Rodent Liver Tumors: NCI/NTP Historic Perspective

Bob Maronpot, Raleigh, NC

STP Annual Meeting - 2023

Rodent Liver Tumors: NCI/NTP Historic Perspective

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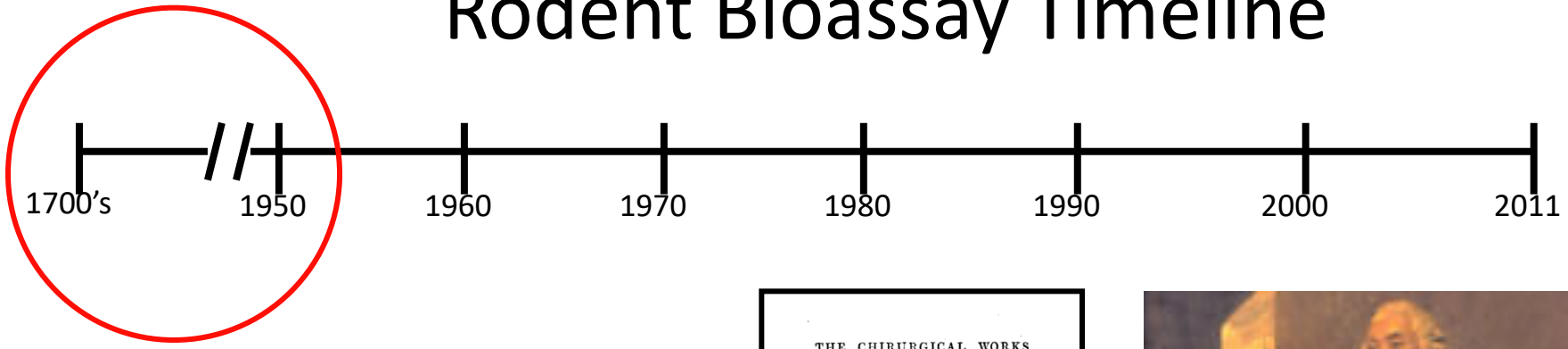
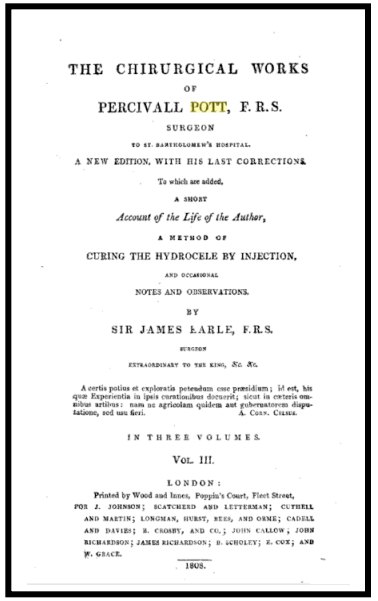
- A little bit of NCI/NTP rodent bioassay history
- NTP liver tumor data
- Liver tumor images
- Current NTP safety assessment perspective

Acknowledgements: Dave Malarkey & Arun Pandiri

Rodent Bioassay Timeline



- Bernardino Ramazzini – 1713
  - Breast cancer in nuns
- John Hill – 1761
  - Snuff & oral/nasal cancer
- Percival Pott – 1775
  - Scrotal cancer
- Elmslie -1866 (Kashmir)
  - Epithelioma of abdomen & thighs
- Rehn – 1895
  - Bladder cancer in aniline dye workers
- Yamagiwa & Ichikawa – 1918
  - Tar & soot on rabbit ears



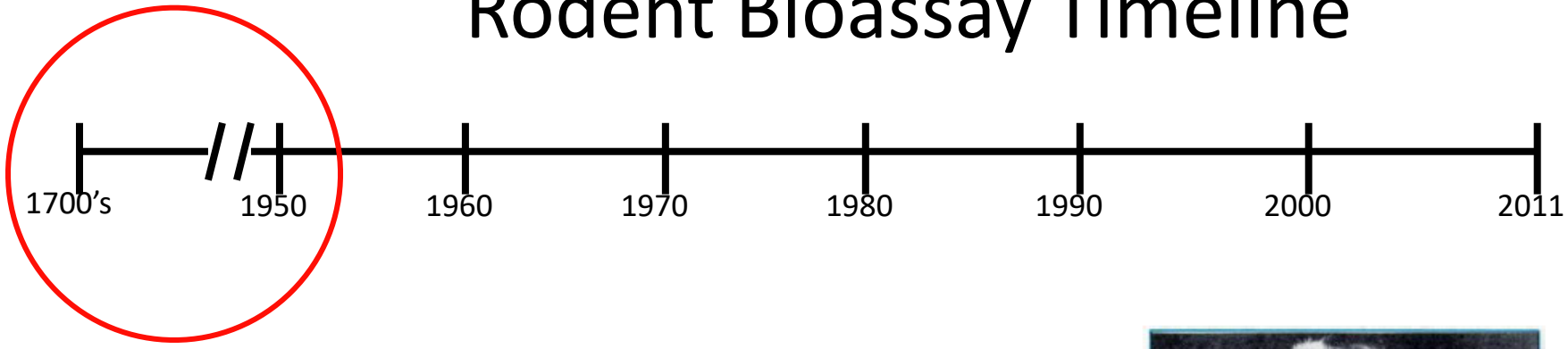
THE CHIRURGICAL WORKS
OF
PERCIVALL POTT, F. R. S.
SURGEON
TO ST. BARTHOLOMEW'S HOSPITAL.
A NEW EDITION, WITH HIS LAST CORRECTIONS.
To which are added,
A SHORT
Account of the Life of the Author,
A METHOD OF
CURING THE HYDROCELE BY INJECTION,
AND OCCASIONAL
NOTES AND OBSERVATIONS.
BY
SIR JAMES EARLE, F.R.S.
SURGEON
EXTRAORDINARY TO THE KING, &c. &c.

A certis potius et exploratis petendum esse præsidium; id est, his quæ Experientia in ipsis curationibus docuerit; sicut in cæteris omnibus artibus: nam ne agricolam quidem aut gubernatorem disputatione, sed usu fieri.
A. CORN. CELSUS.

IN THREE VOLUMES.
VOL. III.
LONDON:
Printed by Wood and Innes, Poppin's Court, Fleet Street,
FOR J. JOHNSON; SCATCHERD AND LETTERMAN; CUTHELL AND MARTIN; LONGMAN, HURST, REES, AND ORME; CADELL AND DAVIES; R. CROSBY, AND CO.; JOHN CALLOW; JOHN RICHARDSON; JAMES RICHARDSON; B. SCHOLEY; E. COX; AND W. GRACE.
1808.

1775 - Scrotal cancer in chimney sweeps. Cancer was attributed to the tar and soot in the chimneys. This is an early and famous example of occupational cancer in humans.

Rodent Bioassay Timeline



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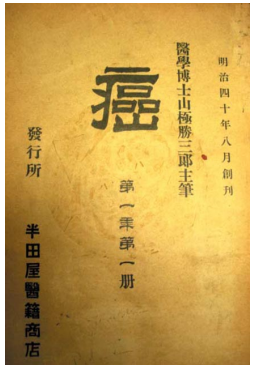
Katsusaburo Yamagiwa (1863-1930)

"Cancer was produced! Proudly I walk a few steps"



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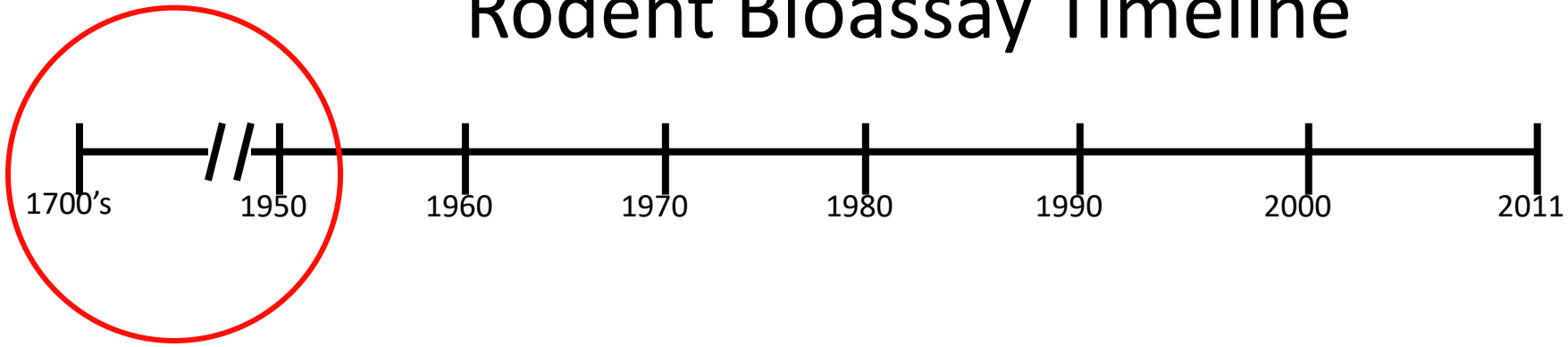

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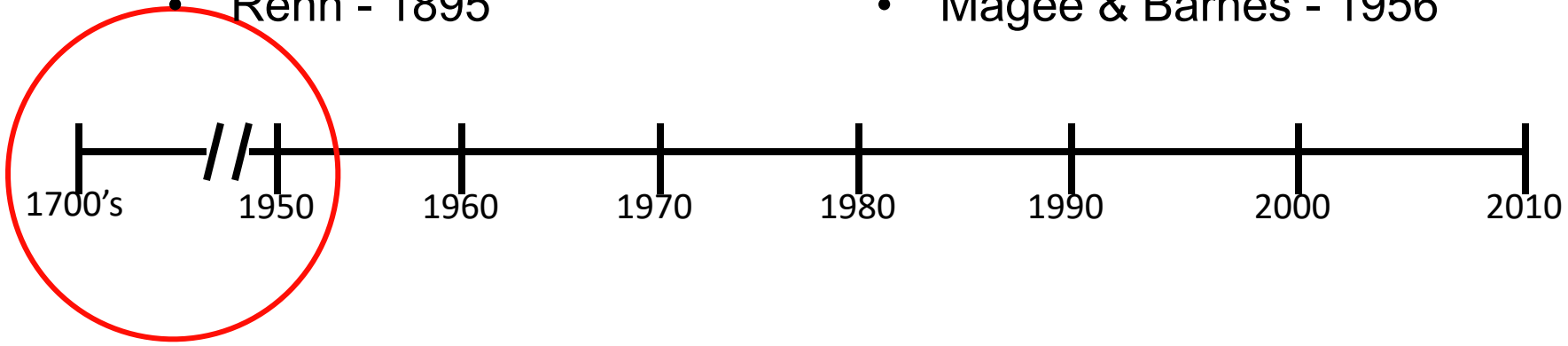
  - Tar & soot on rabbit ears
- Murphy & Sturm – 1925
  - Lung tumors in tar-painted mice
- Cook et al. – 1932
  - Cancer induction by PAHs
- Sasaki & Yoshida – 1935
  - o-amidoazotoluene diet and liver cancer; effects of dose on latacency and use of stop studies
- Berenblum – 1941
  - Concept of co-carcinogenesis
  - Initiation, promotion, progression
- Magee & Barnes – 1956
  - Nitrosamines & liver cancer in rats

- Bernardino Ramazzini - 1713
- John Hill - 1761
- Percival Pott - 1775
- Elmslie -1866
- Jonathon Hutchinson - 1888
- Rehn - 1895
- Yamagiwa & Ichikawa - 1918
- Murphy & Sturm - 1925
- Cook et al. - 1932
- Sasaki & Yoshida - 1935
- Berenblum - 1941
- Magee & Barnes - 1956

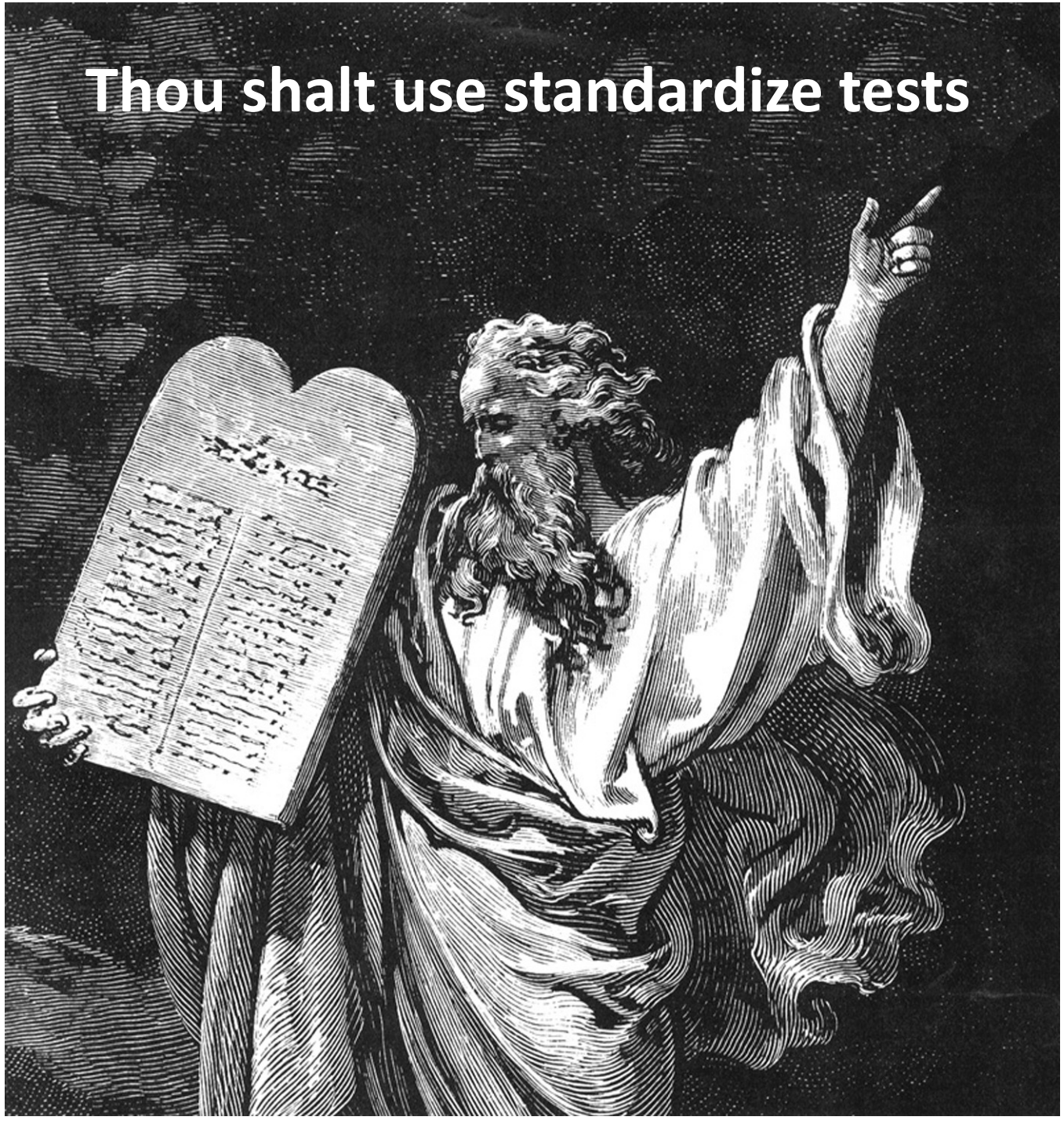


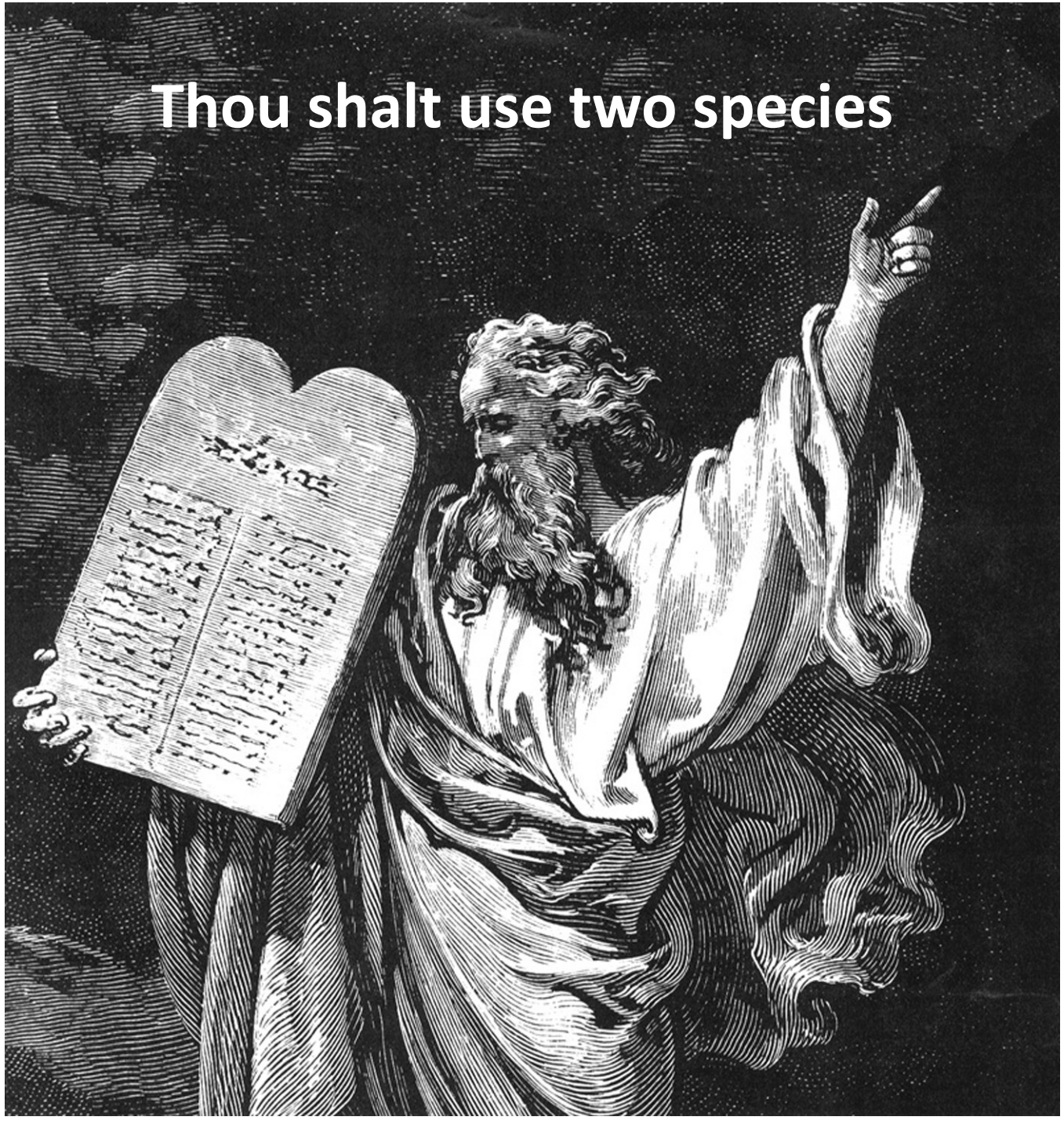
- Realization that chemicals, environmental factors, and aspects of lifestyle cause cancer

