Synthetic individuals
Their role in drug development and toxicologic pathology

10th Digital Pathology & AI Congress: Europe

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Erio Barale-Thomas • Non-Clinical Safety • Janssen R&D

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Johnson & Johnson

Nous nous estimerions trop heureux si les personnes à qui la vie des hommes est confiée, persuadées des progrès que leur art peut attendre encore de la **médecine comparée**, daignaient nous mettre à portée d'**éprouver** […] **sur des animaux** ce que la prudence ne leur permet **pas de tenter sur la nature humaine**

Claude Bourgelat,
Art vétérinaire ou médecine des animaux, 1761
(fondateur de l'enseignement vétérinaire)

We would consider ourselves too fortunate if the persons to whom the lives of men are entrusted, convinced of the progress that their art can still expect from **comparative medicine**, would deign to put us within reach of **testing** [...] **on animals** what prudence does not allow them to **attempt on human nature**.

Claude Bourgelat,
Art vétérinaire ou médecine des animaux, 1761
(founder of veterinary education)

Disclaimer

- The information in this document is based on the presenter's expertise and experience, gathered in view of a training workshop or a scientific conference
- This document may not necessarily represent all of the presenter's personal views nor those of Janssen Research & Development

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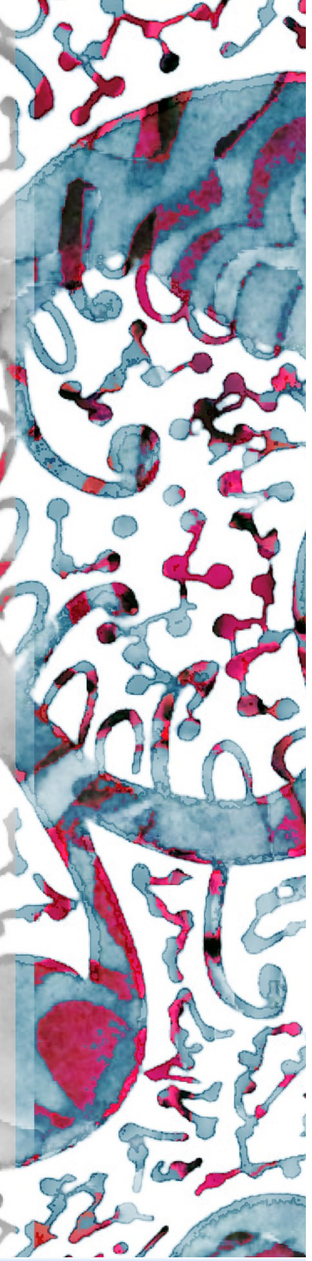
- **STP Working Group**: Reduction in Terminal NHPs and consequences on nonclin safety assessment's ability to protect patients: **Magali Guffroy** (Abbvie), **Robert Jonhson** (Elli Lilly)
- **ViCoG**: **Tinne Boeckx** (Janssen Pharmaceutica), **Thomas Steger-Hartmann** (Bayer), **Frank Bringezu** (Merck KGaA)
  - Involving **Bigpicture** as potential digital slides platform of choice

Other initiatives ongoing

- FDA/BioSafe/**DruSafe** Annual Meeting 2022
- Project **eTRANSAFE** from IMI
- Manuscripts in preparation. **Grevot A et al**. Opinion on the Use of VCGs in Nonclinical toxicity studies: the Anatomic Pathology Perspective. **Mecklenburg L et al**. How important are concurrent vehicle control groups in (sub)chronic non-human primate toxicity studies conducted in pharmaceutical development? An opportunity to reduce animal numbers



Outline

- The nonclinical **toxicologic study**
  - Design, parameters
- **Constraints in drug development** relevant to this talk
  - **Ethics** in animal experimentation
  - Animal & economical **resources**
    - The case of the non-human primates (NHP)
- Applying **the 3Rs**
  - Reduce and refine by **reuse** and **rehoming** of animals
  - Reduce the control **group size** and **replace** animals
- Replacing the concurrent control group (**CCG**)
  - Shared control group (**SCG**)
  - Virtual control group (**VCG**); synthetic control group (**SVCG**)
- The historical control database (**HCD**)
  - **Digital pathology component** of the HCD
- Take-home messages

The nonclinical toxicologic study Design

- **Characterize the toxicity profile of a drug**
  - Potential target organs
  - Dose-limiting toxicities
  - Biomarkers or other translatable monitoring parameters
  - Reversibility of the toxicities
- Standard GLP study design

Studies with typical # animals		1 month Non-rodent		1 month Rodent	
Study phase	Group	Female	Male	Female	Male
Terminal	Control	3	3	10	10
Recovery (opt.)	Control	2	2	5	5
Terminal	Low	3	3	10	10
Terminal	Mid	3	3	10	10
Terminal	High	3	3	10	10
Recovery (opt.)	High	2	2	5	5
Total animals		32		100	
% controls		31,3 %		30,0 %	
Historical control data		–	–	–	–

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Terminal	High	3	3	10	10	65	65
Recovery (opt.)	High	2	2	5	5	–	–
Total animals		32		100		520	
% controls		31,3 %		30,0 %		25,0 %	
Historical control data		–	–	–	–	Yes (>100)	Yes (>100)

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- 4 groups
  - Control = negative control = often **vehicle** used to solubilize test article in treated groups
  - Sometimes positive group and additional intermediate doses are added
  - Rodent 
  - Non-rodent 
  - The **primary reading** by the study pathologist
  - The **peer review** (PR; second opinion) by the reviewing pathologist
    - Subset of the primary reading

What groups are read by the study pathologist?

