

## Symposium 9

# ***Case study: Human relevancy and mode of action of toxic findings observed in GLP toxicity studies***



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The 44th Annual Meeting of the Japanese Society of Toxicology  
July 11, 2017 Yokohama

# Outline

## Candidate selection to IND

- Testicular necrosis in rodents of LH-RH agonists

## IND to NDA

- Thyroid c-cell hyperplasia and tumors in rodents of anti-obesity drugs (GLP-1 agonist)
- Gastric carcinoid tumors in rodents of acid suppressors (proton pump inhibitor)
- Leydig cell tumors of the testis in rodents of acid suppressors (proton pump inhibitor)

## Candidate selection to IND

- Positive outcome of in vivo micronucleus test of anti-obesity peptidic drugs

# Case study 1

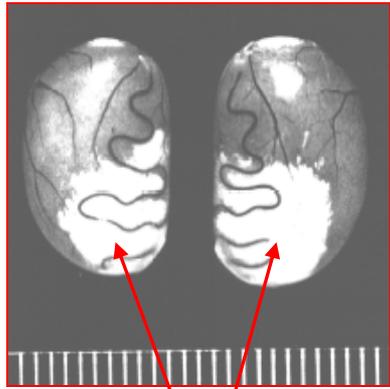
## Candidate selection to IND

### Testicular necrosis in rodents of LH-RH agonists

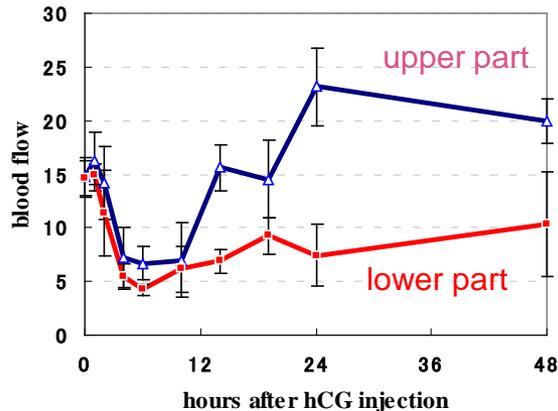
# MOA of testicular necrosis in rodents due to LH-RH agonists

- Single injection of LH-RH agonists causes severe damage to the rat testis within 24 hours  
(Habenicht, Andrologia 17; 440-443, 1985)
- No apparent evidence in mouse, monkey or dog testes after repeated administration of a LH-RH agonist  
(Chatani, Jpn. Pharm & Ther. Suppl, 18; 65-78, 1990)
- No adverse event in human testes of leuprorelin, except for testicular atrophy due to the expected physiologic effect of decreased testosterone levels  
(LUPRON® INJECTION FDA label, reference ID: 2920484, 2012)
- Conclusion: Testicular necrosis in rats represent **a species-specific sensitivity** and does **not** imply **a risk** for clinical use **in man**.

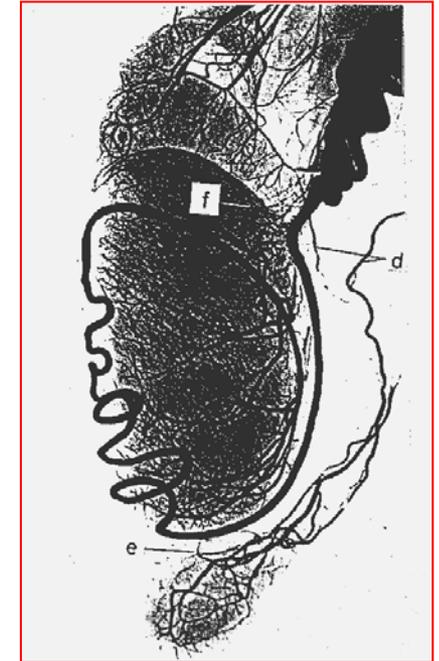
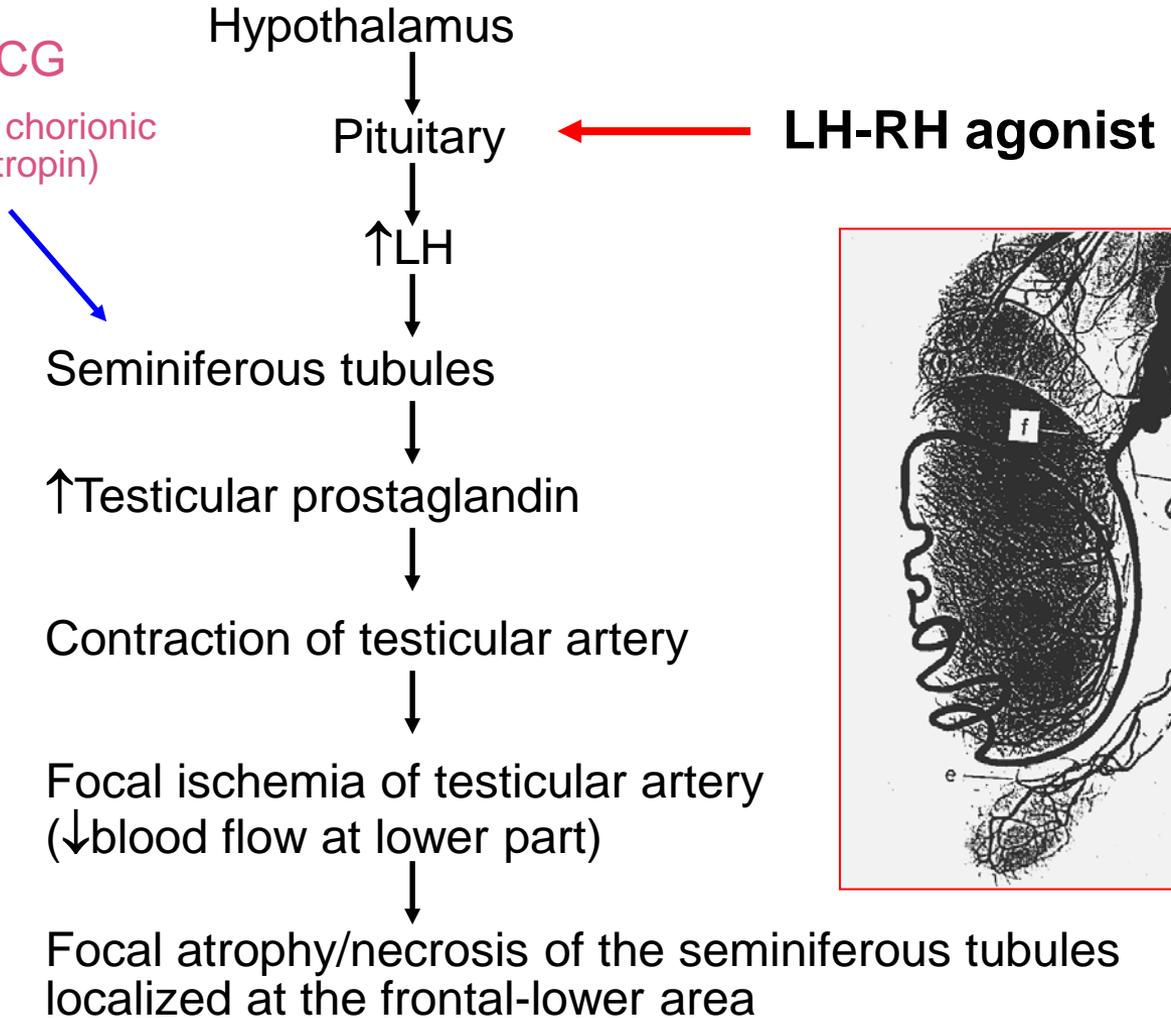
# Possible mechanisms of focal atrophy/necrosis of the seminiferous tubules localized at the frontal-lower area of the testis in rats



