Biomarker Assay Validation – a status update on the EBF Recommendation and discussions in Industry

Marianne Scheel Fjording, on behalf of the EBF

EBF Focus Workshop Biomarkers – Lisbon – 9/10 June 2016

Outline

1. The EBF Recommendation
2. Discussion in Industry
3. Crystal City VI

Biomarker Assay Validation

2015 AAPS Workshop on

Crystal City VI: BMV on Biomarkers

September 28–29, 2015
Renaissance Baltimore Harborplace Hotel
Baltimore, Md.

Pharmaceutical Research, Volume 23, No. 2, February 2006 (© 2006)
DOI: 10.1007/s11095-005-9045-3

Research Paper

Fit-for-Purpose Method Development and Validation for Successful Biomarker Measurement

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

EBF Recommendation on method establishment and bioanalysis of Biomarkers in support of drug development.

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], Ulrich Kunz[11]

[1]Janssen R & D, [2]NovoNordisk, [3]Novartis, [4]Lundbeck, [5]AstraZeneca, [6]Astellas, [7]Abbott, [8]Crucell, [9]GlaxoSmithKline, [10]Quotient Bioresearch, [11]Boehringer-Ingelheim,

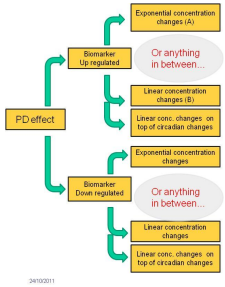
Bioanalysis 4(15), August 2012)

+

Pointer in *Biomarkers in Medicine*

August 2012, Vol. 6, No. 4, Pages 507-509

EBF recommendation



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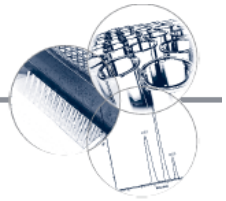

European Bioanalysis Forum recommendation on method establishment and bioanalysis of biomarkers in support of drug development

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 practical implementation of the tiered approach principles. This subteam started their activities in 2008, following up on the publication of the Crystal City III proceedings [1] and have already reported back on a first deliverable: application of the tiered approach applied in the quantification of metabolites in relation to the Metabolites in Safety Testing guideline [2]. In 2010, the EBF wanted to give priority to a recommendation in light of the ongoing Metabolites in Safety Testing discussions after the publication of the related regulatory guidances [101,102].

From 2010 onwards, an EBF biomarker team was formed out of the aforementioned tiered approach team to further investigate how the EBF can contribute to the already intense discussions on biomarker bioanalysis within the global bioanalytical community. We acknowledged the many interesting and important technical papers and White Papers already published on the bioanalysis of biomarkers. Certainly, articles such as the 'fit-for-purpose' paper impacted 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 approach of these 'fit-for-purpose' principles. Consequently, the EBF Biomarker team, consisting of bioanalytical experts from both pharmaceutical companies and CROs, identified the need to contribute to this discussion by integrating the internal EBF knowledge and reflections on the tiered approach with the already existing practices applied for the bioanalysis of a biomarker into an EBF recommendation.

As part of our recommendation and publication strategy, preludes of the insights in this manuscript were already shared for input and socializing at the 4th Open Symposium in Barcelona, Spain [5].

Although all our discussions intended to refer to biomarker analysis requests entering the traditional regulated bioanalysis laboratory, irrespective of size of the molecule of analytical technique involved, the principles of the recommendations we propose in this manuscript may also apply for other areas such as diagnostics or commercial immunoassay kits or similar. We did

Philip Timmerman[a1], 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] & Ulrich Kunz[11]

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[2]NovoNordisk
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[5]AstraZeneca
[6]Astellas
[7]Abbott
[8]Crucell
[9]GlaxoSmithKline
[10]Quotient Bioresearch
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Contributors and other EBF member companies are listed at end of article.

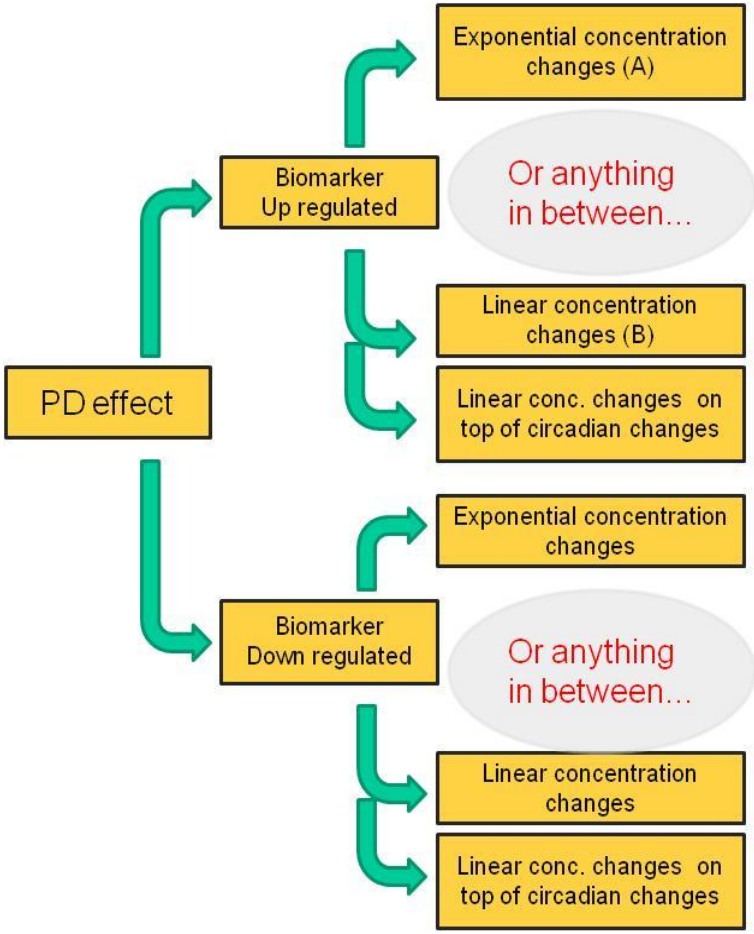
FUTURE SCIENCE part of fsg

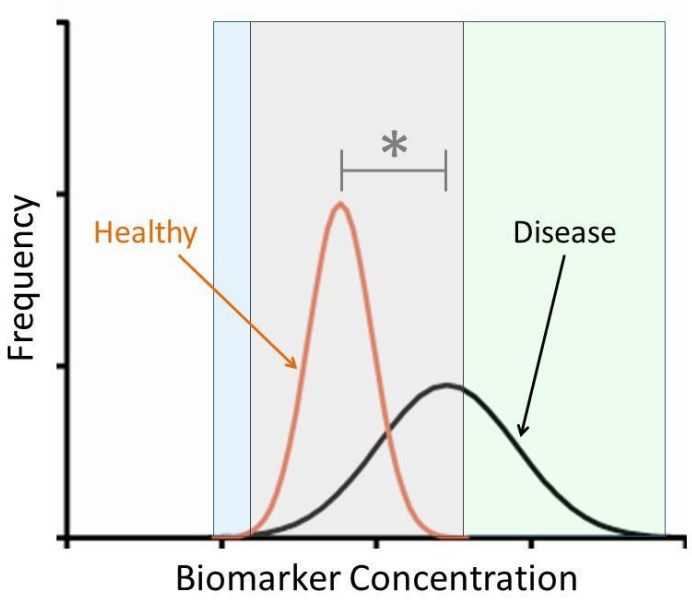
Adhere to Regulated BA guidelines

Nice to have | Need to have

4 pillars
Drivers for BM assay validation

1. Observed or anticipated biomarker level changes





4 pillars
Drivers for BM assay validation

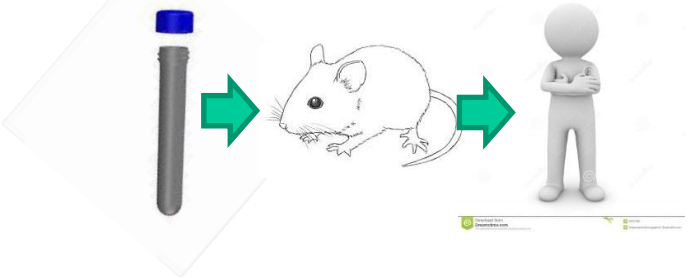
2. Development Phase in which a biomarker is measured

