Victoza® (liraglutide injection): Human Relevance of Rodent Thyroid C-cell Tumors

Novo Nordisk A/S
NDA 22-341

Endocrinologic and Metabolic Drugs Advisory Committee Meeting

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Center for Drug Evaluation and Research

Presentation Overview

- Thyroid C-cell tumors in 2-year rodent carcinogenicity studies of liraglutide (LGT)
- Applicant's proposed mode of action (MOA) for LGT-induced C-cell tumors in rodents
- Evaluation of the MOA
- Relevance of rodent C-cell tumors to human risk

Thyroid C-cell Tumors in Liraglutide Rodent Carcinogenicity Studies

Liraglutide Carcinogenicity in Rodents

- Carcinogenicity evaluated in 2-year lifetime exposure studies in Sprague Dawley rats and CD-1 mice
  - Thyroid C-cell tumors in rats and mice occurred at clinically relevant exposures
  - Fibrosarcomas on the dorsal skin and subcutis (body surface used for drug injection) in male mice at high multiples of human exposure

Liraglutide Carcinogenicity in Rats

Incidence (%) of Thyroid C-cell Findings in SD Rats

Sex	Male				Female			
LGT Dose (mg/kg)	0	0.075	0.25	0.75	0	0.075	0.25	0.75
Multiple of Human AUC	-	0.5	2	8	-	0.5	2	8
Focal Hyperplasia	22	29	40	48*	28	28	55*	48
Adenoma (B)	12	16	42*	46*	10	27*	33*	56*
Carcinoma (M)	2	8	6	14*	0	0	4.1	6
Total Tumors	14	22	42*	56*	10	27	37*	58*

Underlined: Exceeds historical control background incidence maximum for adenomas (21.1% M, 16.0% F), carcinomas (2.1% M, 4.0%, F), and focal hyperplasia (14.3% M, 20.0% F)
*SS different from controls.
Human exposure multiple based on $AUC_{0\text{-}24}$ 816 nM.hr from a clinical dose of 1.8 mg/day
N = 49 – 50 examined/group

Liraglutide Carcinogenicity in Mice

Incidence (%) of Thyroid C-cell Findings in CD-1 Mice

Sex	Male					Female				
LGT Dose (mg/kg)	0	0.03	0.2	1	3	0	0.03	0.2	1	3
Multiple of Human AUC	-	0.2	2	10	45	-	0.2	2	10	45
Focal Hyperplasia	0	0	1.5	16*	38*	0	0	10*	15*	29*
Adenoma (B)	0	0	0	13*	19*	0	0	0	6*	20*
Carcinoma (M)	0	0	0	0	0	0	0	0	0	2.6*
Total Tumors	-	-	-	-	-	0	0	0	6*	22*

Underlined: Exceeds historical control group maximum for adenomas or carcinomas (0% M, F) and focal hyperplasia (0% M, 0.9% F)

* SS different from controls ($p < 0.05$)

Human exposure multiple based on AUC_{0-24} 816 nM.hr from a clinical dose of 1.8 mg/day

N = 75 - 79 examined/ control & HD, 65 – 67 examined / LD & MD groups

Rodent Carcinogenicity Studies of Liraglutide

- LGT is a nongenotoxic, multi-sex, multi-species carcinogen in rodents

- C-cell tumors occur at low multiples of human exposure
  - < 1X in rats
  - ≥ 10X in mice (≥ 10X for adenomas, 45X for carcinomas in females)

Proliferative C-cell Lesions in Mice, Rats, & Humans

C-cell Proliferation	*Mice*	*Rats*	*Humans*	*Human Familial MTC*
Diffuse Hyperplasia	Rare	Common ↑ w/ age	Rare	Common
Focal Hyperplasia	Rare	Common ↑ w/ age	Rare	Common (nodular)
Adenoma (benign)	Rare	Common ↑ w/ age	Rare	Common (MTC in situ?)
Carcinoma (malignant)	Rare	Rare	Rare	Common

Rats are considered a model of human MTC, but the molecular pathology is different (activating RET mutations in humans, but not in rats)

Liraglutide and Approved Drugs Causing Rodent Thyroid C-cell Tumors

Drug	Pharmacologic Class	Rats		Mice	
		Adenoma	Carcinoma	Adenoma	Carcinoma
liraglutide[A]	GLP-1 agonist	2X (M), < 1X (F)	< 1X (M), 2X (F)	10X (M, F)	45X (F)
		< 1X (M, F) combined tumors		10X (F) combined tumors	
Approved Drugs					
exenatide[A]	GLP-1 agonist	< 5X (F)	-	-	-
alendronate[B]	bisphosphonate, osteoclast inhibitor	1X (M)	-	-	-
arformoterol[A]	β_2 agonist	130X (F) combined tumors		-	-
atenolol[W]	β_2 antagonist	-	250X (M)	-	-
colesevalam[W]	Bile acid sequestrant	40X (F)	-	-	-
naratriptan[A]	5-$HT_{1D/1B}$ agonist	180X (M, F)	-	-	-
palonosetron[A]	5-HT_3 antagonist	182X (F)	-	-	-
		182X (F) combined tumors			

Multiple of human exposure at the LOAEL based on plasma AUC([A]), body surface area-based dose comparison ([B]), or weight-based dose comparison ([W])

Liraglutide Causes C-cell Tumors in Rats and Mice

No approved drug caused C-cell tumors in mice
- Drug Facts and Comparison online 2009

- Rodent thyroid C-cell tumors may be a pharmacologic class effect of long acting GLP-1 receptor agonists
  - No other investigational drugs cause C-cell tumors in mice (CDER document databases; DARRTS, DFS)
  - Mechanistic studies using SC infused exenatide performed by Novo Nordisk showed it cause focal CCH in mice
  - Recent final and interim toxicity study reports of other long acting GLP-1 receptor agonists show they cause focal CCH or tumors in mice and C-cell tumors in rats

- GLP-1 receptor mediation of C-cell proliferative effects in rats or mice has not been demonstrated

Mode of Action

Novo Nordisk's Proposed Mode of Action For Liraglutide-Induced C-cell Tumors in Rodents

