Autumn Focus Workshop

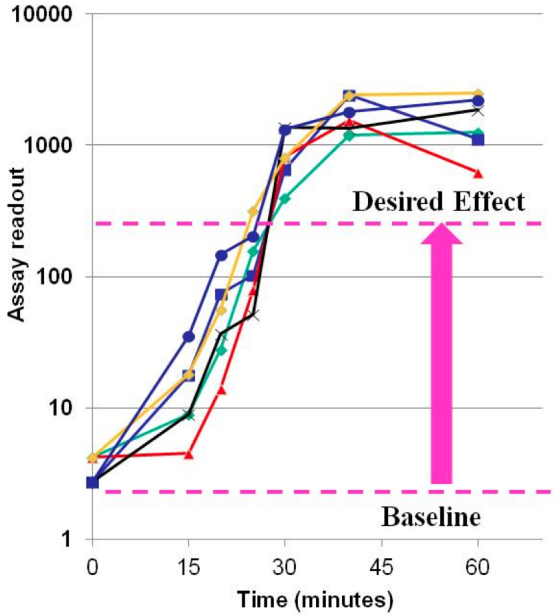
Biomarkers in Pharma R&D

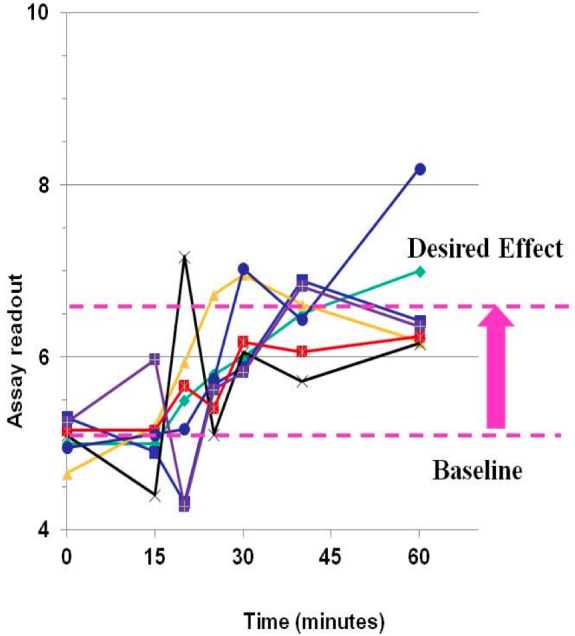
A roadmap from *Context of Use* to *Using the data*

EBF recommendation refining CoU requirements for Biomarker assays
Practical aspect of CoU - a deeper dive into the EBF recommendation

Philip Timmerman, Joanne Goodman, Kyra Cowan
On behalf of the EBF

2011





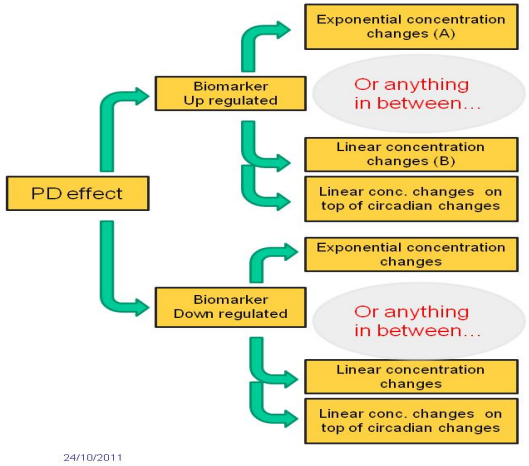
All measured with 4-6-15 “PK” assay, but was this necessary?

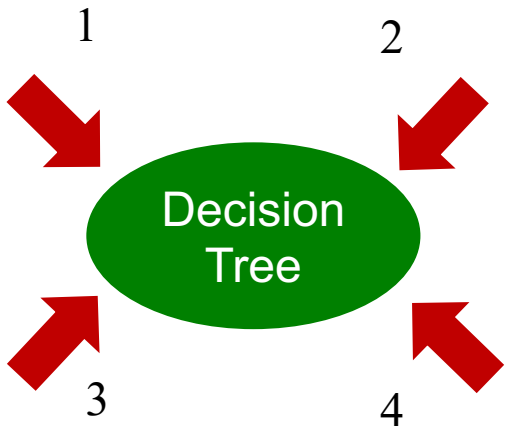
2011 - EBF reflections on biomarker classification

When developing a Biomarker assay, consider:

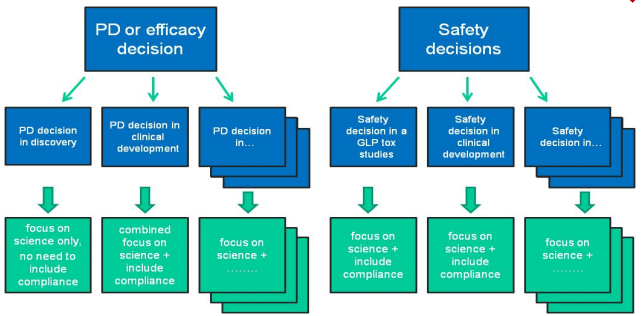
1. Observed or anticipated biomarker levels
2. Development Phase in which a biomarker is measured
3. Decisions taken from the biomarker data, e.g. efficacy, safety…
4. Fit of assay with Regulated Bioanalysis Guidelines

Above classification systems are superimposable and should be applied together to tailor an individual bioanalytical strategy in support of a biomarker assay request





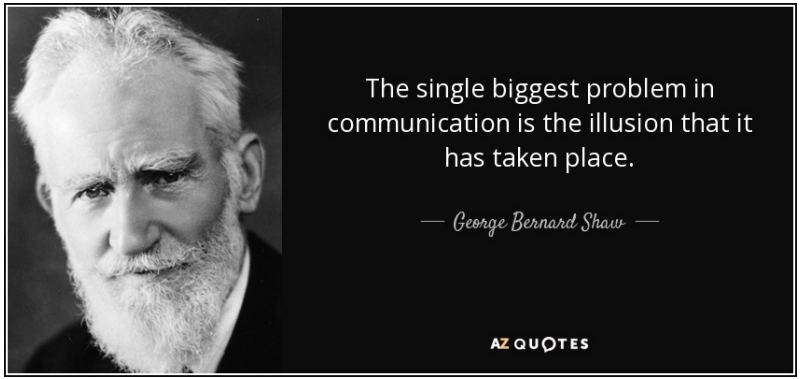
Biomarker measured in Discovery	Biomarker measured Early Development (pre-POC)	Biomarker measured Late Development post-POC)
"Does the biomarker reproducibly and reliably predicts or describes the effect of the drug"	"can I use PK/PD to facilitate compound selection?" "Can I rely on biomarker data for dose selection	"can I rely on the biomarker data to support dose selection?"
Scientific validation of biomarker required. Simple screening assay may not be sufficient.	Does scientific validation from discovery translate into early development	Does scientific validation from discovery and ED translates into Late development clinical studies
Scientific validation ≠ Validated biomarker assay	Qualification of assay for validated biomarker may be required for desired use, validated may not be needed	Qualification of assay for validated biomarker required, if assay format fits, validated assay is desired



2012 added reflection - Inform and be informed

Although included in the flowchart and in order to apply aforementioned classification systems successfully, the EBF also included a 5th principle upon which the overall recommendation is built:

COMMUNICATE



Ensure regular, cross functional and iterative communication with end user or the investigator requesting the biomarker concentration data (e.g. the pharmacologist, PK/TK, Tox-path, clinician or others)

BIOANALYSIS, VOL. 6, NO. 10 | SPECIAL FOCUS ISSUE: BIOANALYTICAL LABORATORY MANAGEMENT - PERSPECTIVE

Full Access

How the bioanalytical scientist plays a key role in interdisciplinary project teams in the development of biotherapeutics – a reflection of the European Bioanalysis Forum

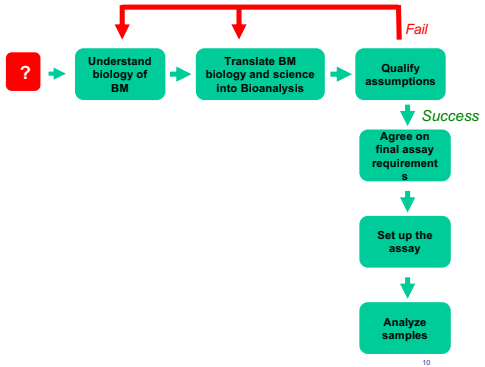
Sherri Dudal, Roland F Staack, Daniela Stoellner, Marianne Scheel Fjording, Eva Vieser, Marie-Hélène Pascual, Margarete Brudny-Kloeppel & Michaela Golob

Published Online: 24 Jun 2014 | https://doi.org/10.4155/bio.14.90

2012: EBF Recommendation

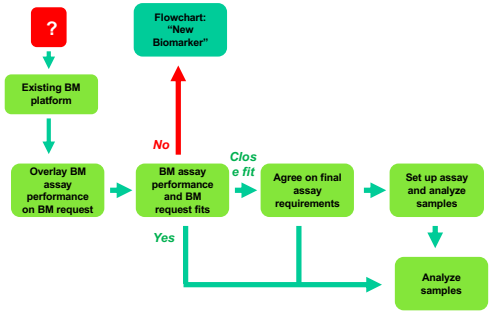
?

