



EBF

European  
Bioanalysis  
Forum

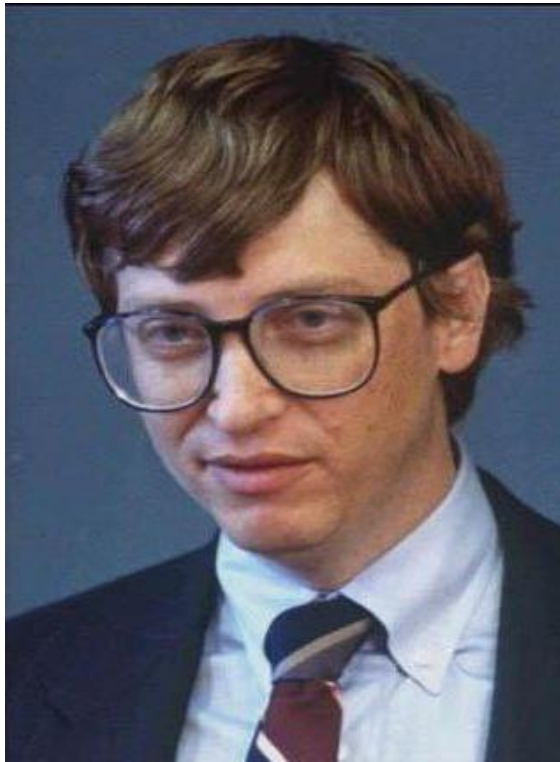
# **Taking tiered approach to the next level**

## **Feedback from the EBF workshop**

**Philip Timmerman**  
on behalf of the EBF

**EBF 7<sup>th</sup> Open Symposium**  
**19-21 November 2014**

# 1990



# 1990

But also...in 1990, the regulatory paradigm for bioanalysis was proposed, discussed and agreed (Crystal City-I)

A paradigm which remains today's solid foundation for regulated bioanalysis

# 2014



# 2014

## Highlights from technology

- Manual low throughput → automated high throughput
- Ng/ml limits of quantification → sub-fg limits of quantification
- Chrom.: Multiple assay formats → Limited (LC-MS/MS) based assay formats
- LBA: Limited assay formats → Multiple (and novel) assay formats
- Paper raw data → Electronic raw data
- PK of unchanged drug → PK/PD, TK, metabolites, biomarkers,..

## Regulatory paradigm

- General validation template → Same general validation template
- One guidance → Many guidances, but generally stating the same

# 2014

## Highlights from technology

Manual low throughput → automated high throughput

Mcg limits of quantification → sub-pg limits of quantification

Chrom.: Multiple assay formats → 1 single assay format (LC-MS/MS)

LA: Limited assay formats → Multiple (and novel) assay formats

**A LOT OF CHANGES**

Paper raw data → Electronic raw data

PK of unchanged drug → PK/PD, TK, metabolites, biomarkers,..

## Regulatory paradigm

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One guidance → Many guidances, but generally stating the same

# 2014

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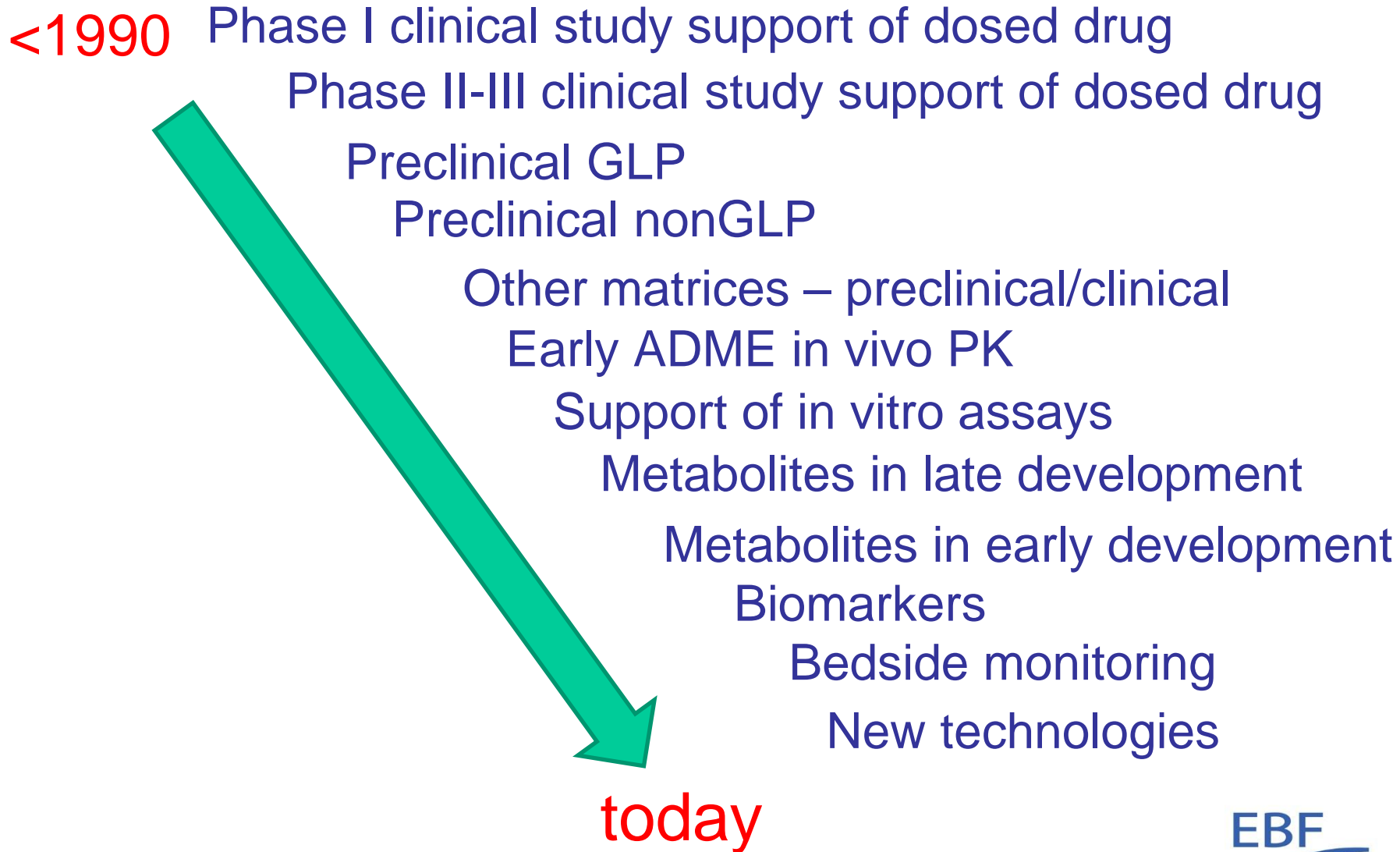
## Regulatory paradigm

