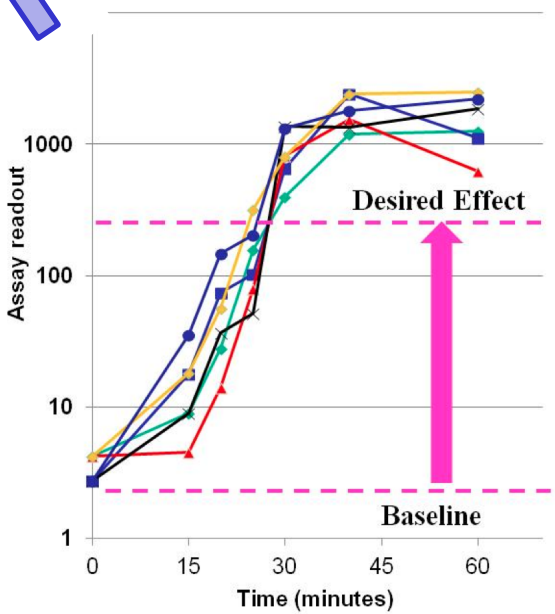
EBF Open Symposium

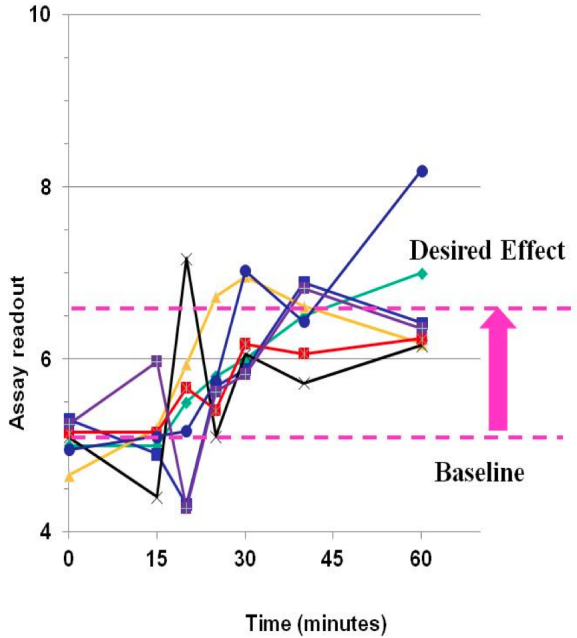
N° 13 From Cyberspace - Staying Connected

The 2020 EBF Recommendation on BM Assay Validation
Key points to consider when implementing CoU practices

Joanne Goodman, Kyra Cowan - On behalf of the EBF

2011





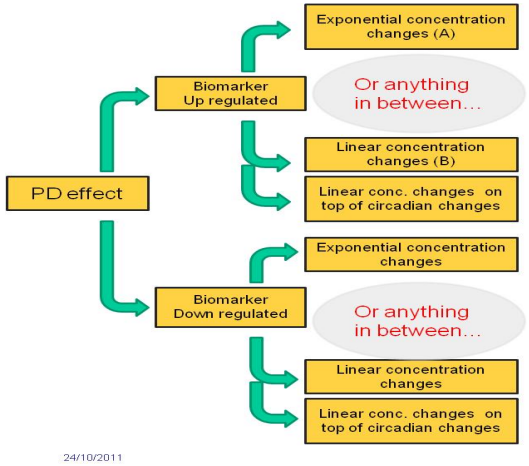
All measured with 4-6-15 “PK” assay, but was this necessary?

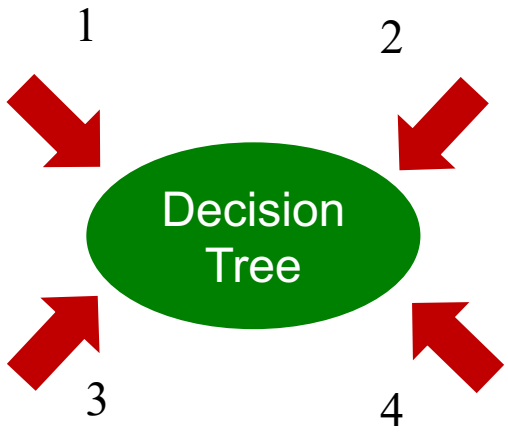
2011 - EBF reflections on biomarker classification

When developing a Biomarker assay, consider:

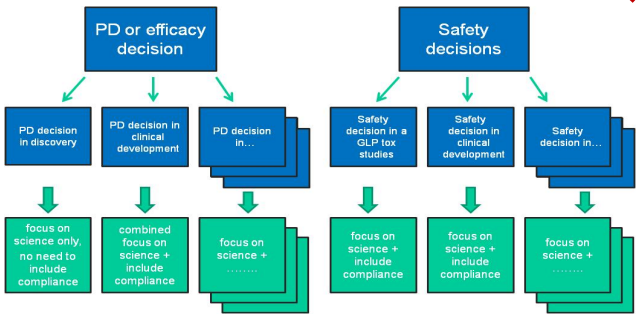
1. Observed or anticipated biomarker levels
2. Development Phase in which a biomarker is measured
3. Decisions taken from the biomarker data, e.g. efficacy, safety…
4. Fit of assay with Regulated Bioanalysis Guidelines

