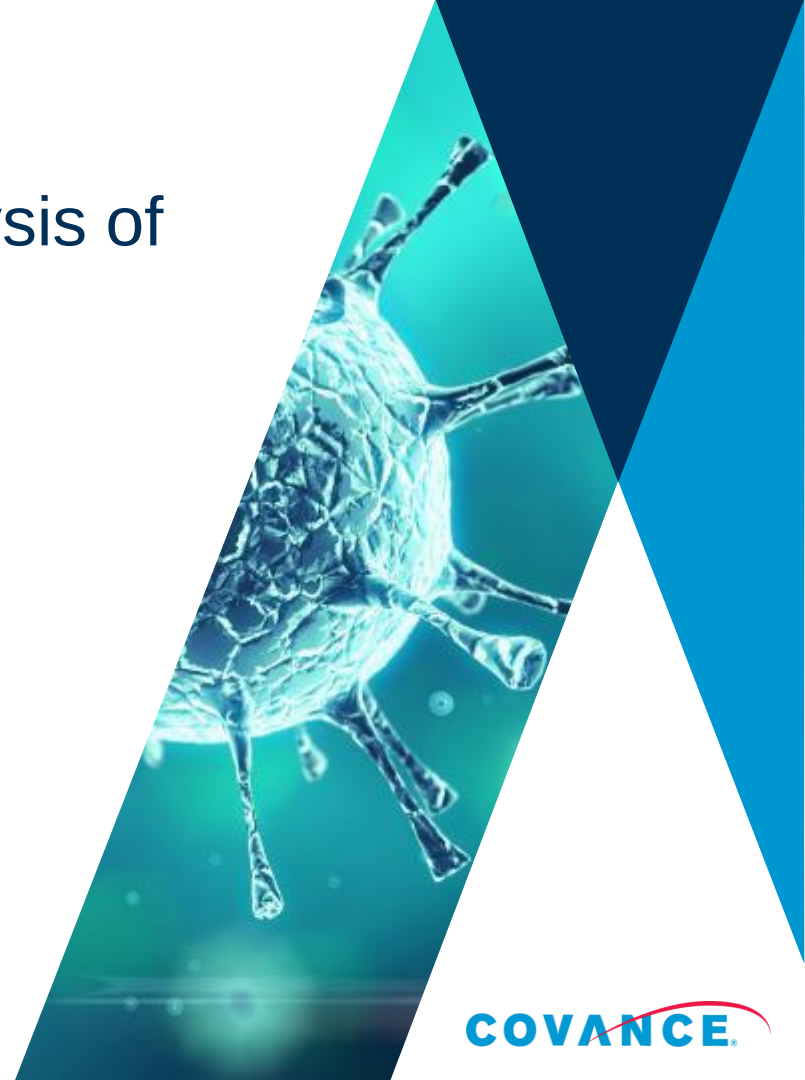
Making Haste, Slowly, in Bioanalysis of Biomarkers

Robert Nelson, PhD



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Overview

1. Introduction: Make haste, slowly
2. Case Study 1: Biomarker of target engagement
3. Case Study 2: Changing use of biomarker data
4. Case Study 3: Assessing parallelism and what it tells us
5. Summary & Conclusions

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Introduction

- In the bioanalytical laboratory, we often receive requests for biomarker assays that come with little to no context as to what questions the biomarker data are expected to answer

- In its simplest form, the request comes as
  - ‘Do you have a validated assay for biomarker X?’

- When faced with this situation, how should we proceed?

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Festina lente

- ‘Make haste, slowly’
  - Classical adage and oxymoron dating back to Roman times
  - Activities should be performed with a proper balance of urgency and diligence
  - If tasks are too rushed then mistakes are made and good long-term results are not achieved

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Making haste, slowly, in bioanalysis of biomarkers

- Make time to understand the background to biomarker assay requests
  - Take the correct path for assay establishment and validation
  - Gives us confidence that the biomarker data our laboratories produce are fit for their intended purpose

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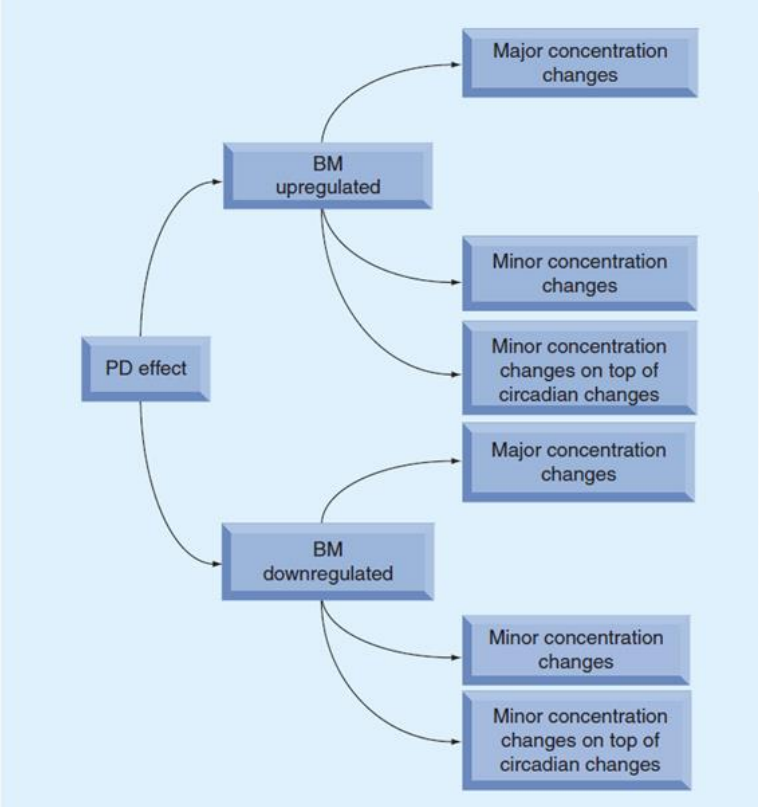
Start from the end

How will the biomarker data be used?

+

Understand the biomarker biology & Mechanism of action (MoA) of the drug



Bioanalysis (2012); 4(15): 1883–94

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2 Case Study 1: Biomarker of target engagement