Groups	Control	Rec. C	Low	Mid	High	Rec. H
Organ 1						
Organ 2						
Organ 3					Target	
Organ 4						
Organ 5						
Organ 6						
Organ 7					Target	
… Organ n						

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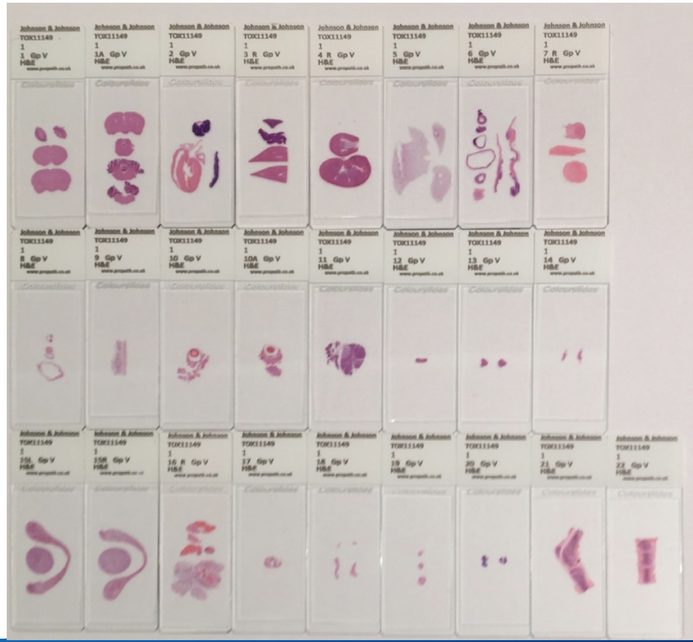
A typical rat (non-rodent have more slides and more tissue surface)



The nonclinical toxicologic study
Sources of variability influencing the study results and the HCD

- Study parameters
  - Animal strain, sex, age, weight
  - Geographic origin/source
  - Animal **supplier**
  - Facility
  - Study duration
  - Year of study conduct
  - Test article information
  - **Vehicle**
- Study personnel & material
  - Necropsy & histology **technicians**
  - **Reagents**, equipment, methods…

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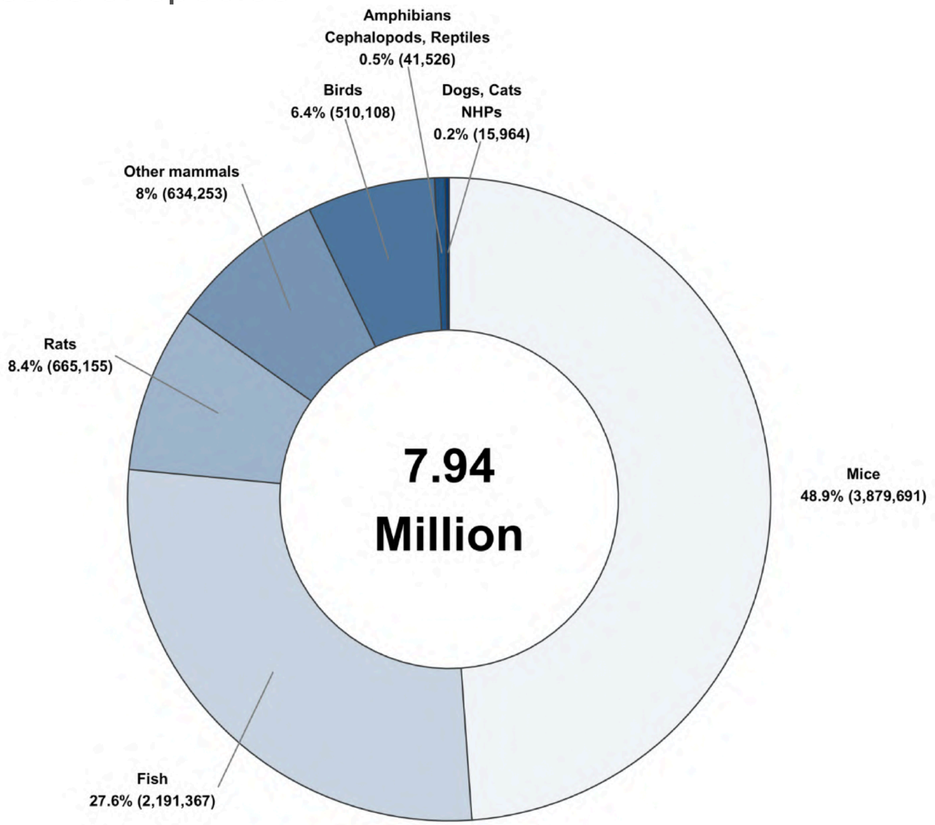
  - **Reagents**, equipment, methods…
- Inlife data
  - Route of administration
    - Restraint
    - Dose frequency/duration
    - Dose volume
  - Husbandry conditions
    - Diet; Bedding
    - Cage; Caging; Enrichment
    - Diurnal/seasonal
  - Clinical observations
  - Body weights; Food consumption
  - Sampling collection
    - Frequency and volume
    - Clinical pathology; Toxicokinetics; Pharmacokinetics
  - **Animal welfare**
    - Study procedure-related stress
- Clinical pathology
  - Fasting status; Restraint; Anaesthesia
  - Sample processing; Analytical laboratory; **Assay**
  - Haematology, clinical chemistry parameters
- Anatomic pathology
  - Mortality: Euthanised moribund; Decedent
  - Macroscopy; Organ weights; Microscopy
  - **Background findings** recorded; Grading; Threshold
- **Controlled vocabulary** and version

The constraints
Ethics in animal experimentation

- The 3Rs (developed in EU Directive 2010/63) for more humane use of animals
  - **Reduce** the number of animals used
    - Reduce the number of control animals euthanised and necropsied
  - **Replace** large (nonrodent) animal [with insensitive material]
    - With smaller (rodent, others) animal
    - With humanised mouse models
    - With animal data with historical control databases (HCD)
    - Keep nonhuman primates (NHP) for critical studies
  - **Refine** procedures
    - HCD to interpret toxicity studies with reduced numbers of concurrent controls

