EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Assessment report for GLP-1 based therapies

Review under Article 5(3) of **Regulation (EC) No 726/2004**

Procedure no: EMEA/H/A-5(3)/1369

Assessment Report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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1. Background information on the procedure

The European Medicines Agency (EMA) was made aware of findings by a group of academic researchers suggesting an increased risk of pancreatitis and cellular changes in patients treated for type 2 diabetes mellitus (T2DM) with GLP-1 based therapies (glucagon-like peptide 1 (GLP-1) agonists and dipeptidylpeptidase-4 (DPP-4) inhibitors)[1]. The findings resulted from the histological examination of 34 pancreata obtained from brain dead organ donors. The pancreata of eight individuals with T2DM who were treated with sitagliptin (n = 7) or exenatide (n = 1) for a year or more were compared to 12 pancreata from individuals with T2DM treated with other therapies and 14 pancreata from non-diabetic individuals. The investigators described a number of findings in the pancreata of the T2DM individuals treated with GLP-1 based therapies which could implicate an association of the treatment with increased risk of pancreatitis and neoplasms.

It was noted that the current product information of all centrally authorised GLP-1 based therapies contains warnings about pancreatitis and that pancreatitis is listed as a reported event. In addition, the incidence rates of pancreatitis and the potential occurrence of pancreatic cancer for authorised GLP-1 based products is being investigated as part of several ongoing studies. However, in view of the new evidence, the Committee for Medicinal Products for Human Use (CHMP) was requested to investigate the emerging data and to give an opinion, under Article 5(3) of Regulation (EC) 726/2004, on the potential impact on centrally authorised GLP-1 agonists and DPP-4 inhibitors products, in consultation with the Pharmacovigilance Risk Assessment Committee (PRAC). In case concerns are identified, the Committees are to indicate whether these should be further investigated at Community level.

2. Scientific discussion

2.1 Introduction

Glucagon-like peptide 1 based therapies are approved for the treatment of patients with type 2 diabetes. These therapies include GLP-1 receptor agonists (exenatide, liraglutide and lixisenatide) and DPP-4 inhibitors (sitagliptin, vildagliptin, saxagliptin and linagliptin) which, albeit in different ways, increase the exposure to GLP-1.

Glucagon-like peptide 1 is a gut hormone secreted by the intestinal epithelial endocrine L-cells as a response to the presence of nutrients in the lumen of the small intestine. Once in the circulation, GLP-1 has a half-life of one to two minutes, due to rapid degradation by the enzyme dipeptidyl peptidase-4 (DPP-4). Due to the short half-life, GLP-1 analogues, resistant to the action of DPP-4, and DPP-4 inhibitors have been developed. The mechanism of these products is to increase the exposure to incretin hormones (mainly GLP-1) which leads to a glucose dependent stimulation of alpha and beta cells. The main actions of GLP-1 are to stimulate insulin secretion (i.e., to act as an incretin hormone) and to inhibit glucagon secretion (the normal glucagon response to hypoglycaemia is not impaired), thereby contributing to limit postprandial glucose excursions. It also inhibits gastrointestinal motility and secretion and thus acts as an enterogastrone and part of the "ileal brake" mechanism. Glucagon-like peptide 1 also appears to be a physiological regulator of appetite and food intake. A number of additional sites with GLP-1 receptors have been discovered including the heart and the nervous system. There are studies supporting that GLP-1 can regulate signaling pathways coupled to cell proliferation and apoptosis.

[1] *Butler et al*, Marked Expansion of Exocrine and Endocrine Pancreas With Incretin Therapy in Humans With Increased Exocrine Pancreas Dysplasia and the Potential for Glucagon-Producing Neuroendocrine Tumors; Diabetes. 2013 Jul; 62(7):2595-604.

The efficacy of GLP-1 receptor agonists and DPP-4 inhibitors has been demonstrated. In terms of safety, the most common adverse events seen in clinical trials with GLP-1 receptor agonists are of gastrointestinal character; mainly nausea, vomiting and diarrhoea. However, the incidence diminishes over time. Other identified risks include pancreatitis, immunogenicity, acute renal failure and rapid weight loss. Identified and potential risks with DPP-4 inhibitors include hypoglycaemia, hypersensitivity, gastrointestinal disorders, pancreatitis, skin disorders, transaminase elevation and infections.

The current review was initiated further to the findings by a group of academic researchers suggesting an increased risk of pancreatitis and cellular changes in patients treated for T2DM with GLP-1 based therapies (*Butler et al*, 2013). The CHMP considered the recently published article on this matter and a review of available pre-clinical and clinical information with respect to pancreatic safety was undertaken. The PRAC was consulted, as applicable. The outcome of an ad-hoc expert meeting held was also considered. Only relevant information for the discussion is presented hereinafter.

2.2 Butler et al (2013)

A summary of the main findings of the publication by *Butler et al*, 2013 is described hereinafter.

Study design and methods

The study examined pancreata from organ donors with type 2 diabetes mellitus (DM) treated by incretin therapy (n=8) or other therapy (n=12) and non-diabetic controls (n=14). All pancreata were procured from brain dead organ donors by the JDRF Network for Pancreatic Organ Donors with Diabetes (nPOD) coordinated through the University of Florida in Gainesville, Florida. The eight subjects who received incretin therapy had been treated for a year or more (seven treated with the DPP-4 inhibitor sitagliptin and 1 with the GLP-1 agonist exenatide).

The subjects characteristics, including age, duration of disease, body mass index (BMI), treatments received and captured cause of death are listed below.