General validation template → Some general validation template

**SAME PARADIGM**

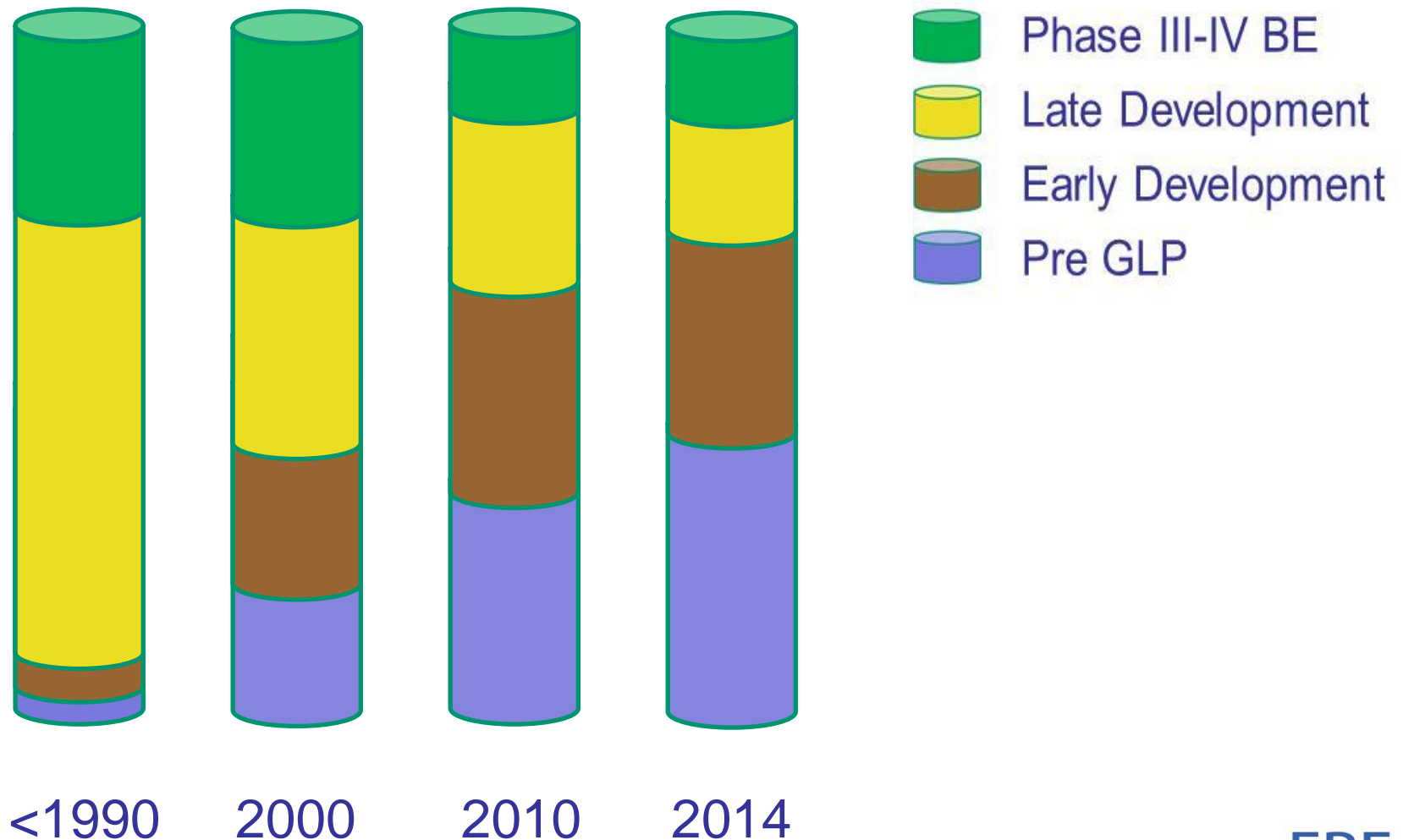
One guidance → Many guidances, but generally stating the same