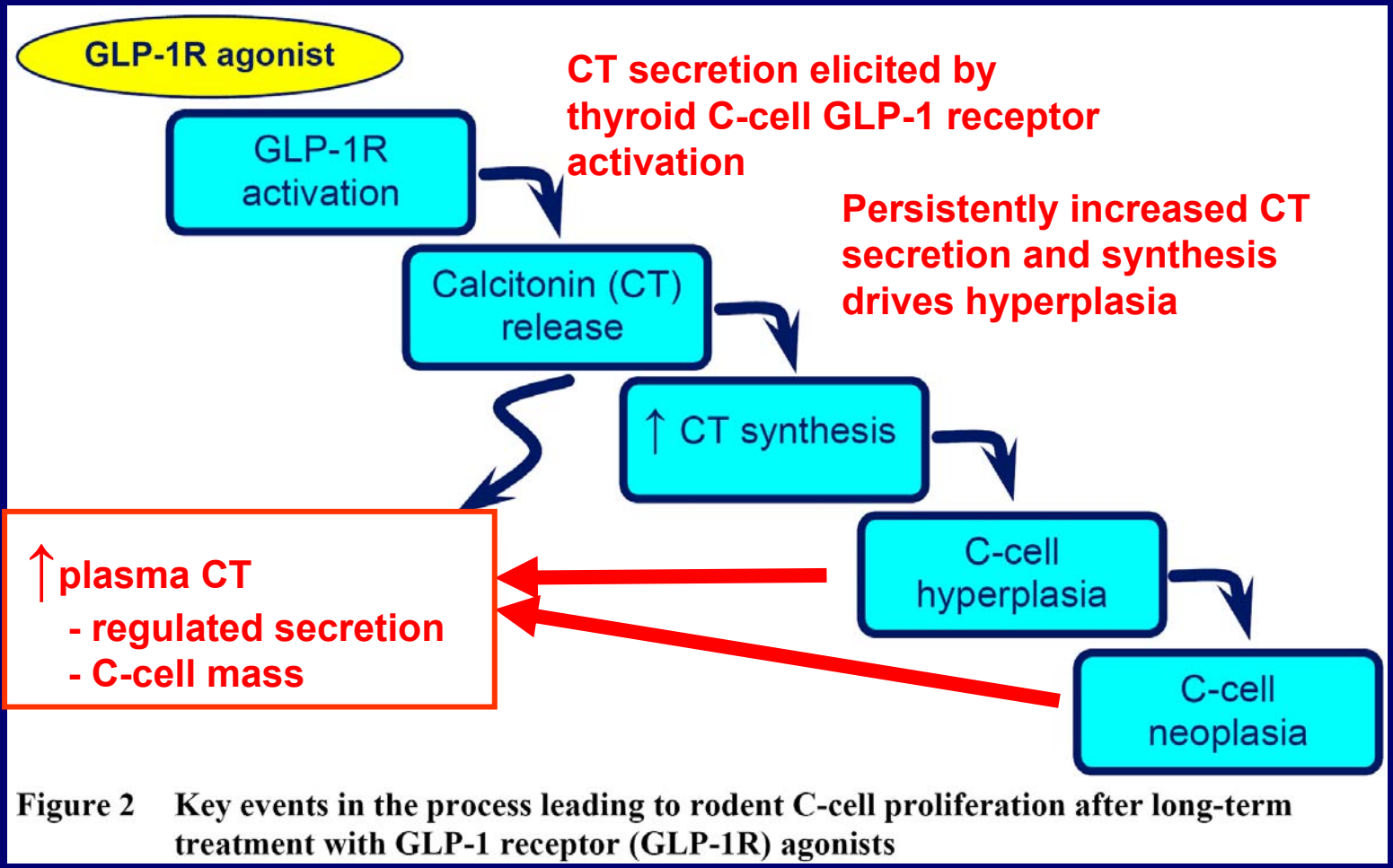


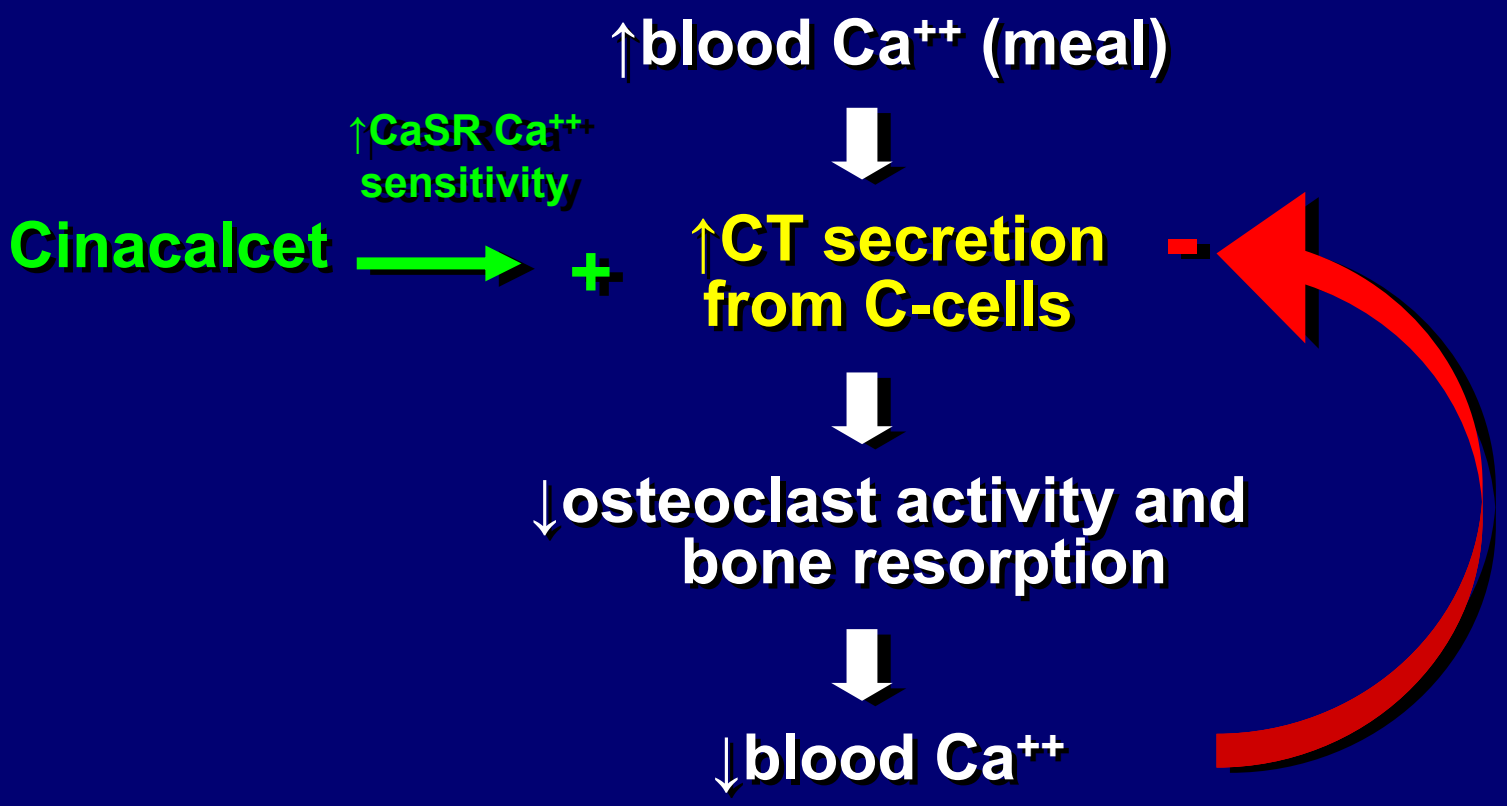
Figure 2 Key events in the process leading to rodent C-cell proliferation after long-term treatment with GLP-1 receptor (GLP-1R) agonists

From Novo Nordisk, NDA 22-341, Rodent C-cell findings: Assessment of human relevance

Sustained Drug-Induced Calcitonin Secretion is a Novel Mode of Action for Causing C-cell Tumors in Rodents

- Pharmacologic class effects linking increased CT secretion to C-cell tumors has not been established for any approved drug causing C-cell tumors in rats
- Cinacalcet, a drug that increases CT secretion in rats, does not cause C-cell tumors in mice or rats
  - Negative feedback inhibits persistent CT secretion

Plasma Calcitonin is Regulated by Calcium



Cinacalcet Increases Plasma Calcitonin, But Not Thyroid C-cell Tumors in Rats or Mice

Cinacalcet

- Transiently increases plasma CT
- Causes sustained hypocalcemia
- Decreases adenomas in male rats
- No C-cell tumors in female rats or mice

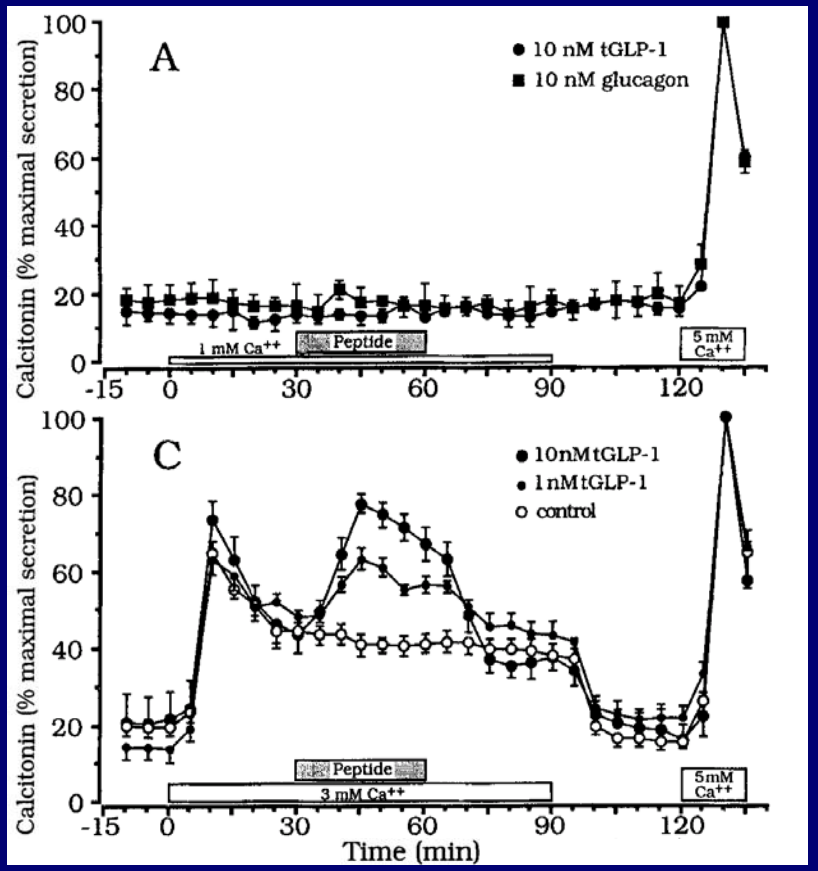
Incidence (%) of Thyroid C-cell Findings in 104-Week Rat Carcinogenicity Study

