# Reverse Transcriptase Inhibitors

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# Reverse Transcriptase (RT)

- **RNA-dependent DNA polymerase**
- **generates complementary DNA (cDNA) from RNA (reverse transcription)**
- **associated mainly with retroviruses (ssRNA viruses).**
- **also in some non-retroviruses:**
  **e.g. Hepatitis B virus (Hepadnaviridae, dsDNA).**

- **RT is involved in the replication of chromosome ends (telomerase)**
- **Replication of retrotransposons**

# Reverse Transcriptase Inhibitors (RTI)

- **Two types of RTI:  nucleoside and non-nucleoside RTI**

- **Non-nucleoside RTI**

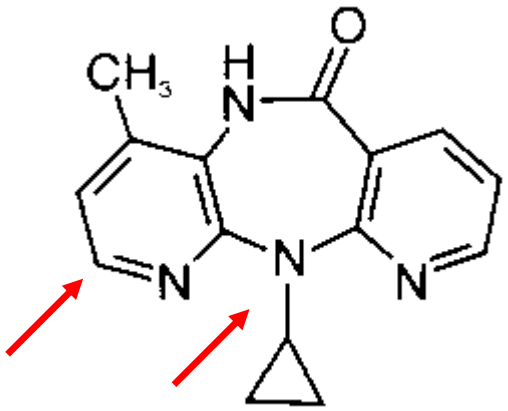
# Reverse Transcriptase Inhibitors (RTI)

- **Two types of RTI: nucleoside and non-nucleoside RTI**

**<u>Non-nucleoside RTI</u>:**

- **Example Nevirapine (11-Cyclopropyl-4-methyl-5,11-dihydro-6H-dipyrido[3,2-b:2,3-e]-[1,4]diazepin-6-on)**

- **Plus Substituents R1, R2**

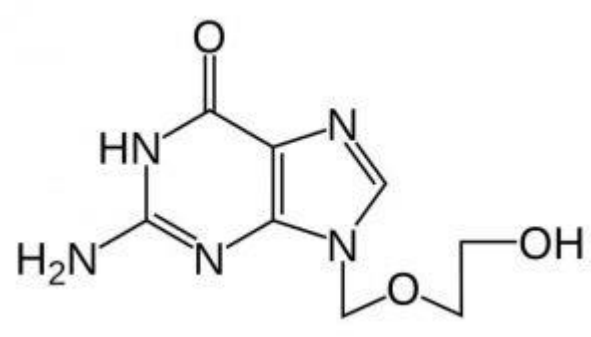



**Pedersen OS, Pedersen EB.**
**Non-nucleoside reverse transcriptase inhibitors: the NNRTI boom.**
**Antivir Chem Chemother.1999 Nov;10(6):285-314.**

# Reverse Transcriptase Inhibitors (RTI)

**<u>Non-nucleoside RTI</u>:**

- **Example Aciclovir (Amino-9-(2-hydroxyethoxymethyl)-3H-purin-6-on, 9-(2-Hydroxyethoxymethyl)-guanin)**

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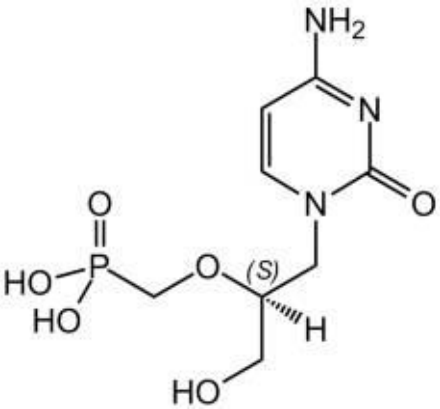
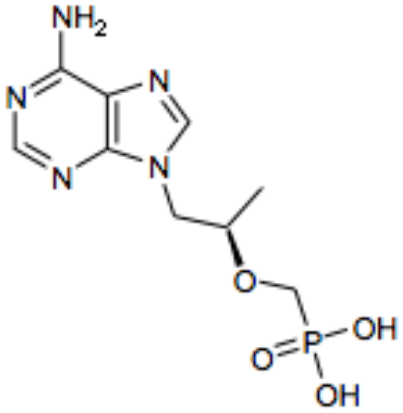
# Reverse Transcriptase Inhibitors (RTI)