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

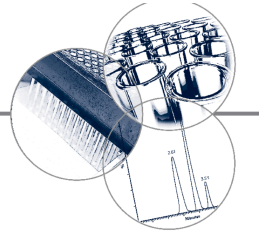
Inform and be informed

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)

WHITE PAPER



European Bioanalysis Forum recommendation on method establishment and bioanalysis of biomarkers in support of drug development

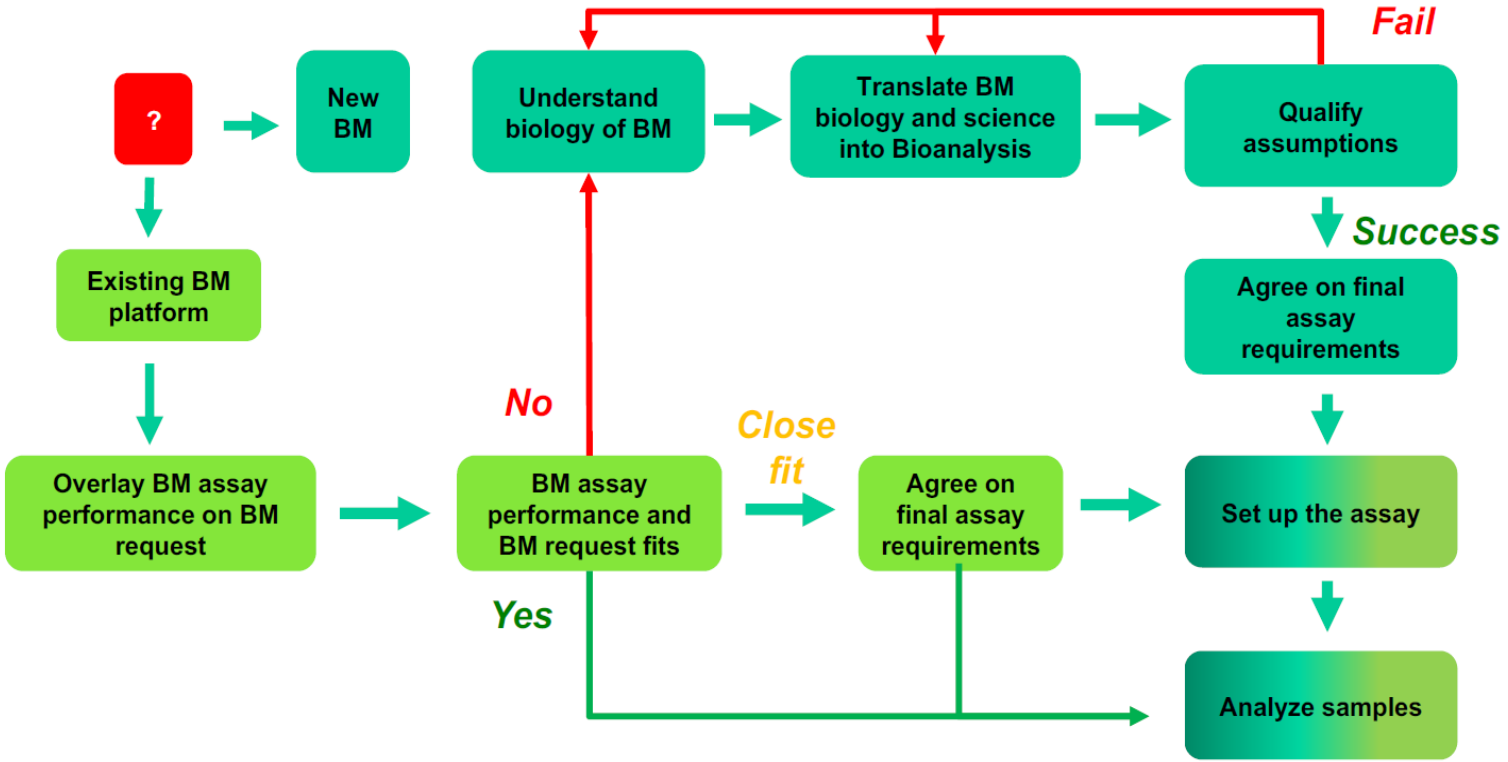
Biomarkers have become increasingly important in drug development and many bioanalysts are getting involved. Consequently, different views on how to approach the bioanalysis of biomarkers have been published or are being developed. The European Bioanalysis Forum has intensively discussed this topic since 2010 and is ready with their recommendation on method establishment and bioanalysis of biomarkers. Acknowledging that the challenges step outside the bioanalytical laboratory is a cornerstone of our recommendation. The importance of integrating all scientific aspects, from purely analytical aspects, all the way to understanding the biology and effects of the biomarker, prior to embarking on method establishment or sample analysis, cannot be underestimated. Close and iterative interactions with the teams requesting the data is imperative to develop a bioanalytical strategy that combines science, analytical performance and regulations. The European Bioanalysis Forum developed a straightforward decision tree to help the scientific community in developing a bioanalytical strategy for any biomarker in drug development.

