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Examining the Use of Virtual Control Groups

The Intersection of Data Science and Biological Relevance

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Agenda

Reduce Control Animal Needs:

1. Data Engineering
2. Data Science
3. Biological Qualification
4. Implementation
5. Next Steps

VCG Data Engineering

- Select animal candidates from historical data

(< 3 to 5 years) to create a Virtual Control Group (VCG) based on selected study parameters, to **Replace and Reduce** some of the Concurrent Control Group (CCG) animals, when feasible

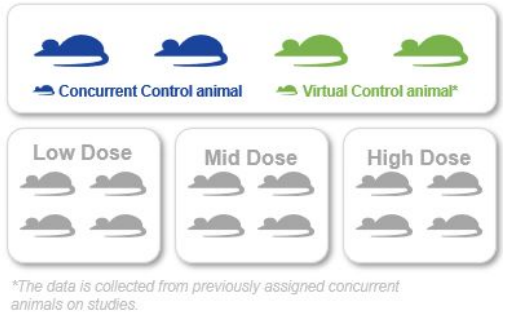
- Combine quantitative and qualitative datasets for all selected animals

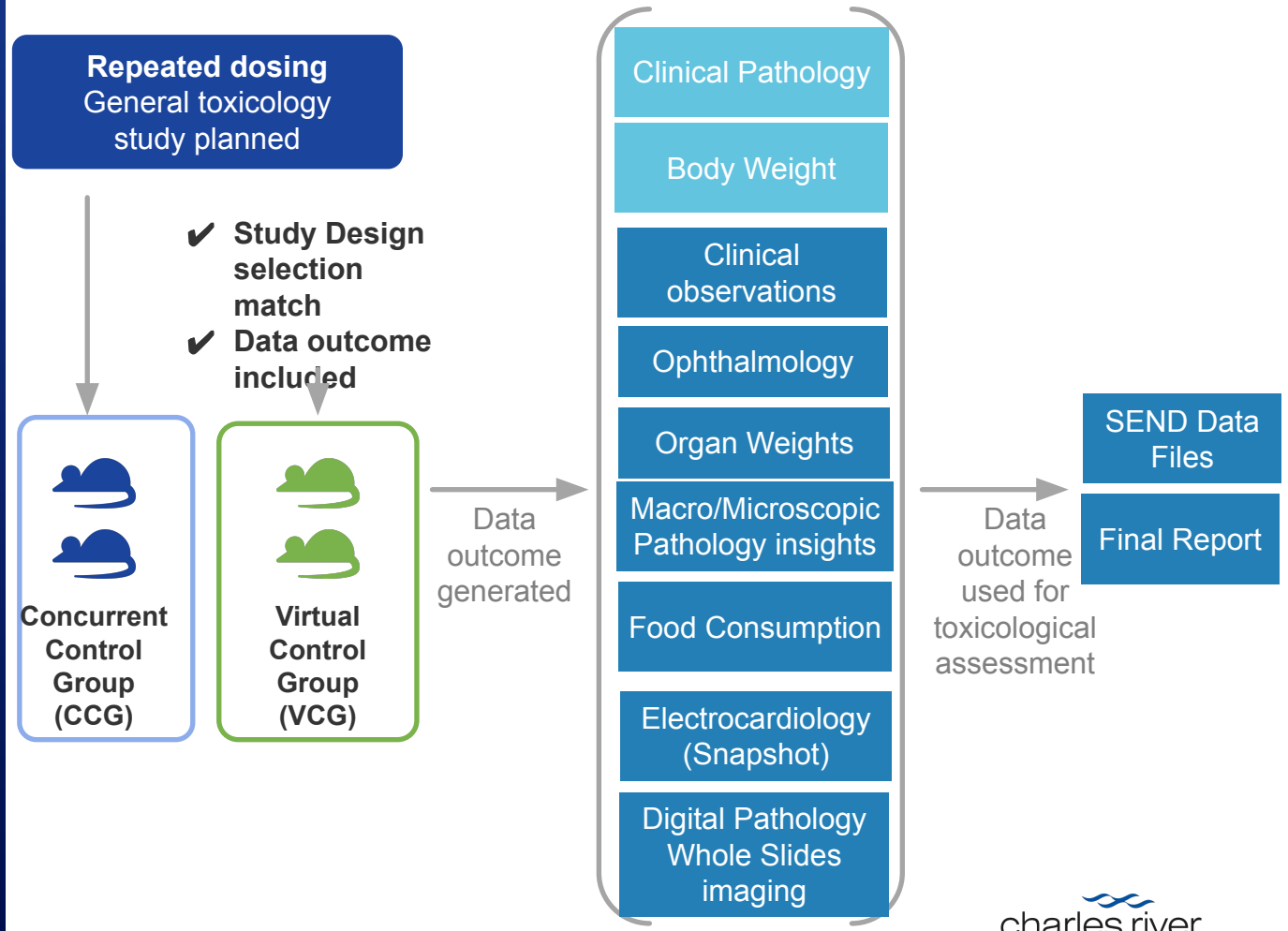
- Target study designs that include:
  - Sufficient data from historical control study animals with common general tox endpoints and study designs

