Development and characterization of a humanized GLP-1 receptor mouse model for translational drug development

Nina Sonne[1], Michael Feigh[1], Micaela Roque[1], Line Fisker Zachariassen[1], Trine Porsgaard[1], Susanne E Pors[1], Michele Cavalera[1], Christopher Jones[2], Olivia Osborn[2], Ditte Dencker Thorbek[1], Malene Lundgaard Riis[1], Kevin Quinn[3], Ibrahim Gaidarov[3], Todd Anthony[3], Henrik H Hansen[1], Marco Tozzi[1]

[1]Gubra, Hørsholm Kongevej 11B, Hørsholm, Denmark

[2]Terns Pharmaceutical, 1065 East Hillsdale Blvd., Suite 100, Foster City, CA

[3]Eurofins Beacon Discovery, 6118 Nancy Ridge Dr, San Diego, California, USA

Corresponding author
Marco Tozzi, mto@gubra.dk

Background & Aim

Injectable peptide-based GLP-1 receptor agonists (GLP1RAs) are effective treatments for obesity and type 2 diabetes, yet present challenges related to patient compliance and scalable production. In contrast, small-molecule GLP1RAs offer significant advantages, including oral bioavailability and scalable manufacturing, but their limited efficacy on rodent GLP-1 receptors poses a significant barrier to preclinical testing and translational research. To address this challenge, we generated a new humanized GLP-1 receptor mouse model.

Methods

The Gubra humanized GLP1R mouse (hGLP1R) model was generated on a C57BL/6NJ background by replacing the murine GLP1R with human GLP1R using CRISPR-Cas9 gene editing, starting from G27 (exon 2). Wild-type (WT) C57BL/6NJ mice served as controls.

Selective expression of human GLP1R was validated by immunohistochemistry using species-specific antibodies targeting both mouse and human GLP-1 receptors. Pharmacological validation was performed using semaglutide (peptide GLP1RA, SC dosing) and orforglipron (non-peptide/small molecule GLP1RA, PO dosing). In lean hGLP1R mice, study endpoints included food intake, glucose tolerance, conditioned taste aversion (CTA), and 3D whole-brain c-Fos imaging for mapping brain activation signatures. Effect of 4 weeks treatment on metabolic parameters was evaluated in diet-induced obese (DIO) hGLP1R mice.

1 Generation of humanized GLP1R mice

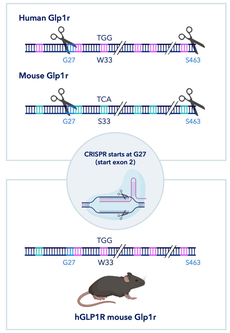

Figure 1. Generation of humanized GLP1R mice. The humanized GLP1R mouse model was generated on a C57BL/6NJ background using CRISPR-Cas9 technology, replacing the endogenous murine GLP-1 receptor (mGLP1R) with the human receptor (hGLP1R) starting from G27 (exon 2) at whole-body level.

2 GLP1R expression in brain and pancreas

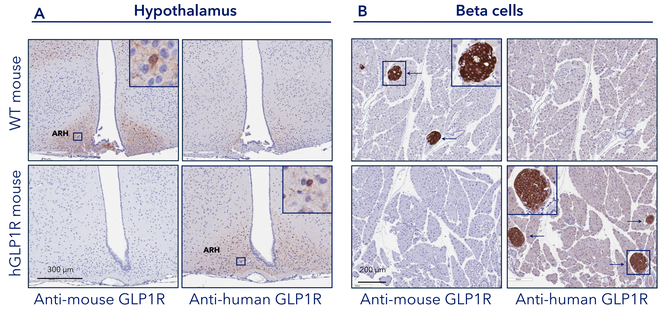

Figure 2. Humanized GLP1R mice only express hGLP1R. Anti-mouse and anti-human GLP1R antibodies were used to profile GLP1R expression in **(A)** hypothalamus (ARH, arcuate hypothalamic nucleus) and **(B)** pancreas of hGLP1R mice and WT littermate control mice. Arrows indicate beta-islets.

3 Acute food intake and conditioned taste aversion

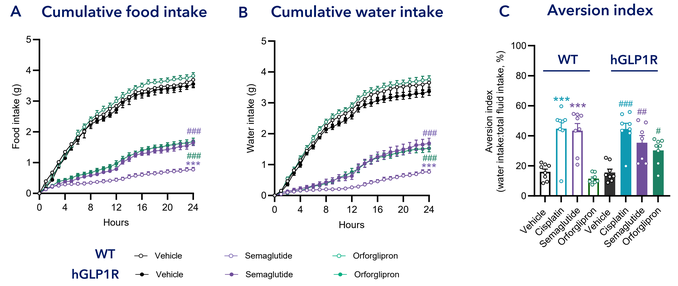

Figure 3. Both semaglutide and orforglipron reduce weight, food intake and induce conditioned taste aversion in hGLP1R mice. (A) Body weight change after single-dosing (% of baseline). **(B)** Cumulative food intake over 24h. **(C)** Condition taste aversion (CTA) was tested in a separate study using cisplatin (3 mg/kg) as positive control. The aversion index was calculated at 72-96h post-dosing. Data are expressed as mean of n=10-12 (panels A and B) and n=8 ± SEM (panel C). ***p<0.001 compared to WT Vehicle; #p<0.05, ##p<0.01, ###p<0.001 compared to hGLP1R Vehicle (Dunnett's test one-factor linear model, only last data point statistically analysed in panel B).