# Workload: a changing landscape - 1

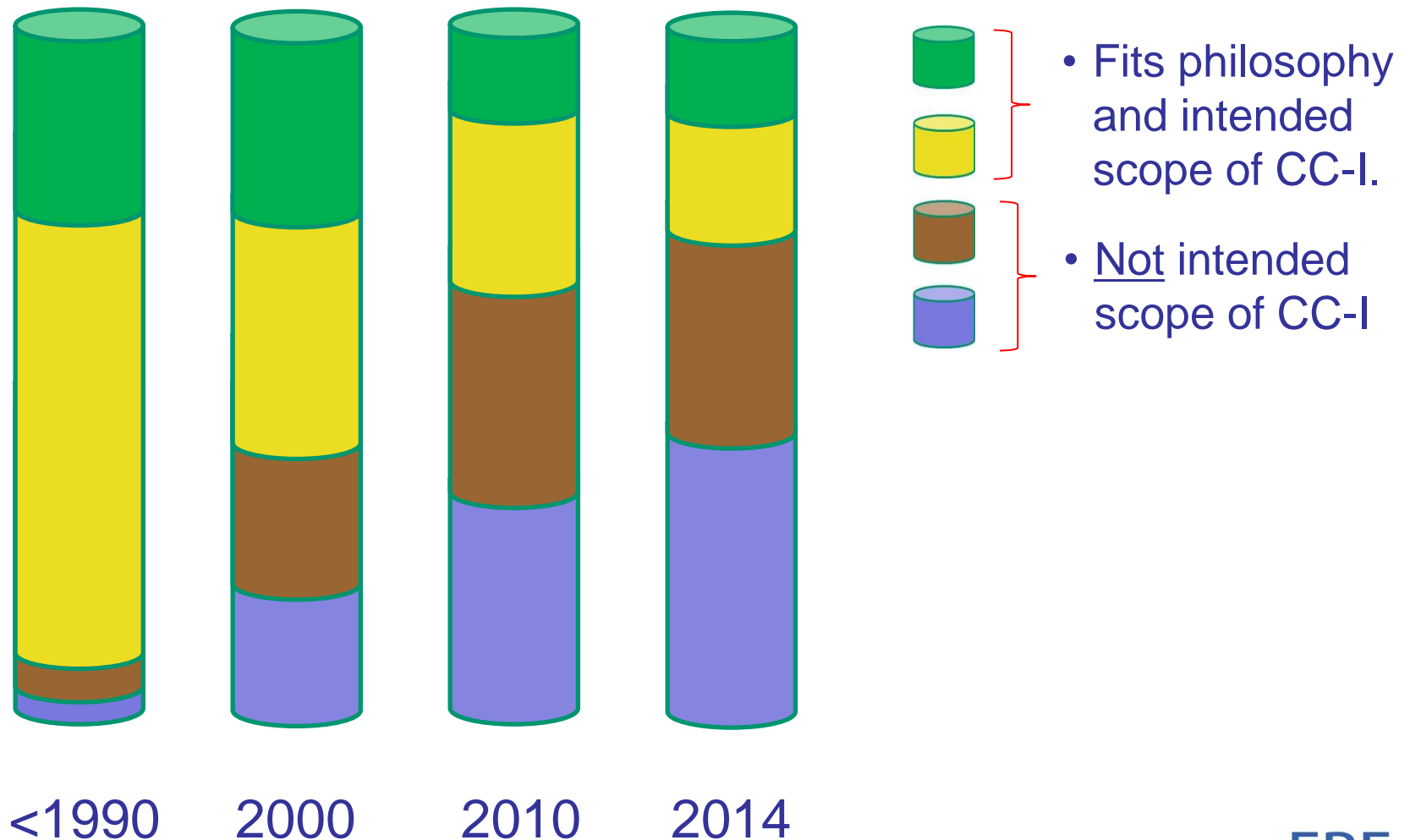




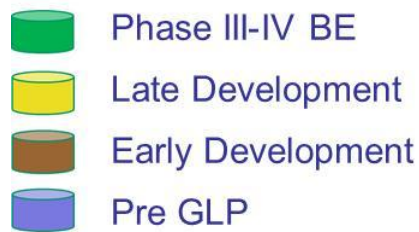
# Workload: a changing landscape - 2



# Workload: a changing landscape - 3



# Workload: a changing landscape - 4



Majority of studies  
will require  
Regulated Validation

Majority of studies  
may fit another  
approach

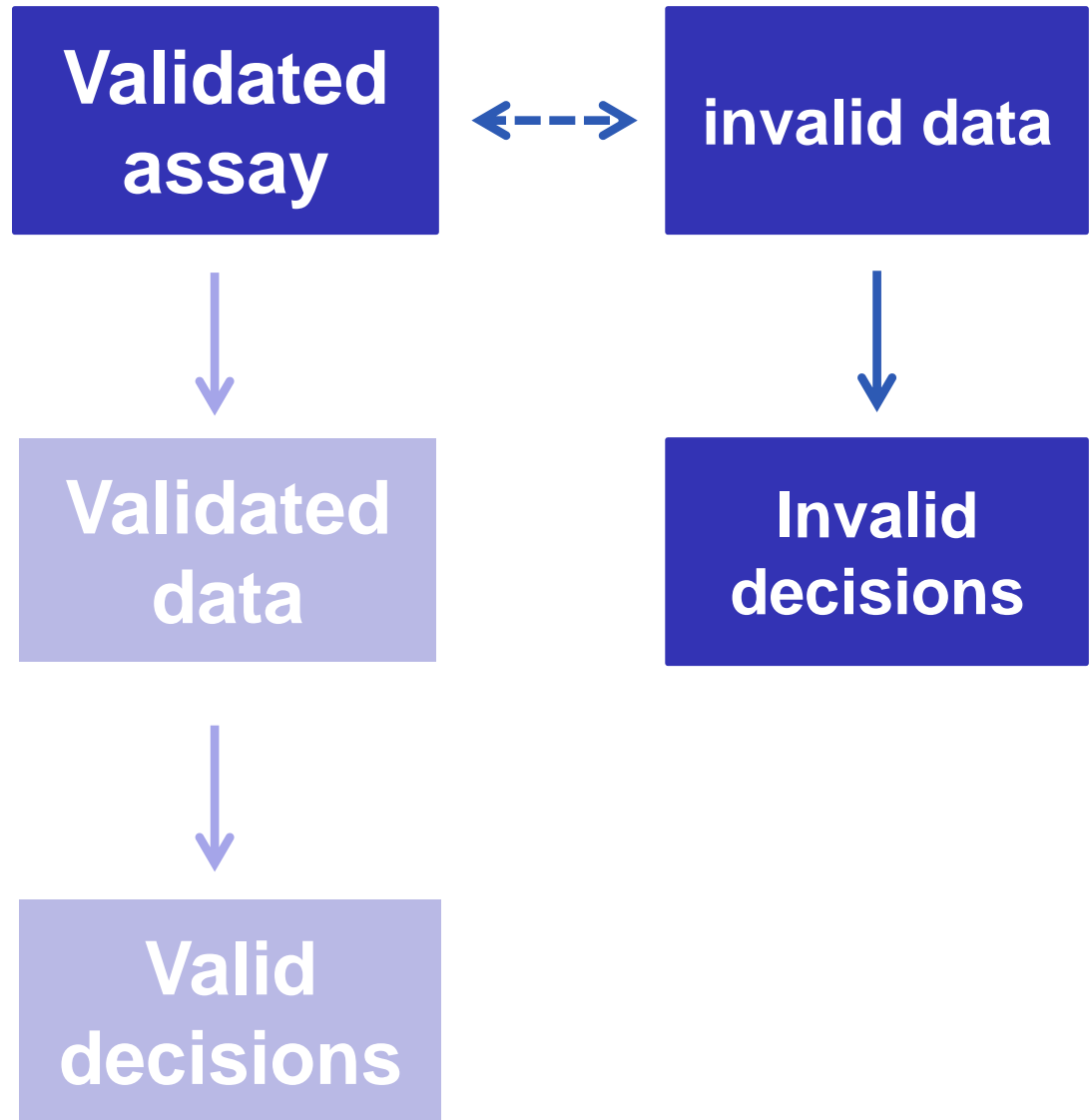
***“another approach.....”***

**What does this mean and.....What  
does quality look like?**

# Today's paradigm



**Today's  
paradigm may  
also result  
in...**



**Valid  
Assay**

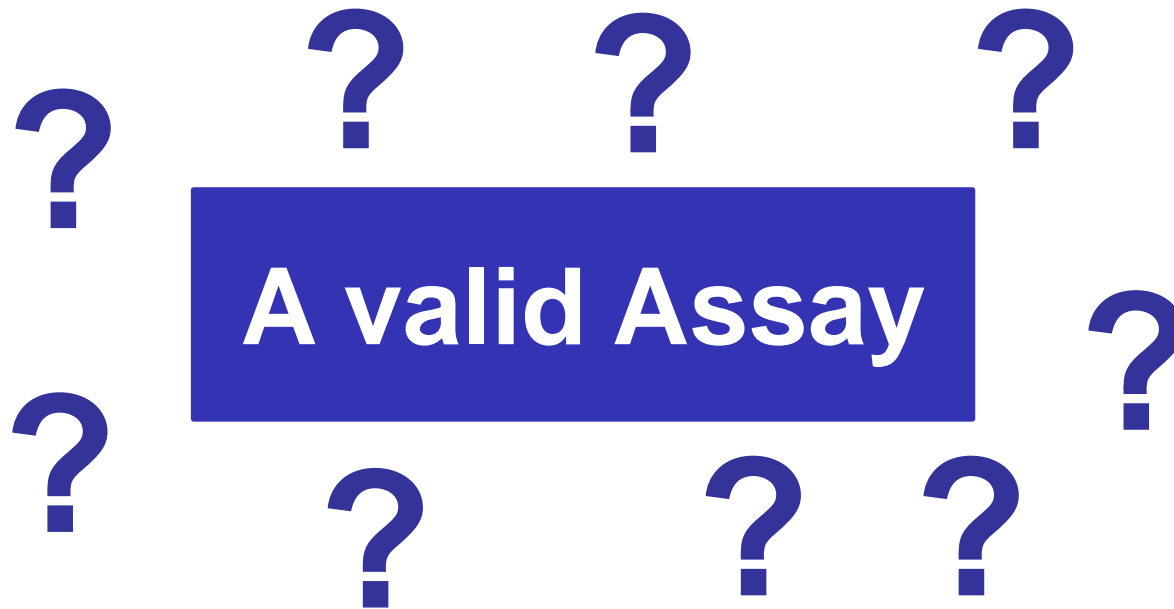


**Valid data**



**Valid  
decisions**

**Tomorrow's  
paradigm**



- How do we define a valid assay today, in view of the increased scope and variety of bioanalysis?
- Can this only be achieved using regulatory standards as per Guidance
- Is there a valid alternative, i.e. less resource demanding, allowing better scientific focus for the intended study purpose ?



