

Species Differences in GLP-1 Receptor in Thyroid Tissue

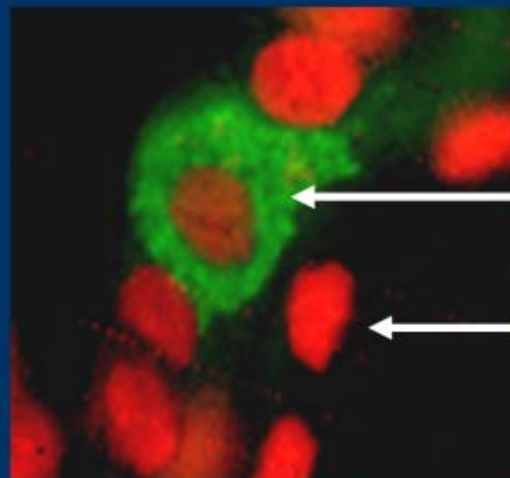
- Markedly lower GLP1 receptor in humans and Non-human primates than in rodents

Technique	Mouse	Rat	NHP	Human
Immunohistochemistry	+	+	+	+
<i>In situ</i> ligand binding	NA	+	NA	-
<i>In situ</i> hybridization	+	+	-	-
RT-PCR	++	+++	NA	+

NHP: Non-human Primate; RT-PCR: reverse transcriptase polymerase chain reaction; NA: Not assessed

No GLP-1R in Human Thyroid - Including Follicular Tumors

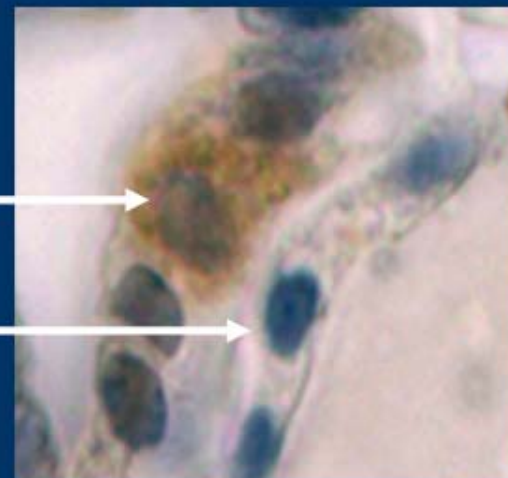
- No GLP-1R expression in follicular cells
 - Human thyroid follicular tumors investigated for GLP-1R
 - Both follicular adenomas and carcinomas examined (N=10)
 - Immunohistochemical double-staining for GLP-1R and calcitonin
- GLP-1R expression only seen in C-cells
 - Consistent with findings in normal thyroid



Green: calcitonin ; Orange: cell nucleus

GLP-1R positive
C-cells

GLP-1R negative
follicular cells



Brown: GLP-1 receptor; Blue: cell nucleus

GLP-1 Receptor Expression in Human Tumors

Tumor (cancer)	Expression (GLP-1 receptor incidence*)	Tumor seen in liraglutide Clinical studies
Phaeochromocytoma	12/20 (60%)	No
Medullary thyroid carcinoma	5/18 (28%)	No
Meningioma	7/20 (35%)	No
Ovarian	2/12 (16%)	No
Prostate	1/20 (5%)	Yes
Breast	0/22 (0%)	Yes
Colorectal	0/21 (0%)	Yes
Gastric	0/20 (0%)	Yes
Renal cell carcinoma	0/20 (0%)	Yes

*Percentage of samples expressing the GLP-1 receptor

Proportion of Subjects with SMQ MACE (Broad) – Population B

Trials	Liraglutide N (%)	Total Comparator N (%)
Total	69 (1.62)	45 (1.89)
LEAD 1–5	49 (1.96)	31 (2.10)
Other Trials	20 (1.14)	14 (1.55)