- Use of animals for scientific purposes in the EU and Norway in 2020
  - Number of animals used for the first time by main classes of species

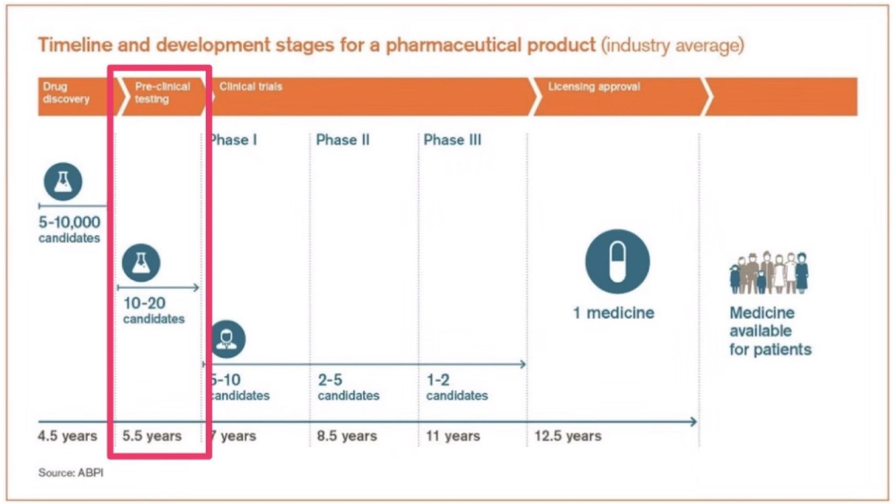


The constraints
Limited animal (esp. NHP) resources

- Geopolitics
  - The Implications of NHP Shortages for US Biomedical Research
  - Chinese ban of non-human primates (NHP) export since 2020
  - **Shortage of NHP** for scientific research
- Biodiversity
  - Many exotic pets suffer or die in transit, and beyond—and the U.S. government is failing to act
  - Mortality: 5-90% (birds imported to US: 83%)
  - Europe **limits the capture of wild animals** to establishing breeding and in most cases **forbids their use** in scientific research

Economics of drug development

- NHP cost forecast
  - ≥ $25,000/monkey in 2023
- Availability of **NHP** & **mature dogs**
  - NHP mature at 2-5 years
  - Dogs mature at ~ 8 months
- Cost and duration of drug development
  - Reduce study cost ⇒ more studies, better characterisation of the test article

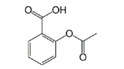


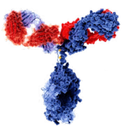
Source: Deciphex

674 M$ | **150 M$** | 273 M$ | 319 M$ | 314 M$ | 48 M$ | Total 1,778 M$

Applying the 3Rs
Reduce and refine by reuse and rehoming of animals

- **Reuse (of NHP)** favoured by EU Directive under strict conditions
  - **Ethical reasons**: 3Rs
    - Proposed cases of reuse
      - Train technical staff for procedures
      - Reuse control animals from a previous terminal or recovery phase in GLP studies
      - Reuse in non-GLP or pharmacokinetic/pharmacodynamic (PK/PD) studies after washout
      - Reuse in non-GLP exploratory studies
      - Devise other novel strategies
    - Inclusion criteria
      - Good health condition, no irreversible pathological changes
    - Exclusion criteria
      - Persisting drug from first study
      - Immunological changes (**non-naïve NHP**)
        - Previous biotherapeutic interferes with measurement of the new test article
        - Homology with previous biotherapeutic (pre-existing antibodies…)
  - **Business justification**: cost and time
    - Logistical challenge
      - Colony maintenance
      - Ability to provide an adequate number of study animals at similar ages and weights
      - Increased need for additional holding space for reuse colonies
  - **Public pressure**
- **Rehoming** when possible
  - Dogs moved to in-house dog adoption programs
- **Euthanasia** for animals which cannot be kept in the husbandry
  - No reuse allowed; age…





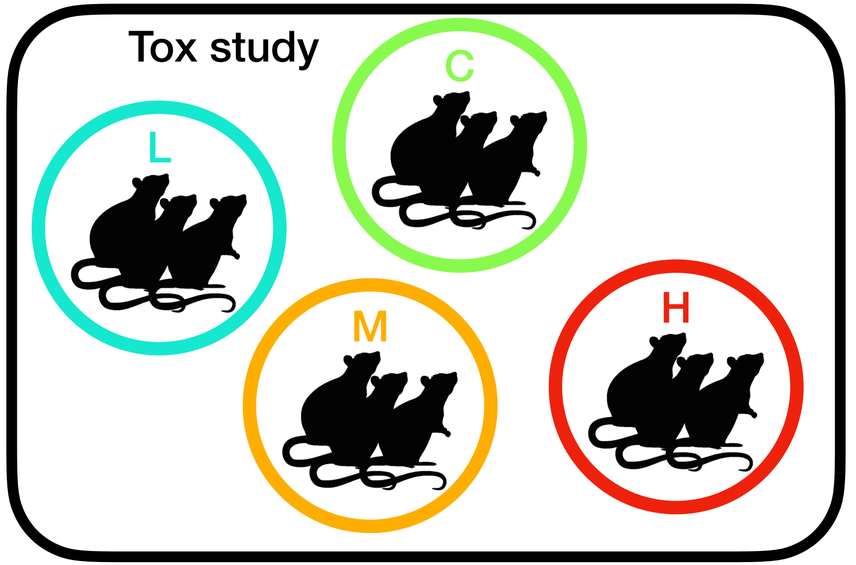
Applying the 3Rs
Reduce the control group size and replace animals

- Potential for **> 25% reduction** of animal use!
- **Reduce use** (of NHPs firstly, but of other species also)
  - Reduce number of treatment groups
  - Reduce number of animals per group
  - Use single sex
- **Refine use** of animals
  - Reduce number of in-study control animals euthanised
    - From both the terminal and recovery phases
    - Used to **maintain the HCD**
    - The others are reused
  - **Replace** in-study concurrent controls with virtual or synthetic control animals

