



# Bioanalytical Issues when Dealing with Phase II/III Studies

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# Agenda

- Different phases of clinical trials
  - Phase I to IV
- Challenges with Phase II/III studies
- Method validation guidelines
  - Additional parameters to be validated
- Reconciliation of samples



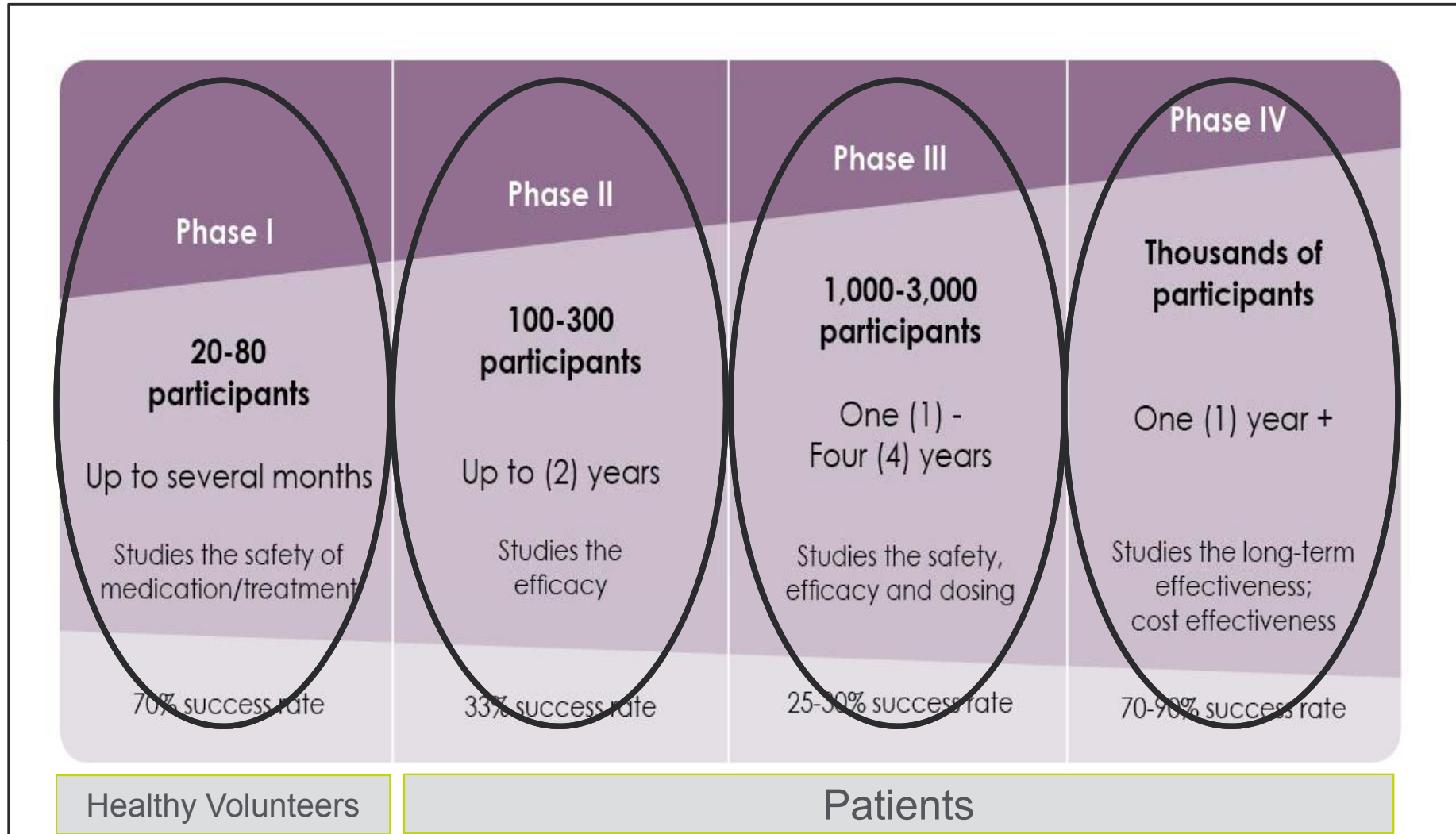
# Different Phases of Clinical Trials

Each phase is designed to answer a separate research question.

