Introduction to Toxicologic Clinical Pathology

Contents

- What is ASVCP-RAC & Toxicologic Clinical Pathology?
- Pharmaceutical Nonclinical Safety Assessment
- Conducting Nonclinical Safety Studies
- Clinical Pathology Interpretation in Nonclinical Studies

What is the ASVCP-RAC?

The Regulatory Affairs Committee (RAC) of the ASVCP is comprised primarily of veterinary clinical pathologists in the biopharmaceutical industry and academia

The ASVCP-RAC strives to improve the quality and reproducibility of nonclinical animal toxicology studies through the following:

- Provides guidance and best-practice policy documents
- Supports training and development of veterinary toxicologic clinical pathologists through funded externships
- Sponsors continuing education
- Advocates for toxicologic clinical pathology through numerous fora, workshops, and publications

What is Toxicologic Clinical Pathology?

- Toxicologic clinical pathology is a scientific/medical discipline that applies the professional practice of clinical pathology—the study of diseases using body fluids—to toxicology—the study of the effects of chemicals and other agents on humans, animals, and the environment.
- Toxicologic clinical pathologists mostly work in the pharmaceutical and chemical industries, contract research organizations, government, or as consultants, and utilize traditional clinical pathology endpoints, as well as contemporary advances in molecular and cellular biology.
- They are dedicated to the integration of toxicologic pathology into hazard identification, risk assessment, and risk communication regarding human, animal, and environmental exposure to potentially toxic substances.

Where is toxicologic clinical pathology used?

- Human and/or veterinary pharmaceutical development
  - Corporations (from to start-ups to “big pharma”)
  - Universities, research institutes
- Medical device, veterinary, food companies
- Regulatory agencies
- Government research

Toxicologic Clinical Pathologists

Training

- DVM or equivalent usually required
- Board certification (ACVP or ECVCP) usually required; Am Board Toxicology (DABT) certification may be helpful
- MS, PhD variably required (research experience is very valuable)
- On-the-job training (incl. GLP training usually)

Professional associations

- ACVP/ASVCP
- Society of Toxicologic Pathology
- Society of Toxicology

Multifaceted roles of toxicologic clinical pathologists in the biopharmaceutical industry

Involved throughout drug development - from drug discovery to market/post-market launch.

LO=lead optimization

CE=candidate evaluation

Veterinary Clinical Pathology
Volume 37, Issue 2, pages 146-158, 2 JUN 2008 DOI: 10.1111/j.1939-165X.2008.00041.x
http://onlinelibrary.wiley.com/doi/10.1111/j.1939-165X.2008.00041.x/full#f3

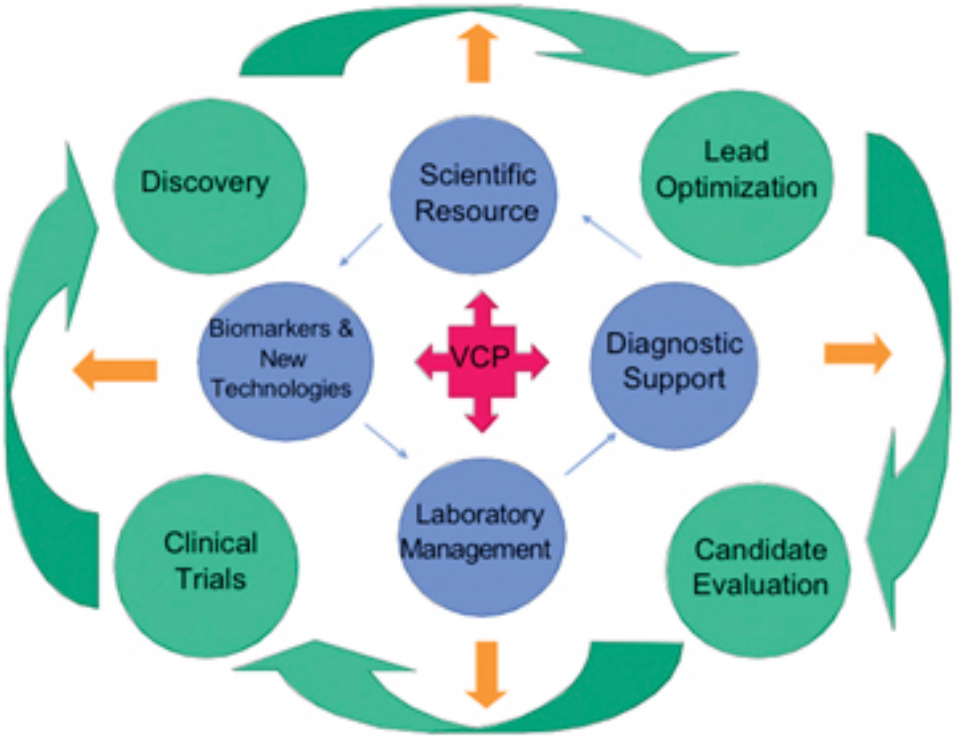
Multifaceted roles of the toxicologic clinical pathologists (VCP) in the biopharmaceutical industry