Table 1 Clinical characteristics of brain-dead organ donors (as presented in the publication)

Case	Age (years)	Duration of DM (years)	Sex	BMI (kg/m^2)	Treatments	Cause of death
DM-I						
6157	74	1	F	39	Januvia	ICH/stroke
6185	46	15	M	41	Januvia, metformin	Anoxia
6186	68	5	M	21	Januvia, metformin	ICH/stroke
6189	49	26	F	36	Byetta, metformin, glipizide	Stroke
6199	53	20	M	30	Januvia, insulin pen	ICH/stroke
6194	47	13	M	24	Humulin, NovoLog, Januvia	ICH/stroke
6203	68	5	M	33	Januvia, metformin	Stroke
6206	59	10	M	42	Januvia, metformin	Stroke
Mean (SEM)	58 (4)	12 (3)		33 (3)		
DM						
6028	33	17	M	30	Insulin	Gunshot wound to head
6059	18	0.3	F	39	None	Cardiovascular
6108	57	2	M	30	Metformin	ICH/stroke
6110	20	0.2	F	40	None	ICH/stroke, DKA
6109	48	—	F	33	None	ICH/stroke, DKA
6114	42	2	M	31	Metformin, noncompliant	Asphyxiation
6124	62	3	M	34	Metformin	ICH/stroke
6127	44	10	F	30	Insulin	ICH/stroke
6133	45	20	F	40	Insulin	Cardiovascular
6139	37	1.5	F	45	None	Seizure
6142	29	14	F	34	None	Bacterial meningitis
6149	39	20	F	29	Insulin	ICH/stroke
Mean (SEM)	40 (4)	8 (3)		35 (2)		
ND						
6009	45		M	31		Anoxia
6015	39		F	32		Anoxia
6012	64		F	31		Cerebrovascular/stroke
6016	42		M	31		Cerebrovascular/stroke
6019	68		F	24		Head trauma
6020	60		M	30		Cerebrovascular/stroke
6022	75		M	31		Cerebrovascular/stroke
6034	32		F	25		Head trauma
6060	24		M	33		Head trauma
6097	43		F	36		Cerebrovascular/stroke
6099	14		M	30		Head trauma
6102	45		F	35		Cerebrovascular/stroke
6158	40		M	30		Head trauma
6165	45		F	25		Cerebrovascular/stroke
Mean (SEM)	45 (5)			30 (1)		

DKA, diabetic ketoacidosis; F, female; ICH, intracerebral hemorrhage; M, male.

In terms of pancreas fixation, embedding and sectioning, the authors described the preparation procedure for pancreata recovered from cadaveric organ donors. Immunostaining was performed in two locations and included: 1) the deparaffinization of serial sections and incubation with primary antibodies to Ki67 and insulin, or CD3 and glucagon with antibody localization visualized with peroxidase-DAB (3, 3'-diaminobenzidine) and alkaline phosphatase-Fast Red polymer systems; 2) staining for Ki67, insulin and Alcian blue by immunohistochemistry and Ki67 and glucagon by immunohistochemistry. A section of pancreas from each of the DM cases treated with incretin therapy and a subset of DM not treated with incretin therapy (5 cases) and non-diabetic cases (6 cases) were stained for insulin and glucagon by immunofluorescence, and additional sections for glucagon, insulin, cytokeratin and DAPI (4',6-diamidino-2-phenylindole).

The stained slides or sections of pancreas were scanned. The morphometric analysis was either through estimating the proportion of insulin and glucagon stained area compared to total tissue area defined by hematoxylin counterstain using an algorithm or measuring the total area of the tissue. Full cross-sections of the pancreas head, body and tail were evaluated for pancreatic intraepithelial neoplasia (PanIN) by a gastrointestinal pathologist blinded to clinical information. The number of PanIN lesions and grade were established per lobular unit and then computed per unit area of pancreas. Using certain stained sections, 100 islets were analysed per section to determine the frequency of Ki67 in the alpha and beta cells of islets and in the non-alpha and non-beta cell compartment of those islets.

A total of 475 alpha cells and 475 beta cells were evaluated. The percentage of beta and alpha cells within pancreatic ducts was determined and the methodology used was described by the authors.

Results

According to the publication, pancreatic mass was increased ($p<0.05$) by approximately 40% in DM patients treated with incretin therapy compared to that observed in subjects with DM and not treated with these medicinal products.

The beta cell mass was decreased by 55% in DM patients not on incretin therapy in comparison to non-diabetic controls (0.29±0.08 *vs.* 0.60±0.10G; $p<0.05$), whilst an increase, mostly on beta cell numbers rather than beta cell size, was noted in incretin treated DM patients compared to the DM group (1.81±0.56 *vs.* 0.29±0.08G, $p<0.01$) and to non-diabetic controls (1.81±0.56 *vs.* 0.60±0.10 G, $p<0.05$).

The pancreatic fractional area immunostained for glucagon was increased in individuals with DM treated with incretin therapy in comparison with those with DM on other therapy (1.65±0.39 *vs.* 0.57±0.12%, $p<0.0001$), as well as compared to non-diabetic controls (1.65±0.39 *vs.* 0.52±0.08%, $p<0.0001$). The glucagon mass pattern was also increased in DM individuals treated with incretin therapy compared to those with DM not treated with these medicines (2.08±0.75 *vs.* 0.45±0.10 G, DM-I vs. DM, $p<0.01$). As for beta cells, the increase in alpha cell mass was mostly due to an increase in the number of alpha cells.

The authors reported a subset of enlarged and peculiar shaped islets, as well as increased numbers of endocrine cells in association with duct structures in DM subjects treated with incretin therapy. Insulin immunoreactive cells were found in individuals from all three groups with no detectable increase between groups regardless of incretin therapy. However, the percentage of cells immunoreactive for glucagon in ducts was increased in DM subjects with prior incretin therapy versus DM subjects not treated with incretin therapy (2.8±0.9 *vs.* 0.5±0.2%, $p=<0.05$). It was noted that the increase in glucagon immunoreactive cells with incretin treatment were mostly observed in the periductal areas whilst the increased numbers of insulin immunoreactive cells with incretin therapy were located in more remote areas from these periductal endocrine complexes.