Discovery → Non-clinical → Phase I → Phase 2 → Phase 3 → Phase 4

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**

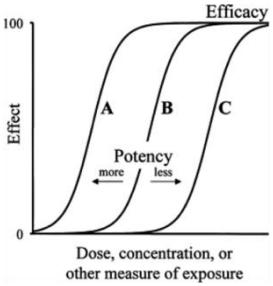
Translational

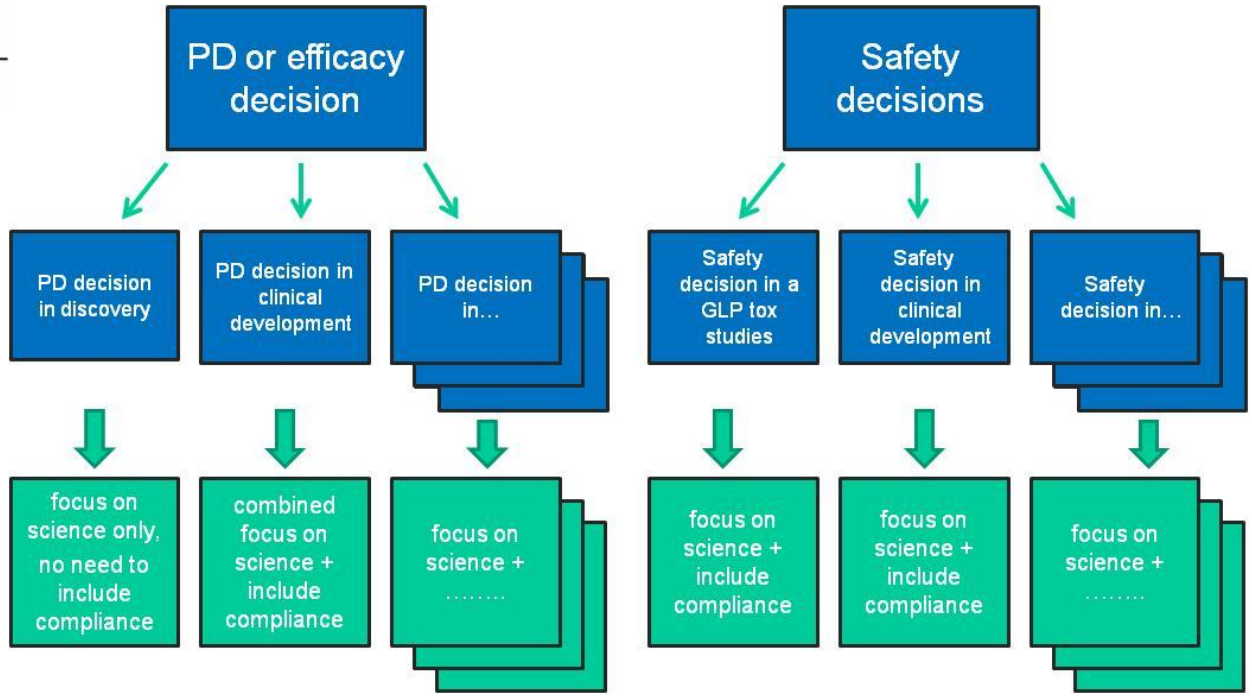
In vitro *In vivo* In man



4 pillars
Drivers for BM assay validation

3. Decisions taken from the biomarker data
   - efficacy decisions
   - safety decisions



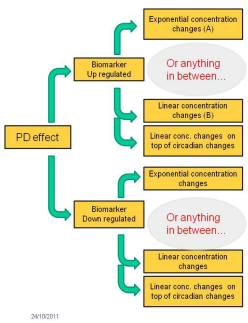


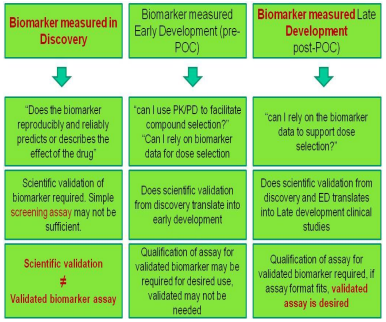
4 pillars
Drivers for BM assay validation

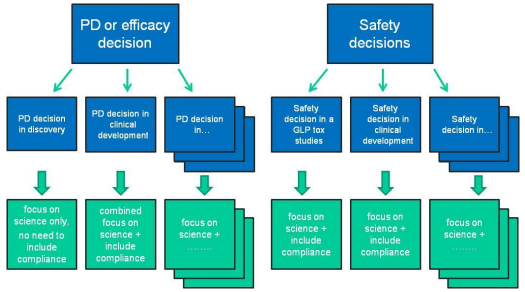
4. Fit of assay with Regulated Bioanalysis Guidelines

4 pillars
Drivers for BM assay validation

1 2 3 4



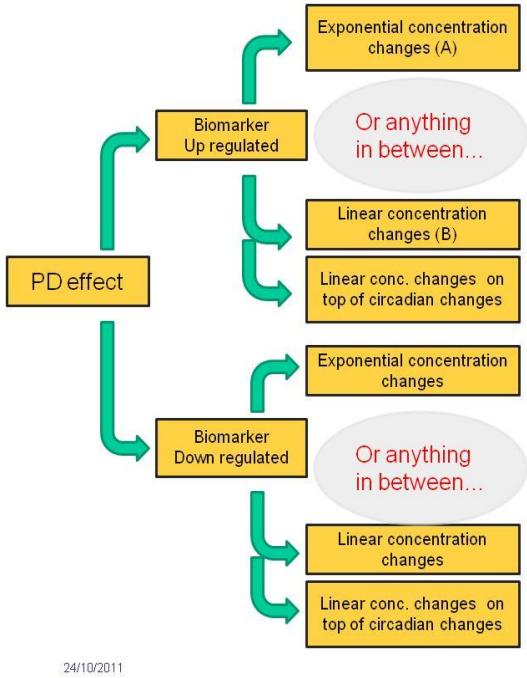




1. Observed or anticipated biomarker level changes
2. Development Phase in which a biomarker is measured
3. Decisions taken from the biomarker data
   - efficacy decisions
   - safety decisions
4. Fit of assay with Regulated Bioanalysis Guidelines

Above classification are superimposable - should be applied in concert to tailor an bioanalytical strategy in support of a Biomarker

Bioanalytical Strategy



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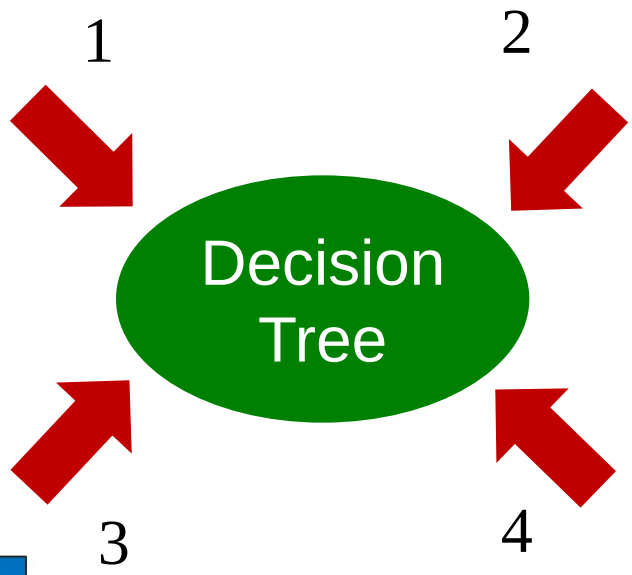
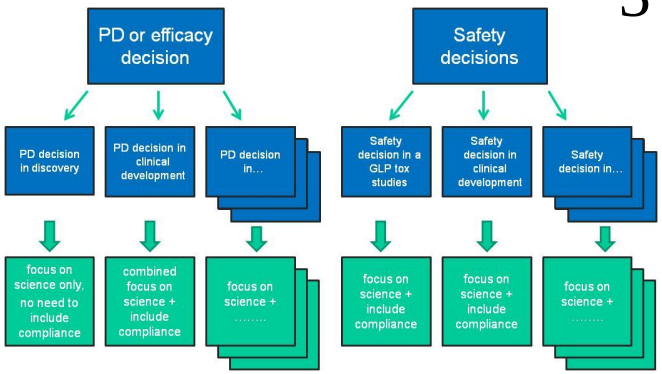




