VICT3R

Developing and implementing Virtual Control Groups to reduce animal use in Toxicology Research

Virtual Control Groups in Toxicity Studies

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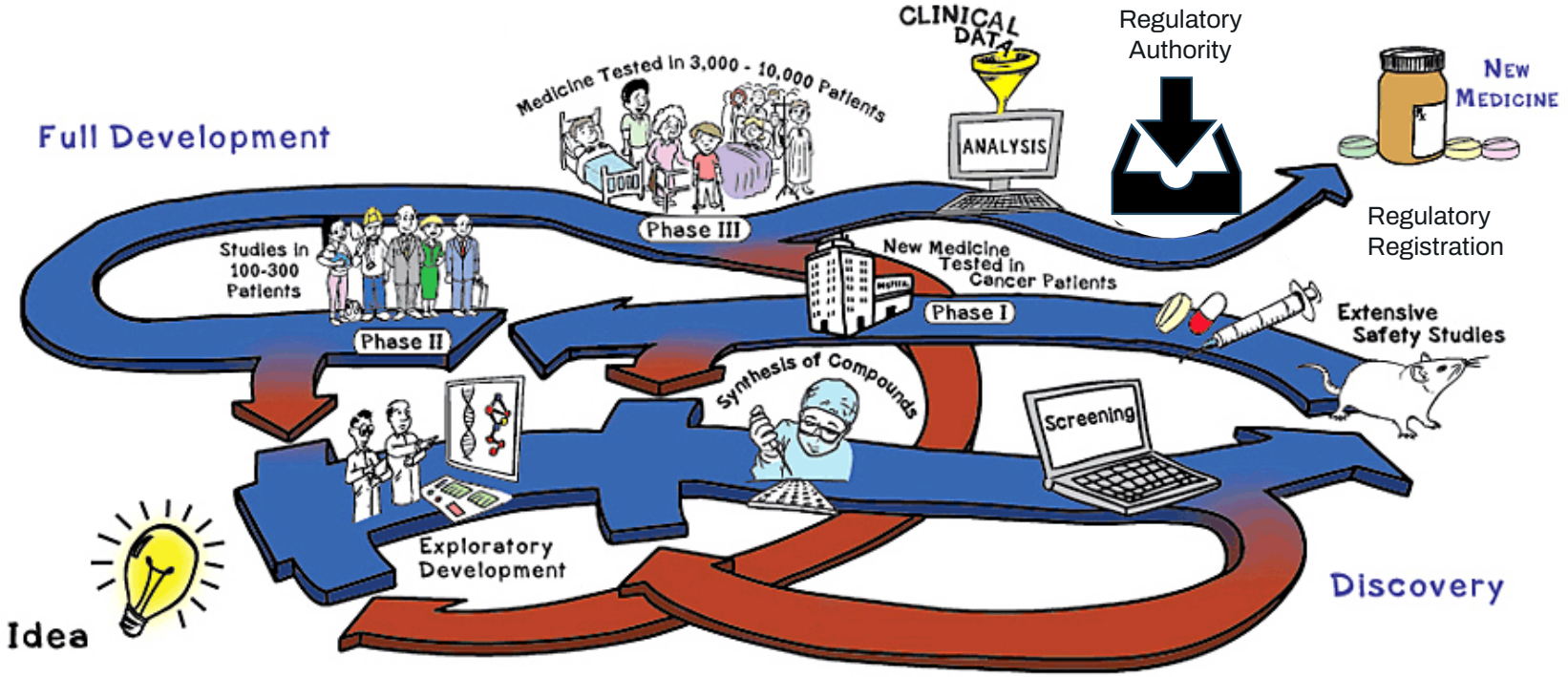
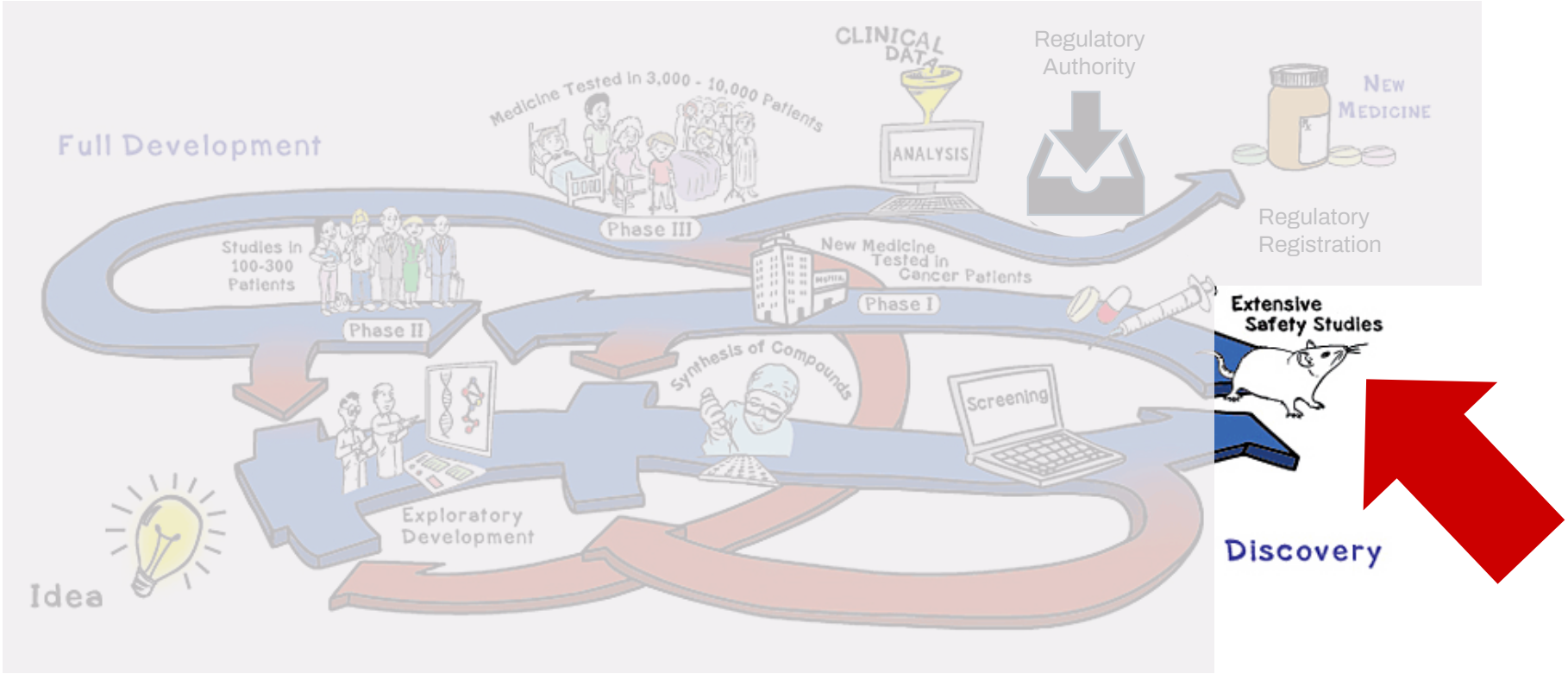
This project is supported by the Innovative Health Initiative Joint Undertaking (IHI JU) under grant agreement No 101172693. The JU receives support from the European Union's Horizon Europe research and innovation programme and COCIR, EFPIA, Europa Bio, MedTech Europe, and Vaccines Europe and Instem Scientific Limited. Funded by the European Union, the private members, and those contributing partners of the IHI JU. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the aforementioned parties. Neither of the aforementioned parties can be held responsible for them.