Alpha cell hyperplasia was reported in one subjected with DM and treated with exenatide. In one individual with DM treated with sitagliptin, an alpha cell/glucagon producing neuroendocrine tumor was identified in the body of the pancreas. Glucagon-producing microadenomas were also detected in the same case and two other incretin treated cases, while hyperplastic islets with predominant glucagon staining were noted in seven of eight of the incretin treated cases. No neuroendocrine tumors or glucagon-producing microadenomas were detected in non-diabetic controls or DM subjects not treated with incretin therapy. The authors indicated that an inspection of pancreatic sections immunostained with either insulin or glucagon from individuals with DM treated with incretin therapy seemed to suggest that several cells within these islets were immunoreactive for both hormones. The percentage of insulin positive cells in incretin treated individuals that were also glucagon immunoreactive were increased when compared to those with DM not treated with incretin therapy (16.8±5.0 *vs.* 3.2±1.4%, $p<0.05$). There was also an increase in double immunoreactive positive cells in individuals with DM not treated with incretin therapy when compared to non-diabetic controls (3.2±1.4 *vs.* 0.4±0.1%, $p<0.05$).

The frequency of Ki67 positive nuclei in islet endocrine cells was extremely rare (all less than 0.01 cells per islet section), with no significant differences between the three groups studied.

Finally, it was noted that the increased pancreatic mass in DM-incretin therapy was accompanied by increased whole pancreas cell proliferation (0.25±0.03 *vs*. 0.12±0.01%, DM-I *vs.* DM, $p<0.0001$) and an increase in the presence of pancreatic intraepithelial neoplasia (PanINs) (11.9±2.6 *vs*. 4.9±1.7, DM-I *vs*. DM, PanINs/mm2 x 103, $p<0.01$). Inspection of pancreas sections in incretin treated

individuals revealed small foci of increased Ki67 immunostaining in and around ducts and sometimes in areas of exocrine dysplasia.

2.3 Preclinical and clinical data on pancreatic safety

Preclinical and clinical information previously available was considered by the CHMP, with a focus on pancreatitis and/or pancreatic cancer. Current pharmacovigilance activities and ongoing studies aiming to collect information on pancreatic events were also considered. A summary for GLP-1 agonists (exenatide, liraglutide and lixisenatide) and DPP-4 inhibitors (sitagliptin, vildagliptin, saxagliptin and linagliptin) is presented below.

Exenatide

In vitro and animal pharmacology studies with exenatide have shown an increase in beta-cell mass following treatment. No adverse effects on the pancreas of healthy animals were observed in any of the toxicology studies included in the initial marketing authorisation application. However, further studies performed by academic groups have demonstrated a potential for other effects in the pancreas. *Gier et al,* 2012 Diabetes 61:1250 showed an increase in pancreatic duct glands in rats treated with exenatide. They also showed that this effect in an oncogene-expressing transgenic mouse could contribute to dysplasia and/or pancreatitis. The relevance of these findings for clinical safety is uncertain. In the non-human primate studies, there was a mild pancreatic hypercellularity in monkeys treated for 3 and 9 months. The effect was only seen at the highest dose, representing an exposure margin to clinical exposure of approximately 1000-fold. There were no suggestions of toxicologically important changes from histopathology. Given that increased beta-cell mass was considered a potentially important mechanism for the adventitious effects of GLP-1 receptor agonists, the mild pancreatic hypercellularity in monkeys was not considered a concern. Moreover, in the carcinogenicity studies in mice and rats, there was no evidence for pancreatic neoplasia.

In the clinical setting, safety data from the clinical trial programm did not suggest an increased risk of pancreatitis with exenatide twice a day (BID) compared to other drugs. However, at the time of approval, spontaneous cases of pancreatitis had been reported in other markets in which the products had already been introduced. The product information therefore contains wording with regards to pancreatitis as a warning and a listed undesirable effect. In clinical trials two cases of pancreatic cancer have been reported. In the Integrated Completed Studies Database supporting the exenatide once weekly (QW) submission, there were three cases of acute pancreatitis (one in a subject receiving exenatide QW and two in subjects receiving pioglitazone). No case of pancreatic neoplasm was reported in the database.

Results from three retrospective studies evaluating the risk of pancreatitis as well as data from a registry with respect to risk of pancreatic neoplasm concluded that the studies did not show a risk difference between current or recent use of exenatide compared to other oral antidiabetic drugs. However, it was also concluded that the evidence needs to be weighed with caution, due to the nature of the data with high risk of residual confounding. However, due to the low number of pancreatic neoplasms, no firm conclusions can be drawn.

In addition to routine pharmacovigilance, ongoing post-marketing initiatives include a trial on the evaluation of cardiovascular outcomes with linagliptin therapy which will also collect information with regards to pancreatic events. Furthermore, observational studies and prescription event monitoring studies are also ongoing.

Liraglutide

Repeat-dose toxicity studies were conducted in CD-1 mice, Sprague Dawley rats and Cynomolgus monkeys. In addition, long-term carcinogenicity studies were conducted in mice and rats. An increased pancreatic weight was observed in the mid and high dose groups of Cynomolgus monkeys at 52 weeks treatment (study duration up to 87 weeks). The weight increase was shown to be related to a balanced increase in exocrine duct and acini mass, however the duct/acinar weight ratio was constant between the control and high dose animals. Normal histological morphology of the pancreas was seen in all studies, no clinical or biochemical changes were seen in any of the non-human studies and there was no histopathology indicative of inflammation. In addition, no macroscopic changes were observed in the 87 week repeat dose toxicity study in Cynomolgus monkeys, therefore the findings at week 52 do not suggest a safety concern for humans with respect to treatment related pancreatitis. Overall the non-clinical data do not indicate that liraglutide treatment is associated with adverse effects on the endocrine and exocrine pancreas. A post marketing authorisation study performed in Zucker diabetic fatty (ZDF) rats also showed that liraglutide treatment was not associated with pancreatitis and no increased exocrine cell mass or exocrine cell proliferation was observed.