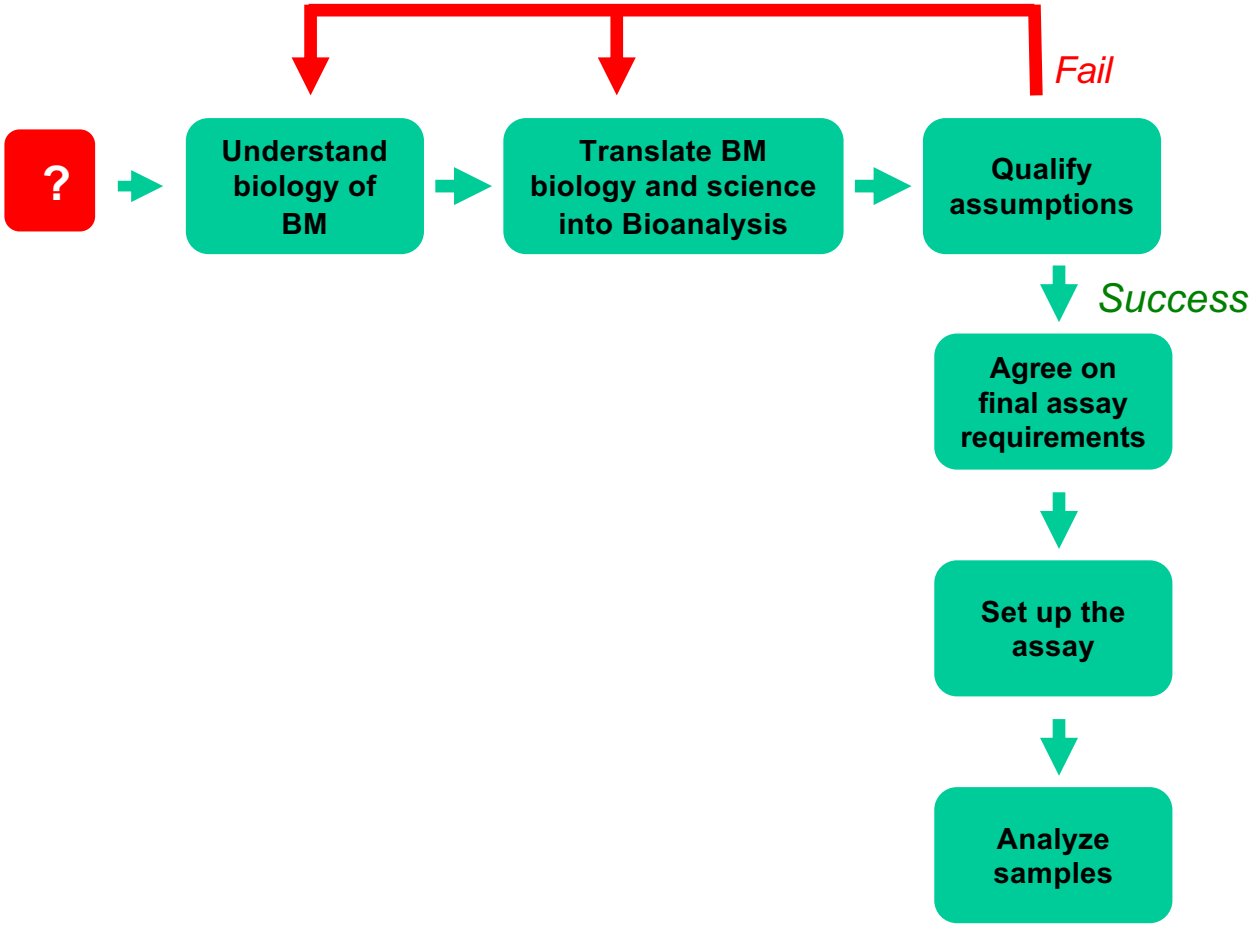
Analysis of BM using a novel assay.



?

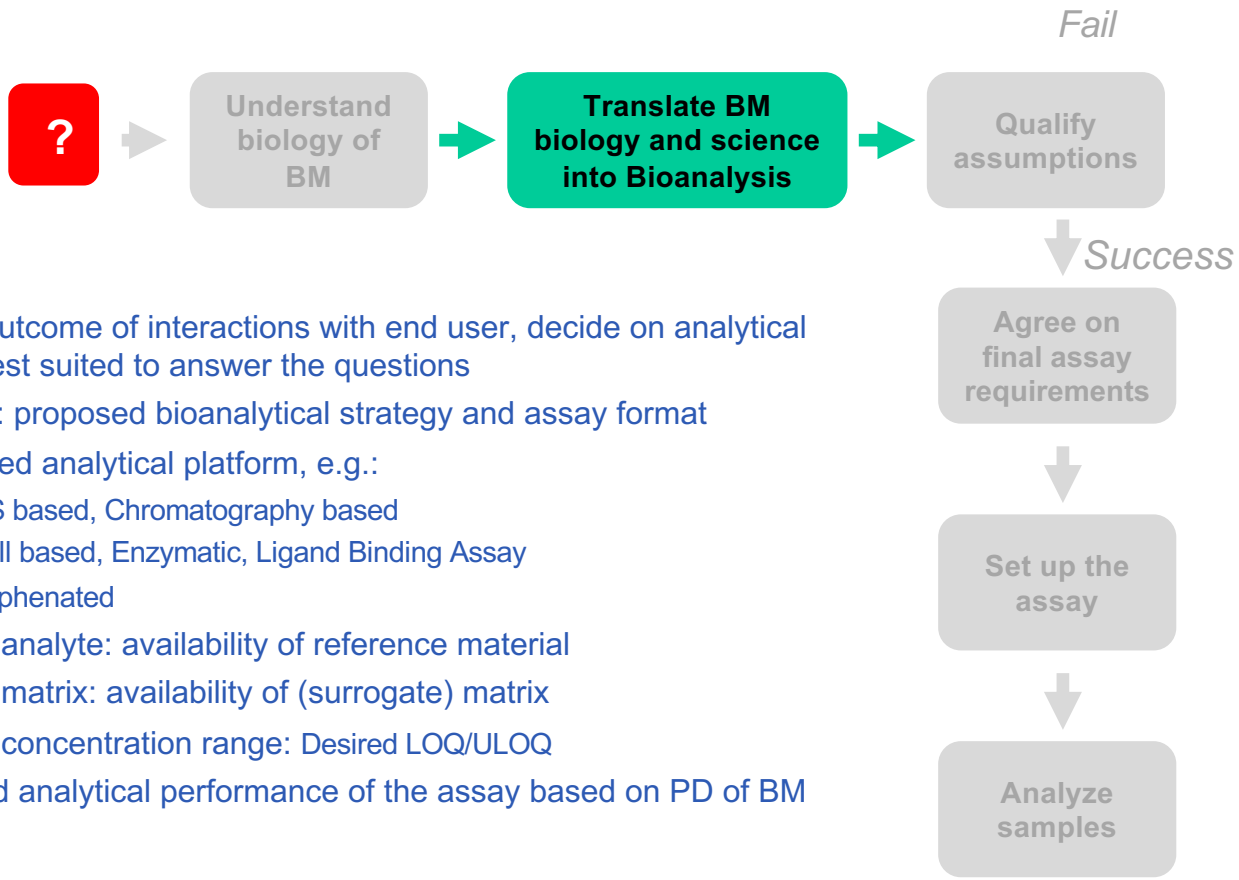
Analysis of BM using an existing assay.