Bumpy road to new medicine



https://www.labroots.com/

Bumpy road to new medicine



https://www.labroots.com/

Role of Safety and Toxicity studies

Regulatory Requirement

insights into how the drug is absorbed, distributed, metabolized, and excreted (pharmacokinetics), as well as its biological effects (pharmacodynamics)

Identifying Adverse Effects

helps in understanding the therapeutic window

understanding the toxicity profile in animal models, scientists can determine the maximum tolerated dose and establish guidelines for dosage in clinical trials

Environmental assessment

Impurity Tox

carcinogenicity

Determining Safe Dosage Levels

evaluate how the drug behaves in biological systems

long term toxicity vs. short term acute

Pregnancy & Lactation

Adequate assessments are key

Some possibilities for repeated dose testing

Table 1: OECD guidelines available for the repeated dose testing

OECD Test guideline	Type of study	
407	28-day oral toxicity study (Rodents)	
408	90-day oral toxicity study (Rodents)	
409	90-day oral toxicity study (Non-rodents)	
410	28-day dermal toxicity study (Rat, rabbit or guines pig)	OECD, 1981a
411	90-day dermal toxicity study (Rat, rabbit, or guinea pig)	OECD, 1981b
412	28-day inhalation toxicity study (Rodents)	OECD, 2018b
413	90-day inhalation toxicity study (Rodents)	OECD, 2018c
452	Chronic toxicity study (Rodents)	OECD, 2018d
453	Combined Chronic Toxicity/Carcinogenicity Studies (Rodents)	OECD, 2018e

Each compound/candidate gets a highly specific set of studies reflecting indication, route of administration, etc.

JRC132210_01.pdf

Jennings, P., Chandrasekaran, V., Hardy, B., Langemeijer, E., Doktorova, T., Madia, F., Prieto, P., Mechanistic Analysis of Repeated Dose Toxicity Studies – Key characteristics of chemical-induced toxicity in the liver, lung, cardiovascular system and kidney, Publications Office of the European Union, Luxembourg, 2023, doi:10.2760/824535, JRC132210.

