Analogues of xenin-25 as promising anti-diabetic therapies
An on going Proof of Concept funded study has led to the preclinical development of novel, stable analogues based on the native peptide Xenin-25, a K-cell derived gut peptide that is co-secreted with glucose-dependent insulino tropic polypeptide (GIP) following a meal. Data generated to date has shown xenin analogues possess enhanced stability, exhibiting improved glucose lowering and insulin-releasing actions across a range of diabetic animal models.

Ulster’s lead analogue, Xenin-25-Gln, has recently been assessed in a chronic (21d), head-to-head study in high-fat-fed mice compared to exendin-4. Studies have revealed Xenin-25-Gln reduces non-fasting blood glucose, improves glucose tolerance and GIP sensitivity. In addition, triglyceride levels were significantly reduced suggesting an improved lipid profile and preliminary assessment of tissue weights, suggest no adverse effects.

**BACKGROUND AND SIGNIFICANCE:**

- Therapeutic peptides are one of the most attractive and emerging therapies currently being developed for Type 2 Diabetes (T2D) due to their numerous advantages over small molecules and/or biologics which include high potency, low toxicity, high specificity, low risk of organ accumulation and drug-drug interaction.
- Xenin, is a naturally occurring, 25 amino-acid peptide originally isolated from human gastric mucosa with known effects on insulin secretion, glycaemic control and satiety.
- Native xenin is currently being assessed for its anti-diabetic actions in an on going clinical trial, sponsored by the Washington University School of Medicine. Despite these early efforts, however it is anticipated that native xenin (like other naturally-occurring peptides such as the incretin mimetics GLP-1 and GIP), will be degraded in the circulation therefore making use of the native form as a new therapeutic agent impossible.
- As a result, Researchers at Ulster University conducted a series of studies assessing the metabolic stability of native xenin against DPP-IV and murine plasma, confirmed rapid enzymatic degradation, identified several key target amino acid residues for substitution and generated a series of modified peptide analogues that are stable, longer-acting and more efficacious than native xenin both in vitro and in vivo.
- Under a recently funded Proof of Concept study, Ulster’s lead analogues including Xenin-25-Gln (K and R substituted with Q) and Xenin-25-[Lys$^{13}\text{Pal}$] (C-16 palmitate linked to $\varepsilon$-NH$_2$ group of K$^{13}$) were evaluated in a comprehensive series of preclinical studies to assess their anti-diabetic potential. Select data for lead analogues from three chronic studies in high fat fed and diabetic (ob/ob) mouse models is presented below. Further data is available upon request.

**COMPETITIVE ADVANTAGES:**

- Xenin offers a new mechanistic approach for diabetes treatment based upon an enzyme-resistant analogue of the endogenous peptide xenin-25, that possesses independent insulino tropic and glucose-lowering actions.
- Xenin analogues are designed to be stable and resistant to degradation and have demonstrated improved efficacy over the native peptide with minimal risk of hypoglycaemia.
- With Xenin, there is also the potential for synergistic effects when combined with other incretin treatments as well as oral antidiabetic therapies.
- Xenin shows promise in potentiating the actin of GIP. In preliminary studies, xenin analogues have been shown to potentiate the biological efficacy of the naturally-occurring anti-diabetic hormone GIP which is severely compromised in diabetes. Thus xenin analogues have potential to overcome metabolic defects associated with diabetes and the deficit in GIP action thereby improving overall metabolic control in this disease.
- Lead analogues exhibit comparable efficacy to that of the marketed drug Byetta® (exendin-4), are weight neutral, and provide for an improved CV profile, all of which make this novel class of drug promising as a potential treatment for Type 2 Diabetes offering an additional step to delay the introduction of insulin.
LONG TERM IN VIVO STUDY ASSESSING LEAD COMPOUNDS IN HIGH FAT FED MICE

Following a comprehensive series of in vitro and acute in vivo testing, high fat fed (HFF) mice with diet induced insulin resistance and obesity were used to assess the chronic effects of lead compounds, Xenin-25-Gln and Xenin-25-[Lys^{13}Pal]. Peptides were administered twice daily at 25nmol/kg body weight for 21 days.

![Graph A](image1.png)

**Figure 1.** Effects of stable Xenin analogues on (A) glucose tolerance and (B) insulin sensitivity in high fat fed mice.

As shown above in Figure 1, both peptides improve glucose tolerance and insulin sensitivity. In addition, analogues were shown to normalize islet morphology, returning pancreatic islet beta- and alpha-cell area towards lean control levels (Figure 2) and restoring the alpha cell mantle in the same manner (Figure 3).

![Graph B](image2.png)

**Figure 2.** Effects of stable Xenin analogues on (A) alpha cell area and (B) beta cell area in high fat fed mice.
Figure 3. Representative micrographs of islets showing insulin (Red) / glucagon (Green) staining in pancreas of high fat fed mice treated with Xenin-25-Gln, Xenin-25-[Lys^{13}Pal], compared with HF saline and lean control.

The above data have demonstrated that, in HFF mice, twice daily administration of stable analogues improve insulin sensitivity, enhance glucose tolerance, and help to restore normal islet architecture in the beta cell.

LONG TERM IN VIVO STUDY ASSESSING LEAD COMPOUNDS IN OBESE DIABETIC OB/OB MICE
To further validate our approach, lead analogues were assessed in a second animal model, namely the genetically induced (ob/ob) diabetic mouse model.