- **Phase I:** Evaluate safety, dosage and identify side effects.
- **Phase II:** Efficacy and safety.
- **Phase III:** Efficacy, monitor side effects, and collect information on safety.
- **Phase IV:** Studies done after drug marketed to gather information on the drug's effect in various populations and any side effects associated with long-term use.



# Different Phases of Clinical Trials





# Bioanalytical Challenges with Phase II/III

- High number of patients
- Length of the studies
- Multicentre



## High Number of Patients

- Various diseases
  - Test selectivity or matrix effect with specific population?
- Use of co-medications
  - Selectivity assessed with co-med during method validation?
  - Effect of co-medications on stability of analyte?
  - Need to have listing of all co-medications



# High Number of Patients

- Study may take place all over the world
  - Samples from different origins / different diets
  - Selectivity assessed during method validation?
  - Shipment of study samples:
    - Regulation / certificate / laws may differ in certain countries
    - Need on-line tracking of study samples
    - Chose carrier which will replenish shipment with dry ice if necessary
    - Custom broker



# High Number of Patients

- Documentation
  - Adequate / clear / precise / readable
  - Sample identification
  - If not, may lead to samples being unacceptable for analysis





## Length of Studies

- Studies may last several years
  - Long-term stability to cover study samples
    - Samples not always sent right after collection
    - Stability needed to cover from collection to analysis
    - May need to cover very long periods
  - Validated methods used for bioanalysis
    - Changes in regulation / new guidance implemented
    - When projects submitted bioanalytical methods may not be updated as per most recent regulations.



# Multicentre Studies

- Trained personnel
  - Importance of proper documentation
- Material to collect samples
  - Collect according to validated method
  - Use of stabilization solution
  - Unstable / light sensitive analyte
- Equipment needed:
  - Centrifuge
  - Freezer



# Validation of Bioanalytical Method at inVentiv Health Clinique

- Located in Quebec City / Canada
- Most of our Sponsors are from:
  - US
  - EU
  - Asia
- BE and Phase I studies
- Clinical projects mostly submitted to:
  - FDA
  - EMA
  - Health Canada
  - ANVISA



# Validation of Bioanalytical Method at inVentiv Health Clinique

- Bioanalytical method validation guidance:
  - FDA
  - EMA
  - Health Canada (follows EMA)
  - ANVISA
  - Deficiency letters, Clarifax



# Validation of Bioanalytical Method at inVentiv Health Clinique

- Parameters evaluated during validation:
  - Between/within-run accuracy and precision
  - Run size evaluation
  - Recovery of analyte(s) and internal standard(s)
  - Selectivity:
    - Matrix selectivity
    - Matrix effect
    - Potentially interfering and commonly used drugs
    - Potentially interfering co-medications
    - Potential conversion of metabolites



# Validation of Bioanalytical Method at inVentiv Health Clinique

- Parameters evaluated during validation:
  - LLOQ
  - Dilution integrity
  - Carryover
  - Reinjection reproducibility



# Validation of Bioanalytical Method at inVentiv Health Clinique

- Parameters evaluated during validation:
  - Stability:
    - Freeze-thaw
    - Short-term in matrix
    - Long-term in matrix
    - Whole blood stability
    - In solution for analyte and IS
    - Post-preparative



## FDA guidance specific to Phase II/III studies

- 2001 Guidance, 2013 Draft Guidance and Crystal City V Conference Report
- 3 Specific Validation Parameters to Re-Assess:
  - Selectivity
  - Matrix effect
  - Stability in matrix with co-meds





## FDA / Selectivity

- Definition:

“Selectivity is the ability of an analytical method to differentiate and quantify the analyte of interest in the presence of matrix components in the sample including endogenous matrix components, metabolites, decomposition products, and in actual study, concomitant medications”. Assess selectivity in target population.
- Routinely we do:
  - 6 different donors (including 4 normal / 1 hemolyzed and 1 hyperlipemic).
- To add:
  - Addition of matrix lots from special population
  - Addition of co-med interference testing. Low and high QC spiked with co-med.



