microbiota, enteroendocrine cells, ICIs and the cancer modulate the efficacy of ICI treatment and incretin hormone secretion, thereby modulating the risk of dysmetabolic disease development. Further studies are required to improve our understanding of these interactions.

Conflicts of interests : none

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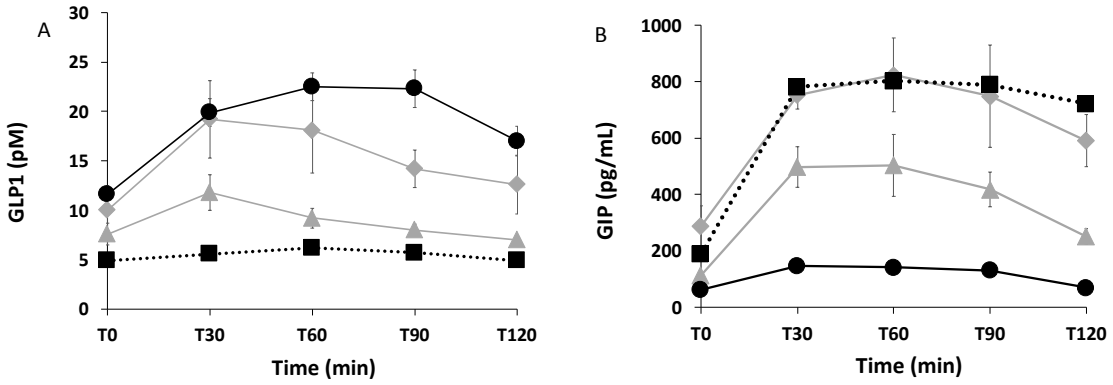

Figure 1: GLP-1 (A) and GIP (B) levels during an oral glucose tolerance test (75 g glucose) in patients with fulminant diabetes (FD, n=3, gray triangles), non-fulminant diabetes (NFD, n=5, gray diamonds), or type 2 diabetes (T2D, n=9, black squares) and in healthy controls (C, n=11, black circles). Data are expressed as the mean ± SEM.