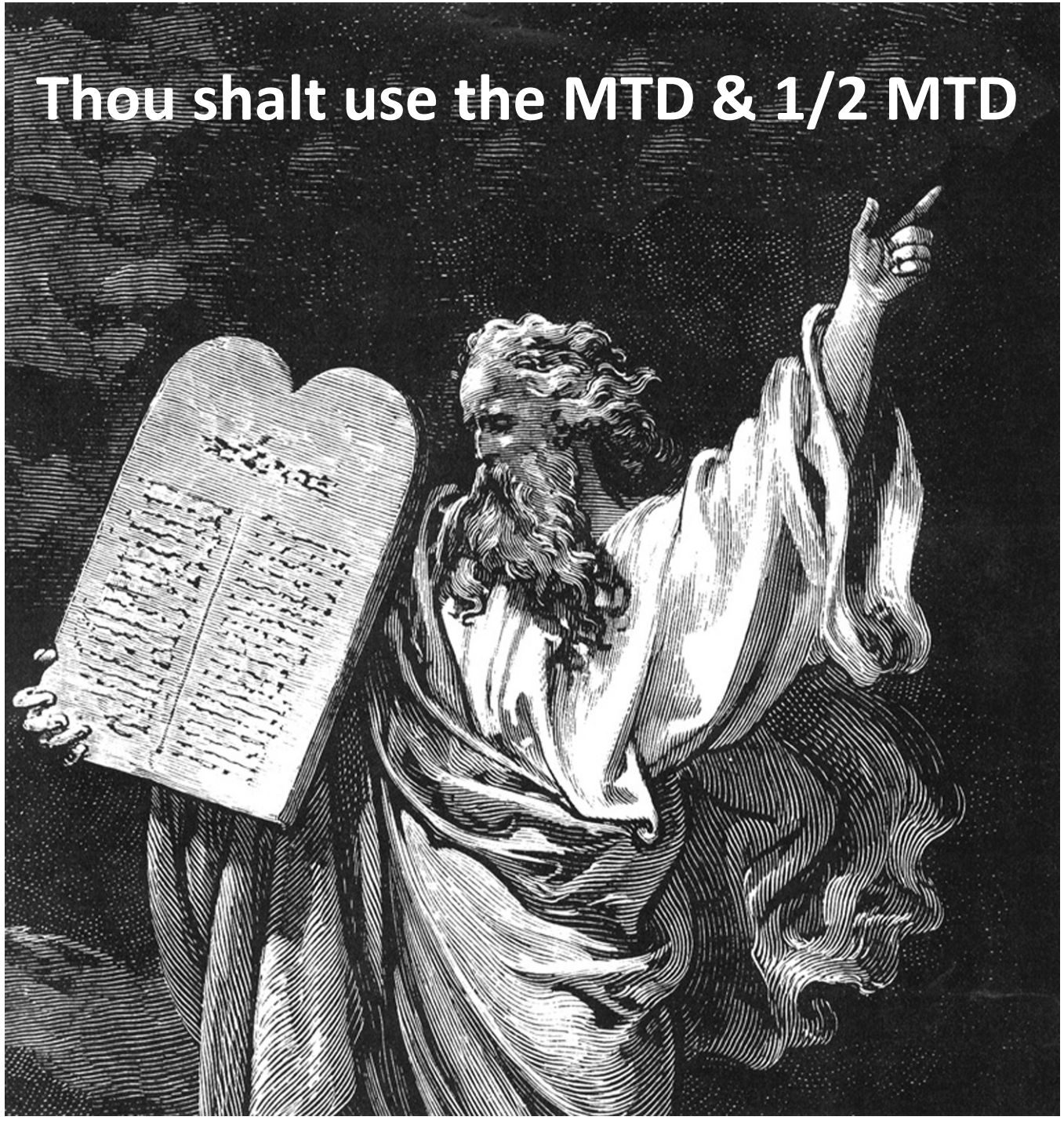
Concept of the rodent bioassay & its establishment by the National Cancer Insititute (NCI)

- 1962 - First contracted bioassay
- 1969 - Innes et al*., study published
  - 20,000 mice; 127 different chemicals; 18-mo studies
  - Selection of B6C3F1 mouse
- 1971 - National Cancer Act
  - Decision made to standardize bioassay testing
- ~1975 – Inbred F344 rat selected
  - Small size, vigor & survival, disease resistance

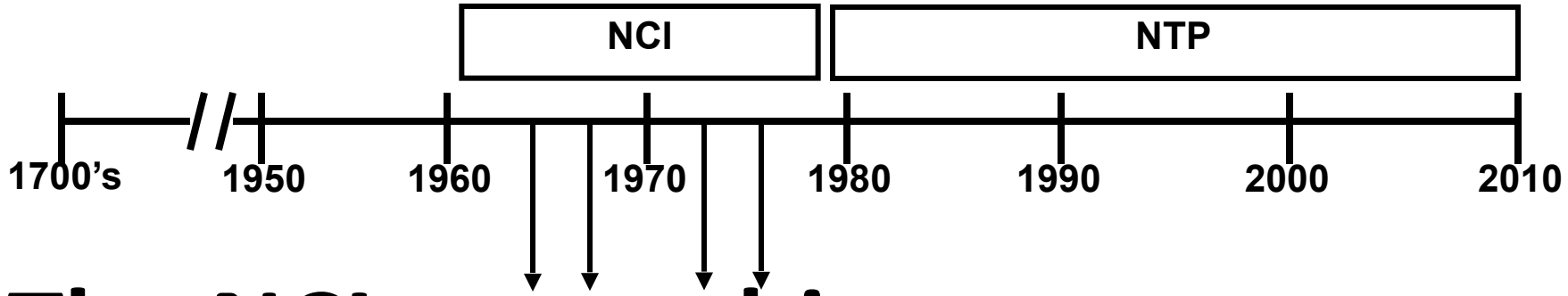
* Innes et al., JNCI 42(6): 1104-1114 (1969)


Thou shalt use standardize tests


Thou shalt use two species


Thou shalt use the MTD & 1/2 MTD

CANCER BIOASSAY TIMELINE



The NCI cancer bioassay

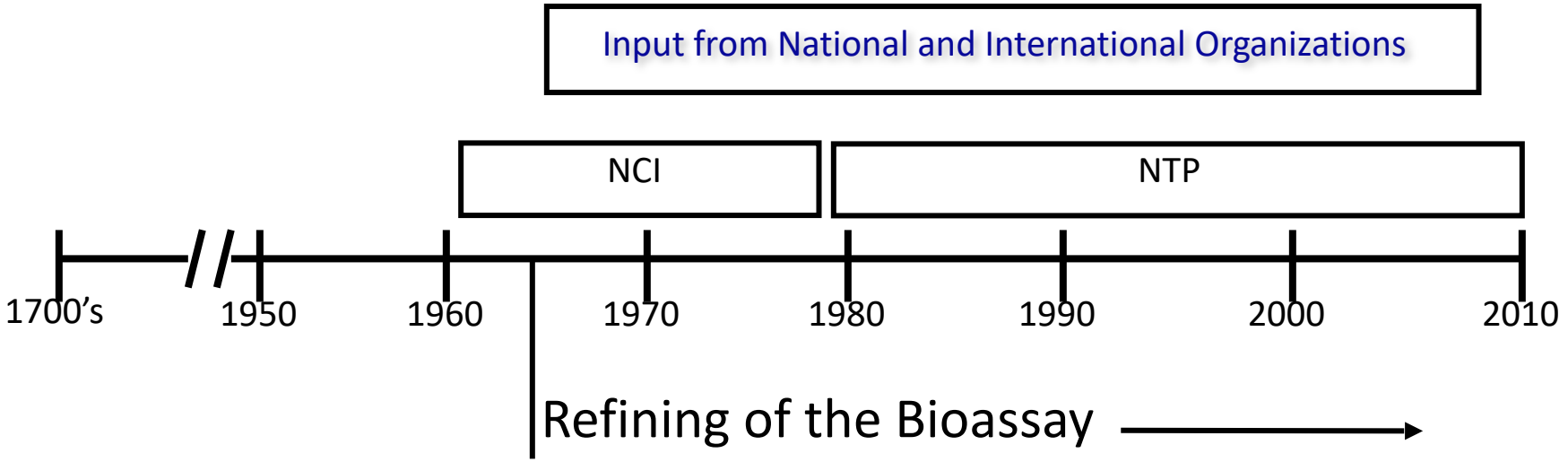
50 Male and 50 female **F344** rats

50 Male and 50 female **B6C3F1** mice

Maximum tolerated dose & 1/2 MTD

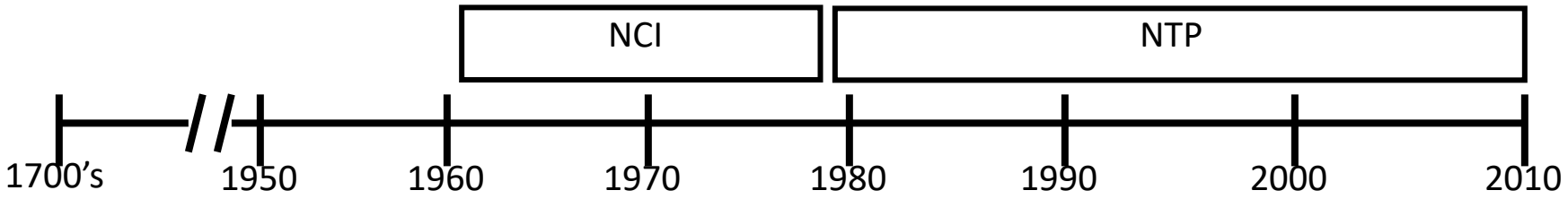
Test duration of 18 months or 2 years

Pathology evaluation



- Standardization of bioassay
  - Originally designed for screening
- Extensive pathology with peer review*
  - Standardization of diagnostic nomenclature
- Statistical evaluation standardized
- Historical control database
- Search for alternative models

*Maronpot & Boorman (1982) Toxicol Pathol 10(2): 71-78



Limitations of the bioassay

- Resource intensive
- Not validated
- Inherent insensitivity for detecting weak or moderate carcinogens
- Single chemical exposure vs "real world"
- Not sure if an agent has carcinogenic potential under actual human exposure conditions
- Debate regarding relevance
  - Rodent-specific mechanisms
  - High doses

Alternative models & ancillary approaches

- Strain A mouse
- Two-stage & neonatal models
- Humanized mice
- Ito medium-term model
- Transgenic models
- Local subcutaneous injection
- Medaka & guppy models
- Genotoxicity batteries

Target organs of chemical-induced carcinogenicity

#	Mouse (%), n=490*	Rat (%), n=490*
1	Liver (27.1)	Liver (10.6)
2	Lung (8.8)	Kidney, tubular cell (9.2)
3	Forestomach (4.7)	Mammary gland (5.9)
4	Hematopoietic system (4.5)	Lung (4.6)
5	Harderian gland Thyroid gland, follicular cell (2.7)	Thyroid gland, follicular cell (4.5)
6	Kidney, tubular cell (2.5)	Forestomach (4.3)
7	Vascular System (Unspecified) (2.3)	Urinary bladder (4.1)
8	Mammary gland (2.2)	Skin (3.8)
9	Ovary (2)	Hematopoietic system (3.7)
10	Skin (1.6)	Adrenal medulla Oral cavity Zymbal gland (3.5)

***n=490 studies where the same chemical was tested in both F344 rats and B6C3F1 mice**

Courtesy of A. Pandiri 2020

Background liver tumor incidence

Historical control incidences of liver tumors in rats (F344/N) and mice (B6C3F1)

Tumor type	Male Mouse % (Range%)	Female Mouse % (Range%)	Male Rat % (Range%)	Female Rat % (Range%)
Hepatocellular Adenoma	54.91 (34-78)	25.68 (10-67)	1.43 (0-6)	0.86 (0-4)
Hepatocellular Carcinoma	30 (16-50)	12.93 (4-20)	0.57 (0-4)	0.14 (0-2)
Hepatoblastoma	3.27 (0-8)	0.55 (0-2)	0	0
Combined	**71.82** (62-84)	**34.43** (16-73)	**2** (0-6)	**1** (0-4)

Mouse, n=550; Rat, n=700

Courtesy of A. Pandiri 2020

Frequency of tissue response in 290 cancer-positive NTP mouse and/or rat bioassays

Liver	*57 %*
Lung	*22 %*
Kidney	*22 %*
Mammary gland	*14 %*
Hematopoietic	*13 %*
Forestomach	*12 %*
Thyroid	*10 %*
Vascular System	*9 %*

Data courtesy of D. Malarkey 2022

Liver tumor incidences based on 490 studies

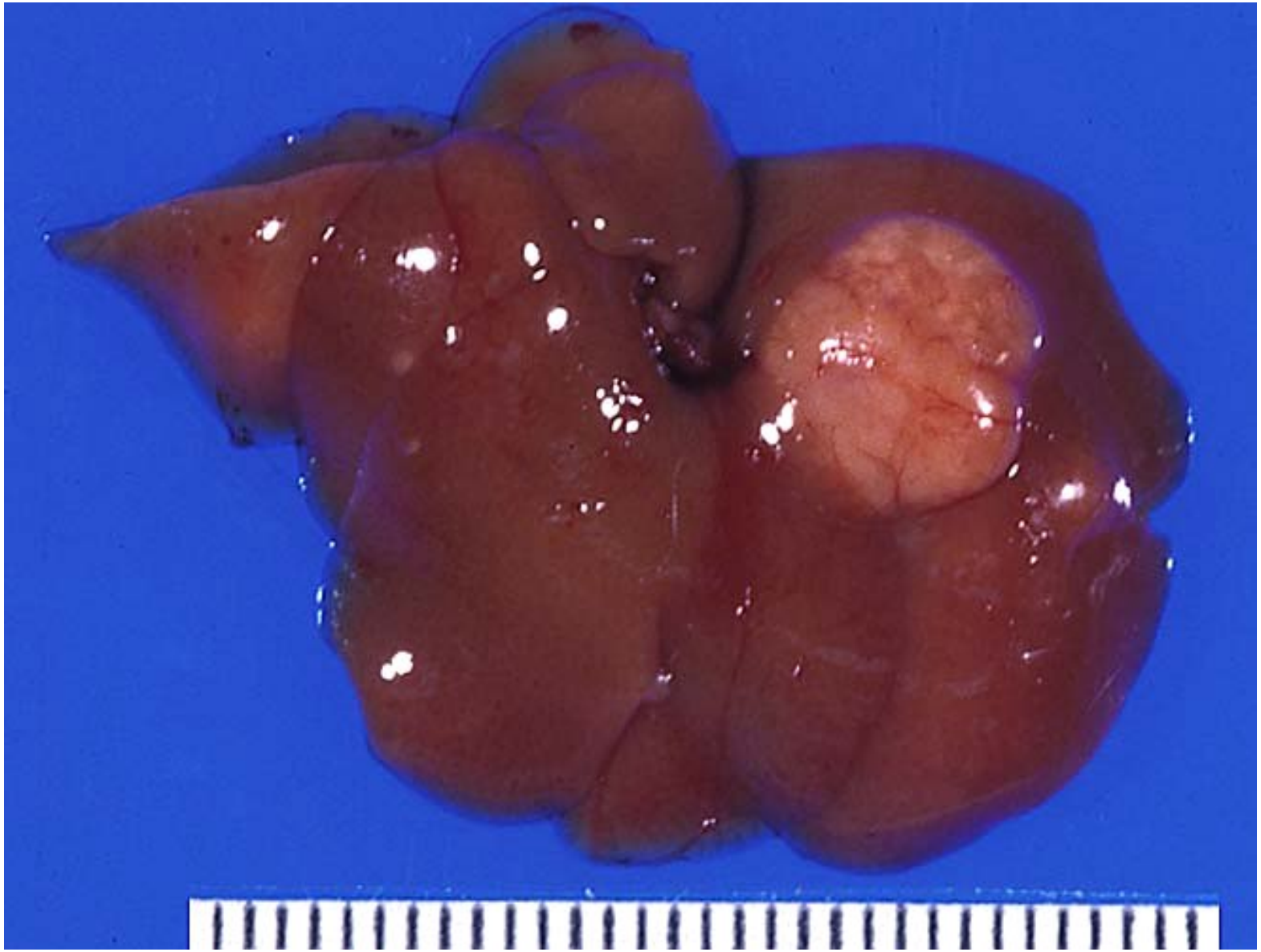
- 30% (146) of 490 NTP studies had an hepatocellular tumor response in rats and/or mice*
- Species dependence: mouse – 95/146 (65%), rat – 14/146 (9.6%), or both species 37/146 (25.3%)