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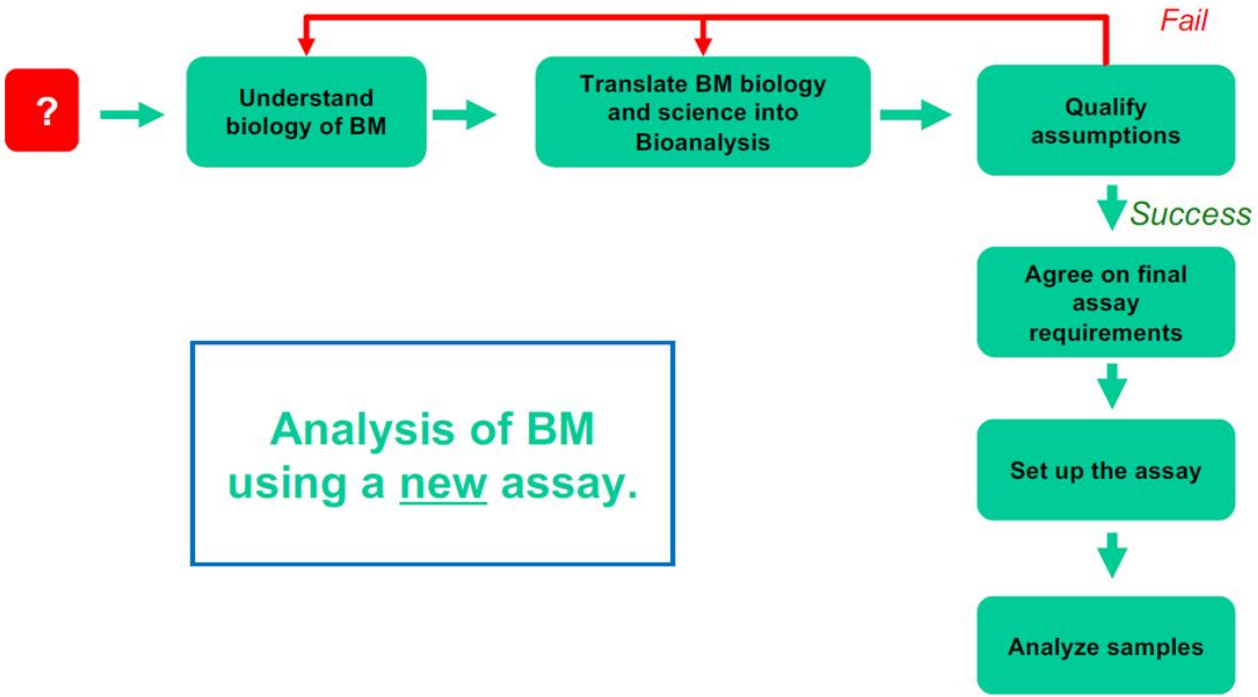
Case Study 1: Enquiry

- We would like to measure the levels of free biomarker X. The endogenous level are very variable so maybe the use of a surrogate will be necessary. We have already established a parallelism between the recombinant biomarker and the endogenous biomarker
  - How will you validate this assay?

Workflow for analysis of a biomarker using a new assay



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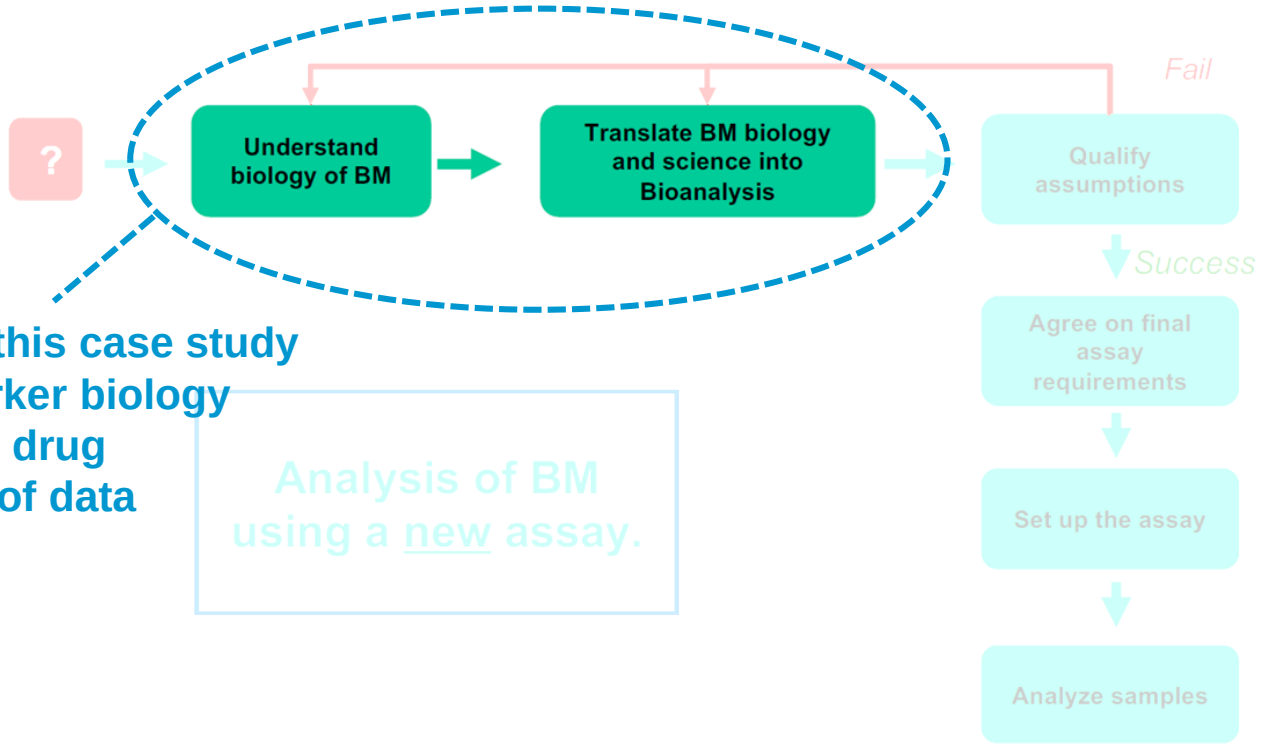
Workflow for analysis of a biomarker using a new assay



Focus for this case study

- **Biomarker biology**
- **MoA of drug**
- **Usage of data**

Bioanalysis (2012); 4(15): 1883–94

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Case Study 1: The biology of the biomarker

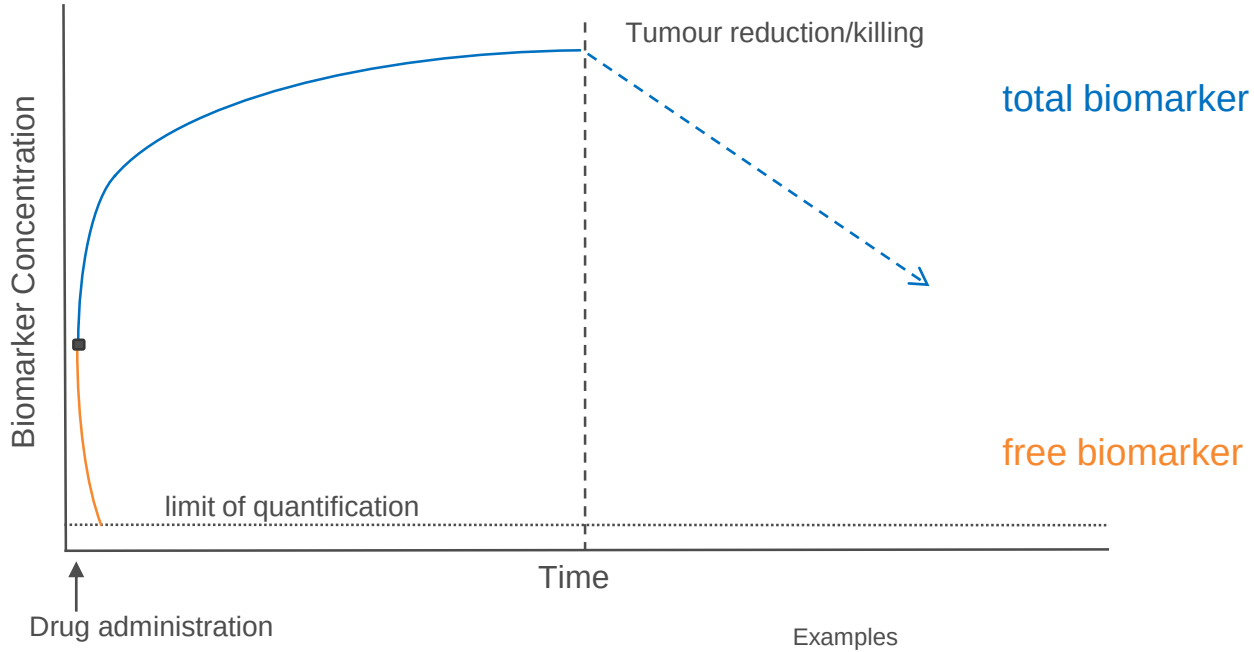
- The biomarker is a membrane-bound extracellular protein involved in modulation of immune function
  - In some cancers, the protein is overexpressed in the tumor microenvironment inhibiting anti-tumor immunity
  - High levels of soluble protein biomarker are associated with poor overall survival and progression-free survival