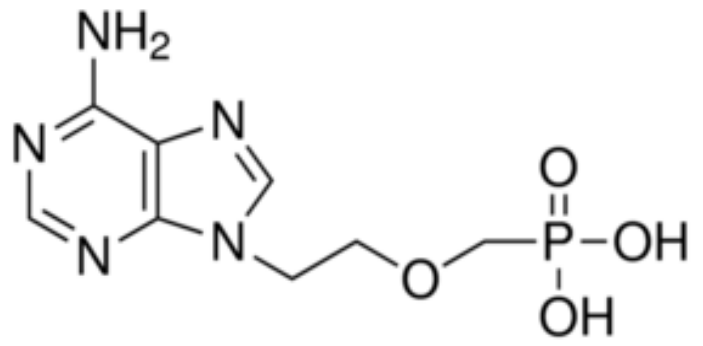
## Nucleoside RTI



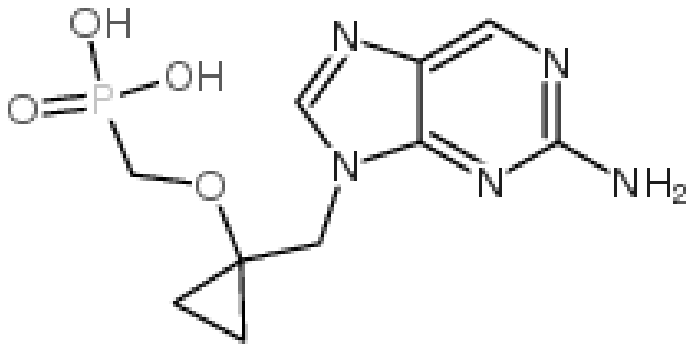

**Cidofovir (Cytomegaly)**




**Tenofovir (HIV)**




**Adefovir (Hepatitis)**

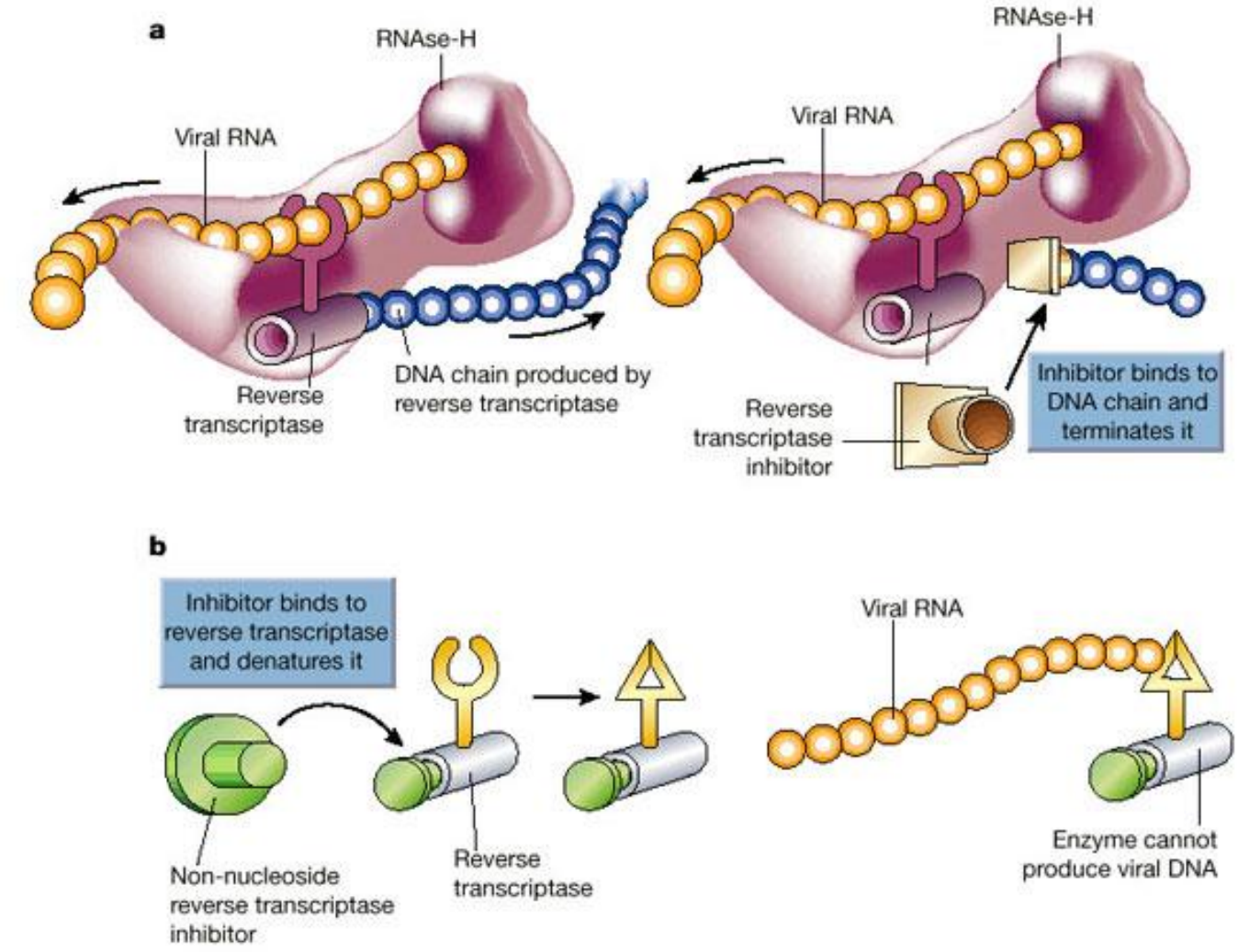



**Besifovir (Hepatitis)**

# RTI - Mechanism

- Virostatics
- Nucleoside analogues; antimetabolites
- Small modifications on ribose
- Lack of 3'-hydroxyl group
- RTI become due to intracellular phosphorylation
- Introduction of RTI into the DNA during the reverse transcription
- Interruption of DNA chain, the stop of the polymerization and hence of reverse transcription.

# RTI - Mechanisms




http://www.nature.com/nature/journal/v410/n6831/images/410995ac.2.jpg

# Study Design

- **Regulatory, 13-Week, Oral (Gavage)**

| Allocation and Dose Levels mg/kg bw/day | | Group 1 Control* 0 | Group 2** 100 | Group 3** 400 | Group 4** 800/600 |
|---|---|---|---|---|---|
| Males | A | 1 - 10 | 14 - 23 | 33 - 42 | 52 - 61 |
| | B | 11 - 13 | 24 - 32 | 43 - 51 | 62 - 70 |
| Females | A | 71 - 80 | 84 - 93 | 103 - 112 | 122 - 131 |
| | B | 81 - 83 | 94 - 102 | 113 - 121 | 132 - 140 |

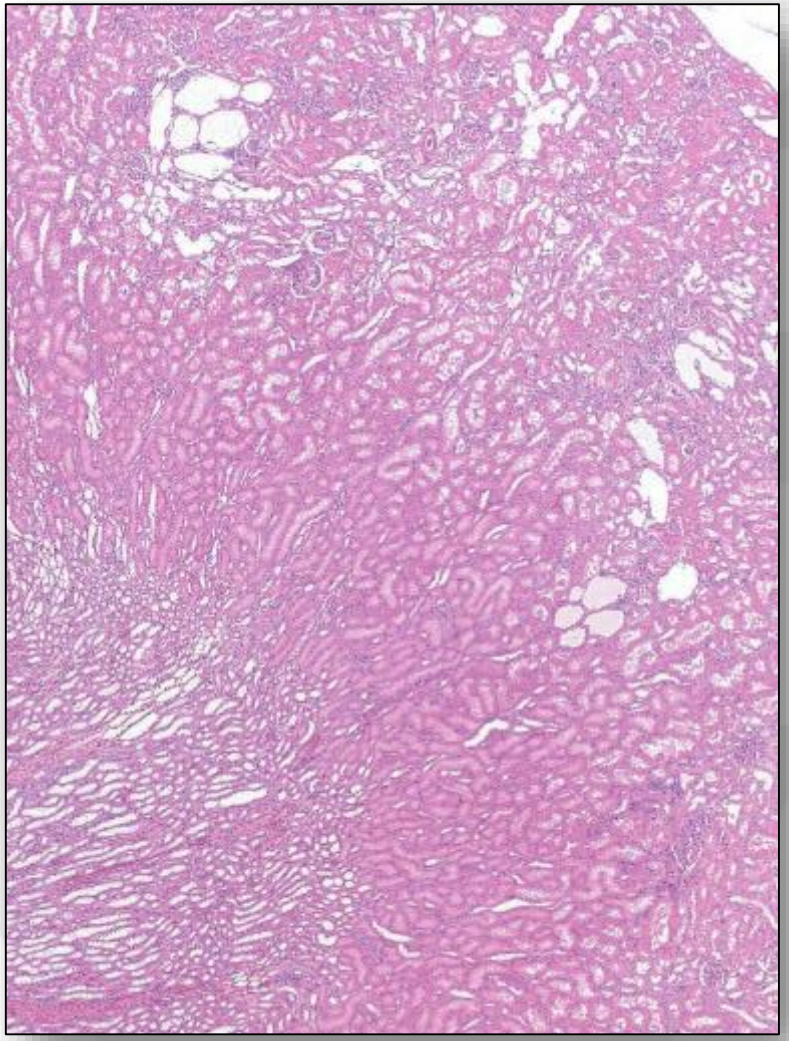
* Control animals were treated with the vehicle, 1% (w/v) Sodium Carboxymethyl Cellulose in water for injection, only