The evaluation process for safety of new drugs utilizes many skills of VCPs.

Our knowledge in core subject areas such as hematology, hemostasis, clinical biochemistry, urinalysis, cytology, histology, and correlative internal medicine is highly utilized, along with assay development and validation.

VCPs contribute to drug development teams by providing valuable insights into basic pathophysiology, elucidation of the mechanism(s) of drug action, determination of biomarker strategies, and excellent written and oral communication skills.

By our unique training and knowledge base, veterinary clinical pathologists are key players in translational medicine.



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Some Terminology in Drug Development

- Nonclinical => in vitro, in silico, and animal studies (not human)
- Clinical => human studies
- Terms for a new drug: therapeutic candidate, compound, test article, new chemical entity, new molecular entity ("small molecule"), biopharmaceutical ("large molecule", ex. antibodies, fusion proteins, RNA-based products, etc.)
- API = active pharmaceutical ingredient
- SA = safety assessment – usually encompasses toxicology and pathology
- FTIH = first time in human; the first human study with a new therapeutic
- GLP = good laboratory practice; a set of rules to ensure stringency and accountability in experimentation, documentation that meet regulatory requirements

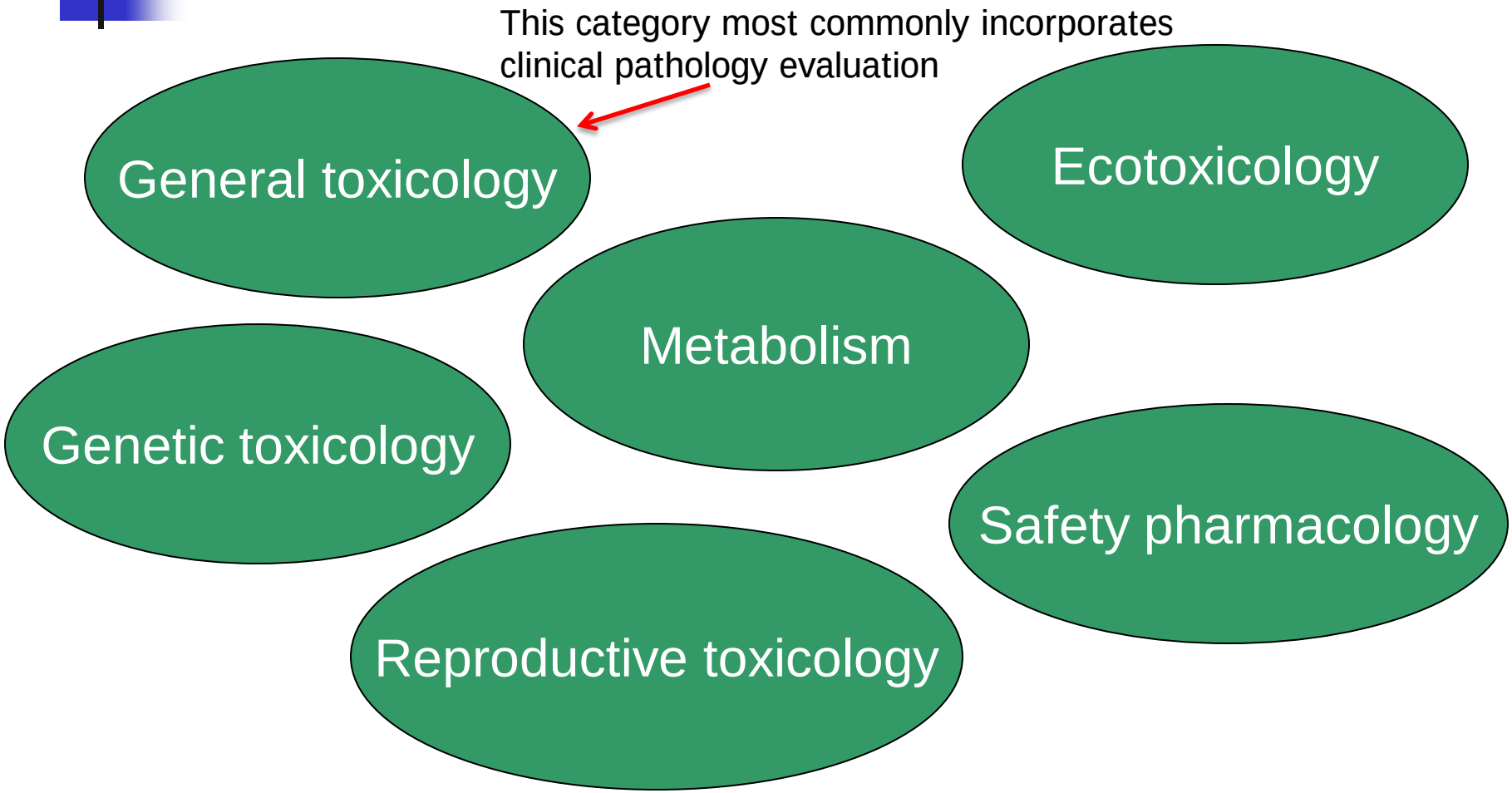
What is Nonclinical Safety Assessment in Drug Development?

- Assessing the toxic effect of a new molecular entity in animals (rodents and non-rodents)
