De-risking Strategies of Pancreatic Effects Induced by GI181771X, a Novel Cholecystokinin-1 Receptor Agonist for Obesity Indication

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Headlines

- **GI181771X (771)** – Background
- **771-Related pancreatic changes**
  - **Molecular mechanism** of pancreatic responses
  - **Species differences** (rodents & nonhuman primates)
  - **Clinical trial results** (pancreatic screens)
- **Rodents to humans translation**

GI181771X (771) – Background

- **771 –** 1,5-Benzodiazepine & orally active
  - Cholecystokinin-1 receptor (CCK1R) agonist &
  - CCK-2 receptor (CCK2R) antagonist
- **771 –** Developed in late 90's for Anti-obesity indication
  - CCK1R's role in mediating **"satiety"** signal to central nervous system (i.e., centrally acting mechanism)

Cholecystokinin (CCK)

- **CCK** – Peptide hormone, originally described as CCK-33 (33-amino acid peptide)
  - Isoforms – CCK-8, CCK-22, CCK-33, CCK-39 & CCK-58
  - CCK-8 → Major active/transmitter isoform
  - CCK-58 → Major intestinal isoform in rats & humans

- **CCK – Synthesized & secreted by**
  1. **Enteroendocrine I cells** (duodenal & jejunal mucosa) → *Intestinal CCK* (secreted in response to fatty acids & proteins in the intestinal lumen)
  2. **Neurons** (enteric nervous system, & brain) → *Neuronal CCK*

Cholecystokinin (CCK)

- **CCK activates CCK receptors & modulates physiological functions –** Pancreatic exocrine secretion, gallbladder contraction & delayed-gastric emptying etc.
- **CCK receptors** (G-protein coupled receptors) – two types:
  - **1. CCK-1 receptor (CCK1R)** – pancreas, gallbladder, stomach, small intestine, vagus nerve, intra-pancreatic neurons & hypothalamus
  - **2. CCK-2 receptor (CCK2R)** – neurons, pancreas, stomach & adrenals

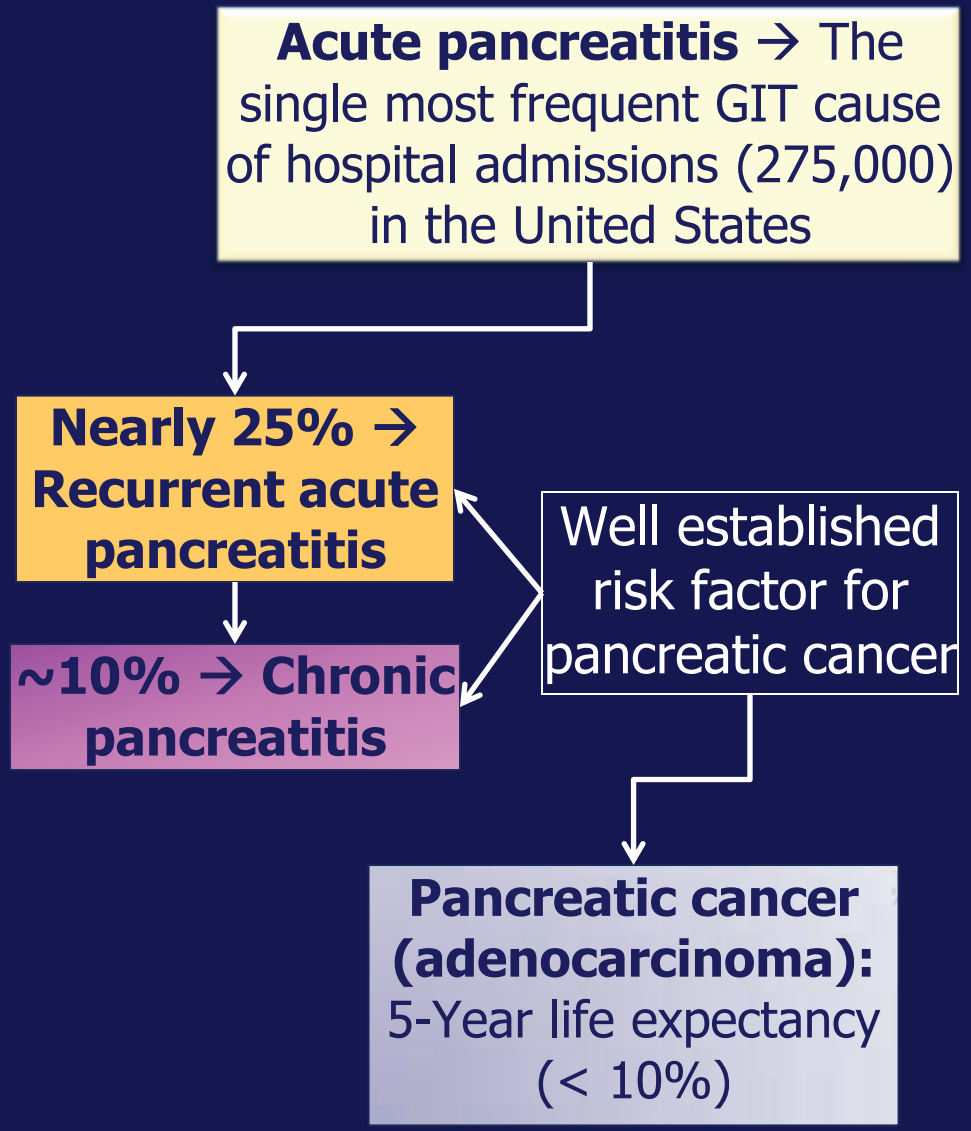
CCR1R mediates "satiety signal" via vagal afferent nerves to central nervous system → **a novel target for obesity treatment**

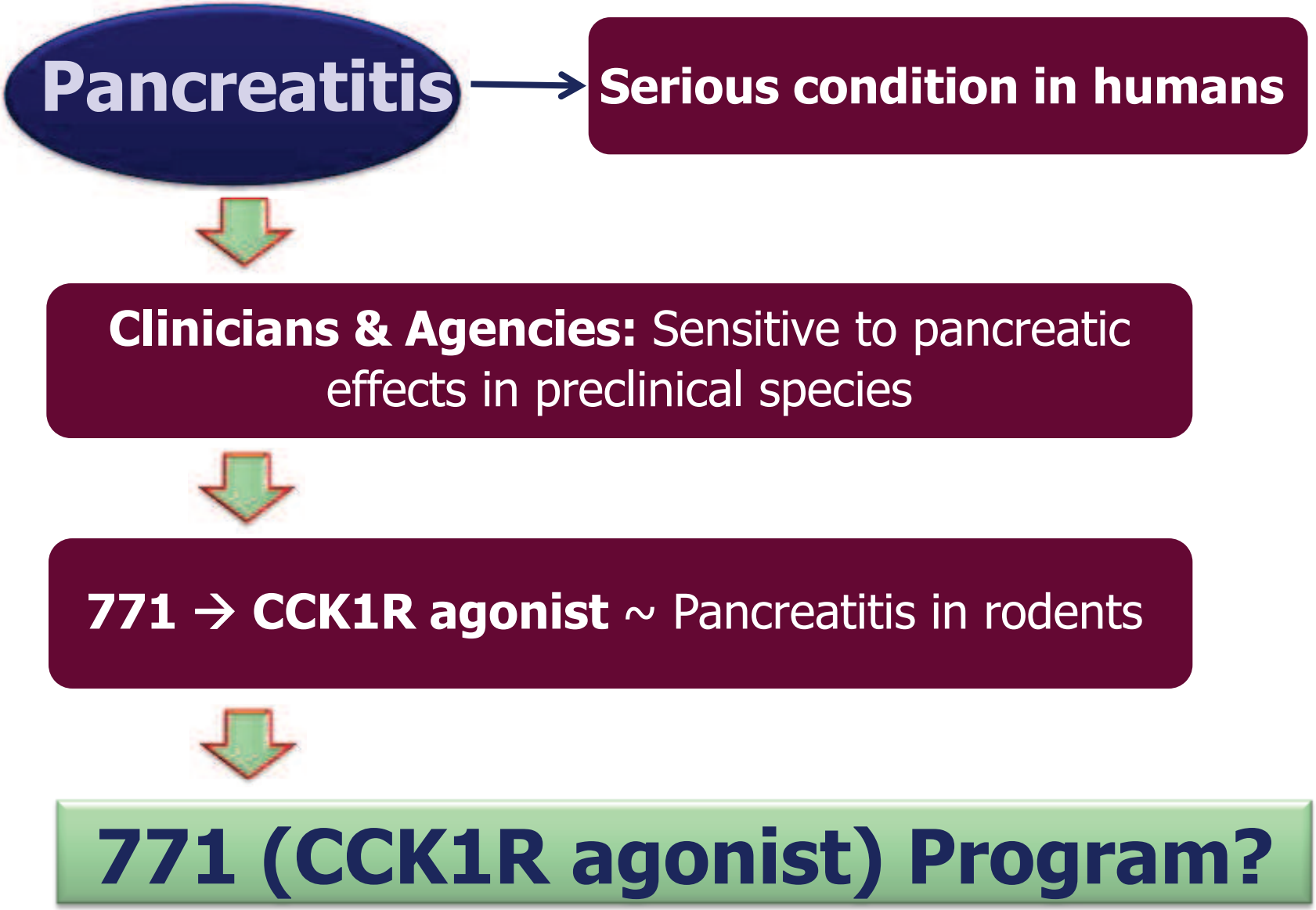
CCK & Rodent Exocrine Pancreas

- **CCK-induced pancreatitis in rodents** via CCK1R hyperstimulation → well known since **1970's**
  - **Rapid & highly reproducible**, histologically quite similar to early phase of acute pancreatitis in humans
  - **Widely used animal model** for experimental pancreatitis
- **771 → CCK1R agonist**
  - **Pancreatitis** – Rodent safety studies
  - **Rodent-specific hurdles** – dose limitation, morbidity & mortality, particularly in long-term & carcinogenicity studies

Pancreatitis – Humans?

The Epidemiology of Pancreatitis & Pancreatic Cancer. Gastroenterology 144: 1252–1261, 2013




Pancreatitis
Serious condition in humans
Clinicians & Agencies: Sensitive to pancreatic effects in preclinical species
771 → CCK1R agonist ~ Pancreatitis in rodents
771 (CCK1R agonist) Program?

771 (CCK1R agonist) Program?