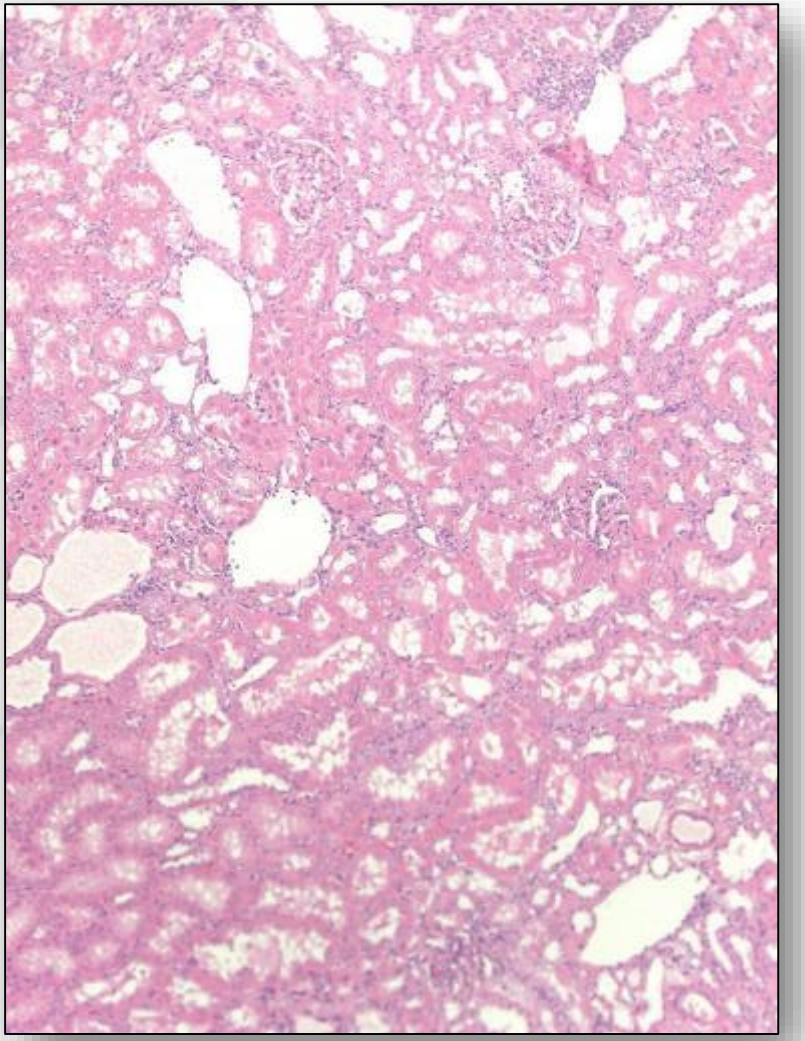
** Dose levels are expressed in terms of free base; a correction factor of 1.22 was applied

A Main study animals

B Animals for toxicokinetic evaluations

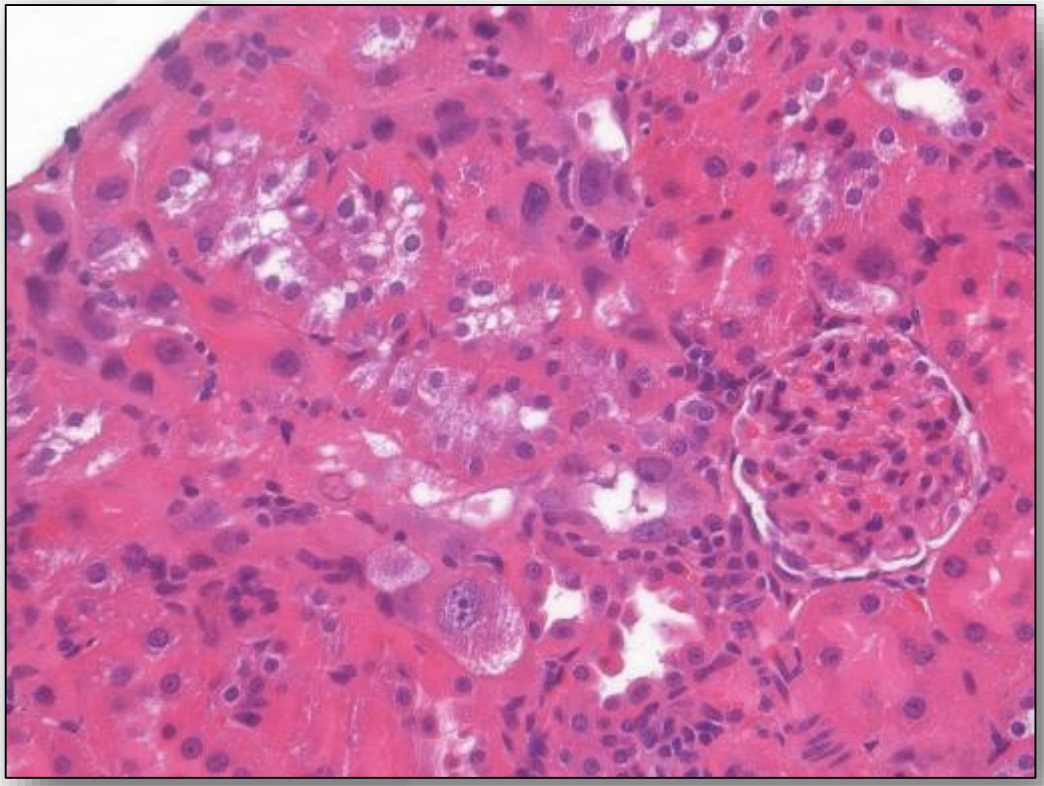
# Kidneys: Overview





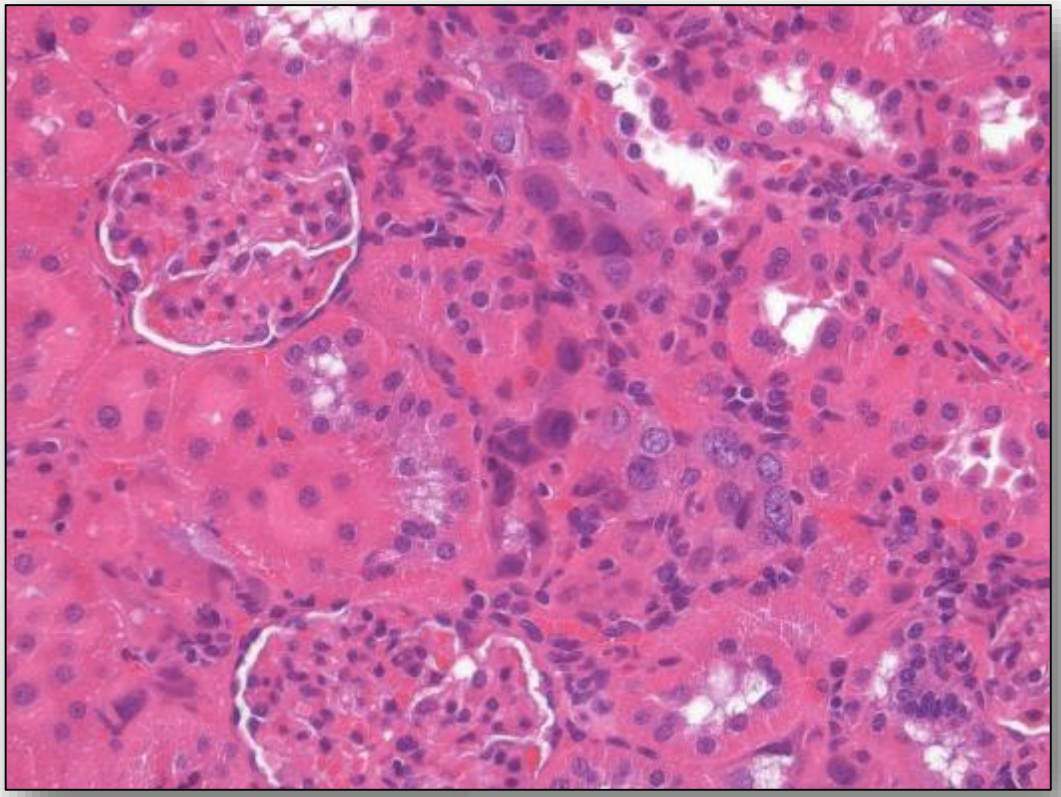
# Major Findings: Kidneys: Karyomegaly

- **enlarged round to bizarre tubular epithelial nuclei with non-condensed chromatin. Mainly proximal tubules were affected, but in cases with higher degrees most cortical structures were involved.**



