EBF Recommendation on method establishment and bioanalysis of Biomarkers in support of drug development.
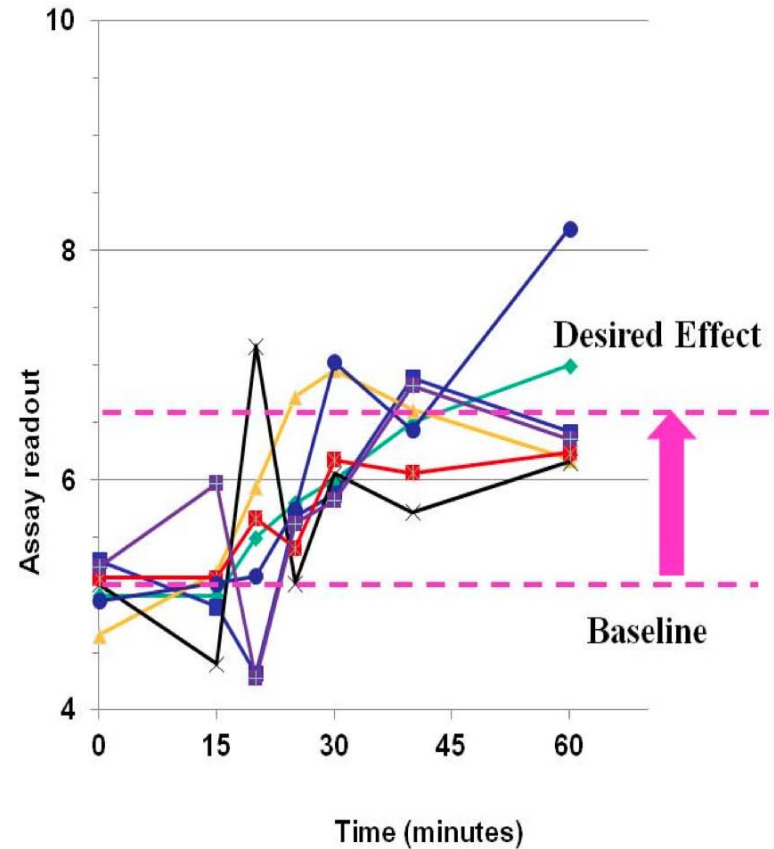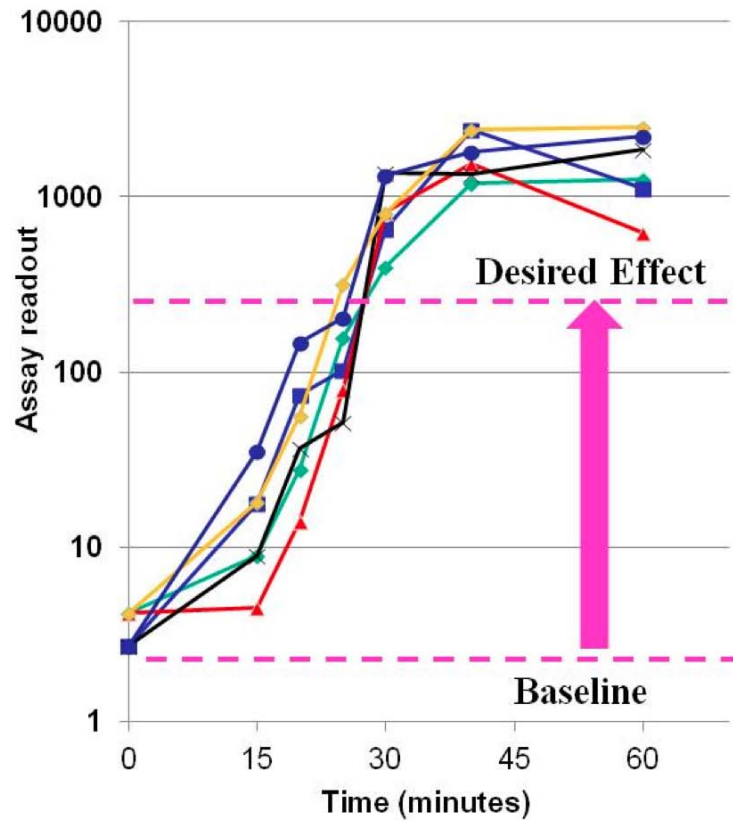
Presenter: Philip Timmerman
on behalf of the EBF

5th EBF Open Symposium
14-16 November 2012
Barcelona, Spain

Reconnect with the EBF presentation from the 4th Open Symposium in 2011
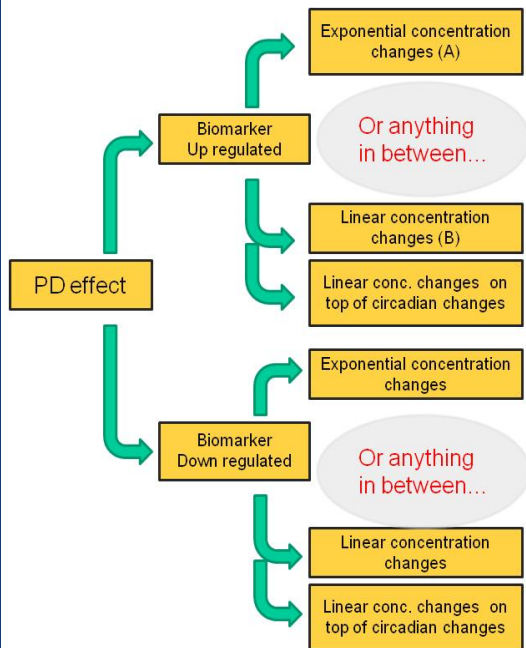
2 examples

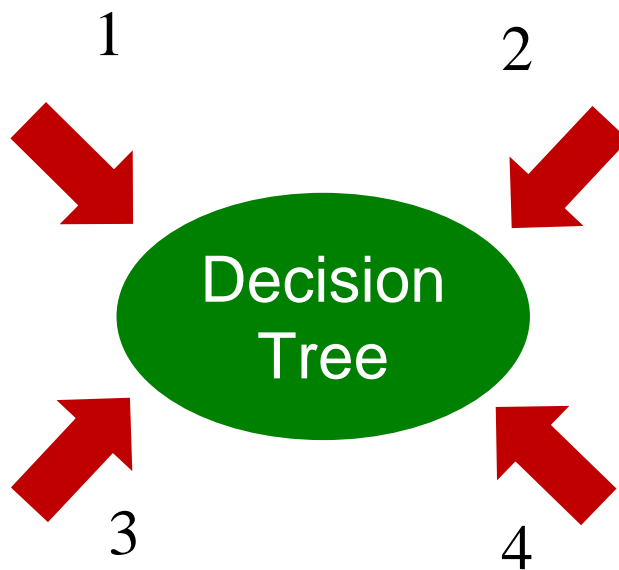## All measured with 4-6-15 assay, but was this necessary?