- What concentration levels do we expect in the study population?
  - How much intra- and inter-individual variability do we expect?

Case Study 1: The MoA of the drug

- Drug is a monoclonal antibody (mAb)
- The biomarker is the *target* of the drug
  - Drug inhibits activity of the protein biomarker to restore a pro-inflammatory microenvironment and help activate an anti-tumor immune response
- What changes do we expect to see in biomarker levels upon drug administration?
  - What assay sensitivity do we require?
  - Should we measure free or total biomarker?

Case Study 1: Expected changes during treatment



Examples
Davis et al. (1999) Drug Delivery 6(3), 171-9 https://doi.org/10.1080/107175499266922
Neubert et al. (2013) Anal. Chem. 85, 1719−26 https://doi.org/10.1021/ac303031q

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Case Study 1: Usage of the biomarker data

- Supporting early clinical trial (phase I/II) evaluating safety, efficacy and optimal dosing regime of the drug

- Biomarker data will be used to evaluate pharmacodynamic (PD) response to the drug
  - Want to achieve target engagement >95%
  - Analysis of PD data at interim and end of study
  - Biomarker data will not support dose escalation

Case Study 1: Biomarker assay establishment

- A number of assay options:
  - Free biomarker assay on standard immunoassay platform
    - Unlikely to detect levels after drug administration
    - Assay results <LOQ may indicate appropriate level of target engagement
  - Free biomarker assay on ultra-sensitive platform
    - Additional investment, but may be capable of measuring free target levels
    - Measurement may be impacted by change in drug:biomarker binding kinetics during sample processing
  - Total biomarker assay
    - Need to identify reagents that are non-competitive with the drug
    - Free biomarker levels can be modelled based on drug concentration measurement (PK) and drug binding kinetics

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Case Study 1: Biomarker assay establishment – path taken

- A number of assay options:
  - Free biomarker assay on standard immunoassay platform
    - Unlikely to detect levels after drug administration
    - Assay results <LOQ may indicate appropriate level of target engagement
  - Free biomarker assay on ultra-sensitive platform
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  - **Total biomarker assay**
    - **Need to identify reagents that are non-competitive with the drug**
    - **Free biomarker levels can be modelled based on drug concentration measurement (PK) and drug binding kinetics**

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3 Case Study 2: Changing use of biomarker data

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Case Study 2: Enquiry

- We would like to measure the levels of biomarker X in our clinical study as part of our patient enrollment
  - Can we move ahead with the current assay you have already validated?

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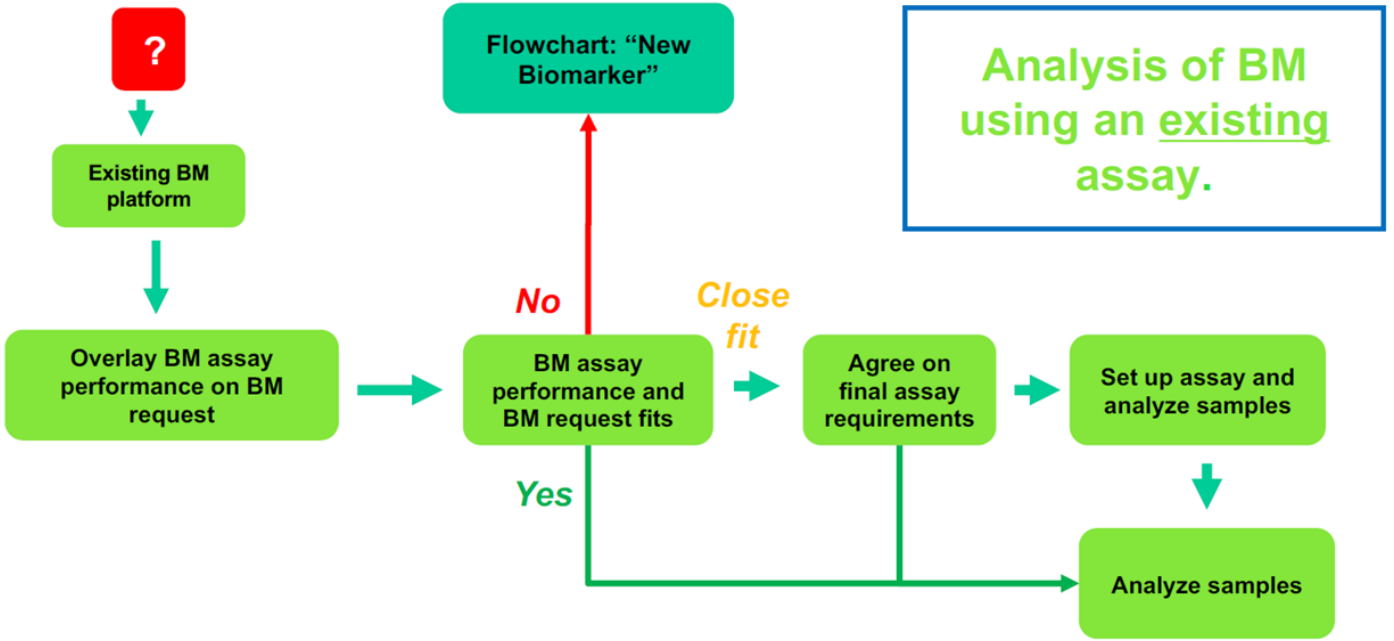
Workflow for analysis of a biomarker using an existing assay