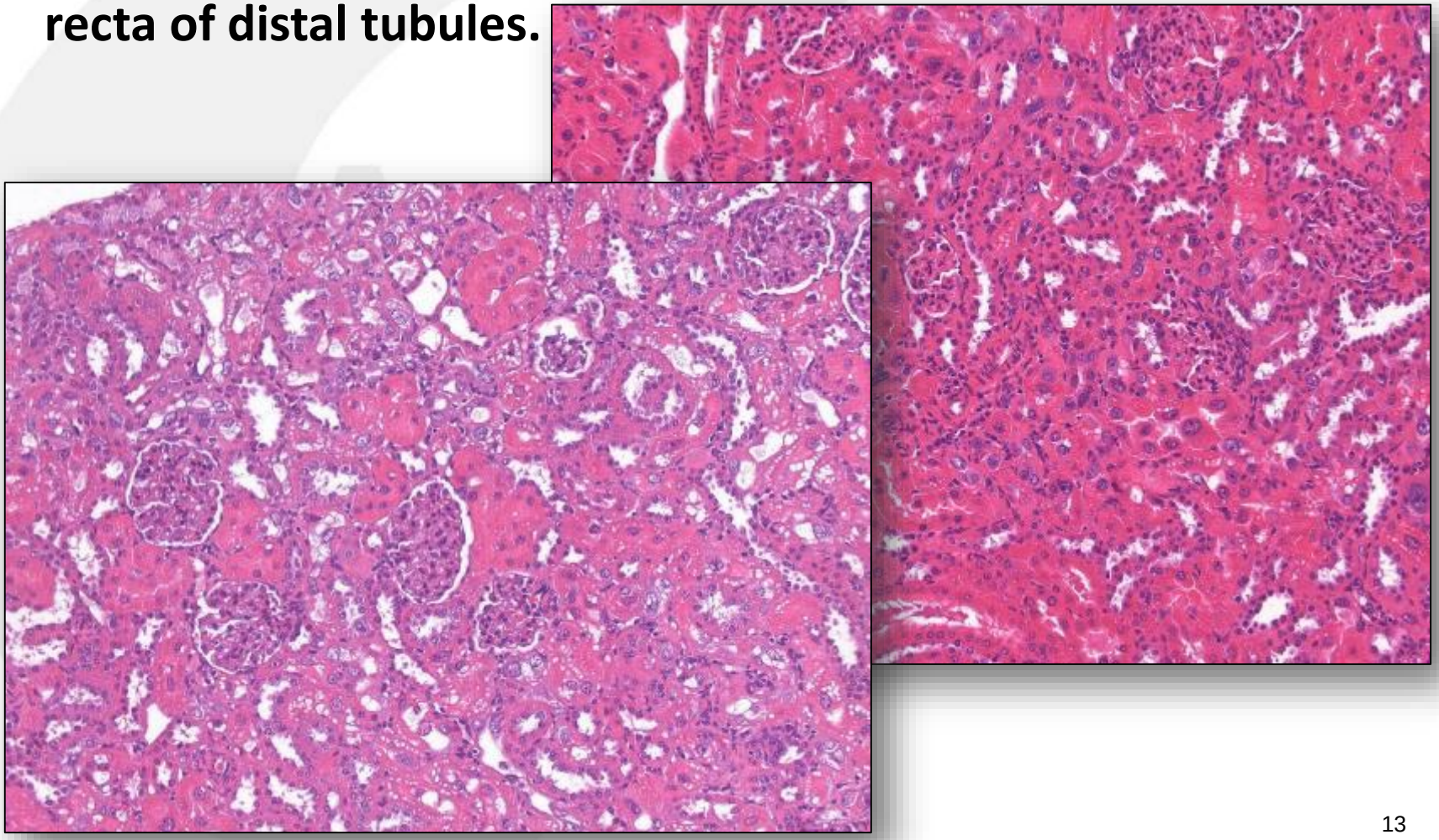
# Major Findings: Kidneys, Polyploidy

- **Enlarged nuclei rich in condensed chromatin (hyper-chromatophilic) exclusively in proximal tubules**



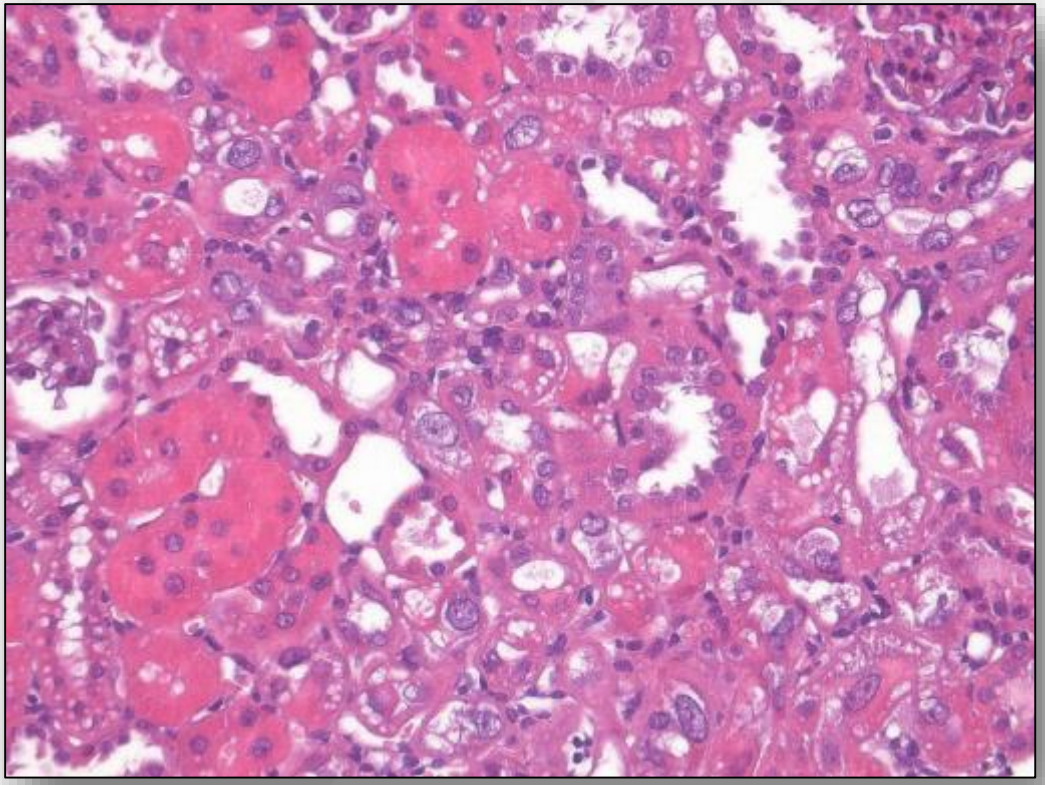
# Major Findings: Kidneys, Tubular Basophilia