- Assisting in the selection of compounds
- Interpreting data from in vitro & animal studies
- Assessing risk before/during human administration of the therapeutic
- Supporting existing marketed products

What do we evaluate in nonclinical studies?

- What did the test article damage?
- At what doses/exposures?
- What expected pharmacology was seen? (pharmacodynamics)
- What happened to the test article? (pharmacokinetics)

Types of Nonclinical Studies



“General" Toxicology Studies

- One goal of general tox studies is to define a “no adverse effect level” (NOAEL), “NOEL” (no effect level) in terms of the test article’s overall toxicity in animals
- Regulatory agencies usually require evaluating test article toxicity in at least 2 animal species – 1 rodent and 1 non-rodent species (“large animal” = dogs, monkeys, pigs)
- Includes single- and repeat-dose studies
- Studies of carcinogenicity potential (6m to 2y studies)
- Type/design of studies depends on the planned marketed application (ex. oral, IV, juvenile, males only, etc.)

General Toxicology Studies

May include many components in order to evaluate a wide range of potential pathophysiologic effects in relation to the test article concentration:

- Clinical observations of the animals, body weight, food consumption
- Clinical pathology
- Anatomic pathology (necropsy, histopathology, organ weights)
- Ophthalmoscopic examination
- Electrocardiogram
- Toxicokinetics of the test article
- Homogeneity and stability of the drug formulation

Designing Studies - Route?