Sex	Male			
Cinacalcet Dose (mg/kg)	0	5	15	35
Focal Hyperplasia	11	3	13	3
Adenoma (B)	21	10	3*	2*
Carcinoma (M)	1	2	0	0

* $p < 0.01$ compared to control group

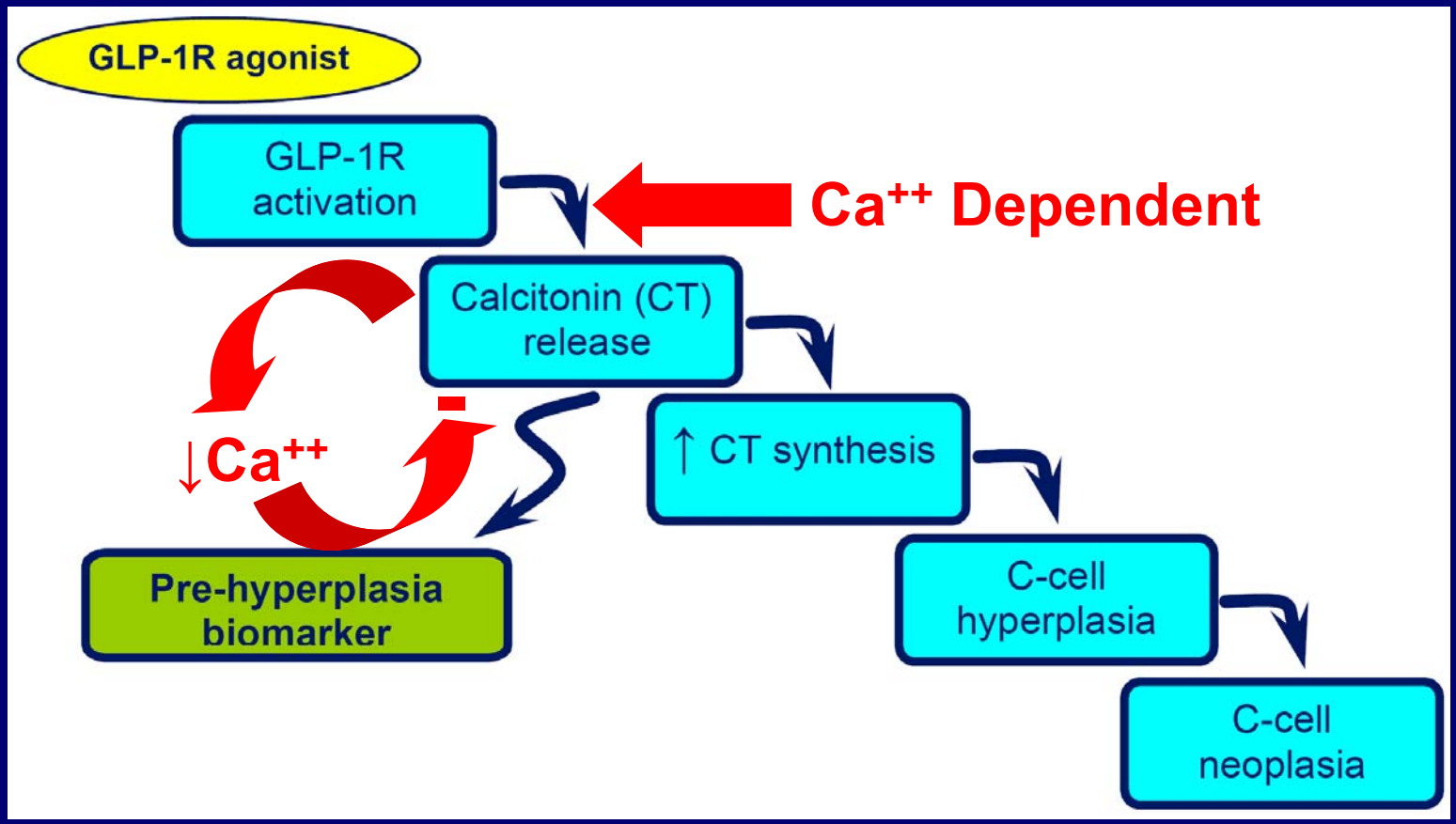
GLP-1 Induced Calcitonin Secretion is Calcium-Dependent in Rats

- Not well characterized *in vivo*
- GLP-1 dose-dependently increased calcium-dependent CT secretion from perfused rat thyroid



Modified Figure 5 from
Crespel et al. Endocrinology 1996;137:3674

Proposed Mode of Action Does Not Account for Calcium Regulation of GLP-1 Receptor-Mediated Calcitonin Secretion

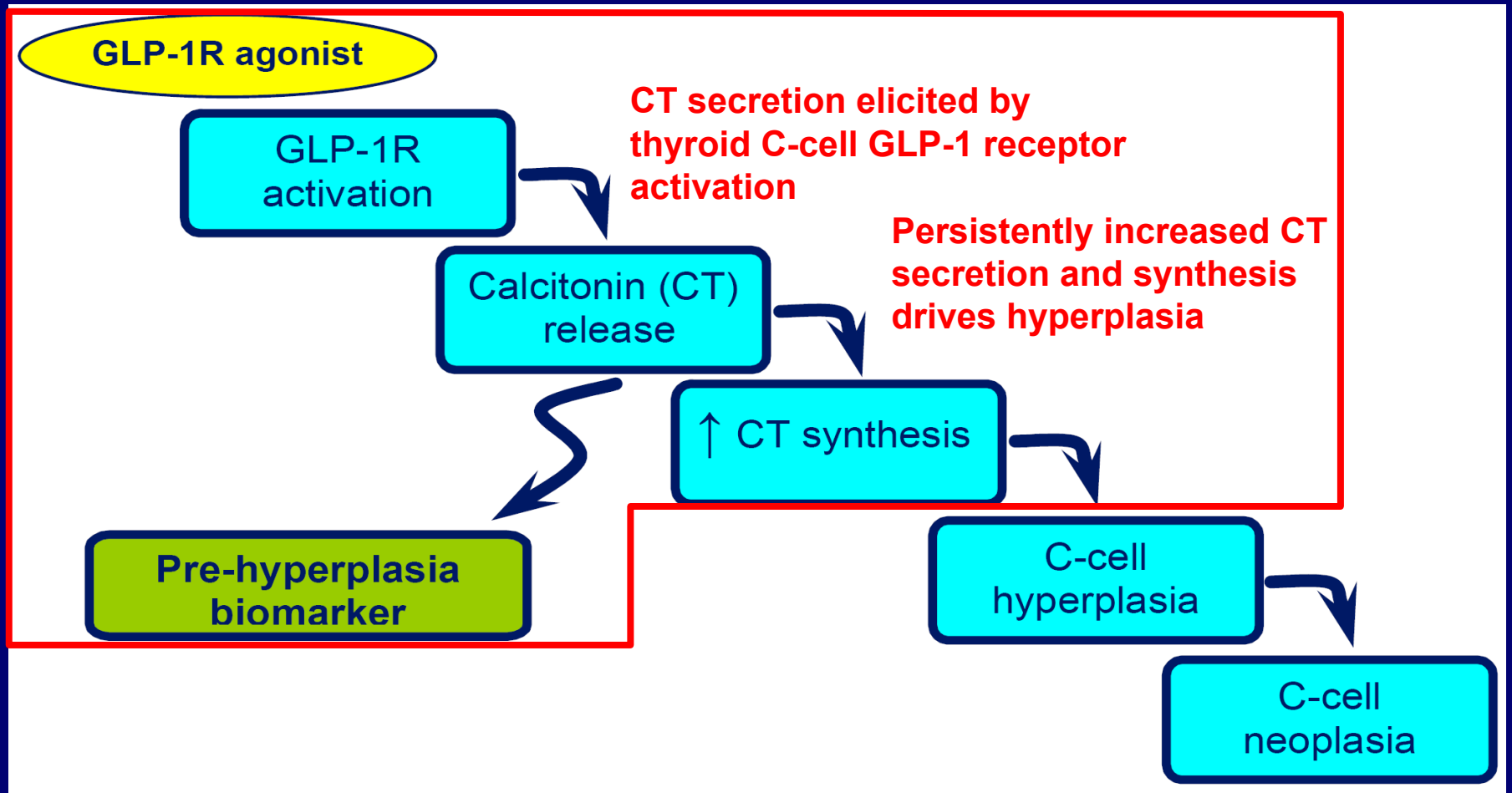


From Novo Nordisk, NDA 22-341, Rodent C-cell findings: Assessment of human relevance

The Mode of Action Does Not Include Regulatory Effects on GLP-1 Receptor-Mediated Calcitonin Secretion