Necrotic area after hCG treatment



hCG  
(human chorionic gonadotropin)



Ref: 1) Chatani et al. J Toxicol. Pathol. 9, 369-379, 1996  
2) Chatani et al. J Toxicol. Sci. 31, 291-303, 2006

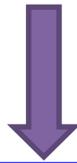
## Case study 2

### IND to NDA

# Thyroid c-cell hyperplasia and tumors in rodents of anti-obesity drugs (GLP-1 agonist)

# Thyroid c-cell hyperplasia and tumors in rodents of anti-obesity drugs (Liraglutide, GLP-1 agonist)

- Rat 13W (0.1, 0.25, 1 mg/kg/day)
  - No c-cell hyperplasia at any dose
  - NOAEL for c-cell hyperplasia=1 mg/kg/day (SM=14X)
- Rat 26W (0.1, 0.25, 1 mg/kg/day)
  - No proliferative c-cell lesion at any dose
  - NOAEL=1 mg/kg/day (SM=8X)
- Mouse 13W (0.2, 1, 5 mg/kg/day)
  - $\geq 0.2$ : c-cell hyperplasia
  - No NOAEL (SM=<2X)



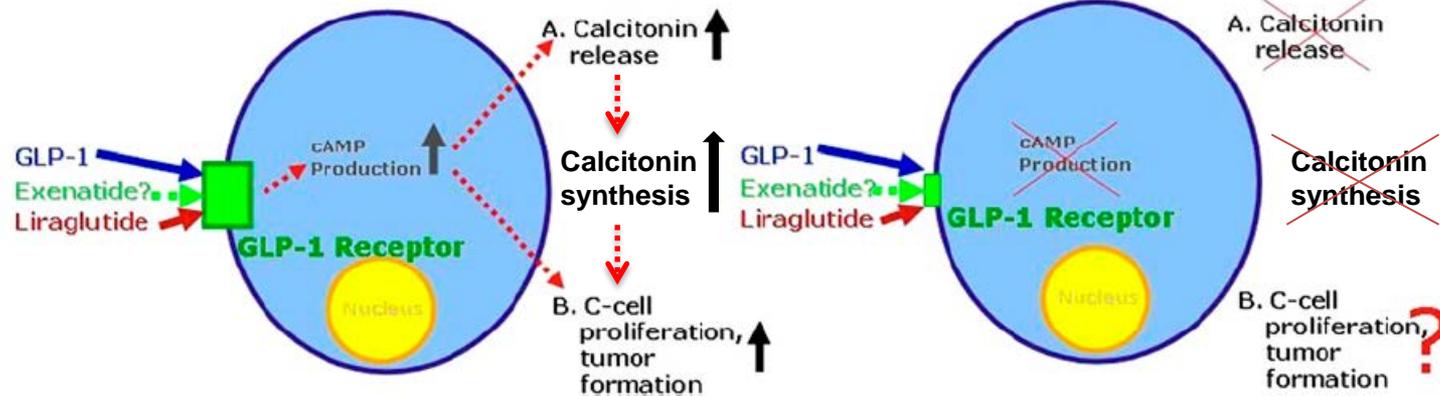
On target or Off target  
Safety margin  
Biomarker, Reversibility

- Rat 2Y carcinogenicity (0.075, 0.25, 0.75 mg/kg/day)
  - No NOAEL for tumor
  - $\geq 0.075$ : c-cell carcinoma (0.5X vs human AUC at 1.8 mg/day)
  - $\geq 0.25$ : c-cell adenoma (2X)
- Mouse 2Y carcinogenicity (0.03, 0.2, 1, 3 mg/kg/day)
  - NOAEL for tumor=0.2 (SM=2X vs human AUC at 1.8 mg/day)
  - $\geq 0.2$ : c-cell hyperplasia (2X),  $\geq 1$ : c-cell adenoma (10X), 3: c-cell carcinoma (45X)

# Possible mode of action for thyroid c-cell tumors in rodents induced by GLP-1 agonist

Rodent

Human



Sustained activation of these receptors caused C-cell hyperplasia and resulted in medullary thyroid cancer. However medullary thyroid cancer also occurred in rodents receiving placebo.