## FDA / Matrix Effect

- Definition:

“The goal of matrix effect is to demonstrate consistent assay performance across multiple sources of matrix that reflect the anticipated population from which samples are expected. Variations of lipid and specific or total protein concentrations which may results from disease indication should be addressed”
- Routinely we do:
  - 6 different donors (including 4 normal / 1 hemolyzed and 1 hyperlipemic).
- To add:
  - Addition of matrix lots from special population



## FDA / Stability in Matrix with Co-Meds

- Aspect still under discussion
- Can the presence of co-med affect stability of analyte?
- Goal: Assure stability in the known presence of two (or more) analytes
- Analytical sites not aware of all co-meds and may differ from study to study.
- To add:
  - Freeze-thaw and long-term stability in matrix with co-medications.



## EMA guidance specific to Phase II/III studies

- Guideline on bioanalytical method validation  
February 2012
- 3 Specific Validation Parameters to Re-Assess:
  - Selectivity
  - Matrix effect
  - Stability in matrix



## EMA / Selectivity

- “Co-medications normally used in the subject population studied should be taken into account”
- To add:
  - Addition of co-med interference testing.



## EMA / Matrix Effect

- “If samples from special population are to be analyzed it is also recommended to study matrix effects using matrix from such populations”
- To add:
  - Addition of matrix lots from special population



## EMA / Stability in Matrix

- “The results of the evaluation of long term stability should be available before the study report is issued”
- “In case of multi-analyte study, attention should be paid to stability in the matrix containing all the analytes”
- May need to cover long storage period.
- Stability with co-meds



## ANVISA guidance specific to Phase II/III studies

- Resolution – RDC N.27 of May 17, 2012 and Technical note 04/2014
- 2 Specific Validation Parameters to Re-Assess:
  - Selectivity (if analogue IS)
  - Matrix effect





## ANVISA / Selectivity

- “Studies using concomitant drugs or in volunteers who have made use of possibly interfering substances should have their samples analyzed only after evaluation of the possible analytical effects”
- To add:
  - Test of selectivity with and without potentially interfering agents.



## ANVISA / Matrix Effect

- Presence of co-med and if analogue IS is used
- To add:
  - Test of matrix effect with and without potentially interfering agents



## Bioanalytical method validation for Phase II/III

- Additional tests to perform if co-meds (selectivity, matrix effect, co-med testing, stability).
- Long-term stability period to cover.
- May need additional stability for clinical sites.
- Update method to latest regulation.



## Reconciliation of Study Samples

- Info at clinical sites on sample ID = analytical site?
- Confirm ID and number of study samples (collected vs received).
- Can be difficult if different clinical sites.
- For long-term studies, need to start the process early.



# Reconciliation of Study Samples

- Resolve issues right away
- If not done adequately, may led to:
  - Results not attributed to correct subject
  - Samples may be judged inadequate for analysis



# Reconciliation of Study Samples

- Examples of problems encountered:
  - Overwriting on labels or shipping document
  - Missing info
  - Tubes with no labels
  - Incoherent info
  - Incorrect ID vs shipping documentation
  - Samples collected not according to protocol
  - Samples with same ID
  - Not sure of matrix sent



## Key to Success from Bio Aspect

- Ongoing reconciliation
- Communications
  - Issues that affect study samples.
  - Co-meds
- Shipment of study samples
- Experienced clinical sites



# Thank you!

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