- The mechanism of persistent CT secretion in LGT-treated mice is unknown
- LGT had no durable effect on CT secretion in rats
- No sustained measurable effects of increased CT in LGT-treated rats or mice
  - Plasma Ca^{++} was unaffected
  - Bone resorption parameters not measured
    - Bone mineral density or content
    - Biomarkers of bone resorption (i.e., urine deoxypyridinoline)

For This Mode of Action, Human Relevance Depends on Species Differences in C-cell GLP-1 Receptor Coupling to Calcitonin Secretion



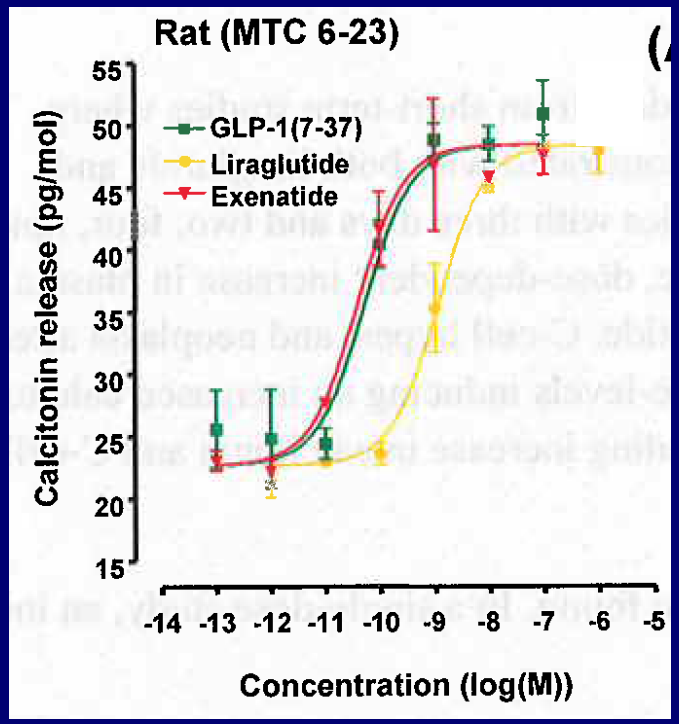
From Novo Nordisk, NDA 22-341, Rodent C-cell findings: Assessment of human relevance

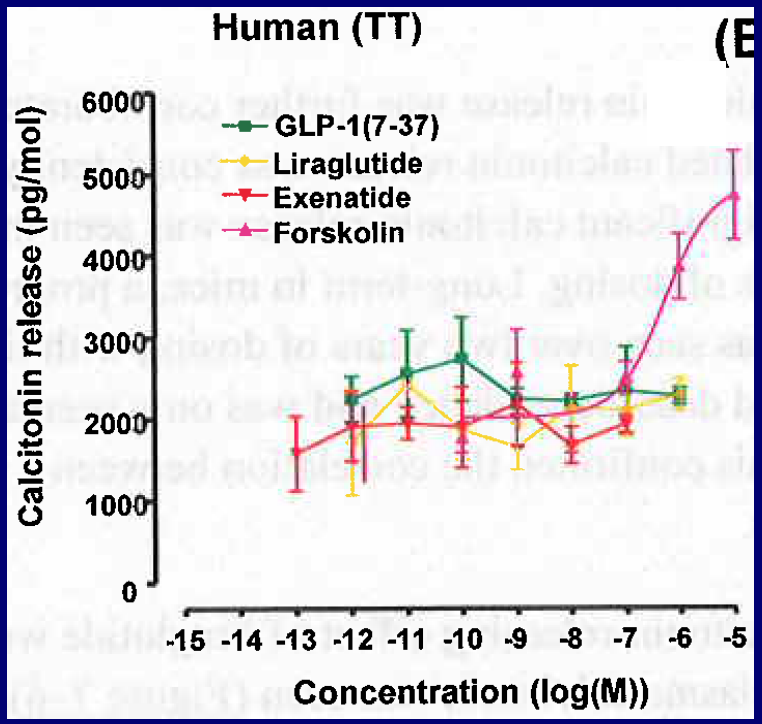
GLP-1 Receptors Occur in Thyroid of Mice, Rats, and Humans, But Cellular Localization was Not Determined

Thyroid GLP-1R Localization Method	*Cell Localization*	*Species*			
		Mouse	*Rat*	*Human*	*Human MTC*
Autoradio-graphic Ligand Binding	Thyroid, but specific type not identified	60% (3/5)	100% (12/12)	6% (1/18)	28% (5/18)
Immunohisto-chemistry	equivocal	Anti-GLP-1R antibody specificity not adequately demonstrated			
In-situ hybridization	equivocal	Low signal/noise ratio			
Ligand Binding	C-cell lines	none tested	+ (CA77, MTC 6-23)	- (TT)	

GLP-1 Receptor-Coupled Calcitonin Secretion in Humans and Rats was Evaluated Using C-cell Lines

GLP-1 agonists elicited CT secretion from a rat C-cell line (MTC 6-23), but not from a human cell line (TT)