- Enable a sustainable current data stream:
  - Ensure that any changes in pre-analytical parameters such as husbandry, in-life processes, and technical methods are reflected in the data.
  - Genetic changes in animals over time may change readouts so it is not feasible to fully eliminate controls.

Selection Data Parameters

Species	Route of Admin
Strain/Origin	Study Type
Sex	Study Duration
Age and weight at initiation Dosing	Dosing Frequency
Vehicle information	Site
Intervals and Parameters	Environmental condition and husbandry





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“Whole Animal” Virtual Control Groups

Leverage all relevant variables and endpoints, from clinical observations to whole slide images (WSI)

Advantages

- Metadata on each animal allows matching of protocol-driven variables (age, facility, strain/source, vehicle, admin route, etc…)
- WSI connected directly with associated individual animal data
- Ability to see a lesion in a control animal and find matching data (macroscopic findings, clin path, organ weights)

Challenges

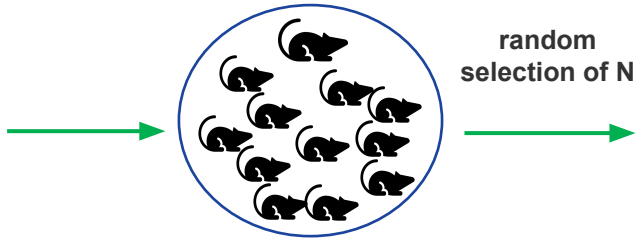
- Not always appropriate or possible
  - Uncommon vehicles, complex study design
- Gaps in some datasets may exist due to variable needs in previous studies
  - Clin path sampling intervals, recovery phase variation
- Producing WSI is time, data storage, and labour intensive
- Keeping VCG data lake up-to-date
  - Genetic drift, environmental changes
  - Match historical control regulatory expectations

VCG Data Science Methodologies and Biological Qualification

Machine Learning, Inferential Statistics, and Biological Validation

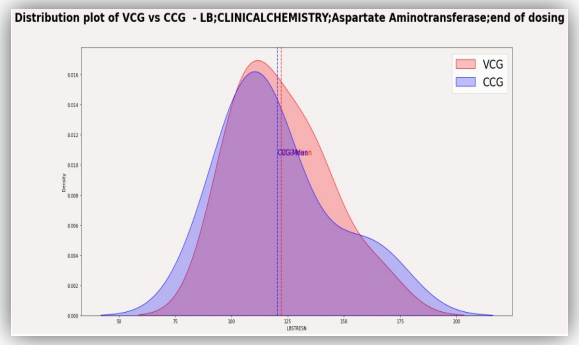
Scope Definition	Feature Selection	Cluster Optimization	ML clustering implementation	Comparison Tool	Toxicology Assessment
How do the selection criteria impact the parameters in scope?	Which parameters are related and important for modelling?	How many unique groups should the animals be placed in?	Which group does each animal fit into best?	Statistical comparison of how the VCG compare to CCG?	Will the VCG lead to the same toxicological conclusion?