**Another approach.....**

**.....tiered approach?**

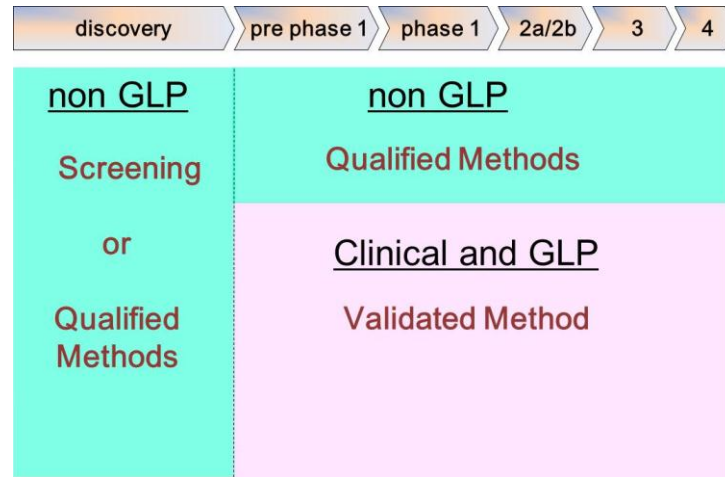
**NOT A NEW  
DISCUSSION**

# “Tiered approach”: A new paradigm ? - 1

1. Fit-for-Purpose Method Development and Validation for Successful Biomarker Measurement, *Pharmaceutical Research Vol. 23, No. 2, Feb 2006, 312-328*
2. Workshop/Conference Report — Quantitative Bioanalytical Methods Validation and Implementation: Best Practices for Chromatographic and Ligand Binding Assays. *Workshop/Conference Report. AAPS J. 9(1), E30-E42, 2007.*
3. Best practices in a tiered approach to metabolite quantification: views and recommendations of the European Bioanalysis Forum, *Bioanalysis, July 2010, Vol. 2, No. 7, Pages 1185-1194*
4. When do you need a validated assay? *Bioanalysis, (2011) Vol. 3, No. 24, Pages 2729-2730*
5. Tiered approach revisited: introducing stage-appropriate or assay-appropriate scientific validation. *Bioanalysis(2014) Vol. 6, No. 5, Pages 599-604*
6. Tiered approach: from plan into practice. *Bioanalysis (2014) Vol. 6, No. 5, Pages 585-586*