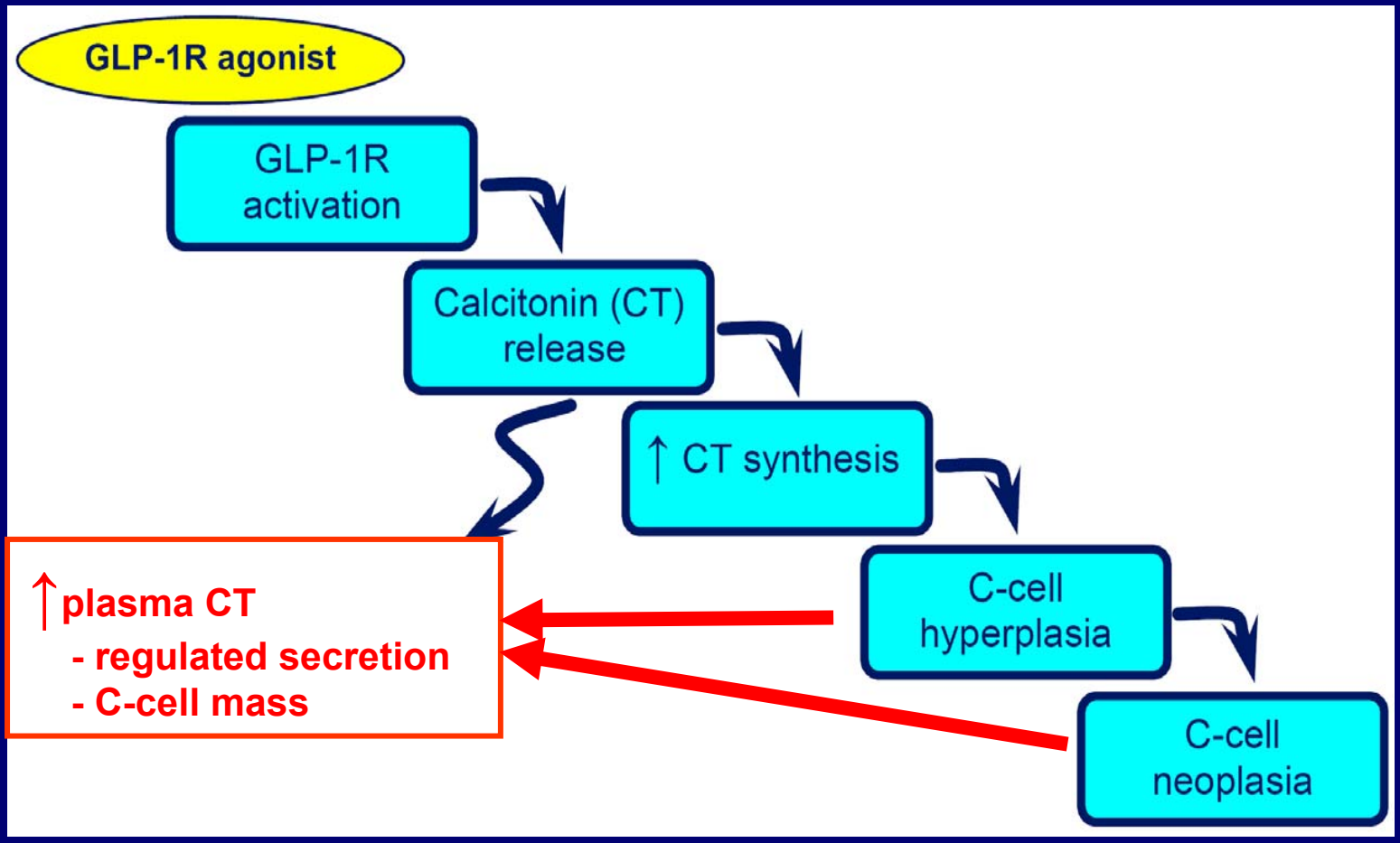




C-cell Lines Are Not Thyroid C-cells

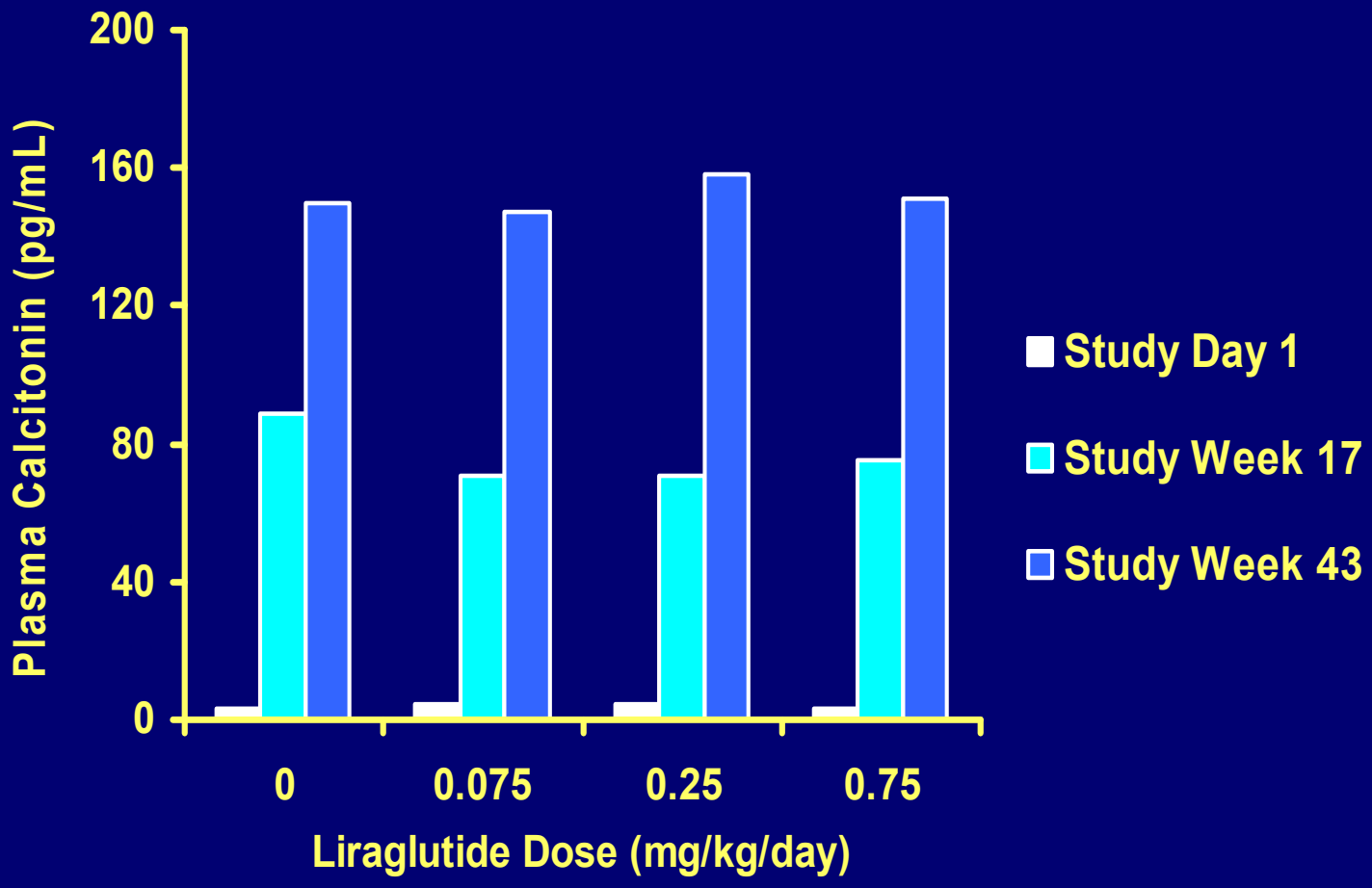
- Receptor expression and coupling to CT secretion in C-cell lines may be different from normal thyroid C-cells *in vivo*
  - Pentagastrin, a CT secretagogue in humans, did not elicit CT secretion from human TT cells
  - Glucagon elicits CT secretion from rat CA-77 cells, but not from rat MTC 6-23 cells
- 28% of human MTCs were GLP-1 receptor positive, so not all human C-cell lines would be expected to express the receptor

Plasma Calcitonin as a Biomarker for C-cell Proliferation

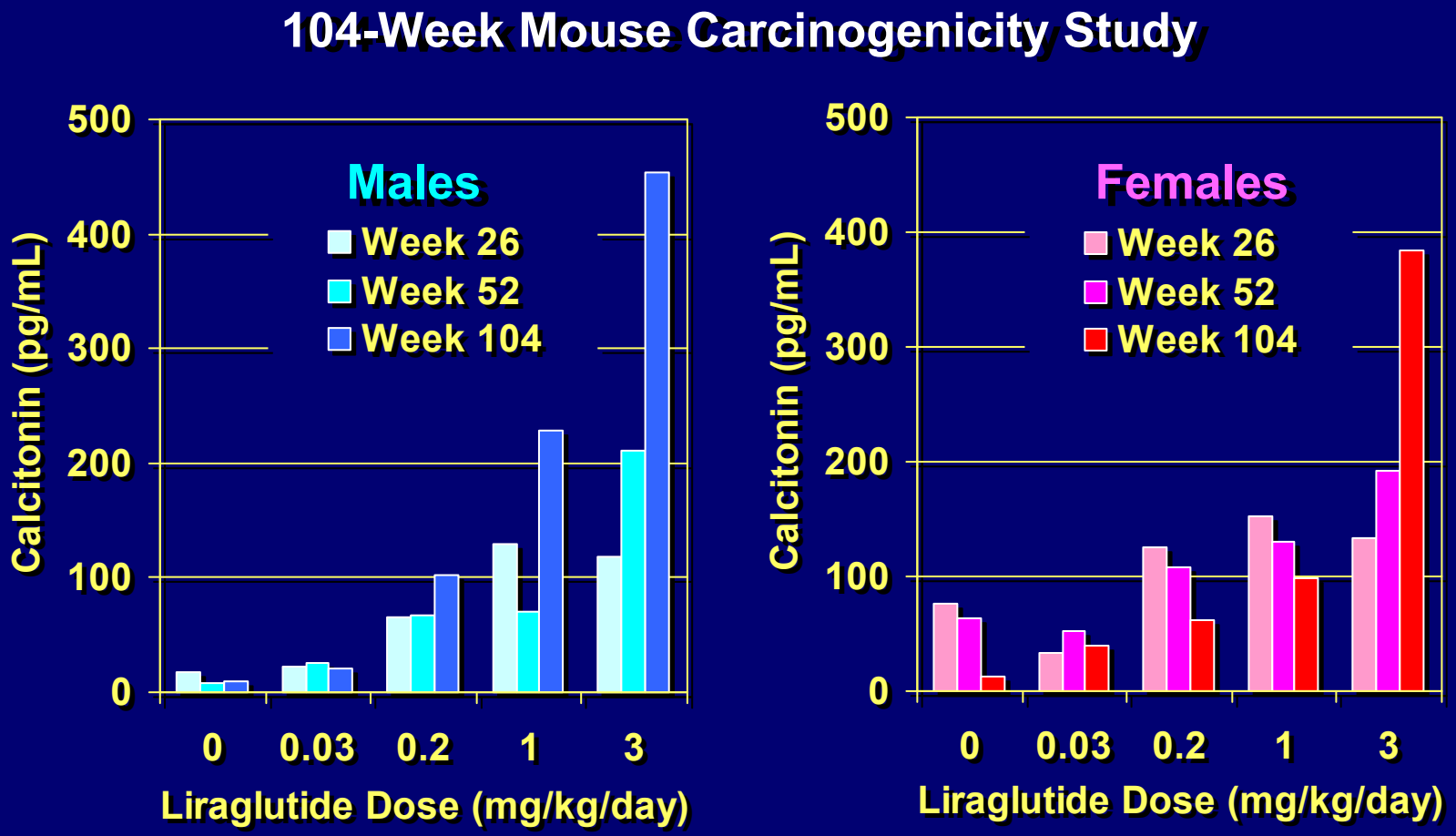


From Novo Nordisk, NDA 22-341, Rodent C-cell findings: Assessment of human relevance