In terms of clinical data, the reporting rates of acute pancreatitis and pancreatitis in Phase IIIa trials was 1.6/1,000 subject years of exposure (SYE) for liraglutide and 1.4/1,000 SYE for oral antidiabetic drugs. One death due to pancreatic carcinoma was also identified and considered as not related to treatment. Cases of pancreatitis and neoplasms are followed up in periodic safety update reports. Based on evidence from clinical trials and the post-marketing phase, the product information has been kept up to date and contains wording with regards to pancreatitis as a warning and a listed undesirable effect.
In addition to routine pharmacovigilance, ongoing post-marketing initiatives include a trial on the evaluation of cardiovascular outcomes with liraglutide therapy which will also collect information with regards to pancreatic events. Observational studies are also ongoing.

Lixisenatide

Repeat-dose toxicity studies were conducted in mice, rats and dogs. The potential effect of lixisenatide on the absolute and relative pancreas weights was not assessed. In two-year carcinogenicity studies performed in mice and rats, some microscopic findings were reported. When histopathological changes were detected in the pancreas (islet cells hyperplasia, islet cells adenoma, acinar cells hyperplasia) they occurred at high exposure levels compared to expected active exposure in clinical practice, in a small number of animals and with a low degree of severity. No gender- or dose-effect relationships were observed. With regards to the incidence of islet cell adenoma/carcinoma seen in rats dosed with lixisenatide, there was no statistically significant difference between these drug-treated rats as compared to the control animals. The microscopic findings were not considered to be indicative of a high clinical safety risk.

In the clinical setting, adverse events specific to pancreatitis were reported in phase II/III studies in nine patients in the lixisenatide group (0.3%) compared to two in the placebo group (0.1%). However, when the events of acute pancreatitis and pancreatitis were confirmed, by either gastroenterological consultation or positive imaging studies, the incidence was found to be similar between treatment groups. Pancreatic carcinoma was reported in three (<0.1%) lixisenatide patients and one (<0.1%) patient in the comparator group (exenatide arm).
Based on evidence from clinical trials, the product information contains wording with regards to pancreatitis as a warning.

In addition to routine pharmacovigilance, ongoing post-marketing initiatives include a trial on the evaluation of cardiovascular outcomes with lixisenatide therapy which will also collect information with regards to pancreatic events. A retrospective database study and a patient registry are planned to monitor occurrences of events of interest, e.g. pancreatitis and pancreatic cancer.

Sitagliptin

In *in vivo* studies, including repeated-dose studies in mice, rats, dogs and monkeys and carcinogenicity studies in mice and rats, no adverse effects on the pancreas were observed. It has also been shown that sitagliptin is not a genotoxic compound *in vitro* and *in vivo*. In non-human primates, potential effects on the pancreas were evaluated in a three month repeated-dose toxicity study. The histopathology data on the pancreas showed no concern. In literature, sitagliptin was observed to cause ductal proliferation and metaplasia in a transgene model of the diabetic rat (*Matveyenko et al* 2009 Diabetes 58:1604), however data from HIP (human islet amyloid polypeptide transgenic) mice and ZDF (Zucker diabetic fatty) rats support the beneficial effect of sitagliptin on beta-cell function, primarily mediated by an improved beta-cell preservation, e.g. by reducing beta-cell death (apoptosis) rather than by expanding of beta-cell mass by cell proliferation of the pancreatic duct. In these studies, cell proliferation of pancreatic duct cells, an important risk factor for the development of pancreatitis and pancreatic cancer, was not increased by sitagliptin as compared to metformin.

Two cases of pancreatitis and two cases of pancreatic carcinoma were reported in the initial clinical trials supporting the marketing authorisation. The data were considered insufficient to draw conclusions. In another trial one case of pancreatic cancer was also reported. Pancreatitis and pancreatic cancer have been reported in the post-marketing setting. With regards to pancreatic cancer, the data do not indicate a true association. A cumulative review of cases has been undertaken and the majority (19 out of 29) had a time to onset < 6 months, a period considered too short to suggest a causal relationship with sitagliptin. Further post-marketing cases did not show any change of pattern or increase in incidence.

Based on evidence from clinical trials and the post-marketing phase, the product information has been kept up to date and contains wording with regards to pancreatitis as a warning and a listed undesirable effect.

In addition to routine pharmacovigilance, ongoing post-marketing initiatives include a trial on the evaluation of cardiovascular outcomes with sitagliptin therapy which will also collect information with regards to pancreatic events.

Saxagliptin

All repeat dose and carcinogenicity studies were performed in non-diabetic animals. No findings indicative of pre-neoplastic lesions or proliferative effects were observed in repeat dose toxicity studies in mouse, rat, dog or monkey at plasma exposure levels adequately above human exposure levels at maximal therapeutic dose. Saxagliptin was non-genotoxic *in vitro* and *in vivo*. At plasma exposure levels adequately above human exposure levels at maximal therapeutic dose, saxagliptin did not lead to pancreatic hyperplasia or neoplasia.

In the clinical setting, there was no evidence for any causal relation between treatment with saxagliptin and pancreatic neoplasms in data from phase IIb and III studies. Four cases of pancreatitis at least possibly related to treatment with saxagliptin were reported. Pancreatitis has also been reported in the post marketing phase. A total of eight cases of pancreatic cancer and two cases of pancreas neoplasm have been reported. Duration of treatment with saxagliptin was known in six cases, ranging from 4-18 months. The short time to event, not expected in drug-induced malignancies, and a lack of sufficient background information makes causality assessment difficult.

Based on evidence from clinical trials and the post-marketing phase, the product information has been kept up to date and contains wording with regards to pancreatitis as a warning and a listed undesirable effect.
In addition to routine pharmacovigilance, ongoing post-marketing initiatives include a trial on the evaluation of cardiovascular outcomes with saxagliptin therapy which will also collect information with regards to pancreatic events.