# “Tiered approach”: A new paradigm ? - 2

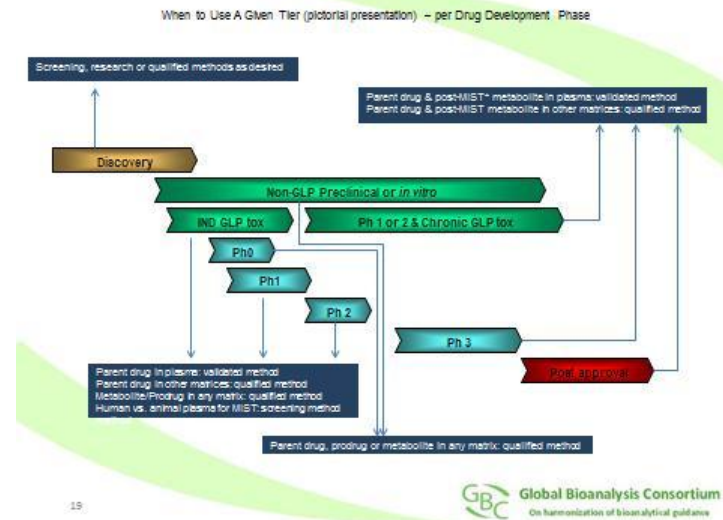
EBF, 2009



But never in enough practical detail



GBC, 2013



No consistent application in industry

# ....MHLW

「医薬品開発における生体試料中薬物濃度分析法のバリデーションに関するガ, 2013

## Annex Application of a tiered approach

Metabolites in human are sometimes unknown at the early stage of clinical trials and the sufficient supply of reference material of the metabolite may be delayed. In such cases, the so-called tiered approach may be applied for analytical method validation for efficient pharmaceutical development.

The tiered approach is a strategy to limit the characterization of an analytical method initially and to gradually expand parameters to be characterized and moving toward a full validation as the development process proceeds. Pharmaceutical research and development could be carried out more efficiently by adopting the tiered approach in the early to mid-stages of the development process, enabling early-stage evaluations and facilitating predictions of future development.

However, even when the tiered approach is used, it is advisable to predefine appropriate criteria for the characterization of analytical method based on scientific judgment in order to improve the reproducibility and reliability of concentration data obtained.

## ...Crystal City - V

draft FDA Guidance 2013:

- “For pivotal studies that require regulatory action for approval or labeling, such as BE or PK studies, the bioanalytical methods should be fully validated. For exploratory methods used for the sponsor’s internal decision making, less validation may be sufficient”

# EBF's anticipated contribution:

- Develop **a strategy** how to identify “*pivotal*”, “*internal decision*”, “*require regulatory actions for labelling*”
- Provide **a toolkit** to allow BA scientist to define:
  - a process ( $\neq$  ‘*decision tree*’) to define scientific assay specifications in support of valid decision making -
  - in practice, and as start, clear templates proposing minimal assay specifications for a selection of study types

# “Tiered Approach” → “Scientific Validation”

The feeling in industry remains:

if not “Validated” → it’s not “Valid”



Validated



Tiered approach

# Quick win?

## Contextualizing the definitions

### Validated → regulatory validation:

- *Assay validations to provide scientifically accurate, reproducible and reconstructable concentration data to allow valid decision making for the intended purpose of the study and comply with regulated BA standards as specified by Health Authority (HA) guidance documents*

### Tiered approach → scientific validation:

- *Assay validations to provide scientifically accurate, reproducible and reconstructable concentration data to allow valid decision making for the intended purpose of the study and can withstand independent review - including scientific review from regulators if so required - although not applying all elements specified by HA guidance documents.*



# Does this mean...

Regulatory validation  $\neq$  science?

Of course not....

- regulatory validation is built on solid scientific principles for studies where the Guidance was the intended scope (i.e. later stage clinical studies).
- However, for an array of study types in general and/or studies in earlier development the Guidance was not the intended scope. Hence, the scientific question may require a different approach, or the scientific principles from the Guidance may only partially meet the needs of those studies.

## Assay-appropriate scientific validation

Irrespective of the development stage in which the study is performed, the proposed validation criteria support valid and documented decisions making from the reported concentrations.

## Stage-appropriate scientific validation

Depending on development stage in which the study is performed, the proposed validation criteria may vary. Valid and documented decisions making from the reported concentrations is warranted in all cases.

# Scientific Validation

## Assay-appropriate scientific validation

### Examples:

#### Preclinical:

- screening PK prior to selection for development
- nonGLP PK during development
- Tissue homogenate analysis

#### Clinical:

- Urine analysis
- Metabolites (excluded: active metabs according to ICH-M3)
- Non pivotal mechanistic human PK-studies (e.g. microdosing,...)

## Stage-appropriate scientific validation

### Notes:

1. When a compound progresses through development, a study may be labeled as non-pivotal (internal decision) or pivotal.
2. 'Regulatory validation' falls within the stage appropriate scientific validation

### Examples:

- Early GLP (28d) = sc. validation → later stage GLP = reg. validation
- SAD/MAD = sc. validation → later stage clinical studies = reg. validation

# We discussed thoroughly

- EBF has been discussing the subject since 2008 and has since published a few papers on the topic to stimulate the discussion in industry:
  - Metabolites in safety
  - Tissue analysis
  - Support of in vitro assays
  - Biomarkers
- Since 2010, EBF supported the GBC A2 team
- Since 2012, EBF re-engaged to provide the proverbial “meat to the bone”- next slide

# An EBF Survey

12 Questions on Current and future practices on applying scientific validation versus regulated validation

Focus on both preclinical and clinical studies in early and late development for small and large molecules

