Application of Veterinary Pathology for Translational Research and Development in the Context of Drug Discovery

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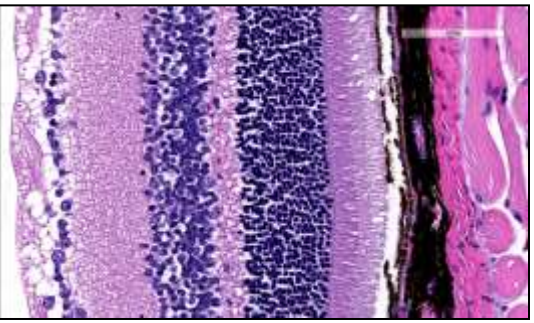
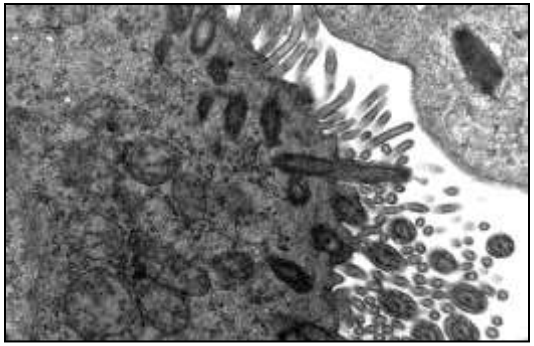
CONFIDENTIALITY DISCLOSURE STATEMENT

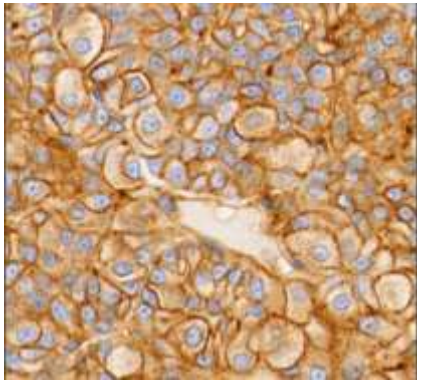
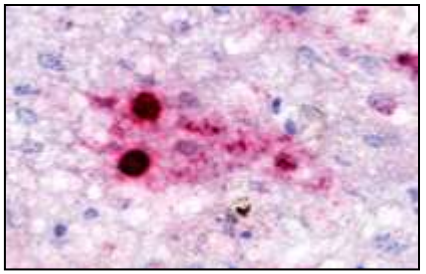
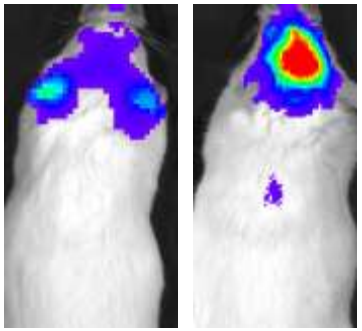
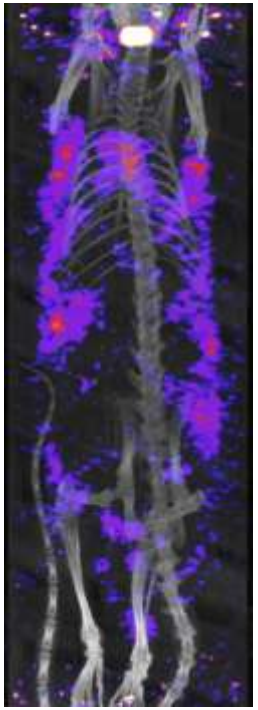
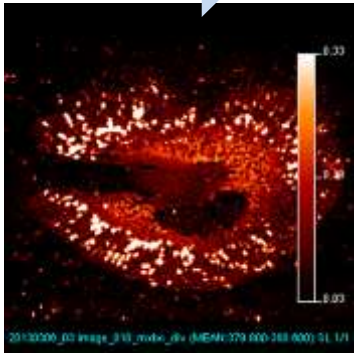
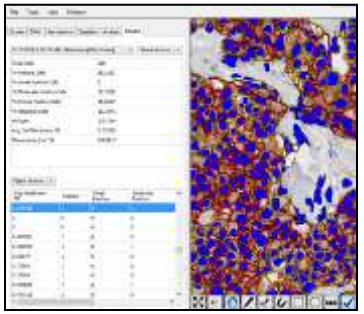
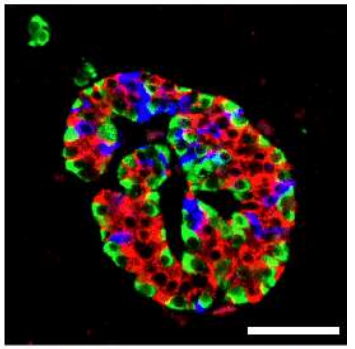
I, Bhanu Singh, will present slides describing my experiences as a pathologist working for various positions within the private sector of commercialized science. Presentation material shown in these slides have already been published or publically presented. None of the slides represent any confidential information pertaining to my current or previous affiliation.

Public Disclosure Date: Oct 23rd , 2016

Evolving Role of Pathologist

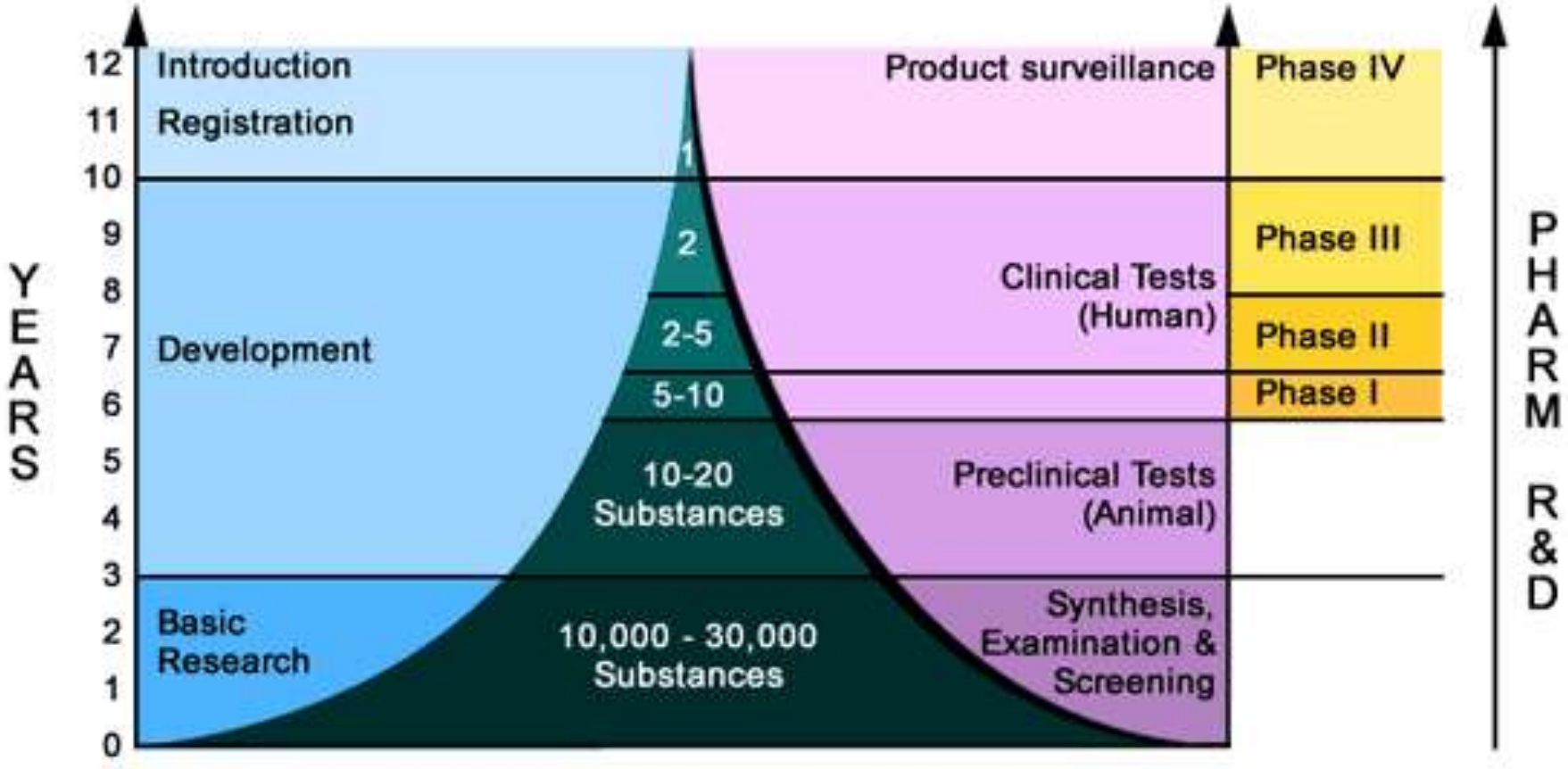


Outline

- Drug Discovery and Development
- Role of Pathologist
- Case Examples



- **Success rate < 1%**
- **Cost of developing one drug ~ 1 B US$**
- **Time ~ 10-12 years**

http://www.msd.com.hk/health_info/drug_education/e_ddp_introduction.html


Found It!!!
Congratulations, it only took you 65298 seconds
www.jolyon.co.uk

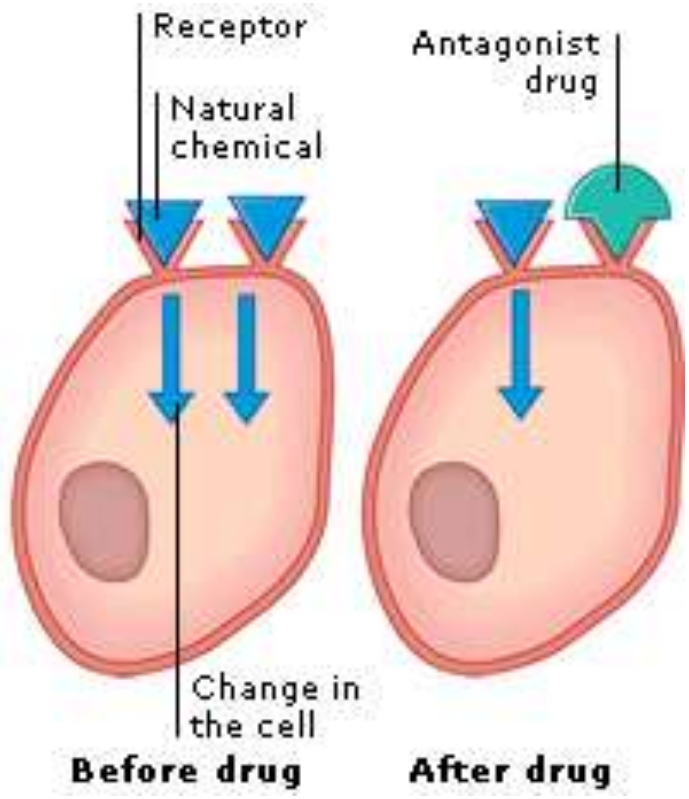
Process: Over View

Find new active structure

Convert it to a useful drug

- ✓ Choose a disease
- ✓ Choose a drug target
- ✓ Choose a compound
- ✓ Identify a “bioassay/biomarker”
  - ✓ bioassay = A test used to determine biological activity.