1. Introduction & scope

In this manuscript, the European Bioanalysis Forum (EBF) reports back from their internal discussions on the method establishment and bioanalysis of biomarkers in support of drug development performed in the regulated bioanalytical environment. Initially, these discussions were an integral part of an EBF subteam assigned to provide a recommendation on the (bio)analytical community's approach to biomarker bioanalysis [3]. Nevertheless, although the latter paper provides excellent insight into the science of how to approach biomarker bioanalysis, the EBF experienced that the industry was moving forward too often to analyze biomarkers using existing regulated bioanalysis standards [4,103–105] or remained confused on fully embracing the opportunities and tiered

Philip Timmerman*[1], Christian Herling[2], Daniela Stoellner[3], Birgit Jaitner[3], Susanne Pihl[4], Karen Elsby[5], Neil Henderson[5], Begona Barroso[6], Stephanie Fischmann[7], Arjen Companjen[8], Amanda Versteilen[8], Stewart Bates[9], Clare Kingsley[10]

Bioanalysis (2012); 4(15): 1883–94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New BM
Understand biology of BM
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Overlay BM assay performance on BM request
BM assay performance and BM request fits
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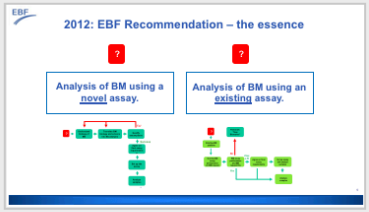
Set up the assay
Yes
Analyze samples

Flowchart detail can be found in the publication or on our website



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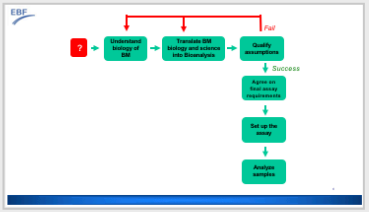

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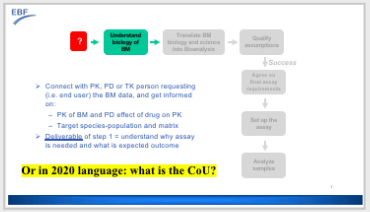

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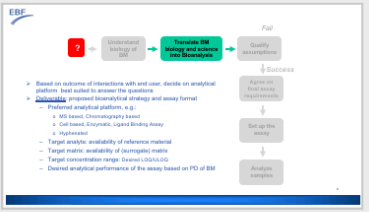

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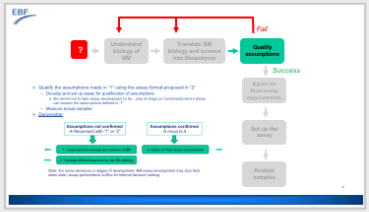

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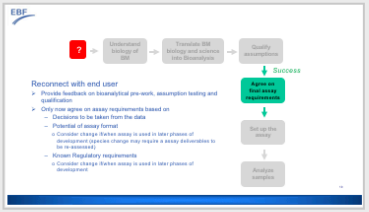

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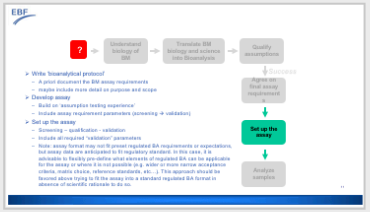

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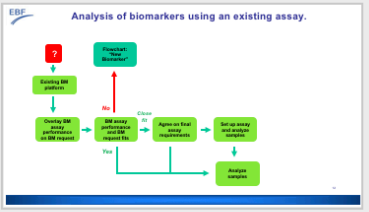

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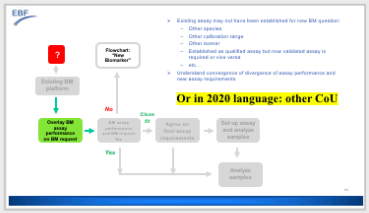

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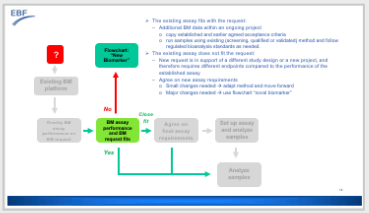

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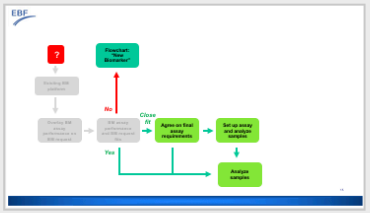

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2019

Autumn Focus & 12th EBF OS

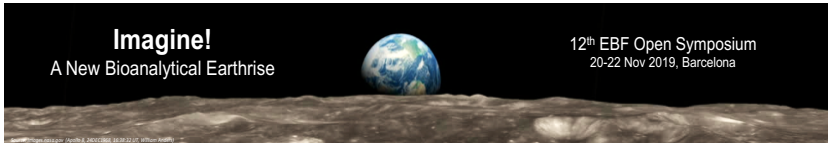
Autumn Focus Workshop

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

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 %

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

12

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

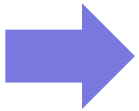
But most of us are on the other island:

- 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)

Actions from the 2019 Focus Workshops

Where can EBF be of help?