Vildagliptin

The influence of vildagliptin on beta-cell regulation was examined in neonatal rats and in streptozotocin (STZ)-induced diabetic mice. Vildagliptin markedly increased replication (>8-fold increase) and inhibited apoptosis (by 65%) on day 7 of treatment. This resulted in a significant increase in beta-cell mass on day 21 (24-h after final dose), which was maintained on day 33 (12-d after final dose). There was no apparent effect of treatment on duct-associated beta-cells (an index of neogenesis) or on glucagon staining in neonatal rats. The vildagliptin inhibition of apoptosis was coherent with the results reported by *Hamamoto S et al*, 2013 in obese diabetic KK-Ay mice, where the authors concluded that in the mouse model used vildagliptin increases beta-cell mass by suppressing cell apoptosis and oxidative stress and by enhancing cell proliferation and differentiation. An effect on the alpha cell mass was not observed. Vildagliptin did not shown genotoxic potential *in vitro* and *in vivo*. The carcinogenic potential was investigated in rats and mice in 2-year carcinogenicity studies. In the rat survival was not affected by treatment. An increased incidence of hemangiosarcoma in male mice treated at ≥ 250 mg/kg/day and in female mice at 1000 mg/kg/day (exposure ratio of 15 at the no observed adverse effect level [NOAEL] of 100 mg/kg/day) was reported, but the findings were found to not represent a significant risk to humans.

In the clinical setting, pancreatitis-related adverse events were reported infrequently with similar incidences across all treatment groups in phase II/III clinical trials. Only a very small number of pancreatic cancer events were reported in vildagliptin and comparator groups (three each), translating into 0.032 cases per 100 SYE vs. 0.046 cases per 100 SYE, respectively. Pancreatitis has also been reported in the post marketing phase, with the majority of cases resolving after drug interruption. In terms of pancreatic cancer, in nine of the 15 cases where time to onset was reported, pancreatic cancer occurred within three months after treatment initiation. This short time does not allow consideration of a direct drug induced neoplasm, although a promoting effect of vildagliptin on preexisting lesions cannot be excluded.
Based on evidence from clinical trials and the post-marketing phase, the product information has been kept up to date and contains wording with regards to pancreatitis as a warning and a listed undesirable effect.
In addition to routine pharmacovigilance, ongoing post-marketing initiatives include a long-term observational study to assess various safety outcomes in association with vildagliptin or the fixed-dose combination of vildagliptin plus metformin, including pancreatic events. A multinational observational study to assess the profile of vildagliptin in a real world setting is also ongoing.

Linagliptin

In non-clinical studies pancreatic morphology was investigated in the mouse, rat, dog and monkey. No consistent findings were obtained, neither in respect to pancreatitis nor in respect to proliferation. Linagliptin did not show a genotoxic potential and did not induce carcinogenic effects in the 2-year carcinogenic mouse study, except for a significant increase in malignant lymphomas in females. This was attributed to a high background of lymphomas in mice. Because linagliptin is not genotoxic and lymphoid hyperplasia in spleen and thymus was not increased in female mice, it was concluded that this finding was not relevant for humans.

Available clinical data from a large number of patients in placebo-controlled clinical trials showed that the incidence of pancreatitis in the linagliptin group is low (0.22 cases per 100 patient years in the linagliptin group vs. 0.07 per 100 patient years in the placebo group; the difference did not reach statistical significance). Cases of pancreatitis and neoplasms are followed up in periodic safety update reports. No conclusions on pancreas carcinoma can be drawn at present due to the low number of cases reported. Based on evidence from clinical trials and the post-marketing phase, the product information has been kept up to date and contains wording with regards to pancreatitis as a warning and a listed undesirable effect.

In addition to routine pharmacovigilance, ongoing post-marketing initiatives include a trial on the evaluation of cardiovascular outcomes with linagliptin therapy which will also collect information with regards to pancreatic events.

2.4 Other initiatives

Ad-hoc expert meeting

An ad-hoc expert meeting was convened on 10 July 2013 on a number of aspects of the *Butler et al* 2013 publication and to inform the CHMP.

Overall the experts considered that there were a high number of methodological issues, confounding factors and potential sources of bias observed in the *Butler et al* 2013 publication and that these precluded any meaningful conclusions to establish a link between the use of GLP-1 based therapies and morphological changes of the pancreas indicating an increased risk of pancreatic malignancies.

With regards to patient selection, the experts considered that the three groups compared in this study (T2DM patients on GLP-1 based therapy, T2DM patients on other or no therapy and the non-diabetic patient controls) were very much mismatched, in particular with regard to age, sex, and to some extent body mass, with all three parameters having variable impact on pancreas findings. Information on previous treatments and the duration of these treatments was also considered to be lacking The mean age of the GLP-1 treated group was 58 years of age, which is significantly higher than the mean age of the non-GLP-1 treated group (40 years) or the control group (45 years), partly due to a number of very young individuals included in the two control groups. The experts agreed that the groups should have been better matched with regard to age through appropriate selection of cases from the nPOD tissue bank. The experts also pointed out that the two diabetic patient groups were mismatched in terms of gender, with the GLP-1 treated group being composed of two females and six males, while the non-GLP-1 group consisting of eight females and four males.

The presence of autoantibody titres (insulin and GAD) in one third of the individuals, a history of diabetic ketoacidosis in one fourth of the T2DM control group and the young age of some individuals in the control groups (18 and 20 in the non-GLP-1 group and 24 and 14 in the n-T2DM group) raised

concerns of a possible misclassification of at least some of these patients as T2DM instead of type 1 diabetes mellitus (T1DM). However, the possibility that all these individuals were indeed T2DM patients was acknowledged, as autoantibodies can be non-specific and ketoacidosis may be observed in some T2DM patients. The experts were of the view that clinical data, including detailed treatment history of the patients, was lacking, although the difficulty in obtaining this data from nPOD due to personal data protection issues was acknowledged.

No concerns were raised regarding the fixation or the embedding and the preservation of the tissues was considered good. However, the experts considered that the substandard staining, the lack of rigorous analysis and the unclear description of the methodological approach raised concerns which could have a major impact on the validity of the conclusions reached by the authors. Issues discussed referred to under-stained and over-stained alpha and beta cells, almost identical compartments within the same islet regions staining positively both for insulin and glucagon, and staining of the acinar area and connective tissue. Consideration should have been given to staining for other types of hormones, such as somatostatin. With regard to sectioning, evidence of a systematic sectioning approach ensuring that samples from all three regions was lacking and variations in sectioning methods and sample selection may have led to biased results. Measuring volume instead of area would have been more adequate with regard to estimation of alpha and beta-cell mass.