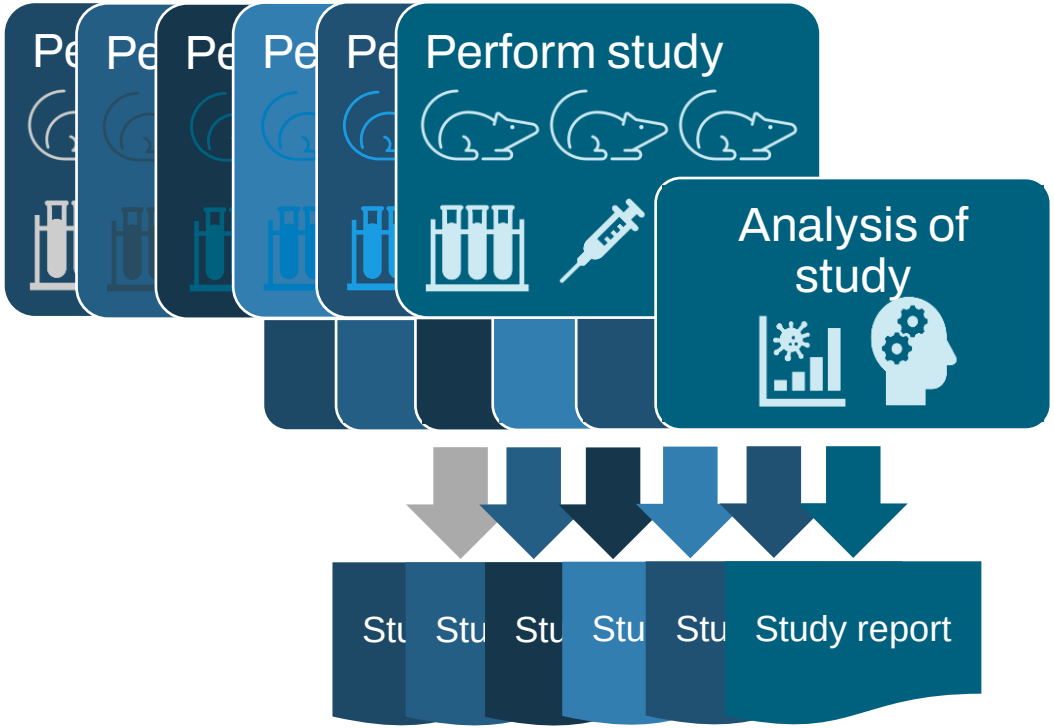
3R

Legislation within the EU

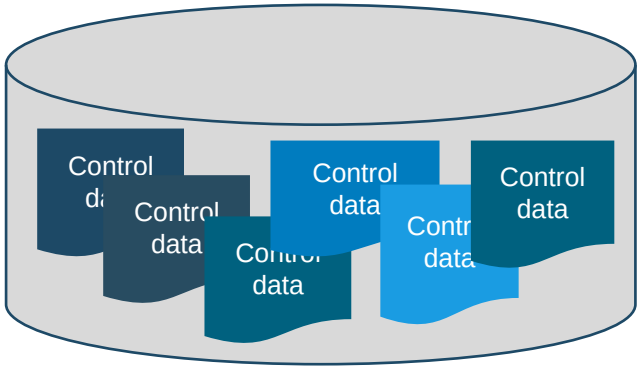
- Directive *2010/63/EU* on the protection of animals used for scientific purposes
- Guideline on the principles of regulatory acceptance of 3Rs (replacement, reduction, refinement) testing approaches (*EMA/CHMP/CVMP/JEG-3Rs/450091/2012*)

https://www.ema.europa.eu/en/human-regulatory-overview/research-development/ethical-use-animals-medicine-testing

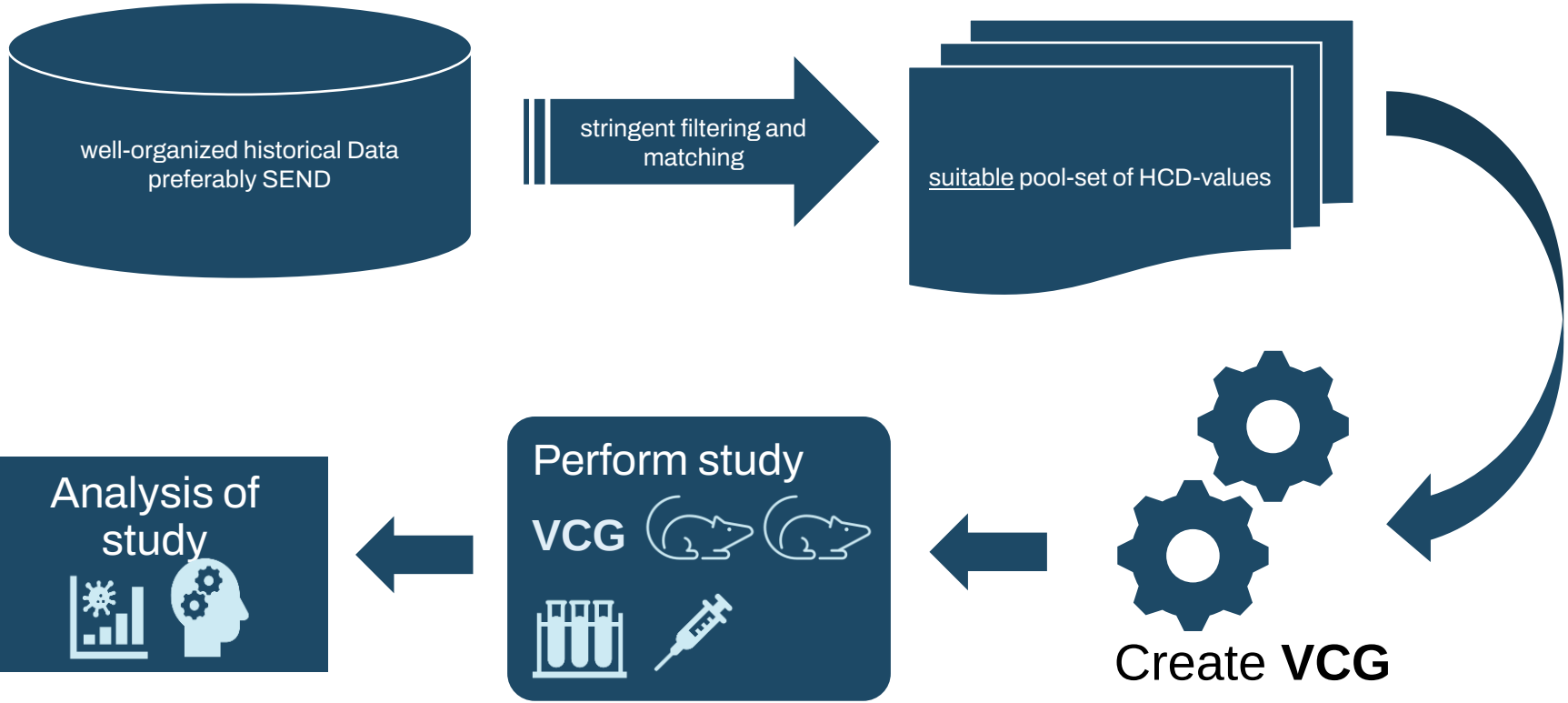
Current situation



- Use of historical controls:
  - Recommended/advised in guidelines
  - Rarely!
  - Simple!
  - Mainly assay quality and validity