Liver tumors N=146/490*	Mouse Male n (%)	Mouse Female n (%)	Rat Male n (%)	Rat Female n (%)
Nodule	0	1 (0.6)	10 (6.8)	6 (4.1)
Hepatocellular Adenoma	6 (4.1)	14 (9.5)	1 (0.6)	5 (3.4)
Hepatocellular carcinoma	64 (43.8)	88 (60.2)	31 (21.2)	30 (20.5)
Hepatoblastoma	20 (13.7)	13 (8.9)	0 (0)	1 (0.6)
Combined	**90 (61.6)**	**116 (79.5)**	**42 (28.8)**	**42 (28.8)**

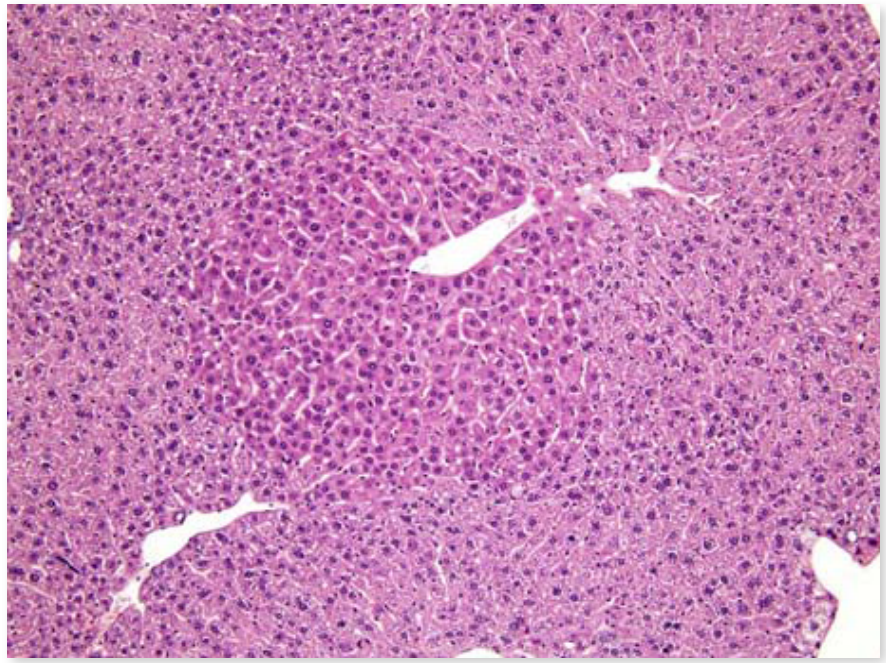
* 490 studies with same chemical tested in both rats and mice

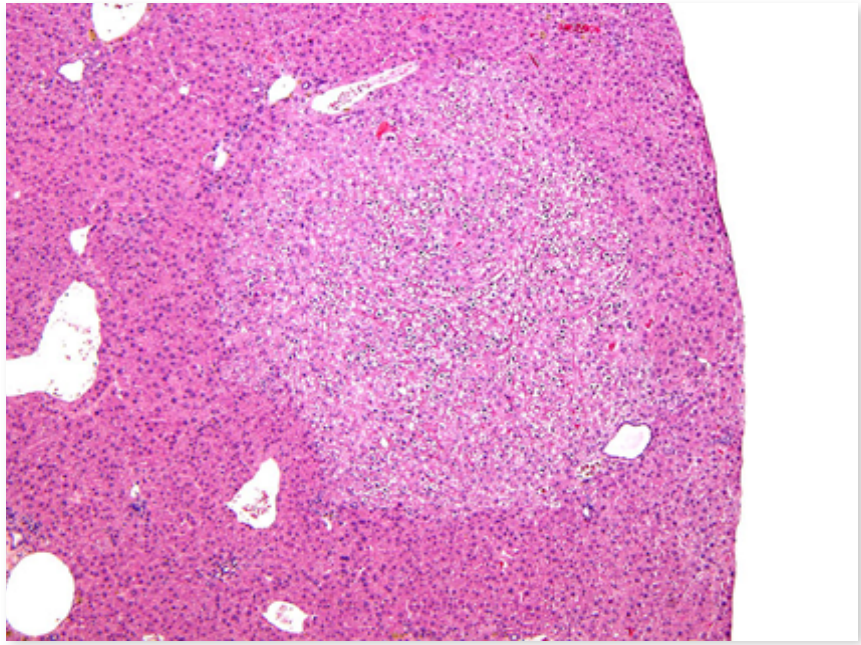
Data courtesy of A. Pandiri 2022

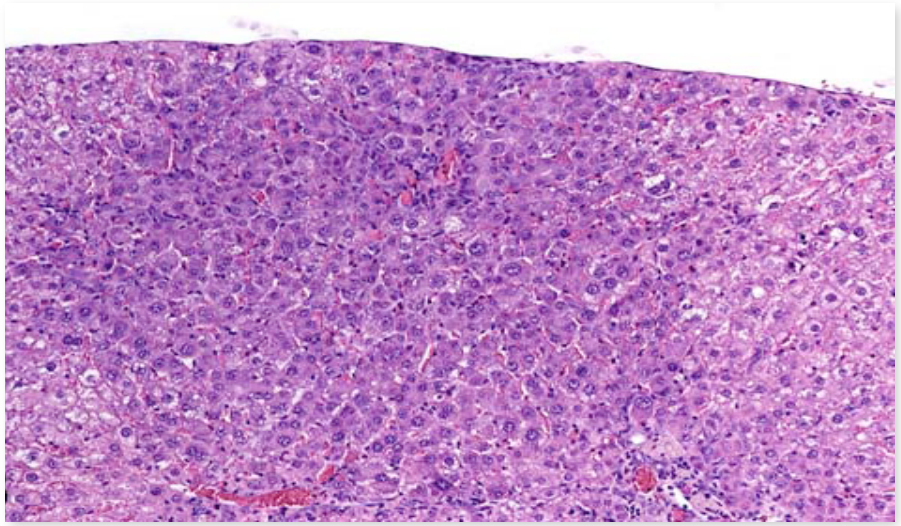
Hepatocellular Adenomas and Carcinomas

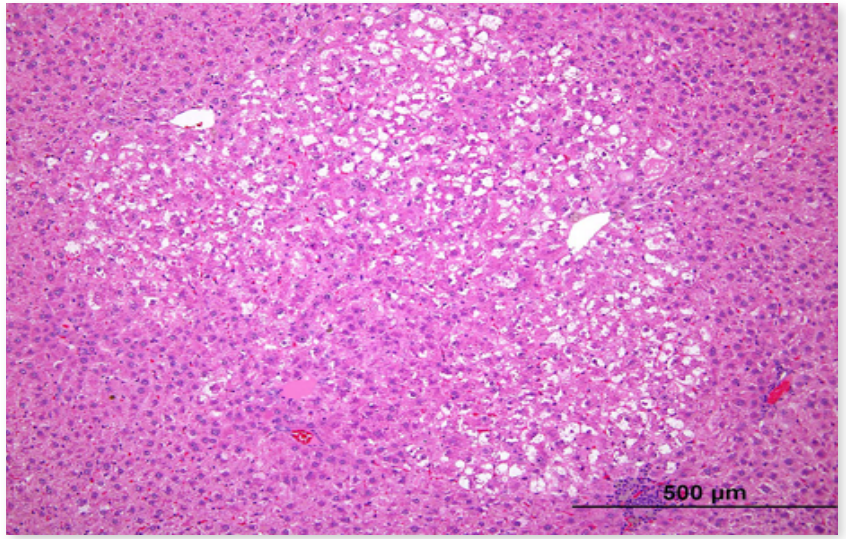


Hepatic Foci of Cellular Alteration

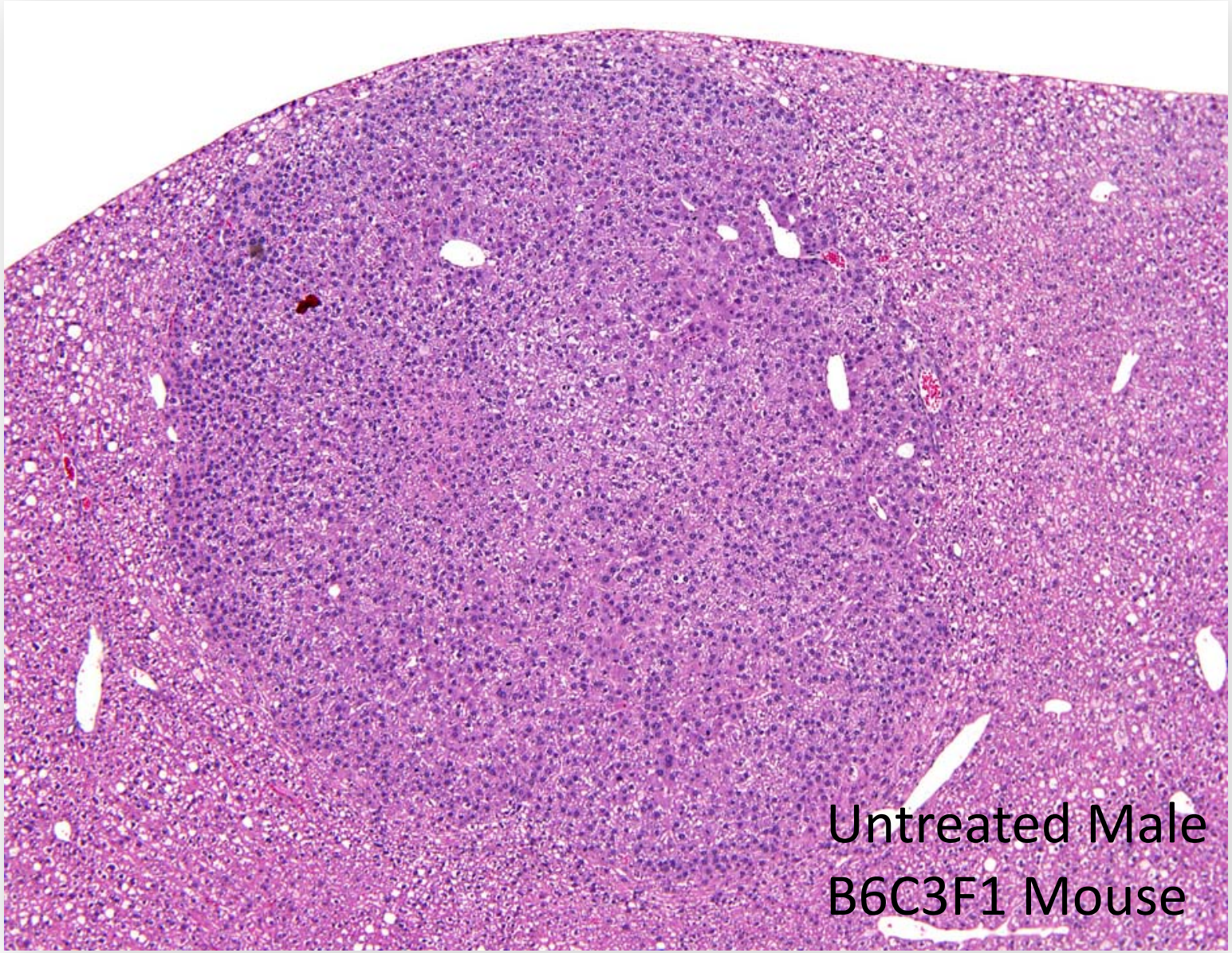








Hepatocellular Adenoma



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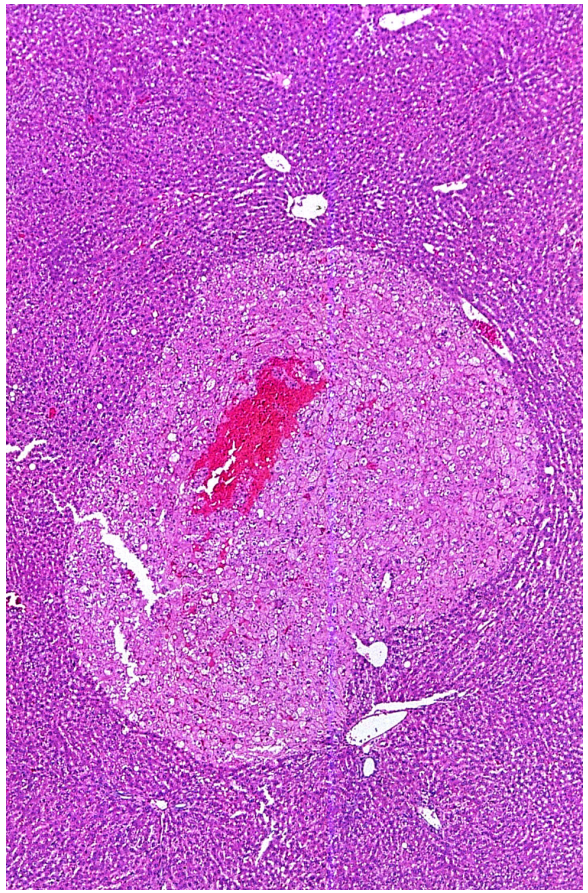