The experts considered the results identified in the publication with regard to changes in alpha and beta cell mass and in overall pancreatic mass to be inconclusive, given the uncertainty raised by major study deficiencies regarding the patient selection and the morphometric analysis. Pancreatic weight should have been adjusted for the height, weight, age and gender of the individual donor, according to available algorithms. Changes in the fat content of the pancreas (in particular in obese individuals) should have been considered as a cause for differences in pancreatic weight.

Overall, the experts considered that the presented evidence did not support the view that GLP-1 based therapies resulted in histological changes of the pancreas in these individuals indicating an increased risk of pancreatic adenocarcinoma. No reports of clinical symptoms for glucagonoma were available and it was noted that patients with glucagonoma tend to lose weight due to wasting, rather than being obese, as observed in the GLP-1 group (the three individuals in which the glucagon-positive neuroendocrine tumour and microadenomas were observed had BMI values of 39, 41 and 42 respectively). The presence of cells staining positive for glucagon would also not necessarily indicate secretion of glucagon by these cells. Moreover, the reliability of the staining was considered questionable, as mentioned above. It was noted that glucagonomas are rare tumours with an incidence of approximately one in 200.000, and that given the widespread use of GLP-1 based therapies, any increase in the incidence of clinically relevant glucagonomas should have been noticed by now.

A study by *Kimura et al* (1991) reviewing pancreata from 800 consecutive autopsies, identified endocrine tumours (including microadenomas) and islet hyperplasia in 10 percent of adult patients, with most of these lesions staining positive for glucagon. The study also indicated that the detection of such lesions depends heavily on the level of scrutiny and that significantly more tumours are found when larger numbers of slides are examined. In view of the apparent relatively high prevalence of small clinically asymptomatic endocrine tumours in the general population and the lack of information on the screening methodology use in the Butler study, the experts found the true significance of their finding of three cases with one or more clinically asymptomatic (micro)adenomas difficult to evaluate. More detailed histopathological studies on larger patient groups would be necessary to address this issue.

Discussion

Glucagon-like peptide 1 based therapies [GLP-1 receptor agonists (exenatide, liraglutide and lixisenatide) and DPP-4 inhibitors (sitagliptin, vildagliptin, saxagliptin and linagliptin)] are approved for the treatment of patients with type 2 diabetes mellitus (T2DM).

The efficacy of GLP-1 receptor agonists and DPP-4 inhibitors has been demonstrated. In terms of safety, the most common adverse events seen in clinical trials with GLP-1 receptor agonists are of gastrointestinal character; mainly nausea, vomiting and diarrhoea. However, the incidence diminishes over time. Other identified risks include pancreatitis, immunogenicity, acute renal failure and rapid weight loss. Identified and potential risks with DPP-4 inhibitors include hypoglycaemia, hypersensitivity, gastrointestinal disorders, pancreatitis, skin disorders, transaminase elevation and infections.

The current review was initiated further to the findings by a group of academic researchers suggesting an increased risk of pancreatitis and cellular changes in patients treated for type 2 diabetes mellitus with GLP-1 based therapies (Butler et al, 2013). The findings in this study were based on histological examinations of 34 pancreata obtained from brain dead organ donors. The pancreata of 8 individuals with T2DM who were treated with sitagliptin (n = 7) or exenatide (n = 1) for a year or more were compared to 12 pancreata from individuals with T2DM treated with other therapies and 14 pancreata from non-diabetic individuals. In their publication, the investigators describe a number of findings in the pancreata of the T2DM individuals treated with GLP-1 based therapies which could implicate an association of the treatment with increased risk of pancreatitis and neoplasms.

An ad-hoc expert meeting was held on 10 July 2013 to discuss the publication and inform the CHMP opinion. The CHMP considered, taking into account the experts' opinion, that the comparison between patients with DM with and without incretins was complicated by the fact that those without incretins may not have had type 2 diabetes considering that only three of 10 patients were on metformin (the rest no treatment or insulin). Some patients, in particular the four younger patients on insulin may have had type 1 diabetes, which would have impact on the validity of the comparison of DM patients with and without incretins. In addition, there were substantial differences between the diabetes patients with and without incretins with respect to age, gender and duration of diabetes, factors that are likely to have impact on the pancreatic findings. Thus, it cannot be concluded that differences between the groups are due to the treatment with sitagliptin/exenatide.

In the incretin treated group, there was an increased alpha and beta cell area and mass as well as pancreatic mass compared to the other groups. The authors stated that these findings were consistent with prior rodent studies (Matveyenko, Diabetes 2009, Gier Diabetes 2012) that revealed proliferative actions of GLP-1 on the endocrine and exocrine pancreas, but also that previous reports suggest a wide range of change in alpha and beta cell mass (or pancreatic fractional area) in patients with DM (Rahier, 2008, Diabetes Obes Metab, *Henquin*, 2011 Diabetologia,). Therefore there are uncertainties as to the importance of these findings in the context of what could be expected in patients with type 2 diabetes as well as possible clinical implications. Furthermore, as mentioned above, the difference between the groups with respect to age, gender and duration of diabetes preclude meaningful interpretation of the data.