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EBF reflections on <u>biomarker classification</u>

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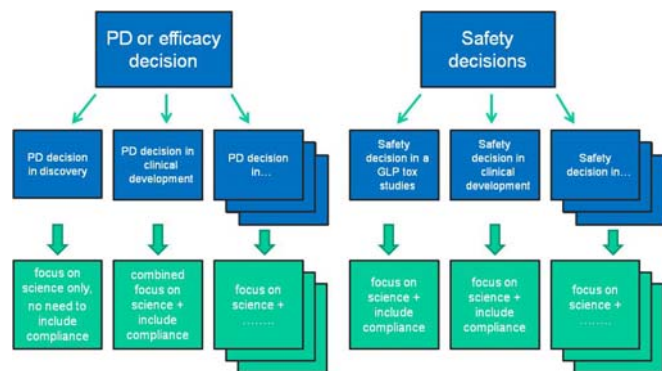4. Fit of assay with Regulated Bioanalysis Guidelines

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

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Decision Tree

1

2

3

4

Exponential concentration changes (A)

Or anything in between…

Biomarker Up regulated

Linear concentration changes (B)

Linear conc. changes on top of circadian changes

PD effect

Exponential concentration changes

Biomarker Down regulated

Or anything in between…

Linear concentration changes

Linear conc. changes on top of circadian changes

24/10/2011

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

PD or efficacy decision

PD decision in discovery

PD decision in clinical development

PD decision in…

focus on science only, no need to include compliance

combined focus on science + include compliance

focus on science +

Safety decisions

Safety decision in a GLP tox studies

Safety decision in clinical development

Safety decision in…

focus on science + include compliance

focus on science + include compliance

focus on science +

Adhere to Regulated BA guidelines

Nice to have

Need to have

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Inform and be informed

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

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

2012: our recommendation

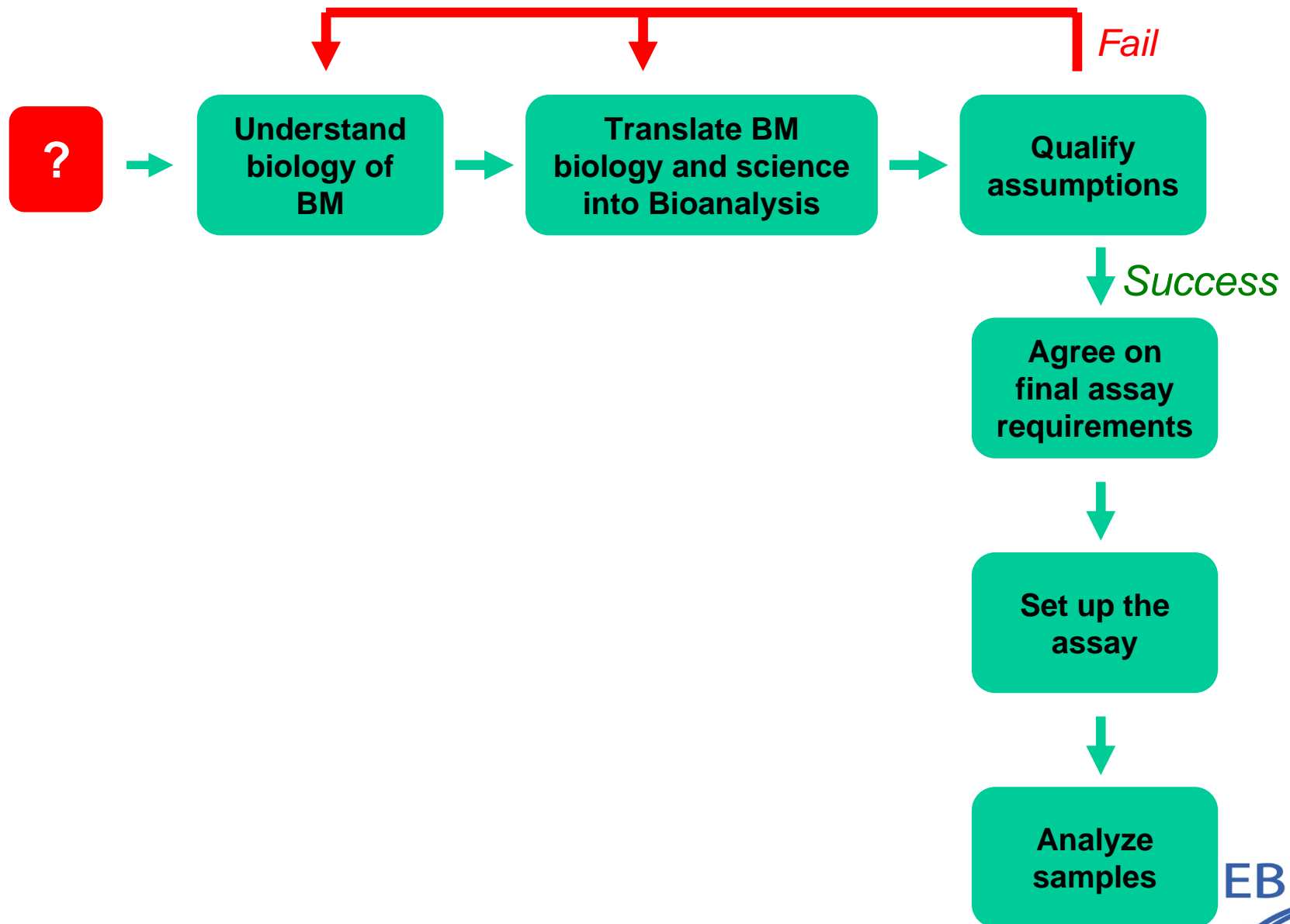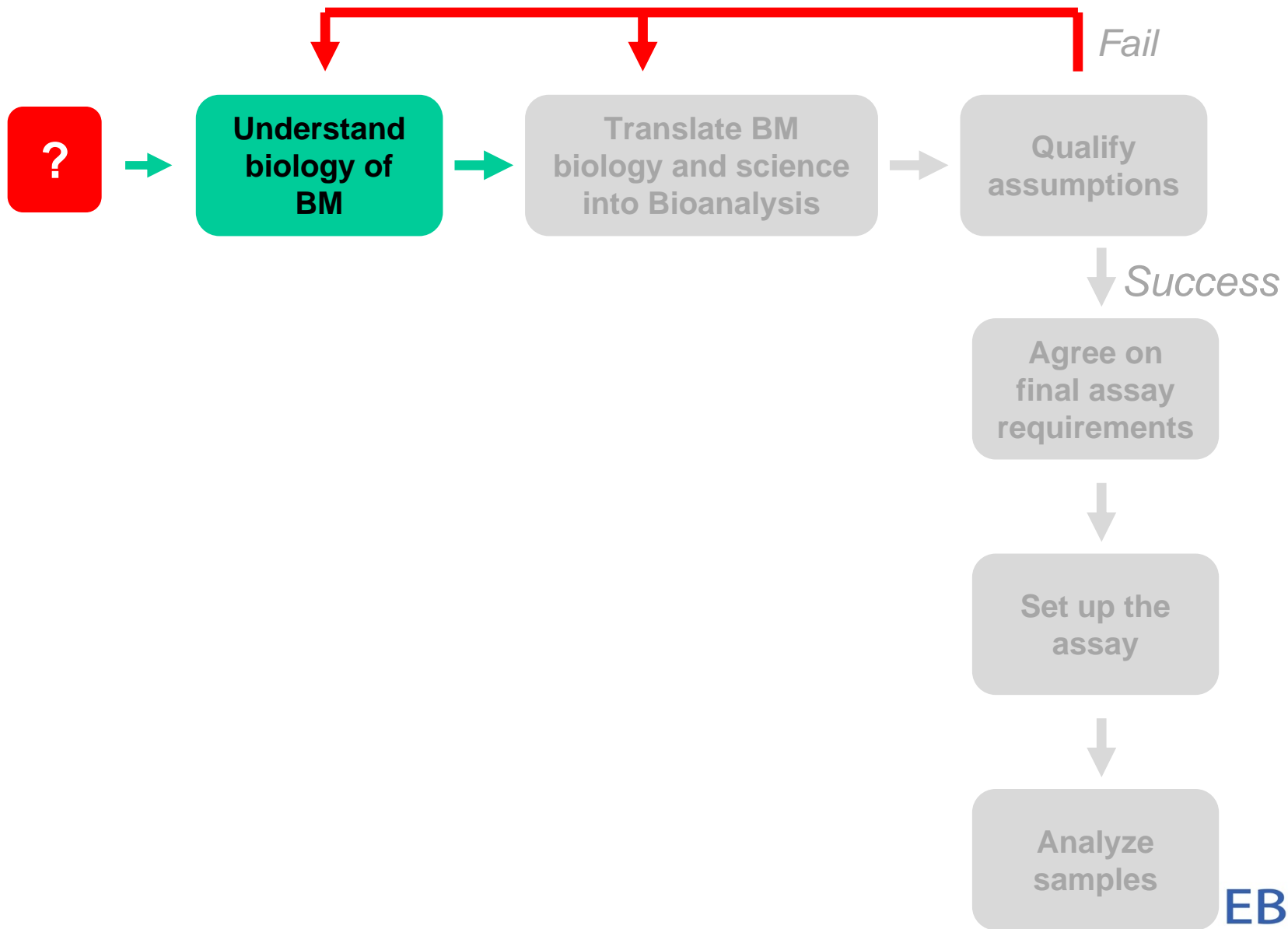➢ Team continued discussions after BCN-2011 and developed 2 flowcharts