- **Scientific rationales:**

  1) **CCK1R-mediated "satiety signal"** – a novel mechanism to treat obesity

  2) **Several efficacy studies in humans** (published – 1981, 1988, 1994 & 2001) with CCK-8 & CCK-33 (iv infusion) → increase perception of fullness, decrease hunger & reduced energy intake

  3) **Inter-species related differences** in CCK1R expression & CCK-induced pancreatic responses (relevant publications & in-house data)

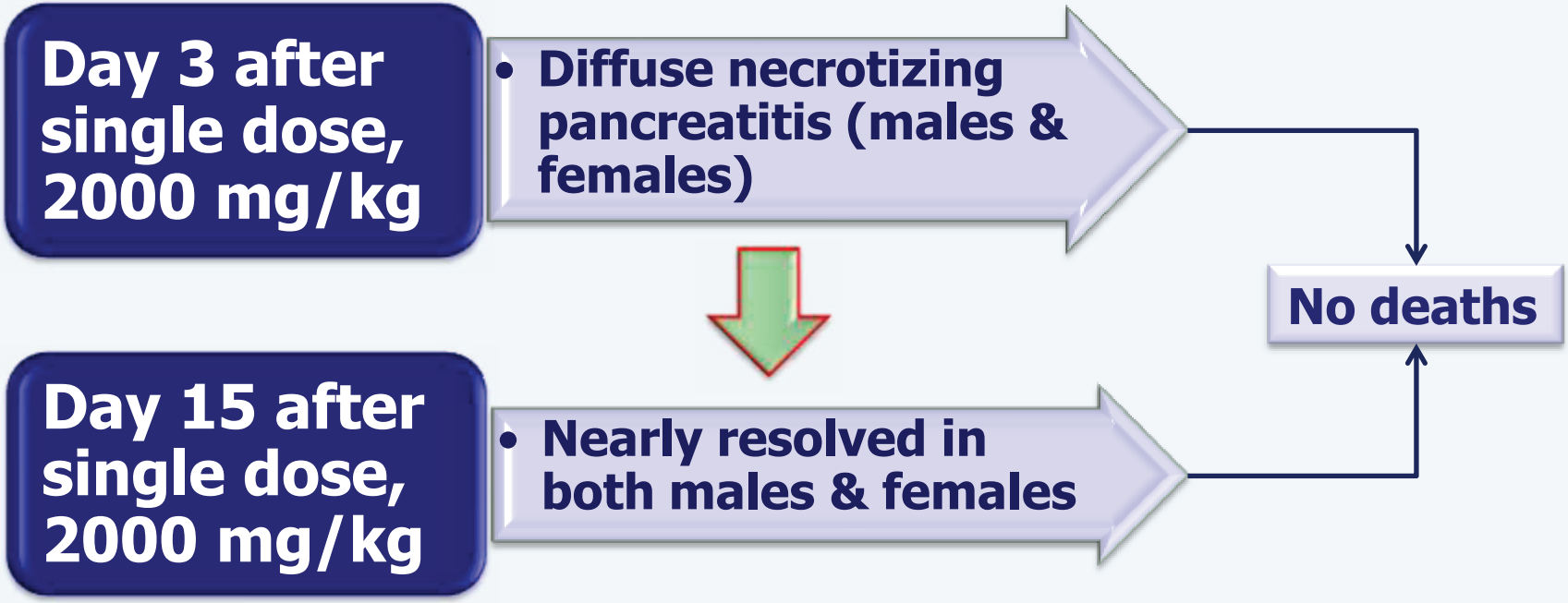
771-Related Pancreatic Responses: Rats, Mice, Monkeys & Humans

Studies:

- **Acute (single) dose study in rats & mice**
- **Repeat dose studies**
  - **Rats:** 7-Day, 4-Week & 26-Week
  - **Monkeys:** 4-Week, 26-Week & 52-Week
  - **Humans:** 24-Week Clinical trial in overweight & obese patients

771: Acute Study in Rats

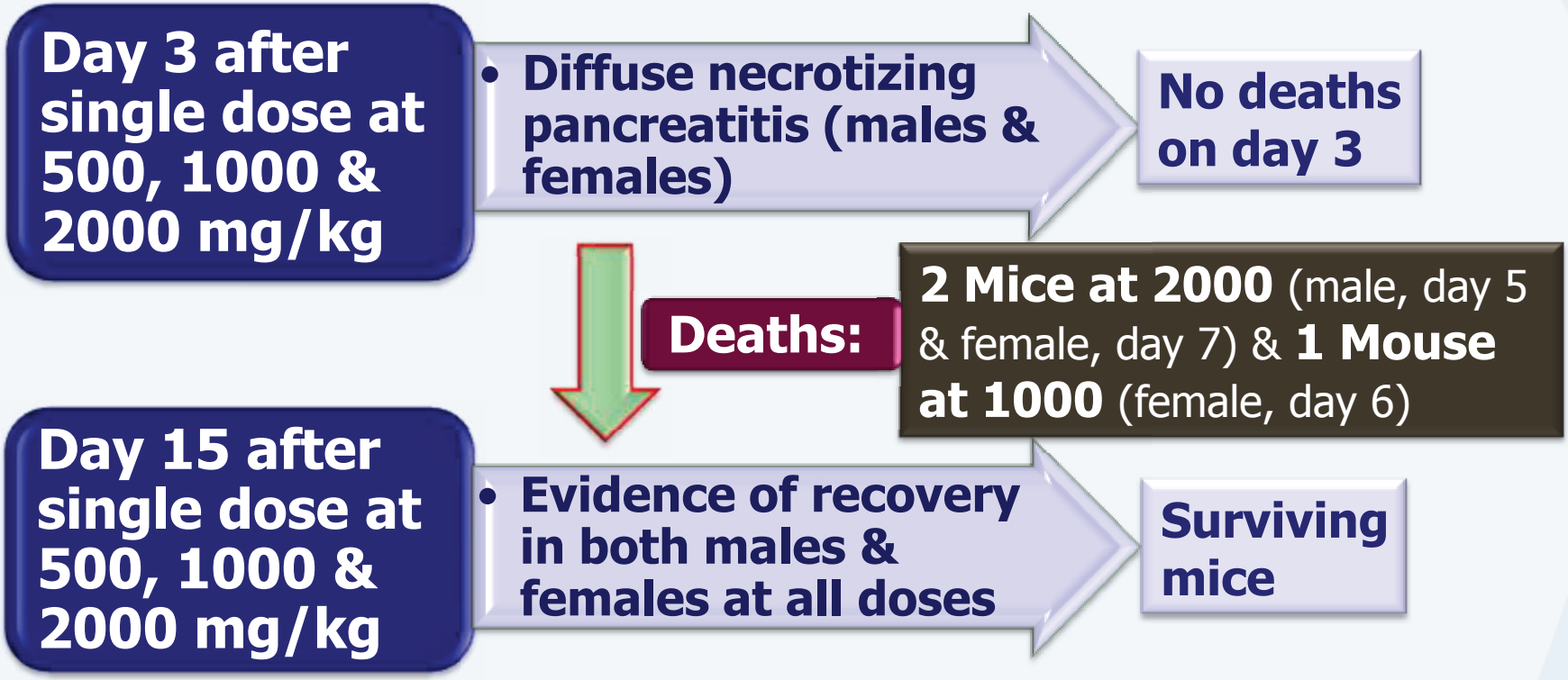
- **12 Male & 12 Female animals: 6 Rats/Sex/Group**
  - **Single oral dose** at 2000 mg/kg 771 & vehicle[1] & sacrificed on Day 3 & Day 15



[1]Polyethylene glycol (PEG) 400

771: Acute Study in Mice

- **24 Male & 24 Female mice: 6 Mice/Sex/Group**
  - **Single oral dose at 500, 1000 & 2000 mg/kg** 771 & vehicle[1]) & sacrificed on Day 3 & Day 15



[1]Polyethylene glycol (PEG) 400

Differences in Pancreatic Responses – Mice & Rats

- **At 2000 mg/kg 771** – Severity/necrosis in mice > rats
- **At 2000 mg/kg 771** – Recovery in rats > mice

  - **Systemic exposure in mice (~ 2x) > rats**

    AUC: 8865 hr.ng/ml (mice) versus 4415 hr.ng/ml (rats)

  - **CCK1R structure/binding differences** between mice & rats*

    - For example, CCK analog JMV-180 → partial agonist (rats) & full agonist (mice)

*Ji et al., 2000. Species Differences between Rat and Mouse CCKa receptors Determine the Divergent Acinar Cell response to the Cholecystokinin Analog JMV-180. J Biol Chem 25:19115-19120.
*Matozaki et al., 1989. A new CCK analog differentiates two functionally distinct CCK receptors in rat and mouse pancreatic acini. Am J Physiol 257:594-600.

Repeat-dose studies:

1. Rats
2. Cynomolgus monkeys
3. Human clinical trial

771: Repeat-dose Studies in Wistar Han Rats

Study	7-Day	4-Week	26-Week
Study type	**Non-GLP**	**GLP**	**GLP**
Number of Rats/Dose	6 Males	10+8[1] Males & 10+8[1] Females	16+8[2] Males & 16+8[2] Females
Doses[3] (mg/kg/day)	0 (Vehicle[4]), 0.5, 10, 50 & 250	0 (Vehicle[4]), 0.25, 0.5 & 50[1]	0 (Vehicle[5]), 1, 15, 50 & 100[2]
Endpoints[5]	Routine non-GLP Partial tissue list	Routine GLP Full tissue list	Routine GLP Full tissue list

[1]2-Week recovery (50 mg/kg/day); [2]4-Week recovery (100 mg/kg/day); [3]Oral dose; [4]Vehicle = Polyethylene glycol (PEG) 400; [5]Vehicle = 0.5% hydroxypropylmethylcellulose & 0.1% Tween 80

[5]Pancreatic effects

Results: Clinical Pathology (Amylase & Lipase)

Amylase (IU/L)