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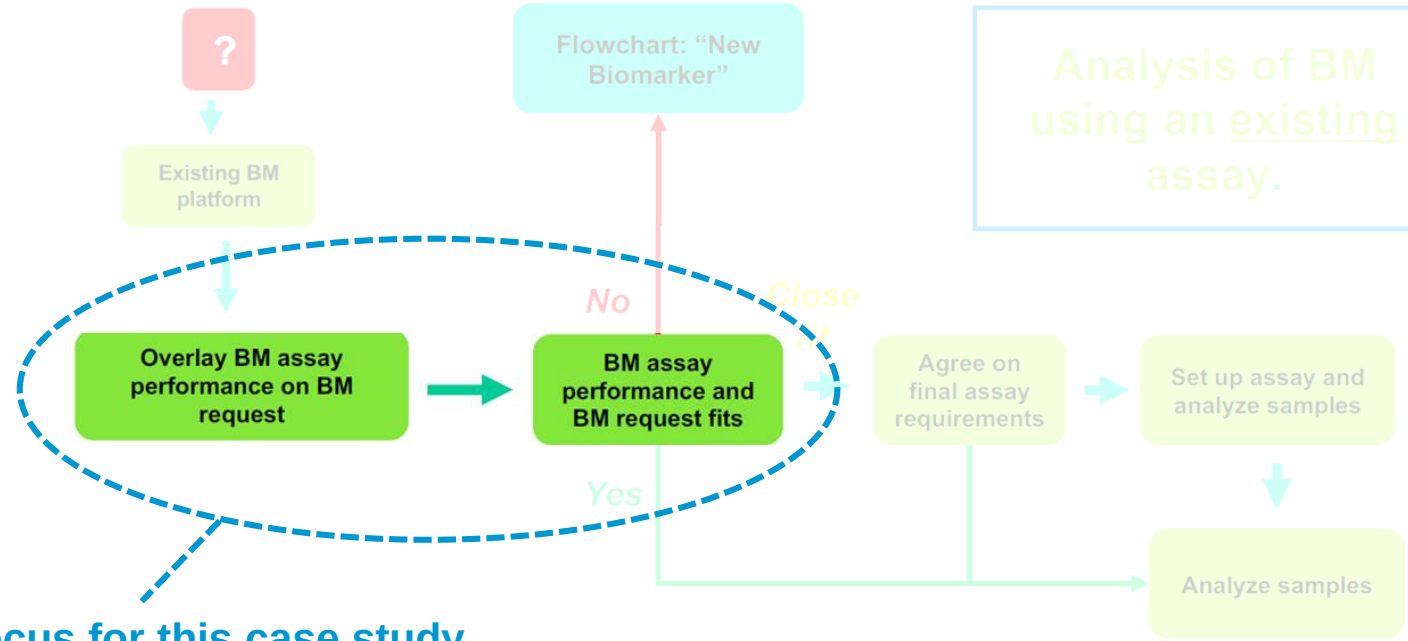

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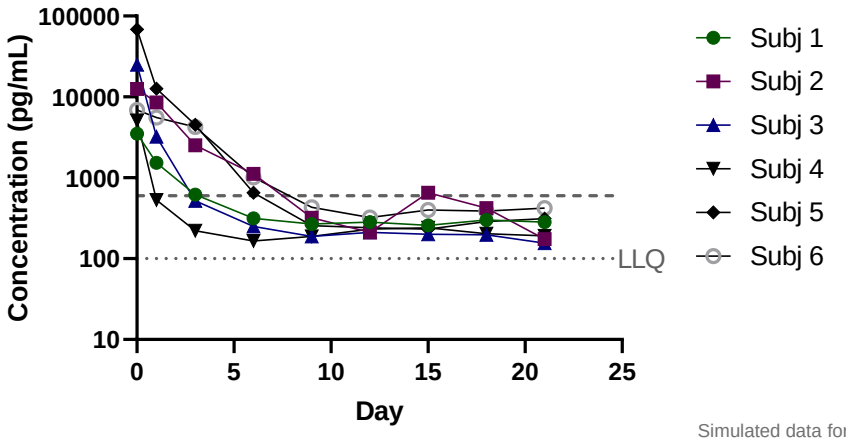
Case Study 2: Biology of the biomarker & MoA of the drug

- The biomarker is an small cytokine important in recruitment of immune cells to sites of infection
  - Production of this cytokine is induced by the target of the drug, and serves as a *surrogate for activity* of the drug target

- The drug is a monoclonal antibody that binds to and neutralizes the activity of its target by preventing it binding to its receptor
  - The target of the drug plays a role in a number of different inflammatory diseases

Case Study 2: Usage of the biomarker data

- Previous clinical studies
  - The biomarker data were used as an pharmacodynamic indicator that drug target was neutralized
  - In patients, biomarker levels were typically high (several thousand pg/mL) at initiation of treatment, returning to ‘normal’ levels (a few hundred pg/mL) when the drug maintained neutralization of its target



Simulated data for illustrative purposes

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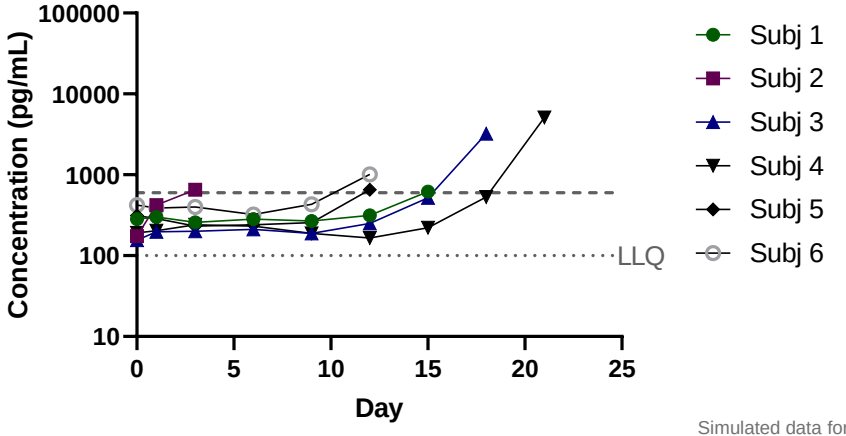
Case Study 2: Usage of the biomarker data

- New clinical study
  - Biomarker data will similarly be as an indicator of drug target activity
  - However, patients expected to have low levels of biomarker initially, with an increase at the onset of an ‘inflammatory episode’
  - If biomarker levels move above a ‘threshold’, it will indicate active disease and patients will be randomized to receive drug or placebo



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Case Study 2: Does the assay fit the biomarker request?

- Do we have data that can be used to set the threshold?
  - Collected data on inter-individual variation during assay development and validation
    - Approx. 50 healthy individuals
    - However healthy individual ≠ subject without disease
  - Data on within individual variation with this method
    - Longitudinal within subject data was collected within previous clinical studies
    - However previous study subject ≠ current study subject
- Initiated collection of samples from this indication to better define subjects with and without active disease

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Case Study 2: Does the assay fit the biomarker request?

- Can the current assay support the new context of use?
  - What level of precision is needed for the end use of the data?