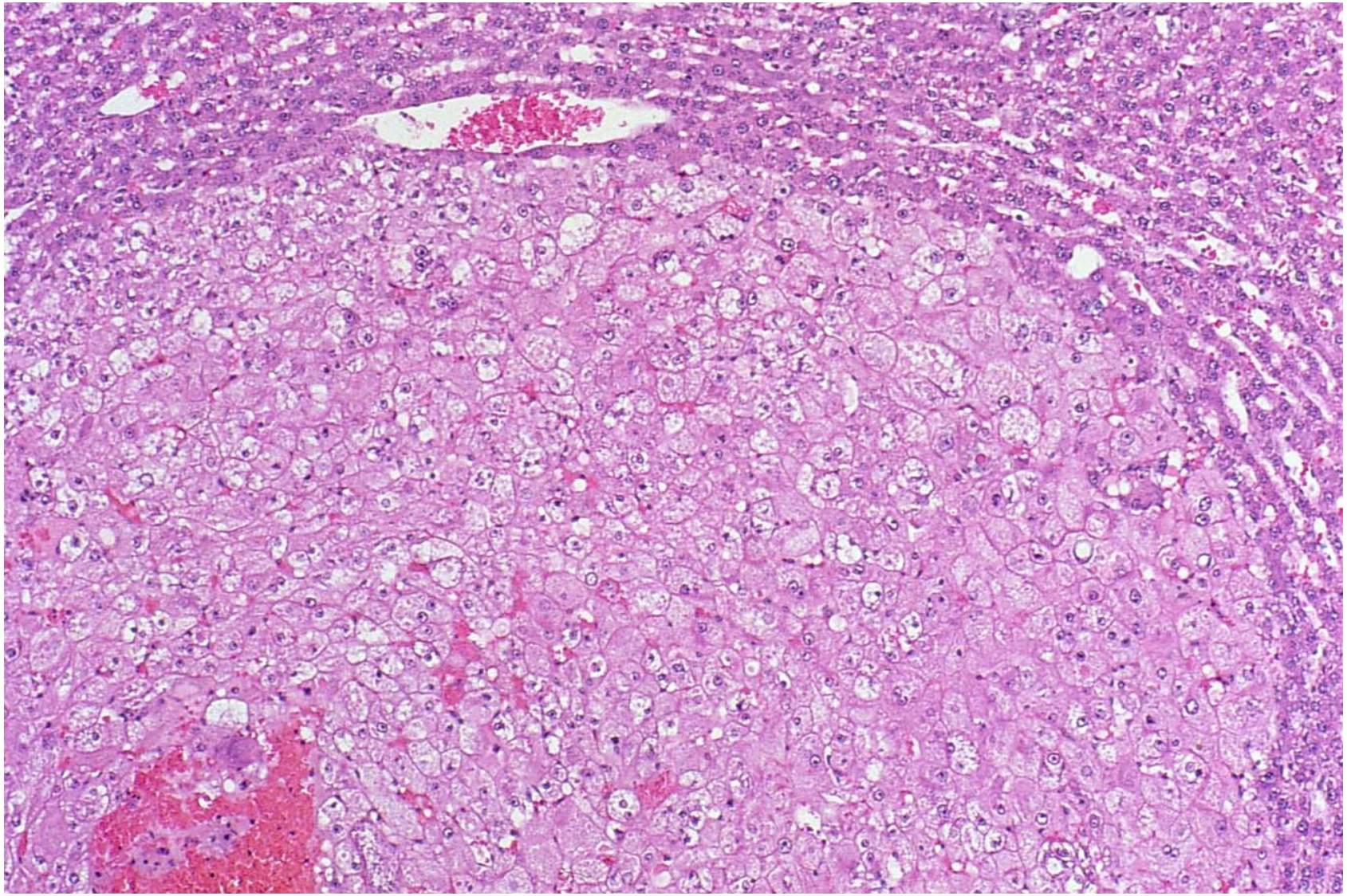

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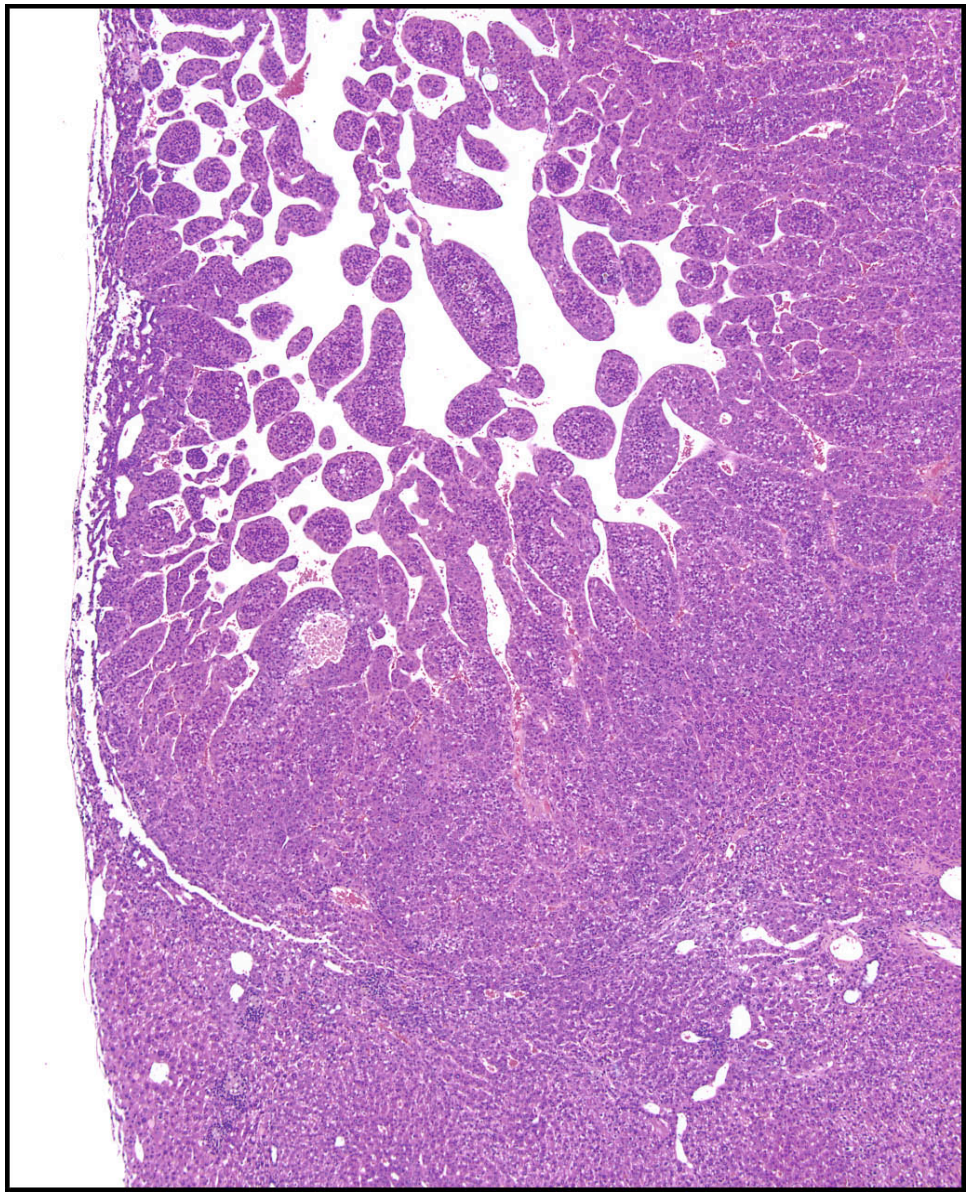

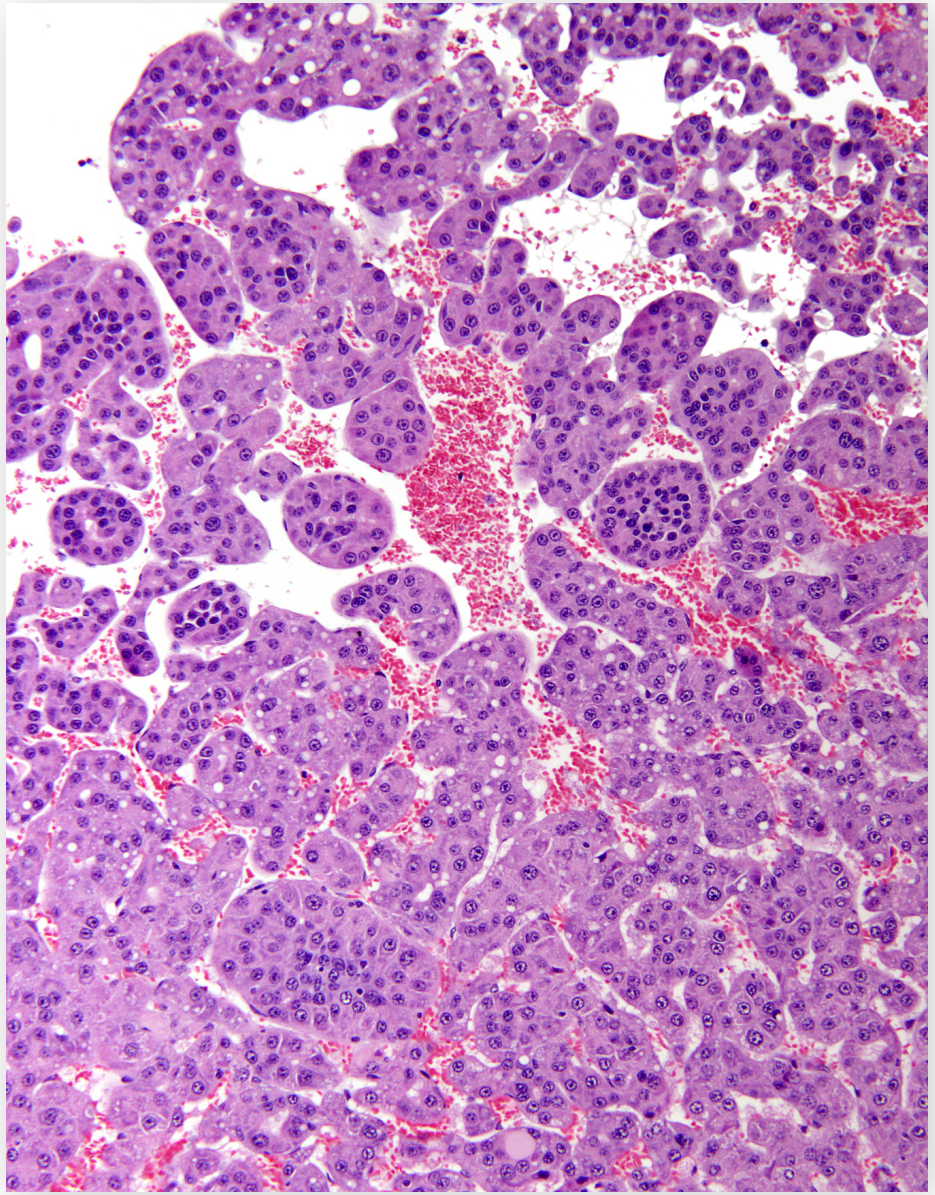

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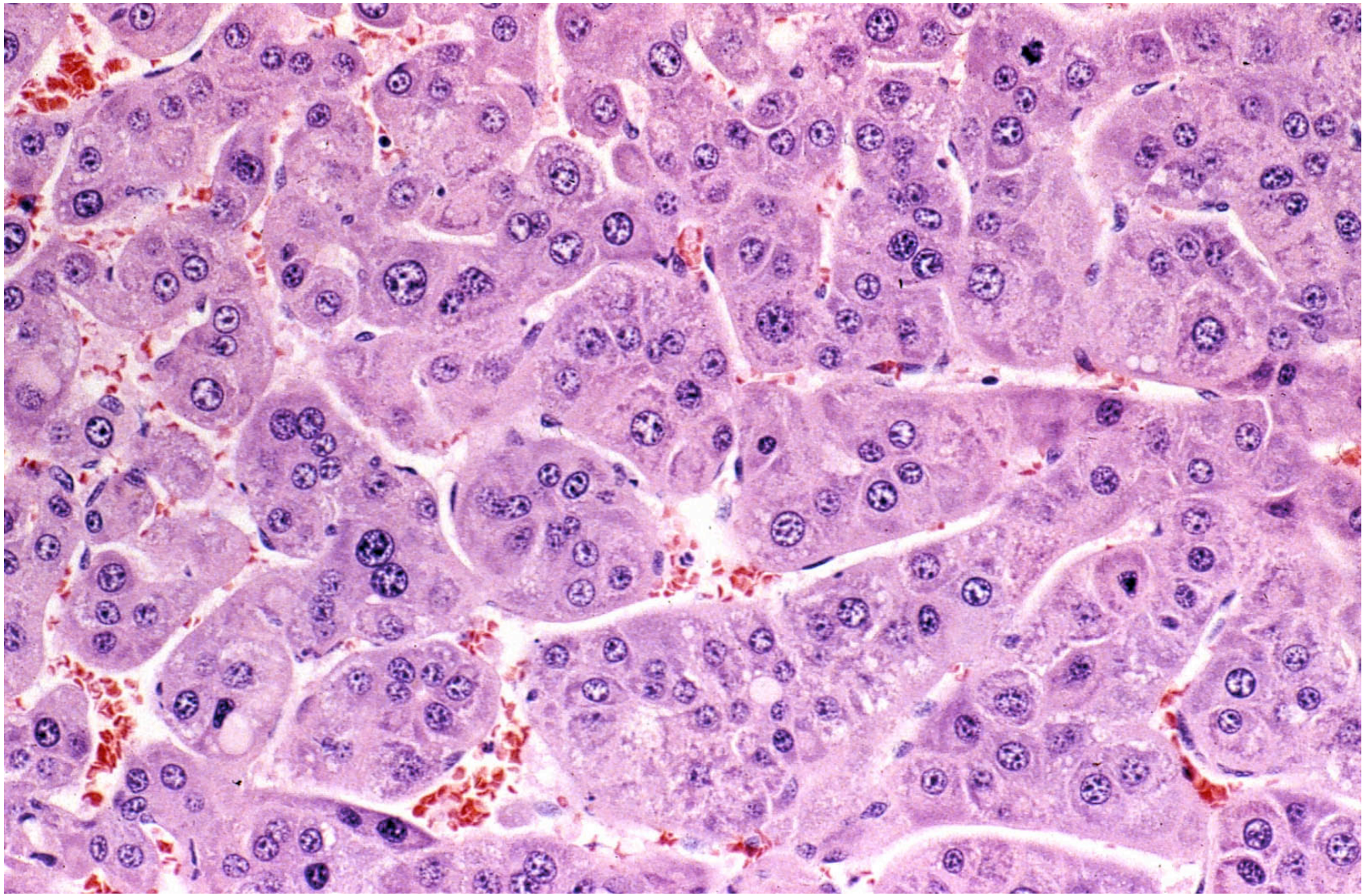
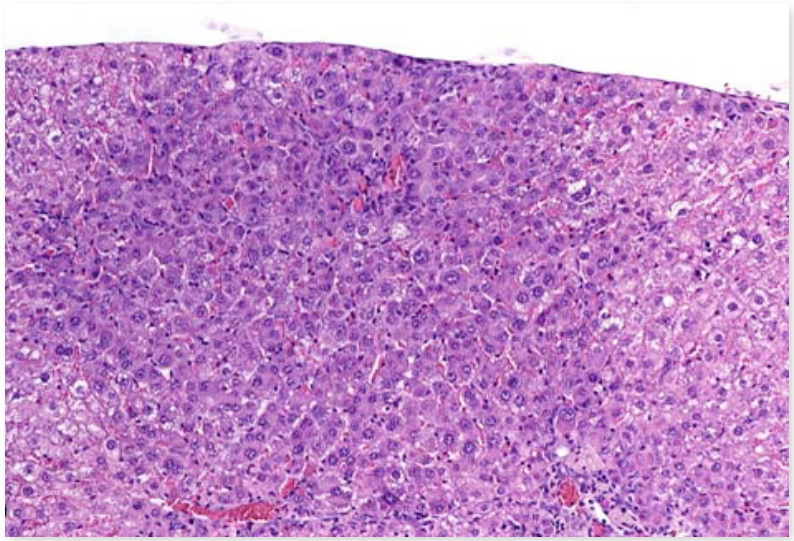
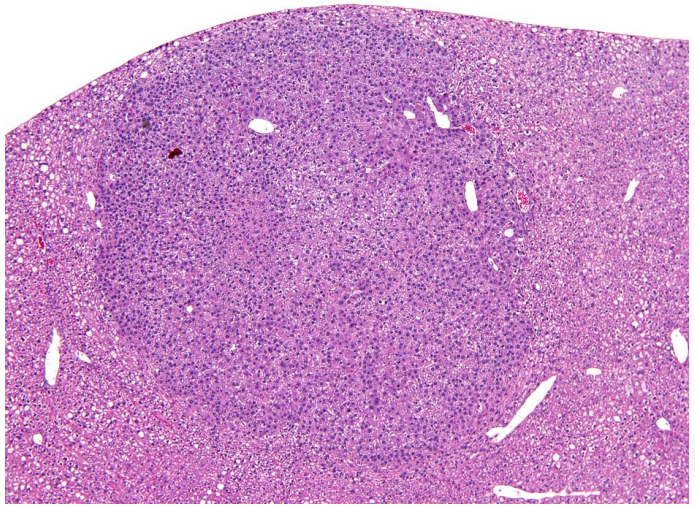
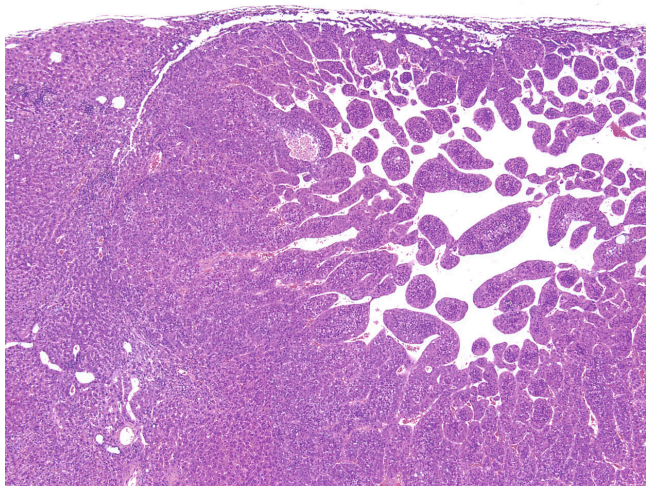