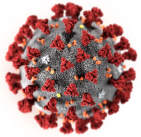
1. Publish recommendation
2. Interact with authorities @ EBF level
3. Provide Training
4. Continue regular meetings as this one
5. Continue to connect with other cross industry groups



2020

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**



EBF

Autumn Focus Workshop

The FW is organised in collaboration with the Biomarker and Precision Medicine Community (AAPS), CBF and JBF

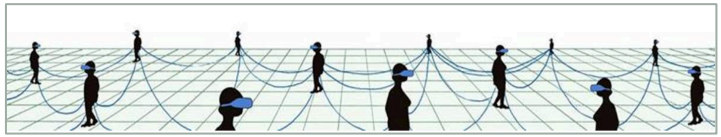
aaps

Biomarkers in Pharma R&D

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

In CYBERSPACE

15-17 September 2020



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aaps

Biomarkers in Pharma R&D

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

In CYBERSPACE

15-17 September 2020

Learnings from 2019 confirmed

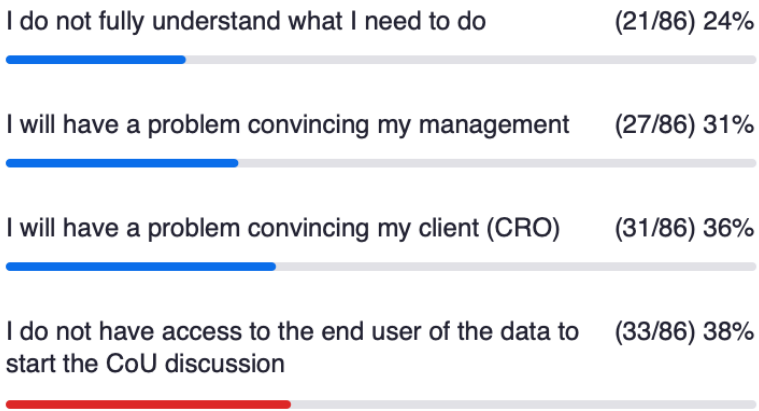
- 2020-FW workshop confirms the community struggles to apply CoU

A lot are still on the other island:

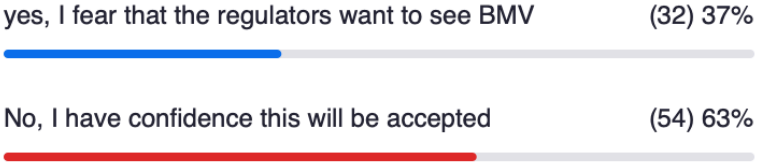
- Hurdles didn't change
  - Difficult to identify or get stakeholder/end-user engaged
  - Fear for 483
  - Fear to leave SOP-comfort zone

We polled the 2020 delegates at the end of the FW

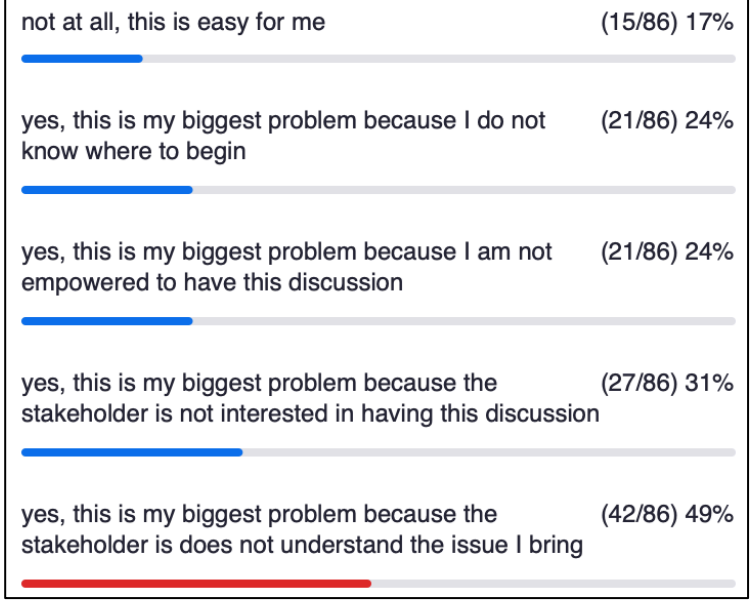
2. What will be the most difficult hurdles for you to take to apply CoU(Multiple Choice)