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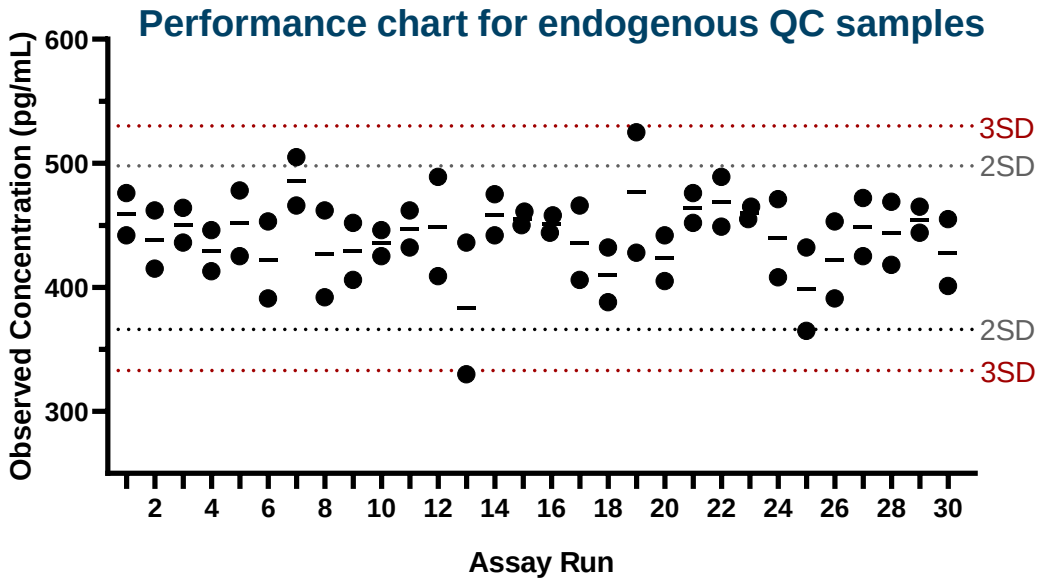
Case Study 2: Does the assay fit the biomarker request?

- The assay is under control for current data usage
  - But level of variation could lead to misclassification of results in new context



Nominal concentration = 432 pg/mL
(36 measurements: 6 replicates x 6 runs)

Inter-assay precision = 7.6%

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Case Study 2: Changes to the assay to meet the new CoU

- Assay moved from manual process to automated platform
  - Improved precision to <5%

- Additional endogenous QC samples included in each run
  - Allowed normalization/correction of study sample data against a reference value

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4 Case Study 3: Assessing parallelism and what it tells us

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Case study 3: Assessing parallelism and what it tells us

- Assay for an inflammatory cytokine was developed and validated:
  - Precision
    - 6 runs with endogenous QC samples
    - Inter-assay precision of 8.0%
  - Stability
    - Stable through 5 freeze-thaw cycles
    - Stable stored at -80°C for 3 months (assessed with freshly collected samples)
  - Parallelism
    - 5 individuals assessed at multiple dilutions
    - Target parallelism results within 3SD (3x inter-assay precision)
    - No pass/fail criteria – assay characterization

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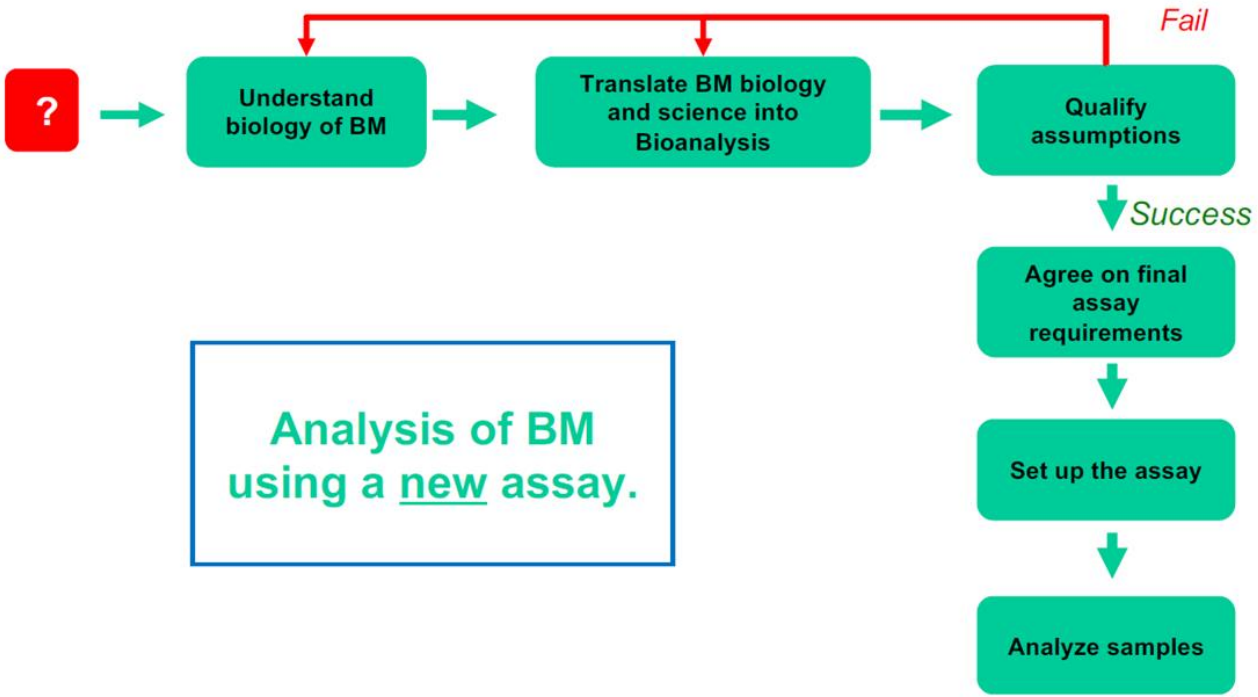
Workflow for analysis of a biomarker using a new assay



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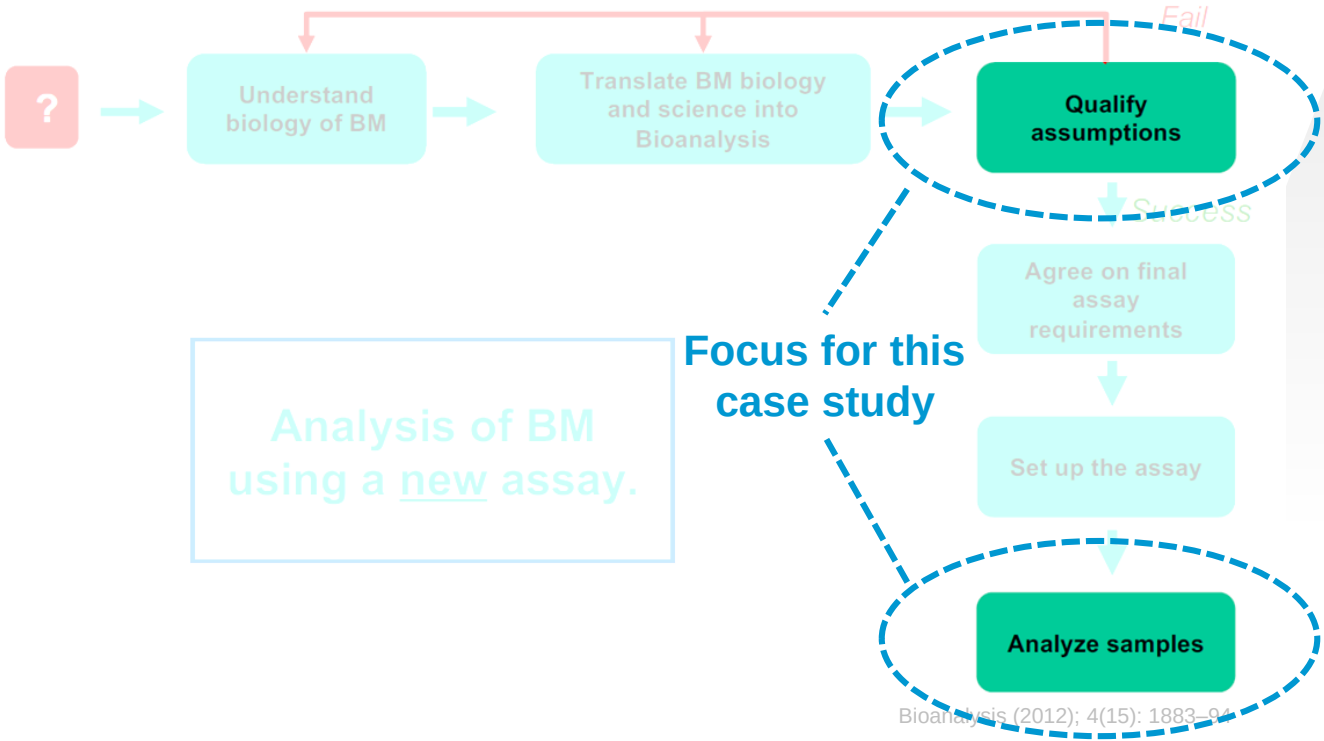