Trials	Liraglutide 0.6 mg N (%)	Liraglutide 1.2 mg N (%)	Liraglutide 1.8 mg N (%)	Placebo N (%)	Active N (%)
LEAD 1–5	12 (2.53)	19 (2.12)	18 (1.59)	8 (1.53)	23 (2.41)
1573-ext	-	5 (1.99)	4 (1.63)	-	3 (1.21)
1572-ext	9 (3.72)	10 (4.17)	3 (1.24)	1 (0.83)	9 (3.72)
1436	3 (1.29)	3 (1.32)	4 (1.71)	3 (2.63)	5 (2.16)
1574	-	1 (0.56)	2 (1.12)	3 (1.71)	-
1697	-	-	5 (2.17)	1 (0.88)	6 (2.59)

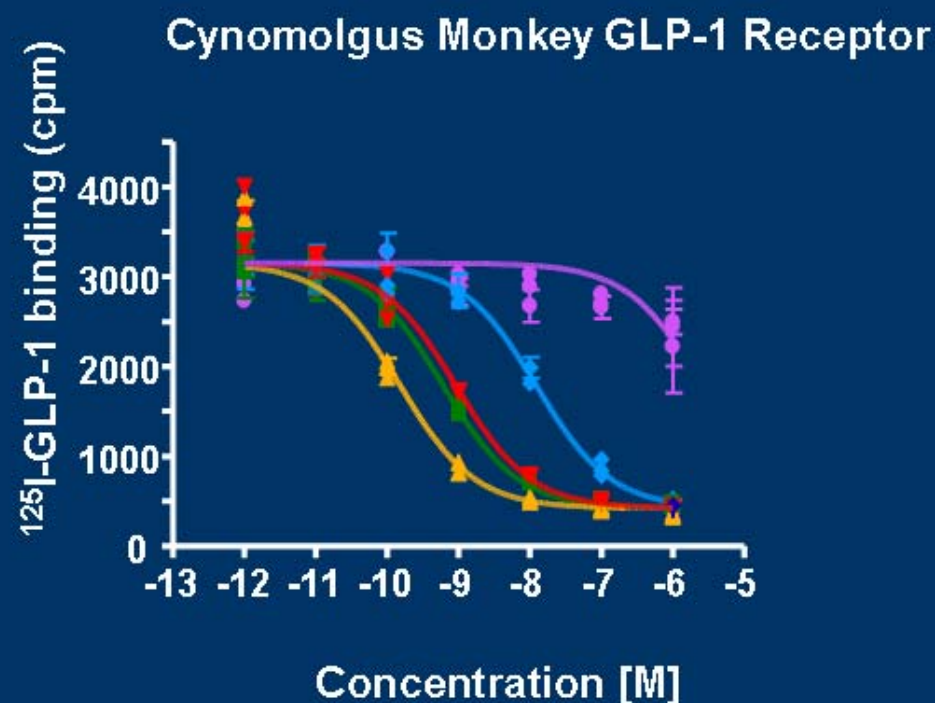
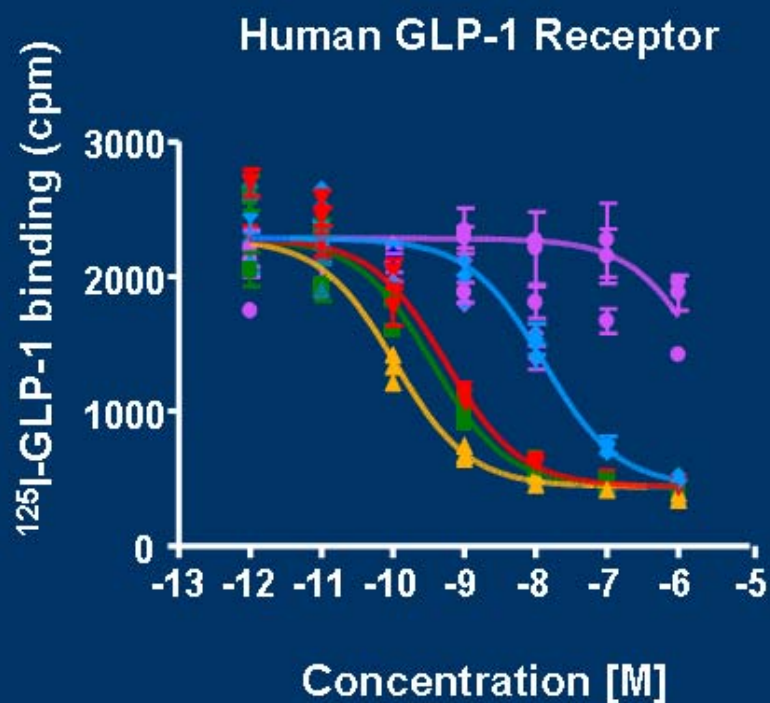
Receptor Specificity – Peptide Receptors