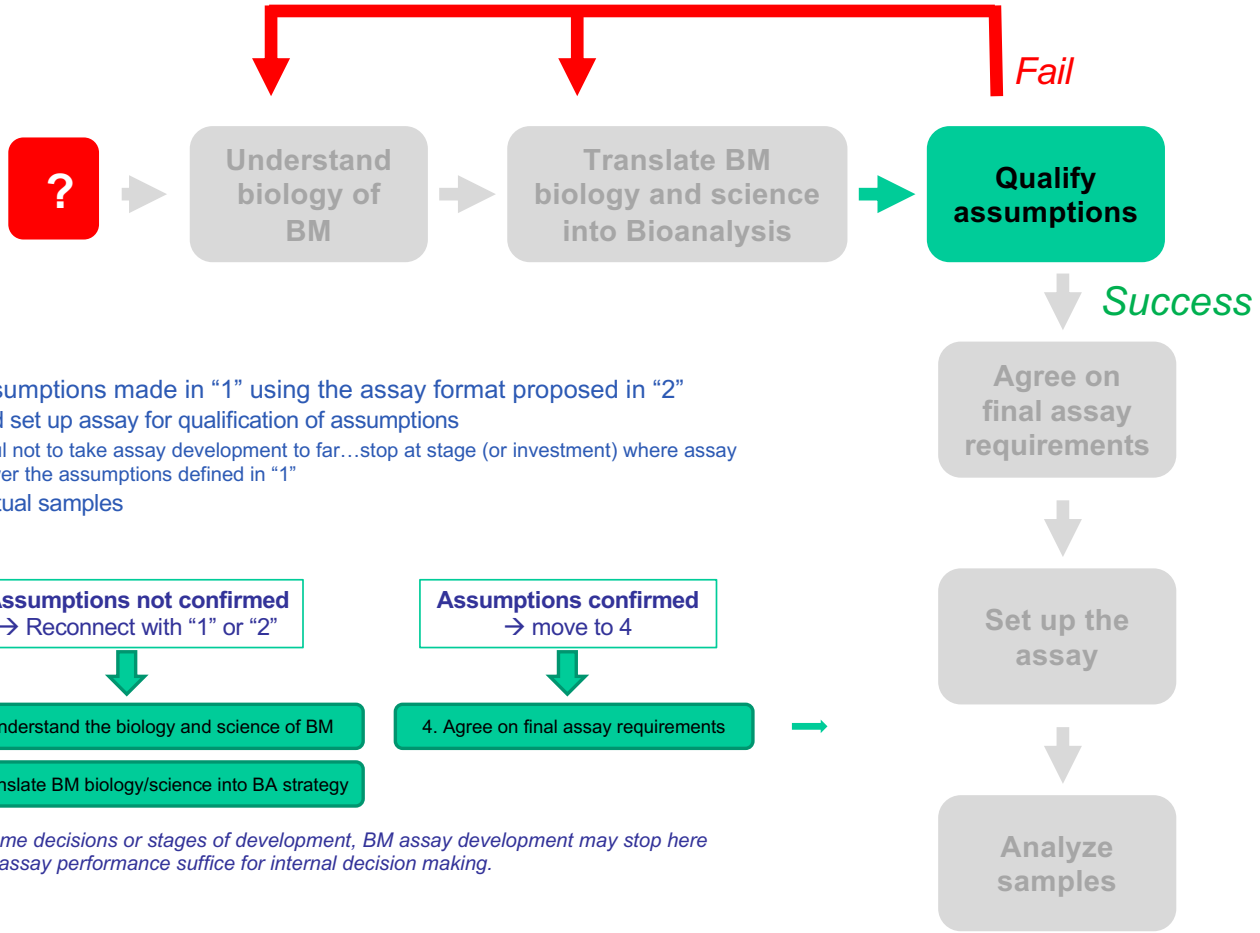

?
Understand biology of BM
Translate BM biology and science into Bioanalysis
Qualify assumptions
Fail
Success
Agree on final assay requirements
Set up the assay
Analyze samples

- Connect with PK, PD or TK person requesting (i.e. end user) the BM data, and get informed on:
  - PK of BM and PD effect of drug on PK
  - Target species-population and matrix
- Deliverable of step 1 = understand why assay is needed and what is expected outcome

Or in 2020 language: what is the CoU?



- Based on outcome of interactions with end user, decide on analytical platform best suited to answer the questions
- Deliverable: proposed bioanalytical strategy and assay format
  - Preferred analytical platform, e.g.:
    - MS based, Chromatography based
    - Cell based, Enzymatic, Ligand Binding Assay
    - Hyphenated
  - Target analyte: availability of reference material
  - Target matrix: availability of (surrogate) matrix
  - Target concentration range: Desired LOQ/ULOQ
  - Desired analytical performance of the assay based on PD of BM



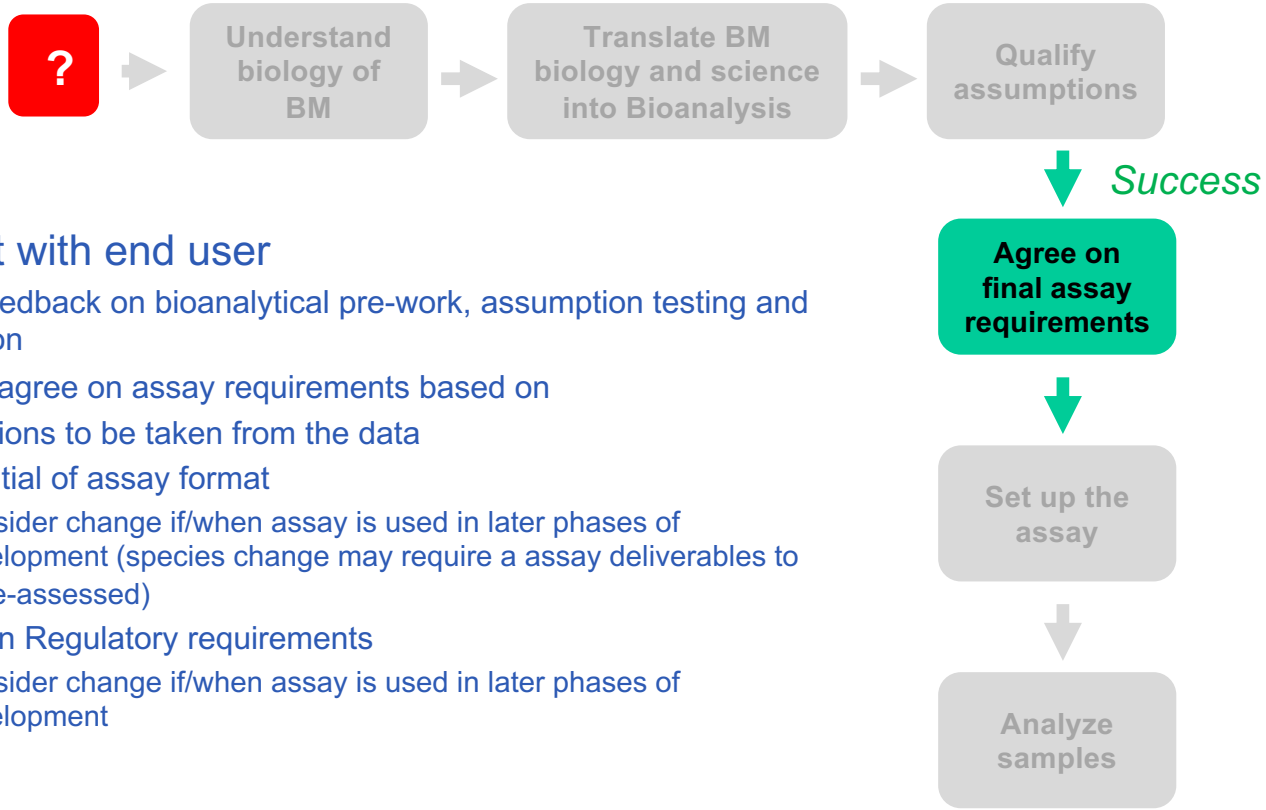
- Qualify the assumptions made in “1” using the assay format proposed in “2”
  - Develop and set up assay for qualification of assumptions
    - Be careful not to take assay development to far…stop at stage (or investment) where assay can answer the assumptions defined in “1”
  - Measure actual samples
- Deliverable:

Assumptions not confirmed
→ Reconnect with “1” or “2”