Progression of Proliferative Liver Lesions

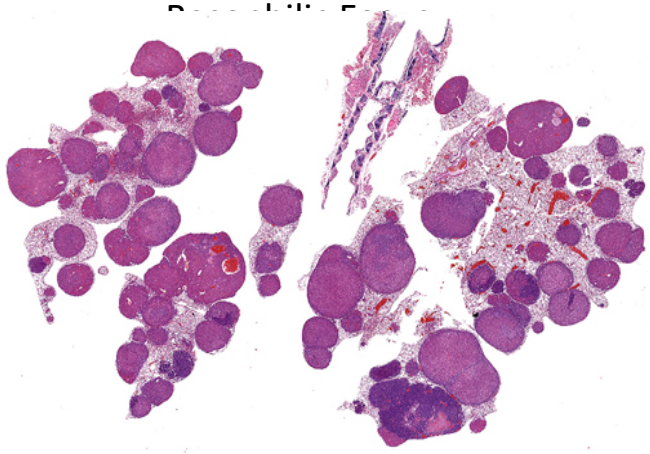




Hepatocellular adenoma

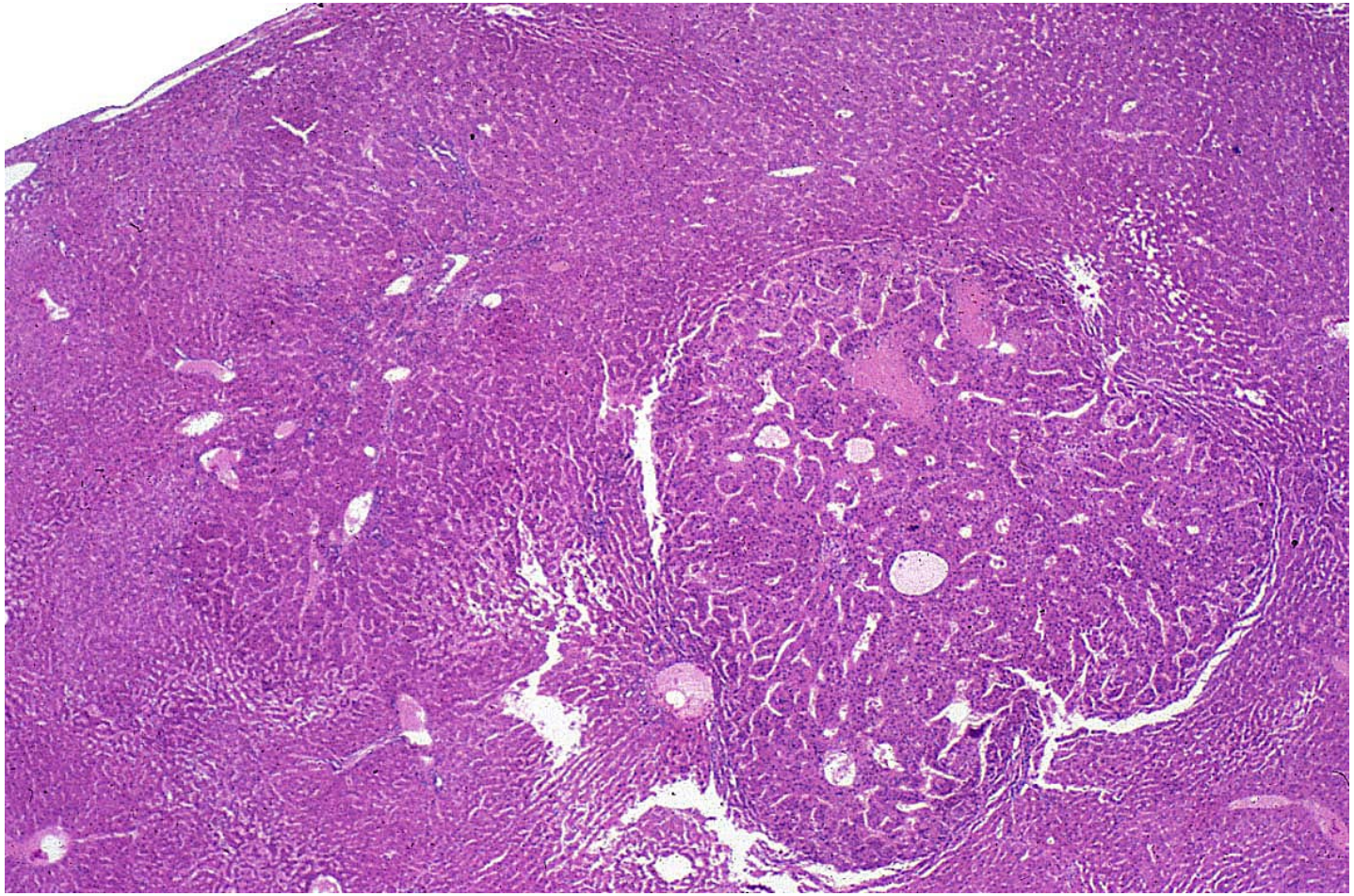


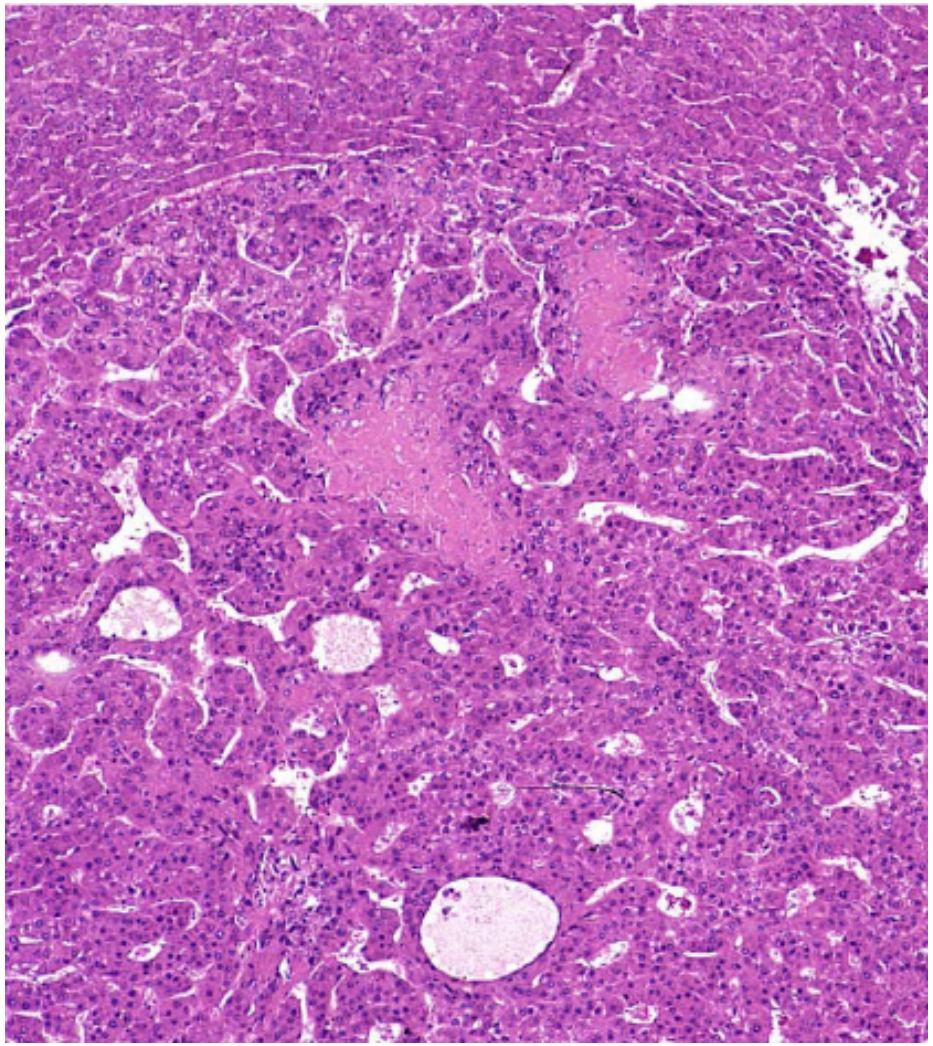
Hepatocellular carcinoma



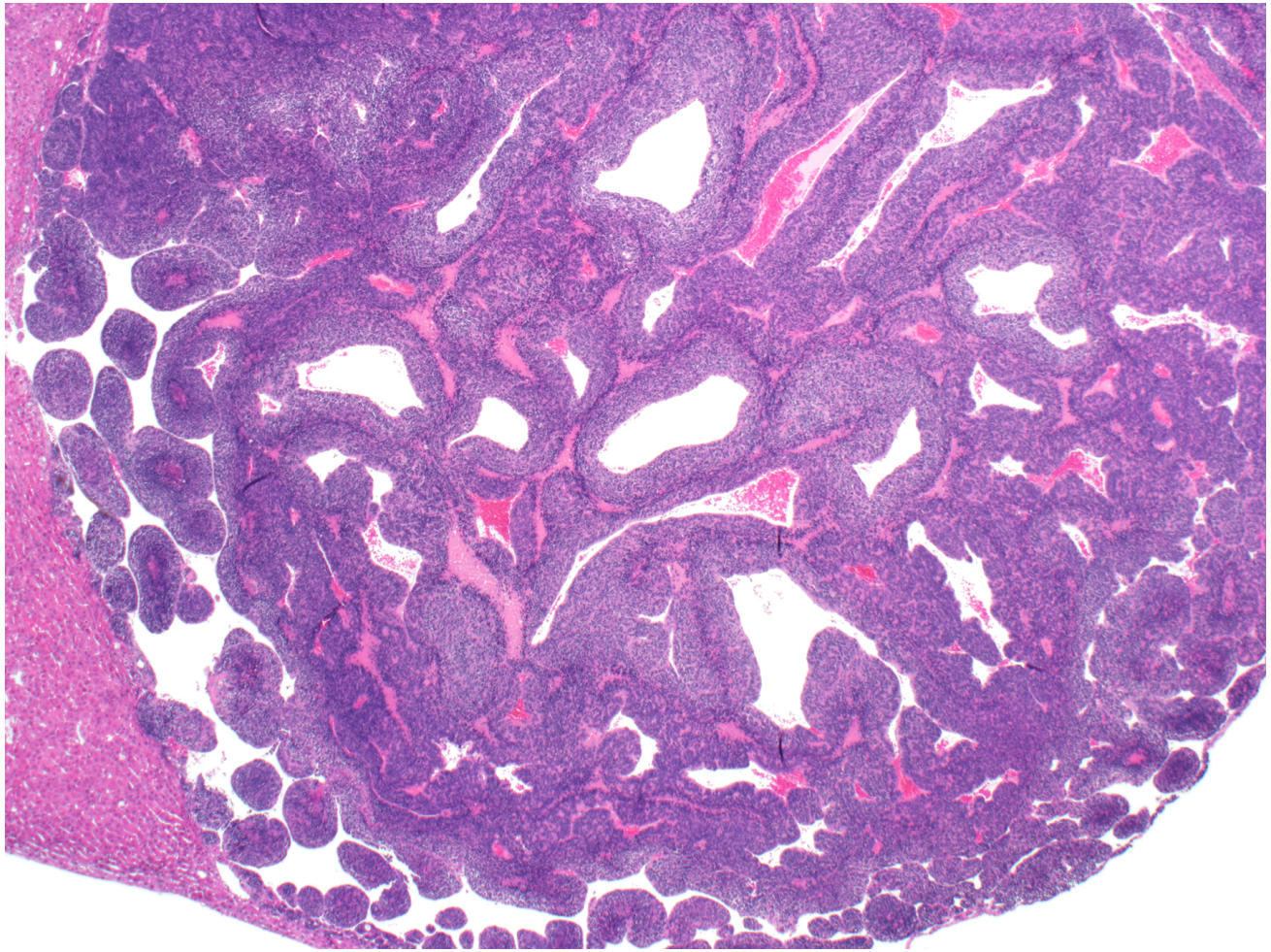
Metastatic carcinoma

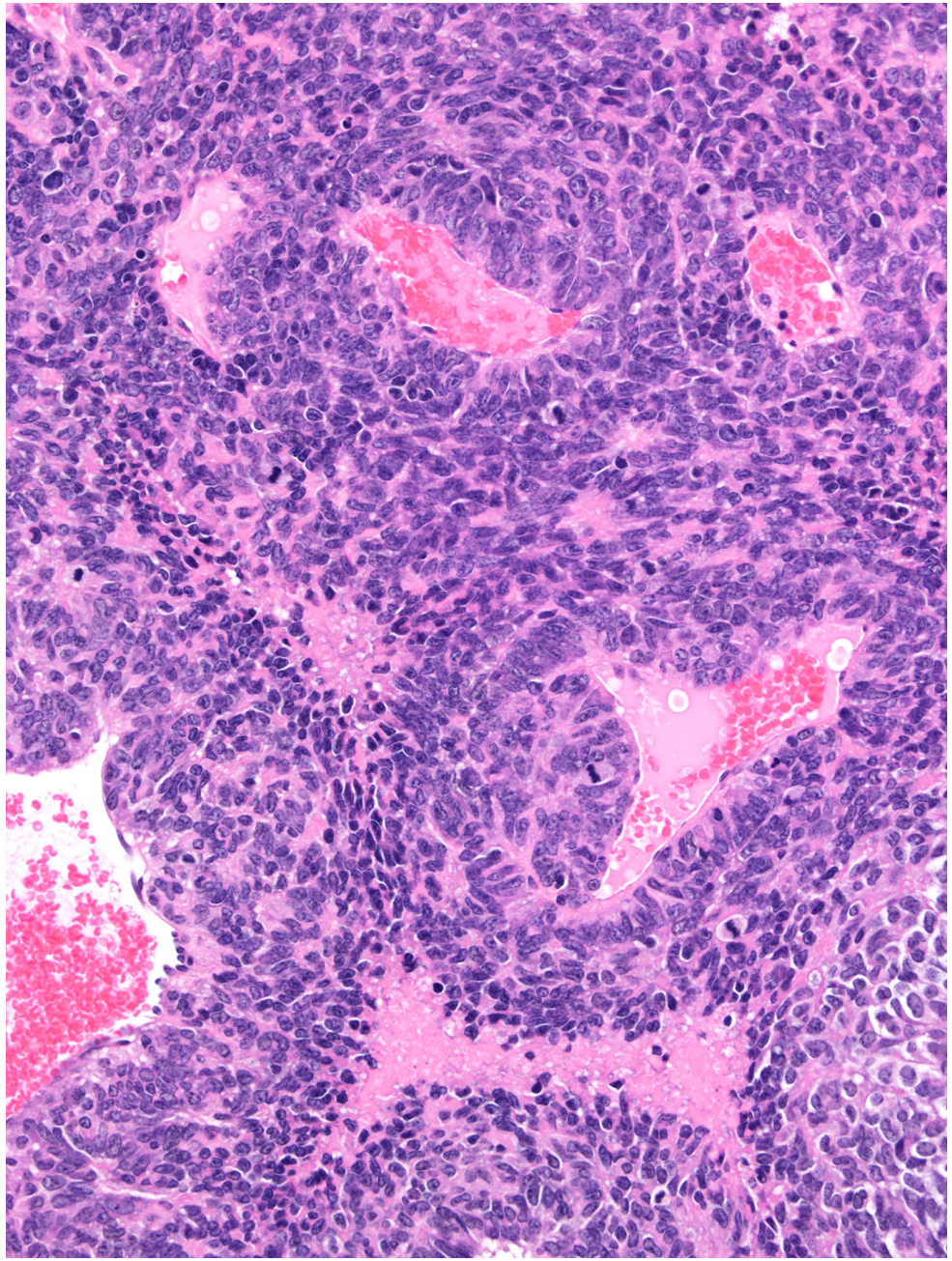
Carcinoma Arising in Adenoma





Hepatoblastoma

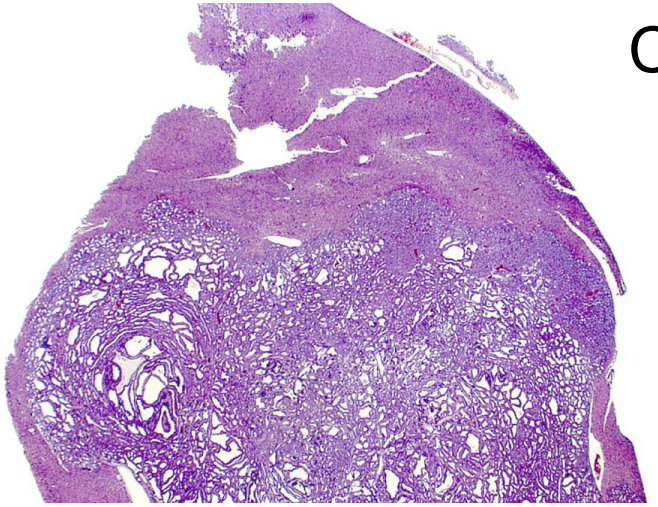


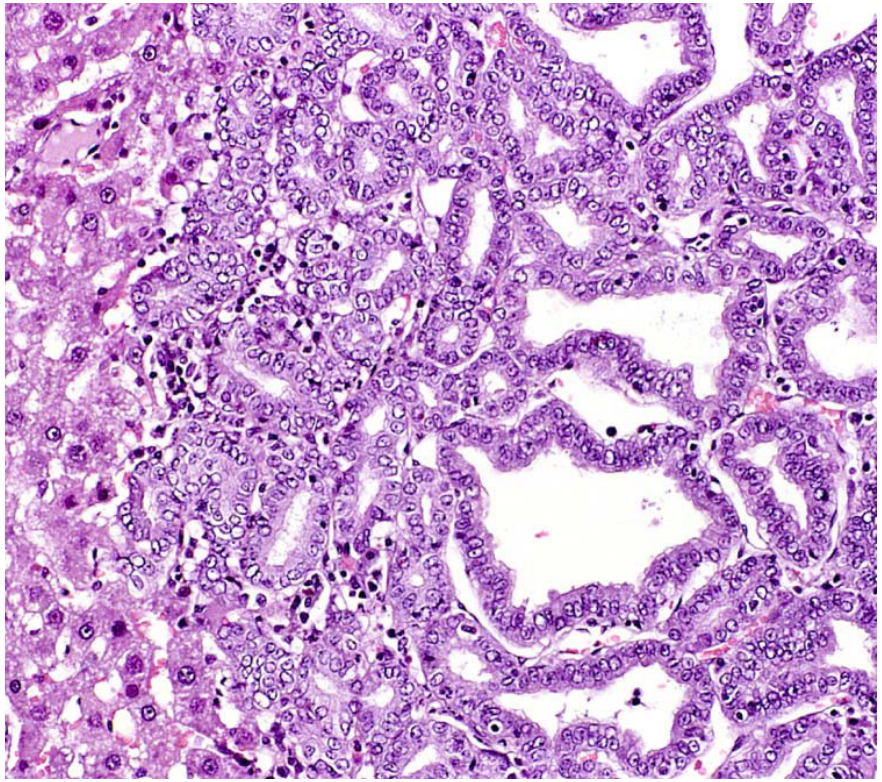


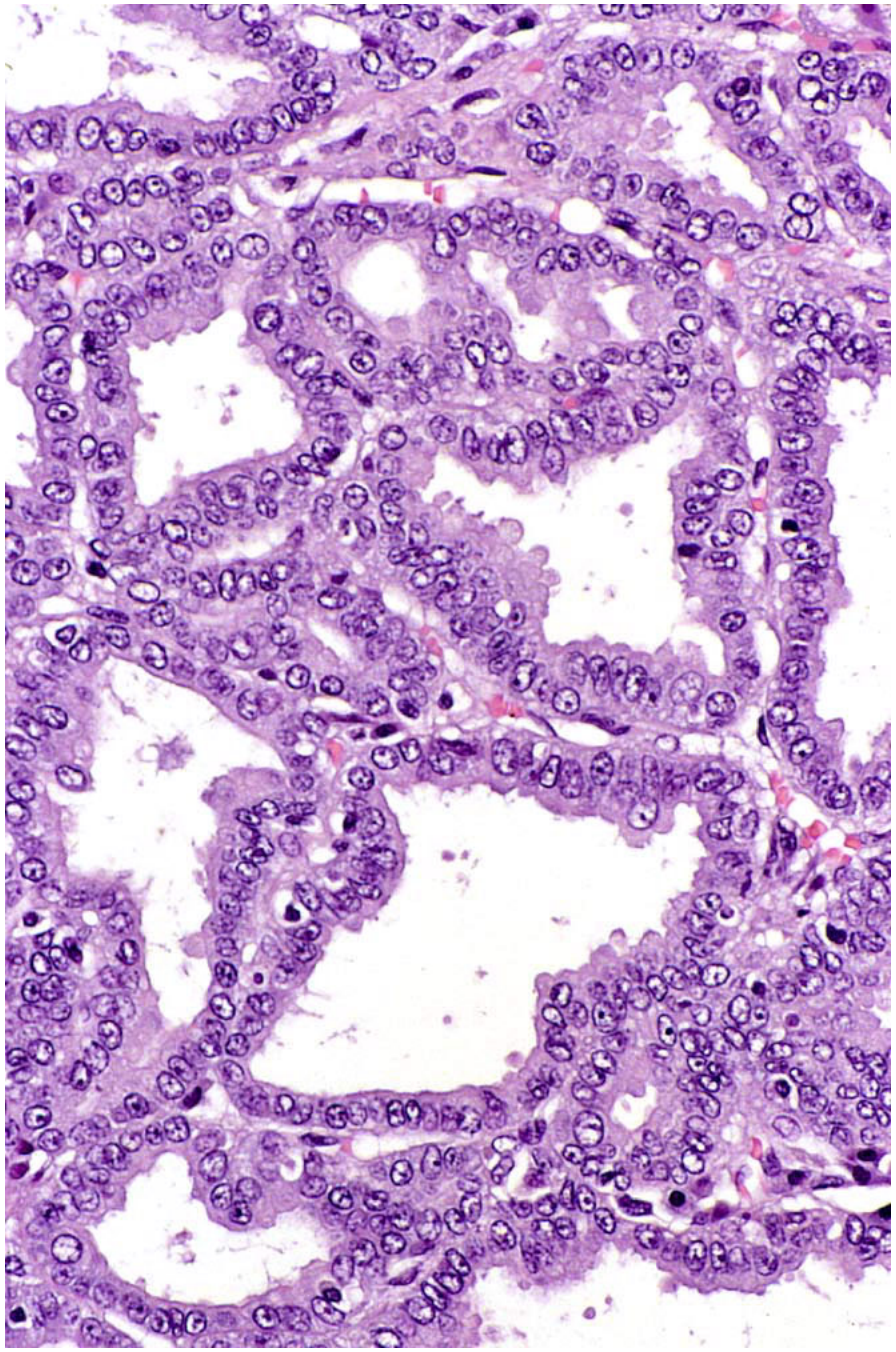
Turusov et al., Tox Path 30(5):580-591 (2002)
(63/140 studies had hepatoblastoma)
(Evaluated 500 hepatoblastomas)