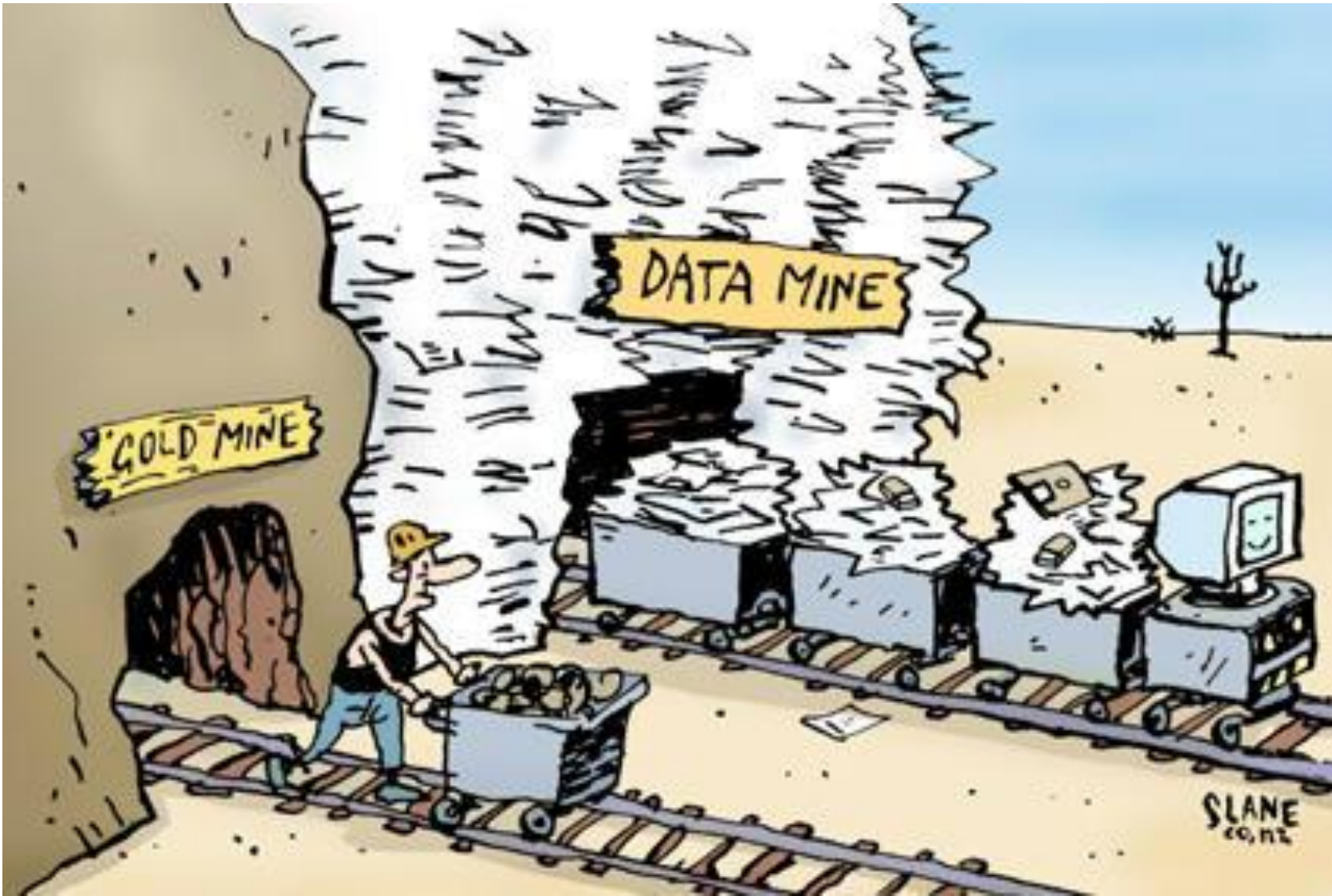


# An EBF Workshop

To discuss a proposed way forward

*Published in recent commentary in Bioanalysis  
Ref. : Bioanalysis, 2014, 6(19), 2593-2599*





# From the Survey, we could identify 4 categories of studies/assays

1. Continue using regulated validation
2. Continue using scientific validation
3. Intensify use of scientific validation
4. Start using scientific validation (instead reg. validation)



# + a Draft consensus on assay criteria for scientific validation, documentation and reporting for:

- 3 types of assay-appropriate scientific validation
  1. Urine analysis
  2. Tissue analysis
  3. Metabolites in relation to ICH M3(R2)
  
- 2 types of stage-appropriate scientific validation
  1. non pivotal clinical studies
  2. Early development GLP

# LBA

For LBA, there was an agreement that the complexity of the macro-molecules (proteins and therapeutic antibodies) and the specificity of the assay reagents are important parameters to take into account for scientific validated assays.

# In summary

## Urine, tissue, metabolites

- Agreement on value to propose minimal assay criteria for scientific validation, i.e. anybody setting up urine, tissue or metabolites assays would get a harmonized view on scientific validation assay criteria

## Early development clinical studies

- Current use of the regulatory assay is a comfort zone, although regulatory framework can jeopardize scientific freedom required in early studies

## Early GLP studies

- Current thinking from the workshop is that, although applying the principles of scientific validation is defensible for GLP purpose, early GLP studies would not be our first area of focus for scientific validation

# In detail

# assay appropriate scientific validation

	Metabolites	Urine	Tissue
<b>Method pre Validation</b>			
CoA with at minimum proof of identity/purity	N	Y or use dosed batch	Y or use dosed batch
Matrix QC identical as study	Y	Y	Y (or matrix matching)
Acceptance criteria QC – mean bias	n/a	n/a	n/a
Inter assay variability	n/a	n/a	n/a
QC levels – replicates	3/2	3/2	3/2
Acceptance criteria QC	4-6-20(25)	4-6-25(30)	4-6-25(30)
Acceptance criteria CAL	75% at least 5 (25% @ LLOQ)	75% at least 5 (30% @ LLQ)	75% of at least 5 (30% at LLOQ), (Surrogate) matrix matching acceptable
QC/Cal from separate stocks	N	Y	Y (unless check equivalence)
Selectivity	n/a (part of in study acceptance of incurred samples ?)		Y, scientific design
Extraction recovery	N	N	N
Carryover	In study	In study	In study
Matrix effect	N	Y (one level only)	Y (one level only)
Dilution integrity	In study	In study	In study
LOQ	As defined by acceptable LLQ QCs (P&A)	As defined by acceptable LLQ QCs (P&A)	As defined by acceptable LLQ QCs (P&A)
Comed selectivity (DDI type)	N	N	N
OTC selectivity	N, s, misc	N	N
FDC stability	N	N	N
Processed sample stability/reproducibility	N	N	Y (processed sample reproducibility)
Stock solution stability	Y/N (20%) minimal assessment performed?	If available previously	If available previously
Permeth stability	N	N	Y
Matrix stability for duration of storage	Y (20%)	N	N
F/T stability	N, consider ISS	? #	Y (1 cycle)
WBS	N	n/a	n/a
Sampling conditions	N	Y, consider including container and adsorption	Y, Specify conditions, consider EBF pap
Hemolytic	N	n/a	n/a
Hyperlipidemic	N	n/a	n/a
Validation plan/protocol	Y, at minimum SOP or short protocol	Y, at minimum SOP or short protocol	Y (if claiming GLP compliance)
Validation report	Y, reporting scientific parameters tested	Y, reporting scientific parameters tested	Y (if claiming GLP compliance)
misc.	Consider longer run time instead of specificity experiment	# = during meth dev. NSB/BTS/sampling conditions & aliquotting	Calibration matrix may be a surrogate