(Nauck, DIABETES CARE 2013)

## Should we be concerned about thyroid cancer in patients taking GLP-1 R agonists?

# Solution 1 for thyroid c-cell tumor

## *In vitro study*

- GLP-1R mRNA expression
  - Significantly higher in rat c-cell line than human c-cell line (RT-PCR)
  - No detection in monkey or human c-cell line (in situ hybridization)
- GLP-1R expression
  - Identification in rat thyroid and thyroid c-cell line but not in human c-cell line (Western blot analysis)
- cAMP accumulation & calcitonin secretion
  - ↑ (Ca-dependent calcitonin secretion from perfused rat thyroid & rat c-cell lines)
  - → (human c-cell line)
- Mitogenic potential in rat and human c-cell line
  - No effect on [<sup>3</sup>H]thymidine incorporation into DNA

## *In vivo study*

- Rat 6W + 2W recovery (0.75 mg/kg/day)
  - ↑ Plasma calcitonin (biomarker), return to normal level during recovery period
- Mice 9W + 6W recovery (0.2, 5 mg/kg/day)
  - ↑ Plasma calcitonin & thyroid calcitonin mRNA (biomarker) prior to c-cell hyperplasia
  - Reversible for c-cell hyperplasia
- Monkey 52W & 87W:
  - SM=73X (52W), 64X (87W)
  - No effect on plasma calcitonin or thyroid c-cell histopathology
- GLP-1R KO mice
  - ↓ Thyroid calcitonin mRNA

Liraglutide NDA No. 22-341 (May 23, 2008)

# Solution 2 for thyroid c-cell tumor

- Human (Phase 3):
  - Plasma calcitonin & thyroid morphology monitored
  - Long-term use of liraglutide in high doses (up to 3 mg per day) did not lead to elevations in serum calcitonin levels
  - Nine patients treated with liraglutide were diagnosed with thyroid cancer, compared with one patient on glimepiride. The odds ratio for thyroid cancer occurrence associated with liraglutide treatment was 1.54, but that was not statistically significant ( $P=0.53$ ).
  - Unclear relationship between  $\uparrow$  calcitonin & carcinoma
  - Clinical significance is unknown because of the small total number of cases in clinical studies

# Liraglutide: thyroid C-cell tumors

- On-target effects of GLP-1 agonists on thyroid C-cells
- In chronic rodent studies, **GLP-1 agonists cause thyroid C-cell hyperplasia, adenomas, and carcinomas at clinically relevant doses.**
- Rats are more sensitive than mice and no C-cell pathology has been observed in dogs or monkeys.
- GLP-1 receptors have the greatest expression in rodent C-cells and both the increased calcitonin and the C-cell hyperplasia caused by GLP-1 agonists are blocked in GLP-1R knockout mice.
- **Calcitonin** is a potential biomarker for medullary thyroid carcinoma (MTC) and **is monitored in humans** given GLP-1 agonists.
- Rodents are more sensitive than other species and humans for the GLP-1 agonist effects on C-cells, GLP-1 agonists carry **a label warning** for the risk of thyroid C-cell tumors.
- Regulatory agencies will be cautious (i.e., black box warning) because of the **irreversible and serious (cancer) nature of the toxicity.**

Toxicologic Pathology, 41: 310-314, 2013

# WARNING: RISK OF THYROID C-CELL TUMORS (April 2017)

- It is unknown whether VICTOZA causes thyroid C-cell tumors, including medullary thyroid carcinoma (MTC), in humans, as the human relevance of liraglutide-induced rodent thyroid C-cell tumors has not been determined.
- VICTOZA is contraindicated in patients **with a personal or family history of MTC** and in patients **with Multiple Endocrine Neoplasia syndrome type 2 (MEN 2)**.
- Counsel patients regarding the potential risk for MTC with the use of VICTOZA and inform them of symptoms of thyroid tumors (e.g. a mass in the neck, dysphagia, dyspnea, persistent hoarseness).
- **Routine monitoring of serum calcitonin or using thyroid ultrasound is of uncertain value** for early detection of MTC in patients treated with VICTOZA.