- Challenges
  - HCD development and maintenance
    - Include digital images
  - **Acceptance by**
    - Pathologists (anatomic, clinical)
    - QA
    - Sponsors, clients, contract research organisation (CRO)
    - Regulatory authorities

Replacing the control group
The Concurrent control group – CCG

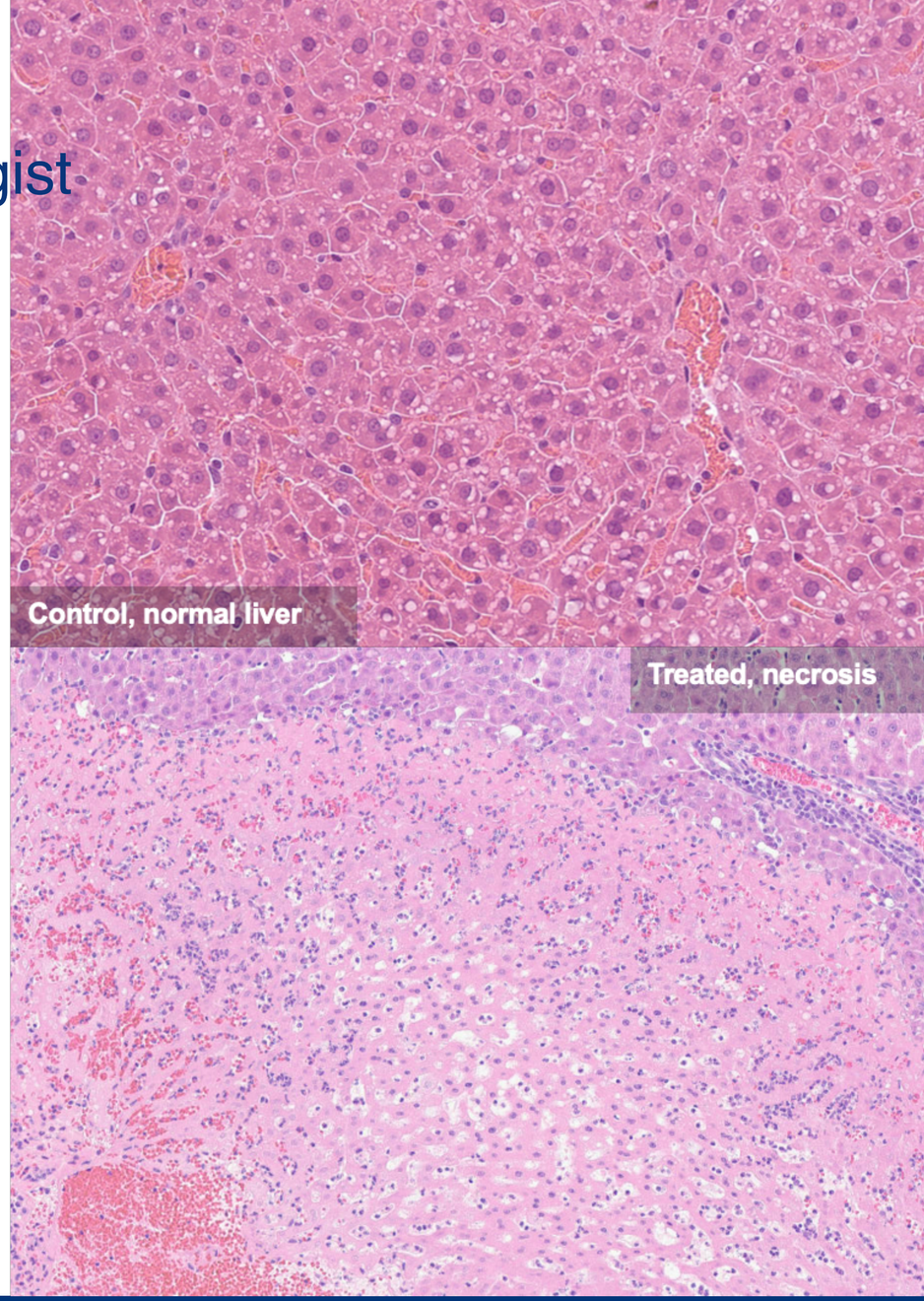
- **Standard control group** assigned to a study
  - Terminal euthanasia in most study designs
  - Standard number of in-study concurrent controls
    - Evaluation of all non-terminal endpoints (in-life, ECG, ophthalmo, clinical pathology…)
- Can be **used for HCD**
- Considered **best scientific practice** and recommended by regulators
- Proposed improvements
  - **Reduce the number** of terminal and recovery animals included in the CCG
  - Refine by **limiting euthanasia** of CCG animals to 1/sex
    - Return non-terminal animals to stock colony
    - Maintain the HCD by expanding the control pool