Virtual Control Groups (VCGs) – the general concept



ViCoG DB Version from 0.1 – 1.7

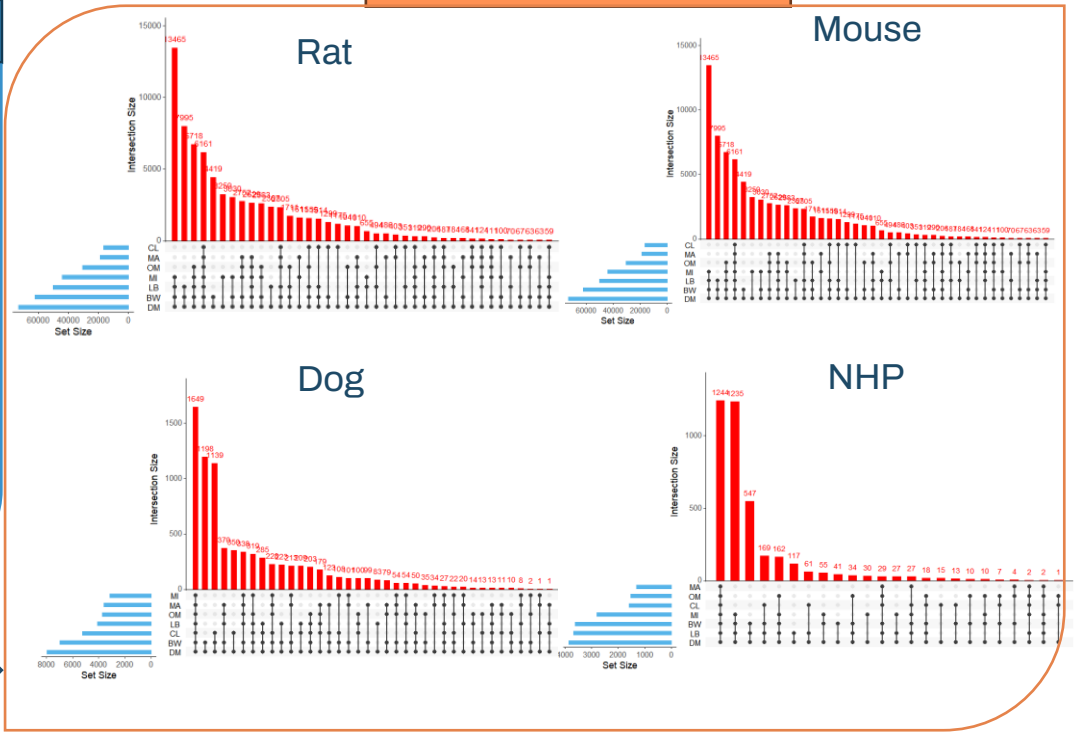
Be not afraid of growing slowly - be afraid only of standing still

The first message shared 2022

Version 0.1 released on May 25, 2022

- Domains: BW, DM, LB
- Contributors: Bayer, Merck, Novartis, Roche
- Comments:
  - Domains were merged in single files in flat file txt format
  - One file per domain
  - DM-decriptive domain
  - BW, LB are data domains
  - DM contains animal information and study specific information
  - DM data should provide information for selection of animals used for analysis
  - These data include Species, Strain, Sex, Vehicle, Route, Duration, Breeder, Company, etc.
  - USUBJIDs an be retrieved and used for data mining for the animal specific data in the data domain

Current Status July 2024



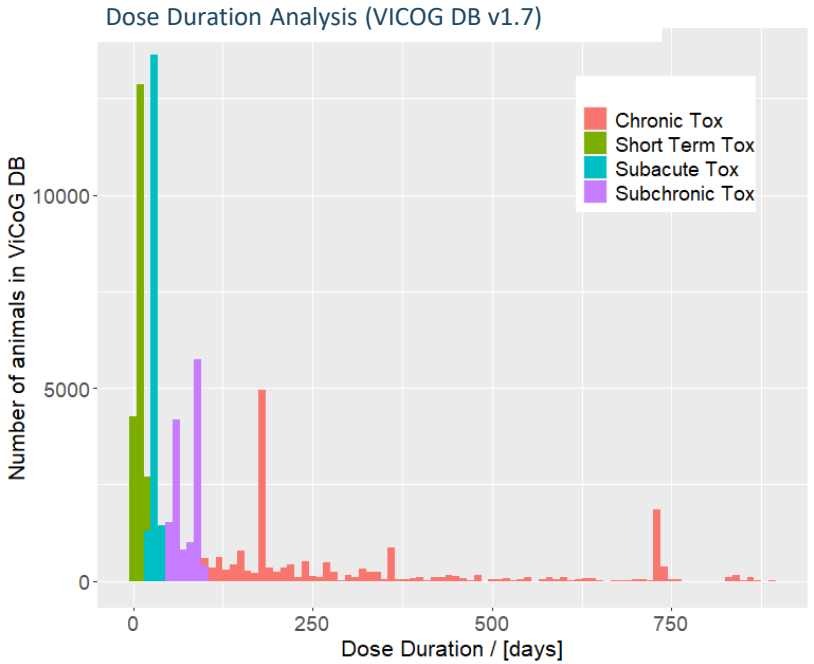
VICOG DB v1.7

Data sets from 91.774 animals donated from Bayer AG, Merck KGaA, Novartis Pharma AG, F. Hoffmann - La Roche AG, and Sanofi compiled and curated by Merck Healthcare KGaA and Fraunhofer Gesellschaft ITEM

Company	Records
Bayer AG	8.451
Merck	10.919
Novartis	16.407
Roche	28.122
Sanofi	27.875

Species	Records
Rat	73.530
Mouse	7.965
Dog	5.856
Monkey	3.860
Rabbit	243
Minipig	50
Guinea Pig	52
Pig	12
other	206

Domain	Records
Demographics (DM)	91.774
Organ Measurements (OM)	599.367
Clinical Observations (CL)	1.914.922
Macroscopic Findings (MA)	567.807
Body Weights (BW)	1.253.281
Laboratory Measurements (LB)	3.853.392
Microscopic Findings (MI)	1.697.546