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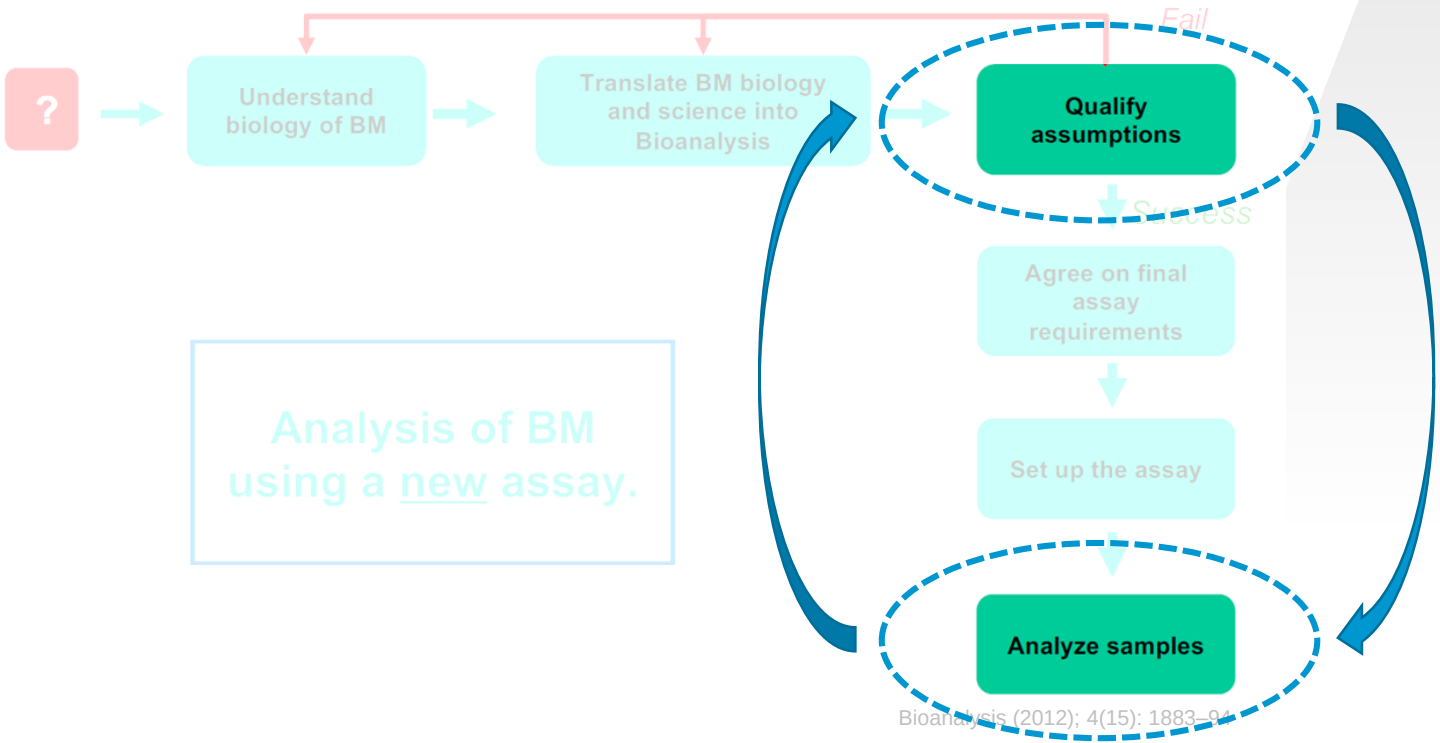
Workflow for analysis of a biomarker using a new assay



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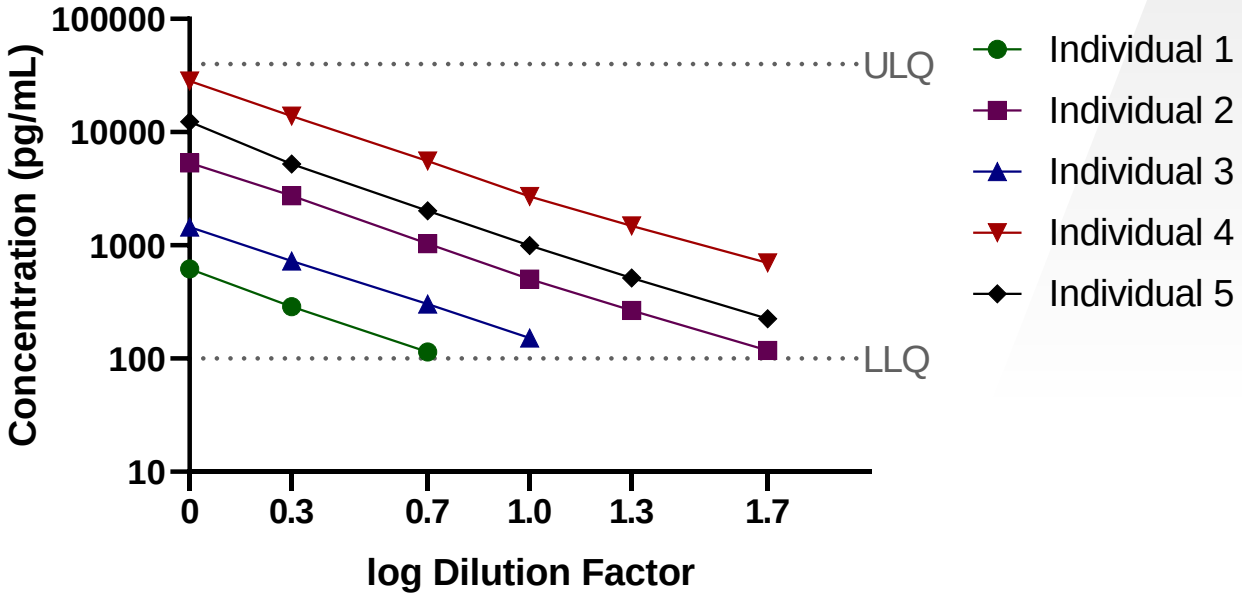
Case study 3: Parallelism assessment in validation