- **Focal to multifocal regenerative basophilic tubules mainly affecting proximal tubules, only occasionally involving pars recta of distal tubules.**



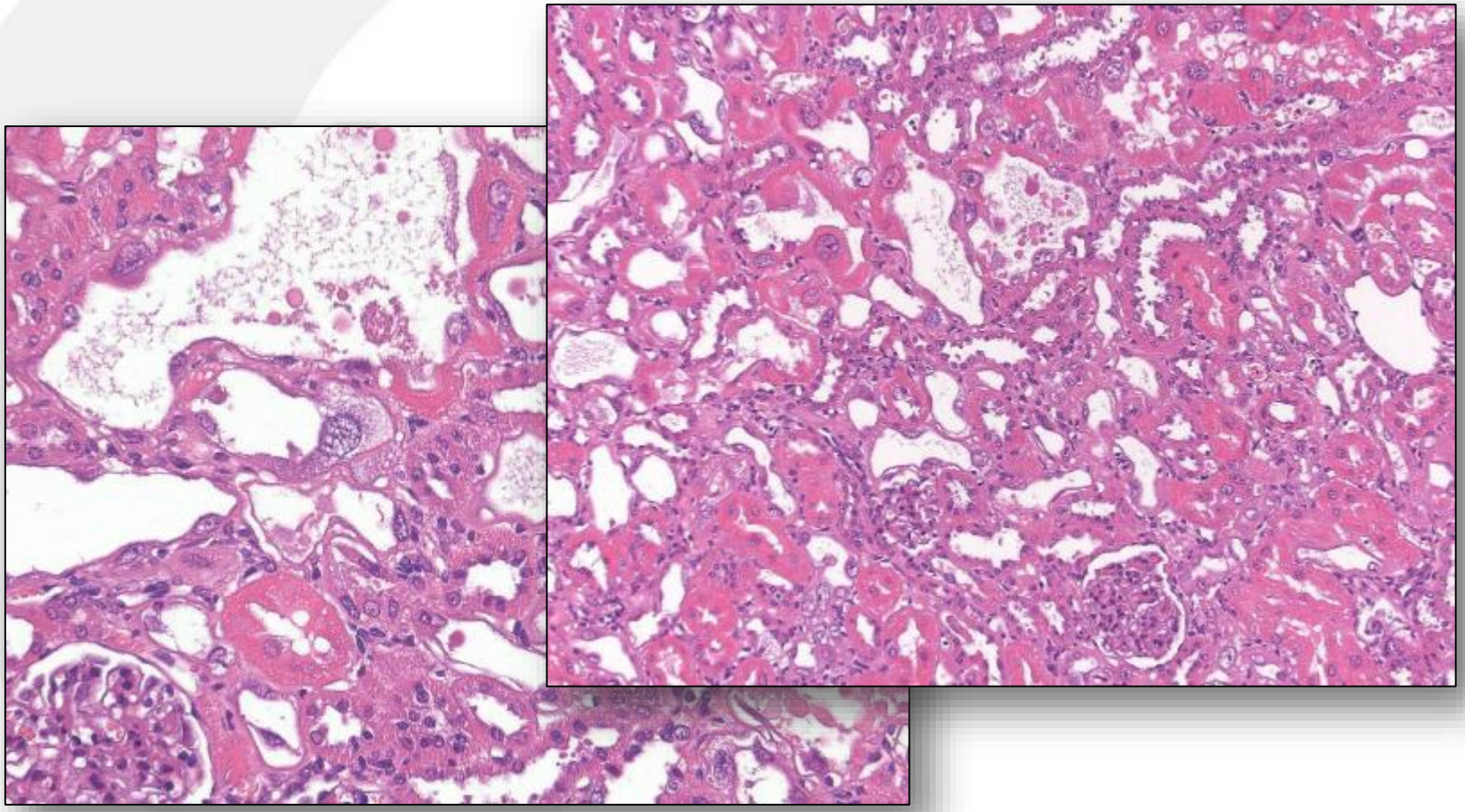
# Major Findings: Kidneys, tubular hypertrophy

**enlarged tubular cells with taller epithelium. The proximal tubules were affected, whereby mainly epithelia with other abnormalities were involved. If in addition 'tubular vacuolation' (fine coarse) was present, all cortical tubular structures were involved. Often with tubular dilatation**