Selection Criteria	
Species	Strain /Origin
Site	Route of Administration
Study Type	Dosing duration and Frequency
Sex	Age and Body Weight at intiation



Virtual Control Animal (VCG)

Concurrent Control Animal (CCG)



Treated Groups

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Inferential Analysis

Inferential Analysis with respect to selection criteria - *POC scope*

Comparison of VCG and CCG using simulation-based inference.

- Our objective is to statistically examine the randomly sampled VCG animals to CCG animals with respect to pooling criteria.

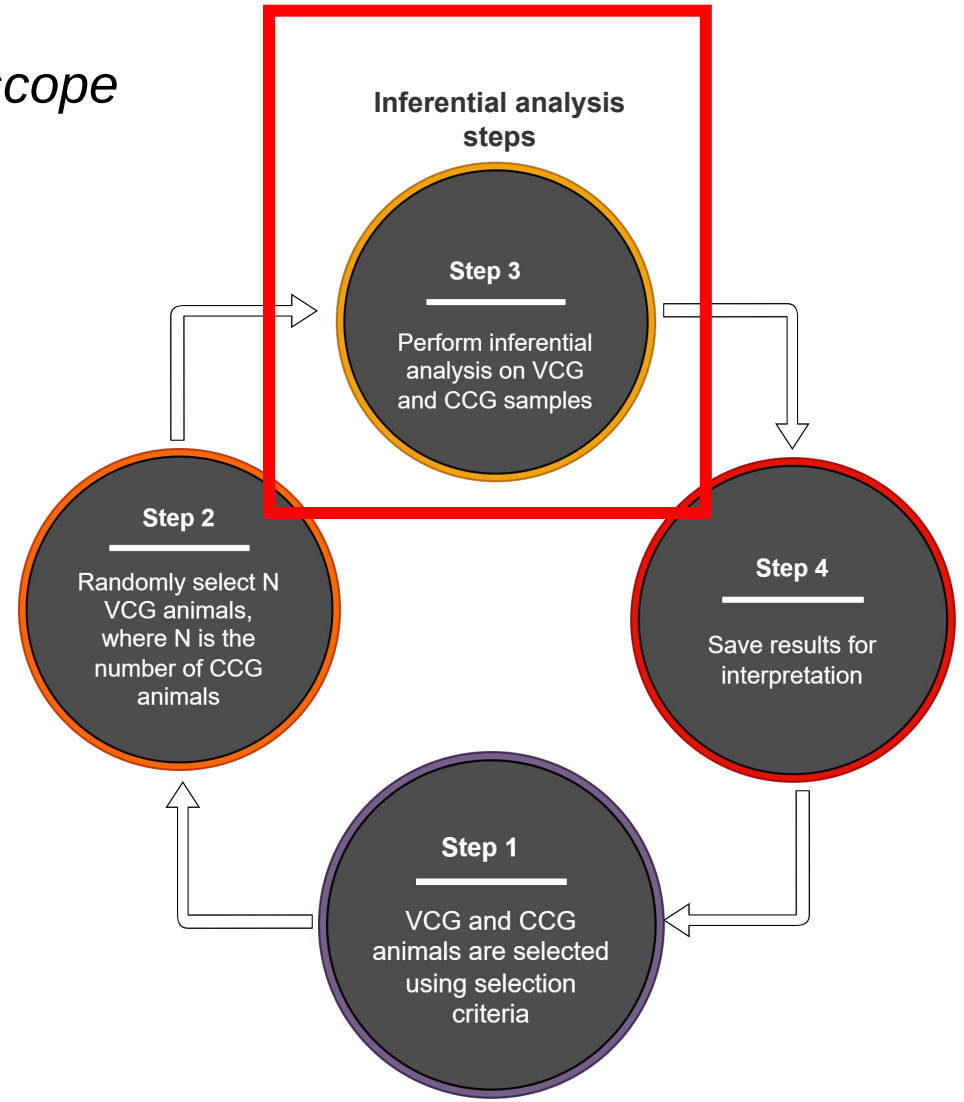
Method:

- A random sample of VCG animals is drawn from the entire VCG population. The sample size matches the number of animals (N) in the CCG.
- A random sampling method is applied to select VCG animals over multiple iterations.
- Conduct the statistical tests for mean and variance on two samples (VCG and CCG)

Comparison tool:

- Statistical testing process on VCG and CCG

The statistical methods used here are aligned with the methods used for inferential analysis conducted on study when we compare the control group data to the treated group data.