- Should reflect what is planned for final marketed drug in humans and/or animals
- Most common routes = oral, intravenous, subcutaneous
- Other routes for drug delivery - inhalation, ocular, dermal, intrathecal, diet, intravitreal, etc.
- Frequency – once, daily, weekly, intermittent, many variations depending on therapeutic strategy

Designing Studies - Dose?

Use experimental modeling data - in vitro models, in silico, and animal models - to select a range of potential doses for animal studies that will ultimately define an acceptable range for the administration to the human (or animal) patient.

By convention, most studies have:

- Controls – vehicle only
- Low dose - no toxic effect expected
- Mid dose - some toxicity expected
- High dose - toxicity expected

Designing Studies - Duration?

Depends upon ultimate clinical use

Duration planned for the human/animal patient	Minimum duration in animal testing studies
1 day	14 day
7-14 days	12-28 days
1 month	1-6 months
1 year	6-12 mo, plus carcinogenicity studies

Terminology:

- Acute Toxicity Studies: Single dose or multiple dose within 24 hrs
- Sub-Acute Toxicity Studies: (14-28 days)
- Sub-Chronic Toxicity Studies: (up to 90 days)
- Chronic Toxicity Studies: 6 months (rodents), 9 months (non-rodents)

Clinical Pathology Endpoints

Routine panel:

- Hematology
  - Complete blood cell count ± blood smear evaluation ± bone marrow evaluation
- Coagulation
  - APTT, PT, ± Fibrinogen

Clinical Pathology Endpoints

- Clinical Chemistry Panel
  - Ex. Glucose, UN, Cr, T.protein, Albumin, Globulin, ALT, AST, T. bili, ALP, Cholesterol, Triglycerides, iP, Ca, Na, K, Cl
- Urinalysis
  - Volume, sp. gravity, pH, reagent strip, ± sediment exam, ± chemistries

Clin Path Collection Timings

- Small animal species (rats, mice, hamsters, guinea pigs, etc.)
  - End of study ± interim sampling depending on study duration
- Large animal species (dogs, monkeys, pigs, etc.)
  - Predose (baseline), end of study ± interim sampling depending on study duration

Regulations: What is “GLP”?

Good laboratory practice or GLP = set of principles intended to assure quality & integrity of nonclinical studies that are intended to support research or marketing permits for products regulated by government agencies.

Most commonly associated with the pharmaceutical industry and the non-clinical animal testing performed prior to approval of new drugs. Also applies to many other non-pharmaceutical agents such as color and food additives, food contamination limits, food packaging, and medical devices.

Governments provide specific regulations which companies are obligated to fulfill.

Quality Assurance

GLP compliance requires an independent quality assurance (QA) staff to review the quality, validity, and accuracy of experimental procedures, data acquisition, result tabulation, and reports

QA also reviews deviations, amendments from the protocol or operating procedures

Ensures that a study can be adequately “recreated” from all the documents collected by people that are unfamiliar with the study

Examples of US Regulations

For each nonclinical laboratory study, a scientist or other professional of appropriate education, training, and experience, or combination thereof, shall be identified as the study director (usu. a toxicologist).

The study director has overall responsibility for the technical conduct of the study, as well as for the interpretation, analysis, documentation and reporting of results, and represents the **single point of study control.**

Nonclinical Study Process

- Protocol development
- Study conduct
- Report
- Archiving

Study Scheduling – Many Parts to Juggle

- Animal availability / ordering
- Animal housing
- Trained staff
- Analytical chemistry
- Clinical pathology sample collection, prep, analysis
- Necropsy, tissue collection, prep
- Reports
- Quality Assurance

Test Article

- When is it available?
- Calculations – How much will you need? Agree to doses
- Analytical Confirmation
  - Certificate of Authenticity, MSDS – purity, stability
- Storage/handling conditions

