Animal Rule Case Study

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CDER Inspections of Good Laboratory Practice, Animal Rule, and Bioavailability/Bioequivalence Study Sites
July 20, 2022

Disclaimer

The opinions and information in this presentation are those of the authors, and do not necessarily represent the views and/or policies of the U.S. Food and Drug Administration.

Learning Objectives

- Describe potential outcomes of an FDA CDER Inspection of studies submitted under the Animal Rule
- Describe two cases of bioanalytical observations from FDA Animal Rule study inspections

Outline

- Observation cases from a natural history study
- Observation cases from bioanalysis in Animal Rule studies
- References

Types of Animal Rule-Specific Studies

- **Natural history studies** that define the animal model in which efficacy will be tested
- Adequate and well-controlled **animal efficacy studies** intended to provide the primary evidence of effectiveness to support marketing approval
- **Pharmacokinetic (PK) and/or pharmacodynamic (PD) studies** in animals used to select a dose and regimen in humans

CDER AR Study Inspections

- Overall, 70% (51/73) of inspected AR studies were conducted in compliance with GLP
  - 100% (15/15) of PK studies were conducted in compliance with GLP
  - 76% (28/37) of efficacy studies were conducted in compliance with GLP
  - 18% (2/11) of natural history studies were conducted in compliance with GLP
  - 0% (0/4) of other studies were conducted in compliance with GLP

Case 1: Study Type Discussed Today

- Natural History Study in nonhuman primates
- Intended to support development of a post exposure prophylaxis treatment (efficacy study)
- Challenge agent - Aerosolized *V. badbug*
- Model characterization

Natural History Study

- Biocontainment Level 3
- Test system, Groups
  - Cynomolgus macaque, experimentally naïve, Vietnamese origin
  - 3 males/3 females in Sac 1, Sac 2, Sac 3 and Sac 4 groups and 6 males/6 females in the Day 28 euthanasia group
- Target presented dose – 1000 CFU *V. badbug*
- Aerosol challenge on Day 0
- Scheduled euthanasia of 3 males/3 females on Days 2 (Sac 1), 4 (Sac 2), 5 (Sac 3), and 6 (Sac 4) and 6 males/6 females in the Day 28 group

Natural History Study

Objectives

- Determine the natural course of disease in cynomolgus macaques of Vietnamese origin challenged with *V. badbug* via aerosol
- Identify a biomarker as a reliable indicator of disease
- Forms the basis for the animal model as predictive of the disease pathogenesis in humans
  - A requirement for animal models under the Animal Rule

Natural History Study

- Assessments included
  - Pathogen load (blood and tissue)
  - Body weights
  - Clinical observations
  - Telemetry monitoring of temperature, respiration rate and heart rate
  - Clinical pathology (hematology, clinical chemistry)
  - Necropsy (macroscopic findings), Histopathology (microscopic findings)

Natural History Study

- Challenge Presented Dose
  - Animals in serial euthanasia groups
    - Ranged from 183 to 6300 CFU
  - Animals in Day 28 euthanasia group
    - Ranged from 43 to 627 CFU

Natural History Study

- Clinical observations
  - Clinical observations were given scores based on severity
    - Scores began to increase on Days 2 and 3 and progressed in those animals that proceeded to MSAC
    - Scores were used to determine moribundity based on the protocol-defined euthanasia criteria

Natural History Study

Mortality for animals in Day 28 group

- Males (6 animals)
  - 2 on Day 6 (MSAC), 3 on Day 7 (MSAC) and 1 on Day 8 (MSAC)
- Females (6 animals)
  - 1 on Day 8 (FD), 4 on Day 9 (MSAC), 1 on Day 28 (TSAC)

Natural History Study

- Telemetry
  - RR increased 48 hours post exposure, dissociated from the circadian rhythm and remained elevated in those that proceeded to MSAC
  - HR increased 49 hours post exposure, dissociated from the circadian rhythm, followed by a decline in those that proceeded to MSAC at around 144 hours post exposure
  - Elevated body temperature (≥ 2°C) and disruption of diurnal body temperature pattern at 47 hours after exposure marked shift to disease BIOMARKER!