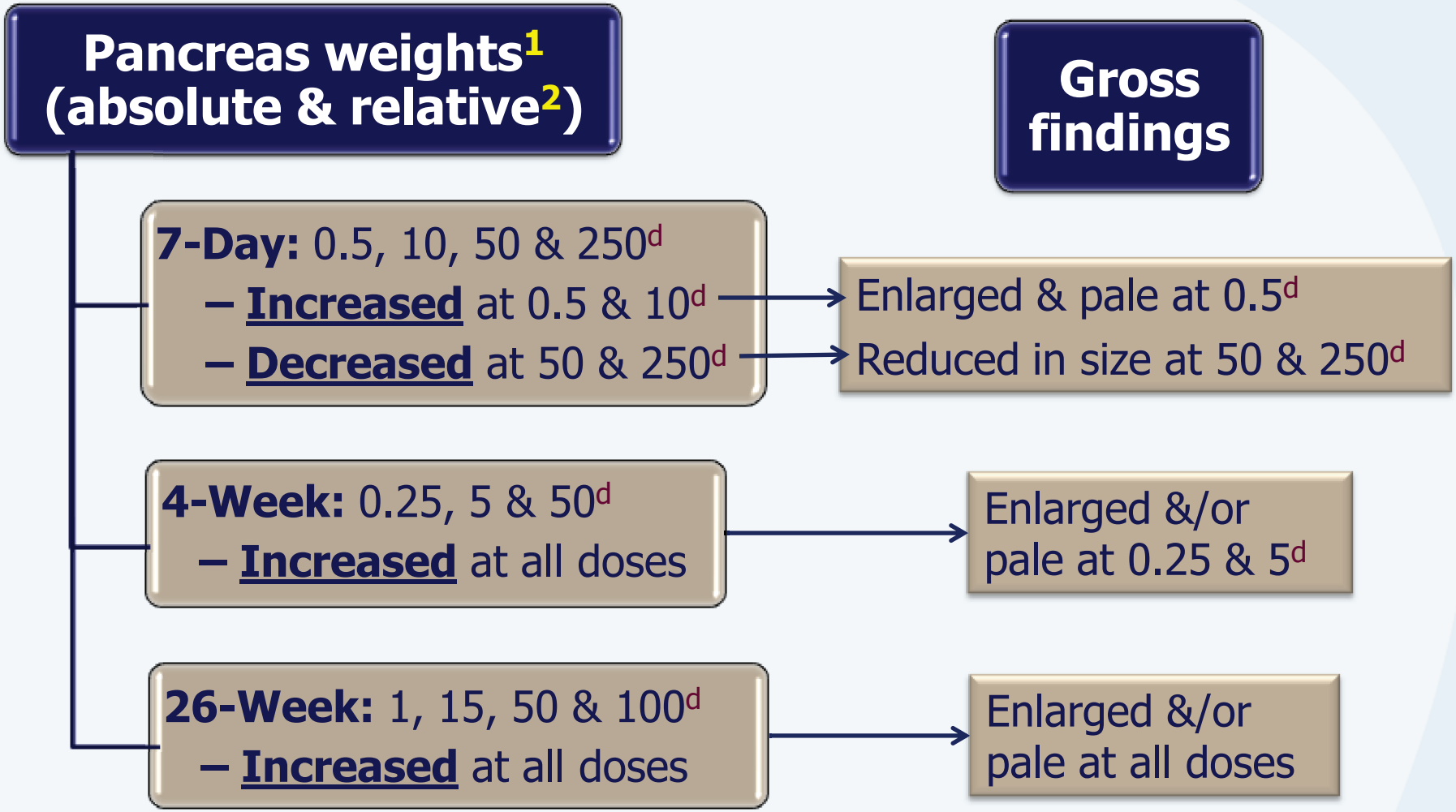
- **7-Day:** 10-23% decreased at all doses (0.5, 10, 50, 250 mg/kg/day)
- **4-Week:** 15% decreased at 50 mg/kg/day (males only) (0.25, 5 & 50 mg/kg/day)
- **26-Week:** 9-15% decreased at 15, 50 & 100 mg/kg/day (males & females); & no change at 1.0 mg/kg/day)

Lipase (IU/L)

- **7-Day & 4-Week:** No test article-related changes
- **26-Week:** Minimal increase at all doses but highly variable