Analysis of BM using a <u>novel</u> assay.

Analysis of BM using an <u>existing</u> assay.

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# Analysis of biomarkers using a novel assay.

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

➢ <u>Deliverable</u> of step 1 = understand why assay is needed and what is expected outcome

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Type of information from step 1: 4 examples

➢ BM is a safety marker amenable to LC-MS. We need assay in human CSF. BM has a basal level of a few ng/ml with natural circadian concentration changes of 2-3 fold from basal. (Expected) PD effect of the drug on the BM (= PK of the BM) is an up regulation to 5-fold of basal. Up regulation maximum concentration exceeds the natural fluctuations.

➢ BM is a compound amenable to LC-MS. We need assay comparing plasma ratios of 2 BMs. Basal value BM1/BM2 ratio is 0.01. BMs can interconvert ex-vivo. (Expected) PD effect of the drug on the BM ratio is expected to see a 2-fold increase of BM1/BM2-ratio.

➢ BM is a compound amenable to LBA. We need assay in serum, BM has a basal level of a few hundreds ng/ml with limited natural concentration changes. (Expected) PD effect of the drug on the BM (= PK of the BM) is a 10 fold down regulation.

➢ Etc….

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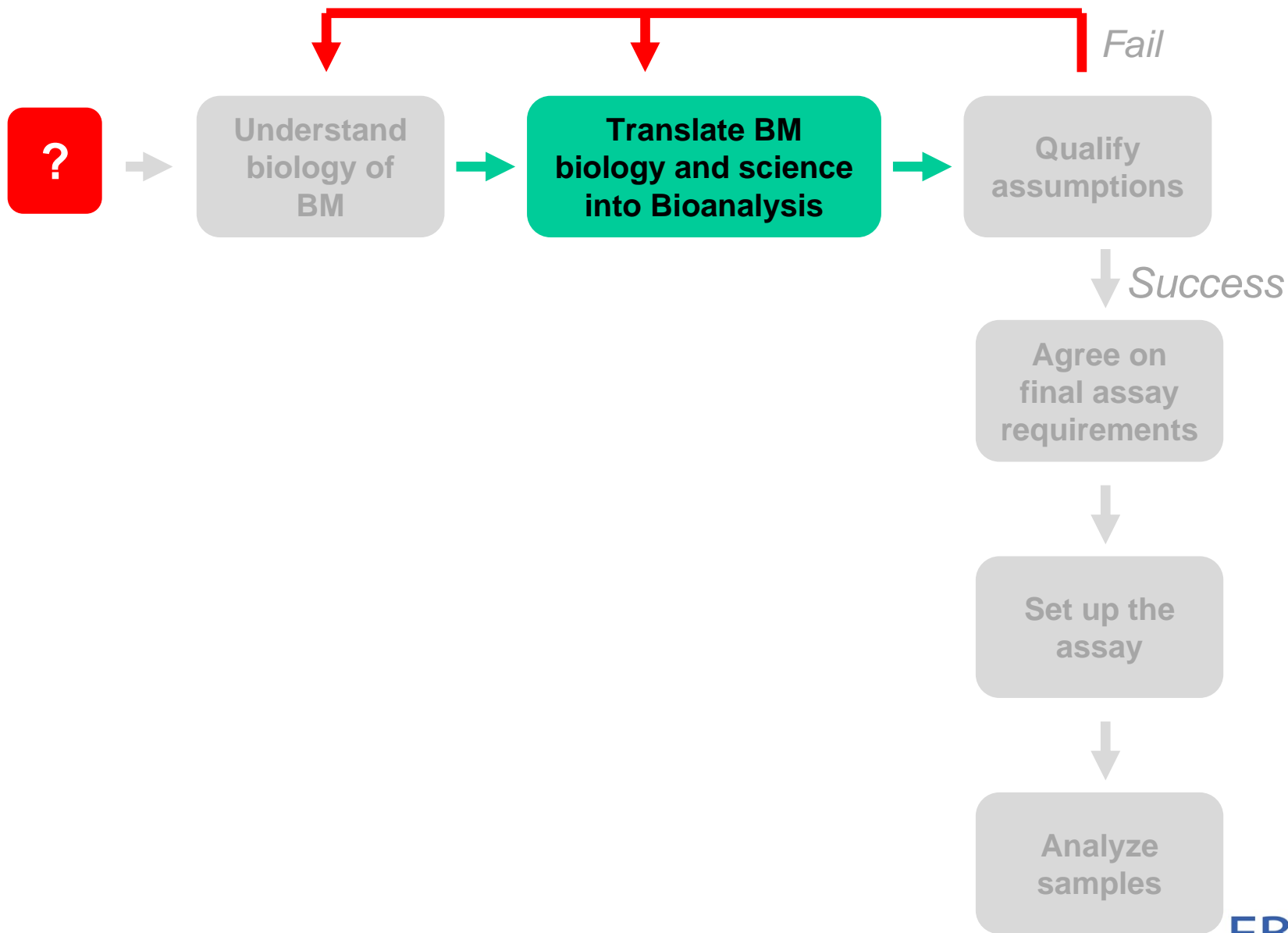
2. Translate BM biology and science into Bioanalytical strategy

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

➢ <u>Deliverable</u>: proposed bioanalytical strategy and assay format

– Preferred analytical platform, e.g.:
  o MS based, Chromatography based
  o Cell based, Enzymatic, Ligand Binding Assay
  o 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 PK behavior of BM

Note: An assay may be available for the BM. However, this assay may not be suited to provide the required answers in the specified situation (accuracy, precision, range,…). In this case, the assay may serve as a basis on which the actually needed assay is build.