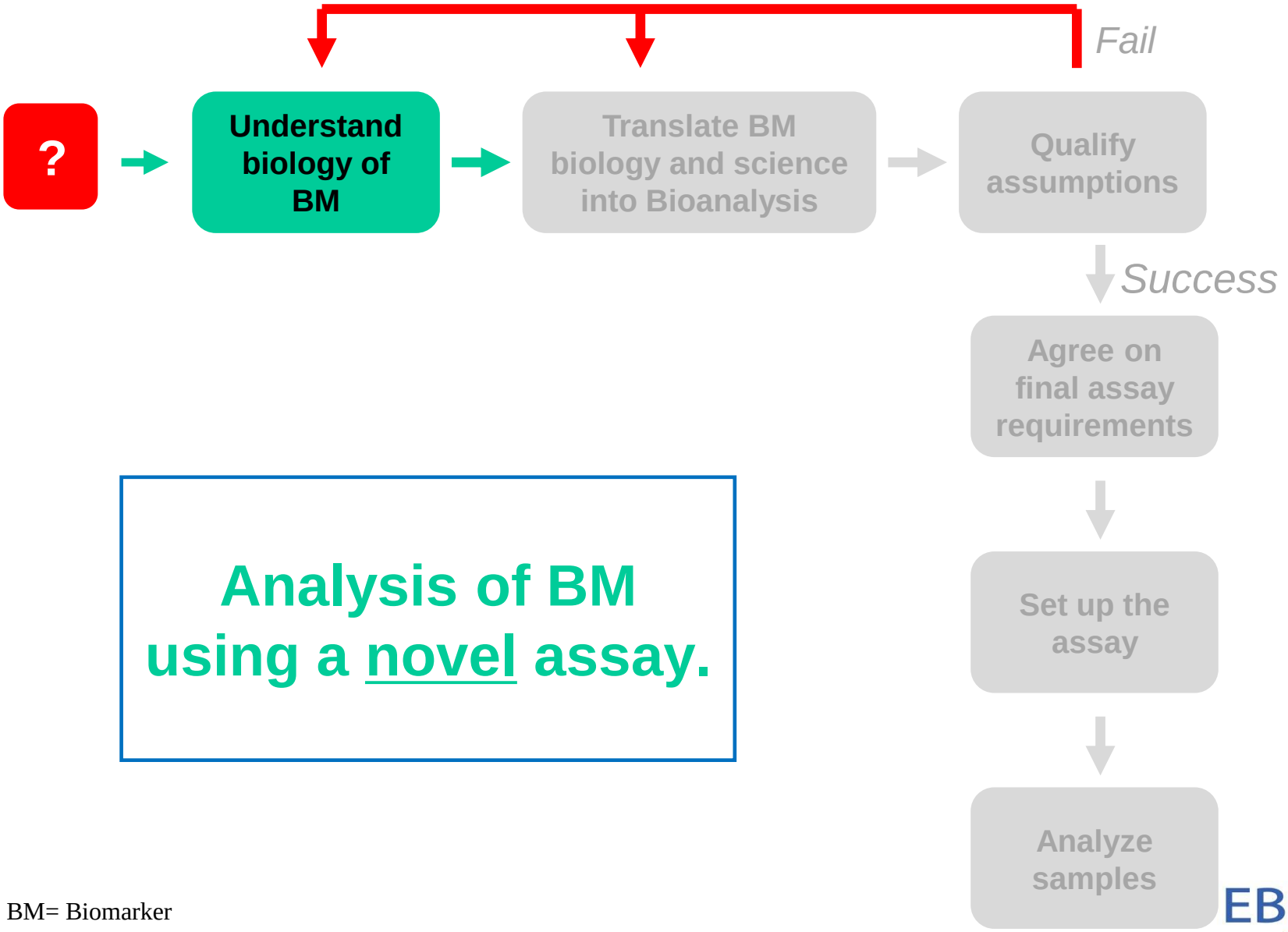
2012: EBF recommendation

Analysis of BM using a novel assay.

Analysis of BM using an existing assay.

BM= Biomarker

Analysis of biomarkers using a novel assay.



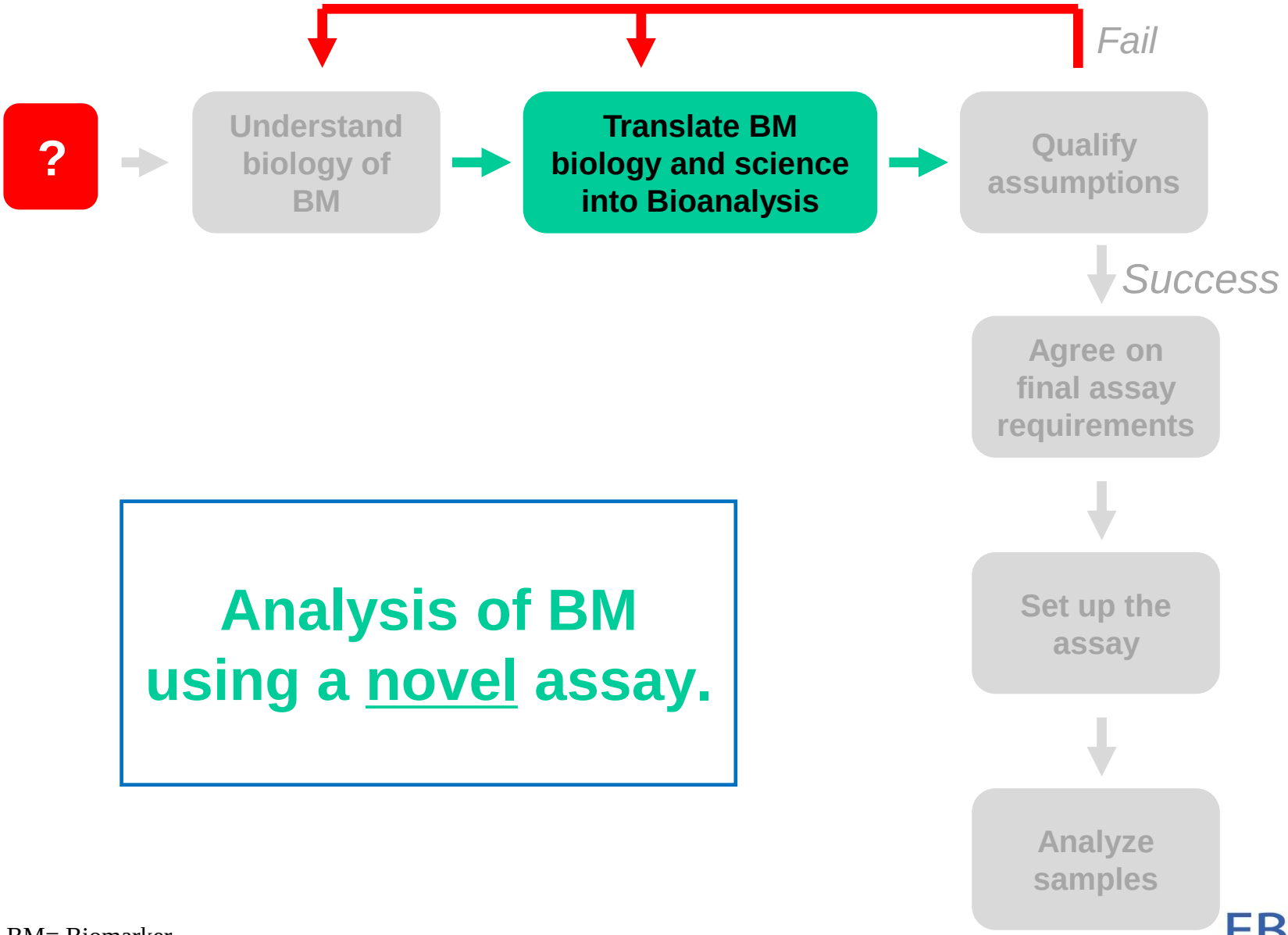
BM= Biomarker

1. Understand the biology and science of BM

- Connect with PK, PD or TK person requesting (='requester') 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. The intended use of the data