Amylase & lipase values: Not consistent & highly variable

Results: Pancreatic Weights/Gross Findings

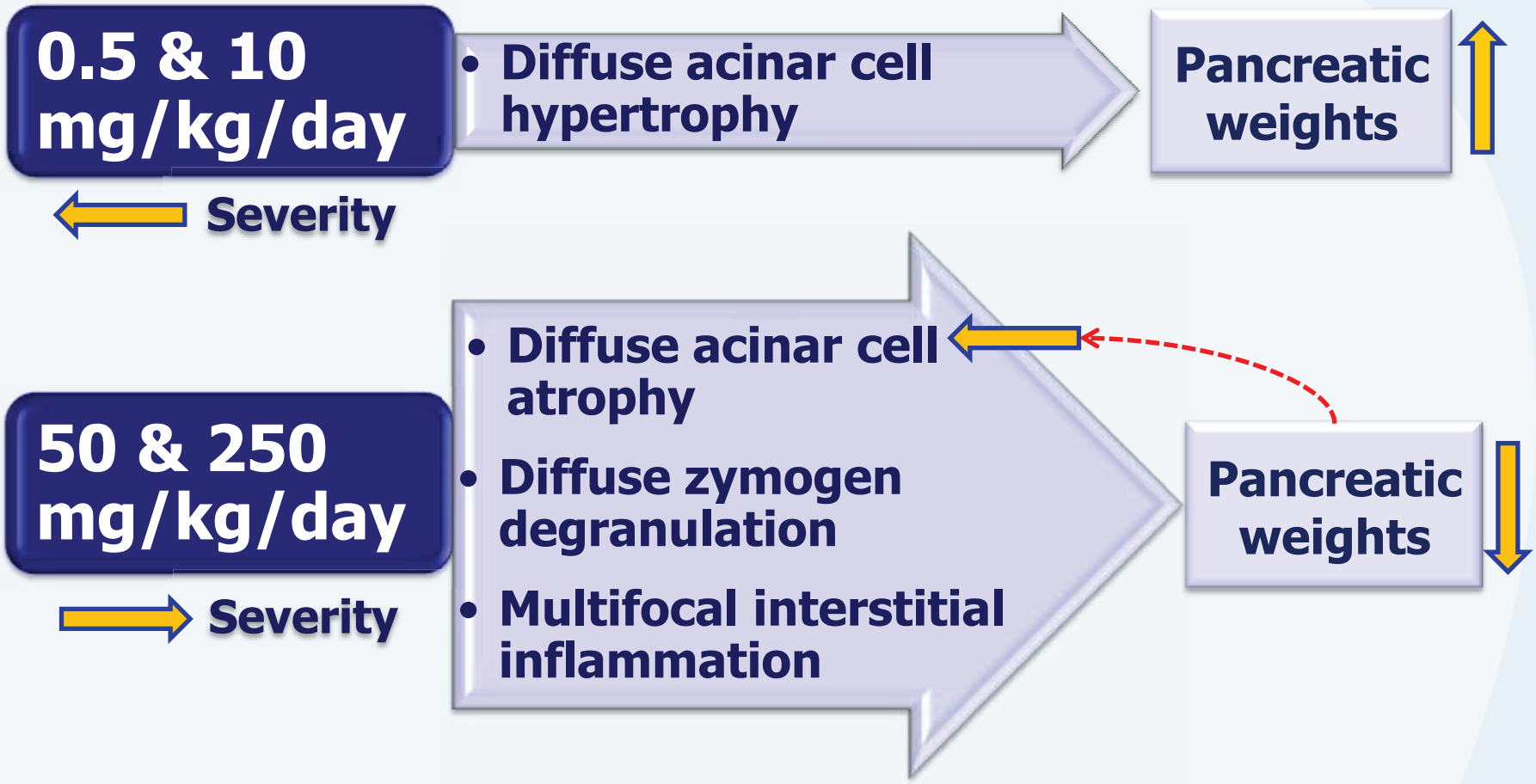


[1]P ≤ 0.05 or 0.01; [2]Relative to terminal body weight & [d]mg/kg/day

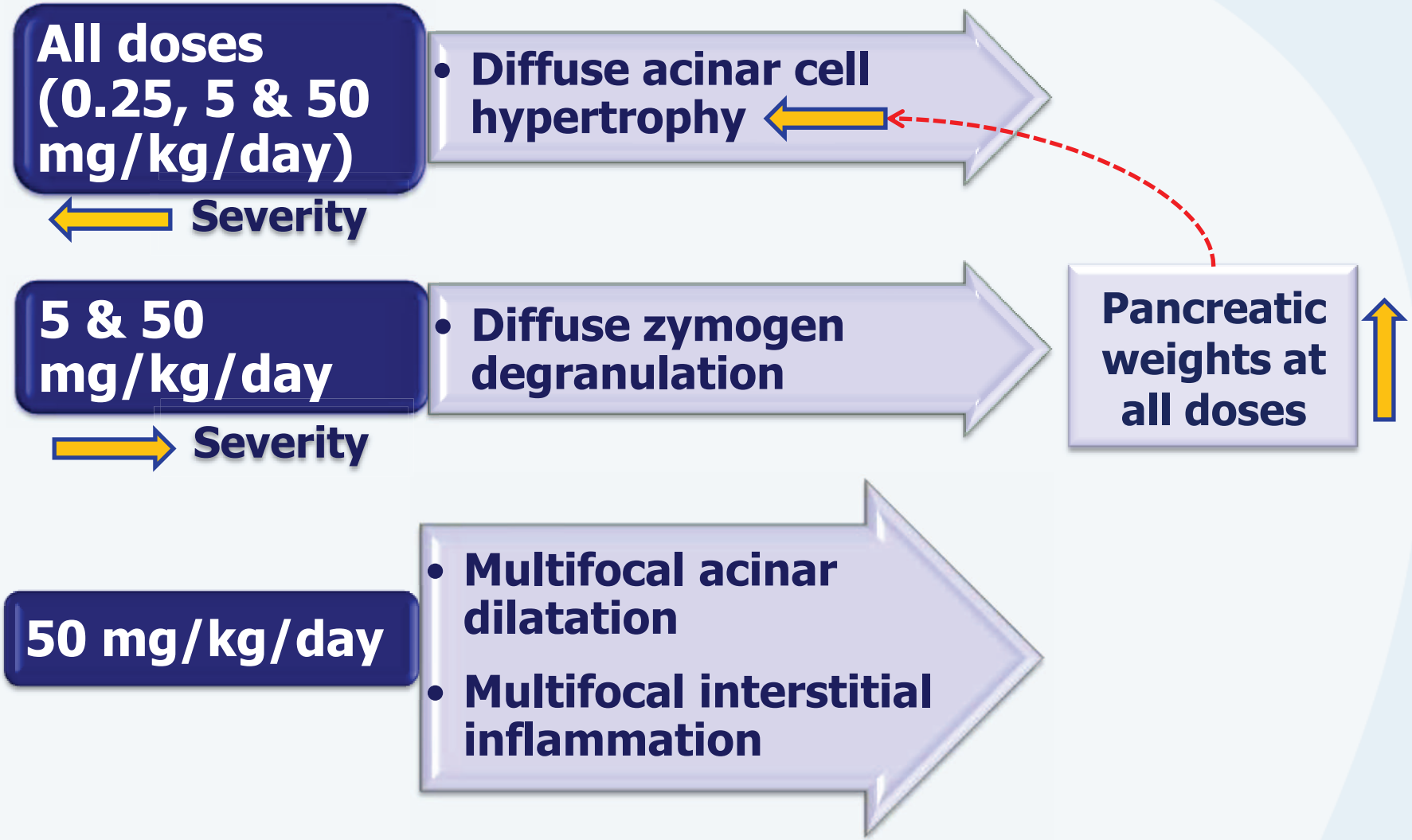
Results: Pancreatic Pathology in Rats

- **Major histopathological findings (7-Day, 4-Week & 26-Week):**
  - **Acinar cell hypertrophy** – enlarged cells with increased zymogen granules &/or decreased zymogen granules plus cytoplasmic basophilia
  - **Zymogen degranulation** – decreased cytoplasmic zymogen granules with or without cytoplasmic vacuolation
  - **Acinar cell atrophy** – decreased cell size with relatively less zymogen granules & cytoplasmic basophilia
  - **Interstitial inflammation** (primarily, mononuclear cells) &/or **interstitial fibrosis**
  - **Focal acinar cell hyperplasia** – well demarcated area (< 3 mm in diameter), tubular-glandular pattern with occasional mitotic figures & some degree of compression of adjacent parenchyma

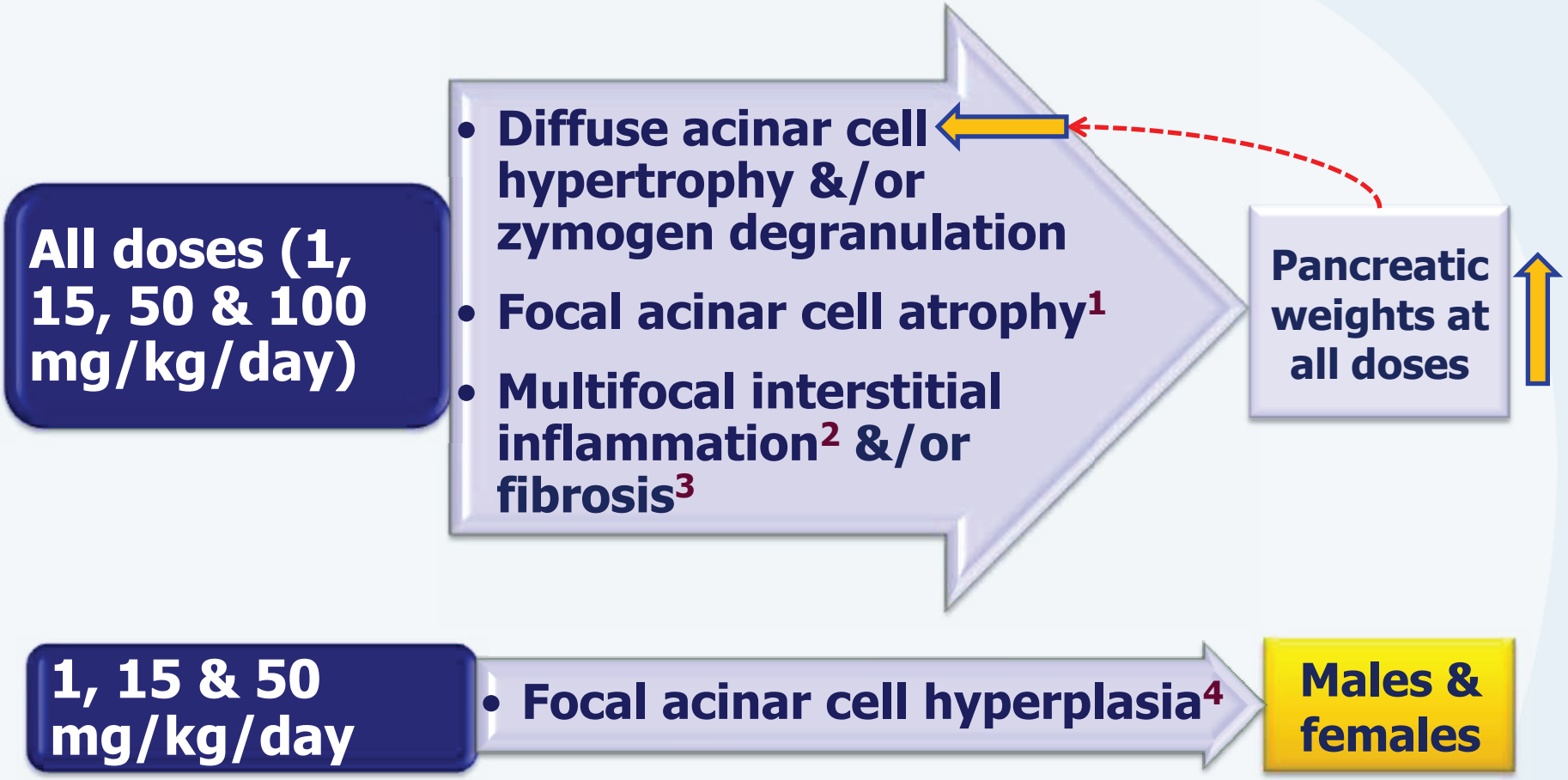
7-Day Rat Study: Dose-related Pancreatic Findings
(Doses: 0.5, 10, 50 & 250 mg/kg/day)



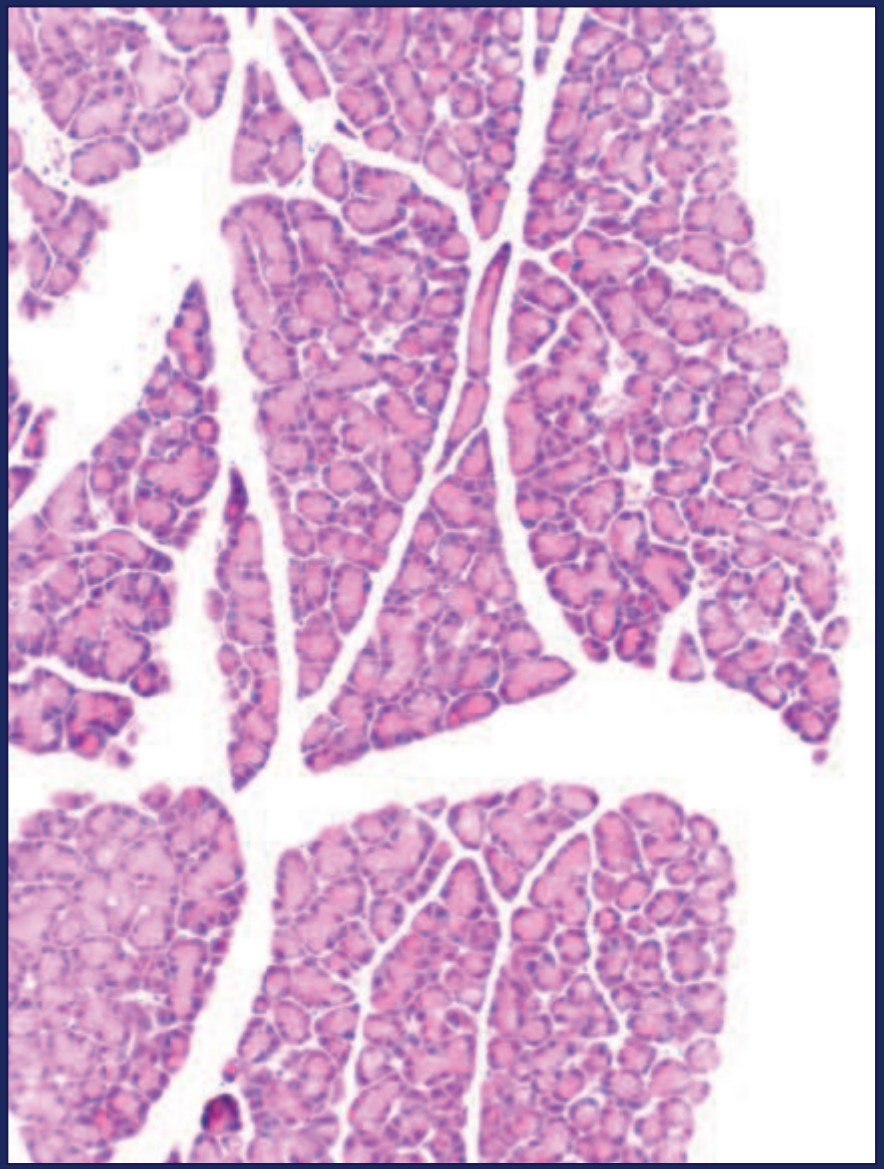
4-Week Rat Study: Dose-related Pancreatic Findings
(Doses: 0.25, 5 & 50 mg/kg/day)