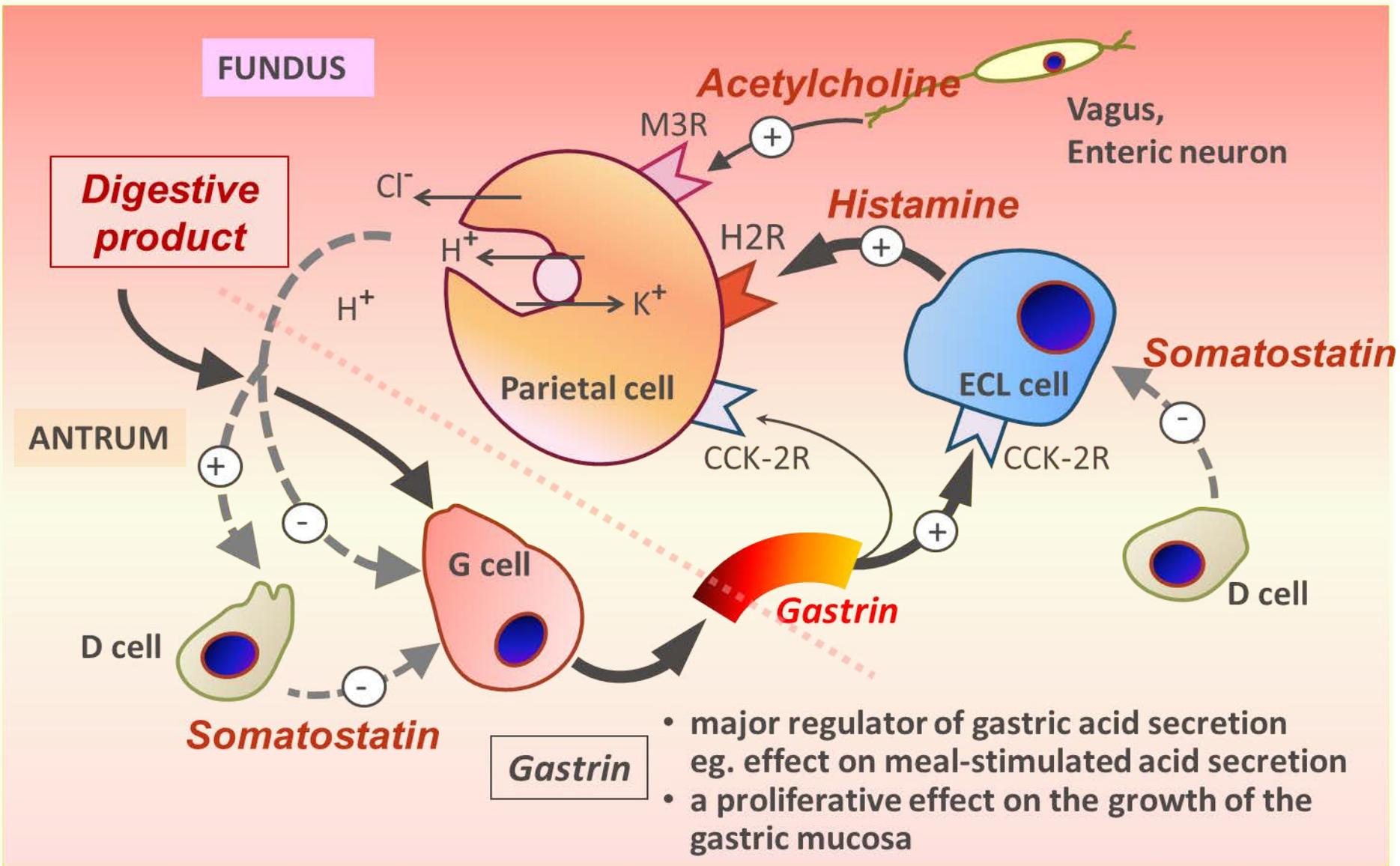
Risk for thyroid c-cell tumor in patients should be discussed based on the future outcomes from post-marketing surveillance studies.