Still draft – fine-tuning ongoing

# assay appropriate scientific validation

	Metabolites	Urine	Tissue
<b>Method pre Validation</b>			
CoA with at minimum proof of identity/purity	N	Y or use dosed batch	Y or use dosed batch
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QC/Cal from separate stocks	N		Y (unless check equivalence)
Selectivity	n/a (part of in study acceptance of incurred samples ?)	Y	Y, scientific design
Extraction recovery	N	N	N
Carryover	In study	In study	In study
Matrix effect	N	Y (one level only)	Y (one level only)
Dilution integrity	In study	In study	In study
LOQ	As defined by acceptable LLQ QCs (P&A)	As defined by acceptable LLQ QCs (P&A)	As defined by acceptable LLQ QCs (P&A)
Comed selectivity (DDI type)	N	N	N
OTC selectivity	N, see misc	N	N
FDC stability	N	N	N
Processed sample stability/reproducibility	N	N	Y (processed sample reproducibility)
Stock solution stability	Y/N (20%) minimal assessment performed?	If available previously	If available previously
Prep stability	N	N	Y
Matrix stability for duration of storage	Y (20%)	N	N
F/T stability	N, consider ISS	? #	Y (1 cycle)
WBS	N	n/a	n/a
Sampling conditions	N	Y, consider including container and adsorption	Y, Specify conditions, consider EBF pap
Hemolytic	N	n/a	n/a
Hyperlipidemic	N	n/a	n/a
Validation plan/protocol	Y, at minimum SOP or short protocol	Y, at minimum SOP or short protocol	Y (if claiming GLP compliance)
Validation report	Y, reporting scientific parameters tested	Y, reporting scientific parameters tested	Y (if claiming GLP compliance)
misc	Consider longer run time instead of	# = during meth dev. NSB/BTS/sampling	Calibration matrix may be a surrogate

Still draft - fine-tuning ongoing

# assay appropriate scientific validation

	Metabolites	Urine	Tissue
<b>Study sample analysis</b>			
Acc&Prec	4-6-20(25)	4/6/20 (25)	4/6/25 (30)
Acceptance criteria QC – mean bias (for n>1 batch study sizes)	20	25	25
ISR	N	N	N
Carry over	Y, Assess impact	Y, Assess impact	Y, Assess impact
Dilution integrity (may be QC)	If required	If required	If required
IS variability with criteria	Scientific judgment	Scientific judgment	Scientific judgment
Number of cal points	75% at least 5 within 20 % (25% @ LLOQ)	75% at least 5 within 25 % (30% @ LLOQ)	75% at least 5 within 25 % (30% at LLOQ), (Surrogate) matrix matching acceptable
Number of QC levels/replicates	3 - 2		3 - 2
Hemolytic	N	n/a	n/a
Hyperlipidemic	N	n/a	n/a
anomalous result repeats	N	N	N
Extrapolation beyond curve	Dilute into range, no extrapolation below	Dilute into range, no extrapolation below	Dilute into range, no extrapolation below
Drug in placebo/control misc.		Selectivity by dilution of incurred samples	Protocol & report QA'd if claiming GLP

**In-study validation as part of study sample analysis** if only in-study validation is performed (no pre-validation), include (with predefine acceptance criteria) those relevant missing parameters from scientific validation above mentioned (e.g. relevant stability)

Still draft – fine-tuning ongoing

# stage appropriate scientific validation

	Clinical non pivotal	Preclinical early GLP (nonPivotal)
<b>Method pre Validation</b>		
CoA with at minimum proof of identity/purity	Y or use dosed batch	Y or use dosed batch
Matrix QC identical as study	Y	Y
Acceptance criteria QC – mean bias	n/a	n/a
Inter assay variability	Use scientific judgment based upon P&A of 1-run validation	Use scientific judgment based upon P&A of 1-run validation
QC levels – replicates	low mid high 4-6 or 1QC-pair per decade, not LLOQ	low mid high 4-6 or 1QC-pair per decade, not LLOQ
Acceptance criteria QC	4-6-20(25)	15 (20 @ the high end) for non P&A runs, align with clinical preferred
Acceptance criteria CAL	75% and at least 6 points within 20% (25% @ LLOQ)	75% and at least 6 points within 15% (20% @ LLOQ) Surrogate matrix acceptable
QC/Cal from separate stocks	N, if other checks are in place	Y, unless accuracy of stock proven
Selectivity	6	n=1 matrix source is still relevant
Extraction recovery	N	N
Carryover	N	In study
Matrix effect	N, assess within study runs via IS response	N, assess within study runs via IS response
Dilution integrity	In study	In study
LOQ	As defined by acceptable LLQ QCs (P&A)	As defined by acceptable LLQ QCs (P&A)
Comed selectivity (DDI type)	n/a or In silico	N
OTC selectivity	N	N
QC stability	N	N
Processed sample stability reproducibility	scientific judgement	scientific judgement
Sample collection stability	Use historical data	Y, unless prepared the same day.
Bench top stability	Total stability expt include historical preclinical data at one level (mid) 20%	Consider combined stability experiment to cover unknown samples
Matrix stability for duration of storage		
F/T stability		
WBS	N, unless for known problem scaffolds, cfr EBF paper	N, unless for known problem scaffolds, cfr EBF paper
Sampling conditions	Cover via a combined stability expt (see above)	Cover via a combined stability expt (see above)
Hemolytic	N	N
Hyperlipidemic	N	N
Validation plan/protocol	Y, predefined scientific parameters tested	Y, predefined scientific parameters tested
Validation report	Y, report scientific parameters tested	Y, report scientific parameters tested
misc.		