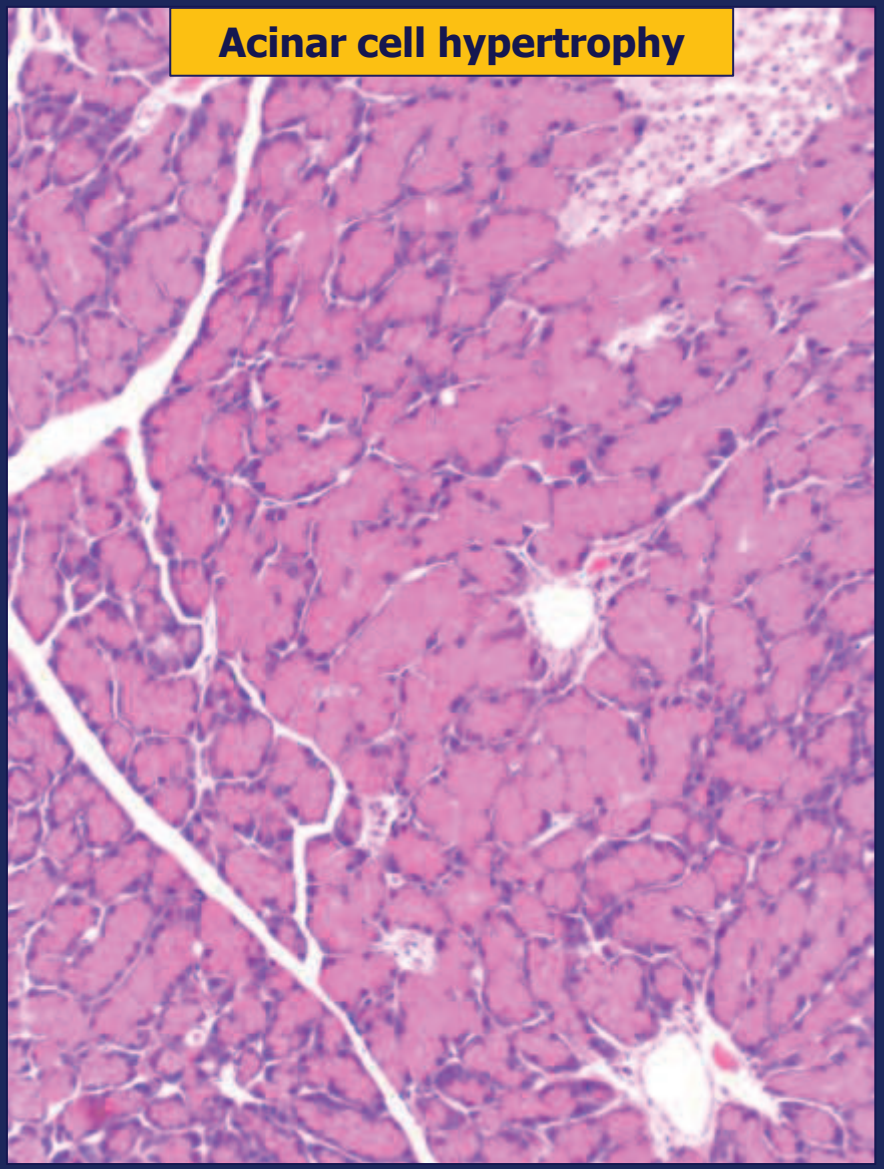
26-Week Rat Study: Pancreatic Findings (Doses: 1, 15, 50 & 100 mg/kg/day)



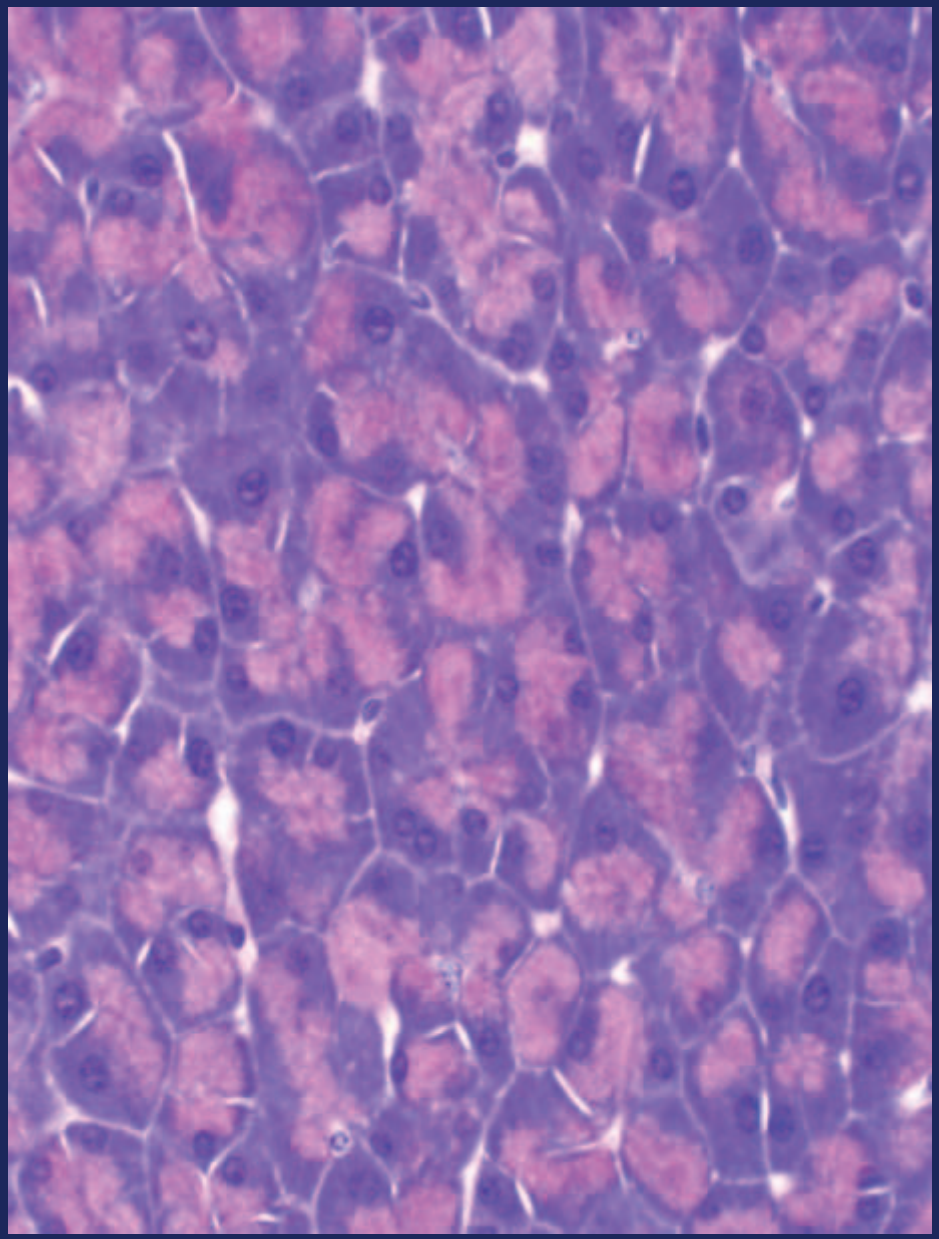
[1]Females all doses; males ≥ 15 mg/kg/day; [2]Males all doses; females ≥ 50 mg/kg/day; [3]Females at 100 mg/kg/day; [4]3/16 Males & 3/15 females at 15 mg/kg/day & 1/11 & 2/15 females at 1 & 50 mg/kg/day, respectively



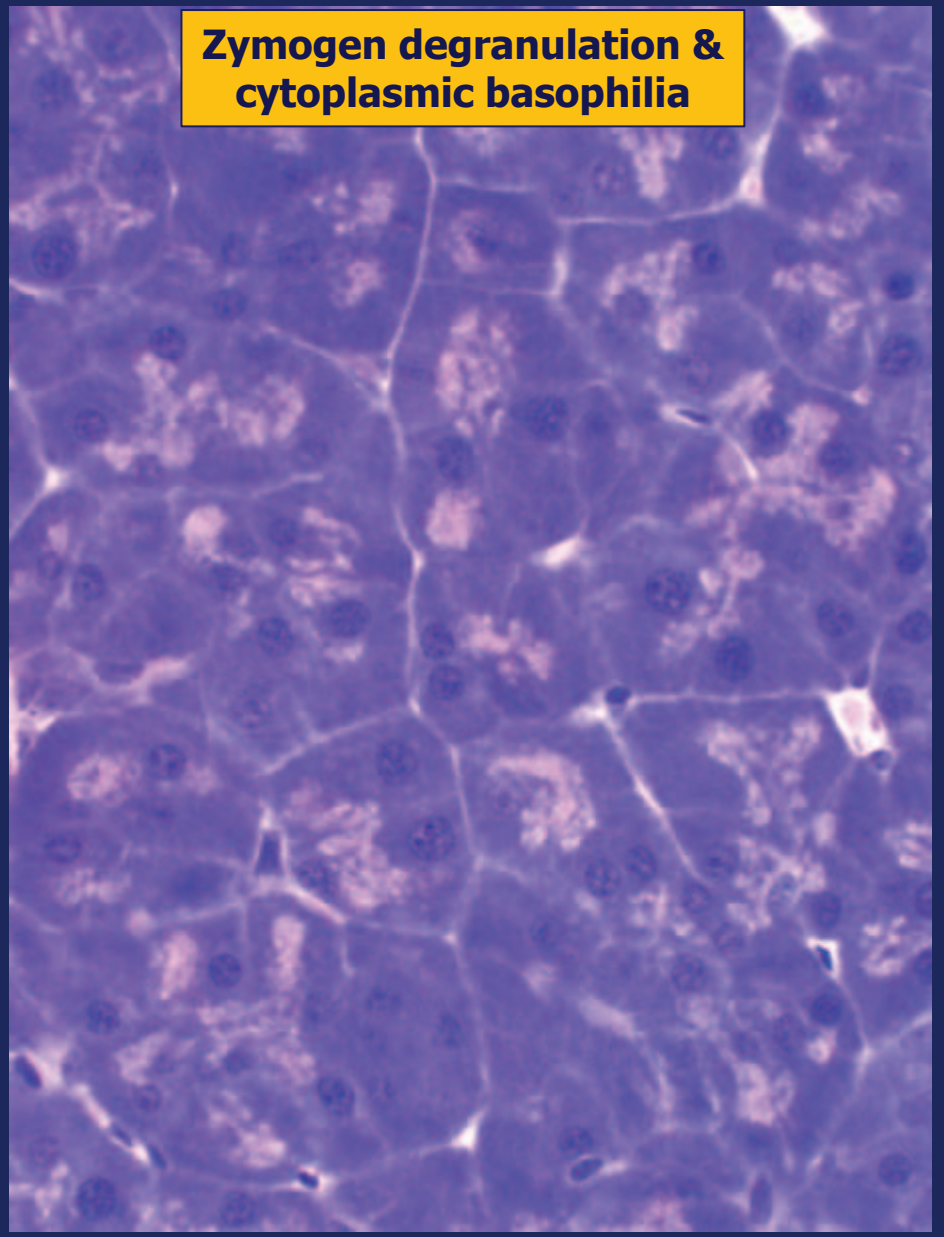
Pancreas from a **control rat**, H&E 100X