Animal Welfare

- Animal Welfare Act
  - Enforced by the USDA
  - Includes warm-blooded animals (not rats and mice)
  - Sets minimum standards for animal housing, care, treatment, exercise, enrichment, recordkeeping, reporting and transportation.
  - Requires oversight by an Institutional Animal Care & Use Committee
- AAALAC
  - Provides objective, peer review of animal care program
  - Uses *The Guide for the Care and Use of Laboratory Animals* as a basis for the assessment
  - Requires triennial site visits and annual reports

Institutional Animal Care and Use Committee (IACUC)

Appointed by chief executive officer of the company

Must have at least five members

- *chairperson*
- *veterinarian*
- *an individual not otherwise affiliated with the institution*
- *a practicing scientist experienced in animal research*
- *a nonscientist*

Reviews and approves activities/protocols in which animals are used

The 3 R's Should Always Be Considered

- **Replacement**
  - Substitute non-animal systems or lower species when possible.
- **Reduction**
  - Reduce number of animals used to achieve scientific goals.
- **Refinement**
  - Improve methods and procedures used in animal experimentation.

Example Animal numbers – Rat Studies

Study duration	**No. males and females in each group**
CS/DRF – 7 day	4 plus 3 TK satellites
1 month	10 plus 3 TK satellites (if recovery group; n=6 in the control and high dose only)
3 or 6 month	12 plus 3 TK satellites (if recovery group; n=6 in the control and high dose only)
24 month	60 plus 3 TK satellites

CS=candidate selection
DRF=dose range finding study
TK= toxicokinetic “satellites” – dedicated animals for toxicokinetic sampling

Ex. Animal numbers - Mice

Study duration	**No. males and females in each group**
CS/DRF – 7 day	6 plus 3 TK satellites/timepoint
1 month	10 plus 3 TK satellites/timepoint
3 or 6 month	12 plus 3 TK satellites/timepoint
24 Month	60 plus 3 TK satellites/timepoint

Ex. Animal numbers – Monkey/Dog Studies

Study duration	**No. males and females in each group**
MTD	1
7 day	1 or 2 depending on design
1 month	3 (if recovery group; n=2 in control & high dose only)
6 month	4 (if recovery group; n=2 in the control and high dose only)
12 month	4 (if recovery group; n=2 in the control and high dose only)

MTD=maximum tolerated dose

Animal Welfare in protocols

Protocol should contain justification for –

- Performing study
  - assurance that study does not duplicate previous experiments
- Route, duration, frequency
- Test animal selection
- Number of animals
- Doses
- Housing (group or individual)

Oversight of Study Conduct

- Study director observe animals and procedures
- Reviews data
- Communicates with technical staff
- Quality Assurance (QA) audits - internal and external, dedicated personnel not directly involved with the study conduct; their role is to review and report on study procedures, documents, changes,
- Interact with contributors / principle investigators
  - Submission of samples
  - Receipt / review of reports
- Study director must respond to/document unexpected events

End of the Study

- Terminal/recovery necropsy
- Terminal status report
- Materials, data, reports are securely archived for future retrieval if needed

GLP – Reports

For each study, a final report will be prepared & includes:

- Testing facility and study personnel
- Objectives and procedures/methods
- Test article and test system information
- Description of circumstances that may have affected the quality or integrity of the data
- Data, analysis of the data, conclusions
- Signed/dated sub-reports from contributing scientists (i.e., clinical and anatomic pathology data interpretations, TK analysis, etc.)
- Storage location for specimens, raw data, and final report
- Quality Assurance statement

Final report must be signed and dated by the study director

Reporting Process

Draft Report

Study Director drafts report with contributing authors' or scientist's input

Initial Review

Peer Reviewer, Project Representative, Study Pathologist, Clinical Pathologist

Management Review

Quality Assurance Review

Editing/Publishing

Signatures and Finalize

Archive

Interpretation of Clinical Pathology Data in Animal Safety Studies

First, review:

- ✓ Study protocol (objective?), amendments, deviations
- ✓ Background info on the test article when available
- ✓ In-life findings – clinical observations, weight, food consumption
- ✓ Anatomic path findings (if available)

Second, review the clinical pathology data and make notes:

- ✓ Individual results
- ✓ Summary table (group means, stats)
- ✓ Unscheduled sacrifices, “health check” data

Is a Change in a Clinical Pathology Endpoint Important?