3. I fear the regulators will not accept a CoU based assay validation



6. But my main problem is identifying the stakeholder and get him/her engaged..(Multiple Choice)



- From here - Part 2 → 2nd presenter

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

2020

White Paper

Bioanalysis

For reprint orders, please contact: reprints@future-science.com

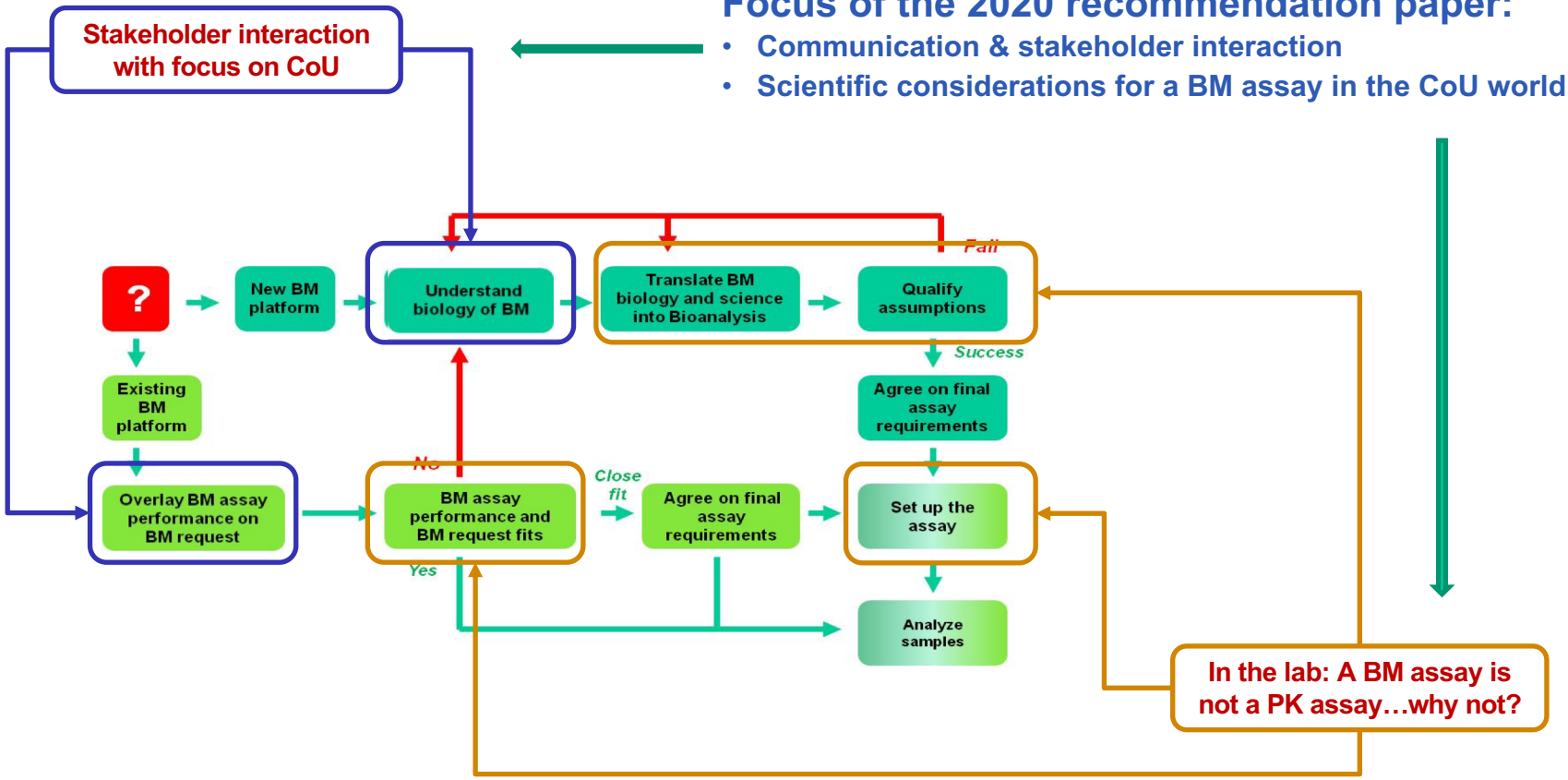
Update to the European Bioanalysis Forum recommendation on biomarkers assays; bringing context of use into practice