- 5 individual analyzed and multiple dilutions across the assay range

Dilution Factor	Log Dilution Factor
1 (MRD)	0
2	0.3
5	0.7
10	1.0
20	1.3
50	1.7



Simulated data for illustrative purposes

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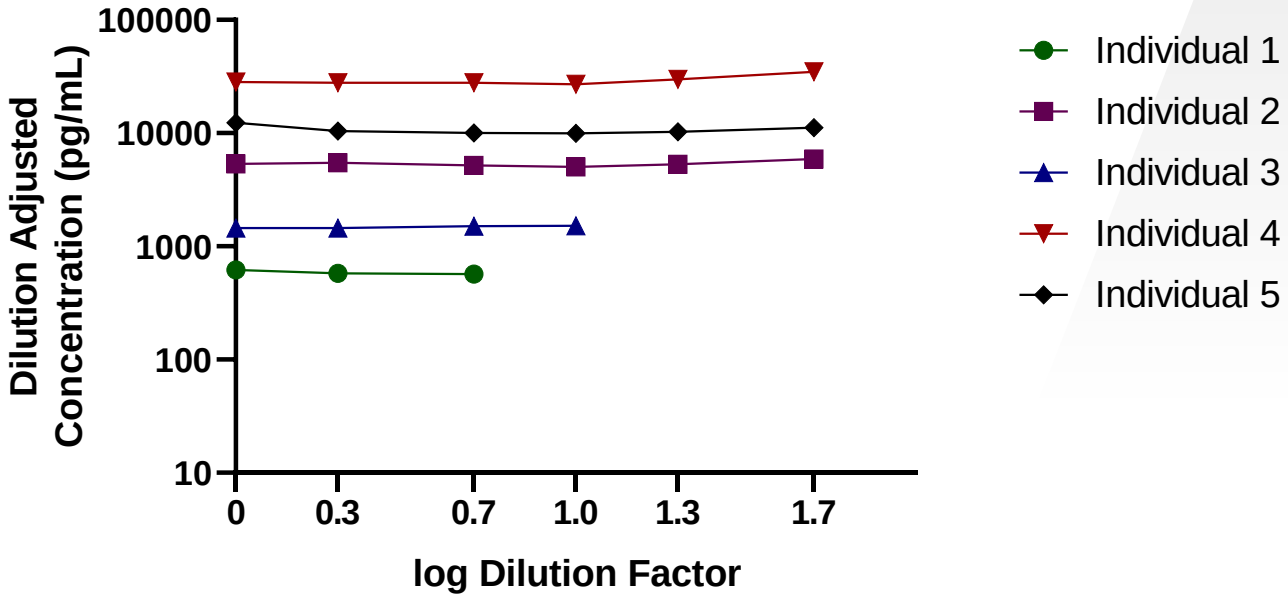
Case study 3: Parallelism assessment in validation

- Dilution-adjusted concentration calculated

Dilution Factor	Log Dilution Factor
1 (MRD)	0
2	0.3
5	0.7
10	1.0
20	1.3
50	1.7



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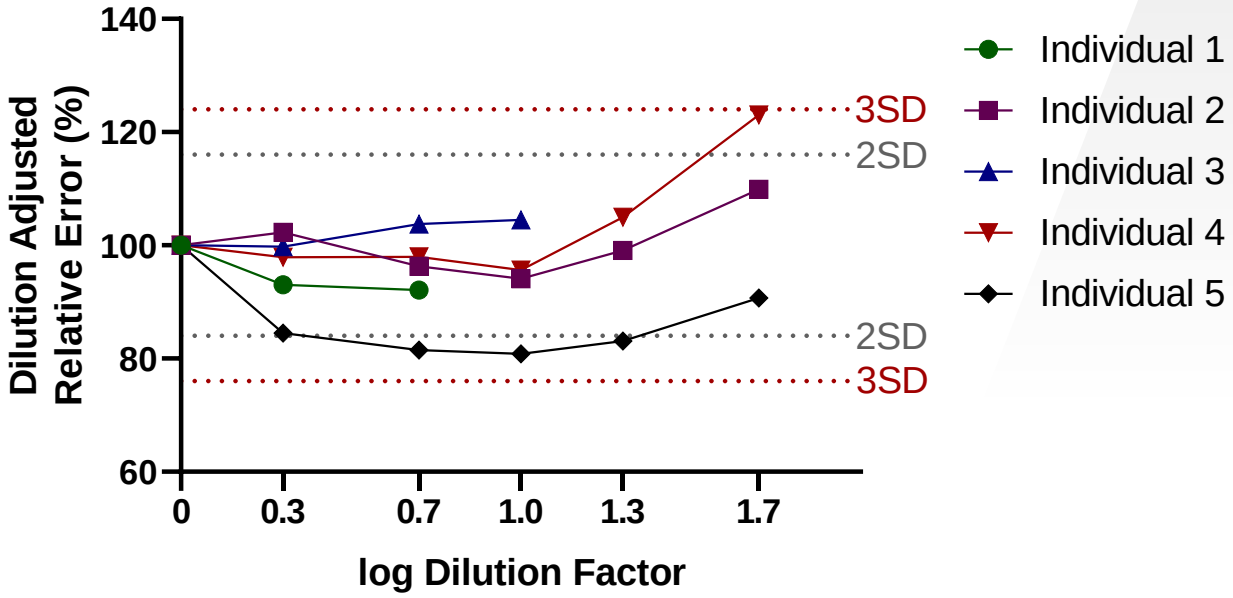
Case study 3: Parallelism assessment in validation

- Dilution-adjusted relative error calculated (vs MRD result)

Dilution Factor	Log Dilution Factor
1 (MRD)	0
2	0.3
5	0.7
10	1.0
20	1.3
50	1.7



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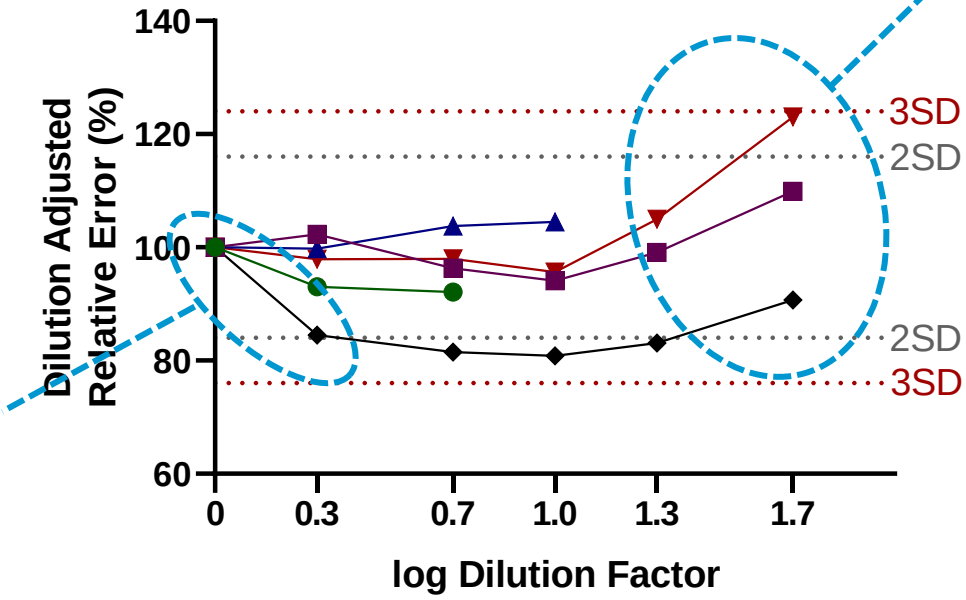
Case study 3: Parallelism assessment in validation

- All 5 individuals have dilutions within 3SD
  - But potential concerns…?