Assumptions confirmed
→ move to 4

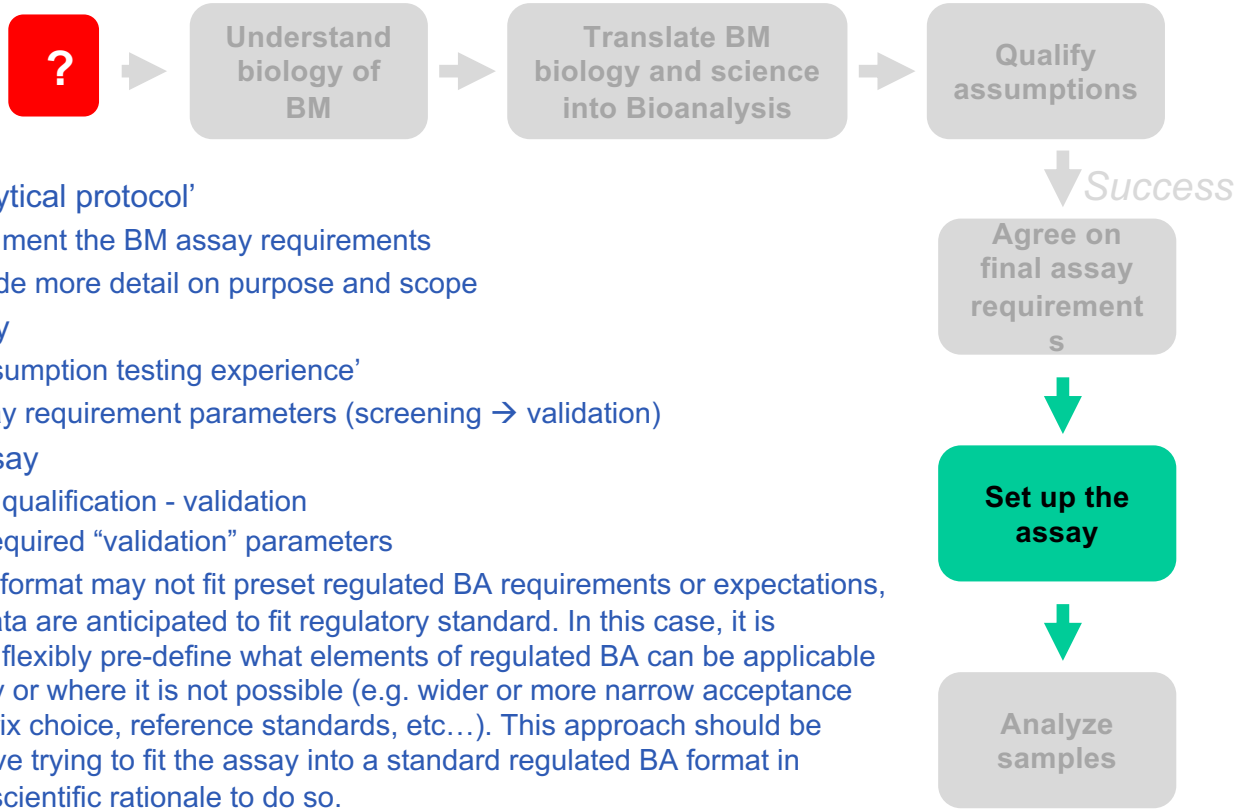
1. Understand the biology and science of BM
2. Translate BM biology/science into BA strategy
4. Agree on final assay requirements

Note: For some decisions or stages of development, BM assay development may stop here when data / assay performance suffice for internal decision making.



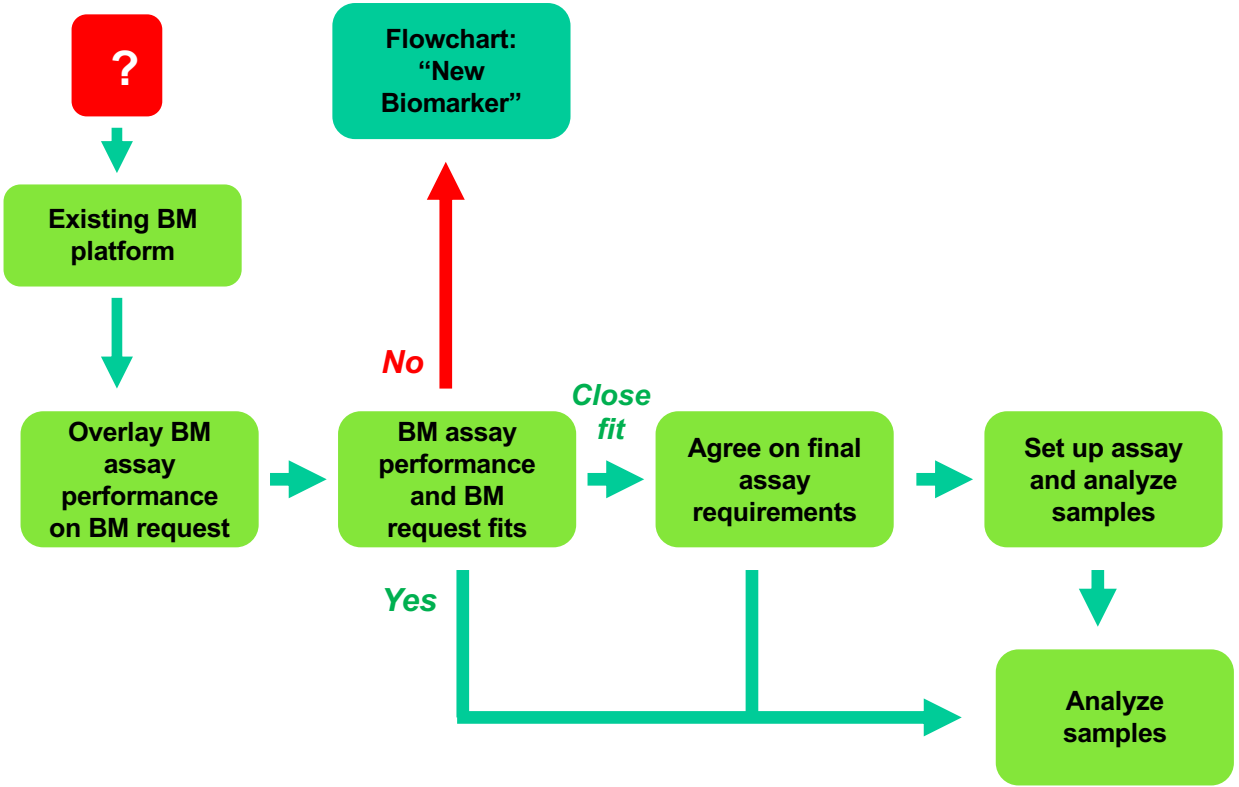
Reconnect with end user

- Provide feedback on bioanalytical pre-work, assumption testing and qualification
- Only now agree on assay requirements based on
  - Decisions to be taken from the data
  - Potential of assay format
    - Consider change if/when assay is used in later phases of development (species change may require a assay deliverables to be re-assessed)
  - Known Regulatory requirements
    - Consider change if/when assay is used in later phases of development



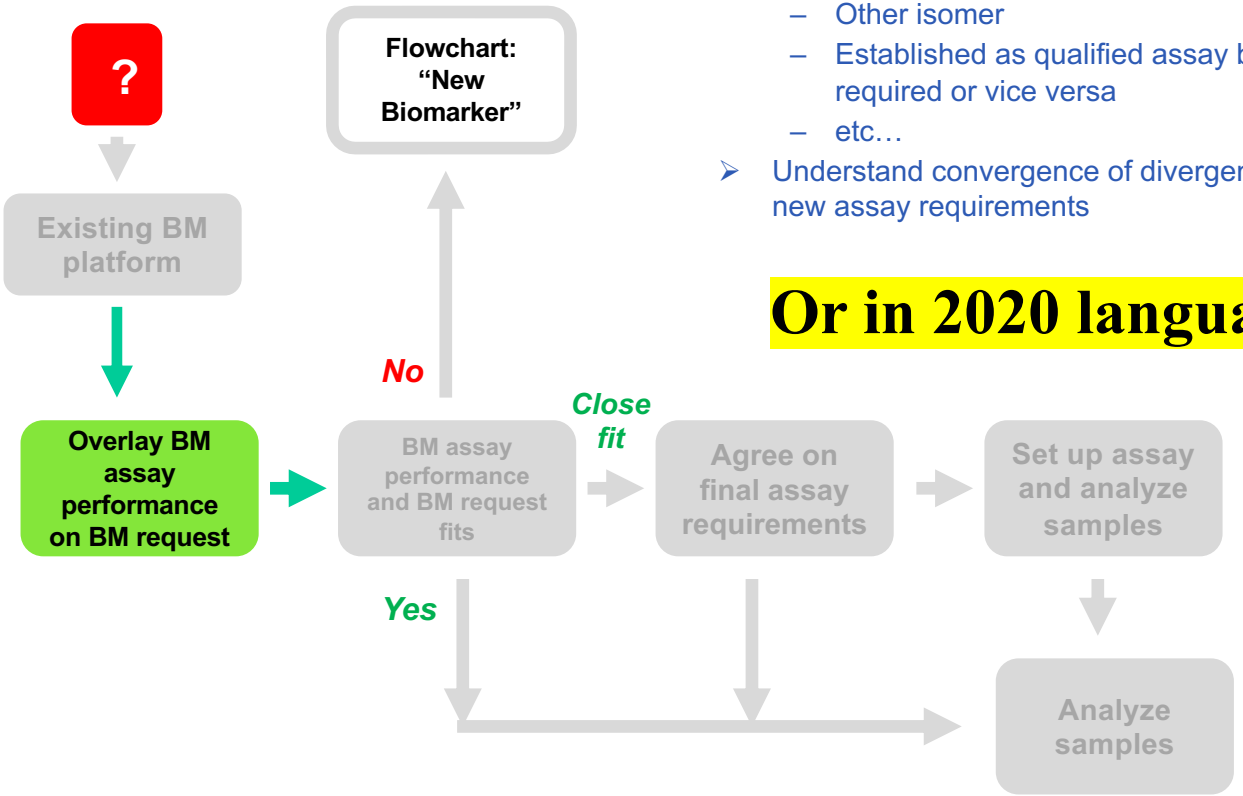
- Write ‘bioanalytical protocol’
  - A priori document the BM assay requirements
  - maybe include more detail on purpose and scope
- Develop assay
  - Build on ‘assumption testing experience’
  - Include assay requirement parameters (screening → validation)
- Set up the assay
  - Screening – qualification - validation
  - Include all required “validation” parameters
  - Note: assay format may not fit preset regulated BA requirements or expectations, but assay data are anticipated to fit regulatory standard. In this case, it is advisable to flexibly pre-define what elements of regulated BA can be applicable for the assay or where it is not possible (e.g. wider or more narrow acceptance criteria, matrix choice, reference standards, etc…). This approach should be favored above trying to fit the assay into a standard regulated BA format in absence of scientific rationale to do so.