Cholangioma

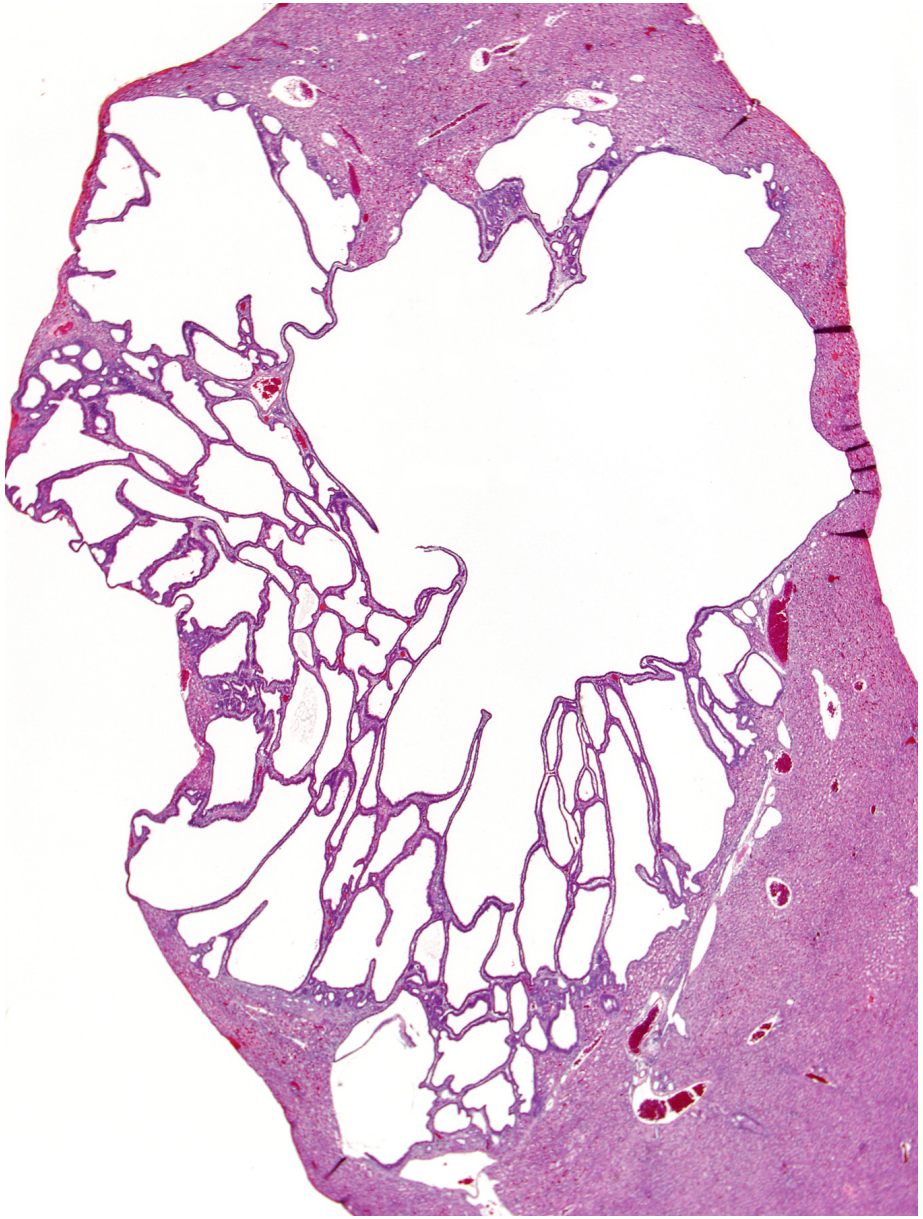
Sprague Dawley
Male

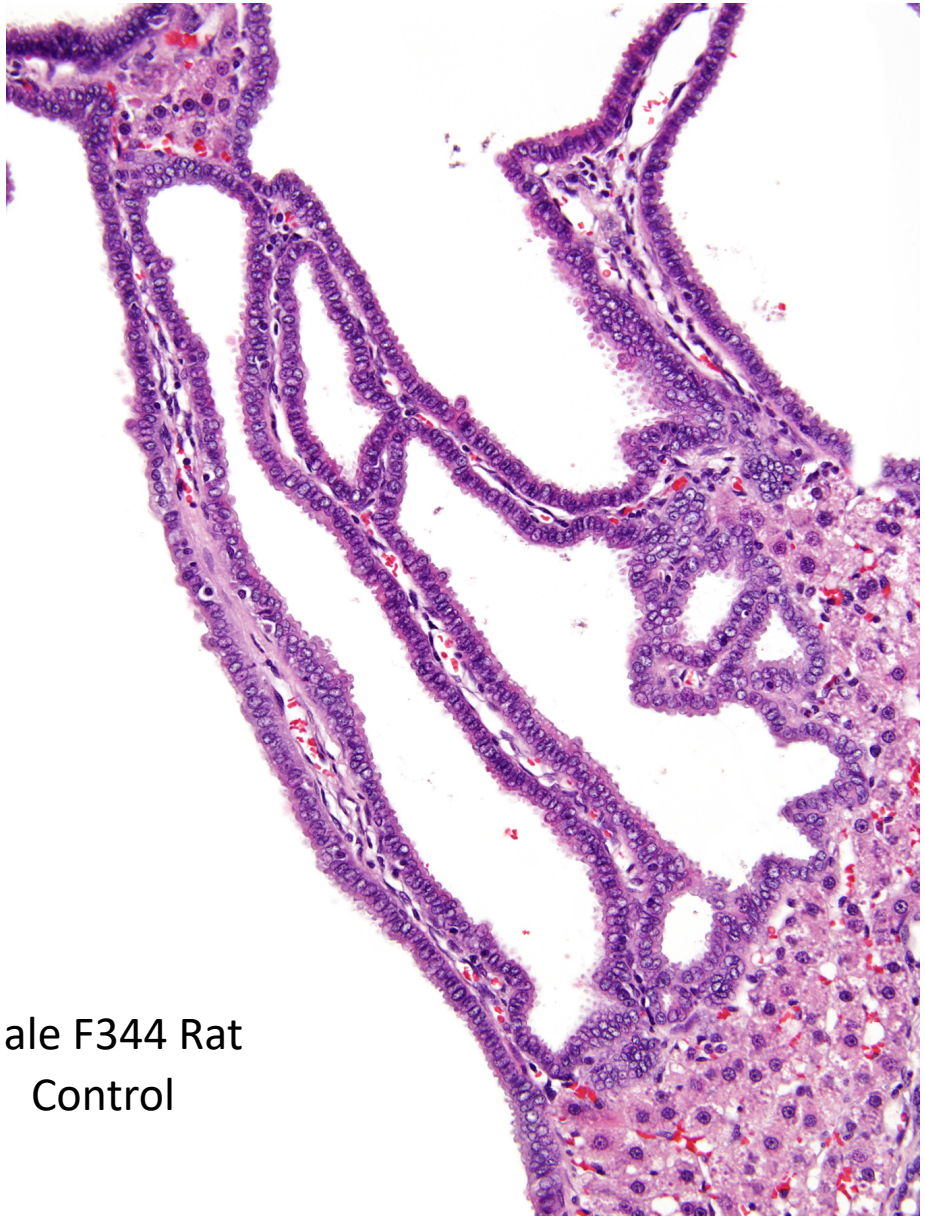






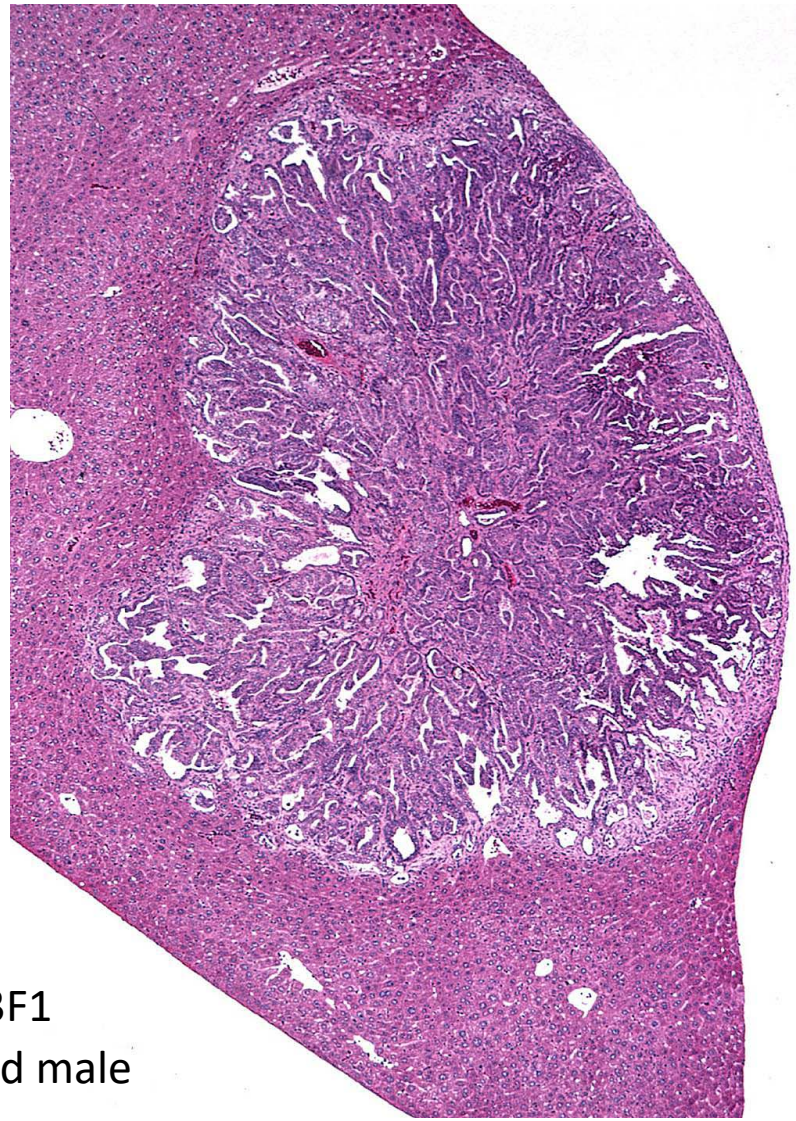
Cystic Cholangioma



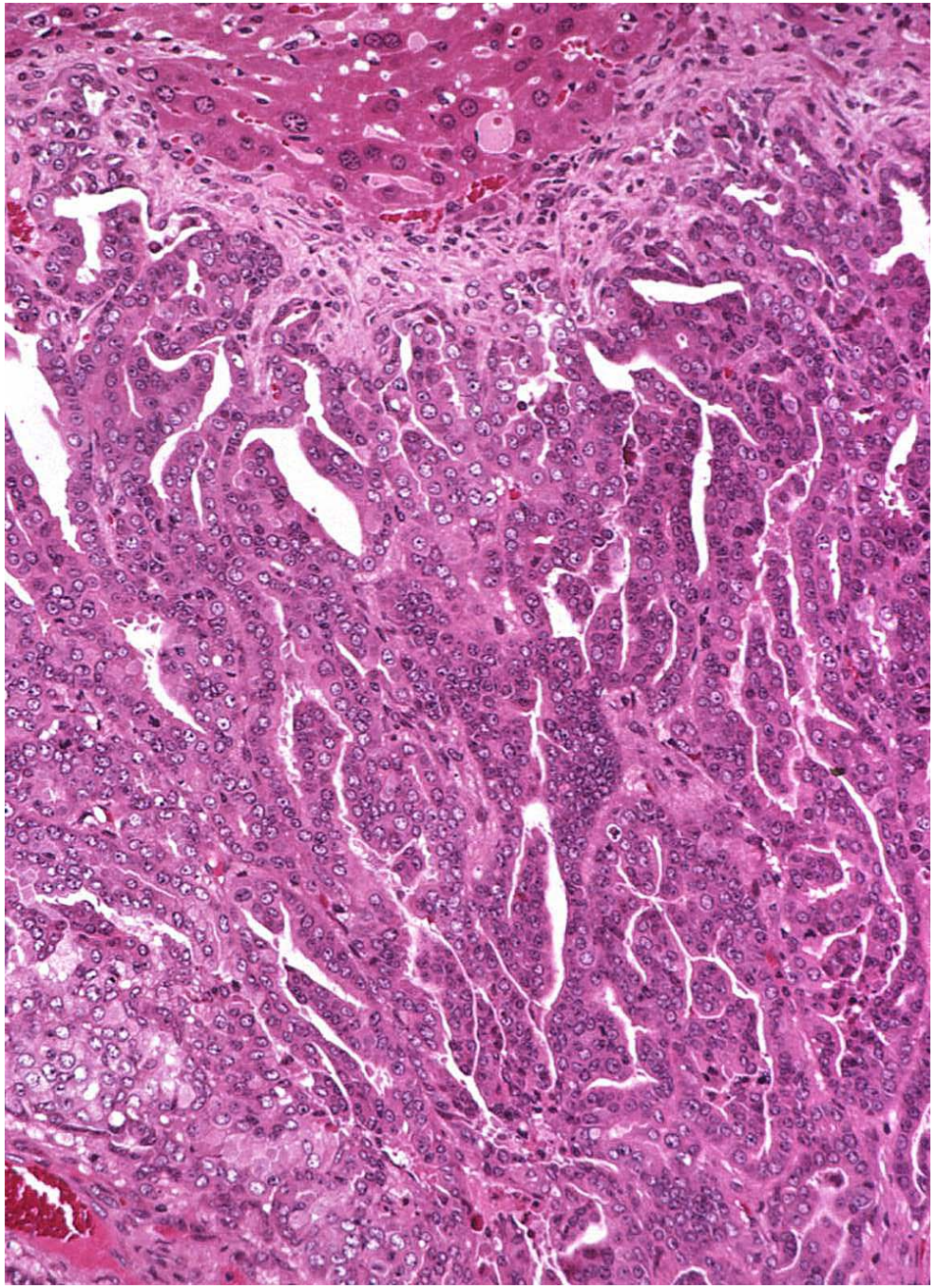


Male F344 Rat
Control

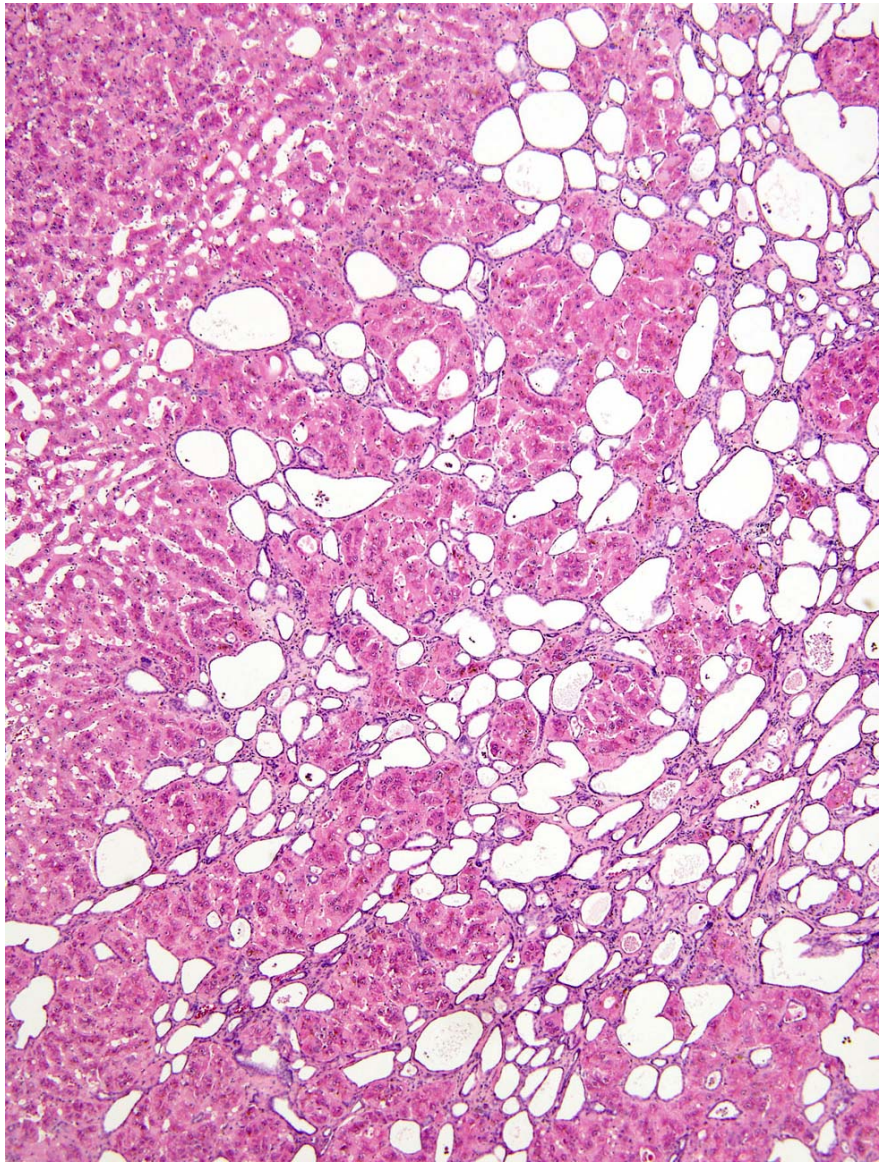
Cholangiocarcinoma



B6C3F1