Natural History Study

- Bacterial Load
  - Bacteremic between Days 3-8; increased in proportion to disease progression
  - Bacteria recovered from all tissues; highest in lung
  - Animal that survived to Day 28 scheduled euthanasia did not have any bacteria found in tissues (received the lowest presented challenge dose)

Natural History Study

- Hematology
  - Increase in total circulating white blood cells (primarily due to increases in neutrophils and monocytes) which peaked by Day 2 post exposure
- Clinical chemistry
  - Marked increase in C-reactive protein between Days 2-3 post exposure
  - Increase in creatine kinase and lactate dehydrogenase starting on Days 4-5 post exposure

Natural History Study

Histopathology

- Lung lesions progressed in severity from Day 2 to Day 6 post exposure
- Inflammation and necrosis in the spleen, regional lymph nodes, liver appear at Day 6 and increased in severity
- Resolved pulmonary lesions (minimal) in animal that survived to Day 28
- Very similar to the autopsy findings in humans with this disease

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Pause for Discussion

Natural History Study

Conclusion

- Aerosol exposure to *V. badbug* in naive cynomolgus macaques of Vietnamese origin was successfully modeled.
- Pathogenesis of the disease in cynomolgus macaques correlated to human disease in clinical signs, hematology and clinical chemistry changes, and histopathology findings in lungs, liver, spleen, and lymph nodes.
- Fever, defined as ≥2.0°C increase in body temperature over baseline, in telemetry-implanted cynomolgus macaques may be used as a real-time trigger for intervention initiation in future efficacy studies.

CDER Inspection

- An inspection of the study was performed per CP 7348.007 (Inspection of Nonclinical Laboratories Conducting Animal Rule-Specific Studies) and reviewed:

Personnel	Test and control articles
Quality Assurance	Challenge agents
Facilities	Protocol and study conduct
Equipment	Records and reports
Operations	Archives
Animal care	

Natural History Study

During the inspection, the following data was not available for review:

- Raw data documenting the challenge exposure to animals
- Raw data documenting the enumeration of bacteria
  - In the challenge agent dose
  - In the biological samples from exposed animals
- Raw data documenting clinical observations
- Raw data confirming the identity of the challenge agent

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Pause for Discussion

Natural History Study

- Form FDA 483 was issued.
- Observations:

1. Not all raw data generated as the result of a nonclinical laboratory study were retained.
2. Testing facility management failure to assure that challenge agent had been appropriately tested for identity.

Natural History Study

As a result, data forming the basis for the study conclusions could not be verified.

Resources

- CP 7348.007 Inspection of Nonclinical Laboratories Conducting Animal Rule-Specific Studies https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/compliance-program-manual/bioresearch-monitoring-program-bimo-compliance-programs
- Product Development Under the Animal Rule, Guidance for Industry, October 2015 https://www.fda.gov/regulatory-information/search-fda-guidance-documents/product-development-under-animal-rule

Types of Animal Rule-Specific Studies

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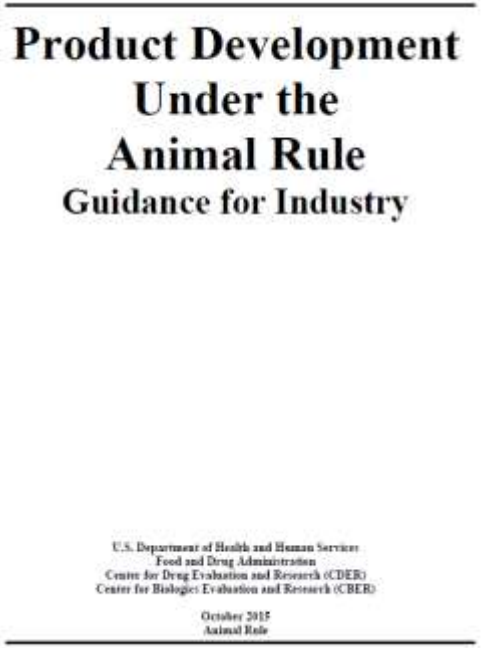
Animal Rule PK/PD Studies


Product Development
Under the
Animal Rule
Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

October 2015
Animal Rule

- *The Animal Rule requires that PK and PD data or information (or other relevant data or information) for the investigational drug be sufficient to permit the selection of a dose and regimen expected to be effective in humans.*
- *Studies should be conducted in healthy animals…… to characterize the PK profile of the drug in each following the administration of a single dose and multiple doses (if applicable).*
- *The assays used for measuring drug concentration in the appropriate body fluids should be validated.*
- *The drug exposures associated with efficacy in the adequate and well-controlled animal efficacy studies should be determined. PK information from affected animals should be compared to PK information from healthy animals to determine whether the challenge agent-induced disease or condition affects the pharmacokinetics of the investigational drug.*

Bioanalysis of Animal Rule PK/PD Studies

- *Informs the development of bioanalytical methods used for nonclinical studies that require toxicokinetic or biomarker concentration data.*
- *Applies to bioanalytical procedures such as chromatographic assays (CCs) and ligand binding assays (LBAs) that quantitatively determine the levels of drugs, their metabolites, therapeutic proteins, and biomarkers in biological matrices such as blood, serum, plasma, urine, and tissue such as skin.*