Actual animal

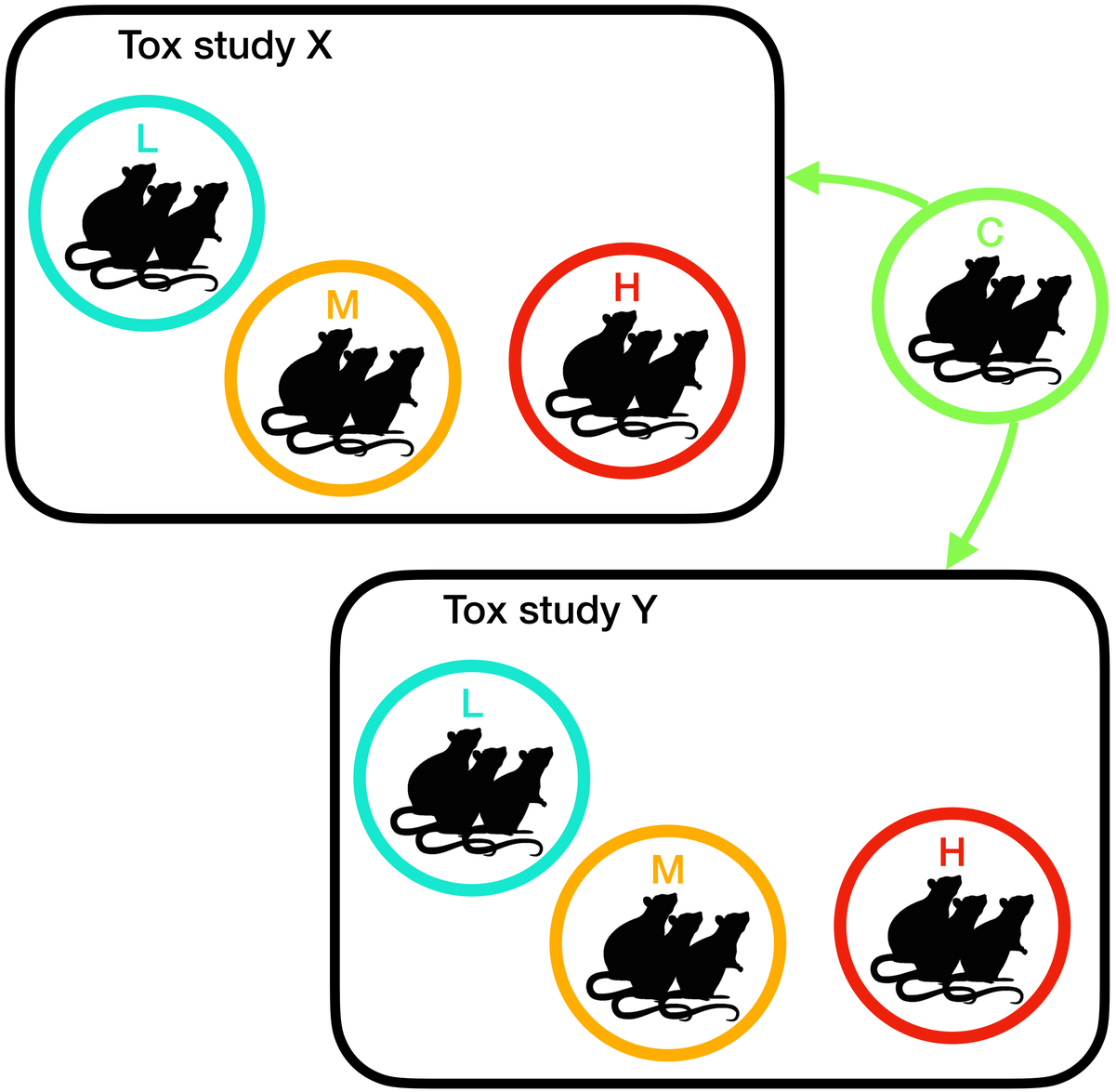
CCG
Control animals are important for the toxicologic pathologist

- Decrease the confounding factors in the study context
  - Subtle finding (liver vacuolation, top)
    - Comparison to controls necessary for **establishing a baseline**/thresholding
- Determine if a test article effect is true or false positive
  - Prominent finding (liver necrosis, bottom)
    - **Lower risk of missing** high-impact safety issues



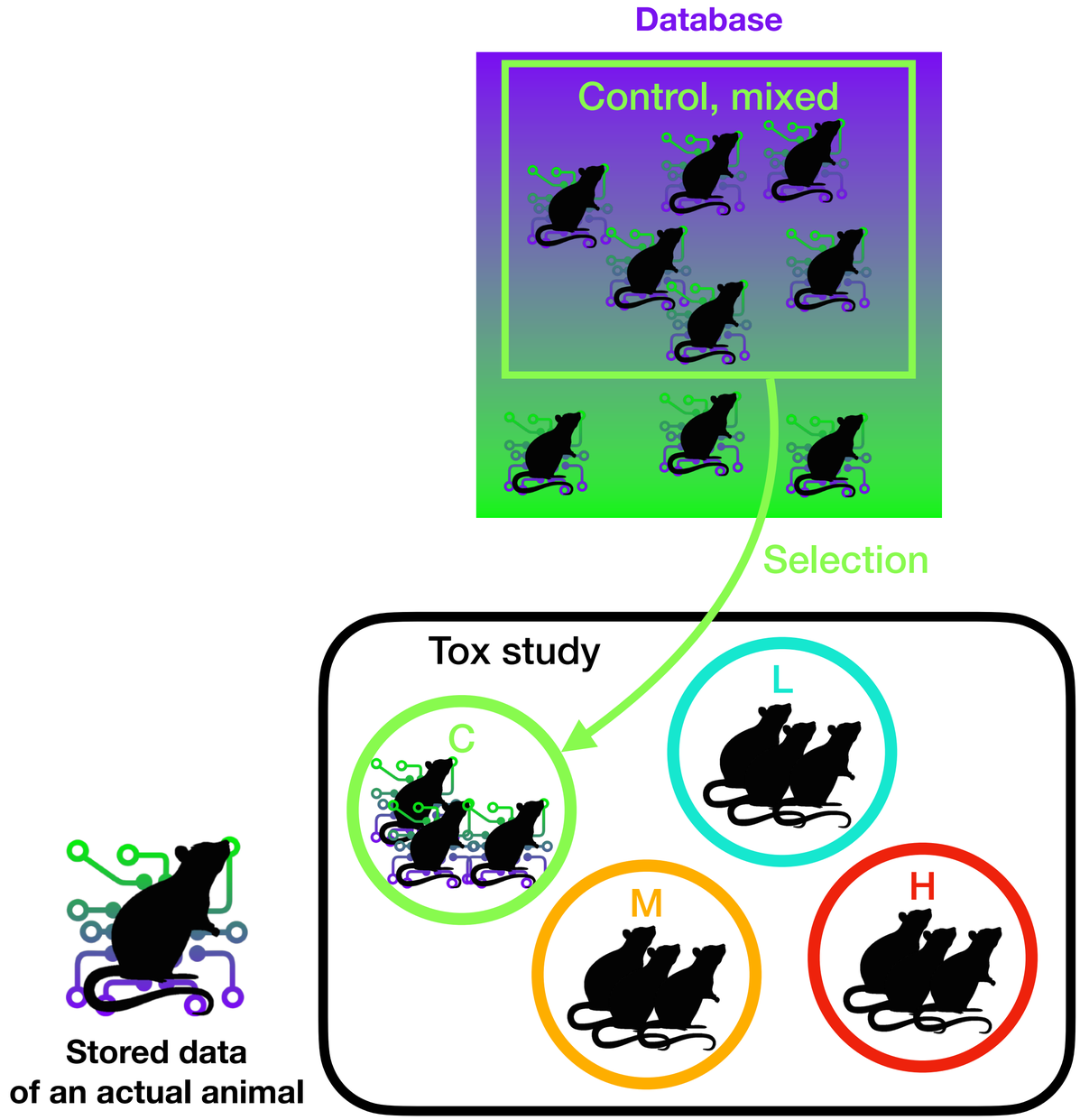
Replacing the concurrent control group
The Shared control group – SCG