Identifying a Drug Target



http://azcache.com/go/img/570e27bddb127b180651657c.jpg

Drug Target = specific macromolecule, or biological system, which the drug will interact with

Selectivity - On target Vs Off Target

e.g. Targeting a bacterial enzyme

- ✓ Not present in mammals, or
- ✓ which has significant structural differences from the corresponding enzyme in mammals

Find a “lead compound”

- “lead compound” = structure that has some activity against the chosen target, but not yet good enough to be the drug itself
- If not known, determine the structure of the “lead compound”

Find a “lead compound”

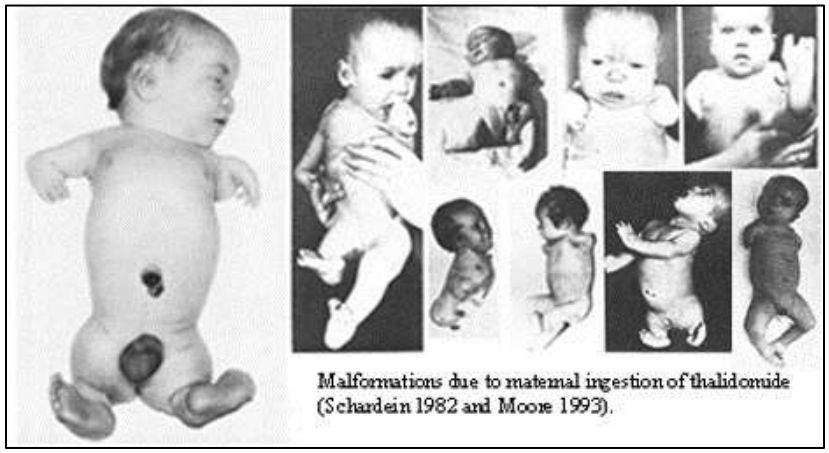
- Synthesize analogs of the lead
- Identify Structure-Activity-Relationships (SAR’s)

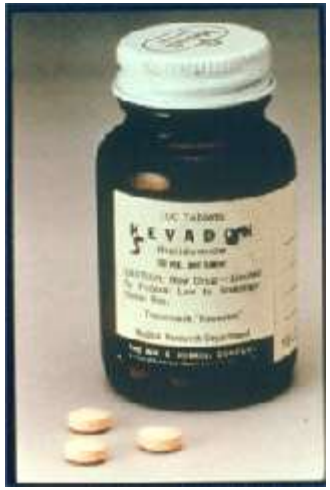
- Identify the “pharmacophore”
  - Pharmacophore = the structural features directly responsible for activity
- Optimize structure to improve interactions with target

Two CRITICAL Questions

- **Is this Compound Efficacious?**
  - Animal models of human diseases
  - Phase 2 clinical trials
- **Is this Compound safe at Therapeutic Dose?**
  - Preclinical toxicity studies (animals)
  - Phase 3 clinical trials

Thalidomide Tragedy (1957-1962)


Malformations due to maternal ingestion of thalidomide (Schardein 1982 and Moore 1993).

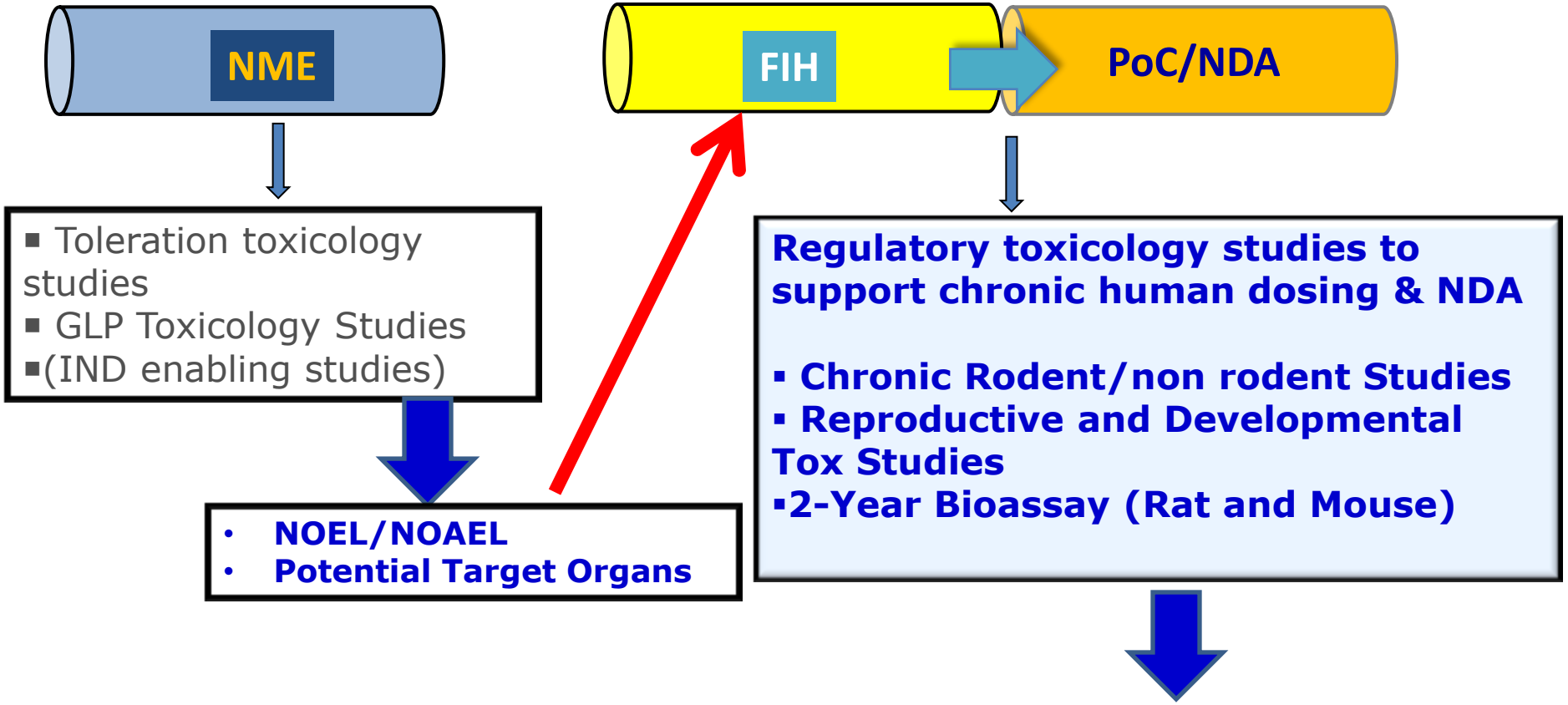


Kefauver-Harris Drug Amendments 1962.

- To ensure drug efficacy and greater drug safety.
- ***For the first time***, drug manufacturers required to prove to FDA the ***Safety*** and *Efficacy before marketing*.

Traditional Role of Pathologist

- **Gross and microscopic examination of animal tissues in drug safety studies**



XARELTO®

(rivaroxaban) tablets, for oral use

Revised: 03/2014
011422-140307

13 NON-CLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Rivaroxaban was not carcinogenic when administered by oral gavage to mice or rats for up to 2 years. The systemic exposures (AUCs) of unbound rivaroxaban in male and female mice at the highest dose tested (60 mg/kg/day) were 1- and 2-times, respectively, the human exposure of unbound drug at the human dose of 20 mg/day. Systemic exposures of unbound drug in male and female rats at the highest dose tested (60 mg/kg/day) were 2- and 4-times, respectively, the human exposure.

Rivaroxaban was not mutagenic in bacteria (Ames-Test) or clastogenic in V79 Chinese hamster lung cells *in vitro* or in the mouse micronucleus test *in vivo*.

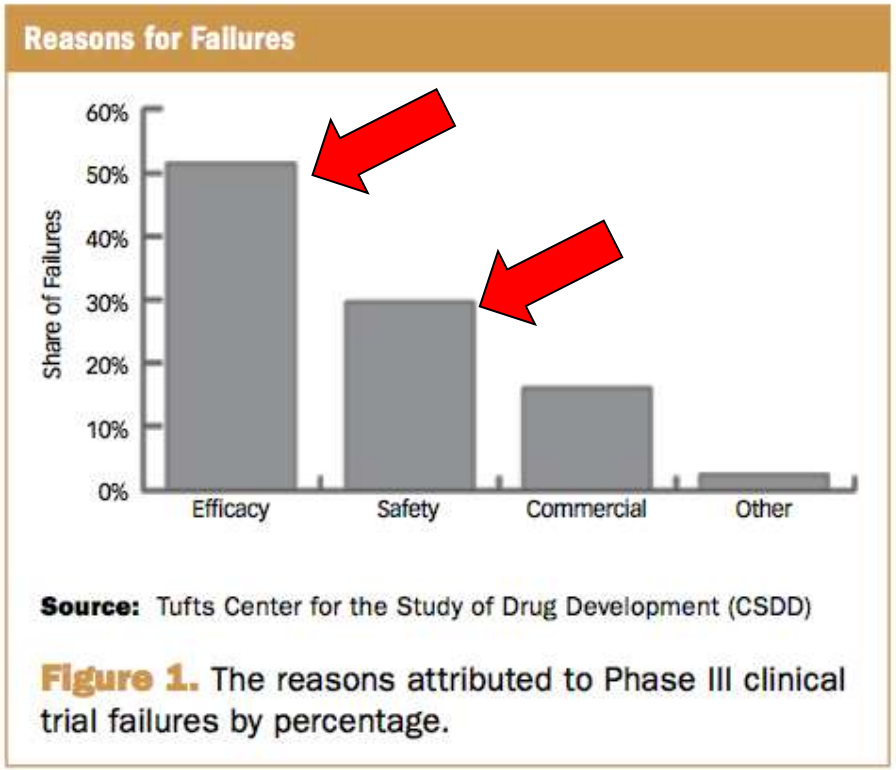
No impairment of fertility was observed in male or female rats when given up to 200 mg/kg/day of rivaroxaban orally. This dose resulted in exposure levels, based on the unbound AUC, at least 13 times the exposure in humans given 20 mg rivaroxaban daily.