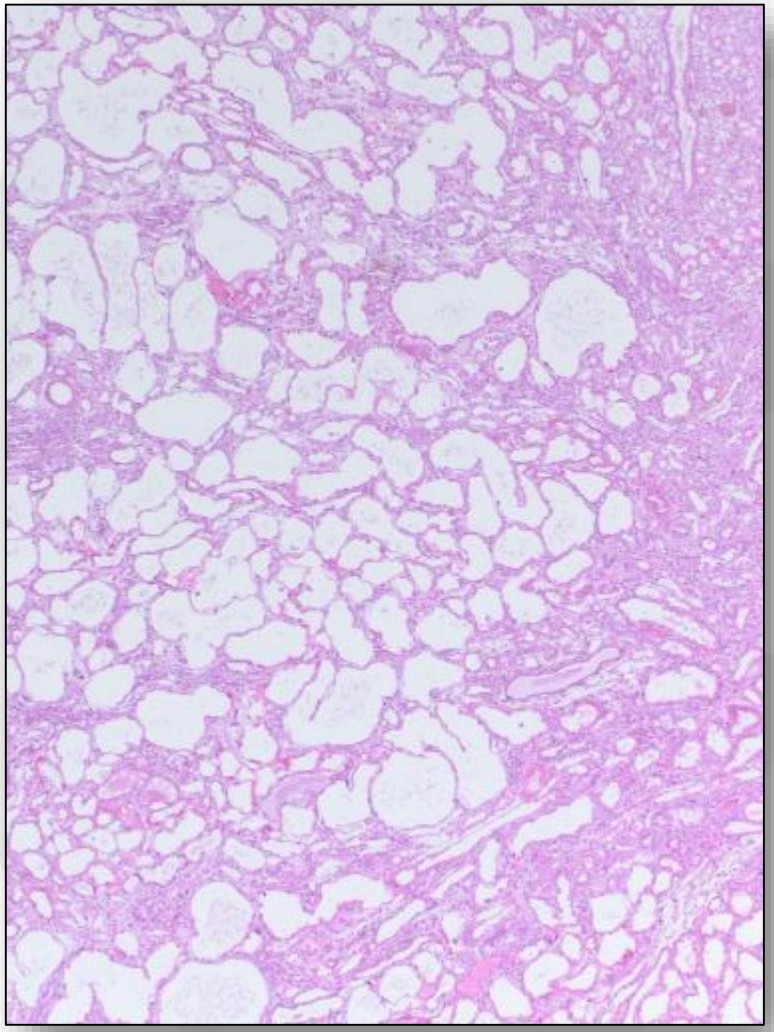


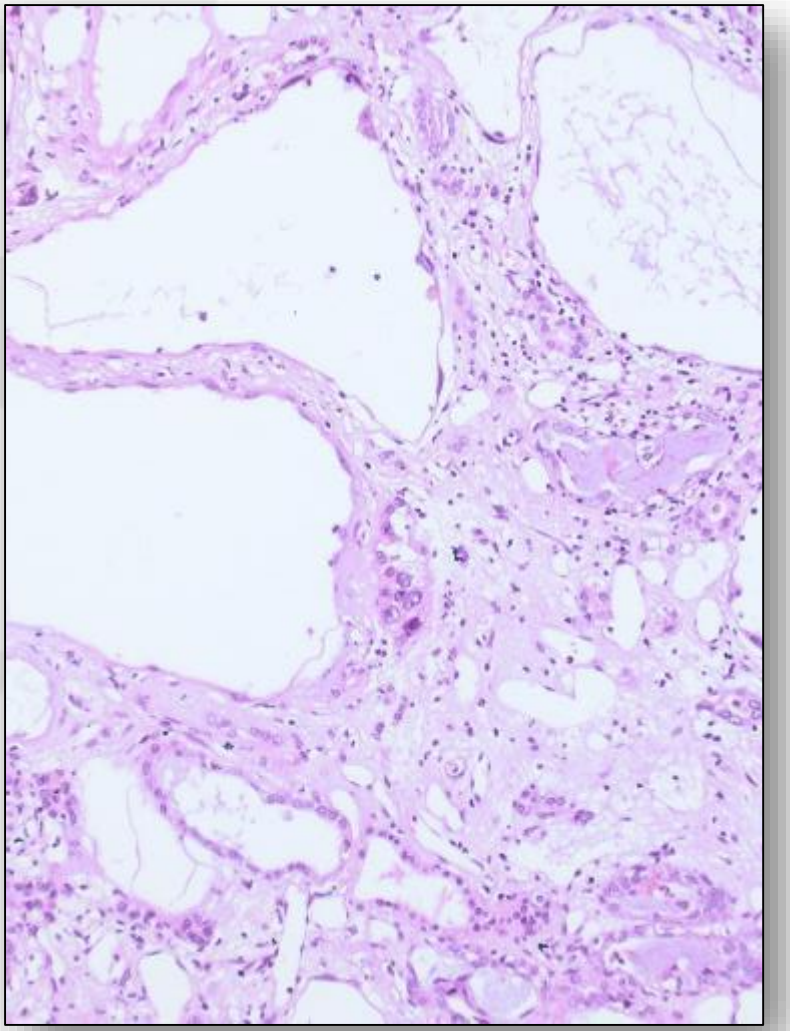
# Major Findings: Kidneys, Single cell necrosis

- **single cell necrosis forming clots and granular hypereosinophilic intratubular casts. Mainly proximal tubules affected.**



# Major Findings: Kidneys, Inflammatory infiltrate and fibrosis

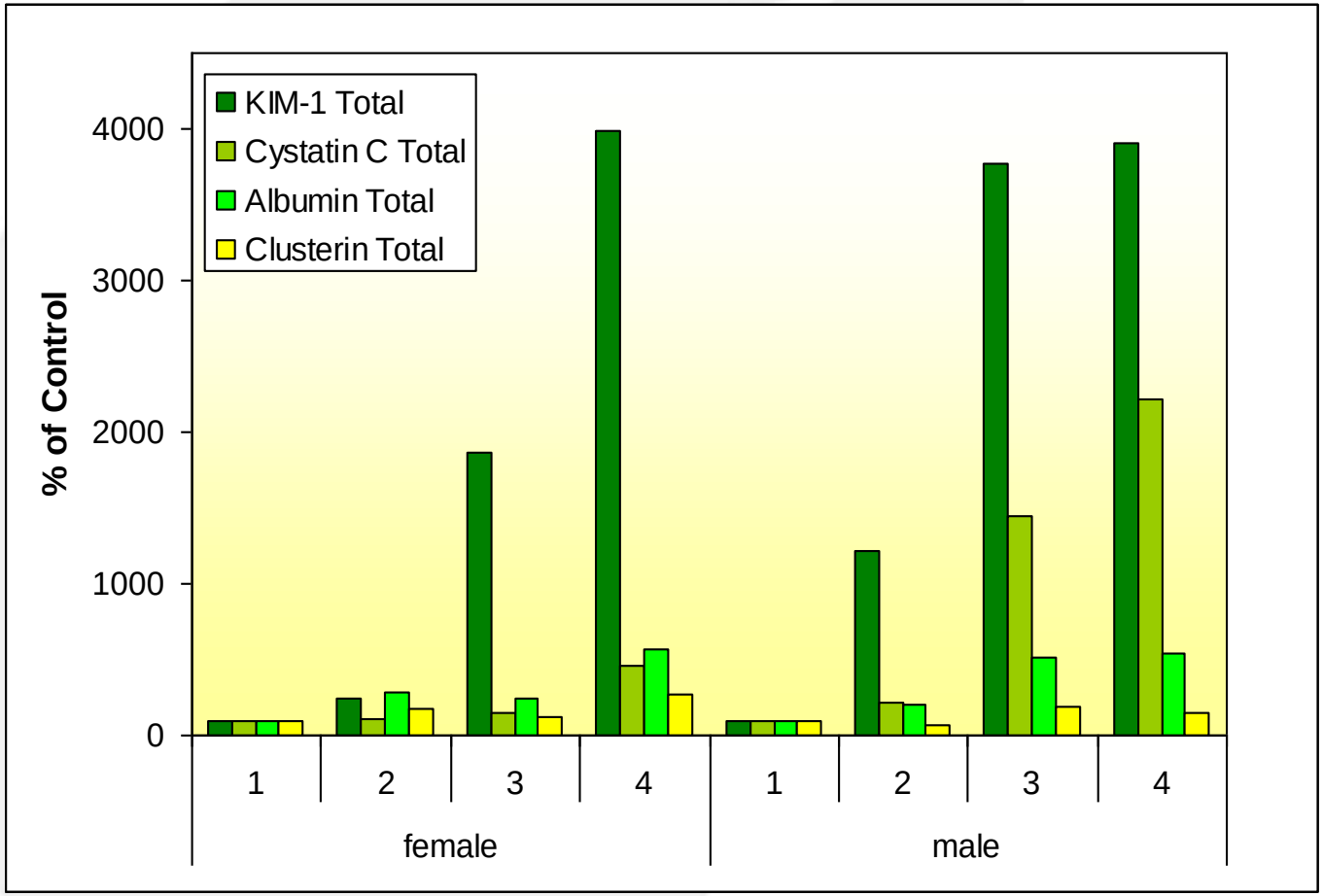




# Major Findings: Kidneys, Summary

| Finding | Group 1 | | Group 2 | | Group 3 | | Group 4 | |
|---|---|---|---|---|---|---|---|---|
| Incidence / Mean Severity | 10 M | 10 F | 10 M | 10 F | 10 M | 10 F | 10 M | 10 F |
| Tubular casts | 0 | 0 | 2/1.0 | 1/1.0 | 0 | 0 | 5/1.0 | 2/1.0 |
| Tubular dilatation | 0 | 0 | 5/1.2 | 0 | 9/1.7 | 2/1.5 | 8/1.6 | 3/1.0 |
| Karyomegaly | 0 | 0 | 9/1.2 | 0 | 10/1.9 | 8/1.6 | 10/2.6 | 7/2.1 |
| Polyploidy | 0 | 0 | 7/1.1 | 0 | 9/1.0 | 5/1.2 | 9/1.7 | 4/1.5 |
| Tubular hypertrophy | 0 | 0 | 5/1.0 | 3/1.0 | 7/1.1 | 2/1.5 | 9/1.6 | 7/1.9 |
| Tubular cell necrosis | 0 | 0 | 0 | 0 | 1/1.0 | 2/1.0 | 9/1.0 | 4/1.0 |
| Interstitial fibrosis | 0 | 0 | 4/1.3 | 0 | 6/1.3 | 2/1.0 | 8/1.0 | 1/1.0 |
| Inflammation, interstitial | 0 | 0 | 2/1.0 | 0 | 7/1.3 | 1/1.0 | 9/1.2 | 4/1.0 |