Analysis of biomarkers using an existing assay.

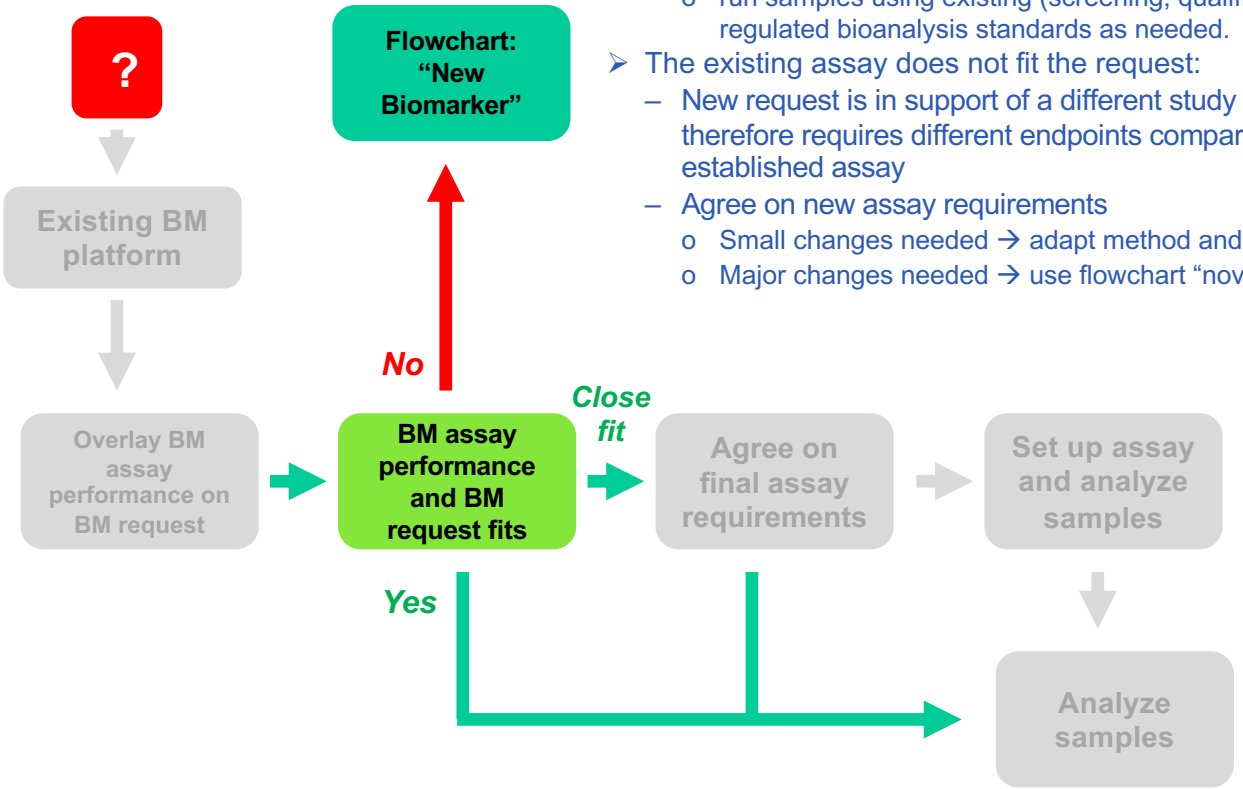


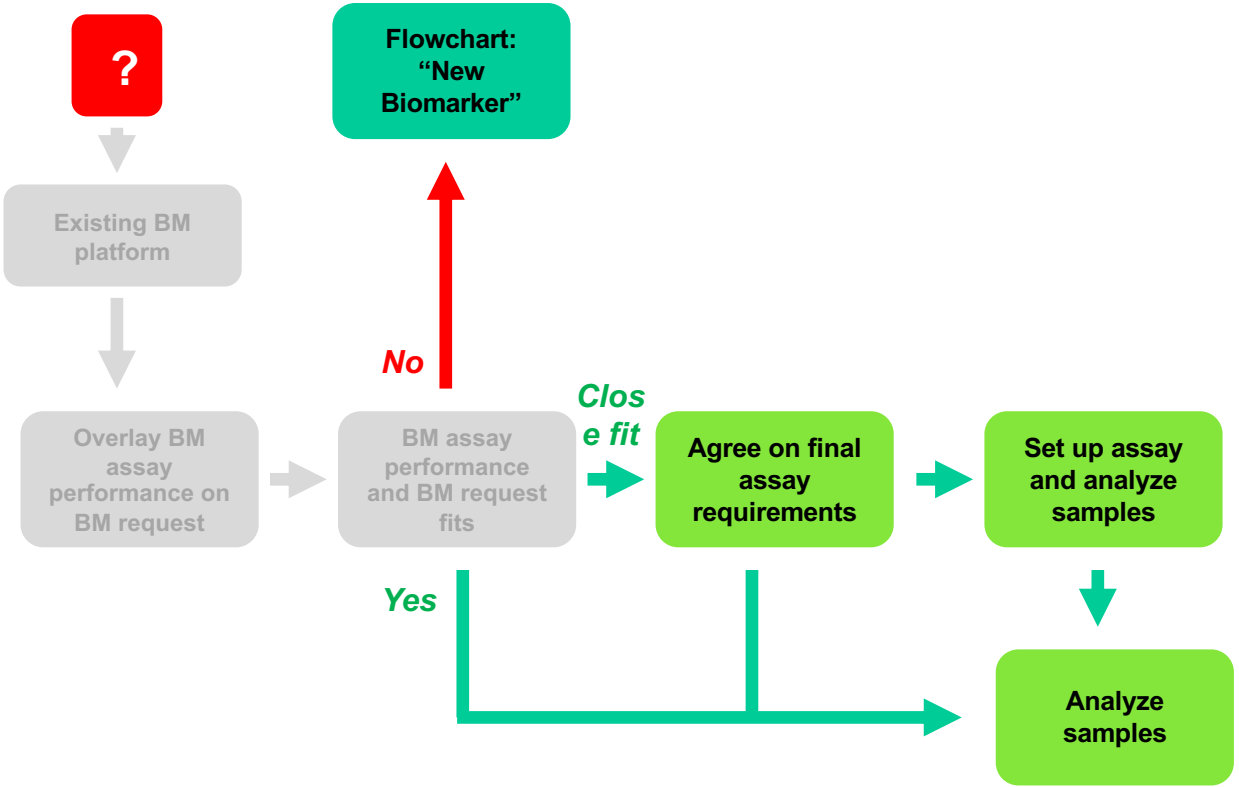
- Existing assay may not have been established for new BM question:
  - Other species
  - Other calibration range
  - Other isomer
  - Established as qualified assay but now validated assay is required or vice versa
  - etc…
- Understand convergence of divergence of assay performance and new assay requirements