Positive bias at higher dilutions…?



Interference…?

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Case study 3: Parallelism assessment in-study

- 3 samples assessed for parallelism from each subject
  - Selected samples from low, mid and high part of observed range

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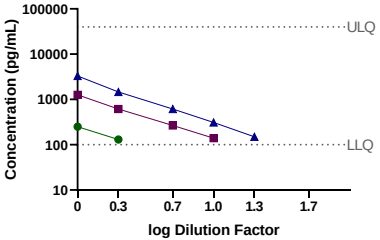
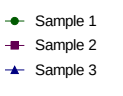
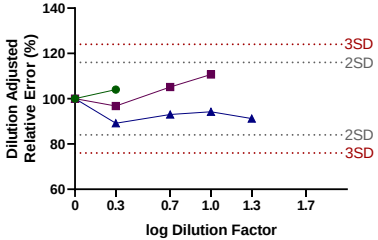
Case study 3: Parallelism assessment in-study

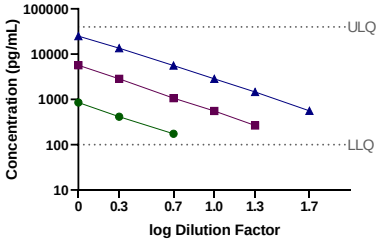


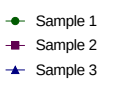


Subject 1

Parallel

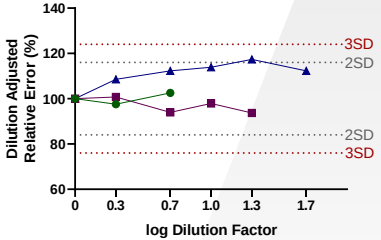


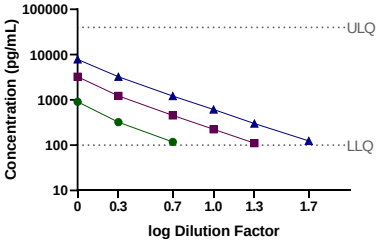


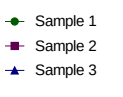


Subject 2

Parallel

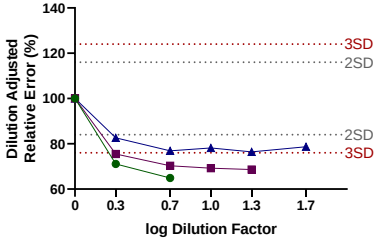


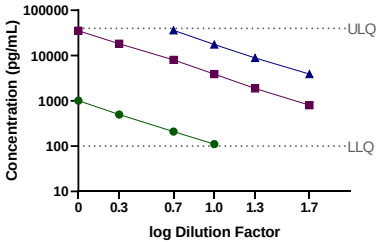


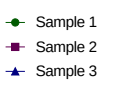


Subject 3

Non-parallel

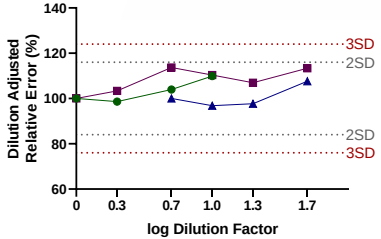






Subject 4

Parallel



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Case study 3: Parallelism assessment in-study

- No indication of positive bias from higher sample dilutions
- A small number of subjects had negative bias at dilutions after MRD
  - Parallelism present at dilutions ≥5
  - Reporting for these subjects amended – reported results from a dilution factor ≥5

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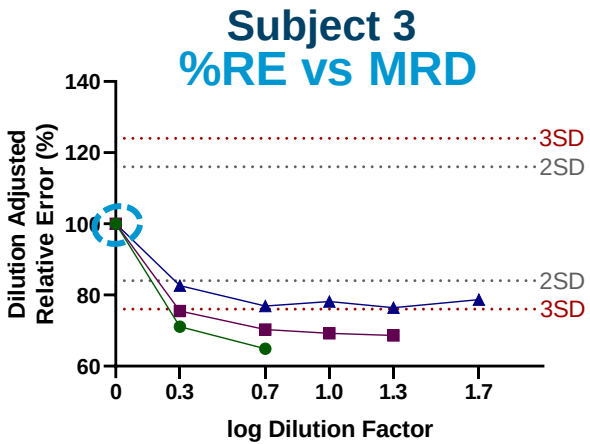

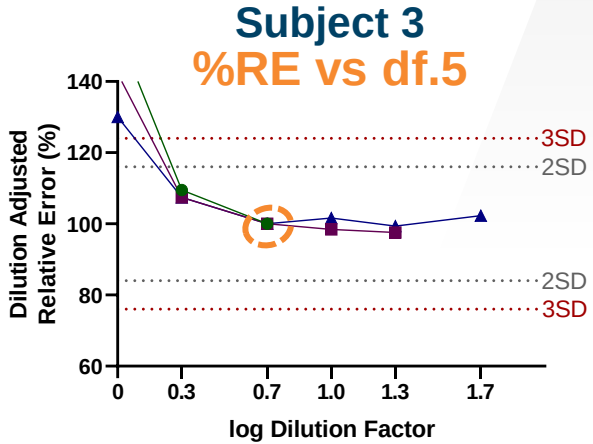

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5 Summary & Conclusions

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Summary & Conclusion (1)

- Establishing a biomarker assay requires an understanding of the biomarker biology, the mechanism of action of the drug, the intended use of the data and the capabilities/limitations of the bioanalytical methods
  - Dialog with stakeholders is essential to understand the biomarker CoU
  - Enables us to adopt a suitable bioanalytical strategy where the appropriate level of assay performance is understood
  - Give us confidence that the data our laboratories produce are fit for their intended purpose

Summary & Conclusion (2)

- Parallelism is a key assessment in understanding biomarker assay performance both in validation and in the study population
  - Determines whether recombinant analyte and/or surrogate matrix are representative of endogenous analyte

- Parallelism is not a straightforward pass/fail assessment
  - We have to evaluated what the data tell us…
    - whether the assay has the appropriate level of performance for the intended use
    - whether we have to put mitigations in place for analysis and/or reporting

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- Covance colleagues
- All colleagues past and present who have played a part in my biomarker education

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Questions

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