In one individual, a glucagon expressing neuroendocrine tumour was detected. Further, glucagon-expressing microadenomas were found in three patients while hyperplastic islets with predominant glucagon staining were noted in seven of eight of the incretin treated cases. In relation to these findings, as well as the findings of increased alpha and beta cell area and mass, the authors questioned the safety of long term suppression of glucagon secretion and action and refer to available preclinical

studies indicating an association between suppressed glucagon secretion or signaling and alpha cell hyperplasia, abnormal alpha cell distribution and predisposition to glucagon expressing neuroendocrine tumours. It is agreed that long term suppression of glucagon represents a non-physiological condition. However, as concluded by the ad hoc expert group, according to literature (Kimura et al, 1991, Digestive disease and sciences, vol 36, No 7), microadenomas can be expected to be found in 10% in the general population. Furthermore, a recent publication by *Drucker et al* (Diabetes online July 1st , 2013), reviewed preclinical studies reporting changes in cell numbers in preclinical studies with DPP-4 inhibitors. One of twenty studies described an increase, six studies reported no change and 13 papers described a reduction in alpha-cell number and/or decreased alpha-cell proliferation. Thus, there seems to be limited support for an alpha-cell promoting effect. Concerning the glucagon expressing tumour, the relevance of this case is questioned considering the lack of clinical data as well as unspecific staining reported in the publication.

The CHMP also noted that there was an increased number of endocrine cells in association with duct structures as well as an increase in the presence of pancreatic intraepithelial neoplasia (PanINs). According to the authors, this was consistent with the prior finding that GLP-1 receptors are expressed not only in the human exocrine pancreas but also in PanINs, and that GLP-1 induces proliferative signaling in human pancreatic duct epithelia cells. According to the expert meeting, PANin 1 and 2 are not considered to be prognostic factors for pancreatic cancer, neither for chronic pancreatitis, and more importantly, the incidence of such findings increase with age.

In addition to the Butler publication, the CHMP also considered other evidence from GLP-1 based therapies with regards to pancreatic events. The GLP 1 receptor is expressed in the pancreas, so some effects on the pancreas upon chronic activation of signaling pathways are to be expected. Studies on normal healthy animals did not show any evidence for toxicological action, but for some of the products and particularly in monkeys, there have been findings on increased weight and hypercellularity of the pancreas. While some data show an increase in beta cells, an expected and potentially advantageous effect in the diabetic patient, these data are not conclusive and an effect also on alpha cells and/or cells in the exocrine pancreas cannot be excluded. Importantly, histological examination of the pancreas did not show any evidence for pathological changes associated with the increased pancreas weight/hypercellularity.

In long-term carcinogenicity studies in mice and rats, the pancreas was not a target organ; no findings on pancreatic neoplasia were observed for any of the products. It is also noted that an extensive analysis of pancreata from mice, rats and non-human primates treated with the GLP-1R analog liraglutide for up to 2 years is published, showing that there was no evidence for treatment-related pancreatitis or pre-neoplastic lesions in any of the studies (*Nyborg et al* 2012, Diabetes 61:1243). The safety studies have been performed in healthy animals, and the interaction of the medicinal product and the underlying disease has not been studied. In the development programs for these products, disease models have been used for pharmacological studies. For some of the products three-month pancreatic toxicity studies in the diabetic ZDF rat have been performed post-approval. In these studies performed with liraglutide (*Vrang et al* 2012 Am J Physiol Endocrinol Metab. 15:E253), exenatide (*Tatarkiewicz et al* 2012 Diabetes Obes Metab. 15:417) and sitagliptin there was no evidence for adverse effects in the pancreas.

Other publications have described potentially adverse effects of treatment. In rats carrying a transgene for human islet amyloid polypeptide, a model for type 2 diabetes, 12 weeks of treatment with sitagliptin resulted in increased pancreatic ductal turnover, ductal metaplasia, and in one rat, pancreatitis (*Matveyenko et al* 2009 Diabetes 58:1604). In another study it was found that in normal rats treated with exenatide for 12 weeks, pancreatic duct glands were expanded. Pancreatic duct glands have been hypothesised to give rise to pancreatic intraepithelial neoplasia (PanIN). In

transgenic mice expressing an oncogenic Kras mutant in pancreas, 12 weeks of exenatide treatment increased duct cell replication, increased the formation of dysplastic PanIN lesions, and accelerated the development of chronic pancreatitis (*Gier et al* 2012 Diabetes 61:1250). The relevance of these findings for clinical safety is uncertain.

Nonclinical animal data may aid in determining the causal relationship between GLP-1 based therapy and development of pancreatitis and/or pancreatic cancer by identifying pharmacological mechanisms and biomarkers that can be studied in the clinical setting. If such biomarkers, shown to be directly related to pharmacological activity in the animal studies, could be correlated with pancreatic adverse events in the clinical setting a causal relationship would be strengthened. At this point of time, it is not considered that available non-clinical data support such relationship.

With regards to available clinical data, overall, there have been very few cases of pancreatitis detected in the phase II and phase III studies. Incidence rates were presented for some products ranging between 1.6-2.6 cases per 1000 patient years. For some products (e.g. exenatide, lixisenatide, linagliptin) there was a numerically higher incidence compared to placebo. According to literature data, patients with type 2 diabetes have an almost threefold greater risk of pancreatitis compared to patients without diabetes (*Noel RA*, 2009, *Whitcomb* 2006, *Forsmark CE,* 2007, *Girman CJ*, 2010). The estimated incidence rate for pancreatitis in the diabetes population is 4.2 to 5.6 per 1000 patient years (*Garg et al,* 2010, Diabetes Care 33(11):2349-2354 and *Noel et al.* 2009, Diabetes care 32 (5):834-838). In the post marketing setting, a significant number of pancreatitis cases have been reported and these need to be interpreted cautiously. Cumulative rates of pancreatitis were presented for some products, with a range from 0.1 to 0.9 per 1000 patient years. It should be noted that these numbers come from spontaneous reporting of adverse events and estimations of exposure based on sale figures, respectively, and thus are associated with great uncertainty. For this reason it is recognised that reporting rates cannot be directly compared to the estimated risk in the general population or in the population with T2DM also due to known under reporting. The reporting rates seem to be consistent over time for the products which has been marketed for the longest time (e.g. exenatide BID and vildagliptin). Having said this, severe and also fatal cases have been reported and a causal relationship between treatment and pancreatitis is possible. The CHMP noted that the product information for all products already contains warnings with regards to pancreatitis and this is included in the risk management plans.