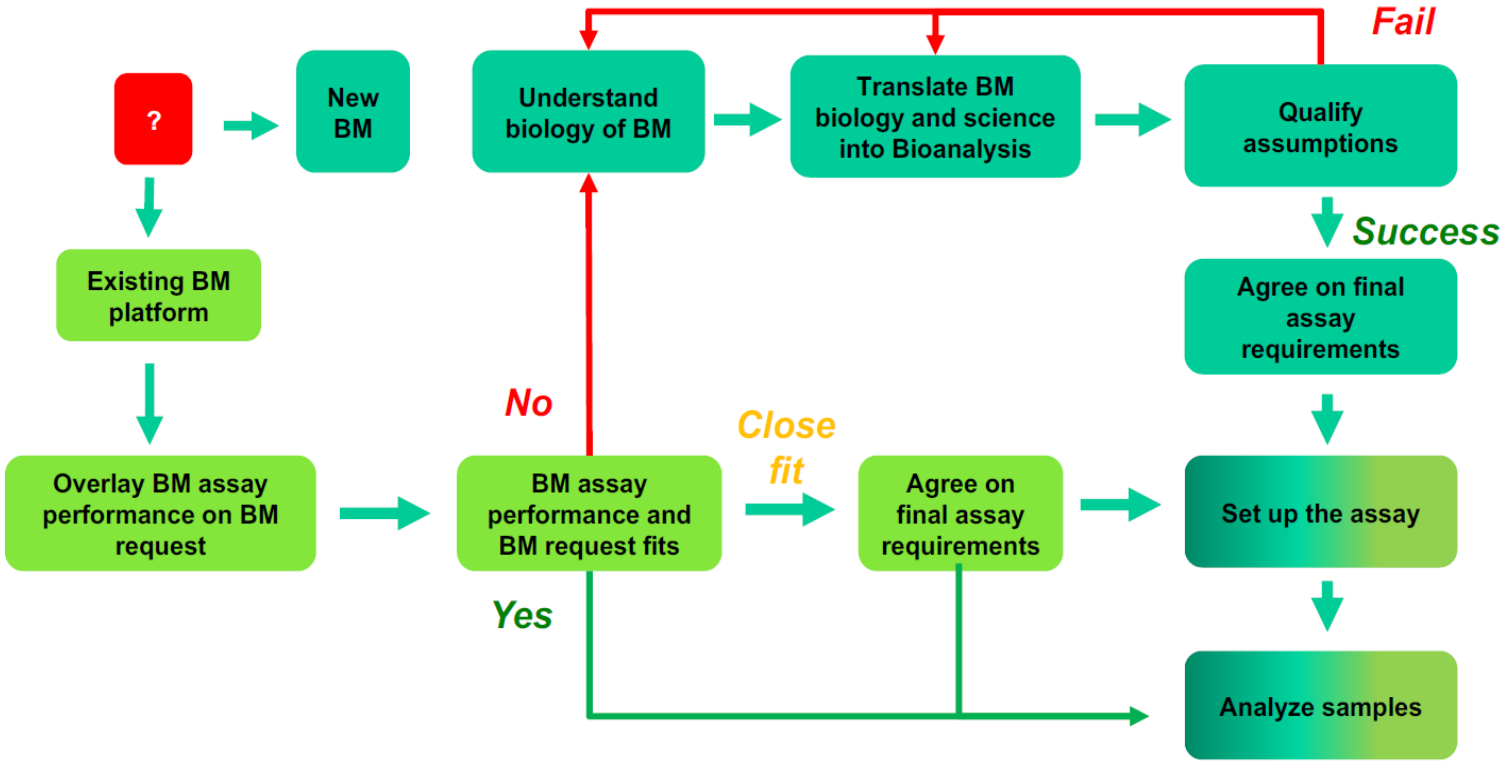
Or in 2020 language: other CoU



- The existing assay fits with the request:
  - Additional BM data within an ongoing project
    - copy established and earlier agreed acceptance criteria
    - run samples using existing (screening, qualified or validated) method and follow regulated bioanalysis standards as needed.
- The existing assay does not fit the request:
  - New request is in support of a different study design or a new project, and therefore requires different endpoints compared to the performance of the established assay
  - Agree on new assay requirements
    - Small changes needed → adapt method and move forward
    - Major changes needed → use flowchart “novel biomarker”




?
Existing BM platform
Overlay BM assay performance on BM request
BM assay performance and BM request fits
No
Flowchart: "New Biomarker"
Yes
Close fit
Agree on final assay requirements
Set up assay and analyze samples
Analyze samples



Bioanalysis (2012); 4(15): 1883–94

2019

Autumn Focus & 12th EBF OS

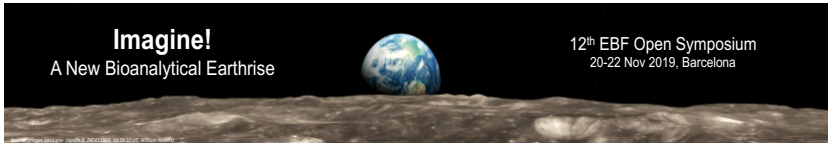
Autumn Focus Workshop

Biomarker Assay Validation

Bringing *Context of Use* into practice

NH Málaga Centro - Málaga, Spain

18-19 September 2019



12th EBF Open Symposium

Hesperia Tower, Barcelona, Spain

Imagine!

A New Bioanalytical Earthrise

Launchpad session

How is the Reg. BA community approaching CoU?

- Depends who you talk to…
- But we had serious doubts
- So…. A survey

Q1: Prior to setting up the assay, I have reached out to the end user of the data to discuss the assay requirements and/or be informed on the “biology”

- o Yes =
- o No =

Q2: Prior to setting up the assay, the end user provided me the precision required for the assay

- o Yes =
- o No =

More details in presentations: https://e-b-f.eu/wp-content/uploads/2019/12/bcn2019-program.pdf and https://e-b-f.eu/wp-content/uploads/2019/05/Final-agenda-17-05-2019.pdf

Yes

- Precision requested was tighter than “4-6-15/20”
- Precision requested was as for “PK assays, i.e. 4-6-15/20
- Precision was looser than 4-6-15/20

Required precision:

No

I validated the assay towards “4-6-15/20” as per PK SOP applicable in my lab

- Yes:
- No:

Required precision:

Q1: Prior to setting up the assay, I have reached out to the end user of the data to discuss the assay requirements and/or be informed on the “biology”

- Yes = 51 %
- No = 49 %

Q2: Prior to setting up the assay, the end user provided me the precision required for the assay

- Yes = 23 %
- No = 77 %

Digging deeper…

Q1: Prior to setting up the assay, I have reached out to the end user of the data to discuss the assay requirements and/or be informed on the “biology”

- Yes = 51 %
  - Pharma = 30
  - CRO = 21
- No = 49 %
  - Pharma = 12
  - CRO = 37

Majority of Pharma (30:12 ratio) speak to the “end user of the data”

Majority of CRO (21:37 ratio) say they don’t

Q1: Prior to setting up the assay, I have reached out to the end user of the data to discuss the assay requirements and/or be informed on the "biology"

- Yes = 51 %
- No = 49 %

Q2: Prior to setting up the assay, the end user provided me the precision required for the assay

- Yes = 23 %
- No = 77 %

And the detailed responses and discussions confirmed that talking to the end user isn't necessarily a CoU discussion…doesn't always result in agreeing CoU inspired assay requirements, but is…

…typically making the "PK-assay" a bit loser by adding 5 or 10% imprecision to the 4-6-xx paradigm

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In summary

Maybe only a few are on the "*Yes, we understand CoU and apply the principles*" Island

But most of us are on the other island:

- Yes, we (think we) understand CoU and apply the principles, but maybe we don't…
- No, we don't understand CoU and want to learn
- Yes, we understand CoU but cannot apply them (Mgtm, stakeholder or other barriers)

From the 2019 Focus Workshops

Round tables consensus: Where can EBF be of help?