BM= Biomarker



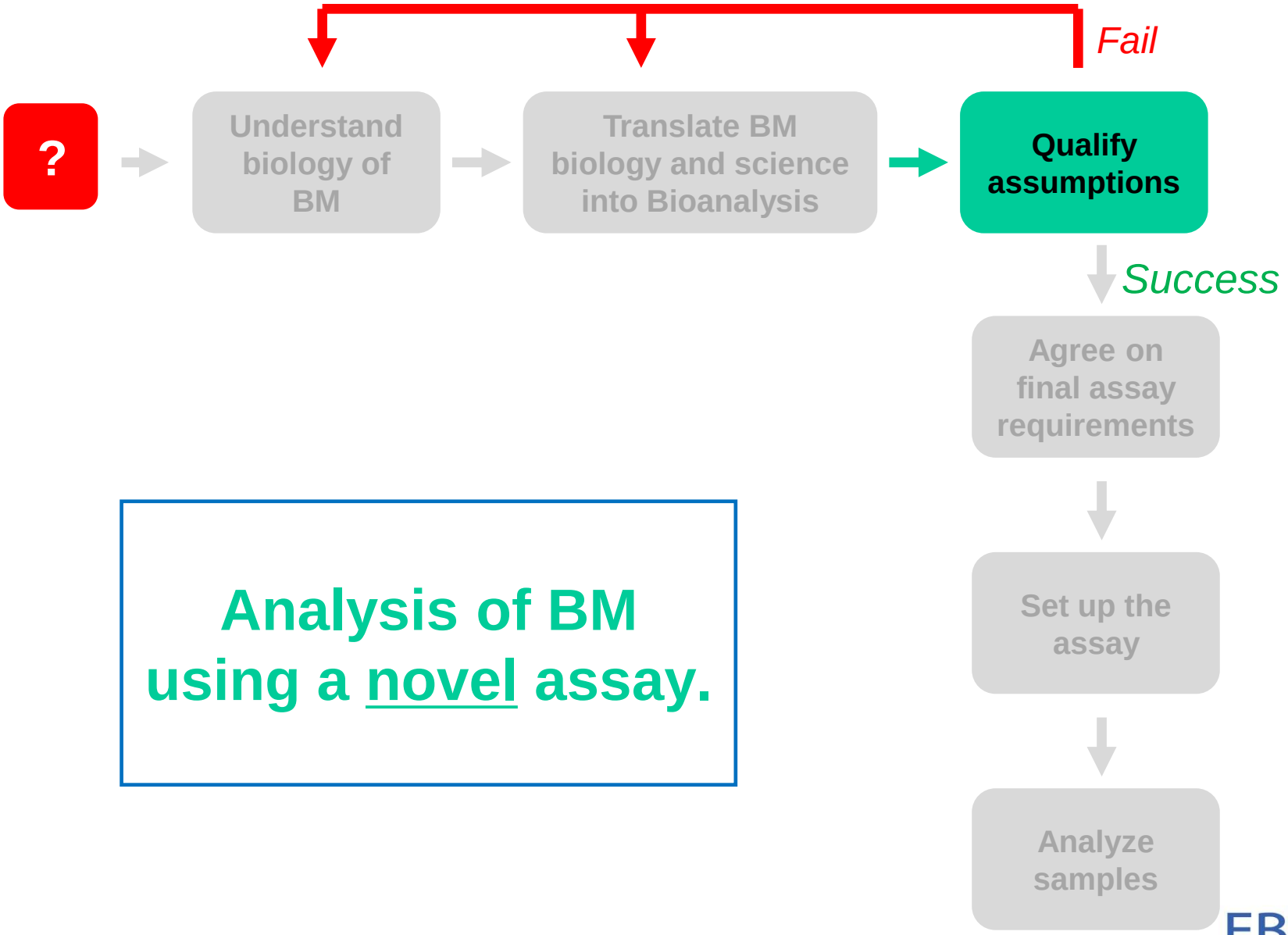
Analysis of BM using a novel assay.

BM= Biomarker

2. Translate BM biology and science into Bioanalytical strategy

- Based on outcome of interactions with requester, decide on analytical platform that is best suited to answer the questions

- Deliverable: proposed bioanalytical strategy and assay format
  - Preferred analytical platform, e.g.:
    - ELISA, Ligand Binding Assay
    - Multiplex
    - LC/MS based, Chromatography based
  - Analyte: availability of reference material
  - Matrix: availability of (surrogate) matrix
  - Target concentration range: Desired LOQ/ULOQ



Analysis of BM using a novel assay.

3. Qualify assumptions

- Qualify the PK(/TK)/PD assumptions made in ‘Understand the biology’ using the proposed assay format
  - Develop and set up assay for qualification of assumptions
    - Make stop/go decisions where assay can answer the intended use of the assay
  - Measure actual samples

- Deliverable:

Assumptions not confirmed

→ Reconnect with requester and assay platform

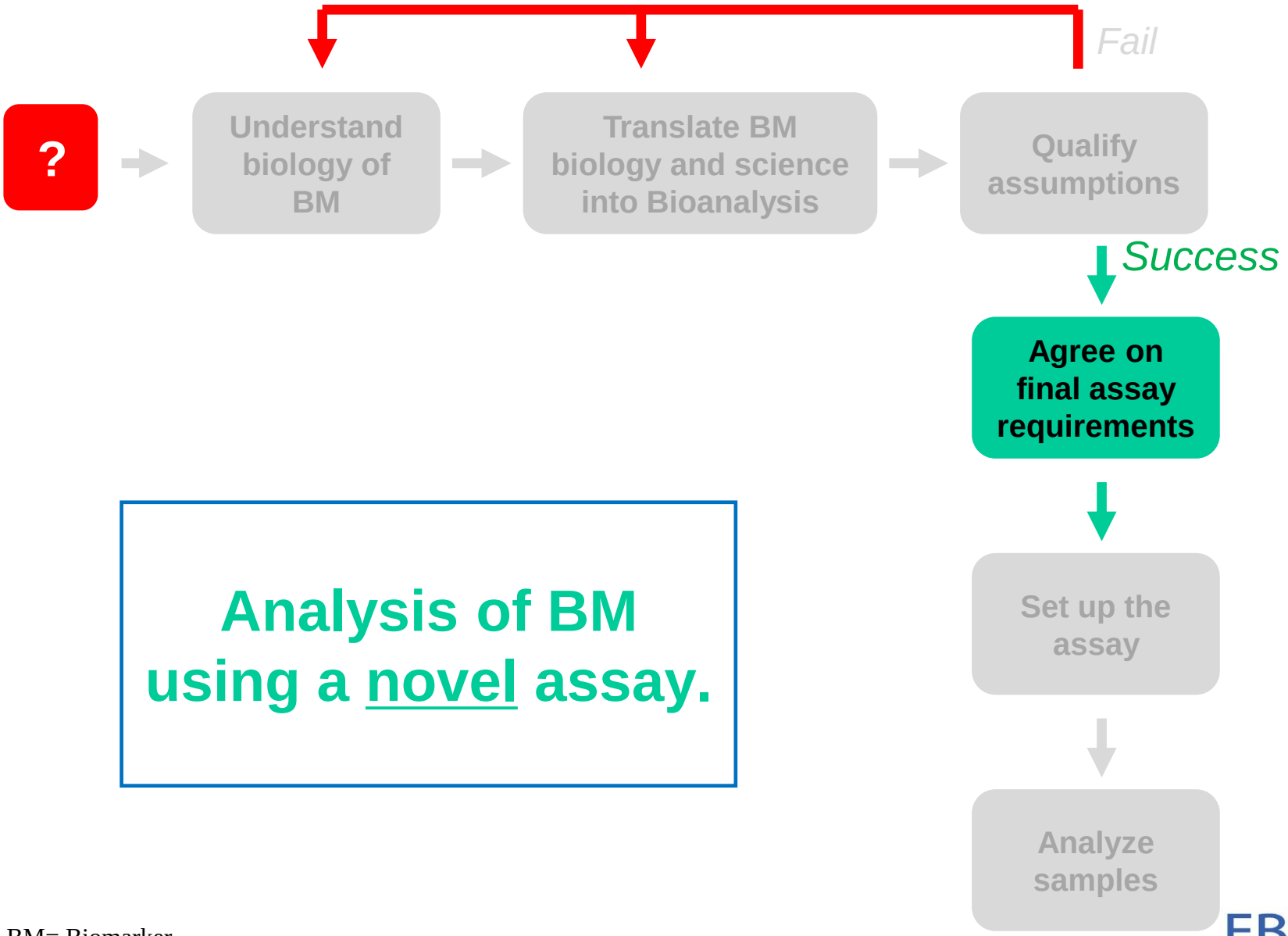
Assumptions confirmed

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

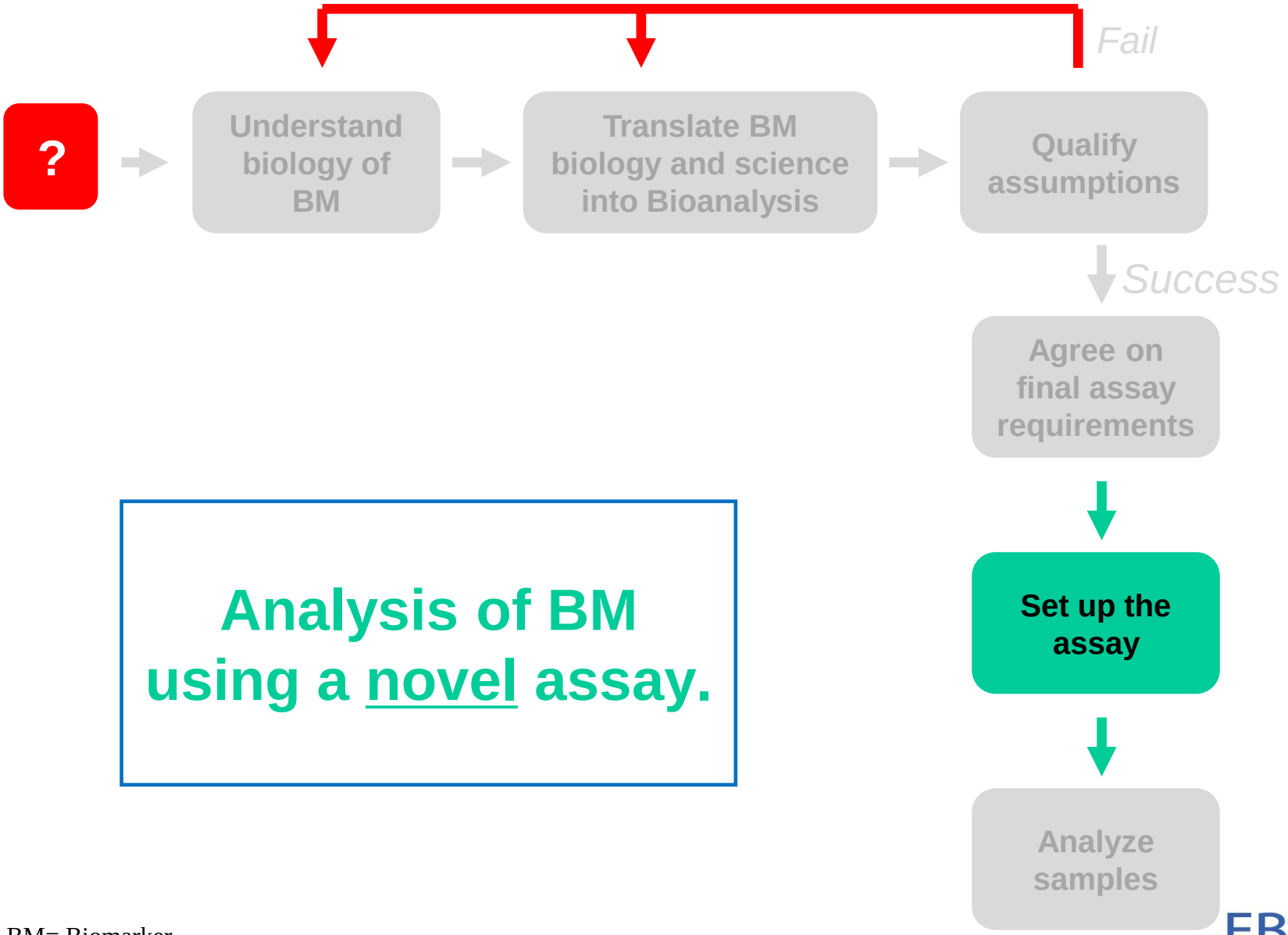


Analysis of BM using a novel assay.

BM= Biomarker

4. Agree on final assay requirements

- Reconnect with requester
  - Provide feedback on bioanalytical pre-work, assumption testing and qualification
  - Make agree now on the 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



Analysis of BM using a novel assay.

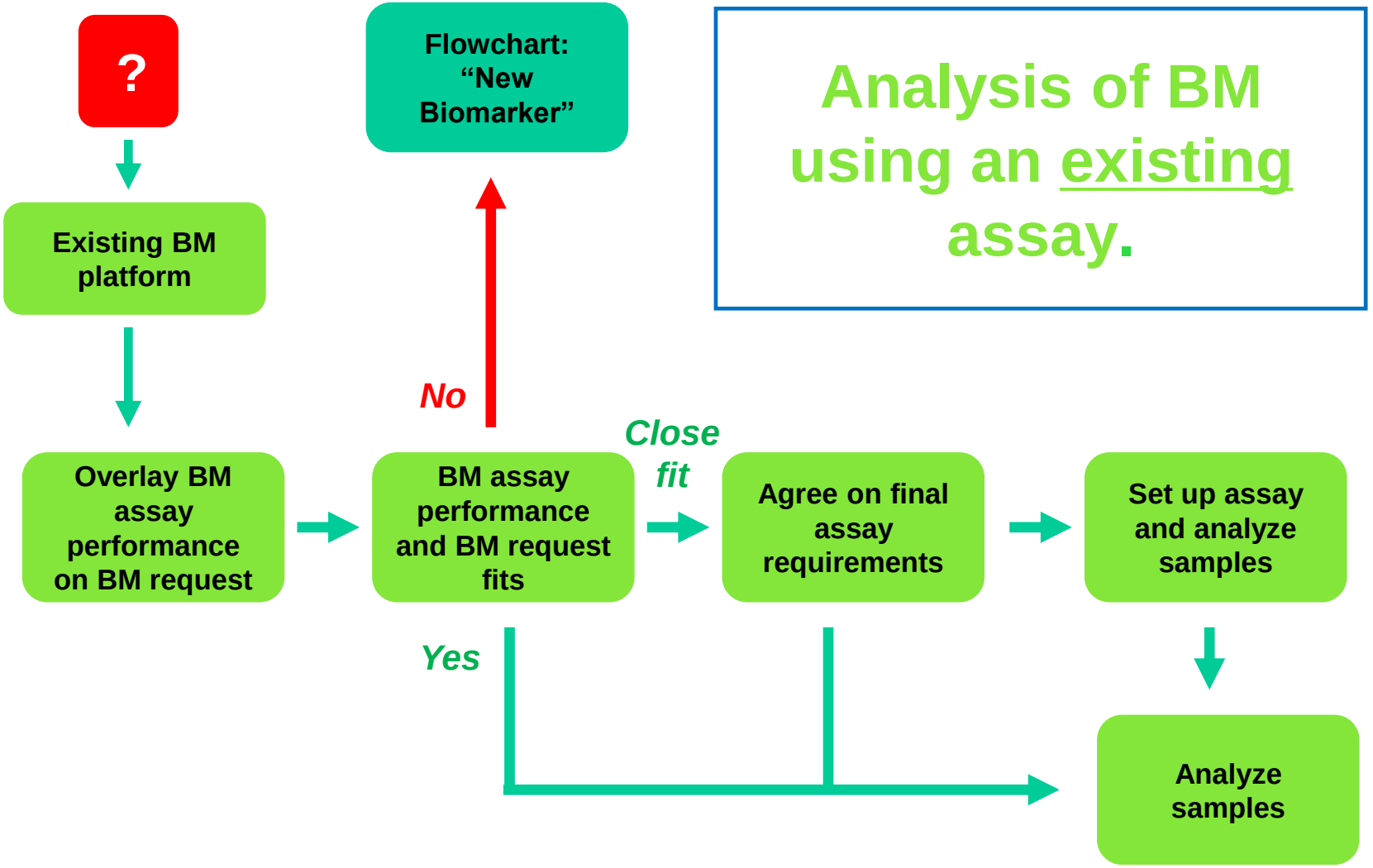
BM= Biomarker

5. Set up the assay

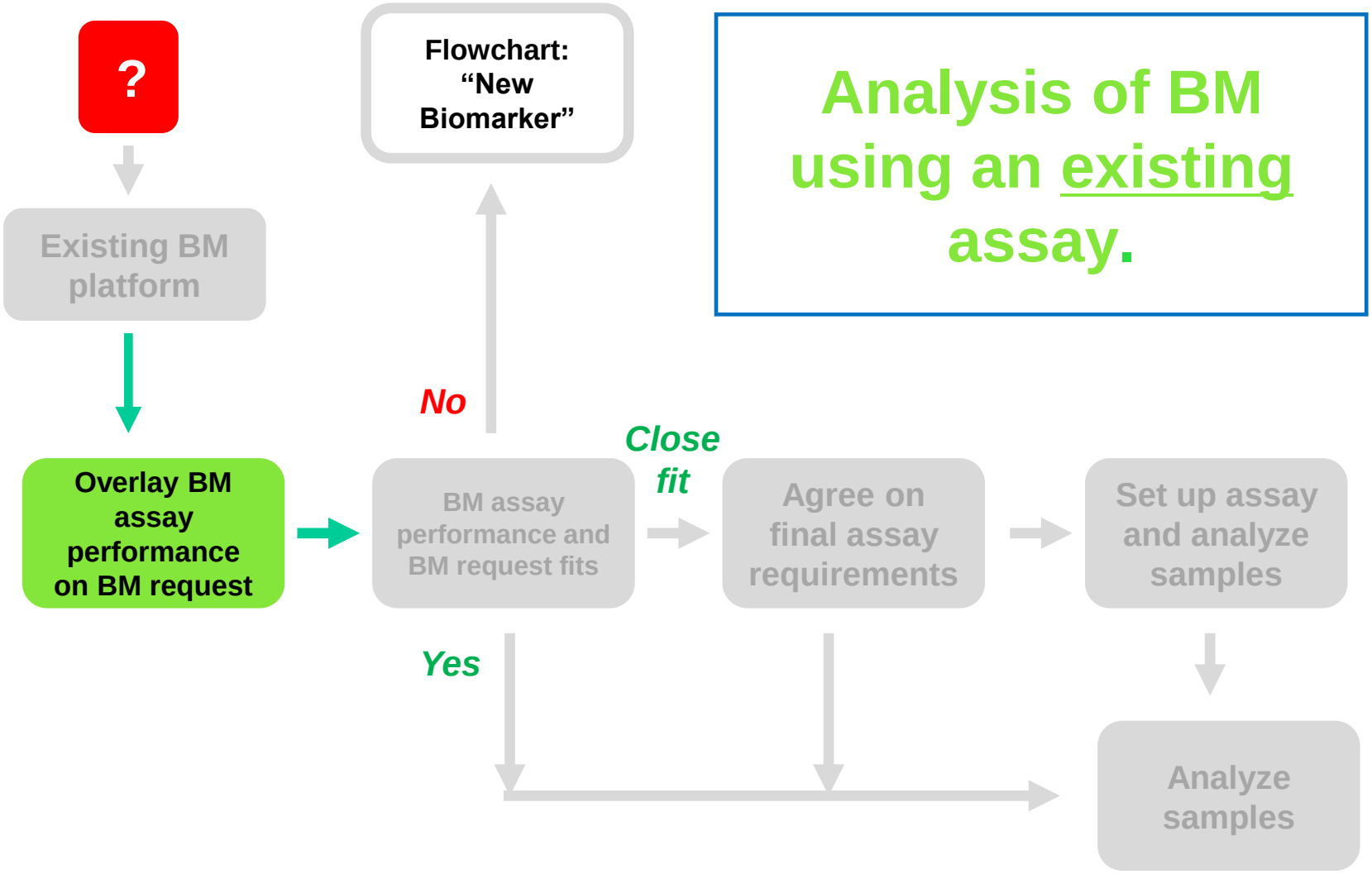
- Write ‘bioanalytical protocol’
  - A priori document for the BM assay requirements
  - maybe include more detail on purpose and scope
- Develop assay
  - Build on ‘assumption testing experience’
  - Include assay requirement parameters (screening → validation)
- Set up the assay
  - Screening – qualification - validation
  - Include all required “validation” parameters