Plasma Calcitonin is Age-Dependent in Male Rats



Increased Plasma Calcitonin in Mice is Liraglutide Dose and Treatment Duration Dependent



Liraglutide Dose-Dependently Increased Plasma Calcitonin in Humans

% Change from Baseline to Week 26/28

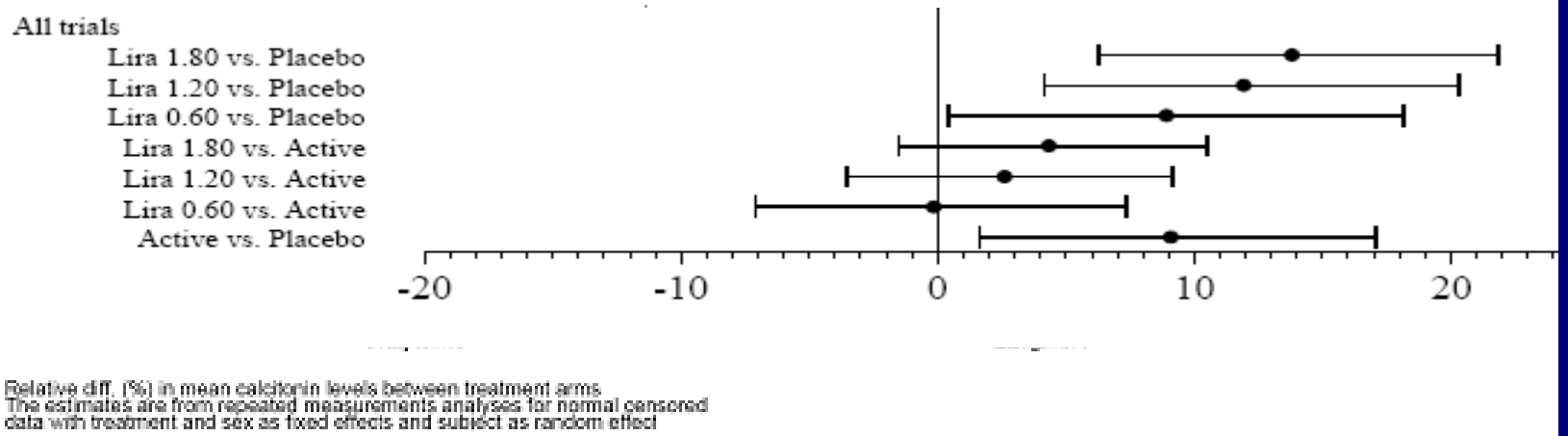
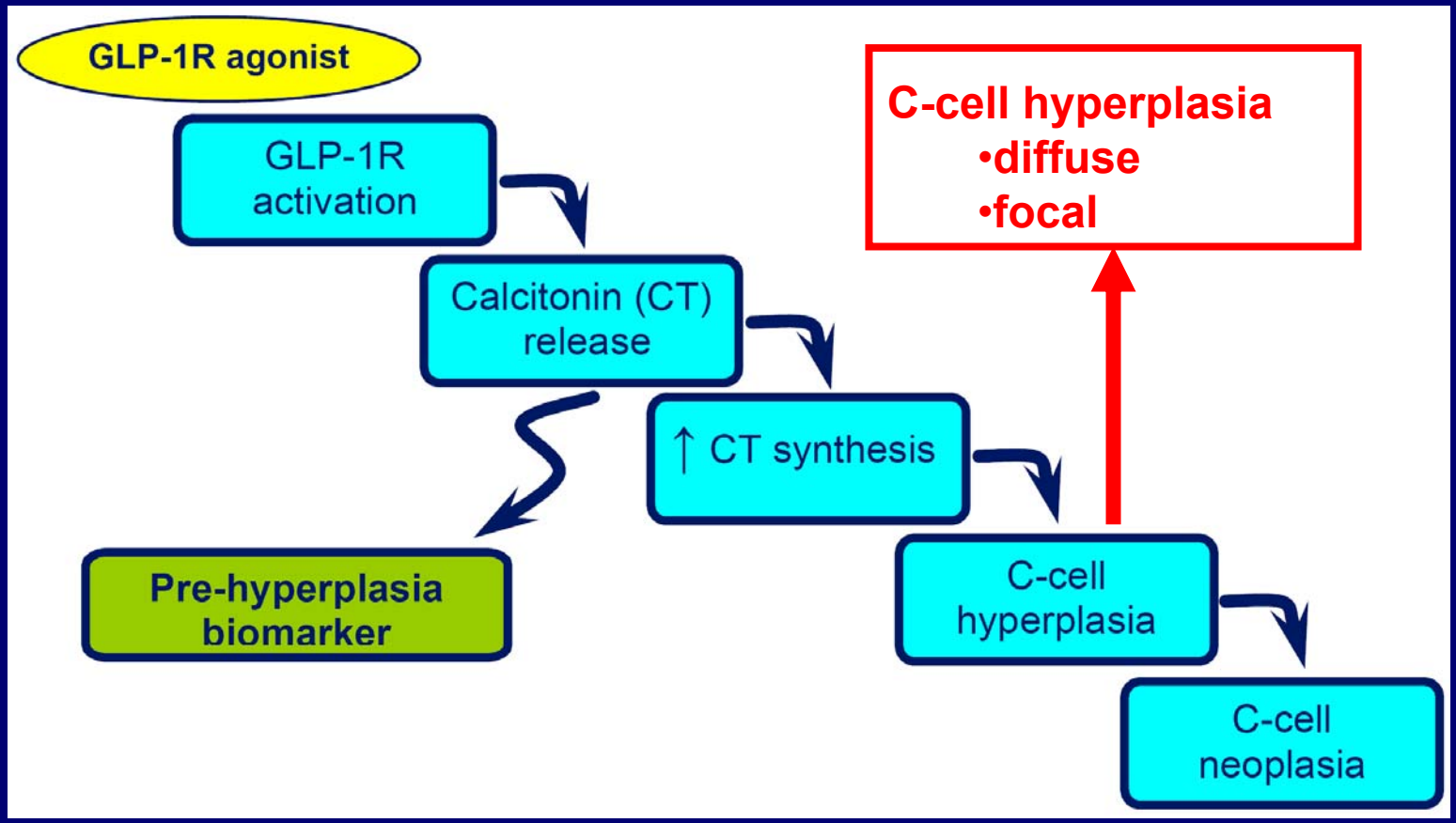


Figure 2 **Forest plot of Calcitonin Continous Analysis – Week 26/28 – All Long-term Trials - Safety analysis set** Figure edited, removed results from individual clinical trials.

Species Differences in Liraglutide Effects on Plasma Calcitonin

- RATS
  - LGT has no durable effects on plasma CT
  - CT not a biomarker for LGT-induced focal CCH/tumors
- MICE
  - CT increased with LGT dose and treatment duration
  - CT is a biomarker for LGT-induced focal CCH/tumors
- HUMANS
  - LGT dose-dependently increased CT
  - CT being used as a biomarker for CCH

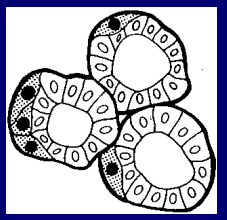
C-cell Hyperplasia Can Be Diffuse or Focal



From Novo Nordisk, NDA 22-341, Rodent C-cell findings: Assessment of human relevance

C-cell Proliferation

normal C-cells



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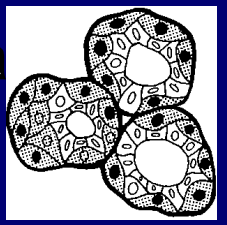
diffuse hyperplasia
(physiologic response)



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focal hyperplasia

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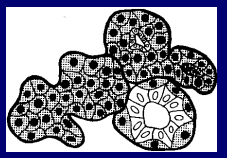

adenoma

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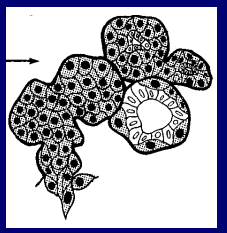