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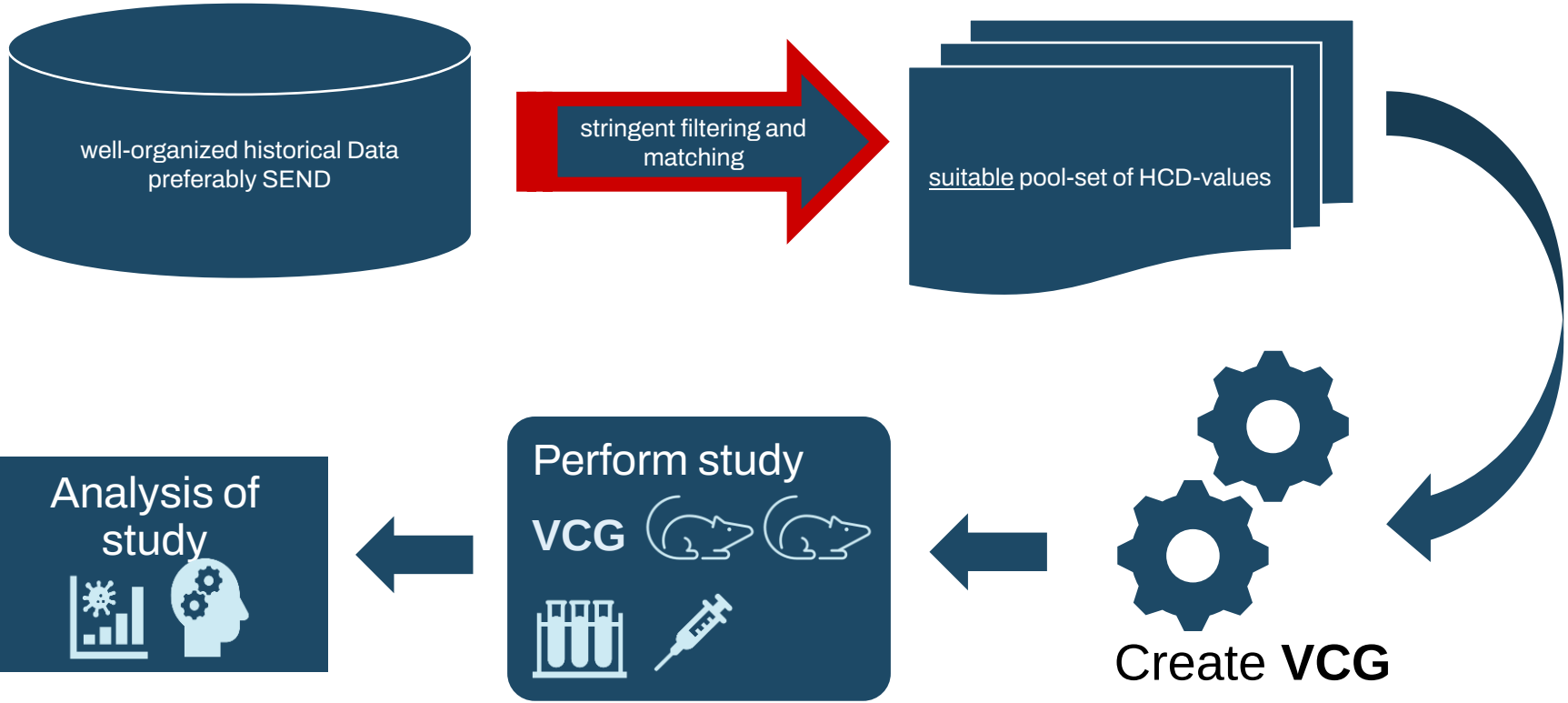
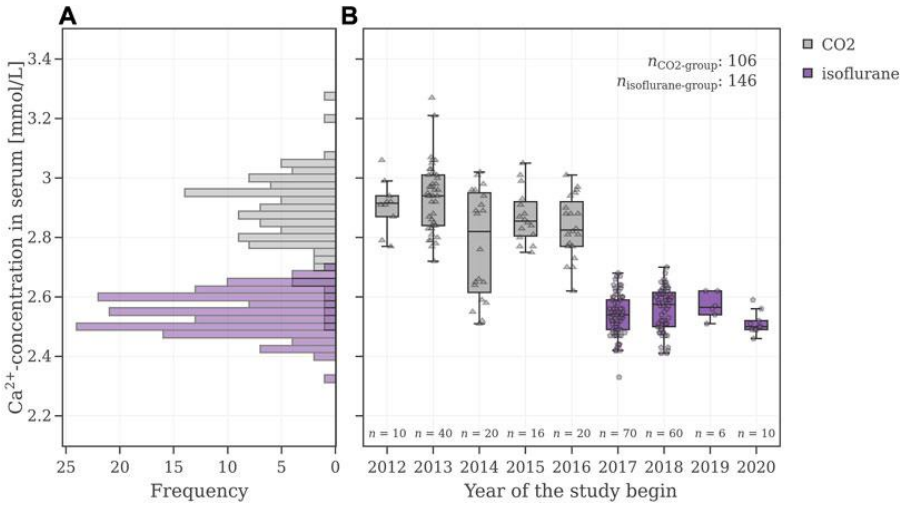


Filtering and Matching

Strategies:

- Filter with available parameters recorded in SEND
  - Sex, strain, supplier, age, housing conditions, route of administration, diet, tissue collection and processing procedures, treatment vehicle



Gurjanov et al. (2023) Front. Pharmacol. Sec. Predictive Toxicology
https://doi.org/10.3389/fphar.2023.1142534

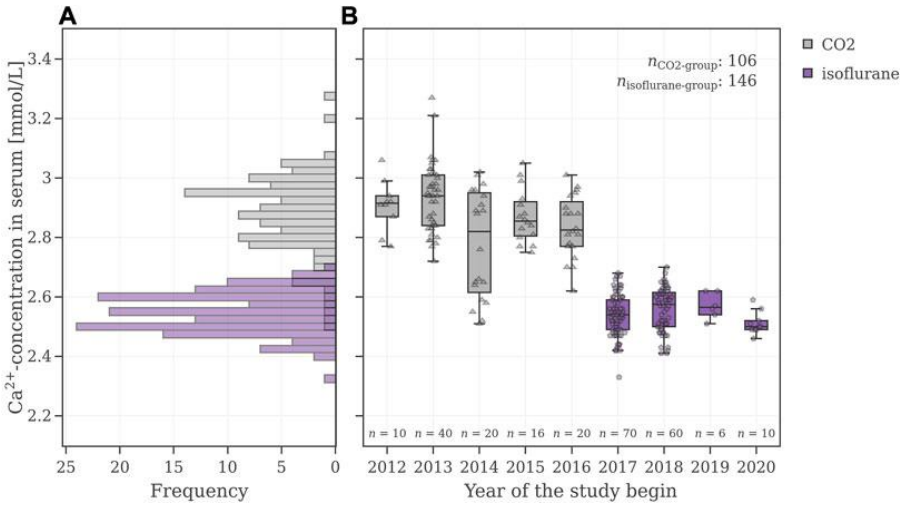
Filtering and Matching

Strategies:

- Filter with available parameters recorded in SEND
  - Sex, strain, supplier, age, housing conditions, route of administration, diet, tissue collection and processing procedures, treatment vehicle
- Limit to characteristics derived from data, e. g. mean +-SD, min/max, etc
- Sentinel animals PLUS characteristics from data

Detour: How similar is similar enough?