- Used in **multiple studies** for the same sponsor
  - Timepoint collected in alignment with the study needs
  - No CCG on the study, could be terminal if needed, mostly used for nonterminal endpoints
- In a CRO, can be used for the same sponsor or for multiple sponsors
- Points of interest
  - Logistics!
  - Can be **used for HCD**
  - Take care of **confounding factors**
    - Vehicle, route of administration, timepoints, parameters collected
  - **Terminal endpoints** are not collected if no euthanasia/ necropsy is performed



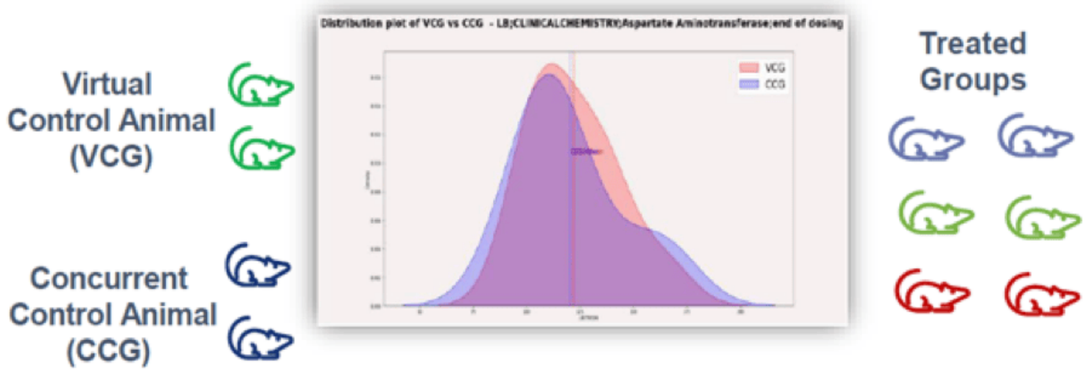
Replacing the concurrent control group
The Virtual control group – VCG

- Include control animal **based on past data** (<3-5 yrs)
  - Can be refined by mixing CCG and VCG in various proportions
- Benefits
  - Maximise **animal reduction**
  - Maximise **HCD use**
    - Large, well-structured, well-annotated data sets and repositories for control animal data
- Challenges
  - **Do not expand** the HCD
    - **Rolling period**: see HCD section
  - Virtual animals used **may not match** timepoints and parameters of the targeted study design
    - Validate the group created scientifically/statistically



Assessing “sameness” is a hard problem

- CRL compared statistical tests to subject matter expertise (SME) judgement
  - Statistical tests led to **high false positives**
  - They propose that declaring a VCG the same than a CCG should be done not with statistical tests, but with an equivalence criteria