Key Driver for Change

- ***Industry-wide high early attrition of novel drug candidates, crippling failures in late stage development or post- marketing withdrawal of approved medicines poses a sustainability challenge to the Bio-Tech and Pharmaceutical Industry.***

Nature Biotechnology 32, 40–51 (2014)

Why Does R&D Productivity Fail?



Aug 01, 2016, Applied Clinical Trials, Volume 25, Issue 8

- **Lack of Efficacy (Poor translation of animal findings to clinical trials)**
- **Safety (toxicology and clinical)**

R&D Failures: “5 R“ Strategies

Lessons learned from the fate of AstraZeneca’s drug pipeline: a five-dimensional framework

David Cook, Dearg Brown, Robert Alexander, Ruth March, Paul Morgan, Gemma Satterthwaite and Menelas N. Pangalos

Nature Reviews Drug Discovery, 13 (6): 419-431

➢ Right Target

- Strong link between target and disease
- Differentiated efficacy
- Available and predictive biomarkers

➢ Right Safety

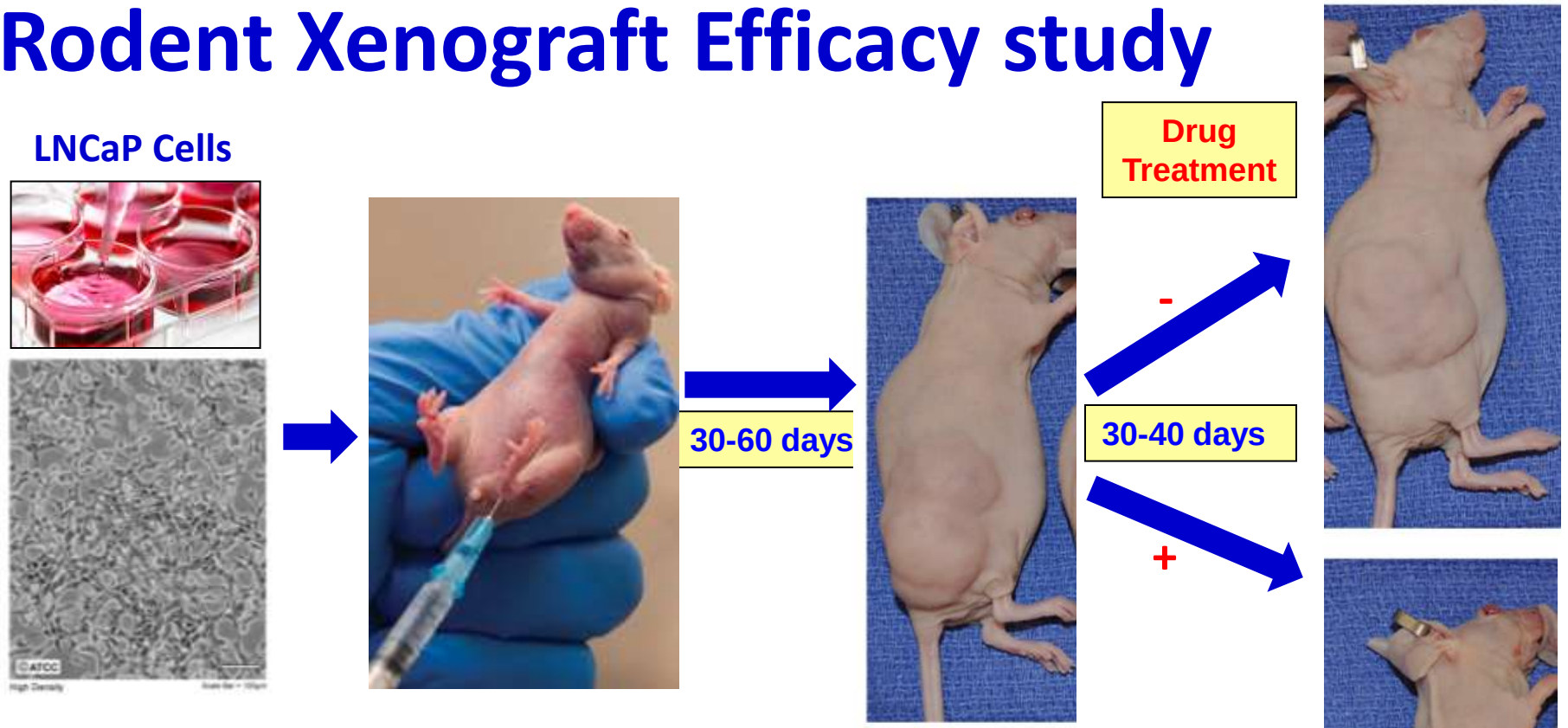
- Differentiated and clear safety margins
- Understanding of secondary pharmacology risk
- Understanding of reactive metabolites, genotoxicity, drug-drug interactions
- Understanding of target liability

✓ Pathologists can play key roles to address these issues.

CASE STUDY - 1

Animal Model Development for Screening anti-androgenic activity

Rodent Xenograft Efficacy study



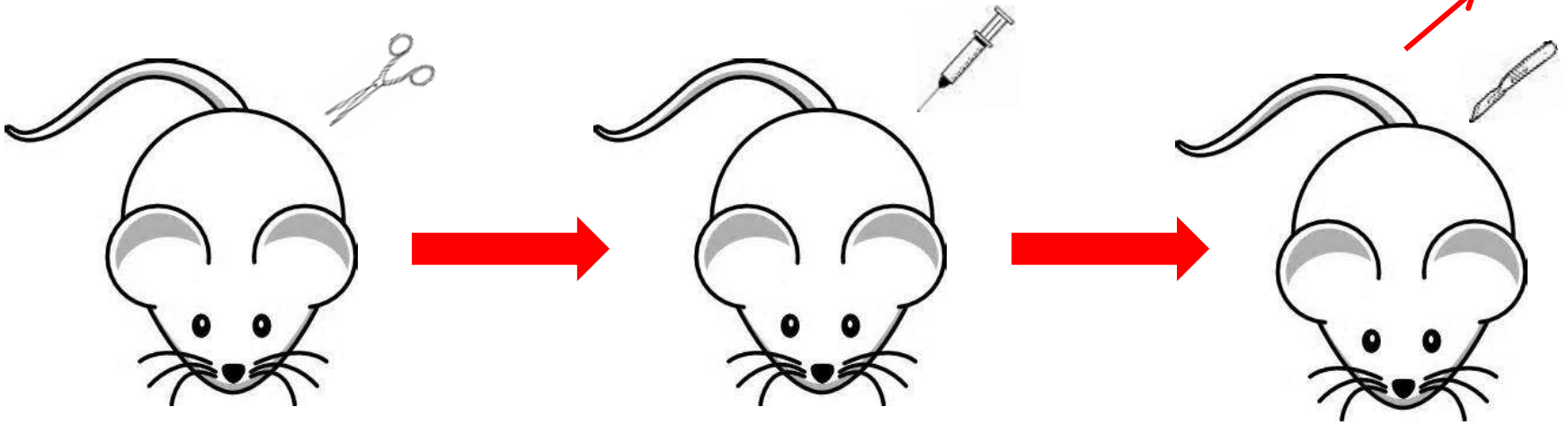
- **Tumor-take rate 50%; need to implant more mice**
- **60-100 days for getting read out**

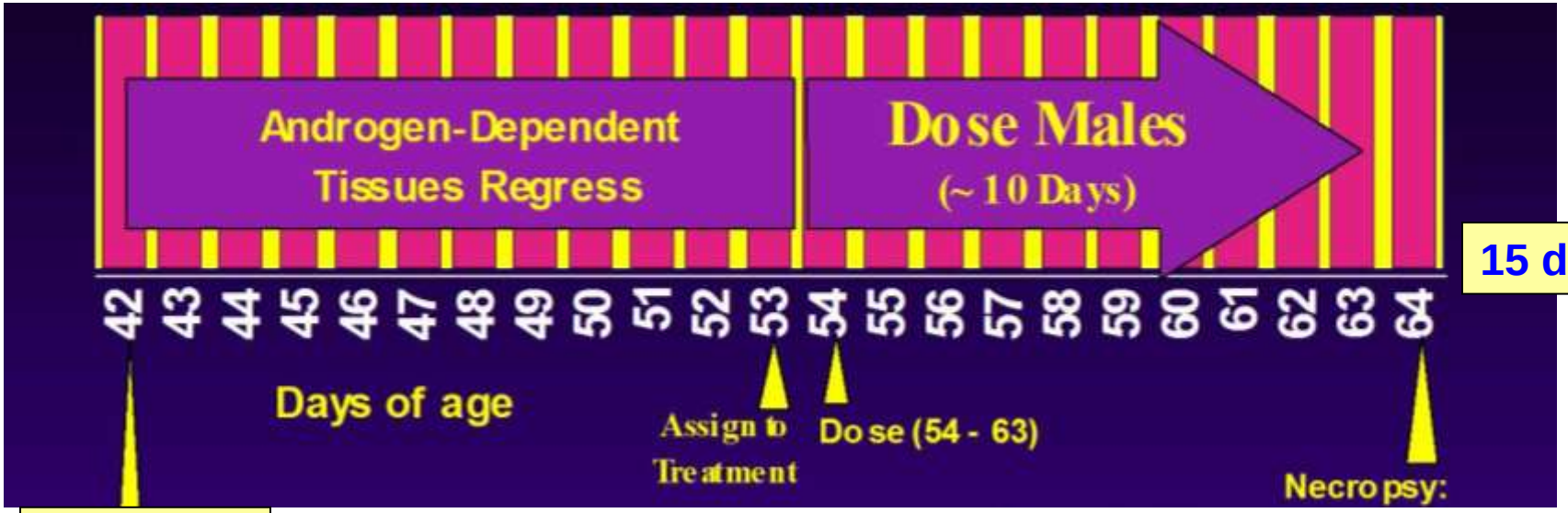
http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0078895

Alterative *In Vivo* Assay for screening Anti-androgenic activity?