Note: assay format may not fit present regulated BA requirements or expectations, but assay data are anticipated to fit regulatory standard.
In this case, it is advisable to 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.



BM= Biomarker

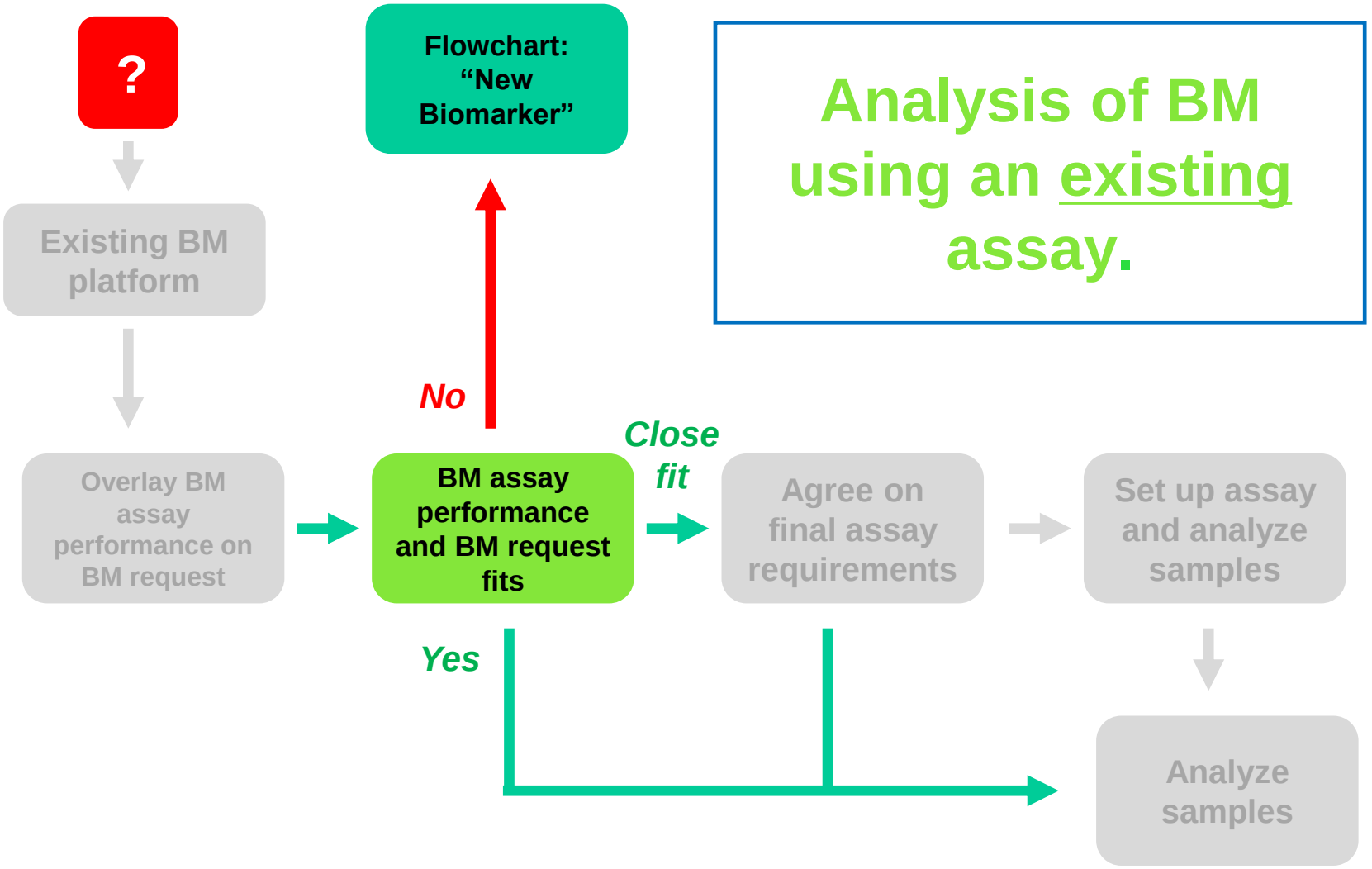


BM= Biomarker

1. Overlay BM assay performance on BM request

- Existing assay may not have been established for new BM question:
  - Established as qualified assay but now a validated assay is required or vice versa
  - Other species
  - Other calibration range
  - Other isomer
  - etc…

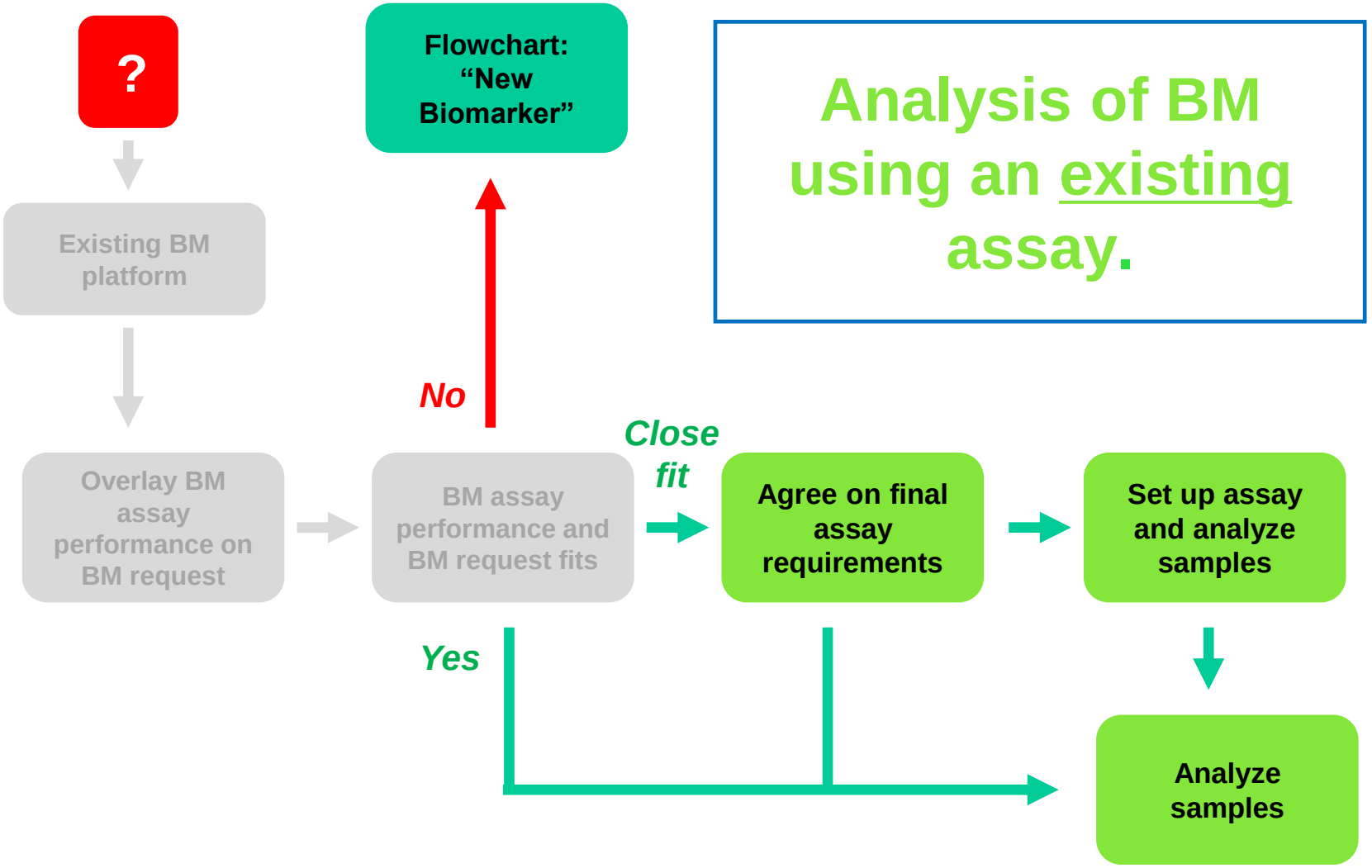
- Understand convergence or divergence of assay performance and new assay requirements