Some points to consider

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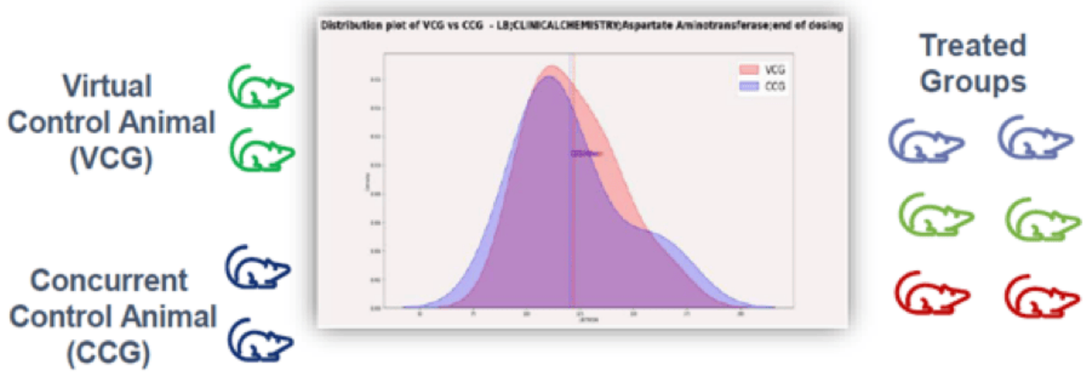
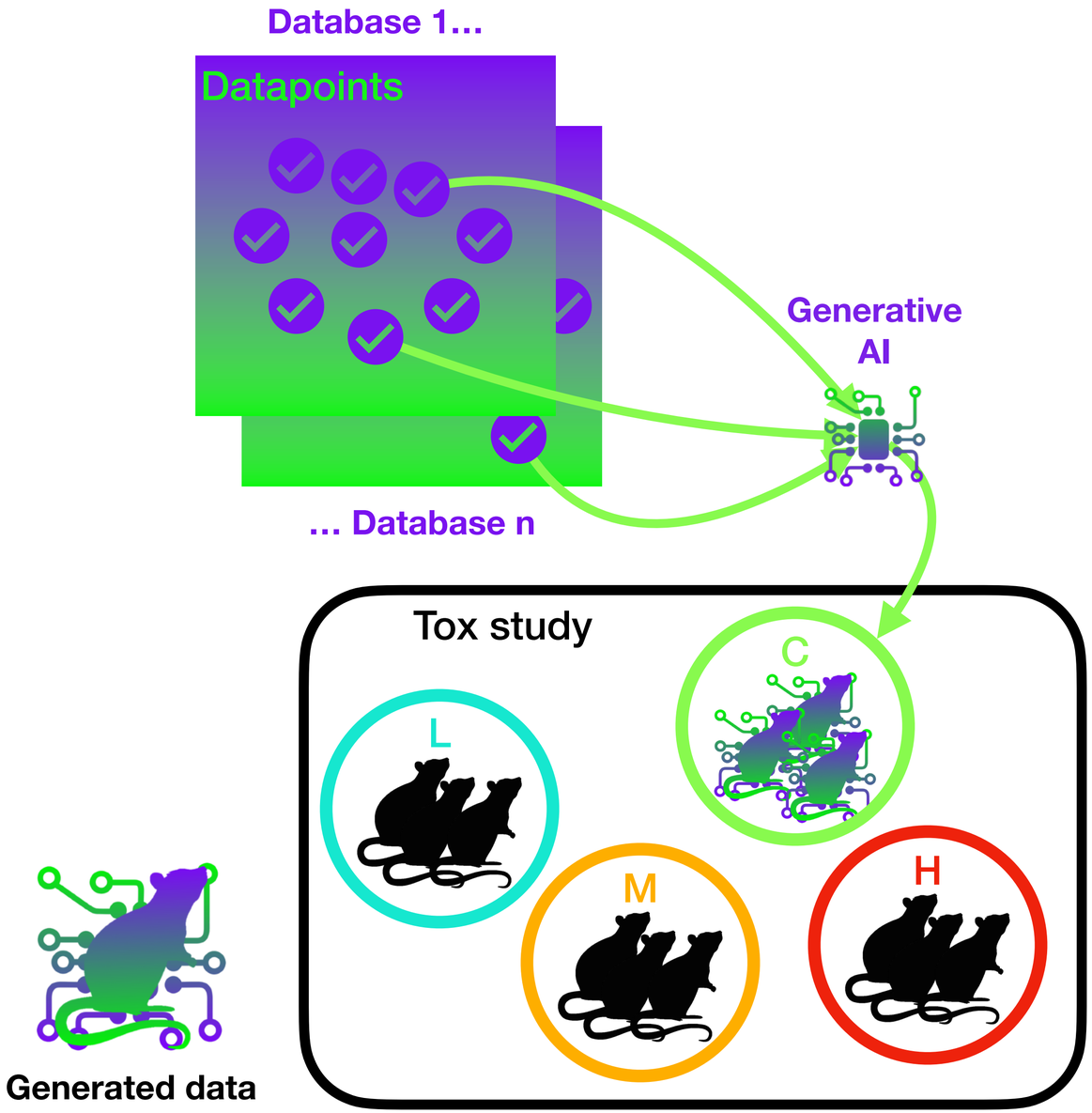


Some points to consider

- Control animals will still be **needed**
  - But reduced in number
- Acceptance of new type of data
  - Different guidelines by regulators
  - **Confidence** of users
- Risks
  - Unforeseen variables **confounding results**
    - Infectious disease, environmental/housing failures, unanticipated temporal impacts, etc.
    - Additional animal use if studies must be repeated
  - False negatives (**patient at risk**) or false positives (**no access** to potentially useful drug)
    - Incorrect interpretations and erroneous assumptions regarding statistical significance

Replacing the concurrent control group
The Synthetic virtual control group – SVCG

- Artificially created, randomised subset of a study population
- Generated through statistical methods or modelling to **mimic the characteristics of a CCG**
  - Constructed from HCD
- Benefits & challenges
  - Those of the VCG
  - Those of an AI model