# Renal Toxicity Parameters (Sampling in Week 13)




**Mean urinary excretion of urinary biomarkers within 18 hours (x-axis: numbers represent the group number**

# Tubulopathy Score

- **Kidney findings were summarized**
- **Tubulopathy Score was applied if at least one of the following parameters was present:**
  **karyomegaly, polyploidy, tubular hypertrophy, and necrosis.**
  **Tubular basophilia was counted only, if combined with one of the other findings (to avoid counting of background)**

# Tubulopathy Score

**Grade 1:**

- **when two of the above parameters were observed in the kidneys, but only at a severity degree grade 1/finding. Tubular basophilia was counted as an indicator for regeneration.**

**Grade 2:**

- **more than two single findings, but each single finding at least severity grade 2**

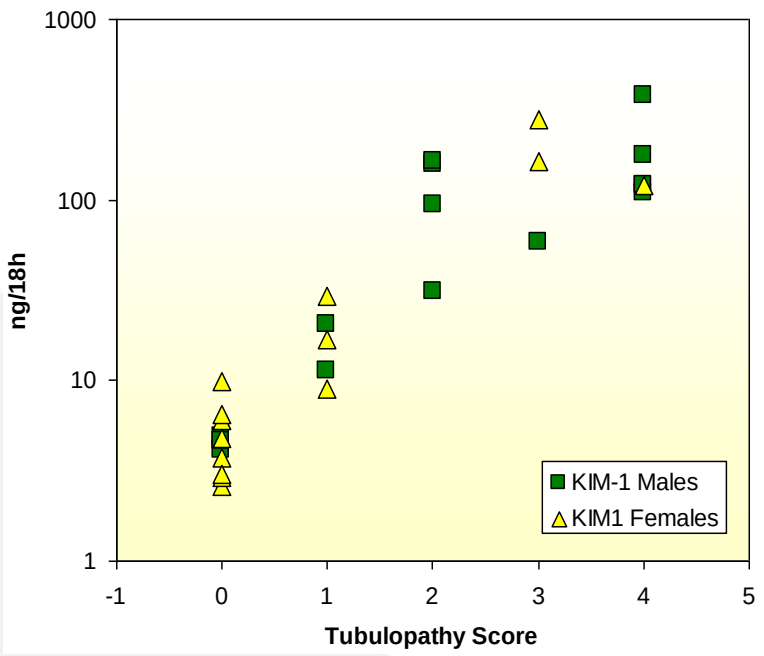
**Grade 3:**

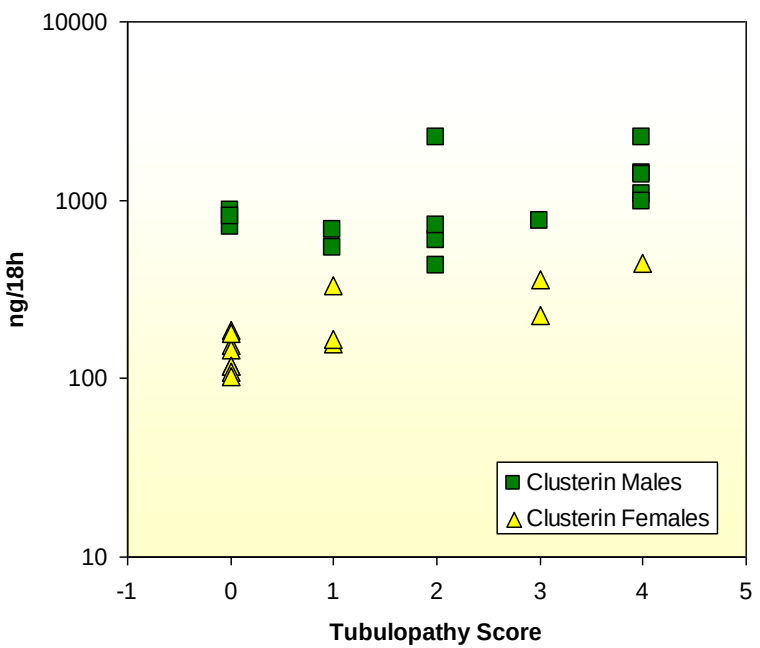
- **more single findings associated with inflammation and/or fibrosis, but each single finding up to severity grade 2**

**Grade 4:**

- **several single findings associated with inflammation and/or fibrosis, but one single finding at least severity grade 3**

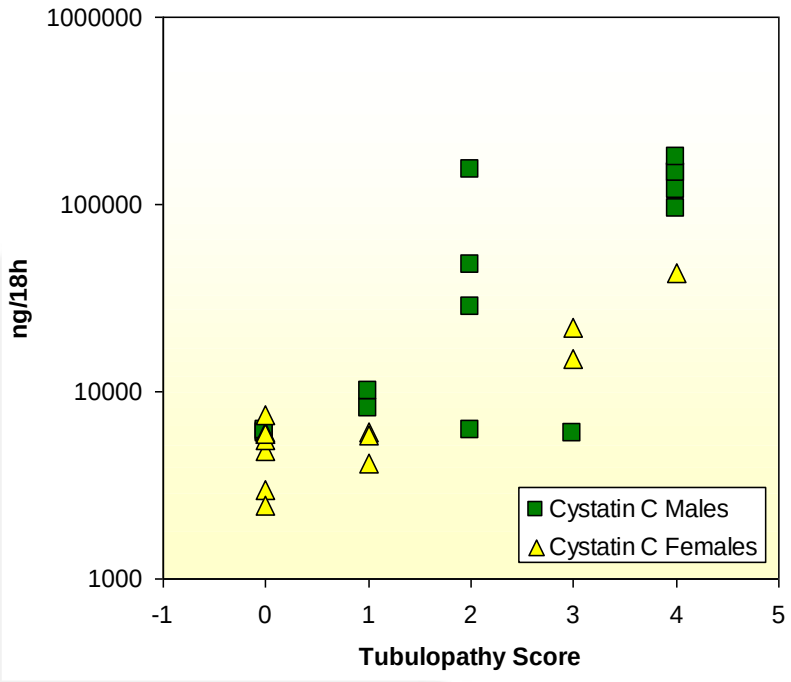
# Tubulopathy Score vs Renal Toxicity Parameters