- Which parameter for filtering are crucial?
- How does data-driven selection help?



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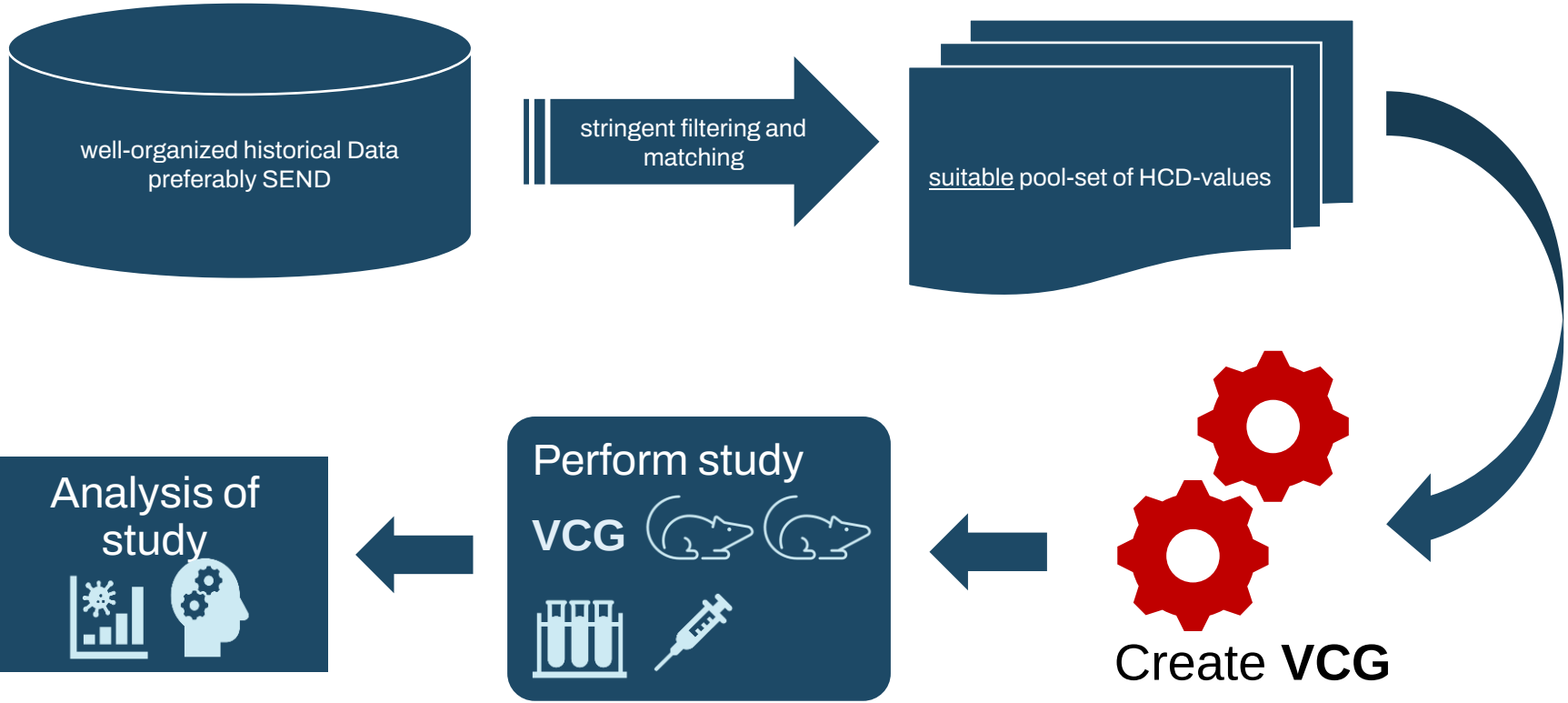
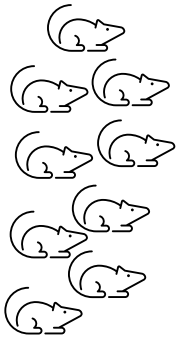


A typical scenario: 28-day toxicity study in rats



Body-weight

Randomisation

- Control
- Low dose
- Mid dose
- High dose

- Mortality
- Clinical Observations
- Food & Water Consumption
- Body weight

In-life parameter

Start of study — End of study

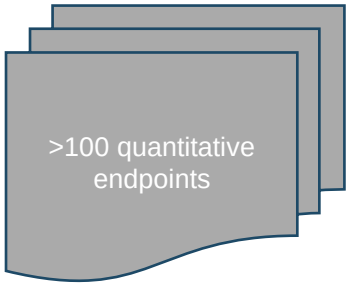
Sampling during study

- Hematology
- Clinical chemistry
- Coagulation
- Urine
- Organ measurements
- Histopathology

Parameters from 28-day toxicity study in rats

Sorted chronologically

	Observation class	Data type
in-life parameters	Mortality	Qualitative
	Clinical observations	Qualitative
	Body weight	**Quantitative**
	Food and water consumption	**Quantitative**
sampled during study evaluated after end of study	Hematology	**Quantitative**
	Clinical chemistry	**Quantitative**
	Urine	**Quantitative**
	Urine	Semiquantitative
sampled at end of study	Organ measurements	**Quantitative**
	Histopathology	Qualitative + Images



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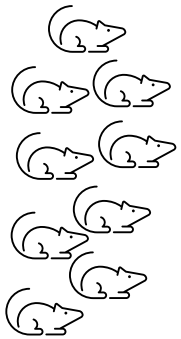


Body-weight

Randomisation

Control

Low dose

Mid dose

High dose

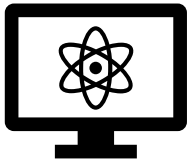
Animals or VCG?