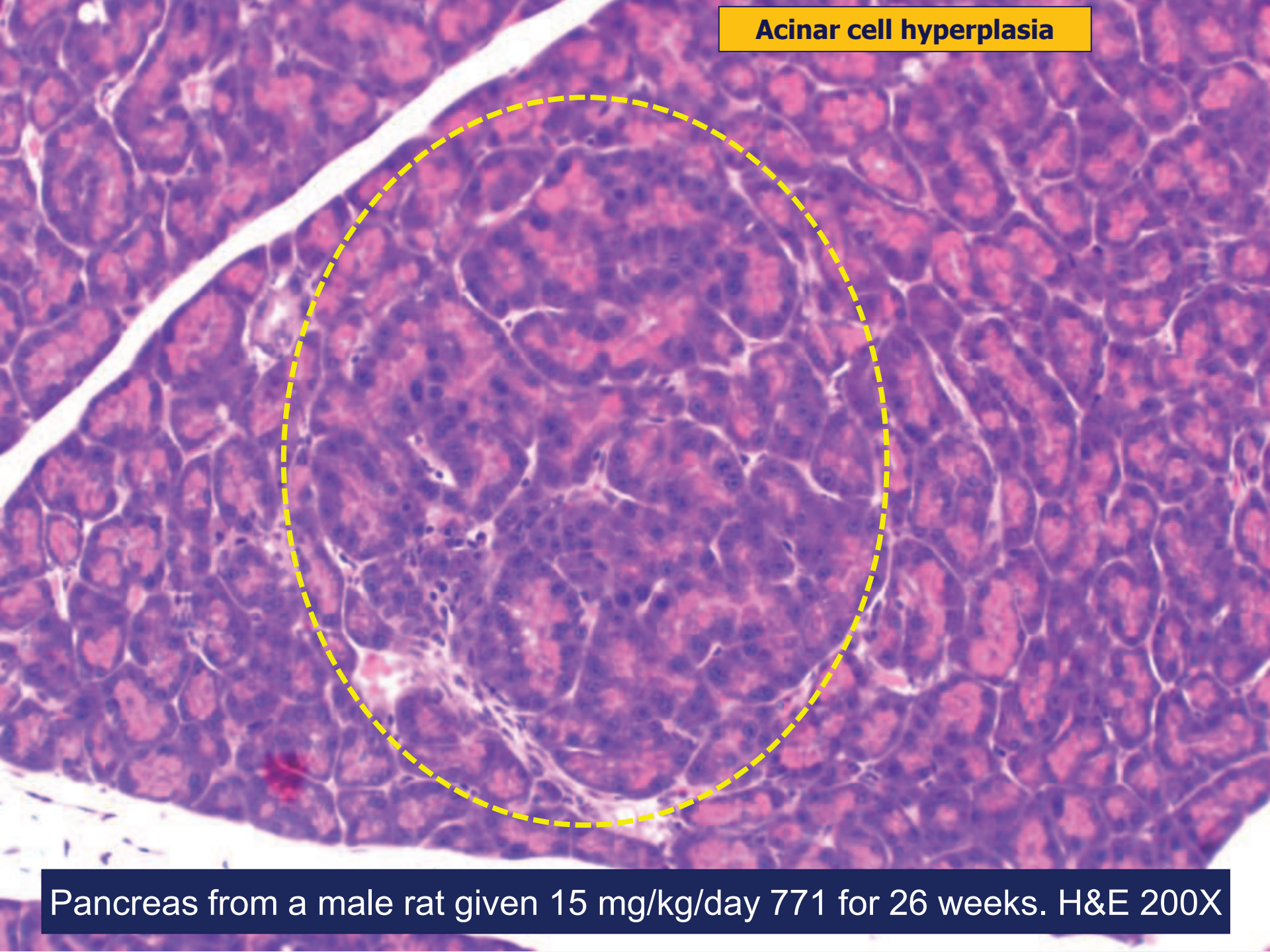
Pancreas from a **rat given 0.25** mg/kg/day 771 for 4 weeks. Note diffuse acinar cell hypertrophy, H&E 100X



Control pancreas. H&E 400X



Note zymogen degranulation &
cytoplasmic basophilia. H&E 400X

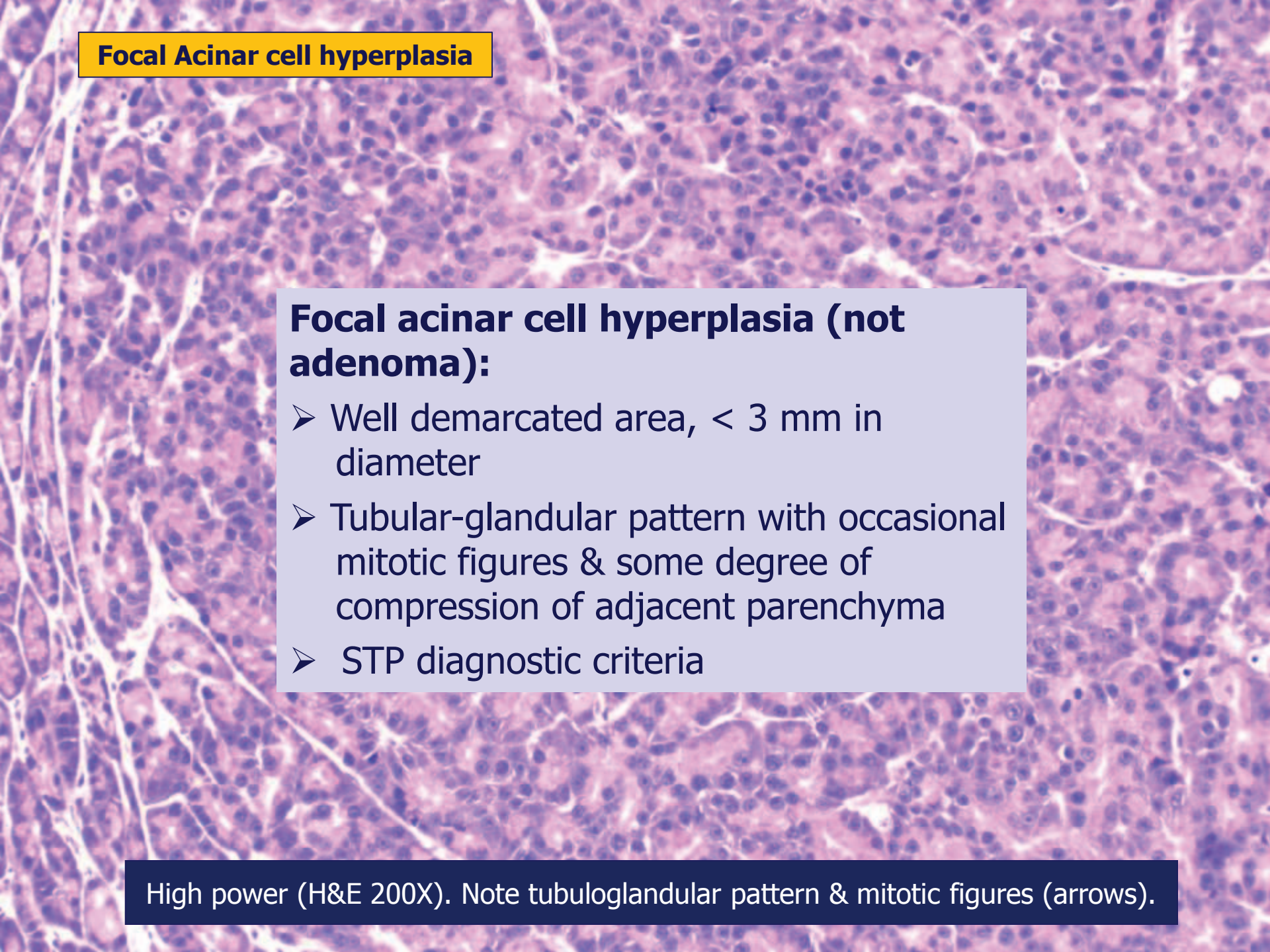


Pancreas from a male rat given 15 mg/kg/day 771 for 26 weeks. H&E 200X

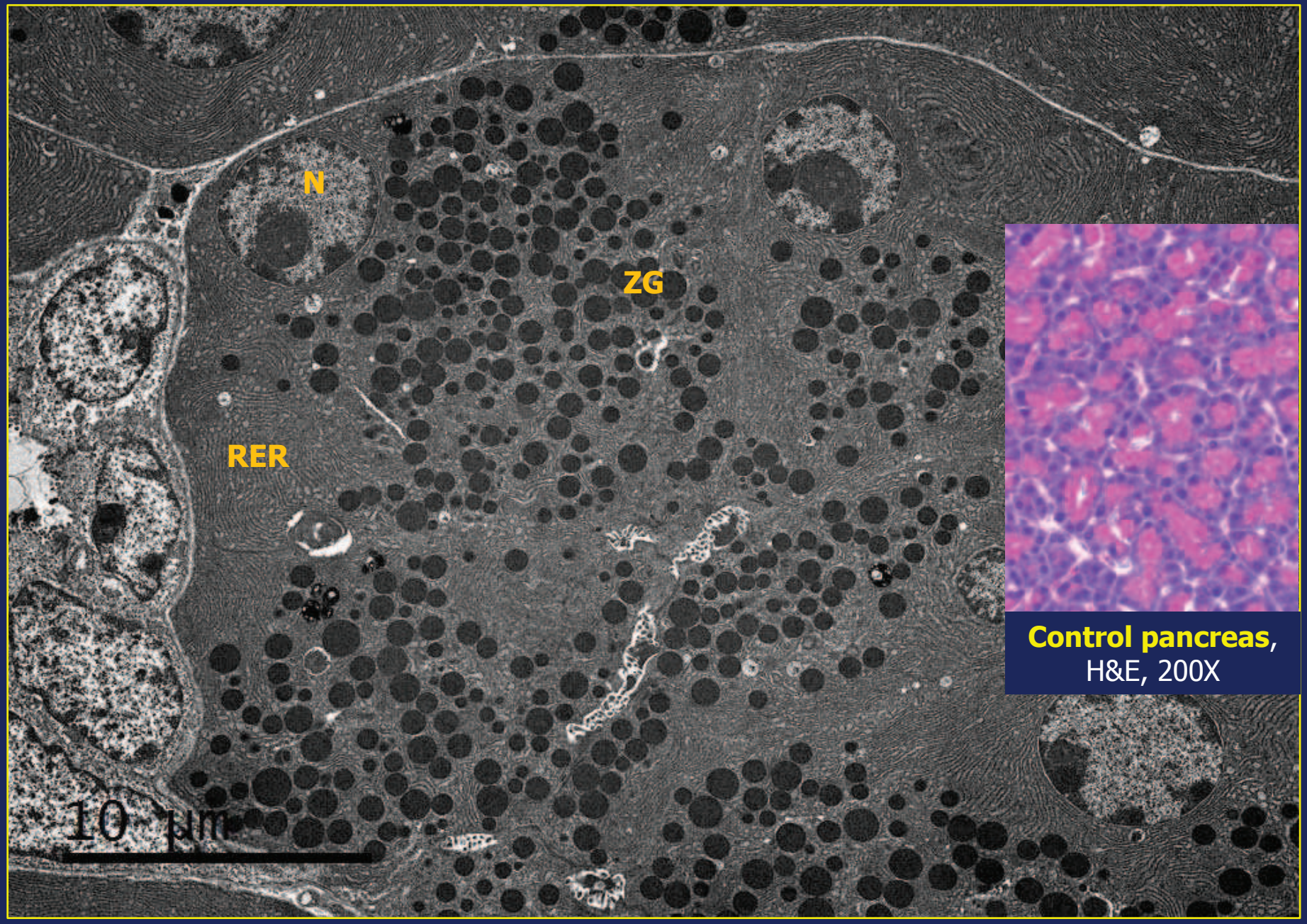
Focal Acinar cell hyperplasia

Focal acinar cell hyperplasia (not adenoma):