Group Id	Sample Id	GLU mg/dl	bHBA mg/dl	ALT U/L	CRE mg/dl	TCO2 mEq/L	BUN mg/dl	LACT mg/dl	Na mEq/L	K mEq/L	Cl mEq/L	NEFA mmol/L	TRIG mg/dl	AST U/L	TBIL mg/dl	CHOL mg/dl
1 Lean	1	190	0.22	39	0.1	21	22	65.77	148	4.7	110	0.96	100	122	0.16	107
1 Lean	2	242	0.42	39	0.1	22	23	51.62	145	4.4	107	1.00	97	133	0.24	**QNS**
1 Lean	3	203	0.24	44	0.1	23	24	57.12	149	4.0	111	0.72	73	45	0.15	98
1 Lean	4	226	0.40	31	0.1	21	18	59.19	148	4.2	111	0.74	74	50	0.13	93
1 Lean	5	274	0.28	36	0.2	22	23	55.95	147	4.7	109	0.68	74	46	0.16	101
1 Lean	6	196	0.27	35	0.1	25	20	43.15	149	4.1	110	0.81	95	51	0.15	89
1 Lean	7	195	0.29	35	0.1	22	21	59.91	151	4.0	113	0.86	85	46	0.14	116
1 Lean	8	186	0.33	28	0.1	23	19	31.98	148	4.5	112	0.94	74	45	0.13	94
	MEAN	214	0.31	36	0.1	22	21	53.09	148	4.3	110	0.84	84	67	0.16	100
	STDEV	31	0.07	5	0.0	1	2	10.79	2	0.3	2	0.12	12	37	0.03	9
	SEM	12	0.03	2	0.0	0	1	4.08	1	0.1	1	0.05	4	14	0.01	4
2 Veh	9	552	0.26	194	0.2	28	25	48.83	150	4.0	104	1.42	269	315	0.20	205
2 Veh	10	654	0.32	155	0.2	25	23	51.62	148	3.7	104	1.29	277	113	0.16	230
2 Veh	11	548	0.26	218	0.2	32	21	39.46	149	3.3	103	1.26	275	135	0.16	256
2 Veh	12	548	0.20	214	0.2	29	25	43.87	148	3.7	103	1.20	332	126	0.17	203
2 Veh	13	498	0.21	178	0.2	37	23	53.51	151	3.1	104	1.00	235	148	0.16	190
2 Veh	14	512	0.23	129	0.2	30	22	64.95	151	3.5	108	1.06	223	158	0.17	189
2 Veh	15	643	0.39	221	0.3	26	27	77.39	149	4.2	107	1.20	213	340	**QNS**	**QNS**
2 Veh	16	421	0.23	184	0.2	36	22	57.03	151	3.3	103	0.94	143	155	0.16	203
	MEAN	547	0.26	187	0.2	30	23	54.58	150	3.6	105	1.17	246	186	0.17	211
	STDEV	76	0.06	32	0.0	4	2	12.07	1	0.4	2	0.16	56	89	0.02	24
	SEM	29	0.02	12	0.0	2	1	4.56	0	0.1	1	0.06	21	34	0.01	10

Is it real? (i.e., related to the test article)

Is it bad? (i.e., toxicologically important)

Is a Change Important?
It Depends….

Understand the assay performance & analytical variation (precision, bias, error)

Compare data with study controls and across individuals: What is the ***variability*** in the study?