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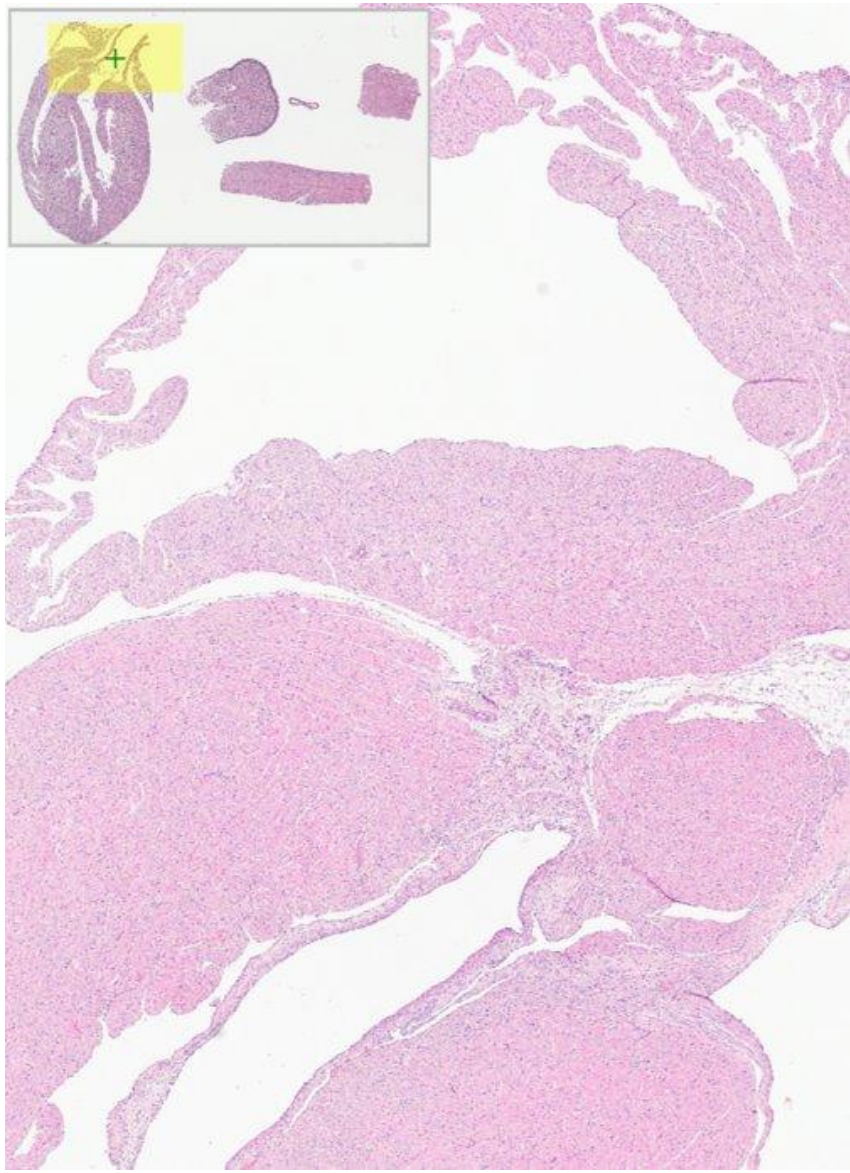
Biological Qualification Process

“Will my study outcome be different with a VCG?”