Hershberger Bioassay

Androgen sensitive organs



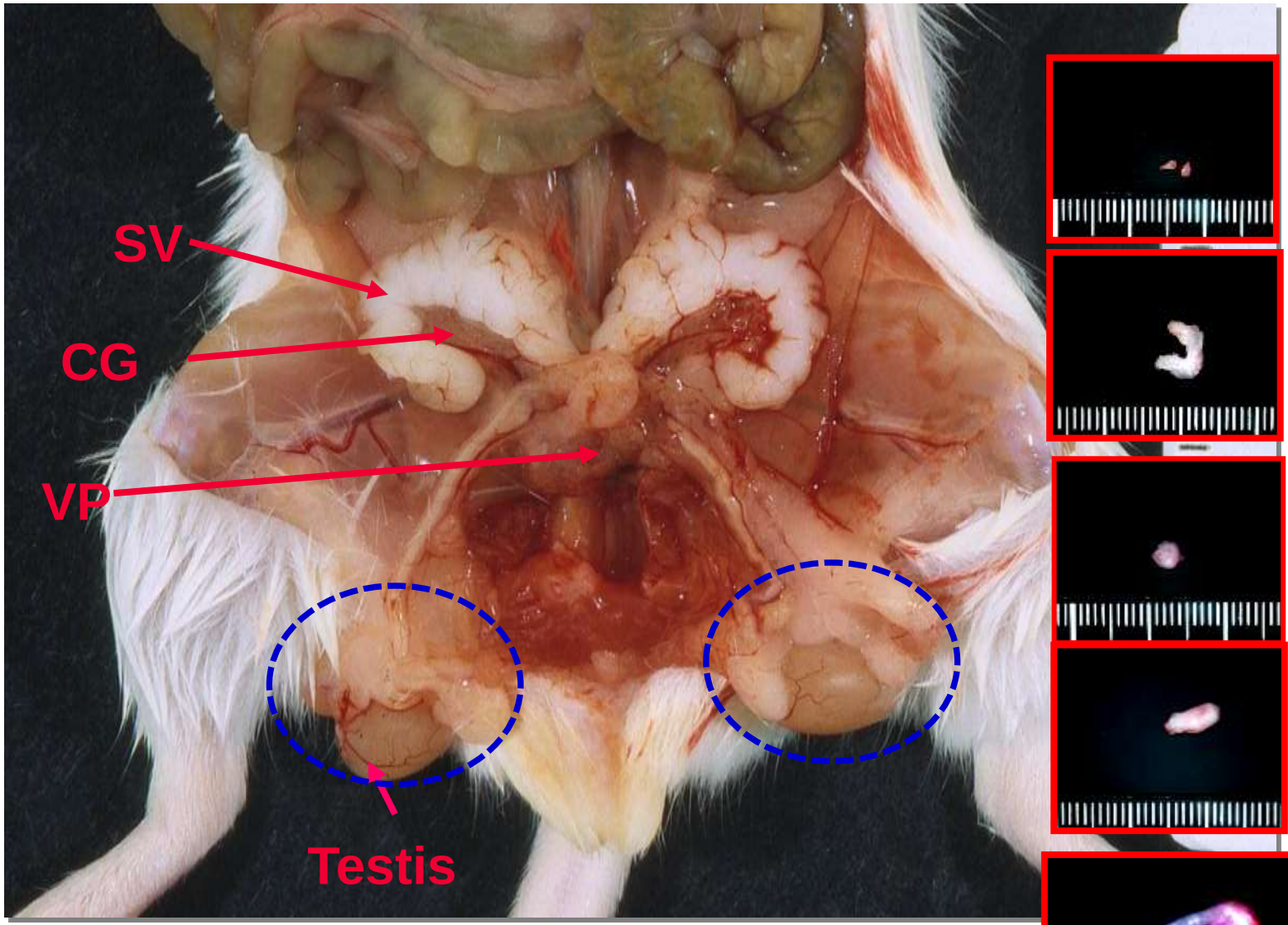


https://www.epa.gov/sites/production/files/2015-07/documents/final_890.1600_hershberger_assay_sep_10.6.11.pdf

Hershberger *in vivo* Bioassay

- Originated in the **1930's**.
- Standardized protocol for endocrine disrupting chemicals in **1962**.
- *In vivo* experiment is performed in castrated male rodents to measure changes in the weight of five (5) androgen-sensitive organs (ASO):
  - Cowper's Glands (CG), Seminal Vesicles with fluids and Coagulating Glands (SVCG), Glans Penis (GP), Ventral Prostate (VP) and Levator Ani-bulbocavernosus (LABC)
  - A positive result is a statistically significant change in the weight of two of the tissues.

https://www.epa.gov/sites/production/files/2015-07/documents/final_890.1600_hershberger_assay_sep_10.6.11.pdf



Seminal Vesicles (SV),
Coagulating Gland (CG),
Ventral Prostate (VP)

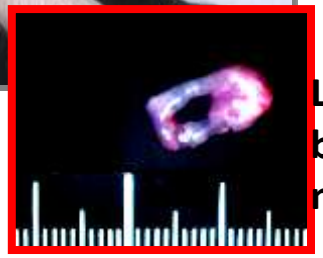
Castrated Rat: Androgen Sensitive Organs

Cowper glands

Seminal Vesicles

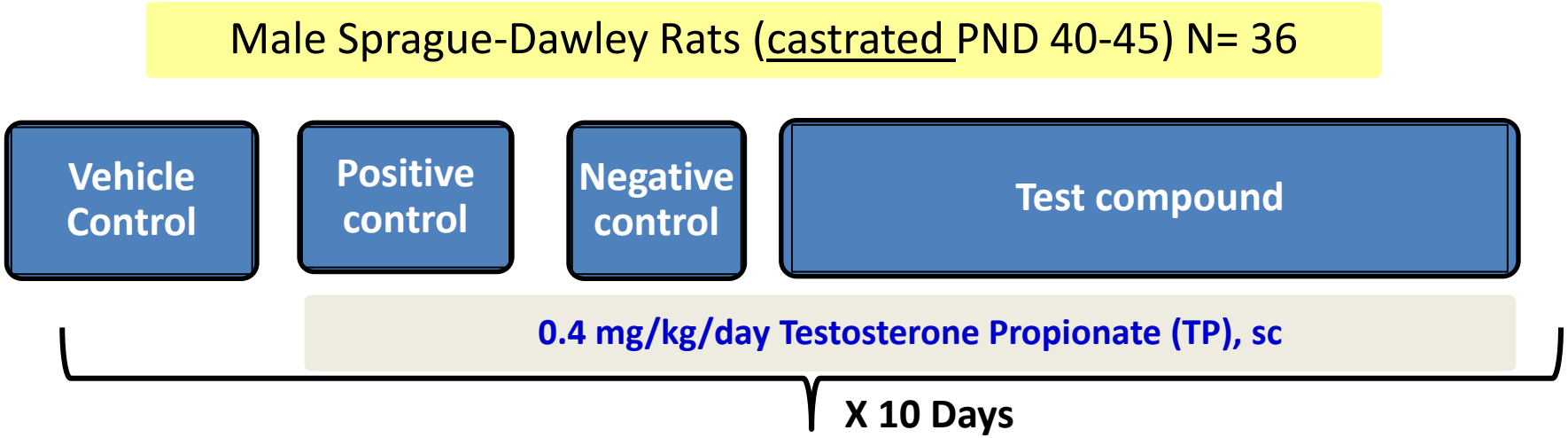
Ventral Prostate

Glans Penis



Levator ani-bulbocavernosus muscle

Study Design: Assay Validation



End Points

- Body weight, Weight of Androgen Sensitive Organs (ASO)
- Gross and Microscopic Examination of ASO, other major organs (Liver, heart, kidneys, Brain, Adrenals)
- Plasma Collection

https://www.epa.gov/sites/production/files/2015-07/documents/final_890.1600_hershberger_assay_sep_10.6.11.pdf

Hershberger Bioassay: Value Addition

- On target PD assay for screening anti-androgens
  Quality read out
  - Gives an idea of dose response
- Significant **Cost** and **Time** Saving
  - 60-100 days Vs 15 days to get read out for screening assay
- Additional endpoints
  - For mechanistic information (AR/ER IHC assay)
  - Opportunity to screen quickly for major safety liability

In Line with 3 Rs- Reduce, Refine and "Rapid"

https://www.epa.gov/sites/production/files/2015-07/documents/final_890.1600_hershberger_assay_sep_10.6.11.pdf

CASE STUDY - 2

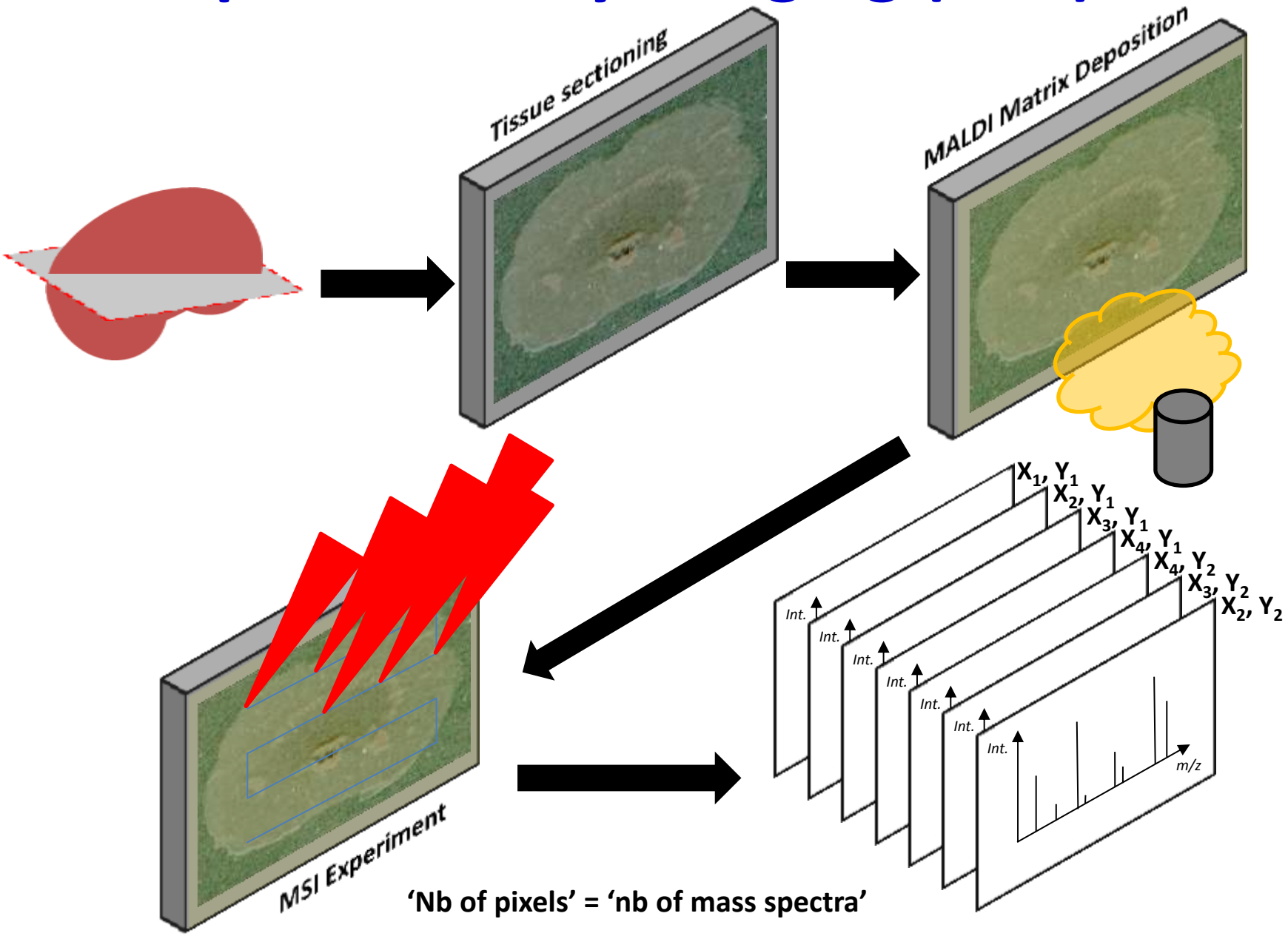
MALDI-ToF Mass Spectrometry Imaging of Rabbit Kidney