Untreated male

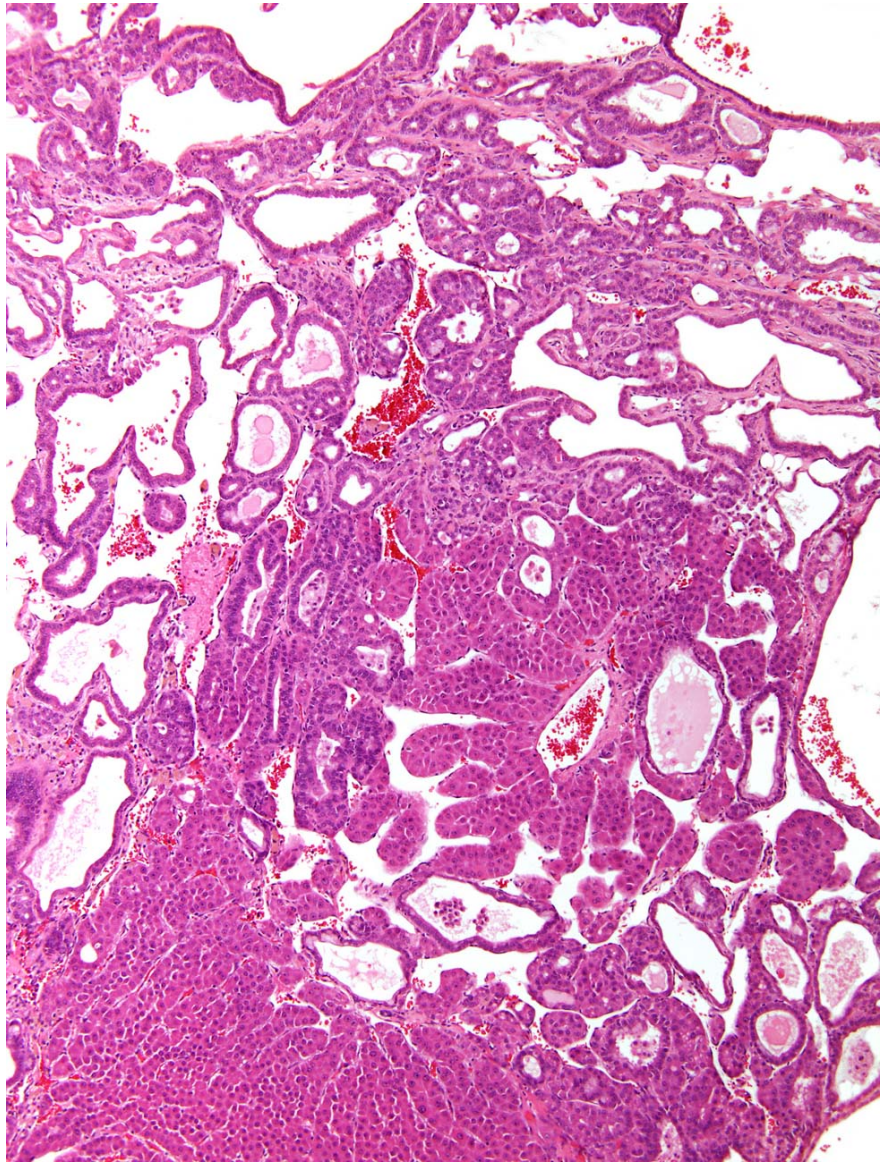


Hepatocholangioma



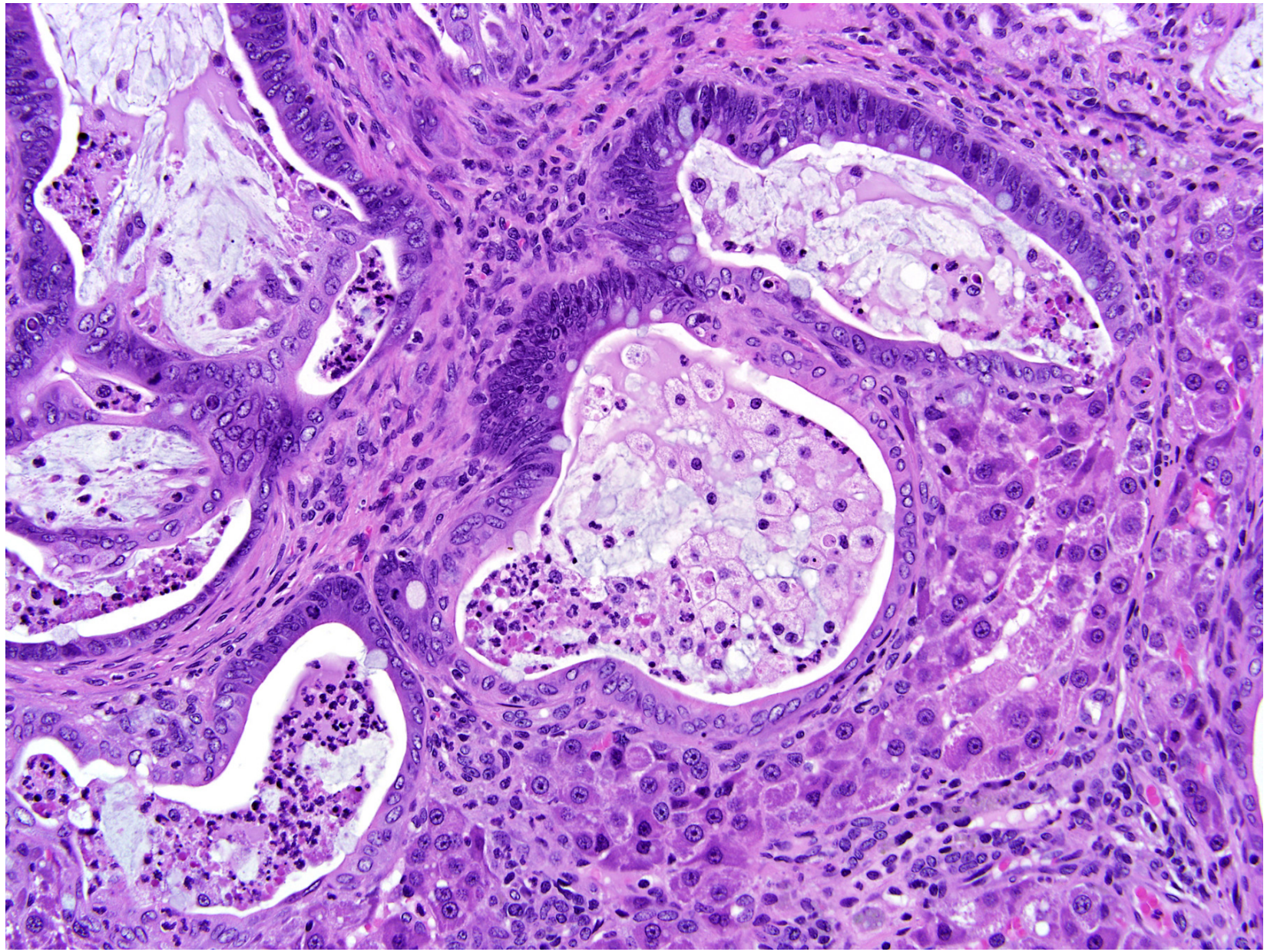
Treated Female Sprague Dawley

Hepatocholangiocarcinoma

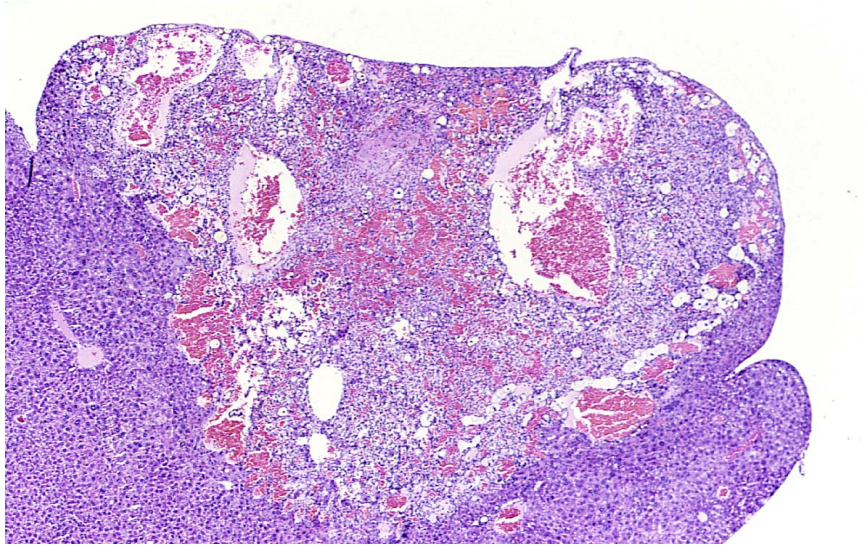


Treated Male F344

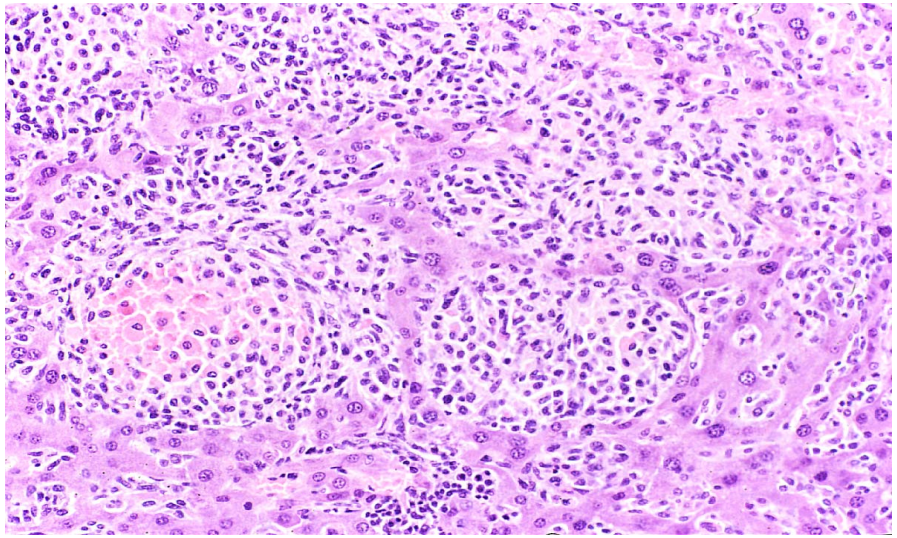
Hepatocholangiocarcinoma with intestinal metaplasia