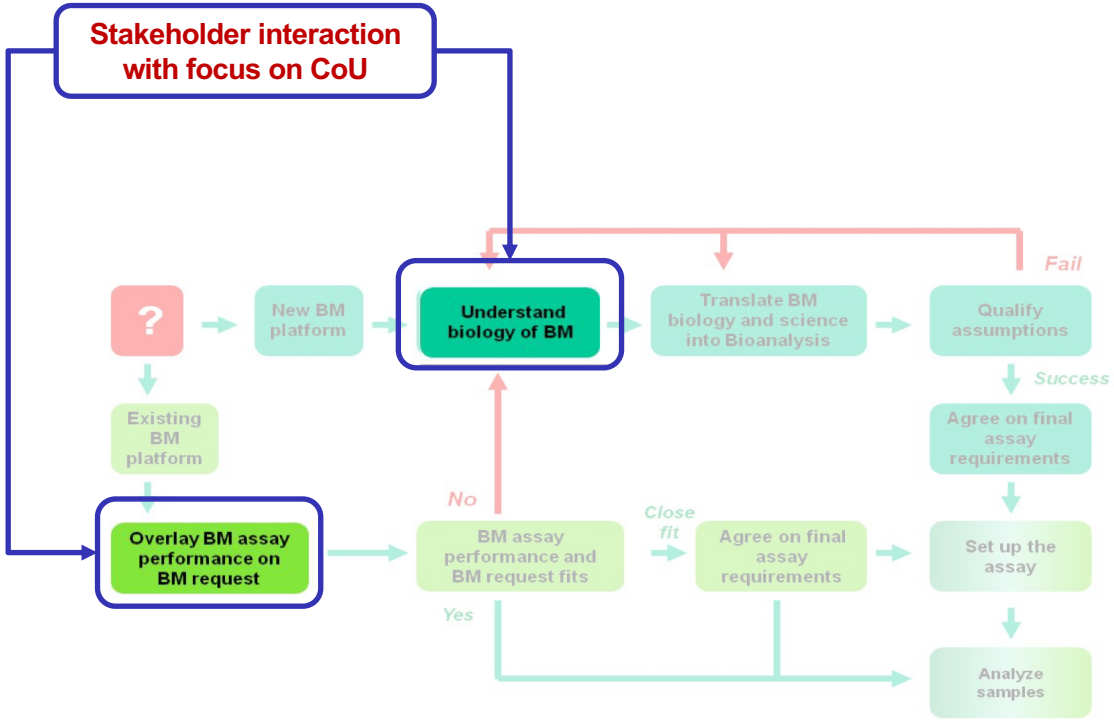
Joanne Goodman[1], Kyra J Cowan[2], Michaela Golob[3], Lars Karlsson[4], Ulrich Kunz[5], Robert Nelson[6], Hans Ulrichts[7], Lauren Stevenson[8], Linda Terry[9] & Philip Timmerman*,[10]

Bioanalysis (2020) 12(20), 1427–1437

Focus of the 2020 recommendation paper:

- Communication & stakeholder interaction
- Scientific considerations for a BM assay in the CoU world