4 Glycemic control

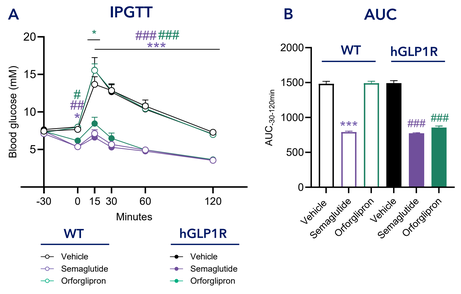

Figure 4. Both semaglutide and orforglipron improve glucose tolerance in lean hGLP1R mice. (A) Intraperitoneal glucose tolerance test (IPGTT). Data are expressed as mean of n=7-9 ± SEM. *p<0.05, ***p<0.001 compared to WT Vehicle; #p<0.05, ##p<0.01, ###p<0.001 compared to hGLP1R Vehicle (Dunnett's test two-factor linear model with interaction). **(B)** Glucose area under the curve (AUC). Data are expressed as mean of n=7-9 ± SEM. ***p<0.001 compared to WT Vehicle; ###p<0.001 compared to hGLP1R Vehicle (Dunnett's test one-factor linear model).

5 Central c-fos activation

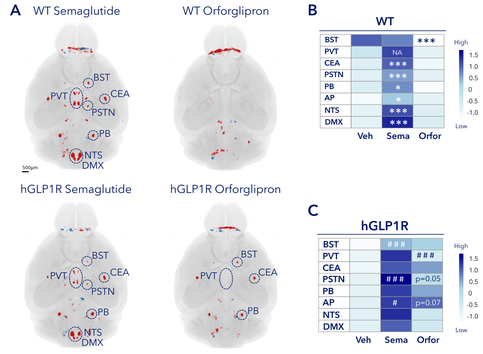

Figure 6. Single dose orforglipron and semaglutide recruit appetite-regulating brain regions. (A) Vehicle-subtracted average c-Fos expression (significant change in c-Fos+ cell counts vs. vehicle controls are indicated in red (increased) or blue (decreased)). Summary of c-Fos responses in **(B)** WT and **(C)** hGLP1R mice (scaled expression). *p<0.05, **p<0.01, ***p<0.001, compared to WT; #p<0.05, ##p<0.01, ###p<0.001 compared to hGLP1R Vehicle. Dunnett's test negative binomial generalized linear model, FDR<0.05 for p-value adjustment) AP, area postrema; BST, bed nuclei of the stria terminalis; CEA, central amygdalar nucleus; DMX, dorsal motor nucleus of the vagus nerve; NTS, nucleus of the solitary tract; PB, parabrachial nucleus; PSTN, parasubthalamic nucleus; PVT, paraventricular nucleus of the thalamus.

6 Chronic metabolic and biochemical parameters in DIO hGLP1R mice

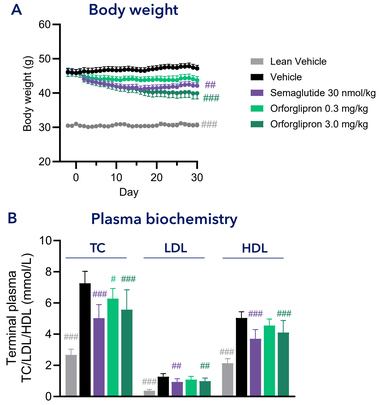

Figure 5. Both semaglutide and orforglipron reduce body weight and plasma biochemistry parameters in hGLP1R DIO mice. (A) Body weight (g) and **(B)** terminal plasma cholesterol (TC), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) following four weeks of treatment (QD). Data are expressed as mean of n=10 ± SEM. #p<0.05, ##p<0.01, ###p<0.001 compared to hGLP1R Vehicle (Dunnett's test one-factor linear model (only last data point was statistically evaluated in A)).

Conclusion

- CRISPR-Cas9 gene editing was used to generate a new humanized GLP1R (hGLP1R) mouse model.
- Immunohistochemical analysis confirmed selective expression of human GLP1R.
- Orforglipron was inactive in WT mice but effective in hGLP1R mice.
- Semaglutide and orforglipron reduced body weight and food intake in hGLP1R mice.
- Both compounds activated canonical appetite-regulating brain regions.
- The Gubra hGLP1R mouse enables preclinical evaluation of small-molecule GLP1R agonists targeting obesity and associated metabolic disorders.

Scan the QR code to download poster.