Concerning pancreatic cancer, in clinical trials, only single cases have been reported for some products and the duration of exposure was in the majority of the cases too short to support a causal relationship or to draw firm conclusions. The clinical trial setting may not be representative for the “real life” scenario (i.e. patients are older, have more comorbidities, among other factors) but the randomised, controlled nature of the clinical studies gives a robust estimate of risk in relation to placebo and other treatments. The data currently available from clinical trials do not indicate an increased risk for pancreatic cancer with these medicines. In the post-marketing setting, cases of pancreatic cancer have been reported for most products, but in a rather large number of cases there were confounding factors or, in general, too short exposure to suspect a causal relationship with the products. Again, data comparing the rate of spontaneous reporting between different products is to be interpreted with care and should always be assessed in the context of other available information (e.g. cumulative data in the periodic safety update reports and results from clinical studies).

It is noted that marketing authorisation holders are closely monitoring for effects on the pancreas. Several initiatives are planned or ongoing which will collect information on pancreatic events, and the potential value of additional studies will also be considered. In particular, cardiovascular outcome studies are ongoing for most products. For some of these studies pancreatitis and neoplasms are listed as adverse events of special interest and/or are adjudicated. The number of subjects planned to be

included ranges between 6000 and 16000 patients and the studies are expected to be finalised in 2015-2017. Results from post-marketing database/registries studies with regards to pancreatic safety will also be considered when available. The data so far has been limited and does not allow conclusions to be drawn.

3. Overall conclusion

The current review under article 5(3) was initiated following the publication by *Butler at al*, 2013 suggesting that histological findings in human pancreata could indicate a possibly increased risk of pancreatic adverse events associated with the use of GLP 1 based therapies.

The CHMP reviewed the publication and considered that differences between the studied groups (diabetes with and without incretins and non-diabetic controls) with respect to age, gender, duration of diabetes and treatments as well as other methodological issues preclude meaningful interpretation of the data. This conclusion was supported by an ad-hoc expert meeting held on 10 July 2013.

Within the procedure, the CHMP was also requested to take other available data into account and a review of submitted clinical and nonclinical data was performed.

With respect to nonclinical data, available studies previously submitted for the approved products have not raised concern with respect to pancreatic safety. Further, published studies have not shown any evidence for treatment-related pancreatitis or preneoplastic lesions, neither in pancreata from healthy mice, rats and nonhuman primates nor in diabetic ZDF rat models. However, studies performed in some other disease models by academic groups may give some plausibility with respect to a possible mechanism for an increased risk of pancreatitis and pancreatic cancer in patients treated with GLP-1 based therapies.

Concerning pancreatitis, the cases in the clinical studies were few. However, when looking at the clinical studies in totality and taking post marketing reports into account, a significant number of cases have been observed and a causal relationship between GLP-1 based therapy treatment and pancreatitis is possible. Warnings are already included in the product information for all products, albeit with small differences in the wording, and pancreatitis is being followed in the periodic safety update reports as well as in observational and randomised clinical trials. These actions are considered as sufficient and no new data has emerged that implies that this risk is higher compared to what has previously been concluded. However, with the next updates of the risk management plans, pancreatitis, which should be already mentioned in the risk management plans as a potential risk should be listed as an identified risk for all products and it would be appropriate to harmonize the wording of the warning with respect to a recommendation to use the products with caution in patients with a history of pancreatitis as well as a recommendation not to resume treatment if pancreatitis has occurred.

Concerning pancreatic cancer, there is currently no support from clinical trials that GLP-1 based therapies increase the risk. The numbers of spontaneous reports are limited and in the cases were information is available, confounding factors and/or short-term exposure is common. However, long term consequences of stimulation of beta-cells and suppression of alpha cells as well as possible effects on exocrine pancreas are largely unknown and therefore some uncertainties exist. Considering that pancreatic cancers are very rare, large populations would need to be studied for a substantial duration to detect a possible increased risk. Observational studies have so far not been able to detect enough cases probably due to the rarity of the condition and, at least in Europe, rather low uptake of the products.

Additional information will be captured in the ongoing cardiovascular outcome studies. Six studies including a large number of patients are ongoing and it is expected that important information can be

collected. The marketing authorisation holders should be requested to confirm that the protocols explicitly include “pancreatic malignancies/neoplasms” as an adverse event of specific interest since this might lead to increased awareness and reporting of this specific type of malignancies/neoplasms. Efforts should be made to capture pancreatic events in a similar way in the studies in order to enable a pooled analysis and consideration should be given to yearly interim reports with respect to pancreatic events (pancreatitis and pancreatic cancer). Furthermore, pancreatic cancer must be included as a potential risk for all products for which it is not already reflected in the risk management plans. Considering the low incidence of pancreatic cancer, results from the ongoing observational studies will also be of importance and therefore marketing authorisation holders should ensure that pancreatic safety is adequately captured in these studies. Other epidemiological approaches to studying this potential risk could also be considered, if appropriate.

Should new evidence indicate an increased risk of pancreatic cancer and/or a higher risk of pancreatitis compared to current estimations (e.g. from clinical studies and periodic safety update reports), the benefit-risk balance of GLP-1 based therapies should be re-evaluated. However, this should be done in a product specific manner considering that the magnitude of the benefits and risk of the products differ with respect to glucose and weight lowering capacity as well as the incidence of gastrointestinal and immunological adverse events. Furthermore, should there be an increased risk of pancreatic adverse events it is not evident that the risk is of the same magnitude for all products considering differences in mechanism of action (i.e. GLP-1 receptor agonists versus DPP-4 inhibitors) and exposure (intermittent versus continuous exposure).

In conclusion, the results of the study by *Butler et al* are not considered to constitute a new safety signal for the GLP 1 based therapies with respect to pancreatic safety. This is further supported by the review of available preclinical and clinical data.

However, due to the mechanism of action, there are still some uncertainties with respect to long term pancreatic safety associated with these products and updates to the risk management plans (including planned and ongoing studies) and harmonisation of warnings in the product information should be taken forward.