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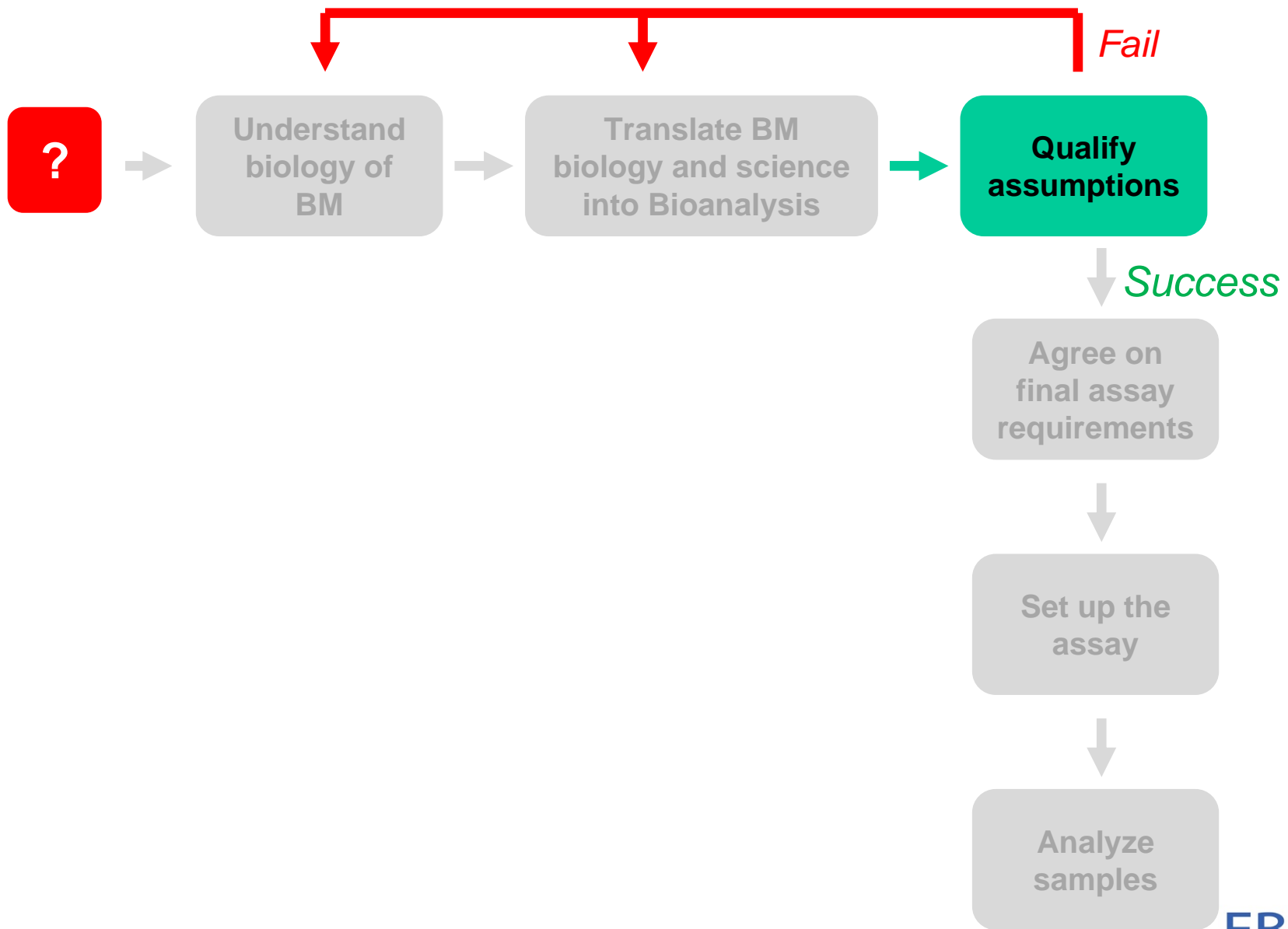
3. Qualify assumptions

➢ Qualify the PK(/TK)/PD assumptions made in "1" using the assay format proposed in "2"

– Develop and set up assay for qualification of assumptions

o 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

➢ <u>Deliverable</u>:

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
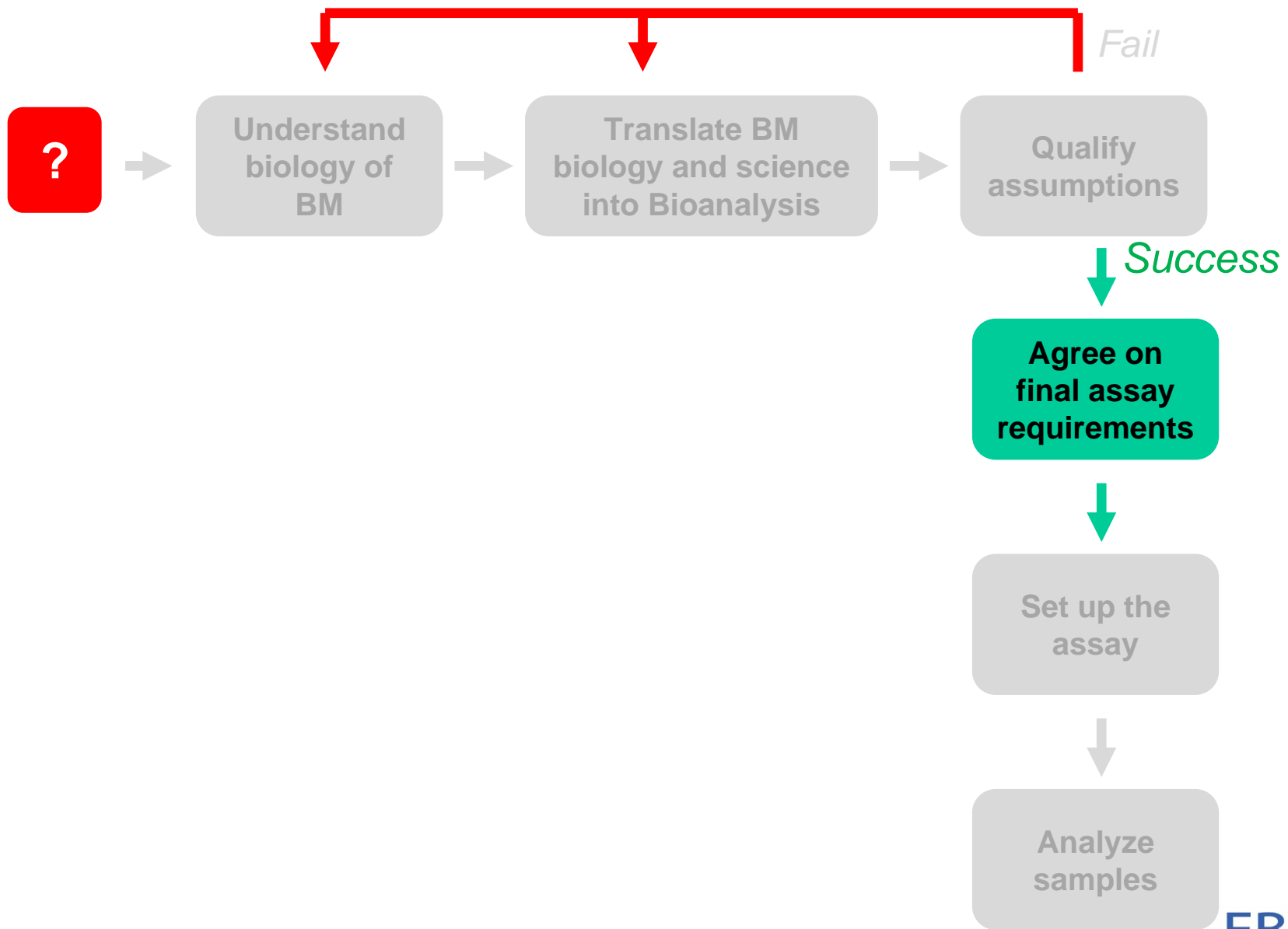
4. Agree on final assay requirements