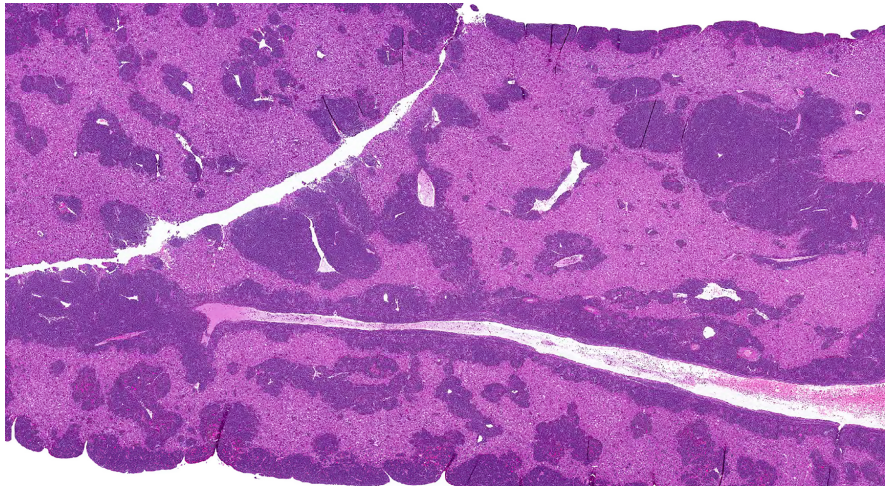
Other types of liver tumors



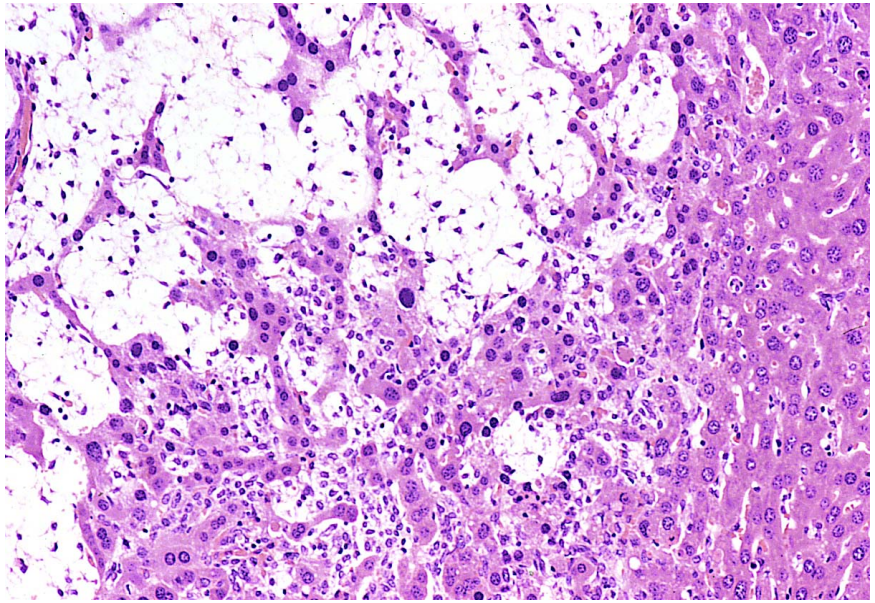
Hemangiosarcoma



Histiocytic sarcoma

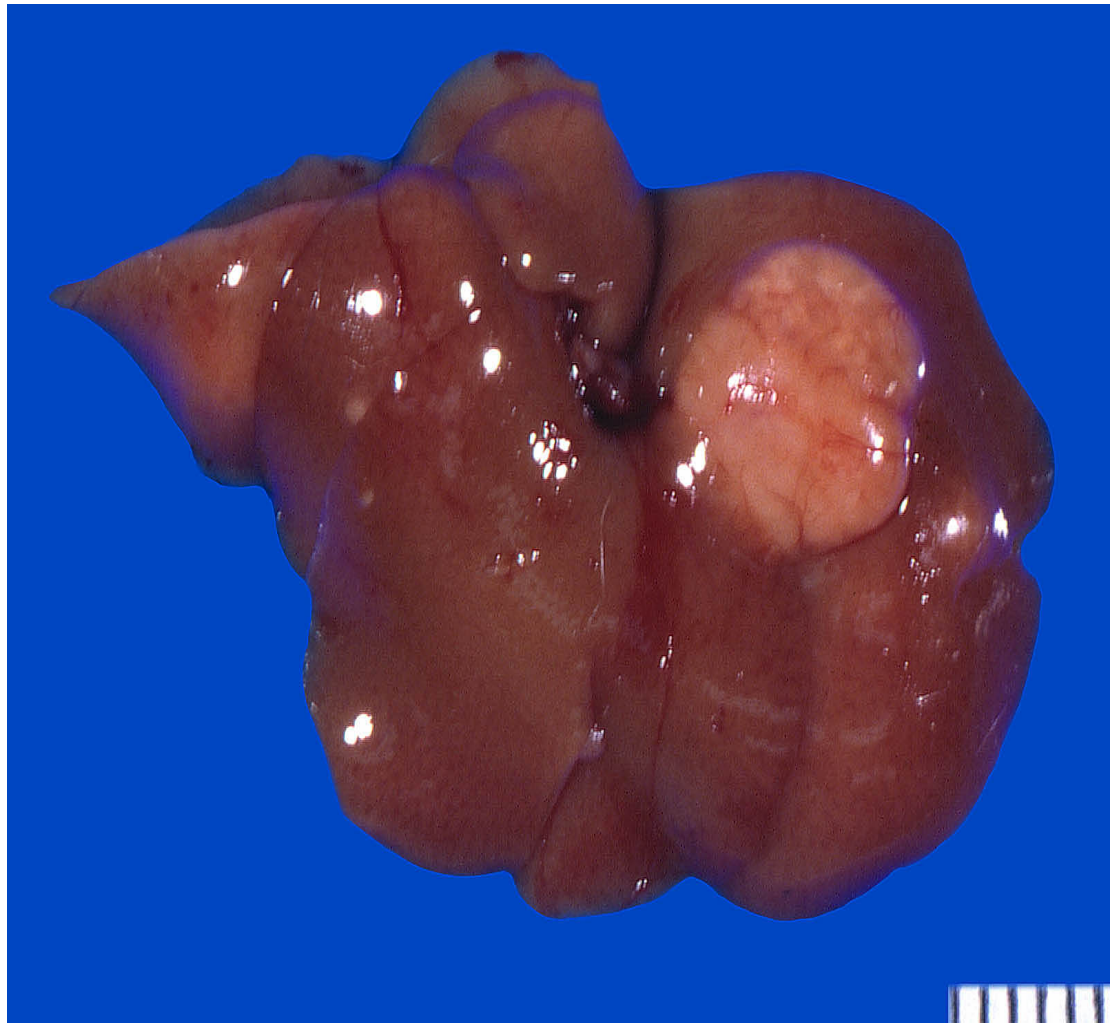


Lymphoma

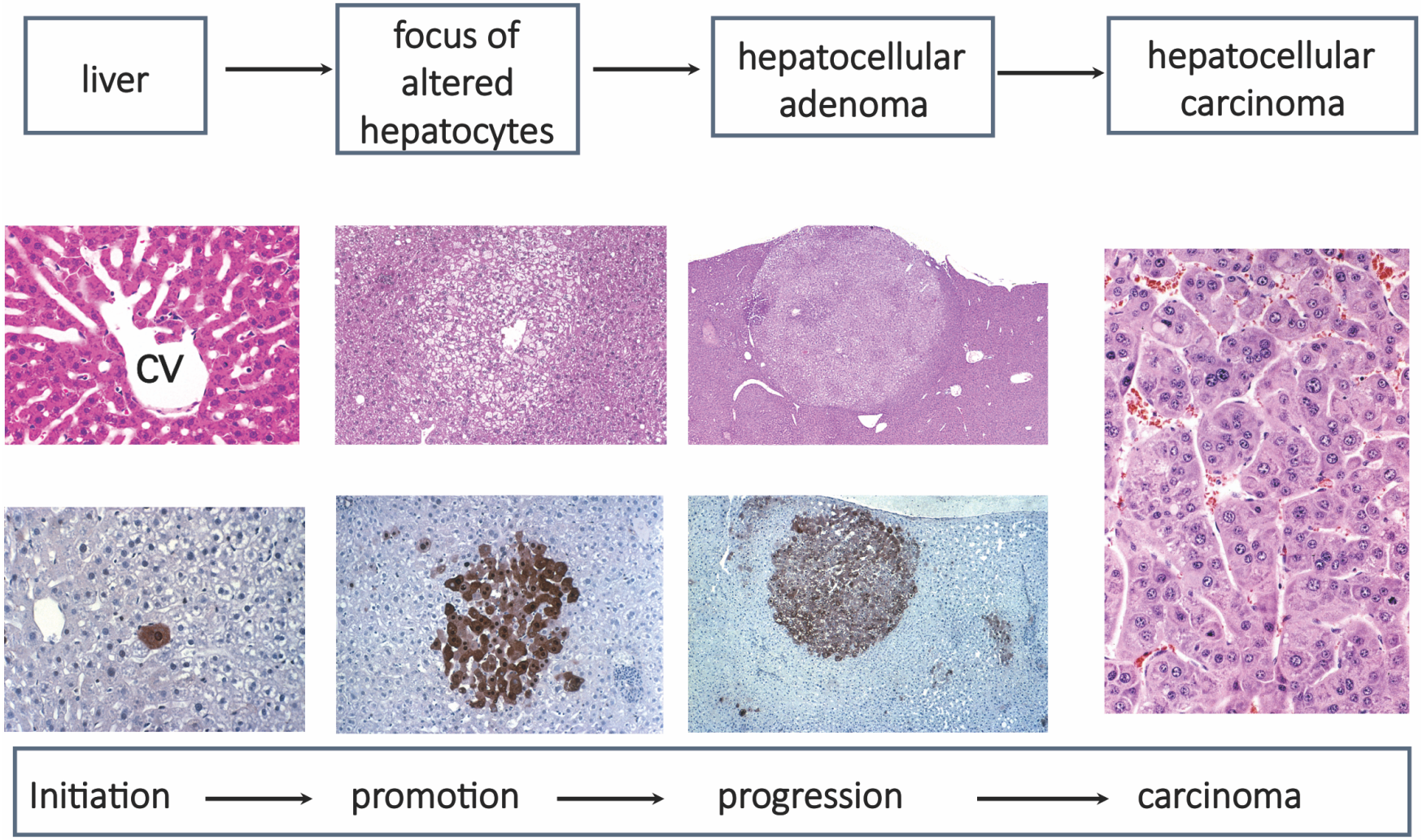


Stellate cell tumor

What have we learned from the conventional bioassay with respect to liver tumors?

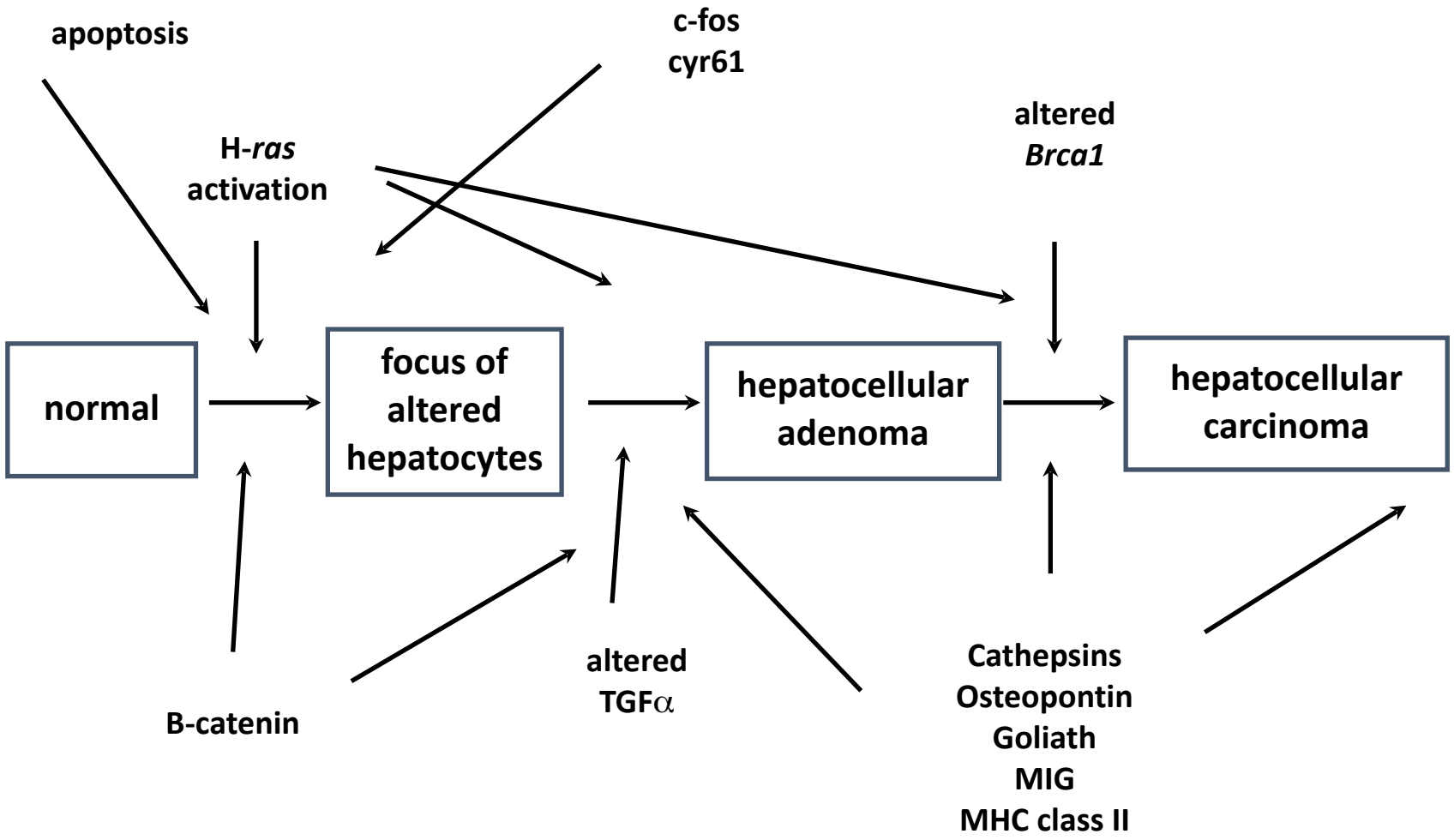


Multistep hepatocarcinogenesis



Malarkey, DE, Hoenerhoff, MJ, and Maronpot, RR. 2018. Carcinogenesis: Manifestations and Mechanisms in Fundamentals of Toxicologic Pathology, 3rd Edition, Wallig, MA, Haschek, WM, Rousseaux, CG, Bolon, B, and Mahler, BW, Editors, Academic Press, San Diego. Pp 83-104.

Multistage hepatocarcinogenesis



There were and still are some strong opinions about the significance of rodent bioassays

Pharmaceutical Pathobiology

A Critical Review of the Effectiveness of Rodent Pharmaceutical Carcinogenesis Testing in Predicting for Human Risk

C. L. Alden[1], A. Lynn[1], A. Bourdeau[1], D. Morton[2], F. D. Sistare[3], V. J. Kadambi[1], and L. Silverman[1]

Veterinary Pathology
48(3) 772-784

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http://vet.sagepub.com

Toxicologic Pathology, 38: 487-501, 2010

ISSN: 0192-6233 print / 1533-1601 online
DOI: 10.1177/0192623310363813

Evaluation of Possible Carcinogenic Risk to Humans Based on Liver Tumors in Rodent Assays: The Two-Year Bioassay Is No Longer Necessary

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ABSTRACT

The PPARα-dependent rodent liver tumor response is not relevant to humans: addressing misconceptions

J. Christopher Corton, Jeffrey M. Peters & James E. Klaunig

Archives of Toxicology **92**, 83–119 (2018) | Cite this article

Review and Evaluation of the NCI/NTP Carcinogenesis Bioassays*

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The Limits of Two-Year Bioassay Exposure Regimens for Identifying Chemical Carcinogens

James Huff,[1] Michael F. Jacobson,[2] and Devra Lee Davis[3]

[1]National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, North Carolina, USA; [2]Center for Science in the Public Interest, Washington, DC, USA; [3]Center for Environmental Oncology, University of Pittsburgh Cancer Institute, Department of Epidemiology, Graduate School of Public Health, Pittsburgh, Pennsylvania, USA

Human relevance of rodent liver tumors: Key insights from a Toxicology Forum workshop on nongenotoxic modes of action

Susan P. Felter [a], Jennifer E. Foreman [b], Alan Boobis [c], J. Christopher Corton [d], Adriana M. Doi [e], Lynn Flowers [f], Jay Goodman [g], Lynne T. Haber [h], Abigail Jacobs [i], James E. Klaunig [j], Angela M. Lynch [k], Jonathan Moggs [l], Arun Pandiri [m]

JOURNAL ARTICLE

Goodbye to the bioassay

Jay I. Goodman

Toxicology Research, Volume 7, Issue 4, July 2018, Pages 558–564,
https://doi.org/10.1039/c8tx00004b
Published: 13 February 2018 **Article history**

*J Toxicol Pathol 2007; **20**: 13–19*

Review

The Two-Year Rodent Carcinogenesis Bioassay — Will It Survive?

Jerrold M. Ward[1]

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Bethesda, Maryland 20892–8135 US

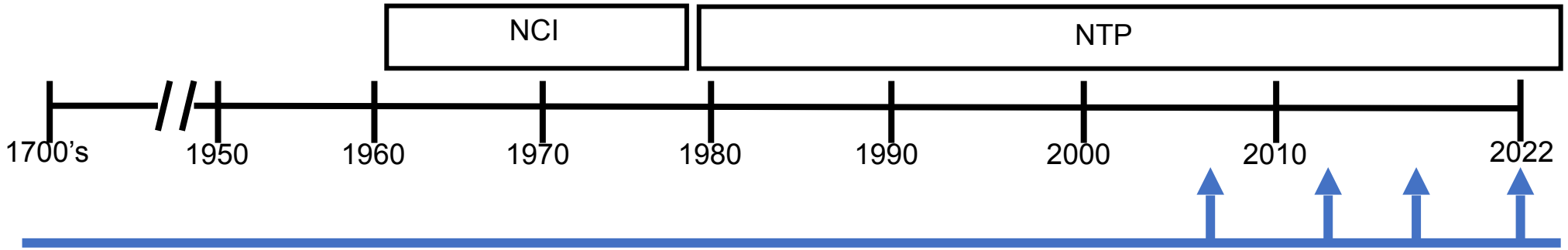
Evaluation of the utility of the lifetime mouse bioassay in the identification of cancer hazards for humans

Thomas G. Osimitz [a], Wiebke Droege [a], Alan R. Boobis [b], Brian G. Lake [c]

Mode of Action in Relevance of Rodent Liver Tumors to Human Cancer Risk

Michael P. Holsapple,*[,1] Henri C. Pitot,† Samuel H. Cohen,‡ Alan R. Boobis,§ James E. Klaunig,¶ Timothy Pastoor,‖ Vicki L. Dellarco,‖‖ and Yvonne P. Dragan‖‖

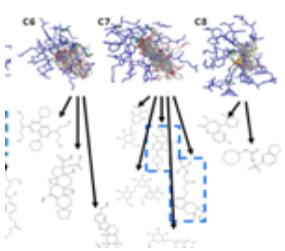
Mechanisms associated with bioassay tumor responses



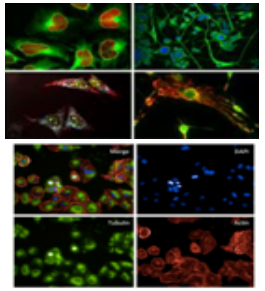
- Nuclear receptor activation
  - CAR/PXR, AhR, PPAR-a
- Cytotoxicity and regenerative hyperplasia
- Endocrine modifiers
- Epigenetic modifiers

- Mitogen/tumor promoter
- Inflammation
- Oxidative stress
- Hormonal perturbation
- Immunosuppression
- Suppression of apoptosis

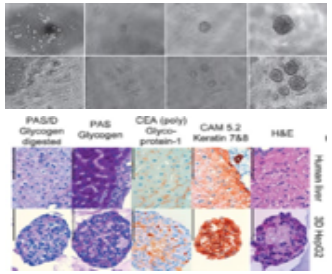
Contemporary NTP efforts



QSAR Relationships



Cell culture and Genetox assays



Organoids, metabolically competent

Lower order model organisms

Rodent models

Throughput

Human relevance

- Core set of mechanistic assays
  - DNA repair & reactivity
  - Receptor-mediated assays
  - Intercellular communication
  - Enzyme induction
  - Cell cycle perturbations
  - Endocrine disruption
  - Effects on methylation
  - Oxidative stress
  - Immunosuppression

- Other contemporary investigative approaches
  - NEGCARC (Genotoxicity, endocrine, histopathology) for pharmaceuticals
  - Tox 21 & high throughput screening assays
  - Genomics, proteomics, metabonomics
  - Mutations in cancer genes
  - Structure activity relationships
  - Epigenetic changes
  - Adverse outcome pathway/MOA

Prechronic liver lesions as predictors of liver carcinogenicity*

B6C3F1 Mouse

- 25 of the 27 (92%) liver tumor positive studies were correctly identified in 90-day studies based on combination of liver hypertrophy, cytomegaly, necrosis and increased liver weight ($p < 0.001$)

- 18 false positives

F344 Rat

- 7 of 11 (64%) liver tumor positive studies were correctly identified in 90-day studies based on combination of liver hypertrophy, cytomegaly, and necrosis ($p<0.01$)

- 16 false positives

*Based on examination of 83 B6C3F1 and 87 F344 90-day studies with corresponding 2-year studies

Allen et al., Toxicologic Pathology 32:393-401 (2004)