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

2. BM assay performance and BM request fits

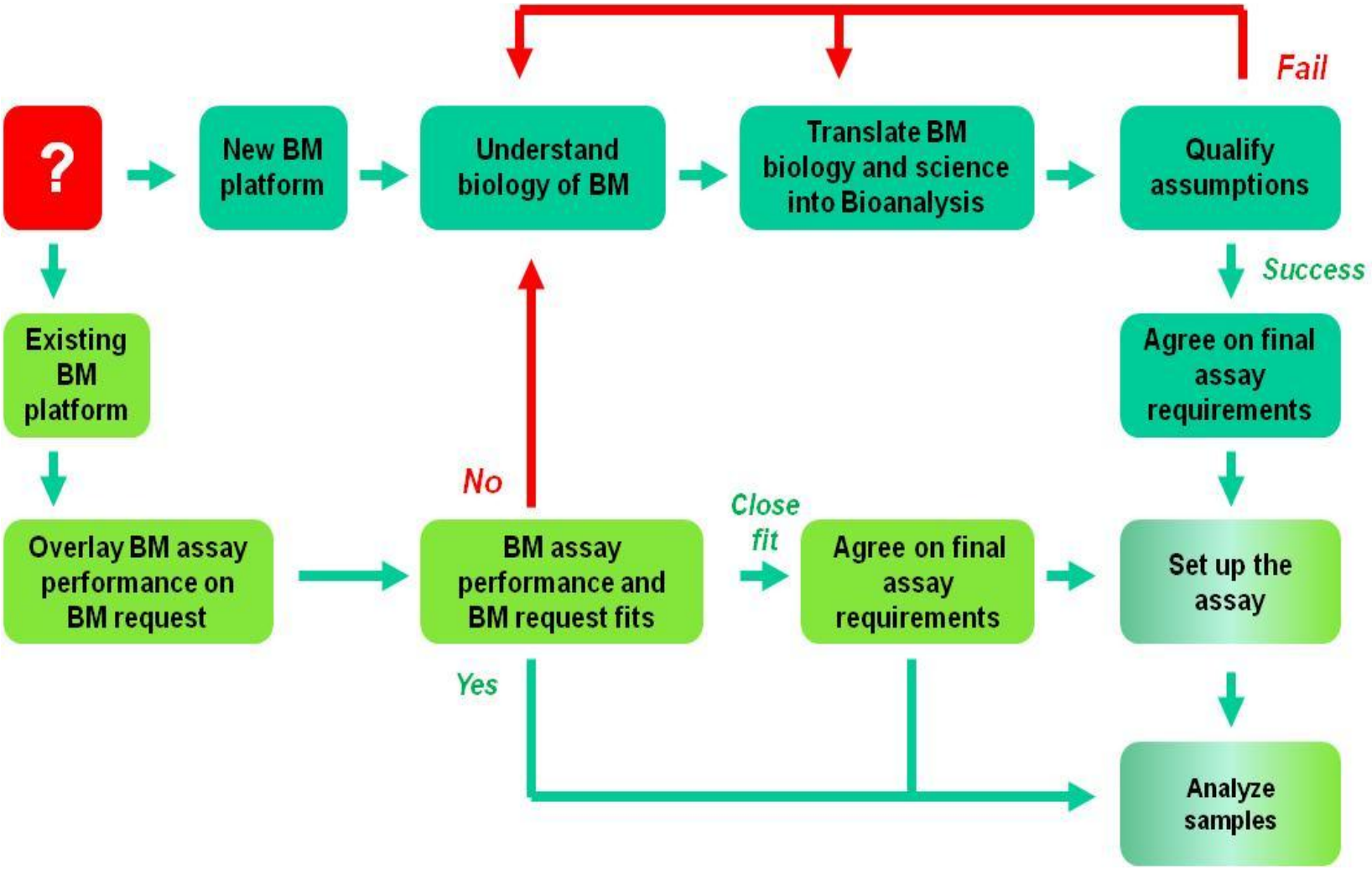
- The existing assay fits with the request:
  - Additional BM data within an ongoing project
    - copy established and earlier agreed acceptance criteria
    - run samples using existing (screening, qualified or validated) method and follow regulated bioanalysis standards as needed.

- 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”



BM= Biomarker

Combined flowchart



BM= Biomarker

Additional Reflections

- Adhere to Bioanalytical guidelines
  - FDA draft BMV guidelines, 2013
- Biological validation versus assay validation
- Stakeholders understanding of how to use biomarker data

Outline

1. The EBF Recommendation

2. **Discussion in Industry**

3. Crystal City VI

2015 – pre-CC-VI discussions in EBF

Survey of 57 statements

- Quick ‘yes/no’ answer
- More a pole than a real survey
- Intended to take the temperature of the EBF community on their feelings on a need for Biomarker BMV
- From the statements we took 5 take-home messages

Industry Take home messages

1. Generic acceptance criteria are difficult for all BM assay
2. Discussion should include all stakeholders
3. Assay validation requirement can change
4. Look for global consensus
5. Use principles of scientific validation

BM= Biomarker

We are passionate about accuracy

And we also want to have guidelines

OECD GLP compliant

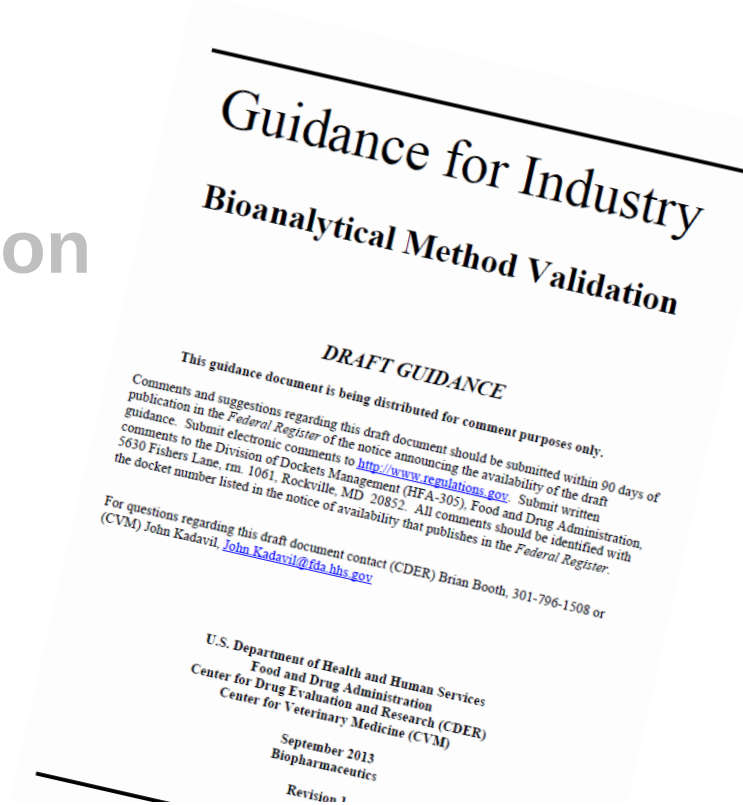
Guidelines

Outline

1. The EBF Recommendation

2. Discussion in Industry

3. **Crystal City VI**



Guidance for Industry

Bioanalytical Method Validation

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

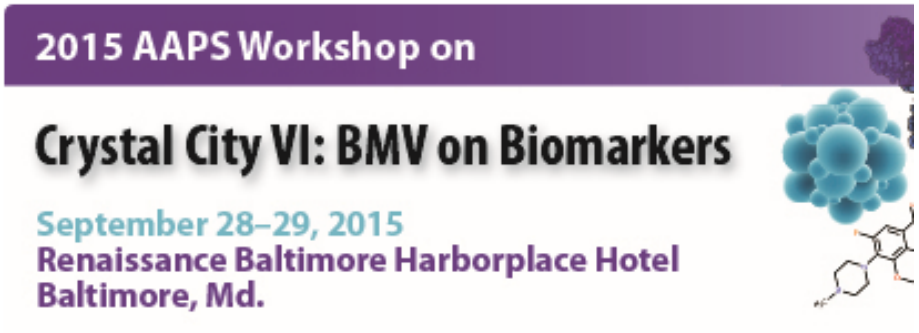
Comments and suggestions regarding this draft document should be submitted within 90 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit electronic comments to http://www.regulations.gov. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. All comments should be identified with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions regarding this draft document contact (CDER) Brian Booth, 301-796-1508 or (CVM) John Kadavil, John.Kadavil@fda.hhs.gov

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Veterinary Medicine (CVM)

September 2013
Biopharmaceutics

Revision 1



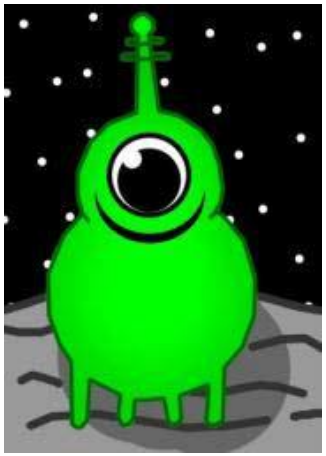
2015 AAPS Workshop on

Crystal City VI: BMV on Biomarkers

September 28–29, 2015
Renaissance Baltimore Harborplace Hotel
Baltimore, Md.