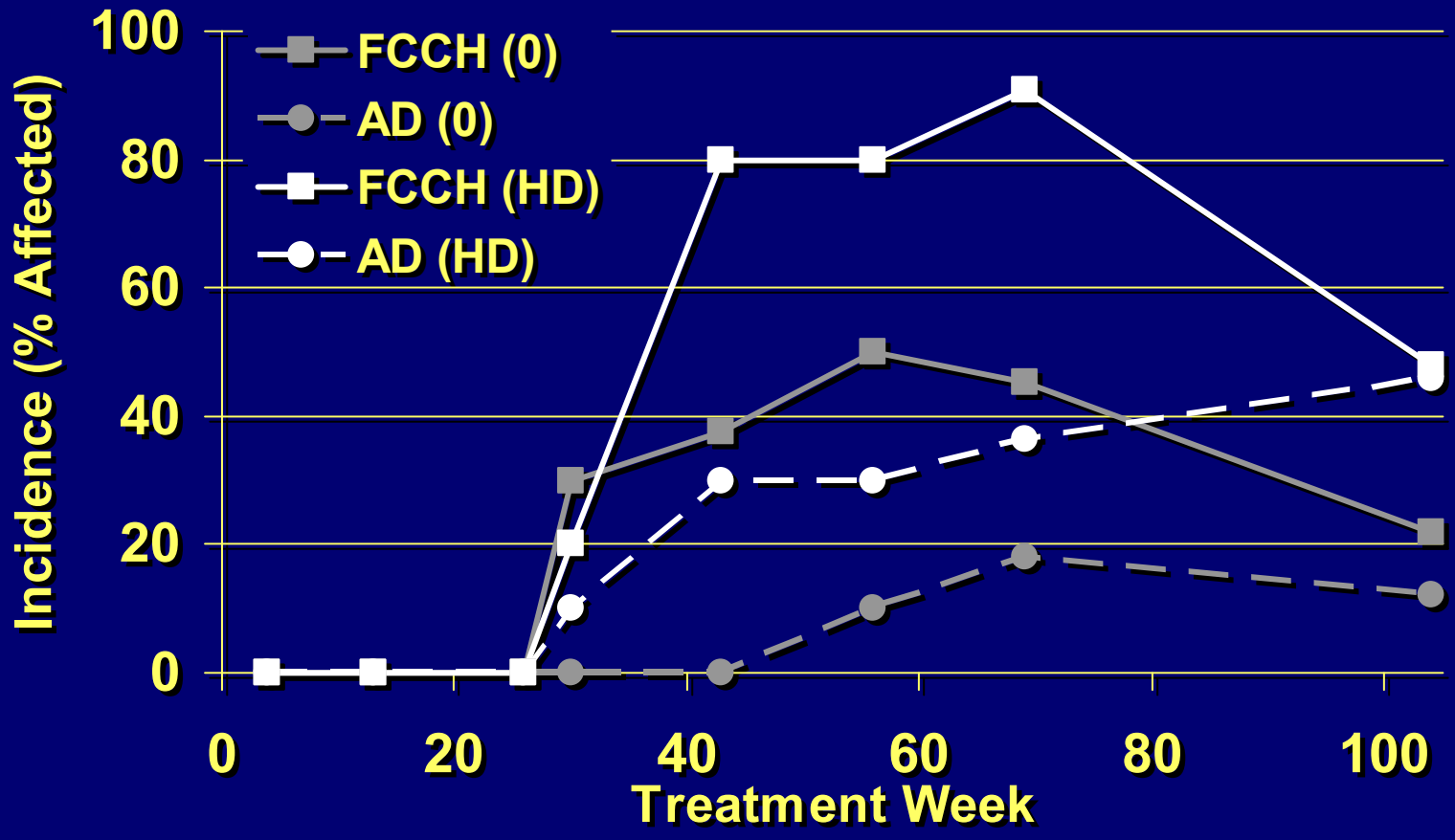
carcinoma

Figures from DeLillis et al., Lab Invest 1979; 40:140-54

Time Course of Liraglutide-Induced C-cell Proliferation in Male Rats

- Time course for the development of proliferative C-cell lesions in control and high dose LGT treated groups was constructed from the following studies:
  - 4-week toxicity (0, 0.75 mg/kg)
  - 13-week toxicity (0, 1 mg/kg)
  - 26-week toxicity (0, 1 mg/kg)
  - Mechanistic studies with sacrifice at 4-, 30-, 43-, 56-, and 69- weeks (0, 0.75 mg/kg)
  - 104-week carcinogenicity (0, 0.75 mg/kg)

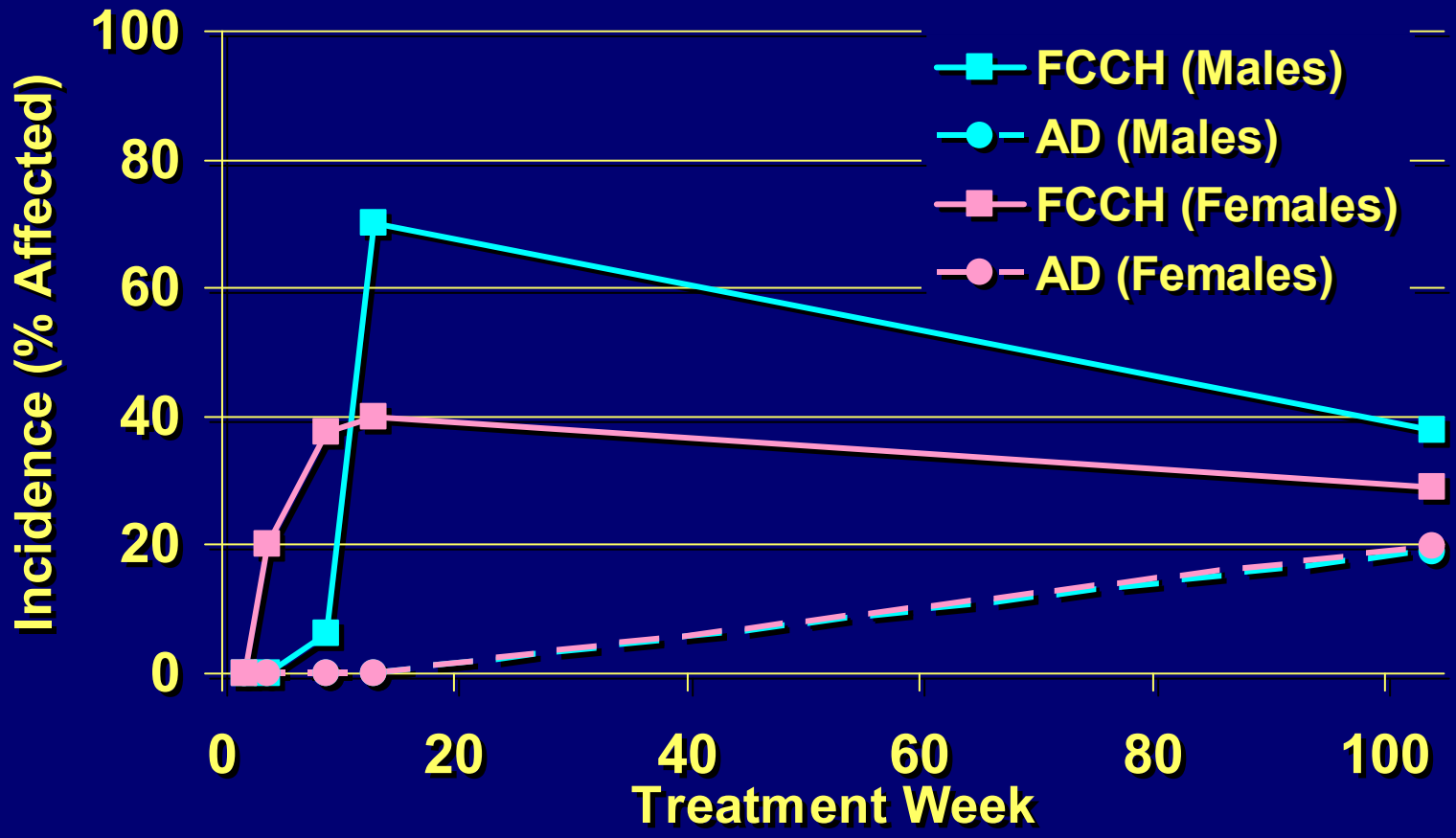
C-cell Focal Hyperplasia (FCCH) and Adenomas (AD) in Male Rats Treated with Vehicle (0) or High Dose Liraglutide (HD, ≥ 0.75 mg/kg/day)



Time Course of Liraglutide-Induced C-cell Proliferation in Mice

- Time course for the development of proliferative C-cell lesions in high dose LGT treated groups was constructed from the following studies:
  - 4-week toxicity (0, 5 mg/kg)
  - 13-week toxicity (0, 5 mg/kg)
  - Mechanistic studies with sacrifice at 2- and 9- weeks (0, 5 mg/kg)
  - 104-week carcinogenicity (0, 3 mg/kg)

C-cell Focal Hyperplasia (FCCH) and Adenomas (AD) in Male and Female Mice Treated with High Dose Liraglutide (HD, ≥ 3 mg/kg/day)