Answering this question by looking at concordance of major study outcomes:

- Clinical Pathology:
  - Hematology, Coagulation, Chemistry, Urinalysis
- Anatomic Pathology:
  - No observable effect level, target organ identification and associated lesions, organ weights
- Toxicological Assessment:
  - No observable adverse effect level, clinical tolerance and survival, clinical + anatomic pathology



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Validation of Virtual Control Data for Implementation

Overview of the Proposed Stepwise Approach

STEP 1

Database Development

Adding additional data/domains/ parameters

STEP 2

Qualification

Visualizing distribution + Time control graphs

Identification of confounders

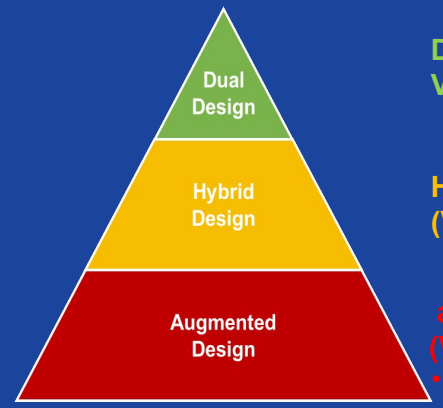
Matching criteria and guidance

Reanalysis using blinded randomly selected legacy studies with the matching criteria

STEP 3

Implementation

nonGLP DRF in rodent/nonrodent with 1 Route of Administration, site-specific approach



Dual Control Groups
VCG ; CCG (Nv=Nc=X)

Hybrid Control = (VCG+CCG) Nc=X

augmented Control = (VCG>CCG)
- **Statistically powered N**

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Implementation

“Gradually and then suddenly…?”

Dual Design

- N = Virtual Control Animals = Concurrent Control Animals
  - Assessment of VCG use
  - Confidence in methodology
  - Regulatory acceptance

Hybrid or Augmented Designs

- Virtual Control Animals > Concurrent Control Animals
  - Reduction in animal use

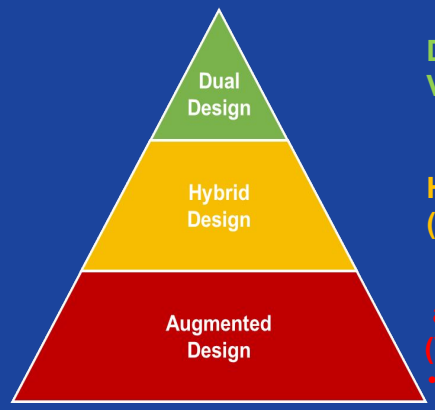
Replacement?

- Virtual Control Animals Only
  - Not suitable for all studies
  - Still need “fresh” inputs for maintenence
  - Benefit for studies that are currently run without controls

STEP 3

Implementation

nonGLP DRF in rodent/nonrodent with 1 Route of Administration, site-specific approach



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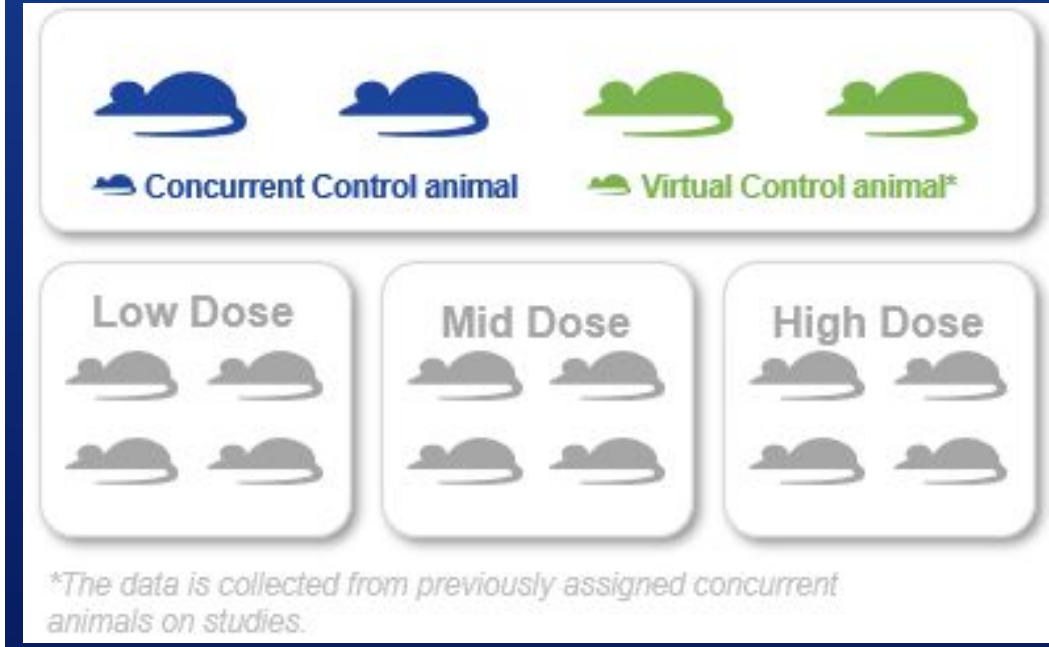
Next Steps