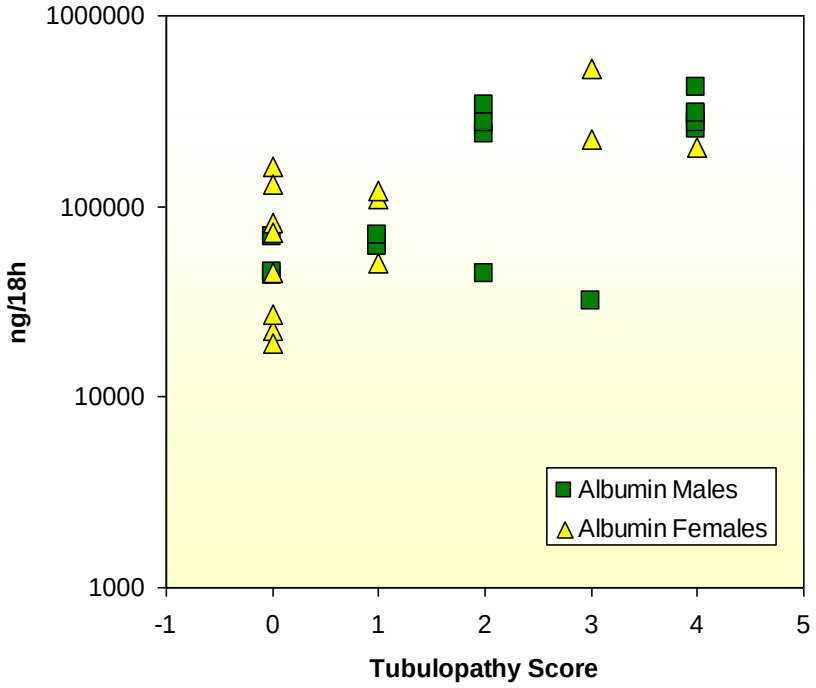



- **KIM-1 excretion increased dose-dependently up to 40 fold, indicative for the involvement of proximal tubules**
- **Clusterin, was only slightly increased (2-3 fold). It is a biomarker with a preference for lesions in the proximal and distal tubules.**

# Tubulopathy Score vs Renal Toxicity Parameters




- **Cystatin C levels increased dose-dependently in males, up to 20-fold, but in females only by 5-fold, It is indicative for damage in the glomeruli and in proximal tubules**
- **Albumin excretion increased dose-dependently about 5-fold in both sexes. It is indicative for glomerular injury, or tubular injury due to disturbed re-absorption.**

# Mechanism of Renal Toxicity

- **Tubulopathy Score correlated with the results of the renal markers.**
- **With increasing score, KIM-1, cystatin C and albumin increased.**

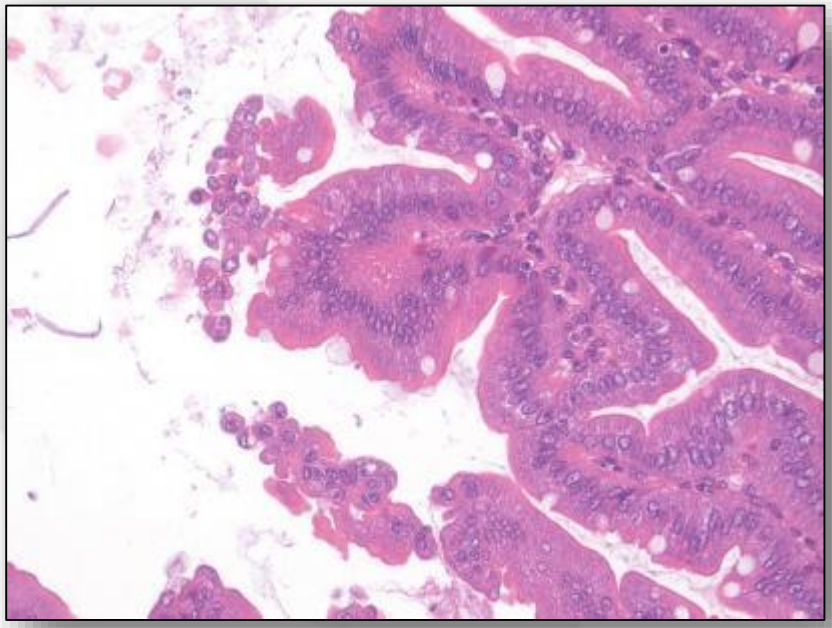
- **Convoluted proximal tubules are considered the target structures (other tubular parts only occasionally involved).**
- **Glomeruli unaffected**
- **The pattern of injury is deemed related to pharmacological action and is a class effect.**

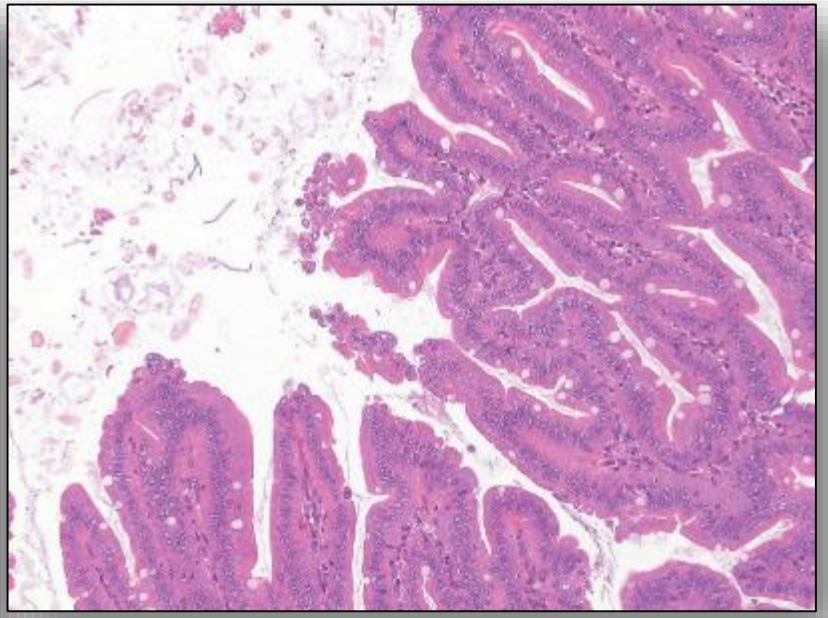
# Mechanism of Renal Toxicity

- **Uptake by the brush border of the proximal tubule**
- **Renal tubular cells contain a high number of mitochondria.**
- **NTI inhibit gamma polymerase, and hence inhibit mtDNA synthesis.**
- **mtDNA depletion or mutation causes insufficient energy production and cell dysfunction.**
- **nRTIs may also be associated with oxidative damage to mitochondria, inhibition of mitochondrial enzymes, uncoupling of the electron transport chain from ATP synthesis, and induction of apoptosis**

# Other Findings: Duodenum

- Minimal regenerative mucosal hyperplasia (high dose only)
- in single cases associated with mucosal erosion, dilated lacteals, vacuolation of villi tips and intra-mucosal fibrosis.
- affection of intestine known from other reverse transcriptase inhibitors. Nelfinavir and Indinavir, e.g., induce apoptosis in vivo in the intestinal mucosa and chloride and water secretion





# Other Findings

- **Liver: incidence and severity of centrilobular hepatocellular hypertrophy increased with dose**
- **Liver: fatty change increased in high dose**
- **Adrenal cortex: hypertrophy**
- **Thymus: atrophy**

# Relevance for Human?

- **Renal changes have been reported from different RTI.**
- **In preclinical trials with Tenofovir, renal tubular epithelial karyomegaly was the most sensitive histological indicator of an effect on the kidney and was observed in rats, dogs, and monkeys.**
- **Similar findings have been reported for Adefovir. They included karyomegaly, cytomegaly, tubular dilatation, degeneration/regeneration, and individual tubular epithelial cell necrosis.**
- **Adefovir dipivoxil: no renal tumors !(Gilead, 2002b).**

**Clinics: adverse effects in patients under Tenofovir causing a warning. They included amongst other symptoms also renal failure.**