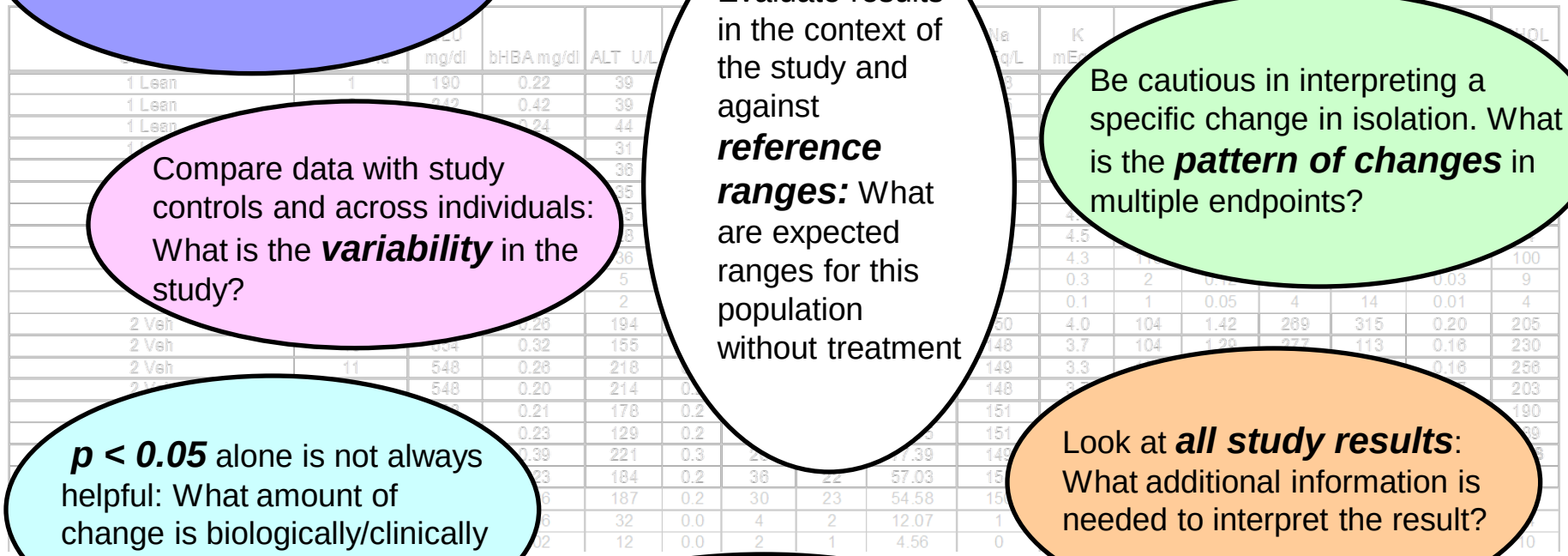
Evaluate results in the context of the study and against ***reference ranges:*** What are expected ranges for this population without treatment

Be cautious in interpreting a specific change in isolation. What is the ***pattern of changes*** in multiple endpoints?

p < 0.05 alone is not always helpful: What amount of change is biologically/clinically meaningful?

Look at ***all study results***: What additional information is needed to interpret the result?

Investigate ***unusual trends or outliers***: Are there non-treatment related factors influencing results?



Is a Difference Between Treated and Control Animals a Real Effect?

Factors to Consider

How many animals were tested?

How much inter- and intra-animal variability is expected?

- ► Species, age
- ► Unique study design conditions
  - staggered start
  - fasted vs nonfasted
  - route of administration
    - Ex. SQ or IV administration of some therapeutics may cause inflammation
  - IM ketamine administration
  - excessive blood collections
  - unusual site for blood collection

How much analytical variability is expected?

Is It Real?