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

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Fail

| ? | → | Understand biology of BM | → | Translate BM biology and science into Bioanalysis | → | Qualify assumptions |

Success

Agree on final assay requirements

Set up the assay

Analyze samples
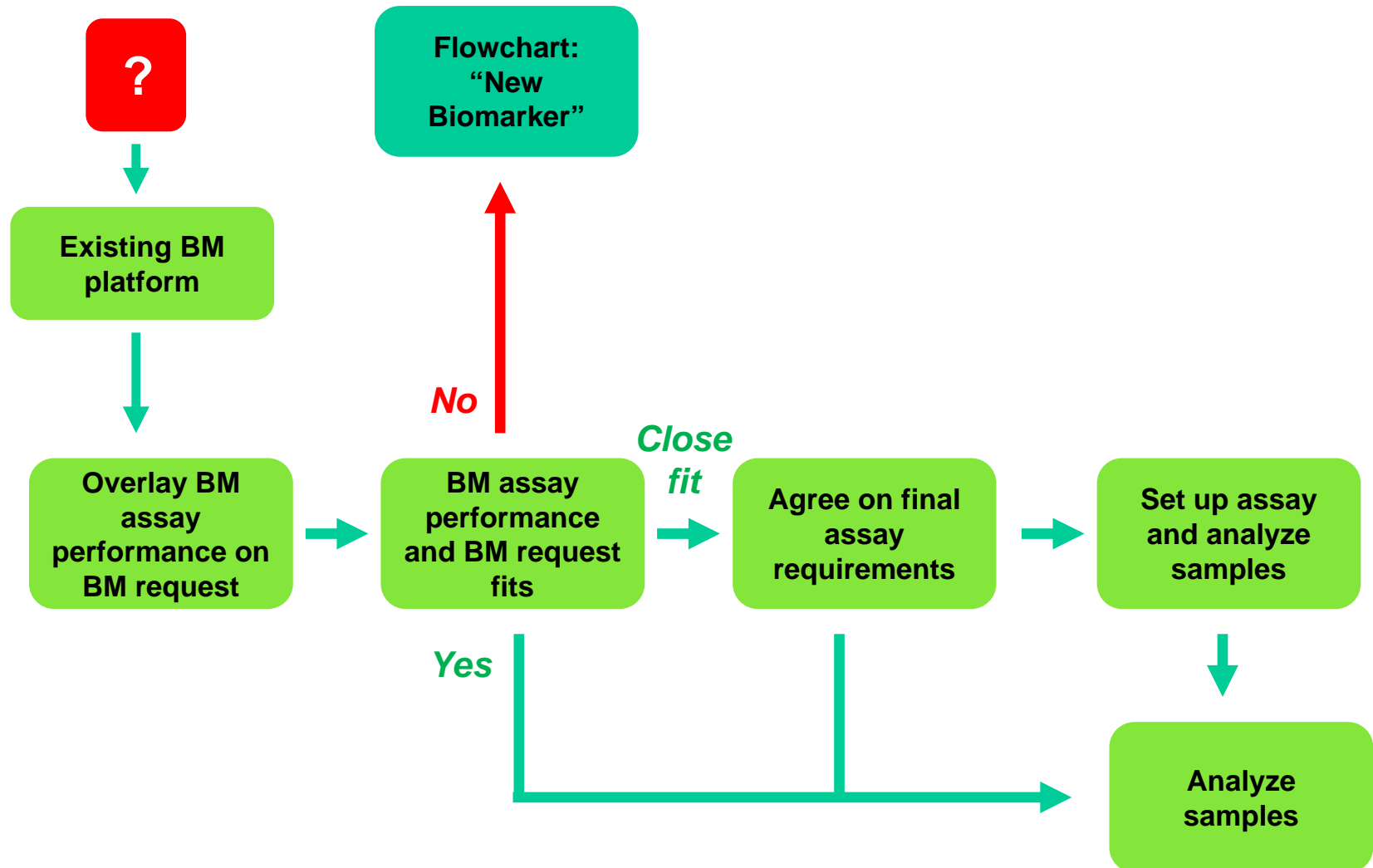
EBF

4. Agree on final assay requirements

> Reconnect with requester

– Provide feedback on bioanalytical pre-work, assumption testing and qualification