- No binding of liraglutide to peptide receptors other than GLP-1R

Angiotensin-II	Bombesin	Bradykinin	Calcitonin	CGRP	Chemokines	CCK
AT1	non-selective	B1	CT	non-selective	CCR1	CCK1
AT2	BB1	B2			CXCR2 (IL-8B)	CCK23
	BB2					
	BB3					
Cytokines	Endothelin	Galanin	Glucagon	Insulin	Melanocortin	Neurokinin
TNF-alpha	ET1	GAL1			MC4	NK1
	ET2	GAL2				NK2
						NK3
Neuromedin-U	NPY	Neurotensin	Opioid/-like	Somatostatin	VIP	Vasopressin
NMU1	Y1	NT1	DOP	non-selective	PAC1	V1a
NMU2	Y2		KOP		VPAC1	
			MOP		VPAC2	
			ORL1			

Human and Cynomolgus GLP-1 Receptor Binding

■ Human GLP-1 ▲ Liraglutide ▼ Exenatide ◆ Exendin-4 (9-39) ● Glucagon



Radioligand GLP-1 receptor binding

Rodent Carcinogens Among Pharmaceuticals

- A large proportion of marketed pharmaceuticals carcinogenic in rodents

n=179-329*	% of compounds			
	Carcinogenic in	Positive	Negative	Discordant
Mice		27	66	7
Rats		30	63	7
Both species		15	51	34

*Not all compounds tested in both species
Data from Brambilla & Martelli, 2008

Concomitant Treatment with Statin And/Or Fibrate at Baseline – Long-term Trials

	Total liraglutide N (%)	Placebo N (%)	Active comparator N (%)
Total n	2501	524	953
Statin* (%)	1119 (44.7)	259 (49.4)	373 (39.1)
Fibrate** (%)	196 (7.8)	54 (10.3)	84 (8.8)
Statin or fibrate (%)	1245 (49.8)	286 (54.6)	435 (45.6)
Statin and fibrate (%)	70 (2.8)	27 (5.2)	22 (2.3)

*ATC: C10AA

**ATC: C10AB

Hyperlipidemia* at Baseline

	Total liraglutide N (%)	Placebo N (%)	Active comparator N (%)
Total n	2501	524	953
Hyperlipidemia*	1404 (56.1)	325 (62.0)	489 (51.3)
Lipid-lowering treatment or hyperlipidemia	1537 (61.5)	355 (67.7)	548 (57.5)

*Hyperlipidemia defined as following PTs: dyslipidaemia, hypercholesterolaemia, hypertriglyceridaemia, lipid metabolism disorder, blood cholesterol increased, blood triglycerides increased, lipids abnormal, lipids increased

Patients with Cardiovascular Disease or Diabetes Duration \geq 10 years or Age \geq 65 Years or Creatinine Clearance $<$ 60 ml/min

	Total liraglutide	Total comparator
Total N	4257	2381
N (%)	1737 (40.8)	1045 (43.9)

All intermediate and long-term trials

C-cell Hyperplasia in Mice Dosed Exenatide or Liraglutide for 12 -13 Weeks

	Method of administration	% animals with focal C-cell hyperplasia			
		0	0.25	1	5
Exenatide	Continuous infusion – daily dose (mg/kg)	0	0.25	1	
		3	31	54	
Liraglutide	Once daily (mg/kg)	0	0.2	1	5
		0	36	38	48

Liraglutide N=46-48 animals/group; exenatide N=36-37 animals/group

Mouse Carcinogenicity Study – Injection Site and Microchips



■ Microchip implanted between the shoulder blades

— Injection areas, used every second day

— Dorsal skin surface