## **Case study 3**

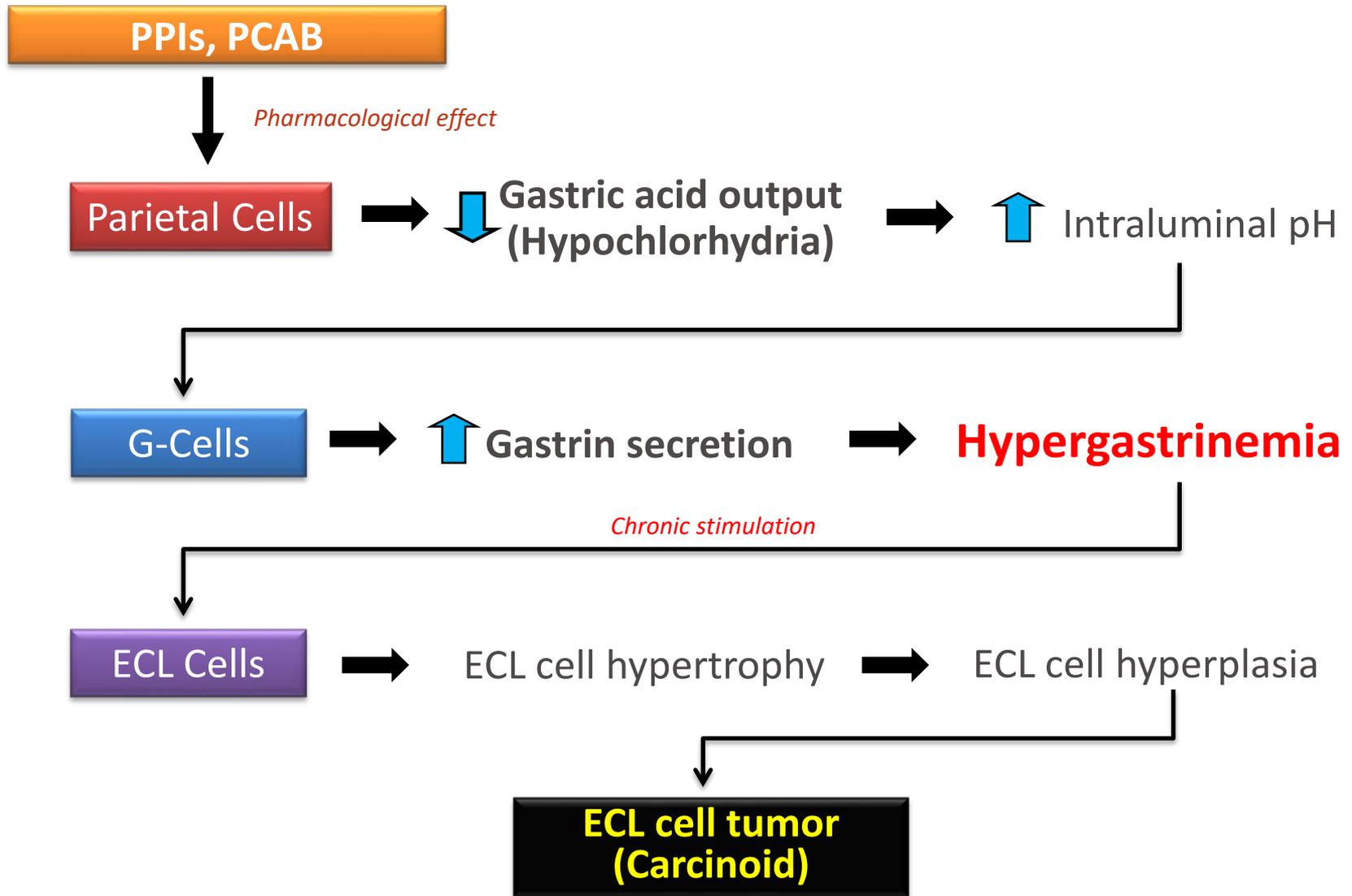
### **IND to NDA**

**Gastric carcinoid tumors in rodents of acid suppressors (proton pump inhibitor)**

# Gastric acid secretion



# MOA of onset of gastric carcinoids in rodents



# Evidence in humans with hypergastrinemia

1. No gastric carcinoid in Zollinger-Ellison syndrome patients which sustained mean gastrin levels **1947 pg/mL (225-5200 pg/mL)** for 4 years (mean, 13-81 months)

(Digest Liver Dis 2002; 34:270-278)

2. No statistical significance in incidence of gastric carcinoid in ZES patients (mean gastrin levels: **3800 pg/mL**)

(Human Pathology 2000; 31(2): 140-148)

3. No evidence of neoplasia or dysplasia has been noted in humans exposed to long-term administration of PPI

(World J Gastroenterol. 2010; 16(19): 2323–2330, Endocr Pathol. 2014 Apr 30.)

# Involvement of MEN1 gene in human carcinoid formation

- Hypergastrinemia alone has never been shown to induce carcinoid formation in humans

(Laine L, Aliment Pharmacol Ther 2000 Jun;14(6):651–68)

- Need mutation/deletion of **MEN1 gene** (Multiple Endocrine Neoplasia syndrome type-1) to produce carcinoid in humans

(Bordi. Endocr Pathol. 2014 Apr 30.)

## Case study 4

### IND to NDA

**Interstitial cell tumors of the testis in rodents of acid suppressors (proton pump inhibitor)**

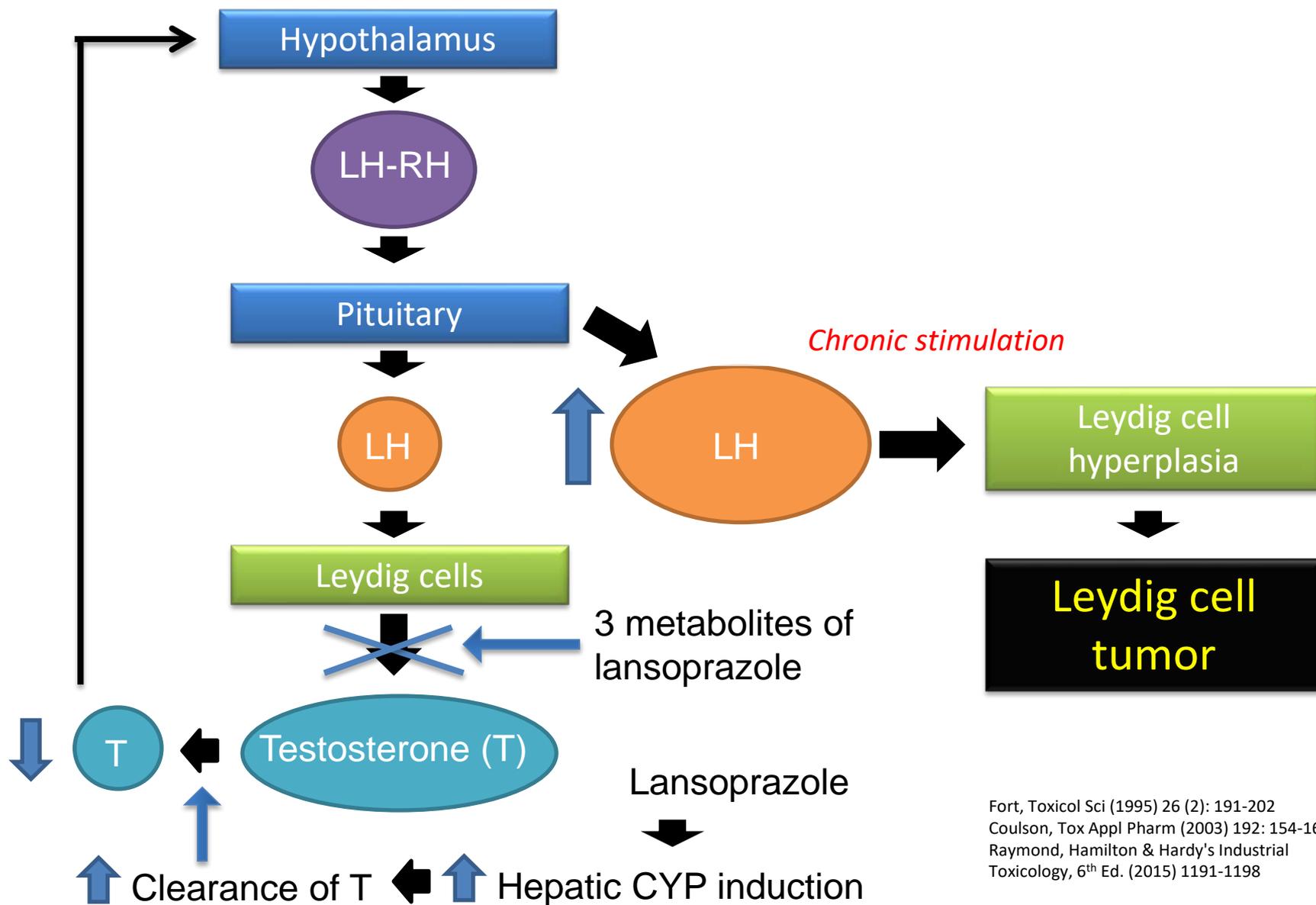
# MOA of Leydig cell tumors in rodents due to PPIs (1)