J Am Soc Mass Spectrom. 2016; 27: 117–123.
Published online 2015 Sep 18. doi: 10.1007/s13361-015-1254-3
PMCID: PMC4686544

Mass Spectrometry Imaging of Drug Related Crystal-Like Structures in Formalin-Fixed Frozen and Paraffin-Embedded Rabbit Kidney Tissue Sections

Anne L. Bruinen, Cateau van Oevelen, Gert B. Eijkel, Marjolein Van Heerden, Filip Cuyckens, and Ron M. A. Heeren

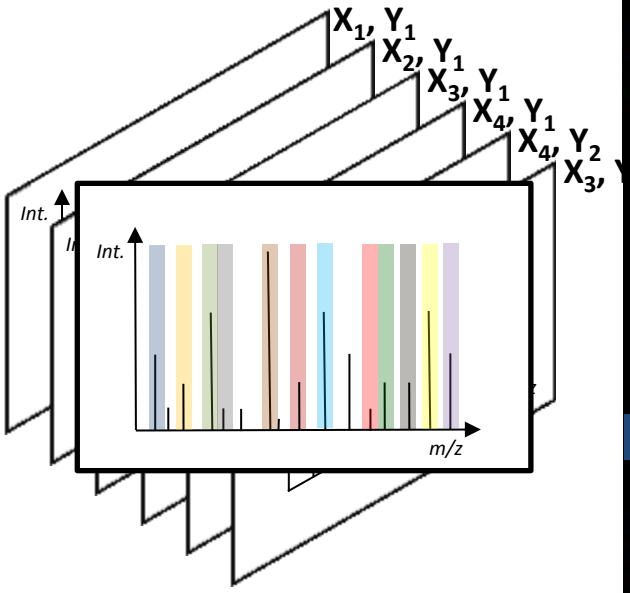
Mass Spectrometry Imaging (MSI)

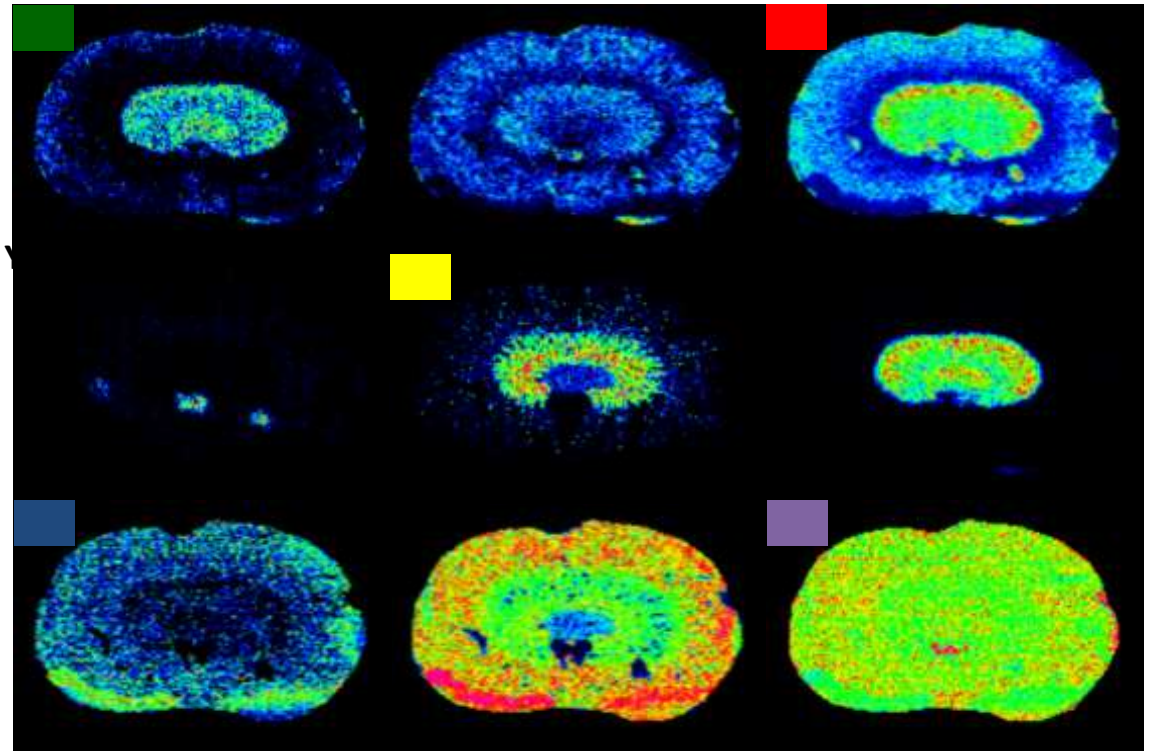


'Nb of pixels' = 'nb of mass spectra'

Alain Héron, Imabiotech

Mass Spectrometry Imaging (MSI)





Each *m/z* signal = One specific MS image

Alain Héron, Imabiotech

Mass Spectrometry Imaging (MSI)

Advantage

- ✓ ***label free technique providing 2D images for potentially every molecule within a tissue at a with a pixel size between 1 and 200 µm***

Limitation

- ➢ Sensitivity depends on molecular nature of the targeted compound (e.g. 10ng/g of tissue), limited Resolution &Quantification

Rabbit- Nephrotoxcity

Histopathology: Crystals in the kidney cortex

Whether the crystal structures are composed of the drug compound, metabolites or an endogenous compound? Test-article relation?

Rabbit Nephrotoxcity

VALUE

- ✓ **Crystal-like structures are related to the drug.**
- ✓ **Crystals contain mainly metabolites and very little parent drug.**
- ✓ **Decision making on safety related Issues**
  - Better understanding of drug/metabolite disposition and site specific toxicity

CASE STUDY - 3

Investigating Cause of Sudden Death and Mechanism of Toxicity in Efficacy Study

Toxicol Pathol. 2015 Jan;43(1):10-40. doi: 10.1177/0192623314555526. Epub 2014 Nov 9.
Proceedings of the 2014 National Toxicology Program Satellite Symposium.
Elmore SA[1], Cora MC[2], Gruebbel MM[3], Hayes SA[4], Hoane JS[4], Koizumi H[5], Peters R[6], Rosol TJ[7], Singh BP[8], Szabo KA[4].

Background

- Context: Experiment aimed at defining the efficacy of "**Wonder drug**" (small molecule) in a mouse model of lymphoma
- Two out of six mouse suddenly died after dosing
- Strain, Age & Sex: 129/SvEv, 6-month-old Females
- Route of compound administration: Intraperitoneally