How does this reduce barriers to drug development?

1. Fewer animals
   i. Commitment to 3R's
   ii. Accompanying resource reduction
2. "Big Data" insights and opportunities
   i. Quantitative AI Tools for decision support?
      a) Abnormality flags -> increased efficiency for PI's
      b) Decreased reporting times
   ii. Predictive analytics/AI?
      a) Incorporation of pre-clinical data with clinical data?
      b) Incorporation of pre-clinical data with drug approval data?
      c) "Fail fast" opportunities
3. Collaboration support?
   i. Anonymization, aggregation, and user interface efforts:
      a) Facilitation of future inter-company, corporate/academic projects



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Terminology Definitions

- **Concurrent Control Groups (CCG)**: a standard control group that is assigned to a study, terminal in most study designs

- **Common Study Control Groups**: a control group that used for multiple studies for the same sponsors with timepoint collected in alignment with the study needs : no CCG on the study, could be terminal if needed, mostly used for nonterminal endpoints

- **Centralized Control Groups**: a common control for multiple sponsors with same/equivalent vehicle grouping, route of administration with timepoints collected in alignment with the study needs: no CCG on the study, only used for nonterminal endpoints (e.g. baseline values for Labsci endpoints)

- **Virtual Control Group (VCG)**: matched control animal candidate constructed through computational techniques and algorithms based on past data (<3-5 yrs) that include the endpoint and timepoints of the targeted study design

  - **Dual Control Groups design** : two control groups VCG with CCG in dual design in R&D design with an equivalent

  $N_{VCG} = N_{CCG}$

  - **Hybrid Control :** one control group with part CCG and VCG with equivalent composition

  $(N_{VCG} + N_{CCG}) = Nc$

  - **Augmented Control** : one control group with wither VCG (when no CCG); or with higher N of the VCG to statistically power the Control group (N_{VCG} when no CCG ; $N_{VCG} > N_{CCG}$, $XN_{VCG} > N_{CCG}$,)

- **Synthetic Virtual Control Group (SVCG)**: A synthetic virtual control group is an **artificially created** subset of a study population that serves as a comparison or reference point for evaluating the effects treatment. It is generated through statistical methods or modeling to mimic the characteristics of a concurrent control group.

N = number of the animals (size of the sample)
X: increased fold

Terminology Definitions

- **Hierarchical clustering** is a method to organize things, like animal data in clinical pathology, into a tree-like structure. It starts by considering each animal as a separate group and then gradually combines similar groups into larger ones. Imagine you're sorting books on a shelf. At first, each book is its own group. As you find books that are similar in topic, you create clusters of related books and keep combining until you have a tree of clusters showing how everything is connected. In the same way, hierarchical clustering helps us see which animals have similar test results and how they group together. It's like a family tree, but for data. This can help scientist understand relationships between animals and their data outcome .