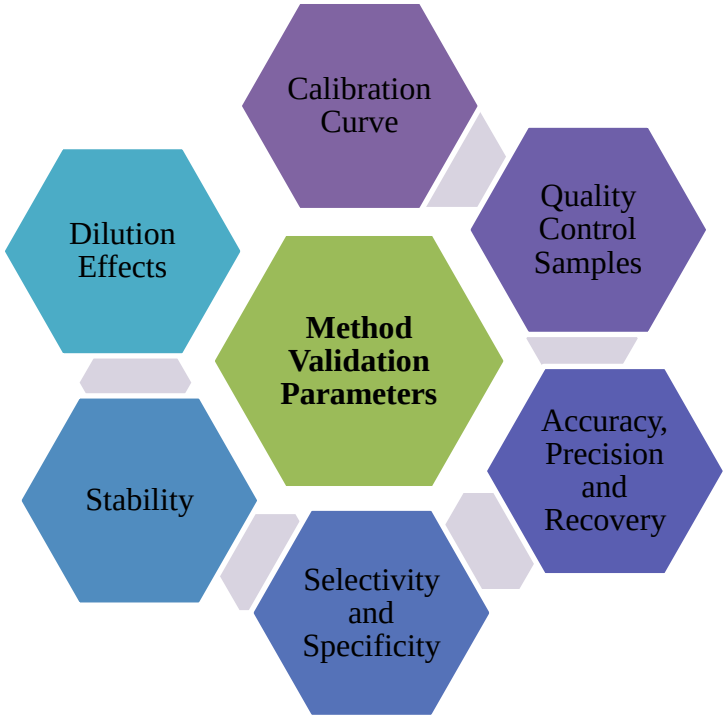

Bioanalytical Method Validation

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Veterinary Medicine (CVM)

May 2018
Biopharmaceutics

Bioanalytical Method Validation



Case Study #2

- **Study Design**: Animal Rule PK Study
- **Species**: Non-human primates
- **Challenge Agent**: Infectious bacteria
- **Therapy**: Antibiotic drug X via inhaler aerosol

- **Bioanalysis:** A high-performance liquid chromatography (HPLC) method for determination of antibiotic drug X concentration in non-human primates' serum

Case Study #2

Inspectional Findings:

Analytical run acceptance criteria of Quality Control (QC) samples

Run ID		LQC	MQC	HQC	Run Outcome
7	Rep 1	Y	Y	Y	Accepted
	Rep 2	Y	N	Y	
8	Rep 1	Y	Y	N	Accepted
	Rep 2	N	N	Y	
9	Rep 1	Y	Y	Y	Accepted
	Rep 2	Y	Y	Y	
10	Rep 1	N	Y	Y	Accepted
	Rep 2	N	Y	Y	
11	Rep 1	Y	N	Y	Accepted
	Rep 2	N	Y	Y	

Y: Within ±15% of nominal values
N: Beyond ±15% of nominal values

** Mock data for illustration purpose only.*

Case Study #2

Inspectional Findings:

Assessment of long-term matrix storage stability (LTS) @ -80℃

Calibration Standards and QC Preparation Records			
Calibration Standards Preparation Date	January 5, 2023	then stored at -80°C	
Stability QC Preparation Date	March 19, 2023	then stored at -80°C	
Run #1 QC Preparation Date (1-month LTS)	April 18, 2023	**Run #1 Date**	April 18, 2023
Run #2 QC Preparation Date (6-month LTS)	October 17, 2023	**Run #2 Date**	October 17, 2023
Run #3 QC Preparation Date (12-month LTS)	April 16, 2024	**Run #3 Date**	April 16, 2024

** Mock data for illustration purpose only.*

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Pause for Discussion

Case Study #2

Observations:

1. The SOP did not define the acceptance criteria of Quality Control (QC) samples for analytical runs. As a result, analytical runs were accepted when less than 67% of the QC samples were within the ±15% of the nominal values and/or less than 50% of QCs per level were within ±15% of the nominal values.

"The analytical run fails if the calibration and/or QC acceptance criteria are not met."

Case Study #2

Observations:

2. Calibration standards were not freshly-prepared for assessment of long-term stability of Antibiotic X in method validation.

"Long-term stability of the sample over a period of time should be equal to or exceeding the time between the date of first sample collection and the date of last sample analysis. The storage temperatures studied should be the same as those used to store study samples. Long-term stability QCs should be compared to freshly prepared calibration curves and QCs."

Case Study #3

- **Study design**: Animal Rule TK Study
- **Species**: Rabbit
- **Challenge agent**: Infectious virus
- **Therapy**: Antiviral drug X via oral administration

- **Bioanalysis**: A liquid chromatography with tandem mass spectrometry (LC-MS/MS) method for determination of antiviral drug X concentration in rabbit plasma

Case Study #3

Inspectional Findings:

Hemolyzed samples were received during sample analysis, but hemolysis effect was not evaluated during method validation.