Start of study

At start of study **only** body weight is available for creation of VCG

Proof of concept

- Body weight is correlated with most other parameters
- → we are scientists, and we are brave, thus, give it a try – there will be a lot to learn



Proof of concept

reproduce results of **entire legacy studies** after replacing CCGs with VCGs

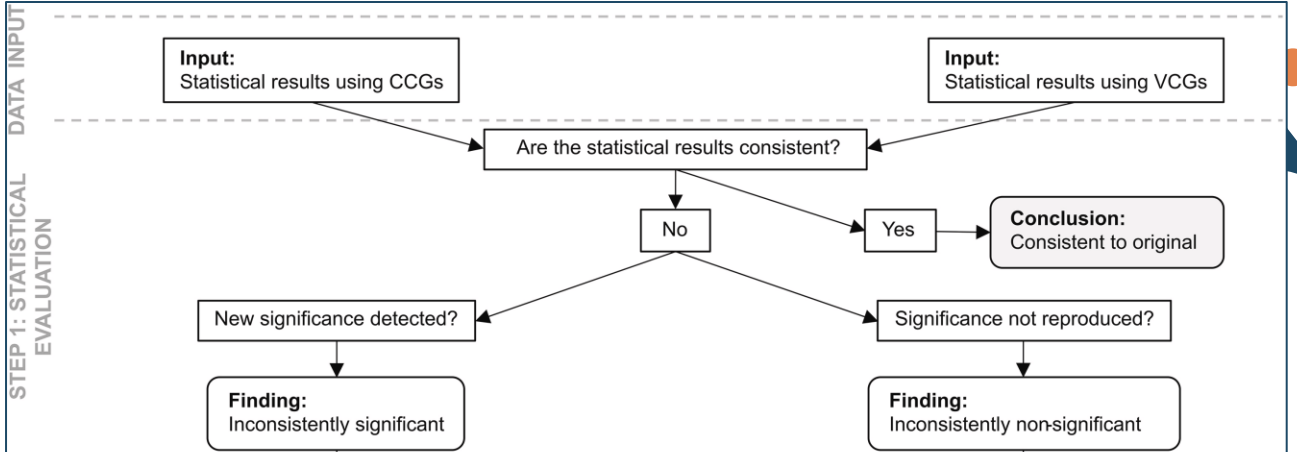
- 3 Legacy studies selected
- Replaced CCGs of these studies completely with VCGs
- Recalculate statistical analysis for
  >100 quantitative parameters

Regulatory Toxicology and Pharmacology
Volume 148, March 2024, 105592

Replacing concurrent controls with virtual control groups in rat toxicity studies

Alexander Gurjanov [a], Carlos Vieira-Vieira [a], Julia Vienenkoetter [b], Lea A.I. Vaas [c], Thomas Steger-Hartmann [a]

Gurjanov et al. (2024) Regulatory Toxicology and Pharmacology, https://doi.org/10.1016/j.yrtph.2024.105592.



Performance evaluation & Benchmarking

Gurjanov et al. (2024) Replacing concurrent controls with virtual control groups in rat toxicity studies. Regulatory Toxicology and Pharmacology, https://doi.org/10.1016/j.yrtph.2024.105592.

Performance evaluation & benchmarking

reproduce results of **entire legacy studies** after replacing CCGs with VCGs

- Benchmark-criterium:
  Reproducibility of overall study outcome

- **No consideration of effect sizes**

- Food for thought:
  - How to formalize “expert-knowledge” from toxicologists?
  - What could be other meaningful characteristics for benchmarking?

Reality-check

reproduce results of entire legacy studies after replacing CCGs with VCGs

- 3 Legacy studies selected
  - Replaced CCGs of these studies with VCGs
  - Recalculated statistical analysis for >100 quantitative parameters
  - Re-assessed treatment-relatedness based on new statistical results (by study director and/or subject matter expert)

Result

- 60 – 70% of test decisions were reproducible
- overall study outcome did not change
  - **N**o **O**bserved **A**dverse **E**ffect **L**evel (NOAEL)
  - **M**aximum **T**olerated **D**ose (MTD)

Gurjanov et al. (2024) Regulatory Toxicology and Pharmacology, https://doi.org/10.1016/j.yrtph.2024.105592.

Where we are…

Experiences so far from 4-week-rat toxicity studies

- VCGs created on baseline body-weight values did the job
- Filtering of HCD-pool and matching towards the cohort at hand is critical
- Re-sampling works reasonably well, method for VCG-creation offers lots of opportunities for improvement
- Benchmarking with test-results is feasible, in future may consider effect sizes and/or reference values/bands
- VCGs offer interesting possibilities to increase sample-size (and thus power) without adding additional animals

Ok. Nice. What's about nonrodents?

ELSEVIER

Regulatory Toxicology and Pharmacology
Volume 154, December 2024, 105733

Statistical applications of virtual control groups to nonrodent animal toxicity studies: An initial evaluation

Dingzhou Li [a], Jeonifer Garren [b], Raja Mangipudy [c], Matthew Martin [c], Lindsay Tomlinson [d], Nichole R. Vansell [c]

ELSEVIER

Regulatory Toxicology and Pharmacology
Volume 150, June 2024, 105632

Points to consider regarding the use and implementation of virtual controls in nonclinical general toxicology studies

Xavier Palazzi [a], Lennart T. Anger [b], Theresa Boulineau [c], Armelle Grevot [d], Magali Guffroy [e], Kristin Henson [f], Natalie Hoepp [g], Matt Jacobsen [h], Vijay P. Kale [i], John Kreeger [j], Joan H. Lane [k], Dingzhou Li [l], Wolfgang Muster [m], Brianna Paisley [n], Lila Ramaiah [o], Nicola Robertson [p], Valerie Shultz [q], Thomas Steger Hartmann [r], Richard Westhouse [s]


… and what's about regulatory acceptance?