- 2Y carcinogenicity study: Leydig cell tumors in rats but not in mice
- 4 week tox study in rats: ↑serum LH, ↓serum testosterone
- 6 month tox study in 9M-old male rats given testosterone supplementation: high incidence of Leydig cell tumors in lansoprazole treated, unsupplemented rats, whereas no Leydig cell tumors in testosterone supplemented rats.
- *In vitro* studies with metabolites of lansoprazole: **three metabolites** were more potent inhibitors of testosterone synthesis than the parent drug, two of them being at least 10 times more potent.

## MOA of Leydig cell tumors in rodents due to PPIs (2)

- **These metabolites** are present **in rats** at substantial levels but are **undetectable in humans**. The lack of induction of Leydig cell tumors in mice, and the absence of testosterone synthesis inhibiting metabolites in man.
- Lansoprazole-treated rats exhibited significant increases in hepatic CYP-dependent testosterone metabolism in vitro and enhanced plasma clearance of testosterone in vivo.
- Conclusion: Leydig cell tumors in rats represent **a species-specific sensitivity** and does **not** imply **a risk** for clinical use **in man**.

# MOA of Leydig cell tumors in rodents due to PPIs



Fort, Toxicol Sci (1995) 26 (2): 191-202  
Coulson, Tox Appl Pharm (2003) 192: 154-163  
Raymond, Hamilton & Hardy's Industrial Toxicology, 6<sup>th</sup> Ed. (2015) 1191-1198

# Case study 5

## Candidate selection to IND

**Positive outcome of in vivo micronucleus test of anti-obesity peptidic drugs**

# Positive outcome of in vitro micronucleus test of anti-obesity peptidic drugs

- Need for genotoxicity testing of peptides has always been discussed controversially, since such compounds per se are **not expected to reach the nucleus and interact with the DNA molecule**.
- However, **synthetic peptides** have usually been subjected to such testing mainly for a genotoxic assessment of the **impurities** carried over from the chemical synthesis processes.
- In a bone marrow micronucleus test of GLP-1/GIP R agonist that had been integrated into a 13-week repeat-dose toxicity study in rats, **small increases in the incidence of micronuclei** had been observed, together with pronounced reductions in food intake and body weight gain.
- However, **negative** in Ames test, micronucleus test in L5178Y tk+/-, chromosome aberration test in human lymphocytes
- **Should we consider mutagenicity risk in patients taking GLP-1/GIP agonists?**

# Reduction of body weight and food consumption induced by anti-obesity peptidic drugs in rats

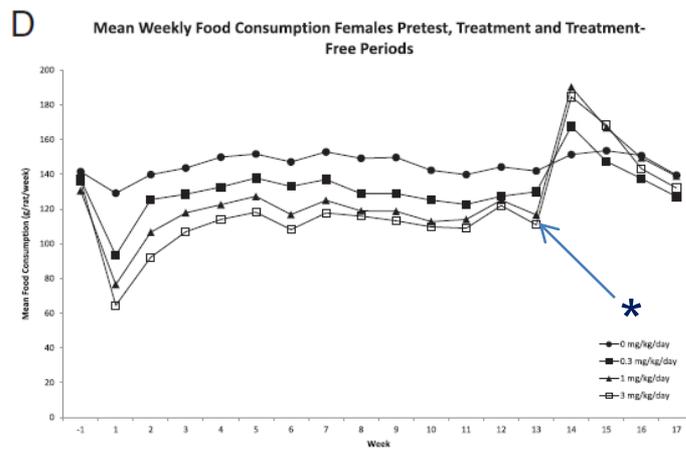
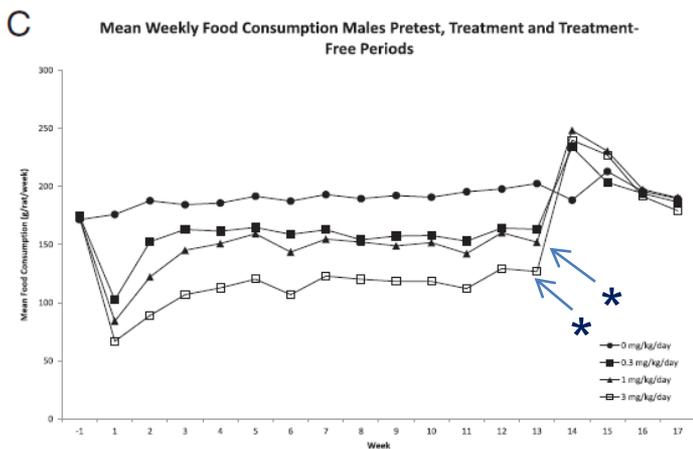
Male body weight