1. Publish recommendation
2. Interact with authorities @ EBF level
3. Provide Training
4. Continue regular meetings as this one
5. Continue to connect with other cross industry groups

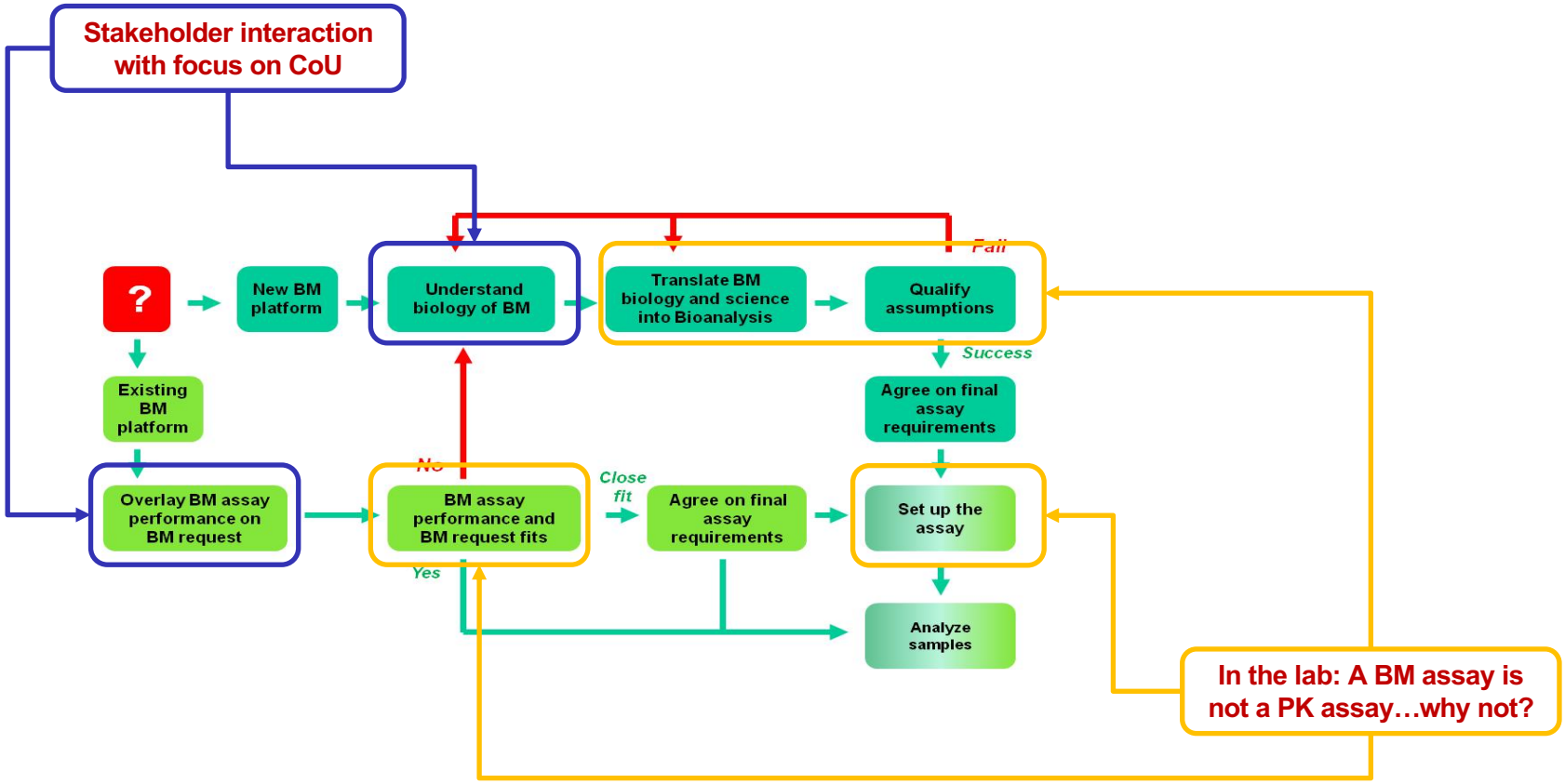
2020

1. Publish recommendation
2. Interact with authorities @ EBF level ? → in cross industry collaborations
3. Provide Training
4. Continue regular meetings as this one
5. Continue to connect with other cross industry groups

Submitted to Bioanalysis on 18AUG2020

Update to the European Bioanalysis Forum Recommendation on Biomarkers Assays; Bringing Context of Use into Practice

Joanne Goodman[1], Kyra J. Cowan[2], Michaela Golob[3], Lars Karlsson[4], Ulrich Kunz[5], Robert Nelson[6], Hans Ulrichts[7], Lauren Stevenson[8], Linda Terry[9], Philip Timmerman[10*]


Stakeholder interaction with focus on CoU
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New BM platform
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No
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Close fit
Agree on final assay requirements
Set up the assay
Yes
Analyze samples
In the lab: A BM assay is not a PK assay…why not?

All on board of the **HMS Biomarker**

Captain Jo and Captain Kyra will now take us to CoU island

Update to the European Bioanalysis Forum Recommendation on Biomarkers Assays; Bringing Context of Use into Practice

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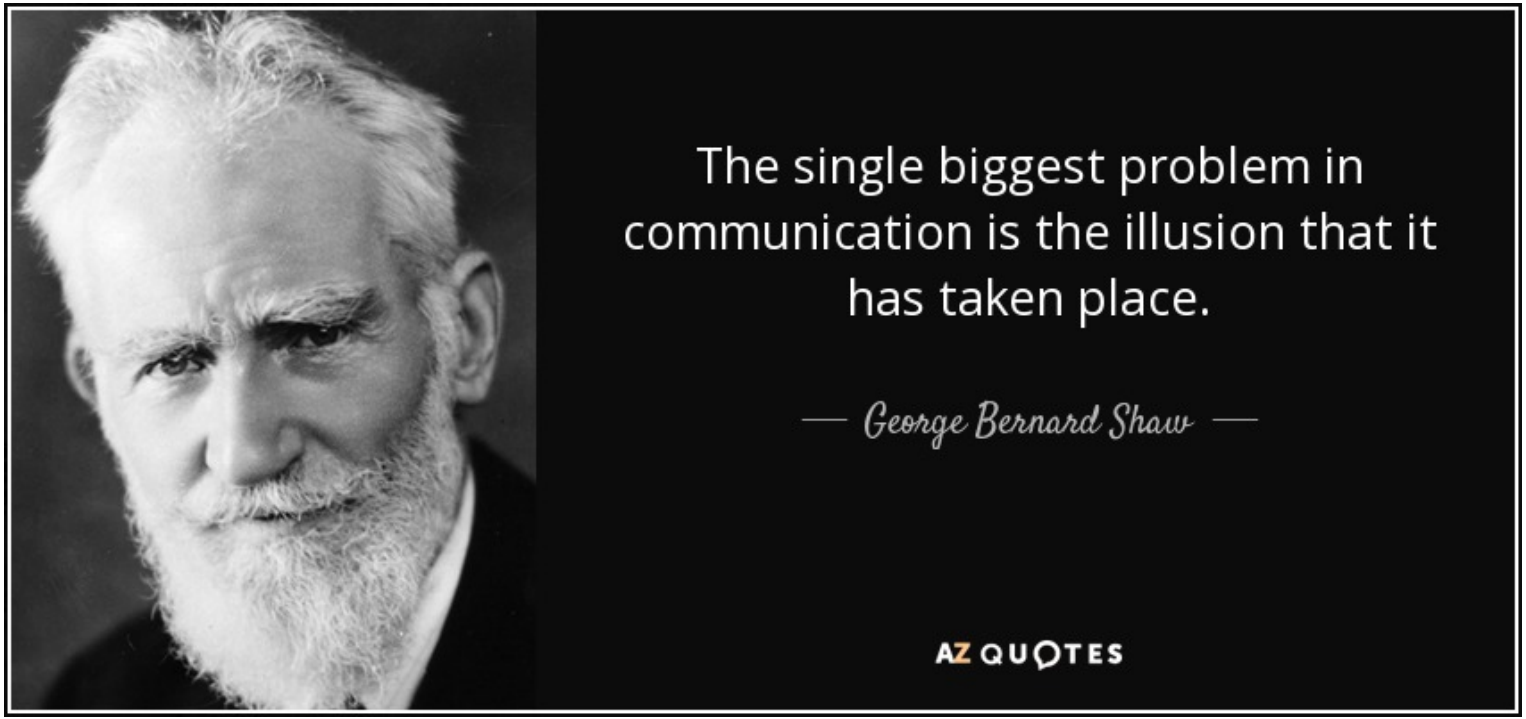
The story continues → 2 areas of focus of the Recommendation 2020

- Stakeholder communication Stakeholder interaction with focus on CoU
- In the lab: In the lab: A BM assay is not a PK assay…why not?