– Only now agree on assay requirements based on
  o Decisions to be taken from the data
  o 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)
  o Known Regulatory requirements
    • Consider change if/when assay is used in later phases of development
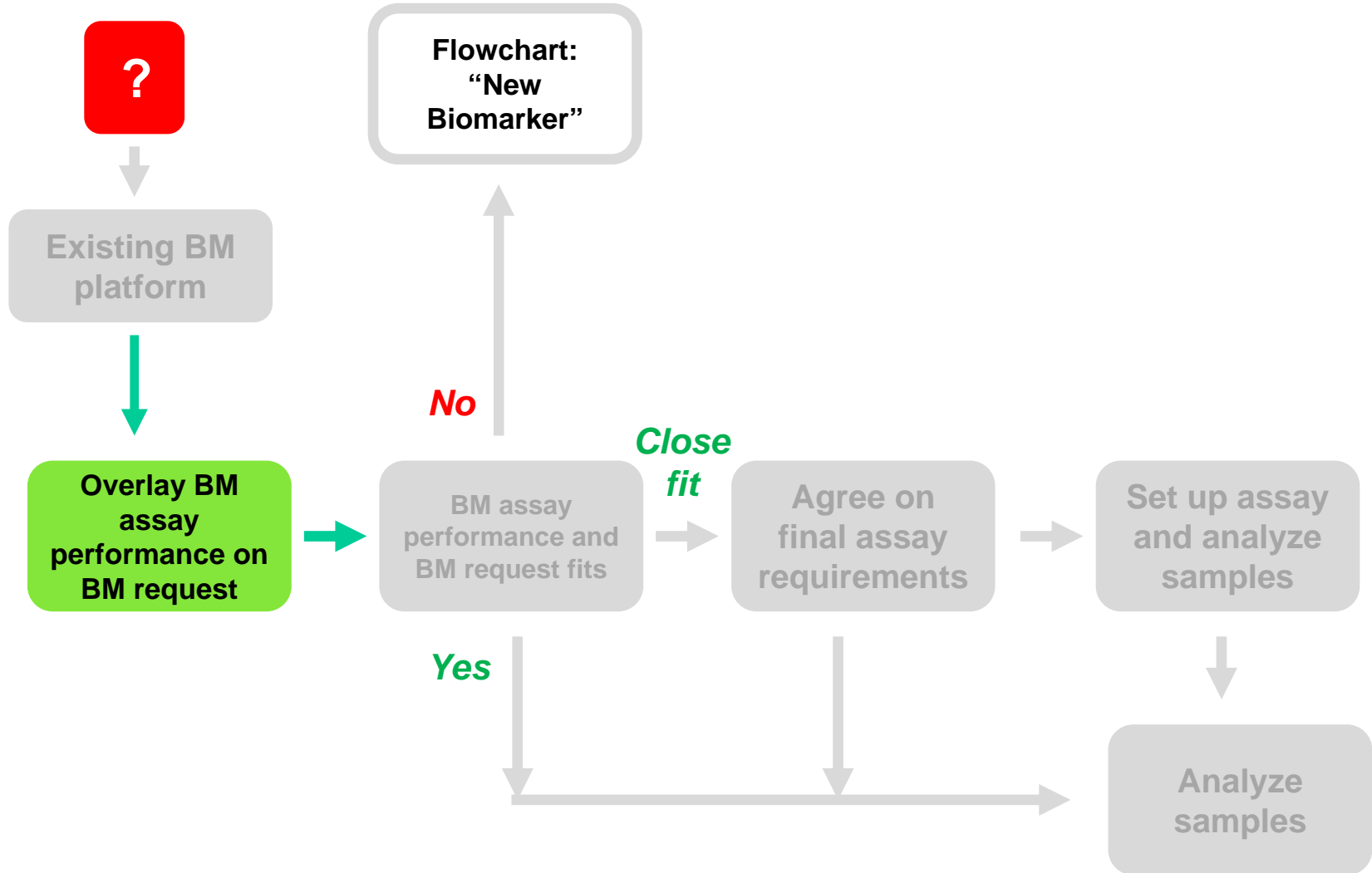
EBF
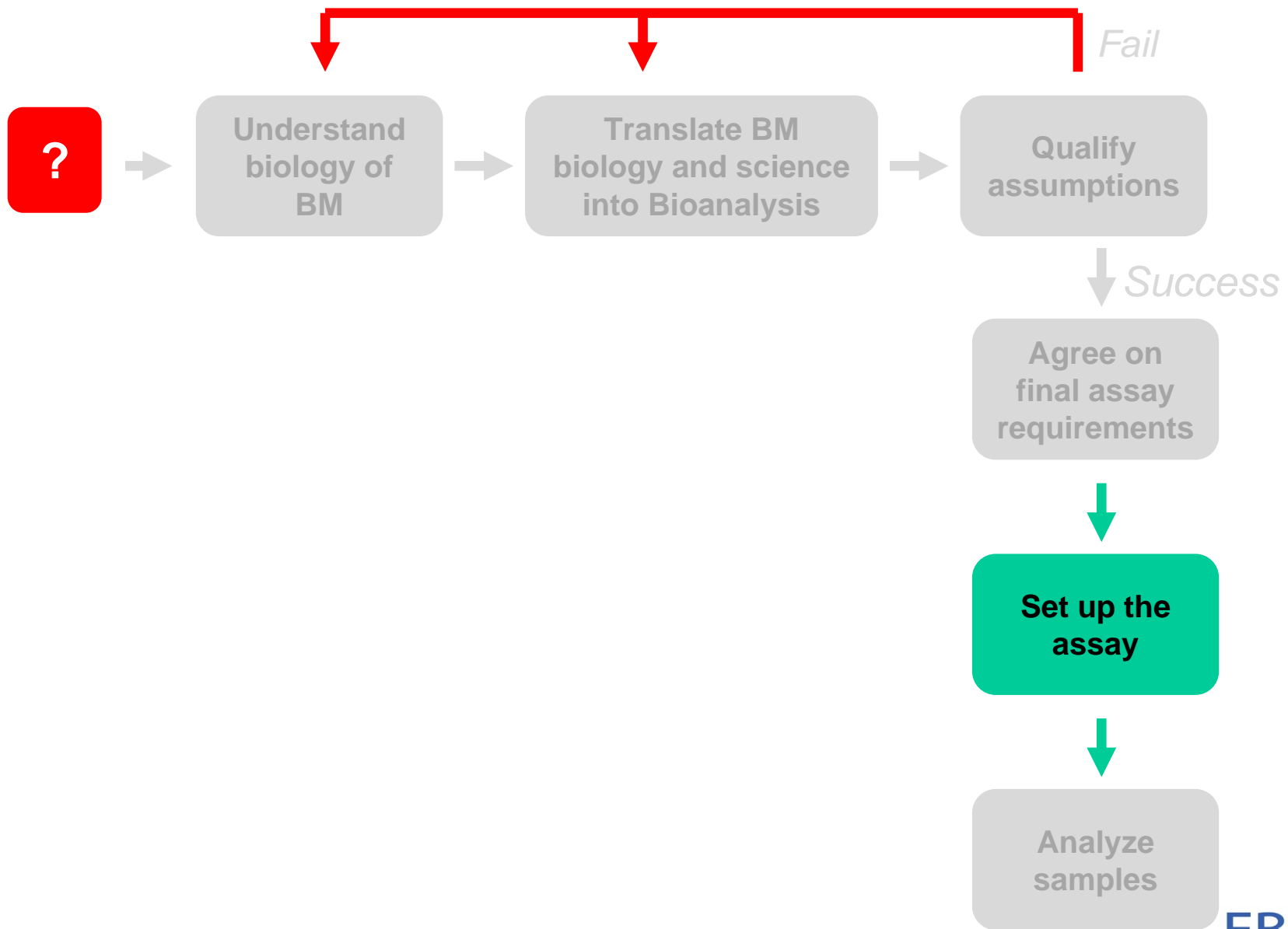
5. Set up the assay

- ➤ 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.