- **Pearson correlation:** is like a friendly way to measure how two things, like clinical pathology parameters, move together (positive) against each other (negative). It's a number that tells us, when its positive: if when one thing goes up, the other tends to go up too, or if one goes down, the other goes down as well or ,when its negative When one thing goes up, the other goes down

- **Decision tree classifier (**using a clinical analogy): Think of the decision tree classifier as a flowchart that doctors use to diagnose a patient's health condition based on their test results. Just like a decision tree, where each branch represents a decision and each leaf represents an outcome, the doctor starts at the top of the flowchart and follows a series of questions based on the patient's symptoms and test values. For example, let's say a patient comes in with symptoms like fatigue, weight loss, and high blood sugar. The doctor starts at the top of the decision tree and asks, "Is blood sugar high?" If the answer is "No," the patient might have a different condition. But if the answer is "Yes," the doctor moves down to the next question: "Is there a family history of diabetes?" Depending on the answer, the doctor continues to follow the path that leads to the most likely diagnosis. Similarly, in clinical pathology, a decision tree classifier analyzes animal data, asking a series of questions about different test results. It then classifies the anima into a specific group or category, based on the patterns it finds in the data. The decision tree mimics how doctors make decisions by using a sequence of test values to arrive at a conclusion about a patient's health condition.

- **Principle Component Analysis (PCA) :** In essence, PCA simplifies the complexity of your data so you can focus on the most impactful aspects, making it easier to interpret and analyze. Imagine you have a complex puzzle made up of many different pieces, each representing a different aspect of clinical pathology data. PCA is like finding a way to simplify that puzzle by highlighting the most important pieces that explain **the most variation**. Think of it as if you're painting a picture. You start with a palette of many colors, but you realize that you can create a beautiful image using just a few key colors. Similarly, **PCA identifies the most significant patterns or relationships in your data and reduces it to a smaller set of "colors" (principal components**). These principal components are like the main themes or trends in your clinical pathology data. They help you see the bigger picture and understand which factors contribute the most to the overall variation in the data.

Terminology Definitions

- **Inferential analysis:** Imagine you have a small sample of puzzle pieces, but you want to make educated guesses about the entire puzzle without seeing it all. Inferential analysis is like using those few puzzle pieces to make accurate assumptions about the entire picture. Similarly, inferential analysis uses a sample of data to draw conclusions about a larger population. In clinical pathology, it's like examining a subset of animals test results to make predictions or conclusions about the outcome of a larger group. Inferential analysis helps you make informed decisions about a whole dataset based on the insights gained from a smaller portion of that data.

- **Variance test:** Levene's test is used to assess the homogeneity of group variances. Variance is a measure of the spread/variability, within a dataset.

- **Equality test**: Parametric/Non-Parametric tests (type of analysis determined by Levene's and normality test) are used to assess if the two independent groups come from a single population.

- **Elbow Analysis**: Imagine you're buying clothes, and you want to decide how many outfits to purchase. You try on different numbers of outfits and note how much your style improves with each addition. The "elbow point" is where adding more outfits doesn't significantly enhance your style anymore. Similarly, in elbow analysis for clustering, you plot how well your data is grouped for different numbers of clusters. The "elbow point" is where adding more clusters doesn't improve the grouping much, helping you decide the optimal number of clusters.

- **K-Means Analysis:** Think of sorting-colored marbles into different groups. You decide on the number of groups you want and then place each marble in the group with the nearest average color. K-Means analysis is like this, but with data points and clusters. It helps you find patterns in data by grouping similar points together and calculating the cluster center (like the average color of marbles).

- **Silhouette Analysis**: Imagine you're at a party and you want to know if you're mingling with the right group of people. Silhouette analysis is like looking at how close you are to the people in your group compared to how close you are to people in other groups. If you're well-connected within your group and far from others, your silhouette score is high, suggesting you're in the right group. In data, it's used to measure how well each point fits into its assigned cluster, indicating how good the clustering is.

- **Descriptive analysis:** is like telling a story about numbers. Imagine you're describing your pet's behavior to a friend who's never met them.You'd talk about things like how often your pet eats, sleeps, and plays. You might mention their favorite toys or quirks. That's exactly what descriptive analysis does with numbers – it summarizes and explains data in a way that anyone can understand.