The 5th pillar - COMMUNICATION

Communicate, communicate, communicate:

- To understand the biology, pharmacological effect ... of the BM
- To understand what the data will be used for
  - Scientific decisions taken
  - Safety decisions taken
  - Other?
- To share what is possible from a BA perspective
- To share what is not realistic from a BA perspective
- To ensure optimal cost/benefit


The single biggest problem in communication is the illusion that it has taken place.
— George Bernard Shaw —
AZ QUOTES

Ensure the right conversation and mutual understanding

Communication can be challenging

- Being able to identify the relevant and appropriate people to truly understand COU
- Industries can be heavily siloed
- May not have embraced matrix teams
- Multiple layers of employees between the relevant individuals
- Vendor-CRO relationship can be challenging if the relevant scientists are not present
- May require education of stakeholders, especially when the main experience is around PK assays and be limited or non-existent for biomarkers
- Ideally sit at the table for project teams or at least have connections back to the team

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Bioanalysis may not be visible on the radar of stakeholders

- Bioanalysis can be an overlooked activity
  - Often only appears on the radar of **stakeholders** when there is a delay or assay challenges during development, validation or study sample analysis
- Many stakeholders may be ill-informed
  - Capabilities
  - Limitations of an assay
  - Data generated
- Bioanalytical scientist takes ownership and accountability to communicate with their stakeholders and provide adequate training

Stakeholder mapping is key

- **Be aware of Proximal and Distal stakeholders**
- **Understand the interactions between the groups**
- **The BioA scientist needs to own and drive the discussions**

- Examples of stakeholders may include:
  - Project Team
  - Clinical Teams
  - Biomarker/Translational Teams
  - Clin Pharm/Pharmacometricians/Modelling and Simulation
  - Biostats/Stats and Programming
  - Project managers – length of time needed and complexity
  - Line Management/Senior Management
  - Outsourcing Experts
  - CRO scientists
  - QA – validation requirements

Agree the COU to develop and validate the right assay for the right data and the right decisions

- COU is an agreement with stakeholders
- Decisions should be documented
  - COU statement
  - Assays may pass through different teams
  - People may leave
- Communication is not a one-time event
  - COU may change over time
  - Different questions and decisions may be needed
  - COU may need to be re-visited regularly
- Without an agreed COU there is a risk that of developing the wrong assay, with inappropriate validation
- Leads to incorrect data and decisions

Every assay needs to be developed and validated for the intended purpose

Update to the European Bioanalysis Forum Recommendation on Biomarkers Assays; Bringing Context of Use into Practice

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The story continues → 2 areas of focus of the Recommendation 2020

- Stakeholder communication Stakeholder interaction with focus on CoU
- In the lab: In the lab: A BM assay is not a PK assay…why not?

A BM Assay is NOT a PK Assay: Why Not?

- Key Challenges:
  - Scientific
  - Analytical
- Key Differences:
  - Starting material:
    - Endogenous vs. Recombinant
    - Platforms and reagents, kits available
  - Development and Validation
    - Parameters
    - Acceptance criteria
  - Regulatory Guidances:
    - Limited
    - Only mentioned in FDA

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Buckets do not address the issues…

A BM Assay is NOT a PK Assay: Why Not?

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Life Jackets do…

Challenges for BM Assays: Scientific

Challenge	Examples

Scientific	• Understanding the biology:
	  • Target population; anticipated biomarker levels for each population
	  • Endogenous form of the analyte (conformational structure, monomeric or multimeric)
	  • Biological mechanism and turn-over rate
	  • Intra- and inter-subject biological variability
	  • Effect of the drug on the biomarker
	  • Decisions taken based on the generated data.

Challenges for BM Assays: Scientific

Challenge	Examples
Scientific	• Sample collection and processing • How the data are being used and by whom • Appropriate assay validation assessments and acceptance criteria • COU changes - new indications, new genotypes, new emergent data - therefore the scientific aspects should be re-visited.

Challenges for BM Assays: Scientific

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Challenges for BM Assays: Analytical

Challenge	Examples

Analytical	• Progress in technology
	• Platform selection:
	  • Plentiful choices, with advantages and disadvantages.
	  • Soluble, on the surface of a cell, a direct marker of target engagement, measuring a downstream event, or genetic level.
	  • One platform may be optimal for one purpose and unsuitable for another.
	• In-house developed assays vs. adaptation of commercial kits
	• Lack of biomarker assay experts or repurposing PK assay experts to develop and validate biomarker assays

Challenges for BM Assays: Analytical

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Analytical variability and the achievable precision for an assay will be affected by assay platform and reagent choices.

A BM Assay is NOT a PK Assay: Starting Material?

- What is the "best" calibrator material for protein biomarker assays?
  - What characteristics are we looking for in a recombinant material?
  - Challenge is to match recombinant material with endogenous
    - Potential post-translation modifications, depending on disease-state, matrix, treatment regimen, genetics, environment…
- Are we measuring what we think we're measuring?
  - Specificity vs. Interference
- Is the reagent reliable as a calibrator?
  - Parallelism – must be assessed early on in assay development/characterization
  - Lot-to-lot variability
  - Stability
- Surrogate matrix?

- Take home message: know your assay and what it can detect.

A BM Assay is NOT a PK Assay: Development and Validation?

- **"Known" biomarker:** available kit and/or published data may not be applicable for the COU, may complicate discussion/agreement with stakeholders. If chosen, will likely need additional characterization.

- **Unknown biomarker:** start assay development, focus on screening individual matrices (healthy & diseased) for biological and physiological variability.

- **Criteria-free analysis** suggested, with retrospective run acceptance:
  - Assess biological variance and the analytical performance of the assay (hypothesis testing).
  - Significant effect must consider the actual performance of the assay.
  - Assay must be specific and sensitive enough to detect the endogenous biomarker of interest.
  - Sufficient precision is the second priority.

A BM Assay is NOT a PK Assay: Development and Validation?

The voyage is ever-changing…

…but some things stay the same:

- **Development**: more or less constant experiments (depending on analytical technique), independent of COU:
  - Parallelism (Selectivity, MRD, LLOQ)
  - Specificity
  - Detectability in target matrix

- **Validation:** a “rubber stamp”, based on previous assay characterization, and not equal to development.
  - Validation purely confirms, in a controlled environment, what is already known from the experiments conducted in method development.

Challenges for BM Assays: Regulatory Guidances?

Challenge	Why categories may not be helpful

Regulatory: In the absence of anything else, there is a default to the misapplication of PK approaches and criteria…	• COU is everything, and may change over time
	  • Diversity and complexity of biomarker assays is wide, a framework may stifle the crucial conversations that are needed for defining the assay purpose.
	  • Wrong COU: inappropriate acceptance criteria, poor use of resources and time, wrong decisions, failed drug development.
	  • COU must be re-evaluated as the „purpose“ changes, will dictate assay characterization and much later validation.
	  • Decisions need to be driven by the science, not a framework or categories.

EBF Recommendations on BM Assay Characterisation

- COU must first be defined and agreed upon by all stakeholders:
  - **EBF recommends** that the requirements for assay validation occurs, and is agreed upon, as part of the COU conversation with the relevant stakeholders.

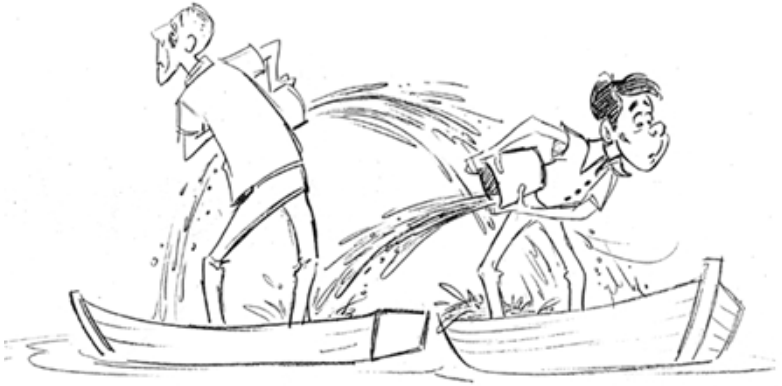
- Key Topics:
  - Type of assay required (e.g. free or total, in-house assay, commercial kit, single analyte, multiplex, research use, diagnostic)
  - Format of the assay and critical reagents
  - Technology choice, with pros and cons
  - Do you have access to biomarker samples that are reflective of the subjects (e.g. commercial or samples from other trials, biobank)?

EBF Recommendations on BM Assay Characterisation

- Several BM assay-specific parameters should be evaluated early on:
  - Precision: one aspect - biological variability in population, as well as analytical variability present within the assay.
  - Parallelism, selectivity, specificity, stability and sample processing must be equally evaluated.

- Avoid categories or buckets for BM assays when starting with method development:
  - **EBF does not recommend definitive terms for dividing up into differing purposes, which may result in inappropriate regulatory hurdles being created around biomarker validation.**

- The term “fit for purpose” or “qualified” rather than “fully validated” can create a perspective that the quality of the assay is somehow inferior. However, in practice this is not the case.



EBF Recommendations on BM Assay Characterisation

Take Home Message:

All BM assays are “fully validated” for the specific COU.

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