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Analysis of biomarkers using an existing assay.

?

Flowchart: "New Biomarker"

Existing BM platform

Overlay BM assay performance on BM request

BM assay performance and BM request fits

Agree on final assay requirements

Set up assay and analyze samples

No

Close fit

Yes

Analyze samples

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1. Overlay BM assay performance on BM request

➢ 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

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?

Flowchart: "New Biomarker"

Existing BM platform

No

Close fit

Overlay BM assay performance on BM request

BM assay performance and BM request fits

Agree on final assay requirements

Set up assay and analyze samples

Yes

Analyze samples

EBF
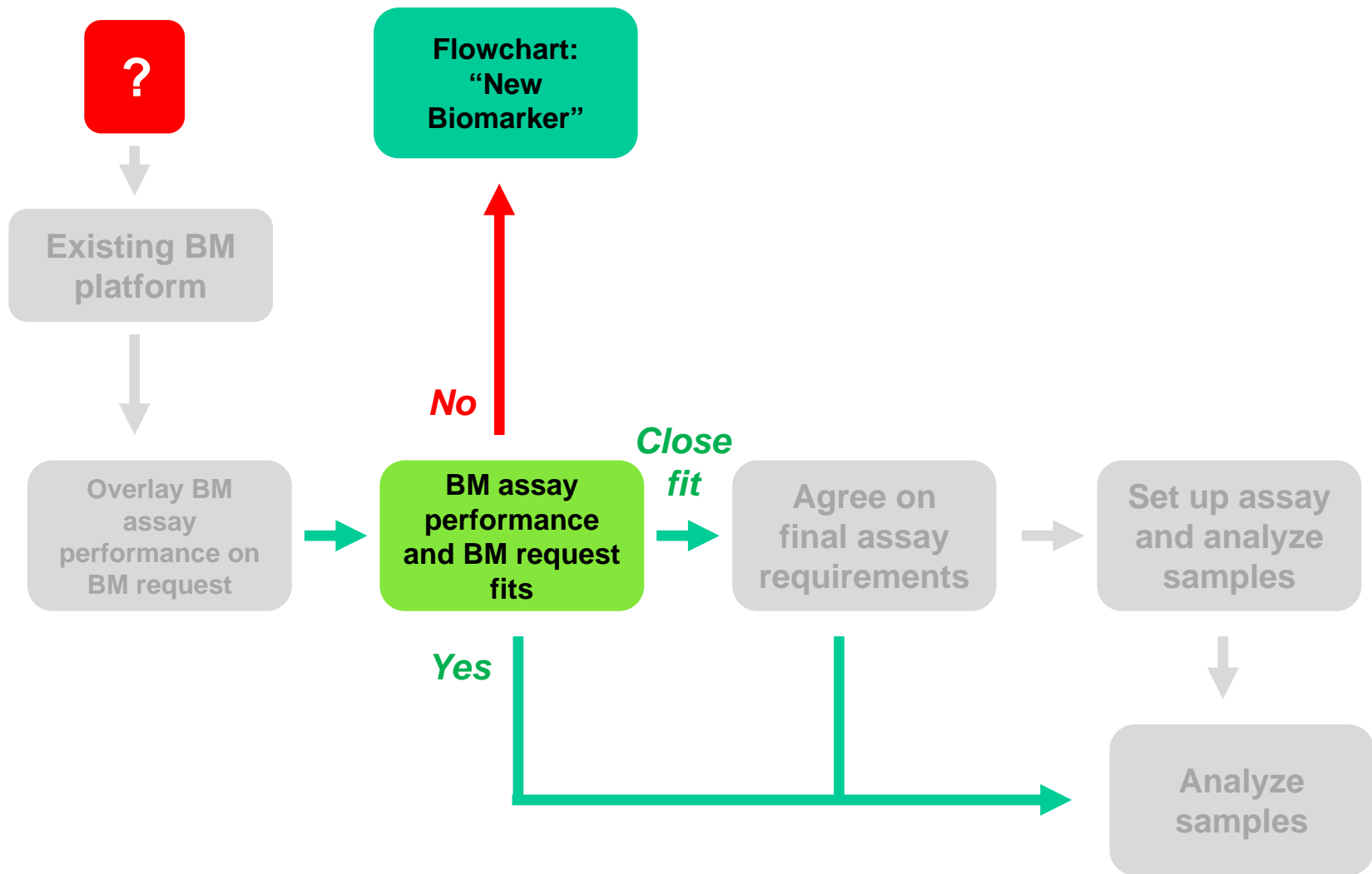
2. BM assay performance and BM request fits

➢ The existing assay fits with the request:

 – Additional BM data within an ongoing project

   o copy established and earlier agreed acceptance criteria

   o 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

   o Small changes needed → adapt method and move forward

   o Major changes needed → use flowchart "novel biomarker"