Feeding a HCD with VCG/SVCG digital slides: some points to consider

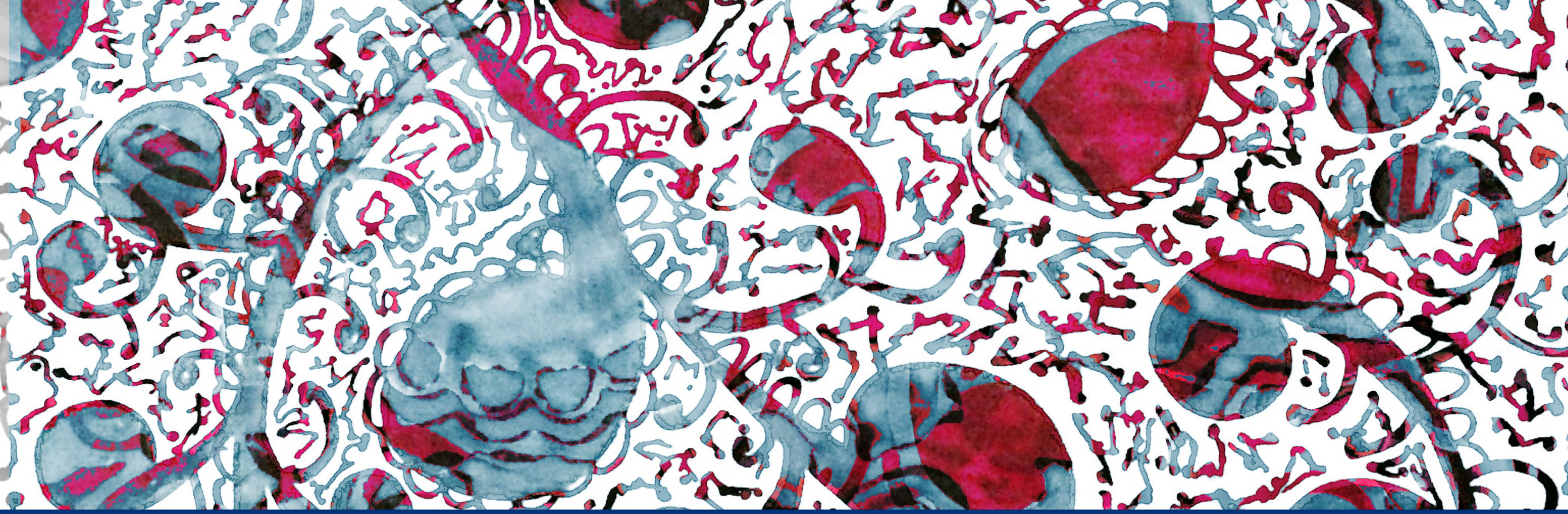
- **FAIR**
  - Findable; Accessible; Interoperable; Reusable
- Benefits
  - **Large** database of retrospective data sets
    - Collect, maintain
      - Weakness if HCD is too small
        - Data gaps; lack of detection of rare events
        - Insufficient for statistical analysis and AI
    - Curate, standardise, annotate
      - Iterative procedure, difficult and **time-consuming**
      - Guarantees **trustworthy data** and robustness of data analysis
    - Quality control
      - Data and metadata are fit for use
      - Risk: data dispersed in several databases are difficult to QC; data/metadata of insufficient quality, esp. for old retrospective studies
  - **Rolling period of 3-5 years**
    - Genetic drift
    - Changes/advancements in data collection and evaluation, animal use, sourcing or environmental
  - **Higher power and confidence** than with small CCG
    - Important for study interpretation (provide evidence for or against test article-relationship)
- Centralised or localised model?
  - **Centralised**: inter-company
    - Weakness: variability in diagnosis and thresholds; access for users and for regulators
    - Likely difficult to achieve
  - **Institution-specific**
    - Weakness: not enough studies/animals/findings

Digital pathology component of the HCD: some points to consider

- High quality **digital slides with metadata**
  - Do not generate new data! (No study report change)
    - Risk: seen as a second PR by study director and regulators
- **Large number** of animals, digital slides, data points (findings) and metadata
  - Connected to all study parameters and covariates
  - The digital slide gives full context, not only textual descriptive data
    - Findings can correlate to matching data (macroscopy, clin path, organ weight)
  - Useful for interpretation of studies
    - **Rare findings**/outliers not present in the CCG or in contemporaneous studies
      - E.g., **proliferative** changes, rare in young animals
    - True incidence and severity of **background findings**
      - E.g., **subtle** changes, test article-related or exacerbated
  - Useful training support for **new pathologists** and for **AI models**
  - Risks
    - Important covariates not captured in searchable DB
    - Loss of HCD reliability, loss of metadata relevant to train AI
- Points of interest in pathology
  - Evolution of **controlled terminology** → versioning
    - Standardisation and harmonisation of findings
  - **Grading of background findings** is variable
    - Background findings frequently unrecorded if below arbitrary threshold
    - Inter/intra-company; inter/intra-pathologist
    - Inter-studies
    - Diagnostic drift

Take-home messages

- In a nonclinical study, **~25% of animals** are untreated with the test article and could be saved
- **Ethical** and **economical** reasons are strong arguments to reduce the number of those animals
- Optimising CCG and SCG allows to **reduce** the number of animals
  - Can be combined with **reuse** of non-euthanised animals
- Using VSG and SVCG allows to **replace** animals
  - Can be combined with **smaller CCG**
- Apply to **nonrodent** and especially to NHP
  - **Generalise** to all laboratory species
- Virtual groups require a **strong HCD process**
  - Difficult to set up
  - Require **validation** and **acceptance** by users and regulators



Thank you for your attention

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Last update: 7 December 2023

Michelle Hammer, Surgical
Michelle Hammer is a NYC native with schizophrenia. At 27, she decided to use her artistic talents and fearless personality, creating a clothing line with the mission of reducing stigma by starting conversations about mental health. Her inspiration comes from her "busy mind." "When you look at these pieces, your eye constantly moves around, and never stays in one spot. One part of the artwork leads you to another area of it and that area also leads you to a different spot"

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2 European grants relevant for virtual control groups

- Bigpicture's vision is to become the catalyst in the digital transformation of pathology. Bigpicture will set up the first European, ethical- and General Data Protection Regulation-compliant, quality-controlled and community-based platform, in which both large-scale data and artificial intelligence (AI) algorithms will coexist. Bigpicture will develop a sustainable secure and scalable infrastructure to store pathology data, collect >2 million nonclinical and >1 million clinical high-quality pathology images with associated technical and biological information, and extensive metadata. It will also develop tools to enable and enhance the use of the repository, such as morphological search tools; and generic AI building blocks to promote the development of AI models. Eventually, it will advance the regulatory, legal and ethical framework around AI in non-clinical safety testing and clinical use

- ViCoG, an EU Innovative Health Initiative grant proposal, aims at reducing the number of research animals used in studies performed for example during nonclinical drug safety evaluation, by replacing physical animals in the control groups by simulating control animals. The simulated, virtual control groups (VCGs) will be obtained by means of state-of-the-art statistical or AI approaches that optimally exploit the wealth of historical animal control data accumulated over decades by the pharmaceutical and other relevant industry sectors. The VCG concept was designed and prototyped during the recently finished eTRANSAFE IMI2 project. The prototyping of the VCG concept demonstrated that it is generally feasible but scientifically and operationally challenging, therefore necessitating dedicated efforts and resources


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