- Well demarcated area, < 3 mm in diameter
- Tubular-glandular pattern with occasional mitotic figures & some degree of compression of adjacent parenchyma
- STP diagnostic criteria

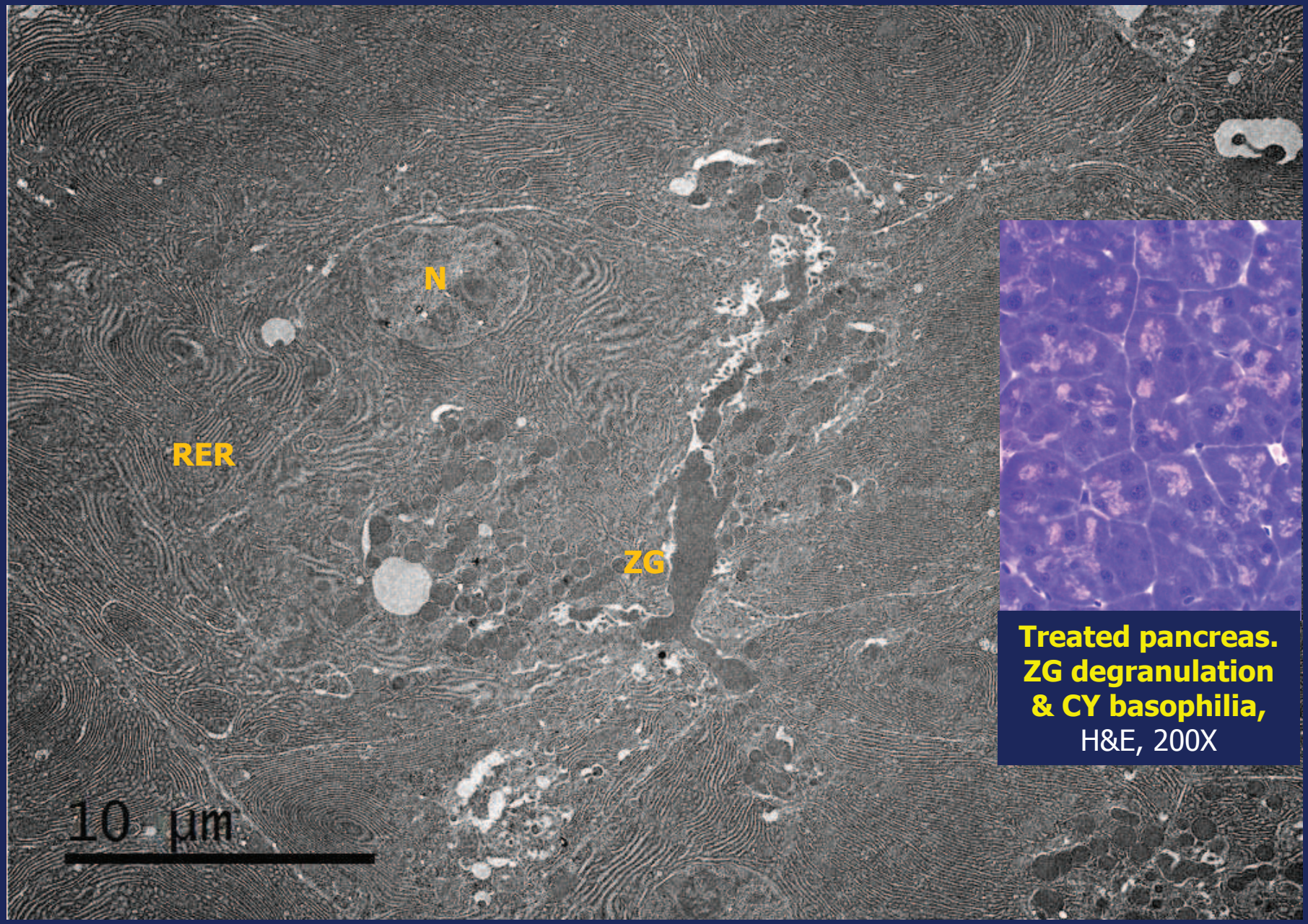


High power (H&E 200X). Note tubuloglandular pattern & mitotic figures (arrows).



Control pancreas, H&E, 200X

TEM of control pancreas – Note acinar cells with zymogen granules (ZG) & rough endoplasmic reticulum (RER)



Treated pancreas. ZG degranulation & CY basophilia, H&E, 200X

TEM of zymogen (ZG) degranulation & cytoplasmic (CY) basophilia.
Note acinar cells with abundant RER & few zymogen granules

Summary of 771-related Findings in Wistar Han rats

Study	7-Day	4-Week	26-Week
Doses (mg/kg/day)	0 (vehicle), 0.5, 10, 50 & 250	0 (vehicle), 0.25, 5 & 50	0 (vehicle), 1, 15, 50 & 100
Amylase & Lipase	Amylase (decreased) & Lipase (increased) changes → Not consistent among studies & highly variable		
Pancreatic weights	Increased (0.5, 10) Decreased (50, 250)	Increased – all doses (0.25, 5 & 50)	Increased – all doses (1, 15, 50 & 100)
Pancreatic changes – microscopic	**0.5 & 10:** DACH **50 & 250:** DACA, Z-D & I-INF	**0.25, 5 & 50:** DACH **5 & 50:** Z-D **50:** A-D & I-INF	**1, 15, 50 & 100:** DACH, Z-D, FACA, I-INF & IF **1, 15 & 50:** ACHP
[1]**Recovery – microscopic**	No Recovery group	2-Week: reversible (except I-INF)	4-Week: reversible (except IF)

DACH = Diffuse acinar cell hypertrophy; **DACA** = Diffuse Acinar cell atrophy; **DACA** = Diffuse acinar cell atrophy; **FACA** = Focal acinar cell atrophy; **A-D** = Acinar dilatation; **Z-D** = Zymogen degranulation; **I-INF** = Interstitial inflammation; **IF** = Interstitial fibrosis;& **ACHP** = Acinar cell hyperplasia

Repeat-dose studies:

1. Rats
2. Cynomolgus monkeys
3. Human clinical trial

Repeat Dose Studies in Cynomolgus Monkeys

Study	4-Week	26-Week	52-Week
Number of animals/Dose	3 Males+2[1] & 3+2[1] Females	4+2[1] Males & 4+2[1] Females	4+2[1] Males & 4+2[1] Females
Doses (mg/kg/day)	0 (Vehicle[2]), 1, 50 & 250/125	0 (Vehicle[3]), 10, 75 & 150[4]	0 (Vehicle[3]), 50, 125 & 300/500[5]
Amylase & lipase	**No change**	**No change**	**No change**
Pancreatic weights	**No change**	**No change**	**No change**
Microscopic findings (pancreas)	**None**	**None**	**None**

[1]Recovery; [2]Vehicle = Polyethylene glycol (PEG) 400; [3]Vehicle = 0.5% hydroxypropylmethyl-cellulose & 0.1% Tween 80; [4]Doses represent 25x, 188x & 375x **anticipated maximum clinical dose** of 24 mg/day (0.4 mg/kg/day, based on a 60 kg individual); Based on body surface area (mg/m^2) 8x, 61x & 122x anticipated maximum clinical dose, 14.8 mg/m^2; [5]Doses represent 167x, 417x & 1000x **anticipated maximum clinical dose** of 15 mg/day (0.3 mg/kg/day, based on a 50 kg individual); Based on body surface area (mg/m^2) 58x, 147x & 353x anticipated maximum clinical dose, 10.2 mg/m^2

771: Clinical Trial in Overweight & Obese Patients*

- **24-Week randomized double-blinded study**
  - **701 (467 Women & 234 men),** overweight (BMI >27 kg/m^2 & <30 kg/m^2) or obese (BMI >30 kg/m^2) patients
  - **Doses:** 0.25, 0.5, 1.0 & 1.5 mg (soft gelatin capsules), t.i.d.
- **Primary efficacy endpoint:** Absolute body weight change from baseline at week 24
  - **Monitor pancreatic & gallbladder effects:** abdominal ultrasound & MRI before & after treatment
  - **Clinical pathology** – extended parameters to monitor hepatobiliary & pancreatic abnormalities

*Jordan et al. Clin Pharmacol Therap 83:281287, 2008.

771: Clinical Trial Results

- **Results:**
  - **No** 771 treatment-associated abnormalities in pancreatic or hepatobiliary structure (ultrasound & MRI)
  - **No** laboratory abnormalities of pancreatic/hepatobiliary disease

771 → Did not reduce body weight & development stopped*

771 Data: Rodents – Monkeys – Humans

*Jordan et al. Clin Pharmacol Therap 83:281287, 2008.