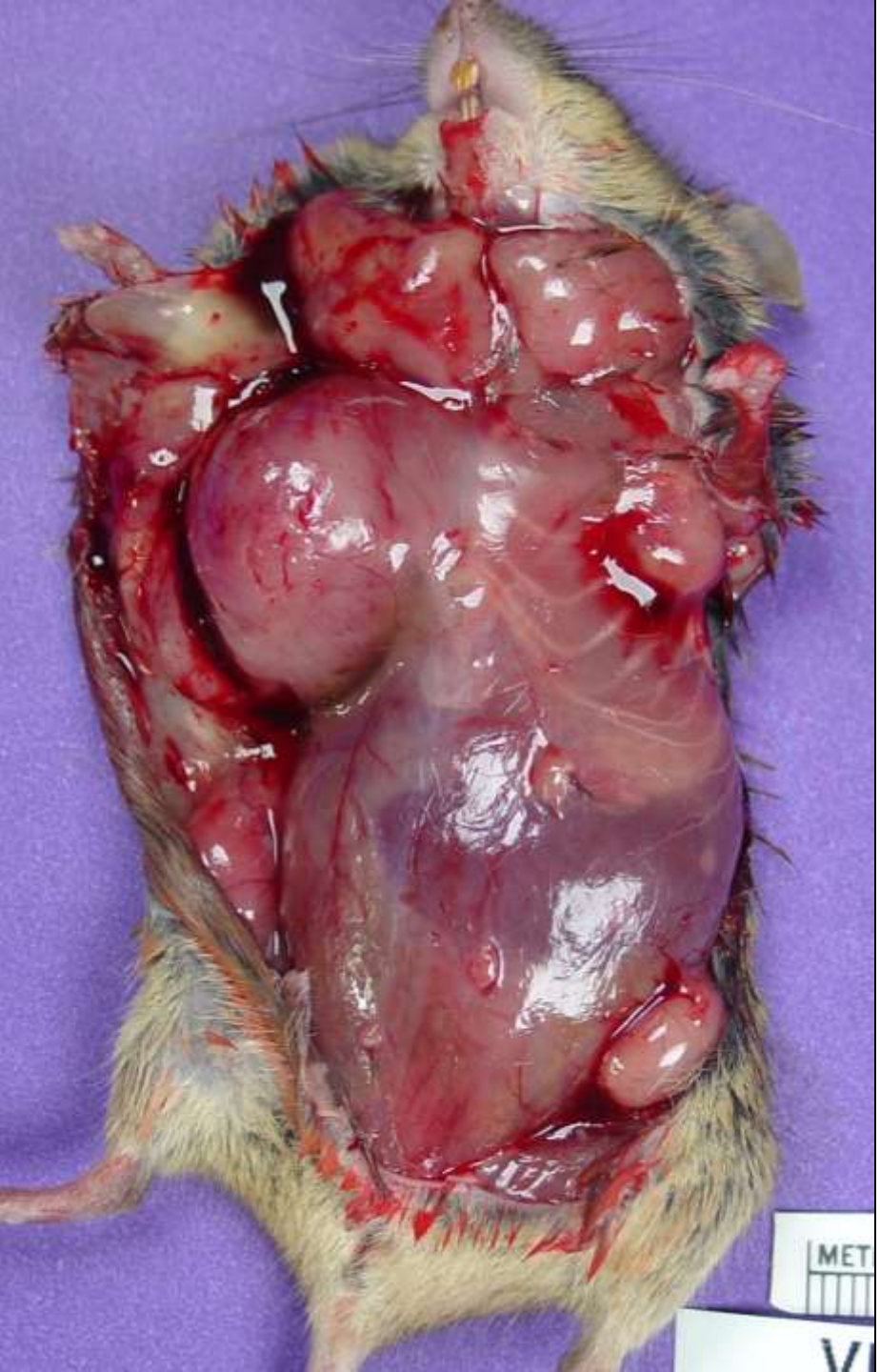
Investigator's Critical Questions

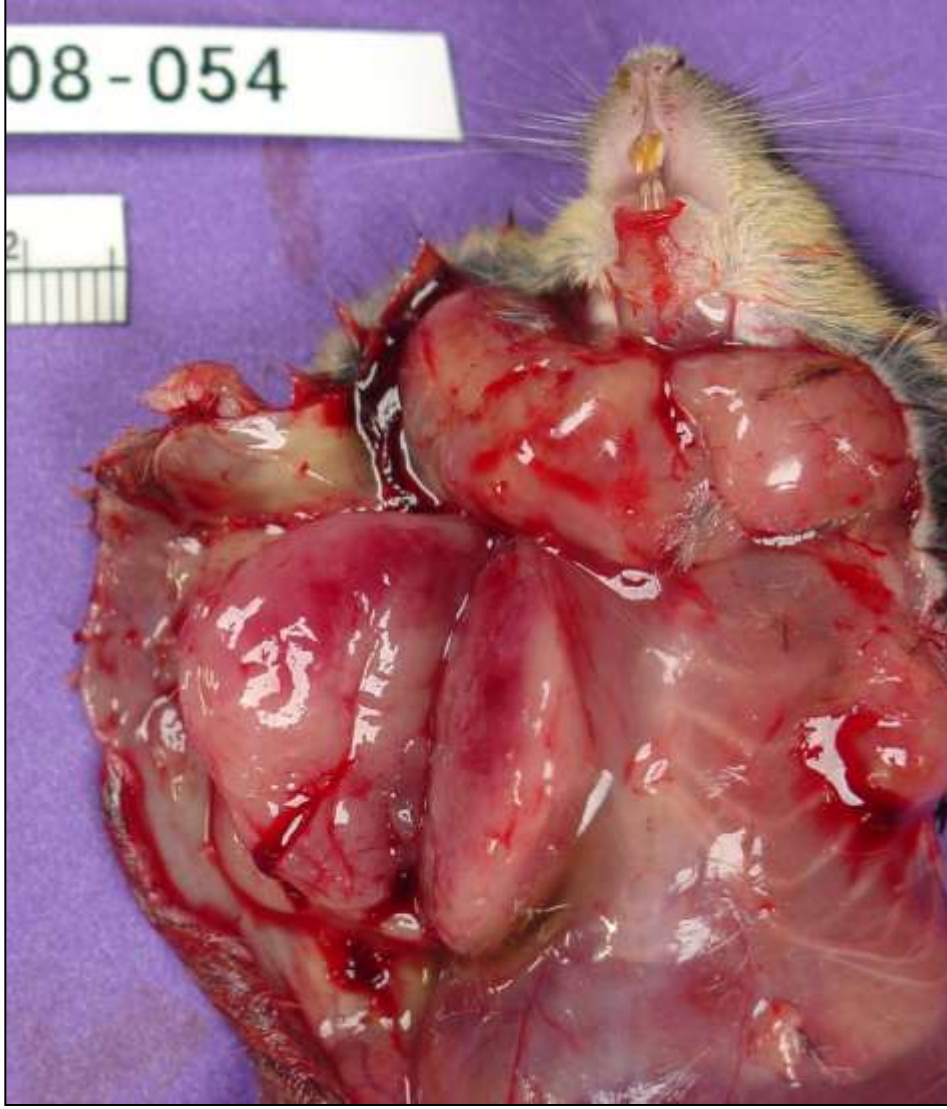
- What is the cause of Death in these animals?
- Is this due to a background change (animal/strain/age specific)?
- Is this a target related toxicity?
- Is this a off target toxicity?
- Is this a chemical based toxicity?

Investigation

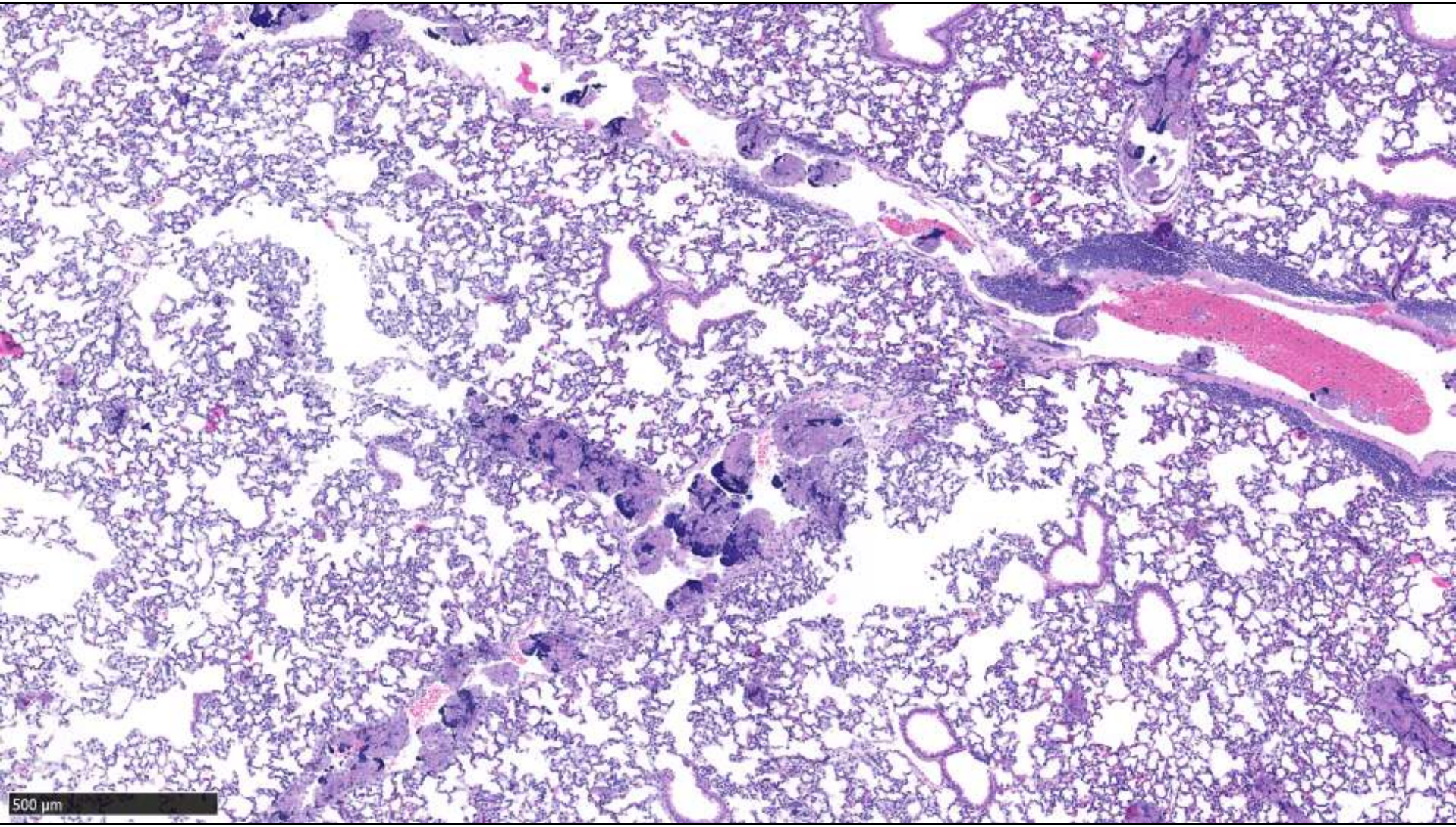
- Gross Examination:
  - Enlarged lymph nodes, spleen, liver, kidneys

- Microscopic Examination:
  - Disseminated lymphoma (expected)
  - Multifocal intravascular clumps (emboli) in small vessels of the lungs, adrenal glands, kidneys, heart and liver .
  - The emboli were most prominent in the pulmonary vessels.