As shown in Figure 4, twice daily administration of Xenin-25-Gln improved glucose tolerance and significantly augmented glucose-induced insulin release, whereas, Xenin-25-[Lys^{13}Pal] did not show any significant improvement.

Figure 4. Effects of stable Xenin analogues on (A) glucose tolerance and (B) insulin sensitivity in (ob/ob) mice.

CHRONIC (21D) HEAD-TO-HEAD IN VIVO STUDY ASSESSING XENIN-25-GILN IN HFF MICE COMPARED TO EXENDIN-4
Following on from the above two long-term studies, Xenin-25 Gln was chosen as the lead candidate to be assessed head-to-head in a final chronic study in high fat fed mice compared with Exendin-4. As shown in Figure 5, Xenin-25-Gln significantly decreased circulating glucose levels and reduced HbA_{1c}. In addition, the peptide was shown to significantly improve glucose tolerance, response to GIP as well as blood triglyceride levels (Figures 6 and 7).
Figure 5. Effects of Xenin-25-Gln on (A) blood glucose and (B) HbA1c in high fat fed mice compared to Exendin-4.

Figure 6. Effects of Xenin-25-Gln on (A) glucose tolerance and (B) GIP sensitivity in high fat fed mice compared to Exendin-4.

Figure 7. Effects of Xenin-25-Gln on triglyceride levels in high fat fed mice compared to Exendin-4.
XENIN AND GIP: POSSIBLE ADDITIVE EFFECTS.
Preliminary research has shown that xenin and analogues have potential to provide additive/potentiating effects when combined with analogues of GIP. As shown in Figure 8 below, administration of Xenin-25 and Xenin-[Lys\textsuperscript{13}Pal] has shown potential to provide for additive/potentiating effects on GIP biological activity both in vitro in clonal pancreatic beta cells as well as in vivo in (ob/ob) mice. While further research is needed, these early studies suggest there is potential for synergistic effects when combined with other incretin treatments.

Figure 8. Effects of Xenin peptides combined with GIP analogue (D-Ala\textsuperscript{2})GIP on insulin secretion in BRIN-BD11 cells and plasma glucose in vivo in an (ob/ob) mouse model.

CONCLUSION:
With current therapies, glycaemic control remains suboptimal and patients often experience weight gain and hypoglycaemia thereby necessitating the need to develop new therapeutic agents to tackle T2DM. In general it is necessary to address insulin resistance and insulin deficiency in the patient and advances in diabetes research have revealed the importance of incretin hormones in maintaining glucose control and patients with T2DM will usually exhibit impaired incretin activity, including impaired secretion of glucagon-like peptide-1 (GLP-1), accelerated metabolism of GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) and defective responsiveness to both hormones.

Recent studies in our laboratory have shown that the relatively unexplored regulatory hormone, xenin, plays a role in enhancing K-cell mediated regulators of insulin secretion. Thus, in acute studies xenin stimulates insulin secretion and lowers plasma glucose concentrations. However, as with a number of naturally-occurring peptides, including the successful GLP-1 compounds currently on the market (e.g. Victoza® and Byetta®), xenin is degraded by circulating enzymes and peptidases therefore making use of the native peptide impractical as a therapeutic.

Proof of Concept studies using these novel analogues has demonstrated that Ulster’s lead candidate, Xenin-25-Gln, has the potential to specifically target the pancreatic beta-cell and restore normal islet architecture, stimulate insulin secretion and lower hyperglycaemia when administered chronically in an animal model of T2DM. Xenin-25-Gln has been shown to reduce non-fasting blood glucose, improves glucose tolerance, GIP sensitivity and triglyceride levels when administered twice daily at 25nmol/kg body weight in high fat fed mice for 21 days.
Ulster’s novel approach using xenin analogues has the potential to revolutionize the industry by providing industry partners with a novel therapeutic opportunity, in particular, those who are interested in investing in new mechanisms of action in order to gain a market share in the space of peptide therapeutics alongside or above the likes of Victoza® and Byetta®.

INVENTORS:
Prof Peter R Flatt (Co-Inventor) is Head of the Diabetes Research Group (DRG) at Ulster and leads a team of over 20 scientists with a strong record of scientific achievement. He has published more than 350 scientific papers, established extensive international networks and served various organizational bodies dealing with diabetes over the past 30 years.

Professor Victor Gaul (Co-Inventor) is a Professor of Experimental Medicine at Ulster University within the DRG and is a Distinguished Research Fellow of Ulster University, a Fellow of the Higher Education Academy and recipient of various external awards for his research. Dr Gault has 15 years’ experience in the field of diabetes research with specific expertise in brain-gut peptides and in particular incretin hormones. He has published 68 full scientific peer-reviewed papers and presented at numerous national and international diabetes congresses and invited symposia (75 refereed abstract communications).

Dr Nigel Irwin (Co-Inventor) is a Lecturer at Ulster University within the DRG and a member of Medefield Healthcare Advisory Board and consultant for Health Advances, LLC. Dr Irwin has 12 years’ experience in the field of diabetes research with specific expertise in incretin hormone action. He has published 63 full scientific peer-reviewed papers and presented at numerous international diabetes conferences. As such, Dr Irwin has extensive experience in intestinal/pancreatic endocrinology and advancement of the understanding of incretin physiology.


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