Female body weight



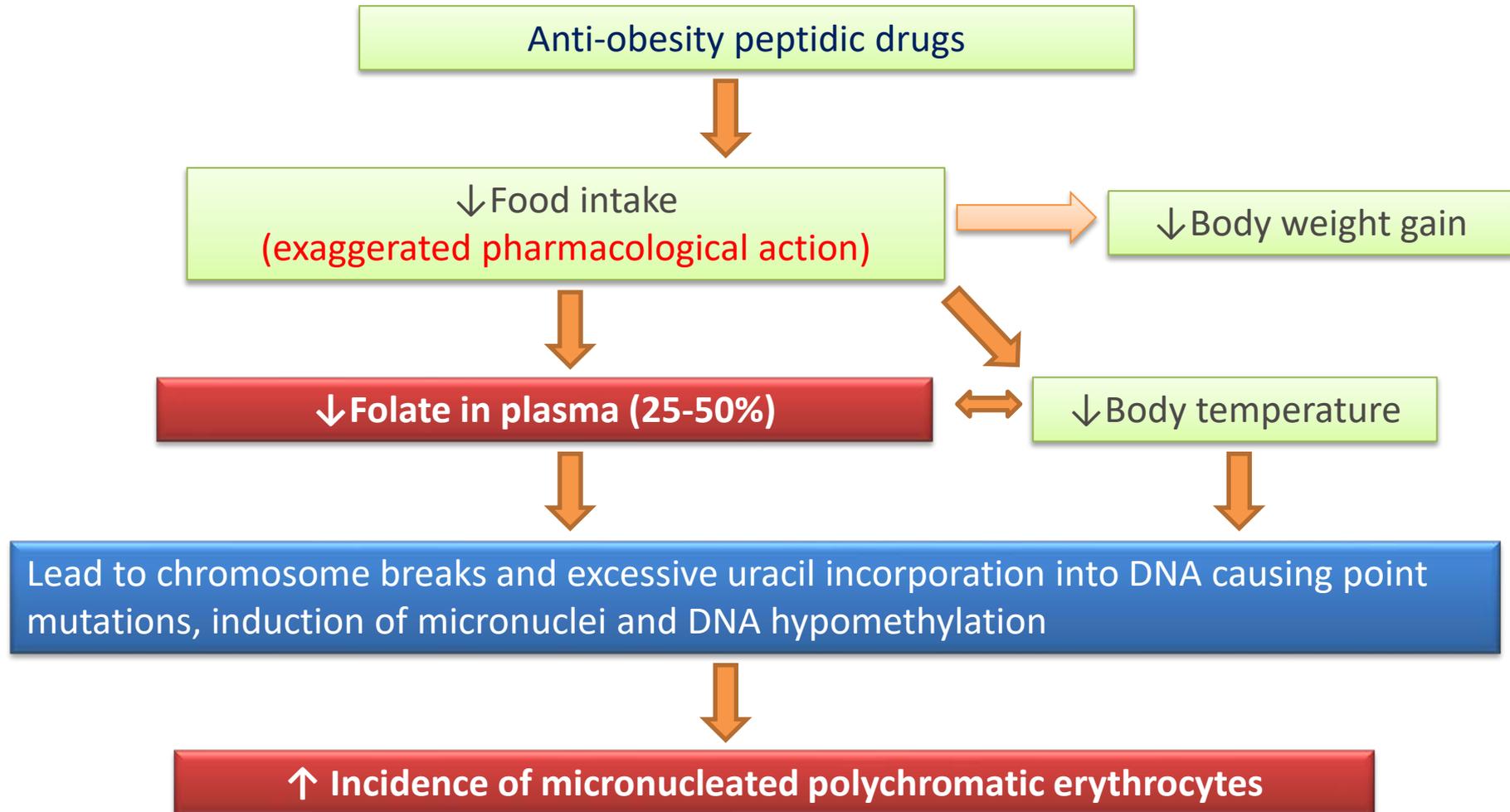
Male food consumption

Female food consumption



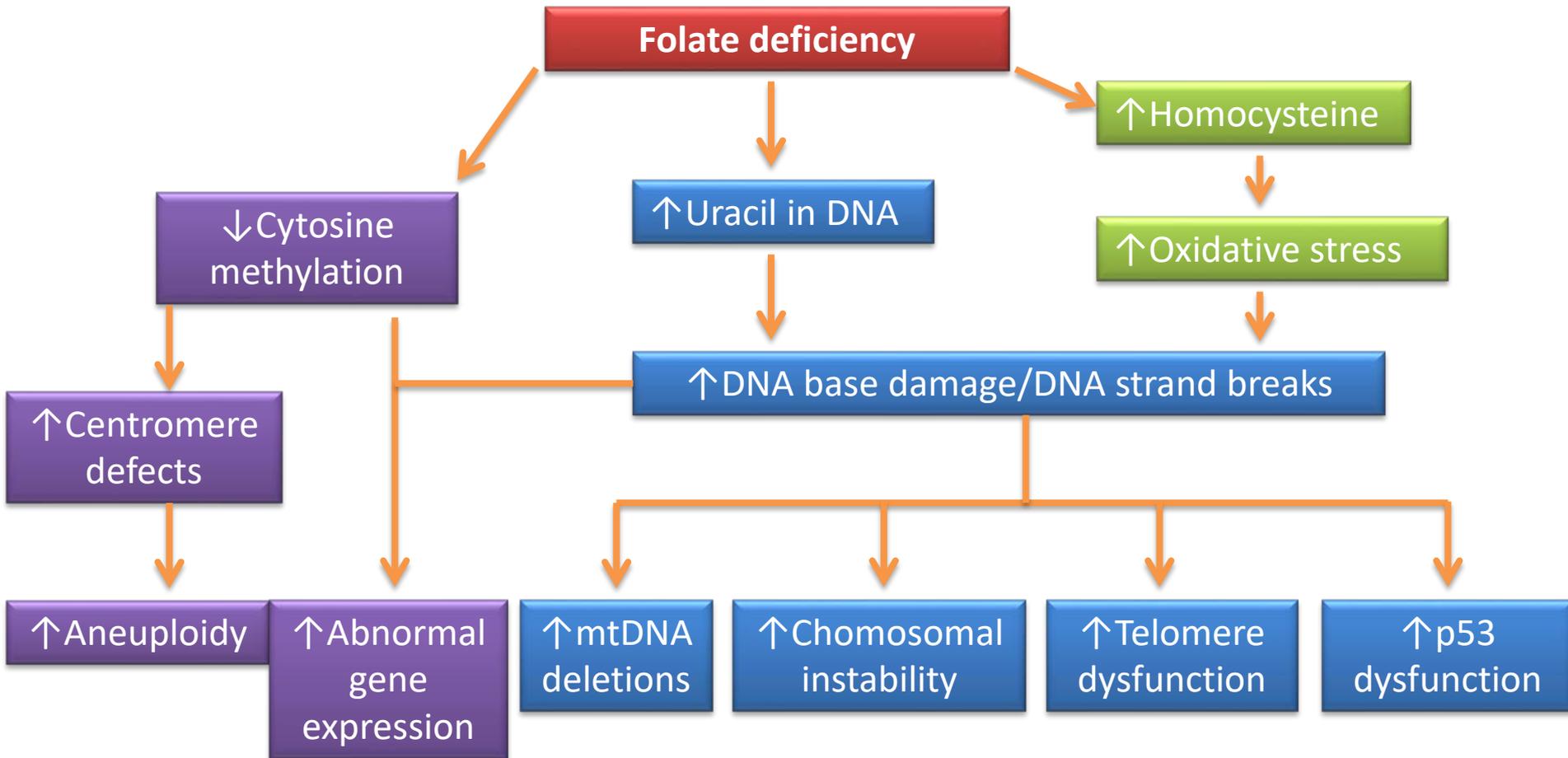
\* Small increases in the incidence of micronuclei in week 13