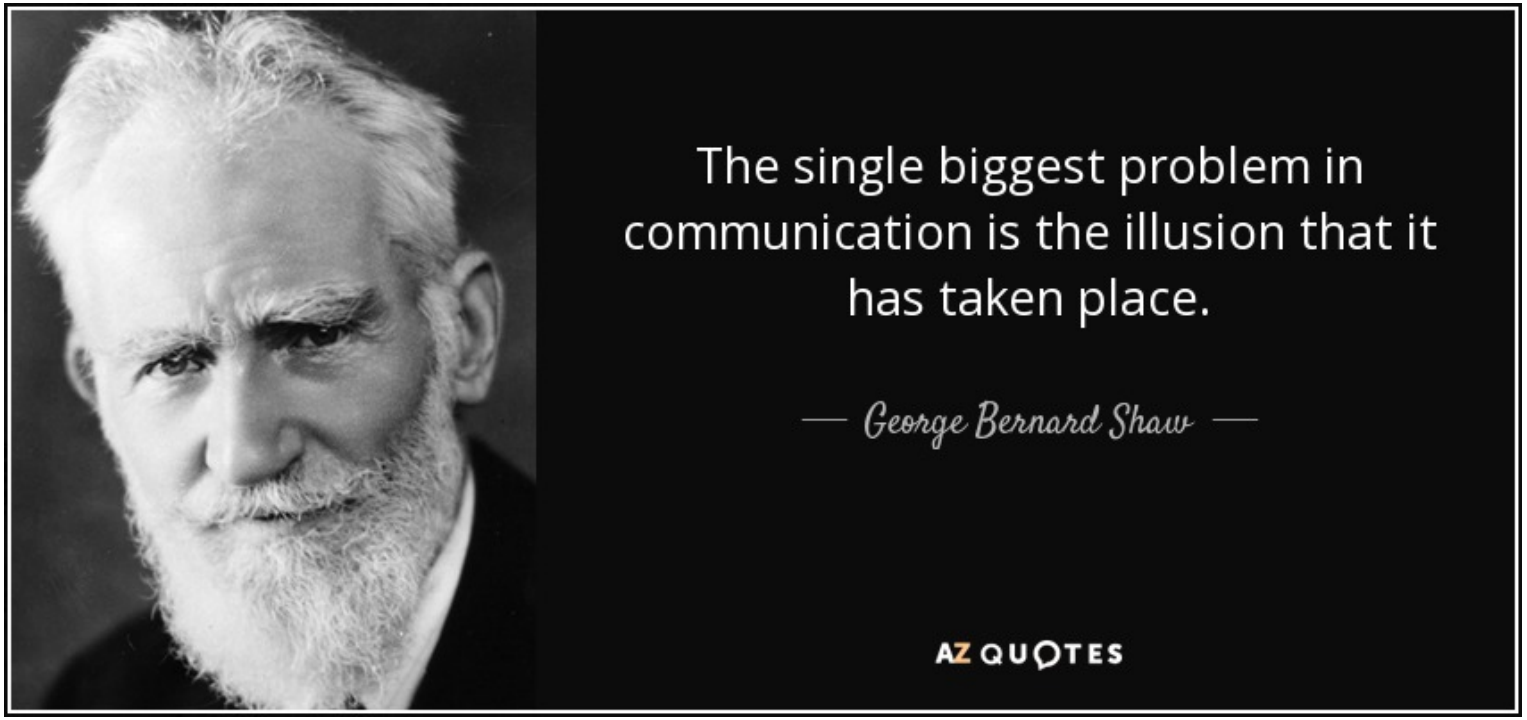



Stakeholder interaction with focus on CoU
?
New BM platform
Understand biology of BM
Translate BM biology and science into Bioanalysis
Qualify assumptions
Fail
Success
Existing BM platform
Agree on final assay requirements
Overlay BM assay performance on BM request
No
BM assay performance and BM request fits
Close fit
Agree on final assay requirements
Set up the assay
Yes
Analyze samples

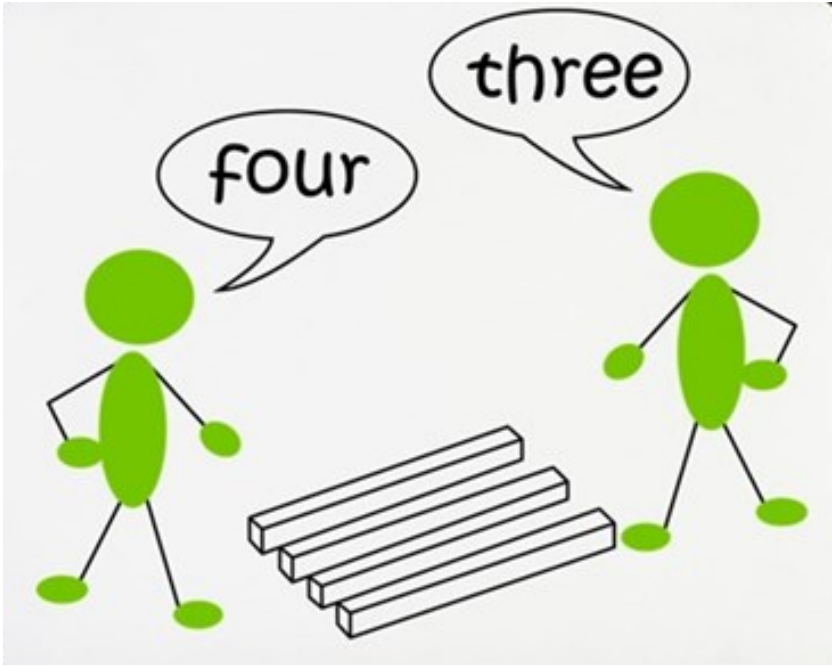
The 5th pillar - COMMUNICATION

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

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

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

Full Access

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

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

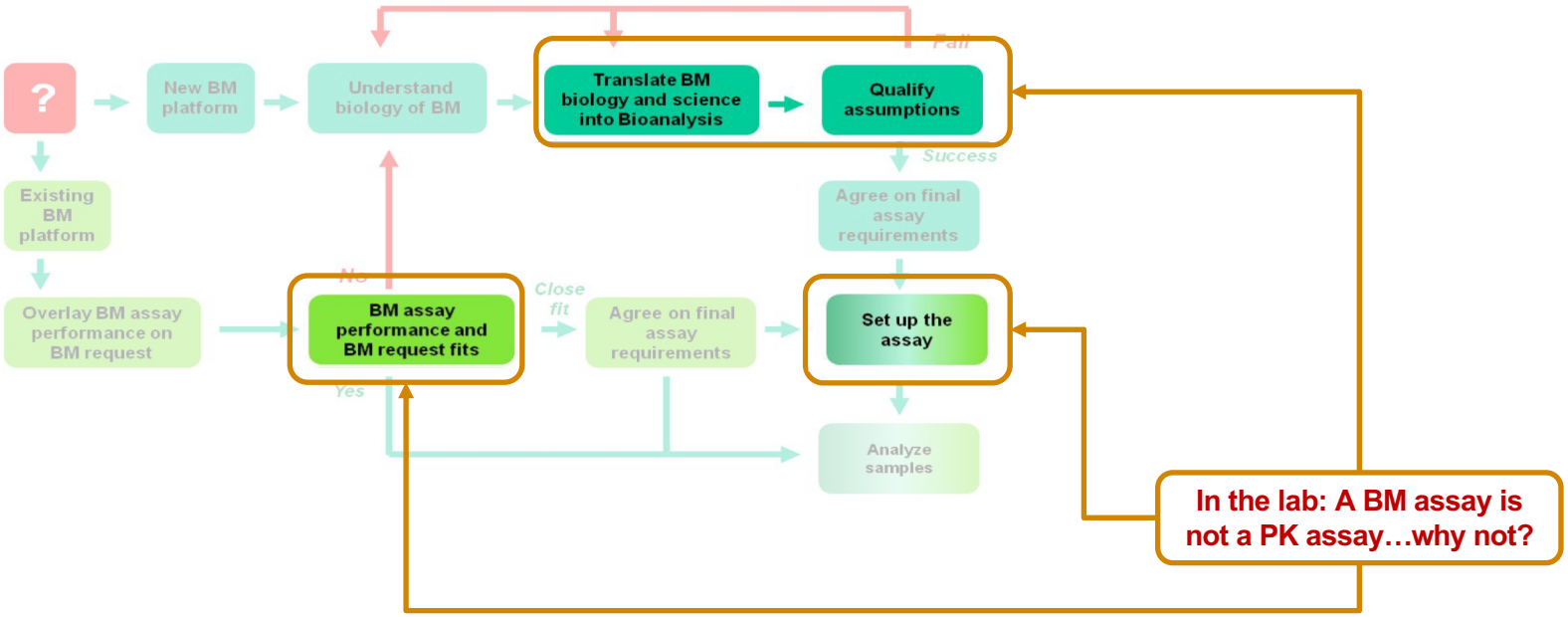
- **Be aware of Proximal and Distal stakeholders**
- **Understand the interactions between the groups**
- **The BioA scientist needs to own and drive the discussions**