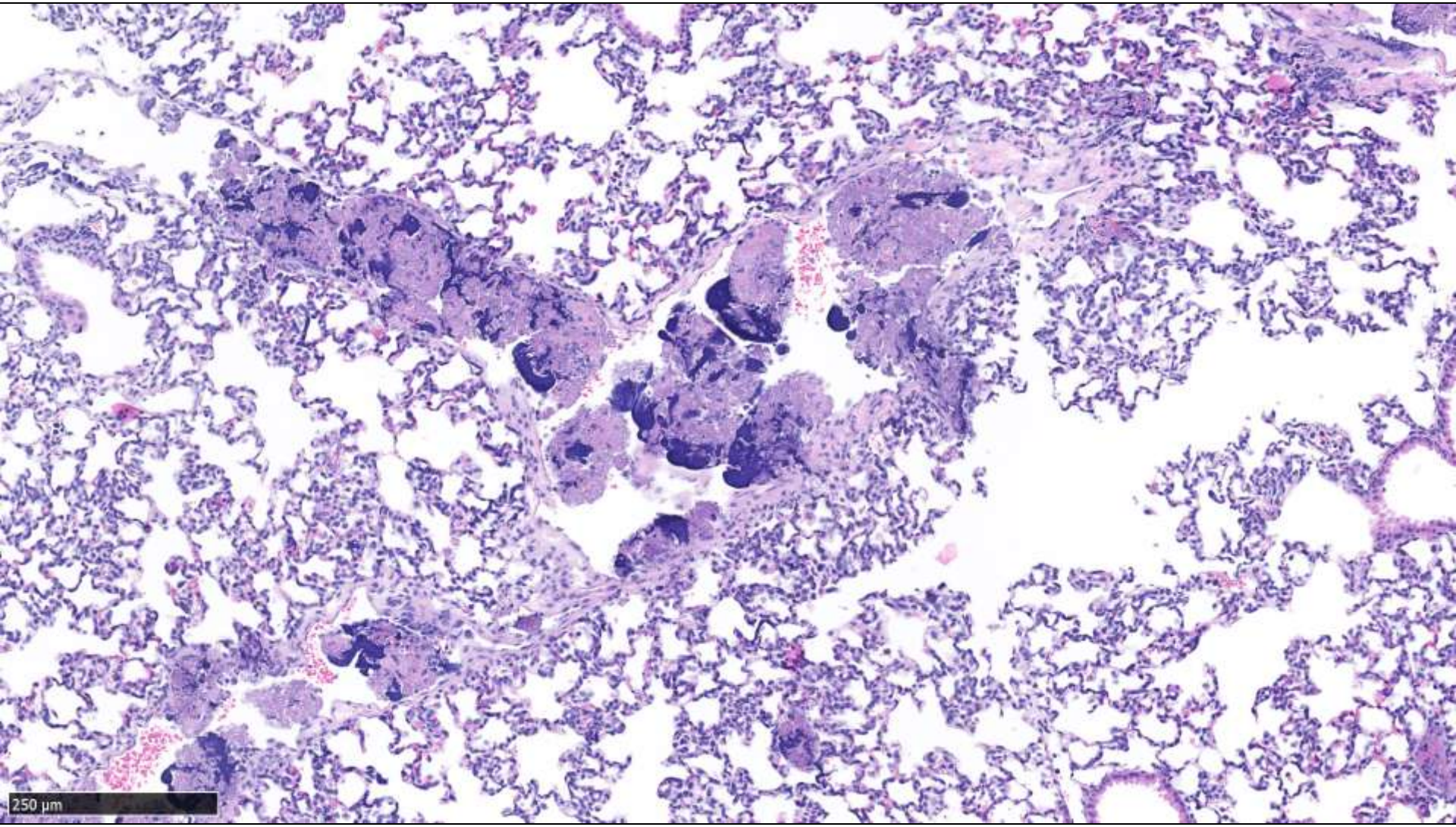



08-054

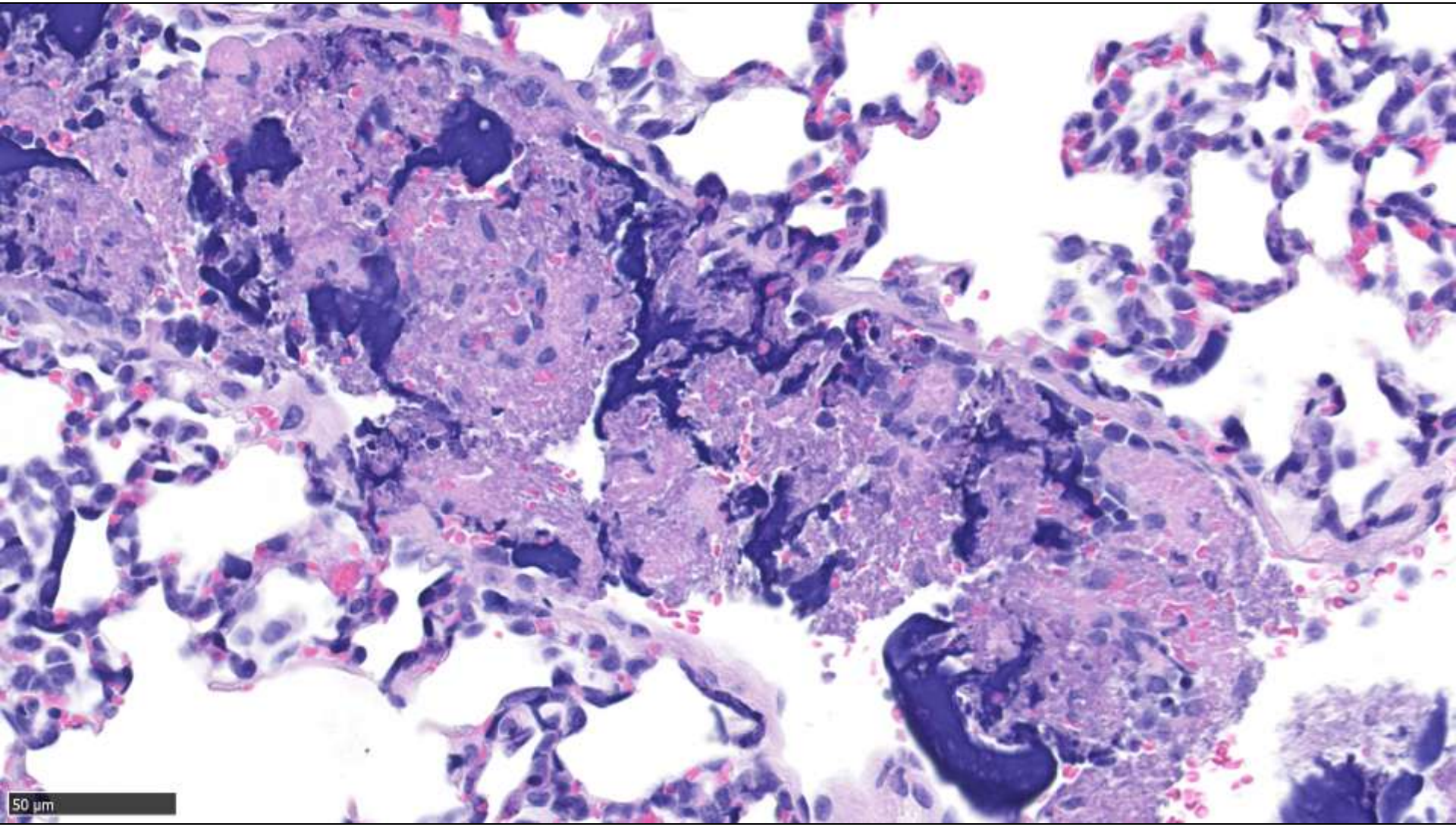
Lung

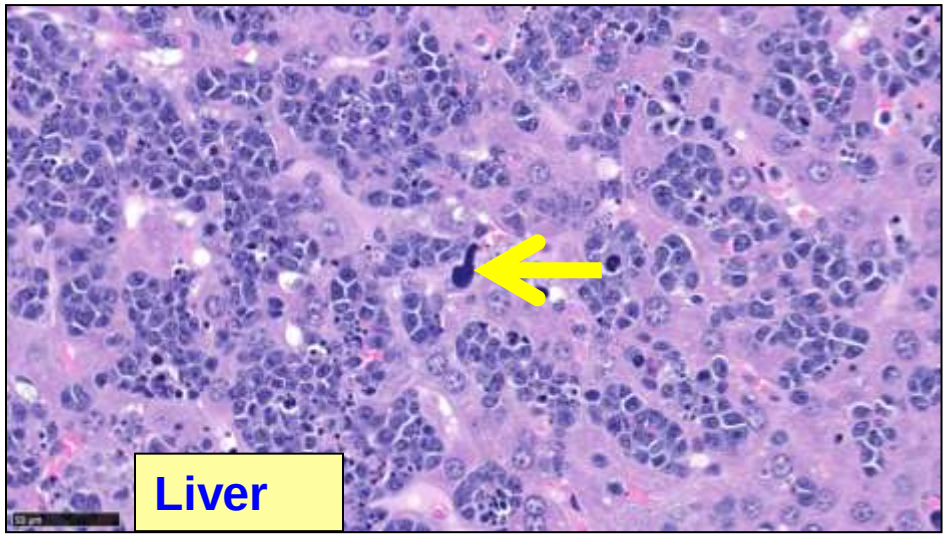


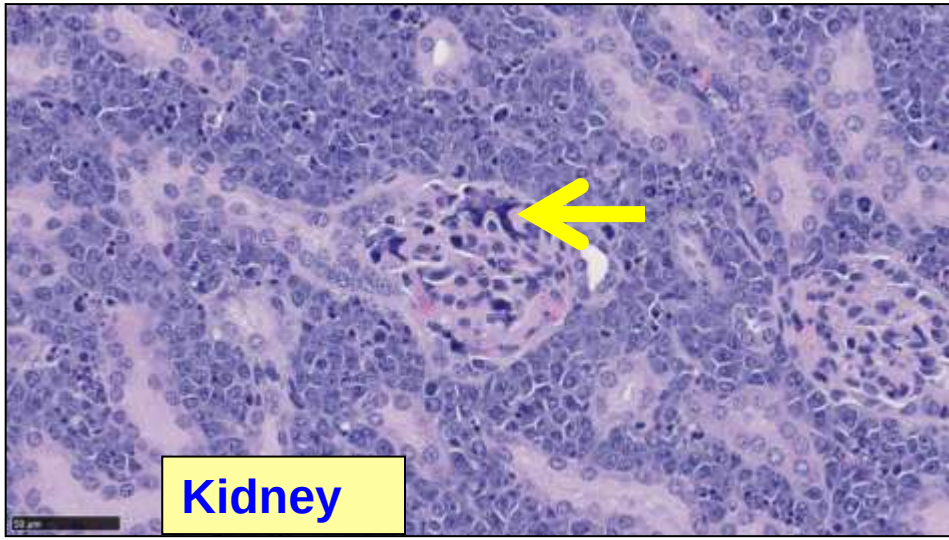
Lung

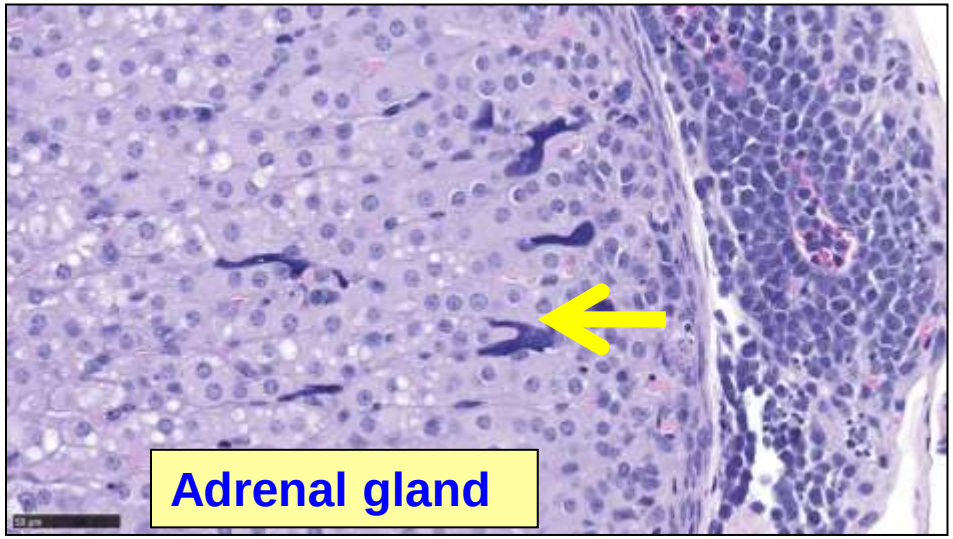


Lung







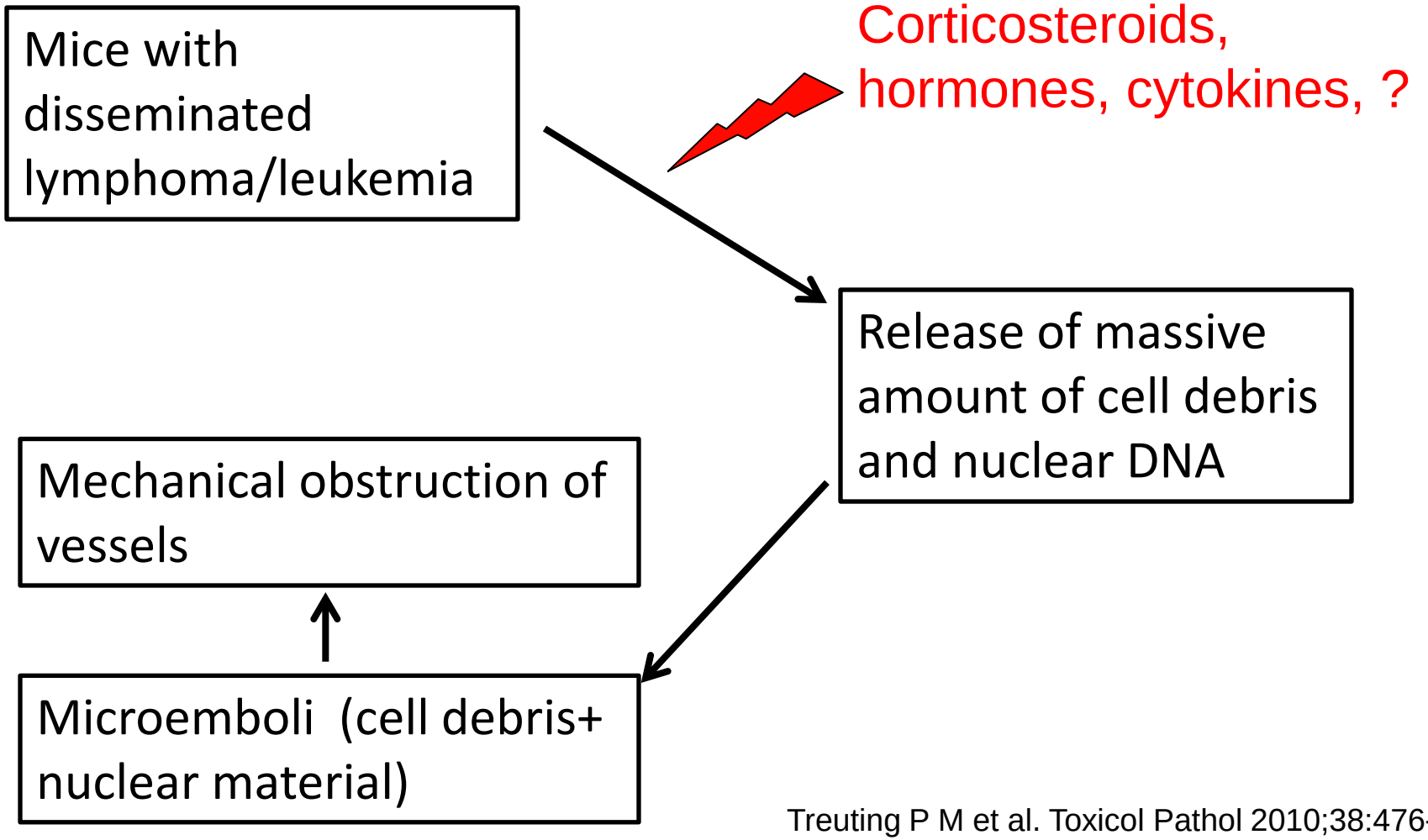


Condition: Acute Tumor Lysis Syndrome

Hallmark finding: Widely disseminated microemboli

Acute Tumor Lysis Syndrome (ATLS)

- Potentially lethal condition



Treuting P M et al. Toxicol Pathol 2010;38:476-485

Value of Diagnostic Exercise

- ATLS has potential to adversely influence the outcome of experimental studies
  - Deaths due to ATLS might mistakenly be attributed to a direct test article effect
  - Misinterpretation of efficacy studies for oncology compounds

Outcome:

- ✓ ***Cause of death: ATLS***
- ✓ ***Mechanism: Secondary to massive tumor cell death***
- ✓ ***Team was advised to reduce tumor burden in animal model and change in dosing schedule and to examine tissues for future studies***

Summary: Case Studies

- ✓ Value of the toxicologic pathologist as part of multi-disciplinary expert team
- ✓ the level of scientific advancement that can be achieved through collaborative research efforts.

Summary: Role of Pathologist

- **Early Discovery Support**
- **Translational Medicine (Science)**
- **Safety**
  - **Continue to play a critical role in Regulatory Toxicology**

Key Message

It Takes A Village to Develop a Drug

-Discovery of a new drug is multidisciplinary effort

Key Message

Pathologists will remain at the heart of disease diagnosis, target organ toxicity evaluation, appropriate risk assessment and risk management, but we must evolve into translational biologists (comparative pathologists) and biomarker scientists.