- How large is the difference?
- Is the difference dose-dependent?
- Consistent over time? Between sexes?
- Statistically significant? Present before treatment?
- When did the difference occur with respect to dosing?
- Correlative findings? In-life, clinical or anatomic path?
- What is known about the test article?
- What is known about the vehicle?
  - Ex. Polyethylene glycol vehicles may cause increased urine output due to osmotic diuresis

Is It Bad? Factors to Consider

- Is affected analyte critical to health or a marker for a process?
  - Ex. Platelet count (critical) v. ALT (a marker)
- Is the direction of change clinically relevant?
  - Ex. Decrease of ALT is not usually worrisome, but an increase often is
- Are there correlative findings?
- Survival
- Histopathology
- Clinical signs, general health of effected animals
- Is it reversible?
- What is the mechanism?
- What is the pharmacologic activity of the test article?
  - Ex. Many therapeutics have a mechanism of action that alters at least some clinical pathology endpoints (ex. glucose, blood cell counts, globulin, etc.)

Is It Bad? Factors to Consider

- How large is the effect?
  - Severity alone may not be relevant to characterizing the adversity of an effect, i.e., markedly decreased ALP is likely not harmful v. markedly decreased glucose which would be considered harmful
- How much inter- and intra-animal variability is expected?
- Species, age
- Unique study design conditions
  - Staggered start
  - Fasted vs nonfasted
  - Route of administration
  - Time bias not eliminated
  - IM ketamine administration
  - Excessive blood collections
  - Unusual site for blood collection

Reference Intervals

Valuable for:

- One sick animal
- Studies with too few animals or no control group
- Lead optimization studies (usually small, short duration)
- Nonspecific measure of quality control
- Nonspecific measure of analyte variability
- Support for “Is It Bad?” interpretation

Remember though, that ~ 1 of every 20 of test results is outside its reference interval

Develop reference ranges that reflect your population, your facility, your lab, your procedures

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INVITED REVIEW

Reference values: a review

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Biological Variation: From Principles to Practice. Fraser CG. 2001. American Assoc. for Clinical Chemistry Press. ISBN 1-890883-49-2

Is It Real?

2-week monkey study; daily dose

cholesterol (89-197 mg/dL)

males; five/group

Dose (mg/kg)	Predose 8	Day 3	Day 14
0		131	143
9.6		142	137
16.8		97*	97*

Is It Real?

2-week monkey study; daily dose

cholesterol (89-197 mg/dL)

males; five/group

Dose (mg/kg)	Predose 8	Day 3	Day 14
0	135	131	143
9.6	134	142	137
16.8	95*	97*	97*

The therapeutic is not effecting cholesterol.

Is It Bad?

Hematocrit (%)

reference interval = 40-50%

Group	Study A	Study B	Study X
Control	48	42	45
High-dose	42*	37*	25*

Minimal, Mild, Moderate, Marked…..

Use of severity grades to characterize test article-related effects may be helpful/required in reports. No hard-and-fast rules:

- Minimal - smallest detectable difference
- Mild - small difference
- Moderate - larger than mild, smaller than marked (Bob Hall's Unabridged Dictionary)
- Marked - large difference

Some sponsors prefer a quantitative value for the magnitude of change, i.e., fold difference (3X) or percent difference (+323%)

Which Finding is Most Important?

Hemoglobin	10% lower
Neutrophil count	10% higher
Creatinine	10% higher
Sodium	10% lower
ALT	10% higher
Urine pH	10% lower

Bottom Line for Data Interpretation

Know the study

Cannot just interpret the numbers or statistics

It’s usually not black and white – get used to it

Clinical Pathology Report Outline - Example

Cover page, signatory page

Summary (like an abstract)

Introduction: Study objective

Methods: Study design, samples collected, timings

Results

- Textual description of test article-related effects (usu. divided into sections for hematology, coagulation, etc.)

Discussion

- Integration of findings, mechanisms where applicable, discussion of test article-related effects, ± adversity if appropriate

References, if applicable

Data Tables

- Individual animal data
- Summary data (means, SDs or SEs) ± statistics

References, resources

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