STUDY NUMBER	SUBJECT	PERIOD NAME	TIMEPOINT	SPECIMEN TYPE	SAMPLE COLLECTION DATE/TIME	SAMPLE ID	SAMPLE RECEIPT DATE	SAMPLE RECEIPT CONDITIONS	SAMPLE STORAGE LOCATION	COMMENT
		P1								Broken tube
18-019	1003	P3	PREDOSE	Plasma	2/22/2019 6:56	650777002514	3/9/2019	Frozen and intact	BA-DEFR-12 (-80ºC)	
18-019	1003	P3	0.5HR	Plasma	2/22/2019 8:00	650777002515	3/9/2019	Frozen and intact	BA-DEFR-12 (-80ºC)	
18-019	1003	P3	2.5HR	Plasma	2/22/2019 10:00	650777002520	3/9/2019	Frozen and intact	BA-DEFR-12 (-80ºC)	
18-019	1003	P3	3HR	Plasma	2/22/2019 10:30	650777002521	3/9/2019	Frozen and intact	BA-DEFR-12 (-80ºC)	Sample hemolyzed
18-019	1003	P3	3.25HR	Plasma	2/22/2019 10:45	650777002522	3/9/2019	Frozen and intact	BA-DEFR-12 (-80ºC)	
18-019	1003	P3	5HR	Plasma	2/22/2019 12:30	650777002528	3/9/2019	Frozen and intact	BA-DEFR-12 (-80ºC)	
18-019	1003	P3	6HR	Plasma	2/22/2019 13:30	650777002529	3/9/2019	Frozen and intact	BA-DEFR-12 (-80ºC)	Sample hemolyzed
18-019	1003	P3	24HR	Plasma	2/23/2019 7:30	650777002534	3/9/2019	Frozen and intact	BA-DEFR-12 (-80ºC)	
18-019	1004	P2	PREDOSE	Plasma	2/15/2019 9:26	650697713814	3/9/2019	Frozen and intact	BA-DEFR-12 (-80ºC)	
18-019	1004	P2	0.5HR	Plasma	2/15/2019 10:00	650697713815	3/9/2019	Frozen and intact	BA-DEFR-12 (-80ºC)	Sample hemolyzed
18-019	1004	P2	1HR	Plasma	2/15/2019 10:30	650697713816	3/9/2019	Frozen and intact	BA-DEFR-12 (-80ºC)	
18-019	1004	P2	3.75HR	Plasma	2/15/2019 13:15	650697713824	3/9/2019	Frozen and intact	BA-DEFR-12 (-80ºC)	Sample hemolyzed
18-019	1004	P2	4HR	Plasma	2/15/2019 13:30	650697713825	3/9/2019	Frozen and intact	BA-DEFR-12 (-80ºC)	

** Mock data for illustration purpose only.*

Case Study #3

Inspectional Findings:

Freeze-thaw stability was established for 3 cycles in method validation. However, a few samples were stored and retrieved more than 3 times.

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Pause for Discussion

Case Study #3

Observations:

1. Hemolysis effect was not evaluated during method validation while several subject samples were hemolyzed.

"Selectivity of the method is routinely demonstrated by analyzing blank samples of the appropriate biological matrix (e.g., plasma) from multiple sources. Depending on the intended use of the assay, the impact of hemolyzed samples, lipemic samples, or samples from special populations can be included in the selectivity assessment."

Case Study #3

Observations:

2. Cycles of sample storage and retrieval exceeded the freeze-thaw stability established in method validation.

"Assays of all study samples of an analyte in a biological matrix should be completed within the time period for which stability data are available. If sample handling conditions are changed or exceed validated stability data, then the stability of the sample should be established at the new conditions."

References

- **21 CFR 314.600 through 314.650 (drugs)**: Subpart I. Approval of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible (www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=314&showFR=1&subpartNode=21:5.0.1.1.4.9)
- **21 CFR 601.90 through 601.95 (biological products)**: Subpart H. Approval of Biological Products When Human Efficacy Studies Are Not Ethical or Feasible (www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=601&showFR=1&subpartNode=21:7.0.1.1.2.8)

- **FDA BIMO program Compliance Program 7348.007**: Inspection of Nonclinical Laboratories Conducting Animal Rule-Specific Studies (www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/compliance-program-manual/bioresearch-monitoring-program-bimo-compliance-programs)

- **Guidance for Industry**
  - Product Development Under the Animal Rule, 2015 (www.fda.gov/regulatory-information/search-fda-guidance-documents/product-development-under-animal-rule)
  - Bioanalytical Method Validation, 2018 (www.fda.gov/regulatory-information/search-fda-guidance-documents/bioanalytical-method-validation-guidance-industry)

Questions?

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