J Pathol. 2014 Jan;232(2):99-102. doi: 10.1002/path.4290.
Pathology in drug discovery and development.
Jubb AM[1], Koeppen H, Reis-Filho JS.

Discovery PreClinical Clinical

Thanks for your attention!!!

Back Up Slides

ATLS: Comparison

Humans

- Mostly associated with aggressive chemotherapy
- Cause of death: cardiac arrythmias, acute renal failure
- ↑K, ↑P, ↑Uric acid, ↓Ca

Rodents

- Sporadic and spontaneous
- Disseminated Microemboli (most commonly in lungs)
- No evidence of renal crystals

Treuting P M et al. Toxicol Pathol 2010;38:476-485

Incidences of Lymphoblastic Lymphoma and ATLS

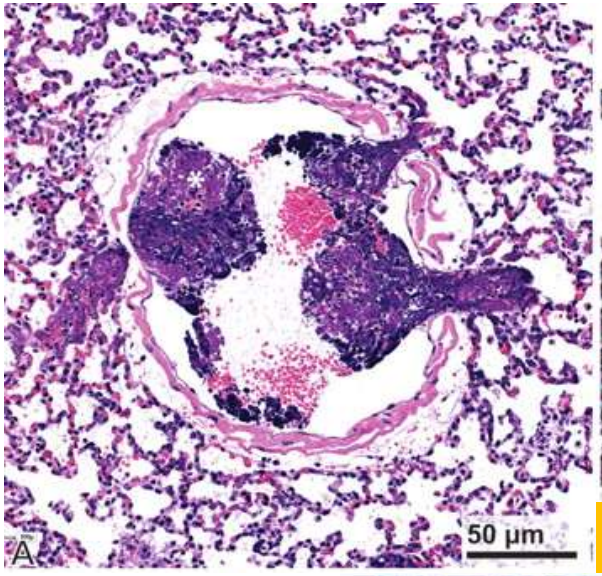
	Number of Mice				
End of Life	Initial	Necropsy	Histology[a]	Lymphoblastic Lymphoma (LL) (% of histology[b])	ATLS (% of LL[c])
Moribund (euthanized)	457	457	457	325 (71)	14 (4)
Expired (found dead)	113	93	42	41 (98)[d]	17 (41)[d]
Total			499	366 (73)	31 (8)

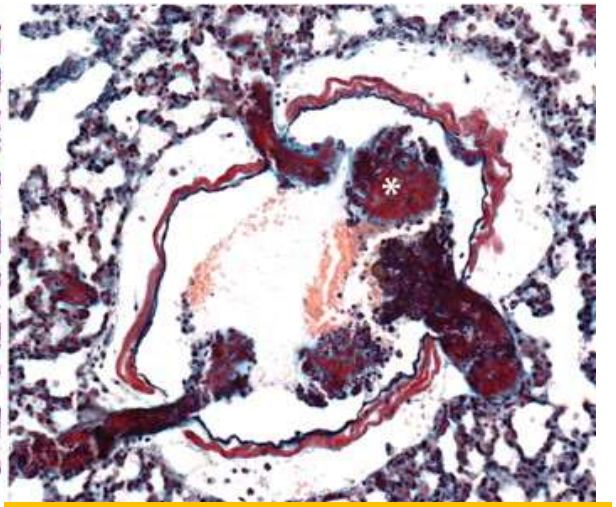
[a] Mice with informative histology.
[b] Percentages of euthanized, expired and total mice with informative histology.
[c] Percentages of euthanized, expired and total mice with lymphoblastic lymphoma (LL).
[d] Statistically different from moribund mice ($p < .0005$; Fisher exact test).

- ATLS - associated with lymphoblastic lymphoma
- No difference in the severity of histologic lesions between mice that were '*euthanized*' and those '*found dead*'

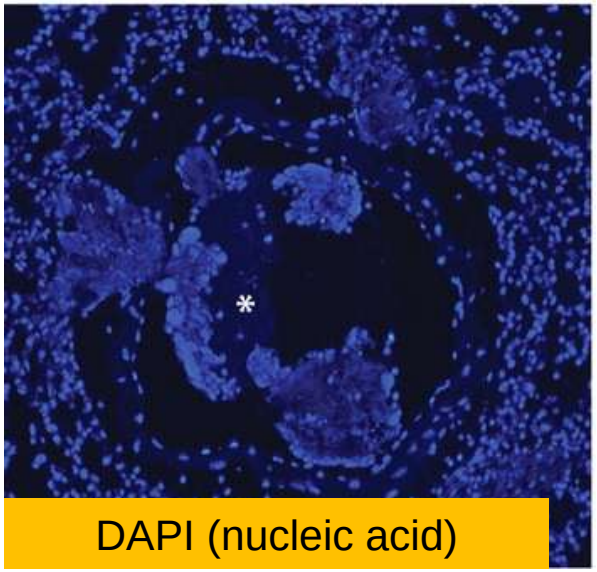
Treuting P M et al. Toxicol Pathol 2010;38:476-485

ATLS- Characterization of Microthromboemboli





Movat's pentachrome (Fibrin)



DAPI (nucleic acid)

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