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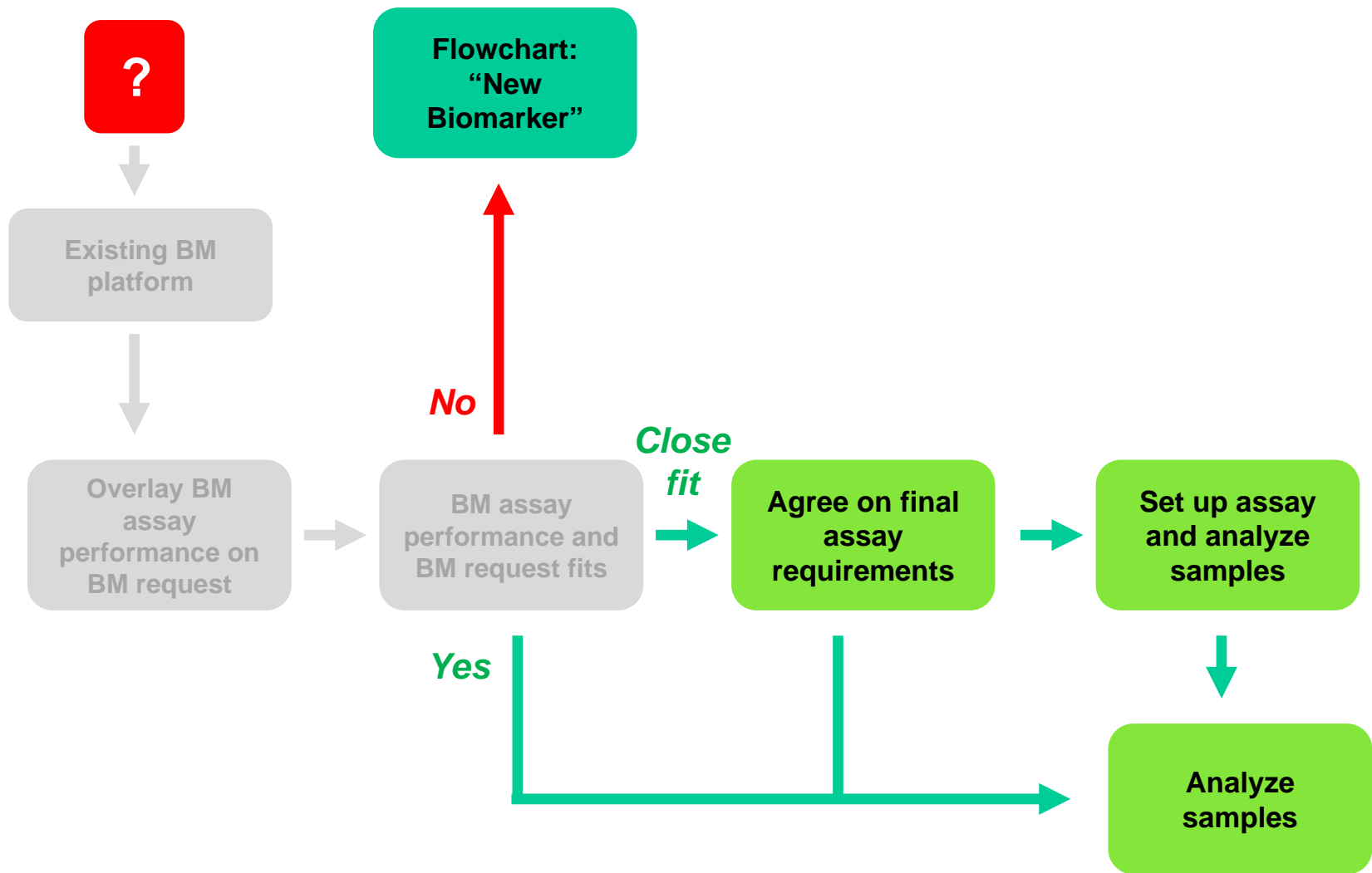
Combined flowchart
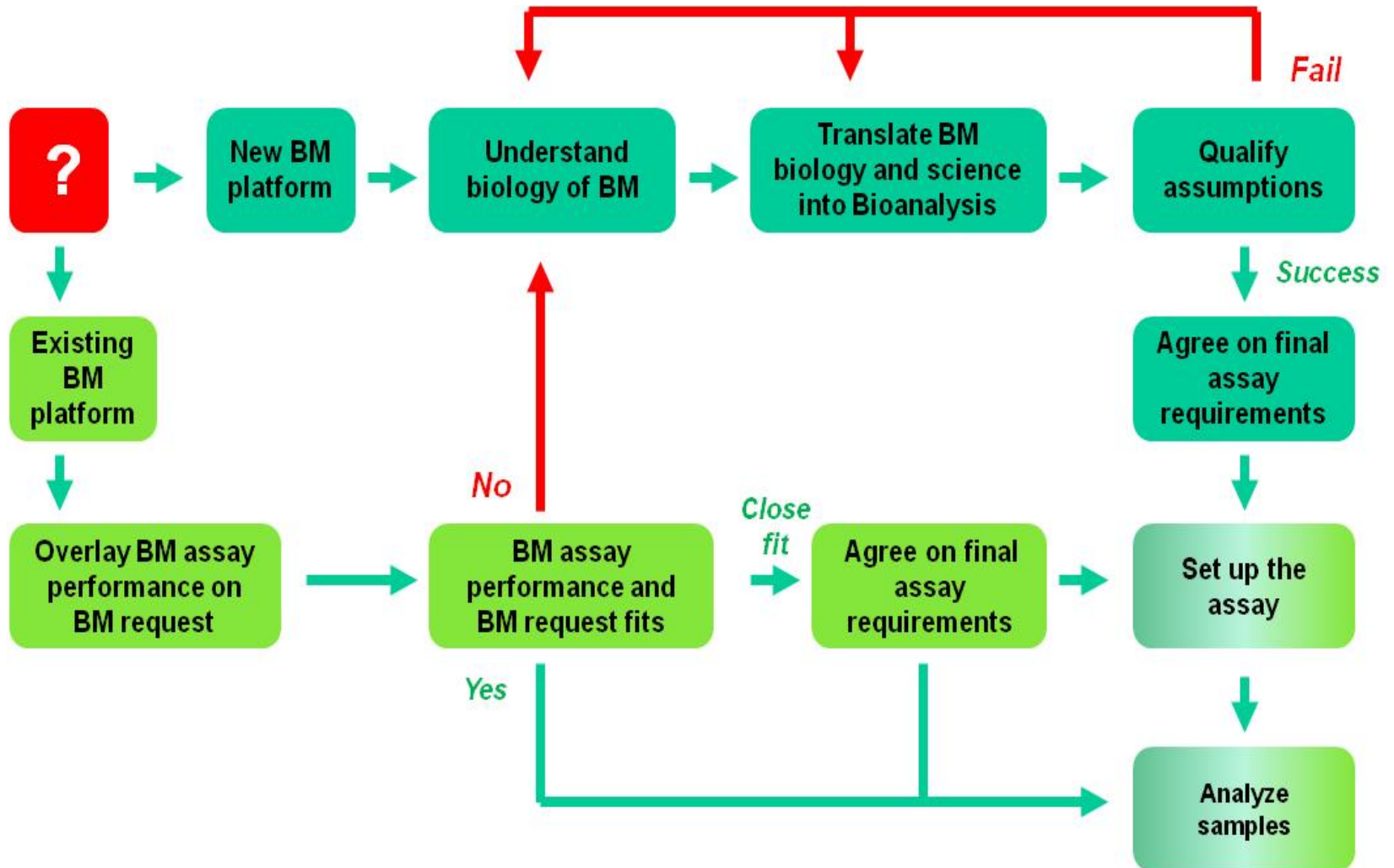
Additional reflections

With more BM analysis amenable to LC-MS, industry may say/think "*we can easily reach 4-6-15 quality*", stimulating regulated bioanalysis standards for BM analysis, but:

➢ A significant number of biomarkers are novel and analysis involves the use of cutting edge or developing science
− High resolution MS
− novel hyphenated techniques
− novel LBA/cell based assay formats
− assays combining LBA and MS technology,…

➢ "***Yes, can do !***" shouldn't mean "***Yes, let's do !***" if there is no scientific driver.
− "Yes, let's do !" may increase cost with no added value for the patient.
− "Yes, let's do !" may jeopardize science to progress.

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Summary and overall recommendation

- ➢ Consider 4 classifications and use them in concert to decide on the assay requirements.
  - Anticipated concentration changes (PD effect)
  - Development phase
  - Decisions taken from the data
  - Fit of assay format with Regulated Bioanalysis Guidelines.
- ➢ Ensure effective/iterative communication between the BA team and the investigator, also when a method is available.
- ➢ Apply tiered approach principles when defining assay performance.
  - If assay performance needs to comply with regulated bioanalysis requirements are needed, allow and document flexible and scientific adaptation of these Guidelines.
- ➢ Follow EBF flowcharts when developing your assay
  - start from understanding the biology of BM, confirm the scientific assumption (a preliminary assay), prior to embarking on final method establishment and sample analysis.

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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)

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Pointer in *Biomarkers in Medicine*
August 2012, Vol. 6, No. 4, Pages 507-509

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