- 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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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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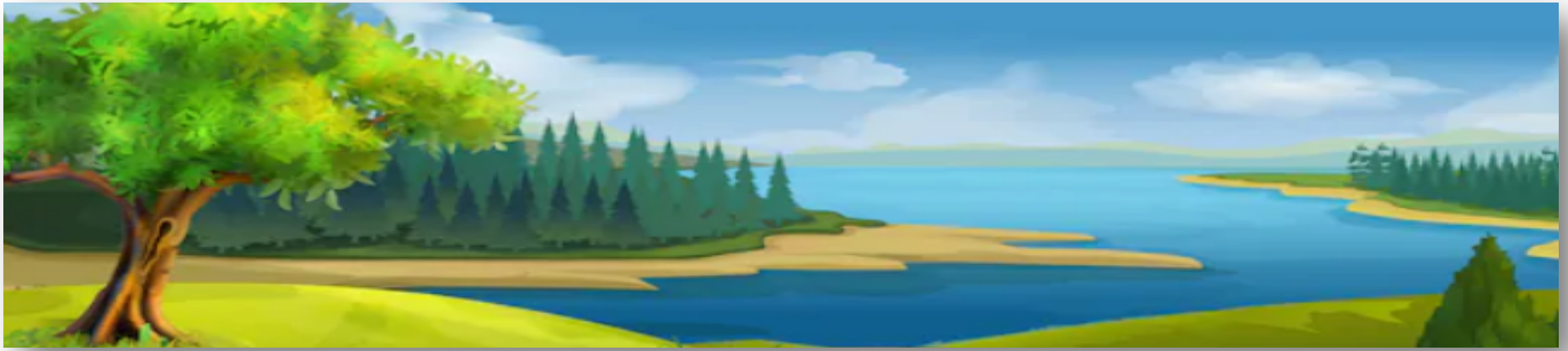

Challenges for BM Assays: Analytical

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

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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

Regulatory: In the absence of anything else, there is a default to the misapplication of PK approaches and criteria…	• COU is everything, and
	  • Diversity and compl framework may stifl needed for defining
	  • Wrong COU: inappr of resources and tim development.
	  • COU must be re-eva dictate assay charac
	  • Decisions need t framework or cat

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.

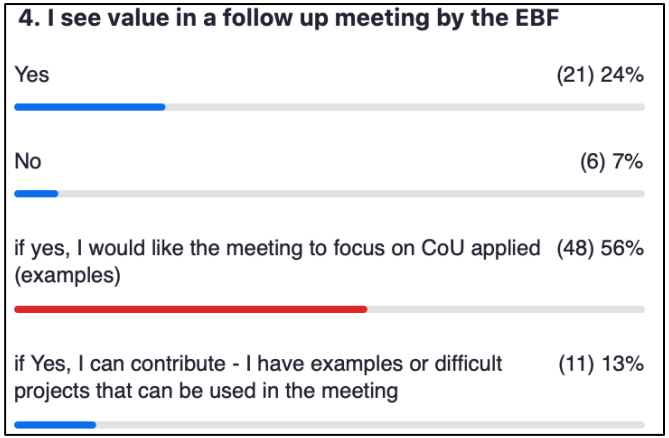
2021

EBF Cyberconnect Event in (e.o.) April 2021

A meeting (two ½ days) providing tools to bring CoU into practice:

- Manage stakeholder interactions in day-to-day practice
- Continue discussions on Scientific value vs. copy from the comport zone/PK BMV, e.g.
  - Don't get dragged into the ISR rabbit hole for BM assays
  - The importance of parallelism
  - Do we understand the matrix
  - The challenge of the reference standard
- Starting from examples
- Bringing stakeholders to the table

From the poll….



EBF Recommendations on BM Assay Characterisation

Take Home Message:

All BM assays are "fully validated" for the specific COU.

Acknowledgment

- Past and current EBF Biomarker team members for driving
- EBF Community for continued input and discussion
- Experts in Partner organisation i.e. AAPS, JBF, CBF
- Delegates 2019/2020 Focus Workshop and 12th EBF Open Symposium