# Solution 1 for in vivo MNT positive results



**Folate plays a crucial role in maintaining genomic integrity**

# Various mechanisms by which folate deficiency can cause loss of genome integrity

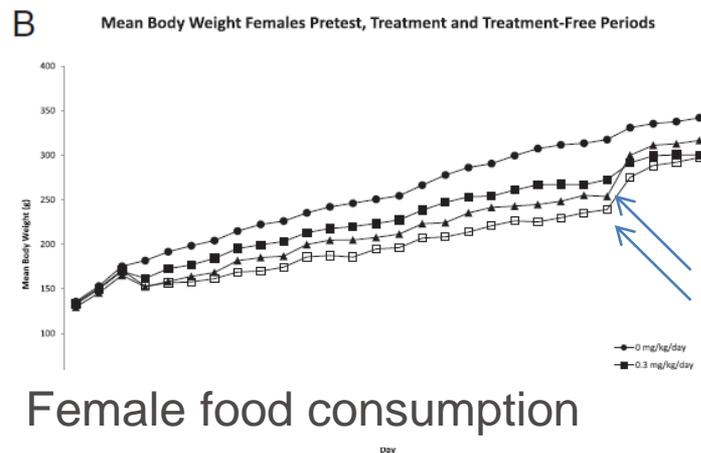


Guérard Tox. Appl Pharm 2014

# Reduction of body weight and food consumption induced by anti-obesity peptidic drugs in rats

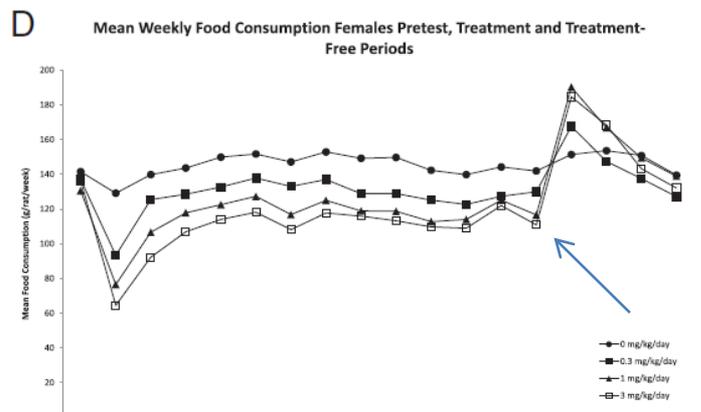
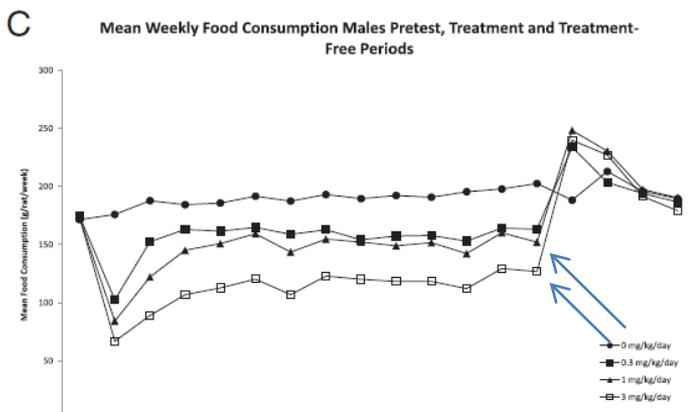
Male body weight

Female body weight



Male food consumption

Female food consumption



**Reduction in food consumption in males (25-35%) & females (15%)**  
 → ↑ incidence of micronucleated polychromatic erythrocytes

# Solution for IND of anti-obesity compound

- Indirect-DNA reactive mechanisms due to secondary to malnutrition, exaggerated pharmacological effects
- Possible to discuss safety margin
- Folate levels in plasma are monitorable in clinic
- Moving forward to IND

Any Question?