Take home messages from CC-VI

- **Biomarker Assays are not PK Assays**
  - Nor are they the sons/daughters of PK assays
  - Or their distant cousins
  - Or even same species



- **Biomarker assays are not PK assays**
- And they don't want to grow up to be PK assays either

Used with permission from Lauren Stevenson

Biomarker Assays ≠ PK assays

- What does this mean;
  - PK assay; validated using pre-defined parameters
  - Biomarkers; validated using pre-defined parameters

  - Are BM different?

Short answer : Yes

- Recombinant protein used as a standard for an endogenous biomarker
- Relative accuracy
- Use of surrogate matrix
- Incurred versus spiked matrix differences
- Stability assessment

Take home messages from CC-VI
Biomarkers & Biology

- Biomarker data are used to make important decisions in your project
  - Assay must be reliable in order to be confident in these decisions
  - Assay validation plays a pivotal role

- Do you know what you are measuring?
- What is the purpose of the assay?
- Assay design – reagents?
- What are the limitations of the assay?
- What is the precision of the measurement?
- How do sample handling conditions affect the measurement?

Take home messages from CC-VI
Consensus

- **Category 1** = most Biomarkers we analyze today
  - Internal decision making
  - Extent of assay validation is up to you!

- **Category 2**
  - Biomarker to support pivotal decision & label claim
  - Assay validation in scope of FDA

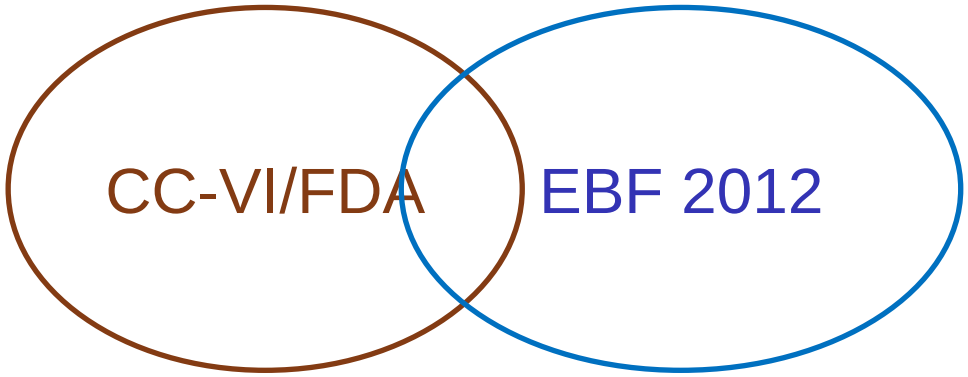
Take home message CC-VI – in summary

- Biomarker Assays ≠ PK assays
- Category 2 Biomarkers are in scope of FDA review

- Scientific reflections when setting up assays
  - Rethink accuracy
  - Emphasis on precision
  - Parallelism is the key analytical validation experiment
  - Endogenous analyte to define performance and manage change
  - Establish reference range for assay
  - Understand the biology!

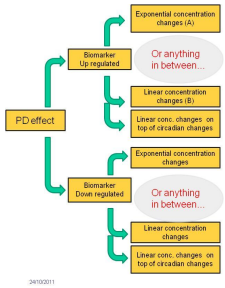
Connecting EBF 2012 and CC-VI 2015

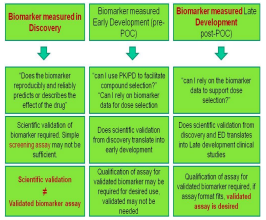
- Meeting discussions were closely aligned with 2012 EBF Recommendation & feedback from 2015 pole

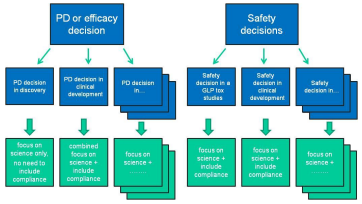


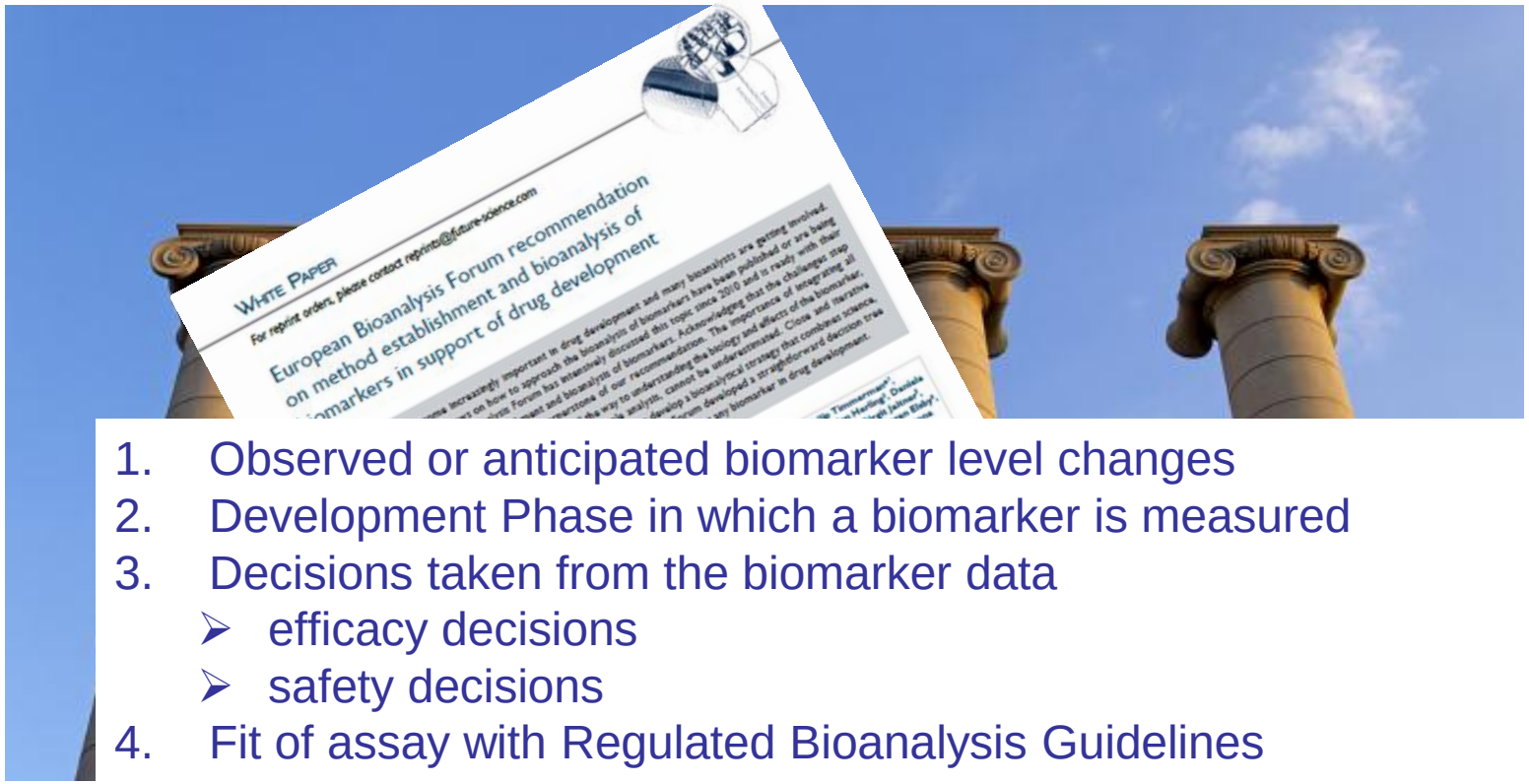
- And…FDA expressed they are in learning mode, and do not expect a Biomarker Guidance in several years to come

EBF recommendation – 4 pillars









1. Observed or anticipated biomarker level changes
2. Development Phase in which a biomarker is measured
3. Decisions taken from the biomarker data
   - efficacy decisions
   - safety decisions
4. Fit of assay with Regulated Bioanalysis Guidelines

THANK
YOU !