Time Course of Liraglutide-Induced C-cell Proliferative Lesions is Different in Mice and Rats

- Rats
  - < 8 months old male rats were insensitive to LGT effects on C-cells
  - LGT induced focal CCH/tumors in the absence of any discernable effect on plasma CT
  - LGT increased C-cell adenomas at 30 weeks and focal CCH at 43 weeks
  - LGT did not accelerate the onset on focal CCH

- Mice
  - Plasma CT increased with LGT dose and treatment duration
  - Focal CCH develops within 4 – 9 weeks of treatment with HD LGT
  - Focal CCH precedes adenomas

Diffuse C-cell Hyperplasia Does Not Precede Focal Hyperplasia in Liraglutide-Treated Mice and Rats

- Diffuse CCH, a physiologic response, was expected to precede focal CCH in mice and rats
- Diffuse CCH assessed in CTRL and HD LGT treated rats and mice terminated prior to the development of focal CCH
  - Focal CCH occurs after 4 – 9 weeks in mice and after > 26 weeks in rats
  - Quantitative analysis of CT immunoreactive cells
- C-cell proliferation assessed by BrdU labeling and PCNA staining in rats

Liraglutide Did Not Cause Diffuse C-cell Hyperplasia in Mice

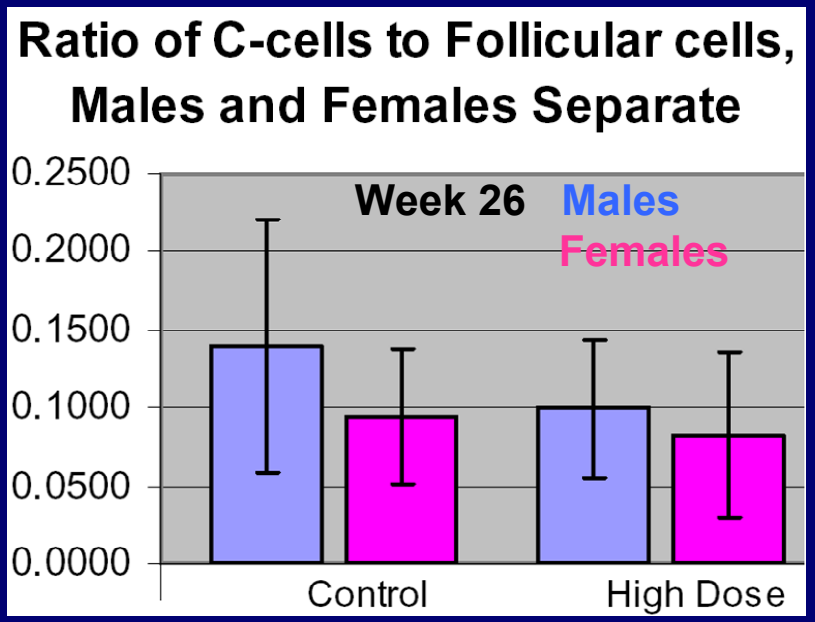
- Mice
  - LGT did not cause diffuse CCH (thyroid CT immunoreactive cells, 5 mg/kg/day for 2 weeks)

Treatment	Control		5 mg/kg	
Sex	Male	Female	Male	Female
C-cells/mm^3	219	214	250	224
C-cells / follicular cells	0.042	0.042	0.048	0.042

N = 15/sex/dose

Liraglutide Did Not Cause Diffuse Hyperplasia in Rats

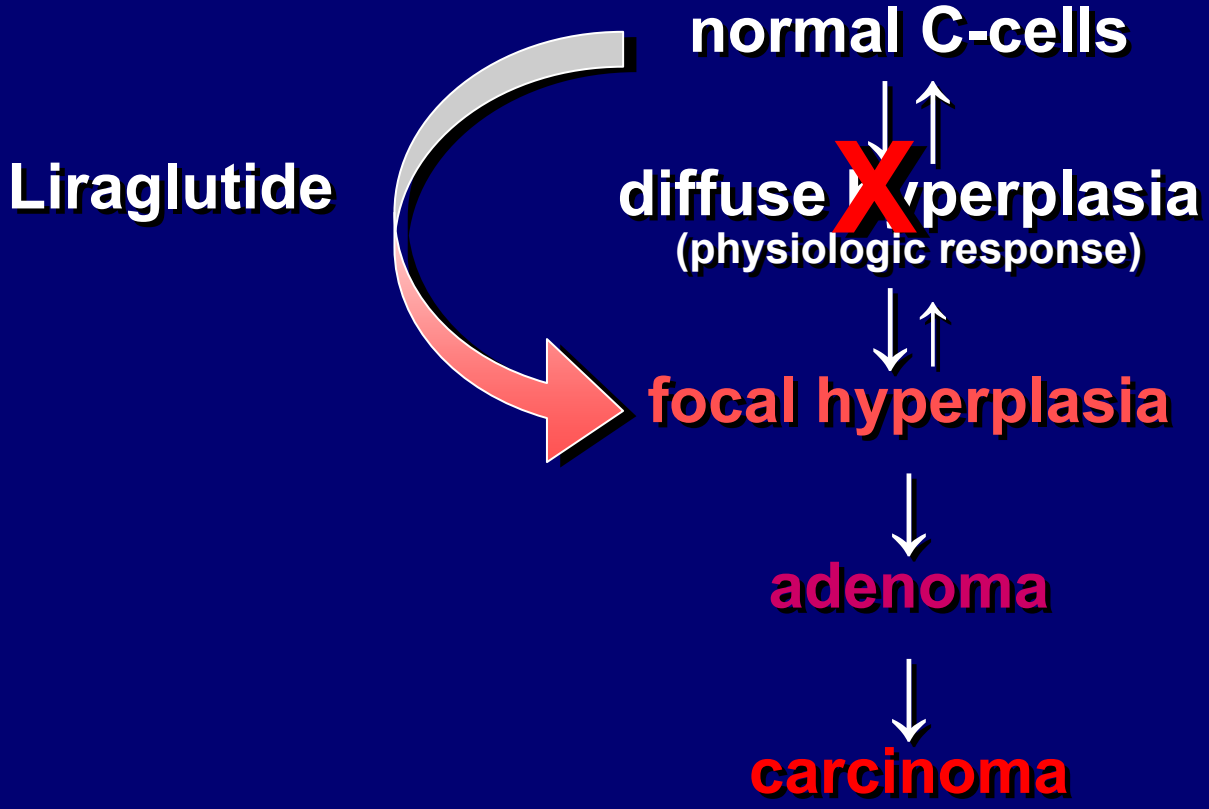
- In rats, LGT did not cause:
  - Diffuse CCH
    - No effect on the ratio of C-cells to follicular cells (1 mg/kg/day LGT for 26 weeks)
  - C-cell proliferation
    - No effect on PCNA labeling index (1 mg/kg/day LGT for 26 weeks)
    - No effect on volume of BrdU labeled cells in thyroid (0.75 mg/kg for 4 weeks)



Liraglutide Did Not Cause Diffuse C-cell Hyperplasia in Mice, Rats, or Monkeys

- Diffuse CCH did NOT precede focal CCH in LGT treated mice or rats
- LGT had no effect on plasma CT or CCH in cynomolgus monkeys treated for up to 87 weeks
- Absence of diffuse or focal CCH in LGT treated monkeys is not reassuring because:
  - Diffuse CCH didn't occur in LGT treated mice or rats, but LGT induced focal CCH and tumors
  - Monkey studies do not adequately assess the risk of focal CCH and C-cell tumors because:
    - Small number of animals treated
    - Short period of dosing compared to their lifespan

C-cell Proliferation in Liraglutide-Treated Mice and Rats



Relevance of Rodent C-cell Tumors to Human Risk

Human Relevance of Liraglutide-Induced Thyroid C-cell Tumors in Rodents

- Mechanistic studies do not support the mode of action for LGT-induced rodent C-cell tumors
  - MOA does not account for calcium regulation of GLP-1 agonist-induced CT secretion
  - Differences between rodents and humans in C-cell GLP-1 receptor coupling to CT secretion not demonstrated
  - LGT-induced focal CCH / tumors occurred in the absence of sustained increased CT in rats
  - Diffuse hyperplasia didn't precede focal CCH

Human Relevance of Liraglutide-Induced Thyroid C-cell Tumors in Rodents

- Mechanistic studies do not adequately support the mode of action for LGT-induced thyroid C-cell tumors in rats and mice
- In clinical studies, LGT increased plasma CT, a biomarker for C-cell proliferation, so even if the mode of action is correct, it may be operable in humans
- There is a potential increase in the risk of thyroid tumors in humans treated with LGT

Acknowledgments

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