Still draft - fine-tuning ongoing



## stage appropriate scientific validation

### Clinical non pivotal

### Preclinical early GLP (nonPivotal)

#### Study sample analysis

	Clinical non pivotal	Preclinical early GLP (nonPivotal)
<b>Acc&amp;Prec</b>	4/6/20	4/6/15
<b>Acceptance criteria QC – mean bias (for n&gt;1 batch study sizes)</b>	20	15
<b>ISR</b>	Y, cfr Rocci et al. (n=20 or so) for 1 <sup>st</sup> study	Y, if first time in species, Asses... proposal
<b>Carry over</b>	Y, assess impact	Y, assess impact
<b>Dilution integrity (may be QC)</b>	If required	If required
<b>IS variability with criteria</b>	EBF: white et al	white et al
<b>Number of cal points</b>	75% at least 6	75% at least 6
<b>Number of QC levels/replicates</b>	3 - 2	3 - 2
<b>Hemolytic</b>	N, is part of IS tracking, observation... clinical Unit - interrogate data for... until	N
<b>Hyperlipidemic</b>	N, is part of IS tracking	N
<b>anomalous result repeats</b>	Yes as requested in duplicate	Yes, as requested in duplicate
<b>Extrapolation beyond curve</b>	Dilute into range, no extrapolation below	Dilute into range, no extrapolation below
<b>Drug in placebo/control</b>	Yes in duplicate	Yes in duplicate
<b>misc.</b>		

Still draft – fine-tuning ongoing

**In-study validation as part of study sample analysis** if only in-study validation is performed (no pre-validation), include (with predefine acceptance criteria) those relevant missing parameters from scientific validation above mentioned (e.g. relevant stability)

# Documentation/reporting

- Include Reference to Study number, Study responsible, Protocol or SOP (?signed by sponsor?)
- Template scope of scientific validation to frame the context
  - maybe with reference to EBF recommendation paper or other?
- Body:
  - (reference to) assay description
  - short summary table providing evidence of assay performance, range and stability
  - More detail in appendix as required or as per company desire (e.g. sponsor-vendor relationship)
- No need to include chromatograms
- No GLP claim on validation
- Signature of study responsible

## Assay type – Ligand Binding Assays

PK

### scientific validation

CoA - Reference Material

Yes, preferred to use dosed batch

MDev = Matrix effect (MRD),

Range, QC levels,

Recovery,

Conc of coat/detect reagent

Method Development (Mdev)

Number of runs

3

QC/Cal from separate stocks

Intermediate Stock solution stability in buffer  
or matrix?

N

Matrix of QC

identical as unknown samples

QC levels – replicates

3/2

Acceptance criteria QC - mean bias %RE

Pre& Acc runs - between 20-40% depend on stage

Inter assay variability (%CV)

Pre& Acc runs - between 20-40% depend on stage

Acceptance criteria QC

4-6- (20-40)

Total Error

N/A

Calibrator %RE

25

Acceptance criteria CAL

75% at least 6

Precision duplicate - CAL/QC/Sample

25

LLOQ

Y, defined by Low Cal point

ULOQ

Y, defined by High Cal point

Still draft - fine-tuning ongoing

## Assay type – Ligand Binding Assays

	PK
<b>scientific validation</b>	
Acceptance criteria LLOQ/ULOQ %RE	%RE: 25% LLOQ /40% ULOQ
Selectivity in individuals	N for preclinical - (depend on Meth Dev)
Specificity: Reagents	Y
Interference -Structural related compound	Y
Dilution Linearity	Y
Bench top stability	N - compound related & matrix
Sample stability for duration of storage (Long-term stability)	N - compound related & matrix
F/T stability	N - compound related & matrix
Whole Blood Stability	N
Description of Sampling	
Hemolytic / Hyperlipidemic	N/N
Validation report	Summary report
Validation plan/protocol	N

<b>Study sample analysis</b>	
Acc&Prec	4-6-(20-40)
ISR	N
Dilution Linearity	Y
Number of cal points	75% at least 6
Number of QC levels/replicates	3 levels - 2 replicates
Hemolytic	Scientific judgment
Hyperlipidemic	N

# What is the gain? a **FRESH** start

## ➤ **F**lexibility

- Allowing in study vs. pre study validation
- More flexible follow up of analytical issues

## ➤ **R**esource

- In general, less time to set up an assay
- Reporting

## ➤ **E**thical

- preclinical... combining, limiting or removing some test affecting the 3R, *being aware that current validation practices copied to earlier studies can consume more animals for blank matrix purposes than the study itself*

## ➤ **S**cientific:

- More opportunity to focus on the scientific questions for the specific question, development stage or assay.

## ➤ **H**armonization of approaches across industry

# What do we not want to lose?

- Quality
- Transparent and documented decisions
- Documentation
- A priori criteria

# “4-6-20” vs. “4-6-15”

- Is anxiety around more liberal acceptance criteria a valid worry or are we over-reacting?
  - For regulated SMOL BA: 4-6-15 is limit, but most studies come in @ better acc&prec.
  - In practice, also with more liberal criteria, most studies will be coming in at better acc&prec, than 4-6-20, and likely even 4-6-15.
  - Having more pre-agreed liberal acceptance criteria, will prevent undue repeat for those studies which fall outside (too) stringent criteria for the purpose of the study

# Next steps

- Your input in the panel discussion
- A 1-day meeting in NA (together with Delaware Valley Drug Metabolism Discussion Group – DV/DMDG) on Jan 20<sup>th</sup> 2015 to allow discussion with our NA colleagues on the subject
- Session @ AAPS Annual Meeting 2015? Proposal submitted
- Feedback in 8<sup>th</sup> EBF Open Symposium (Nov. 215)
- Publish as an EBF Recommendation in 2015.



# Acknowledgment

- The team
  - Magnus
  - Steve
  - Morten
  - John
  - Marianne
  - Stuart
  - Philip
- EBF
- Workshop delegates