Gain regulatory acceptance

Any idea where to start?

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

FDA
U.S. Food and Drug Administration

?

独立行政法人 医薬品医療機器総合機構
Pharmaceuticals and Medical Devices Agency

AUDA-NEPAD
AFRICAN UNION DEVELOPMENT AGENCY

CDSCO
सत्यमेव जयते

Australian Government
Department of Health and Aged Care

NATIONAL MEDICAL PRODUCTS ADMINISTRATION
国家药品监督管理局

EMA support mechanisms for evidence generation strategies

Special case: No product involved and not about marketing authorization

- Innovation Task Force (ITF)

- Qualification of Novel Methodologies
  - Qualification advice
  - Qualification Opinion

- Make yourself familiar with the formats for Academia > Partners and networks
  Academia | European Medicines Agency (EMA)

From Discovery to Regulatory Qualification and Beyond

Scientific Advice is product specific, prospective advice, no assessment of data Confidential up to MAA (M1).

Qualification is related to a specific novel methodology, prospective advice and is including assessment of data.
Open science driven principle. First step can remain confidential.

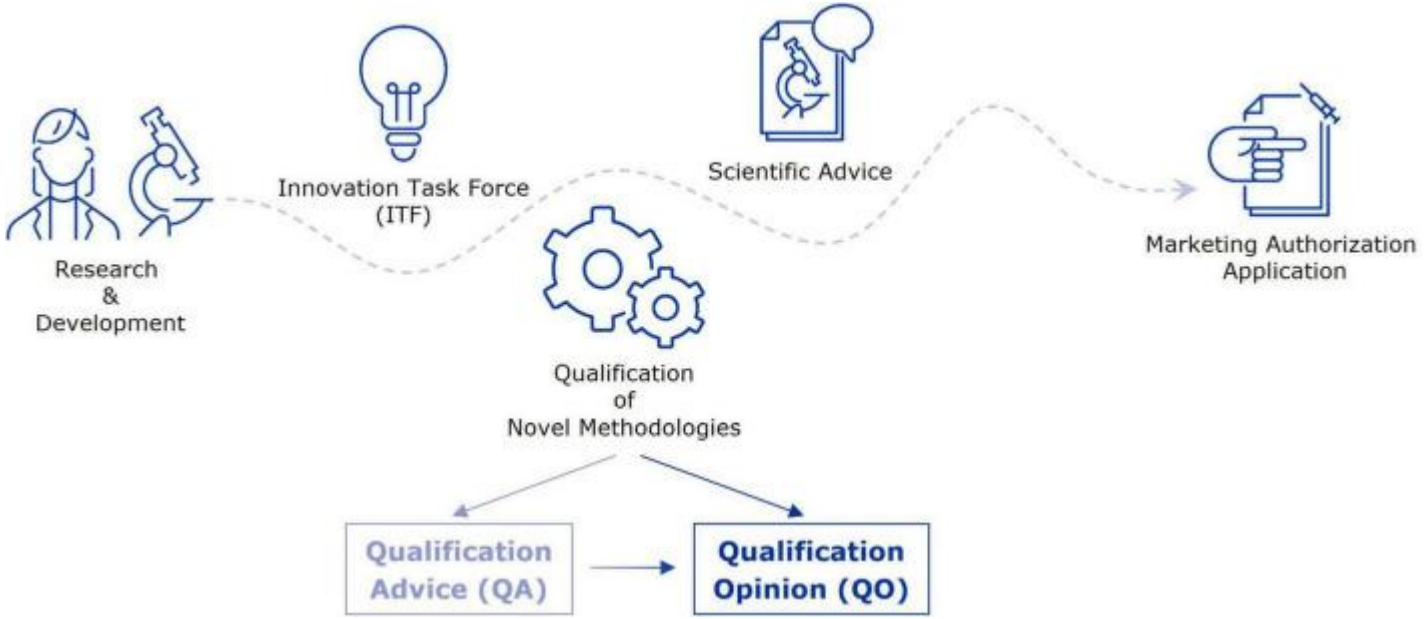


Figure 1: From early-stage research to marketing authorization – EMA support mechanisms for evidence generation strategies.

Hendrikse et al. (2022). Front Med (Lausanne) 26;9:878942. doi: 10.3389/fmed.2022.878942

EMA SAWP for VCGs before start of VICT3R

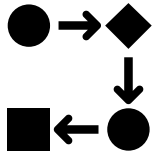
Legacy study re-analyses (Bayer, Merck, Sanofi, Roche)

Request for EMA SAWP **Qualification of Novel Methodology** with provision of

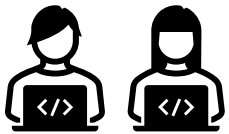
- Information on the VICOG database
- Detailed results for seven re-assessed legacy studies: CCGs were replaced by VCGs
- a series of questions

Response from EMA:

- 11 issues to be answered





Endurance and Scientific Excellence

2010 - 2016

eTRANSAFE

2017 - 2023

Subgroup:

Virtual Control Groups

The described research has been performed under the Innovative Medicine Initiative (IMI) Enhancing TRANslational SAFEty Assessment through Integrative Knowledge Management, (eTRANSAFE) project. eTRANSAFE has received support from IMI2 Joint Undertaking under Grant Agreement No. 777365. This Joint Undertaking received support from the European Union's Horizon 2020 research and innovation program and the European Federation of Pharmaceutical Industries and Associations (EFPIA).

Kick-off September 2024

VICT3R

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Thanks!

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innovative health initiative

Co-funded by the European Union

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