771 (CCK1R agonist) in rats, monkeys & humans

RAT STUDIES
Acute, 7-Day, 4-Week & 26-Week

- Repeat-dose (0.25 to 250 mg/kg/day)
- Profound pancreatic changes (weights, macroscopic & microscopic changes)
- Amylase ↓ & lipase ↑ but not consistent & variable

MONKEY STUDIES
4-Week, 26-Week & 52-Week

- Repeat-dose (1 to 500 mg/kg/day)
- Higher systemic exposure than rats & longer treatment
- No clin-path, weights, macroscopic or microscopic changes in the pancreas

24-WEEK CLINICAL TRIAL*

- No abnormalities in the pancreatic structure (abdominal ultrasound & MRI)
- No laboratory abnormalities

*1.5 mg t.i.d. (4.5 mg per day), weight ~ 100 kg & BMI 35.3 kg/m^2

771 → Inter-species variation

CCK1R expression profiles – Pancreatic Acinar cells

RODENTS

- CCK1R: Abundantly expressed

MONKEYS[1]

- CCK1R: Non-detectable (RPA)
- Weakly positive (RT-PCR) → low CCK1R

HUMANS

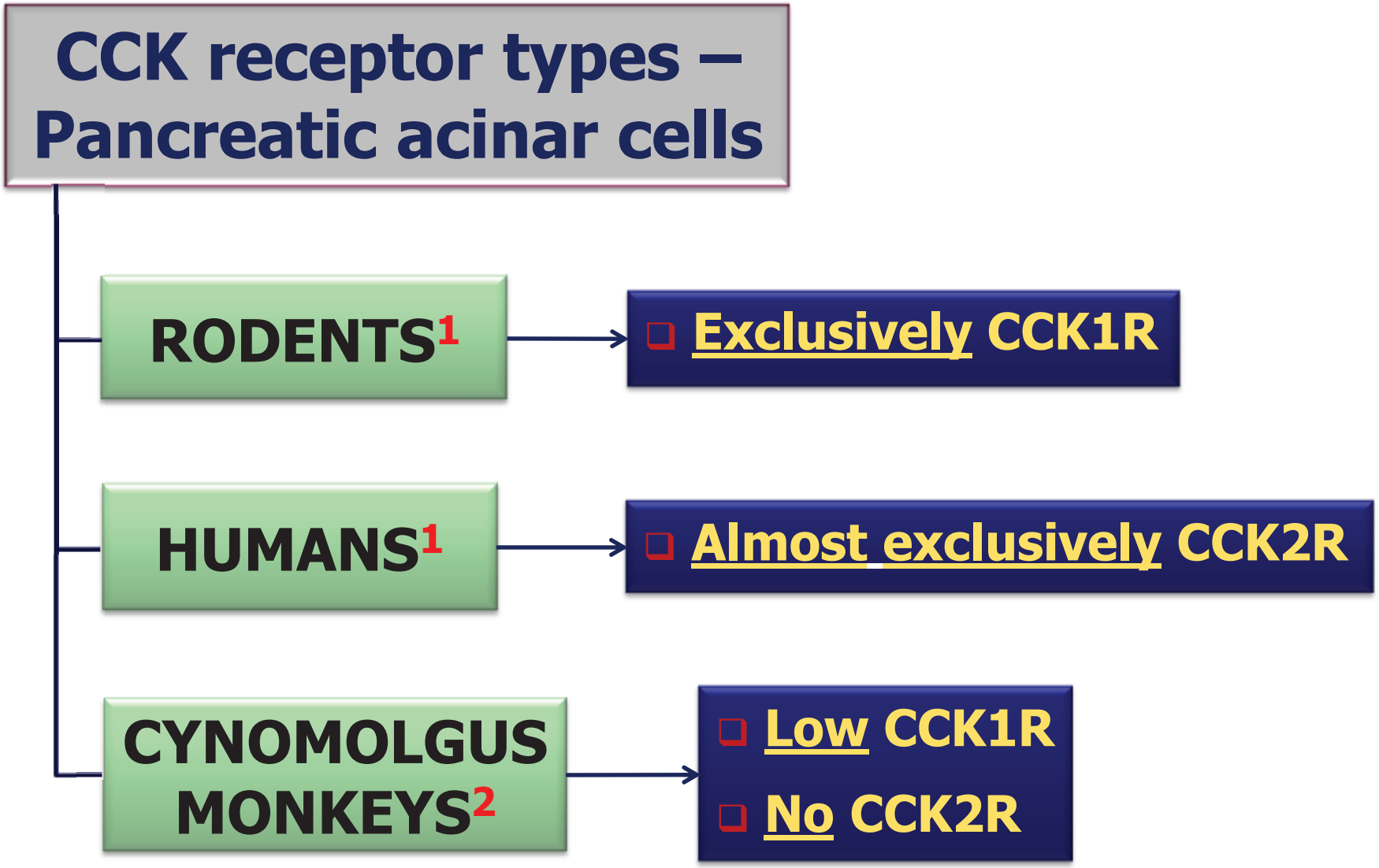
- Lack of CCK1R → several reports[2-5]
- CCK → no functional responses[3,5]
- One report with functional activity by CCK on isolated human acinar cells[6]

Inter-species variation → CCK1R

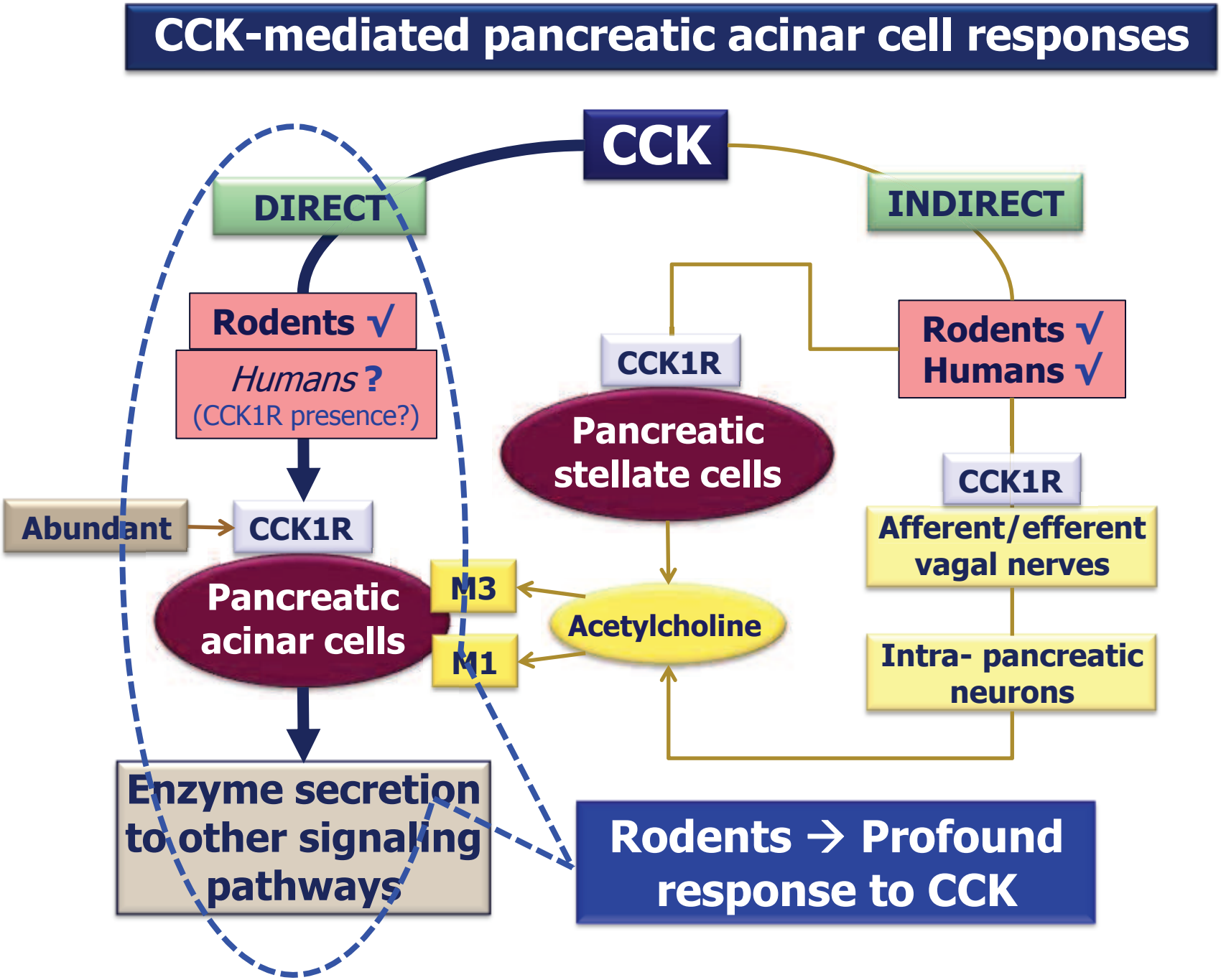
[1]Holicky et al., 2001; RPA = Ribonuclease protection assay; RT-PCR = Reverse transcriptase polymerase chain reaction

[3]Ji et al., 2001, [5]Miyasaka et al., 2002, Wonk et al., 1994;
[4]Morisset et al., 2004; [6]Murphy et al. 2008

CCK receptor types – Pancreatic acinar cells



[1]Berna MJ and Jensen RT. Curr Top Med Chem 1211-1231, 2007
[2]Holicky et al. Am J Physiol Gastrintes Liver Physiol, G507-G514, 2001


CCK-mediated pancreatic acinar cell responses
CCK
DIRECT
INDIRECT
Rodents √
Humans ?
(CCK1R presence?)
Abundant
CCK1R
Pancreatic acinar cells
M3
M1
Acetylcholine
CCK1R
Pancreatic stellate cells
Rodents √
Humans √
CCK1R
Afferent/efferent vagal nerves
Intra- pancreatic neurons
Enzyme secretion to other signaling pathways
Rodents → Profound response to CCK

Conclusion & Translation of Rodent Pancreatic Findings to Cynomolgus Monkeys & Humans

- **Pancreatic responses to 771 (CCK1R agonist):** Different among rodents (sensitive & responsive), monkeys (non-responsive) & humans (no response – 24-Week clinical trial)
- **Relevant inter-species variations**:
  - **CCK1R expression** (abundant in rodents; low in monkeys; & undetectable/absent in humans, except one report showing functional response in isolated human acinar cells)
  - **CCK receptor types** → Rodents (CCK1R); humans (CCK2R) & cynomolgus monkeys (low CCK1R & no CCK2R)
  - **CCK1R-mediated acinar cell response** (direct & indirect pathways in rodents versus mostly indirect in humans)

CCK1R agonist-induced pancreatic findings in rodents – unlikely to occur in monkeys & humans

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