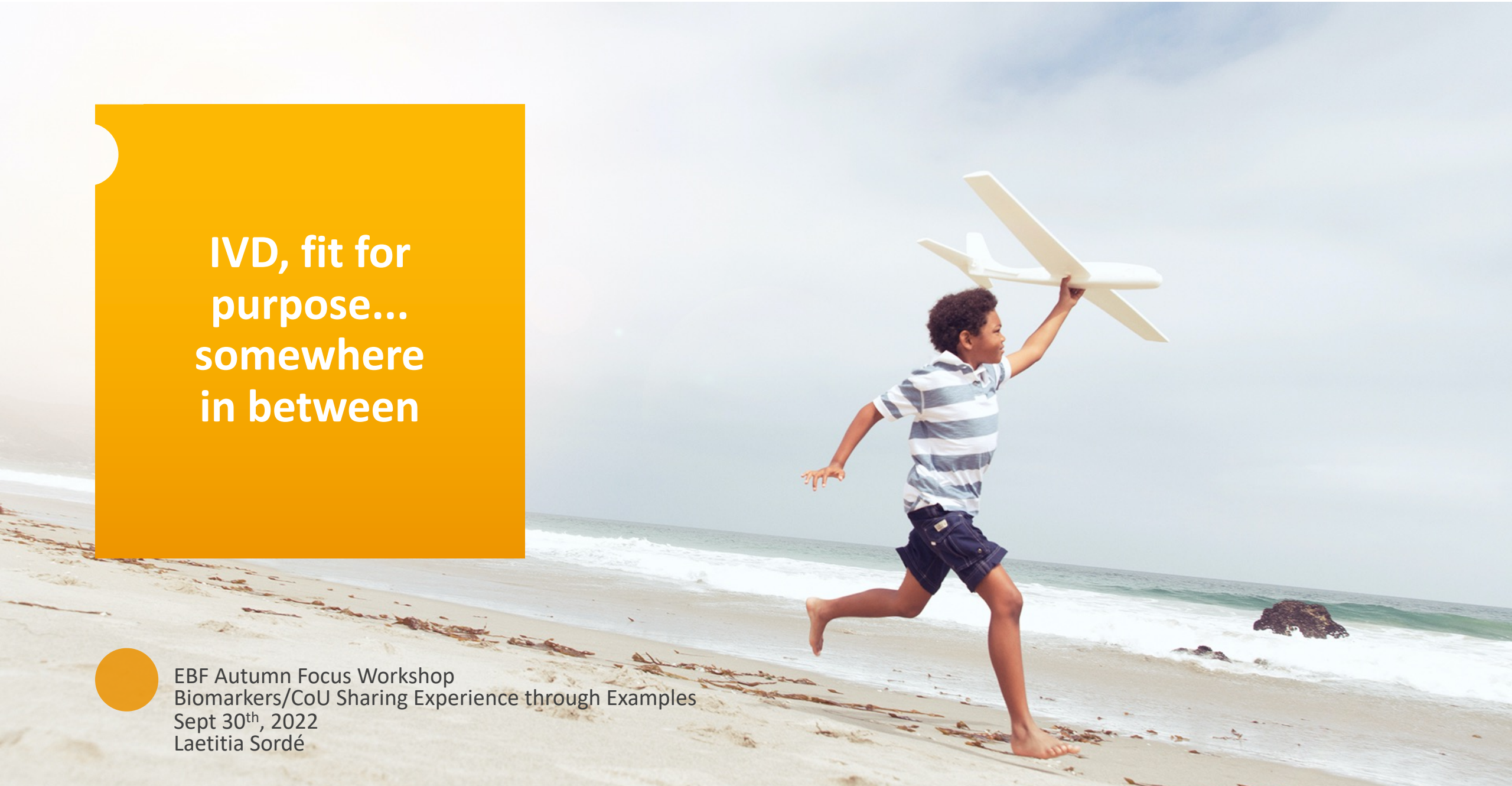
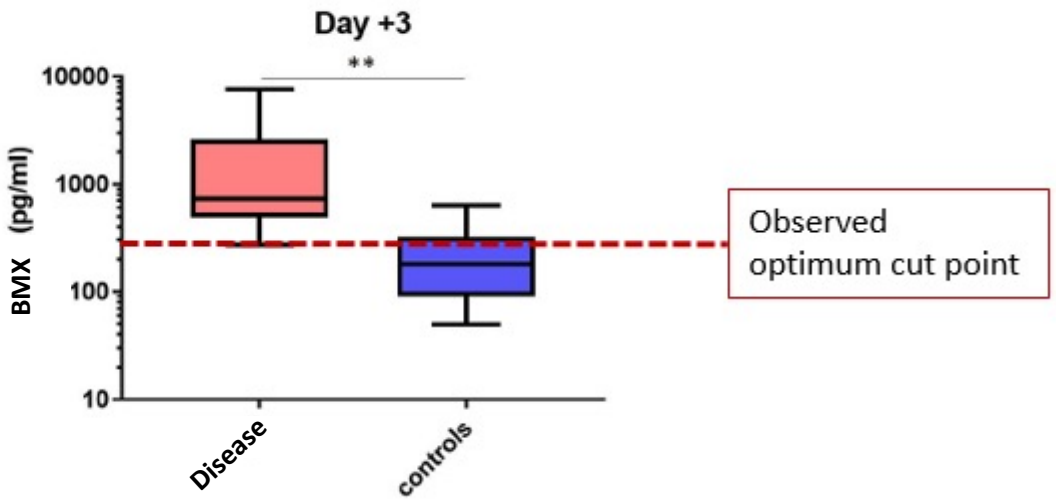
IVD, fit for purpose... somewhere in between



EBF Autumn Focus Workshop
Biomarkers/CoU Sharing Experience through Examples
Sept 30th, 2022
Laetitia Sordé

Biomarker Background

- Biomarker X (BMX) is a Chemokine downstream of target receptor
- Post hoc analysis of an internal clinical trial allowed to identify BMX as a good predictive biomarker of an acute inflammatory condition observed during patient follow up
- This was also observed by an independant group who studied BMX after standard of care intervention in various underlying diseases:



Graph reported in the literature

Study Design

Develop a human therapeutic antibody in patients who have elevated BMX and are at high risk of developing acute inflammatory condition after standard of care intervention

Observational Study

- Provide additional data on the BMX levels in various underlying diseases
- Use **MSD assay** validated at Sobi
- Correlate BMX levels with inflammatory condition to support the determination of a cutoff for Phase 3

Phase 2 PoC

- Establish the appropriate dosing regimen
- Assess preliminary safety and efficacy
- Use a **RUO assay** to assess BMX levels on sites to define patients inclusion

Placebo-controlled Phase 3

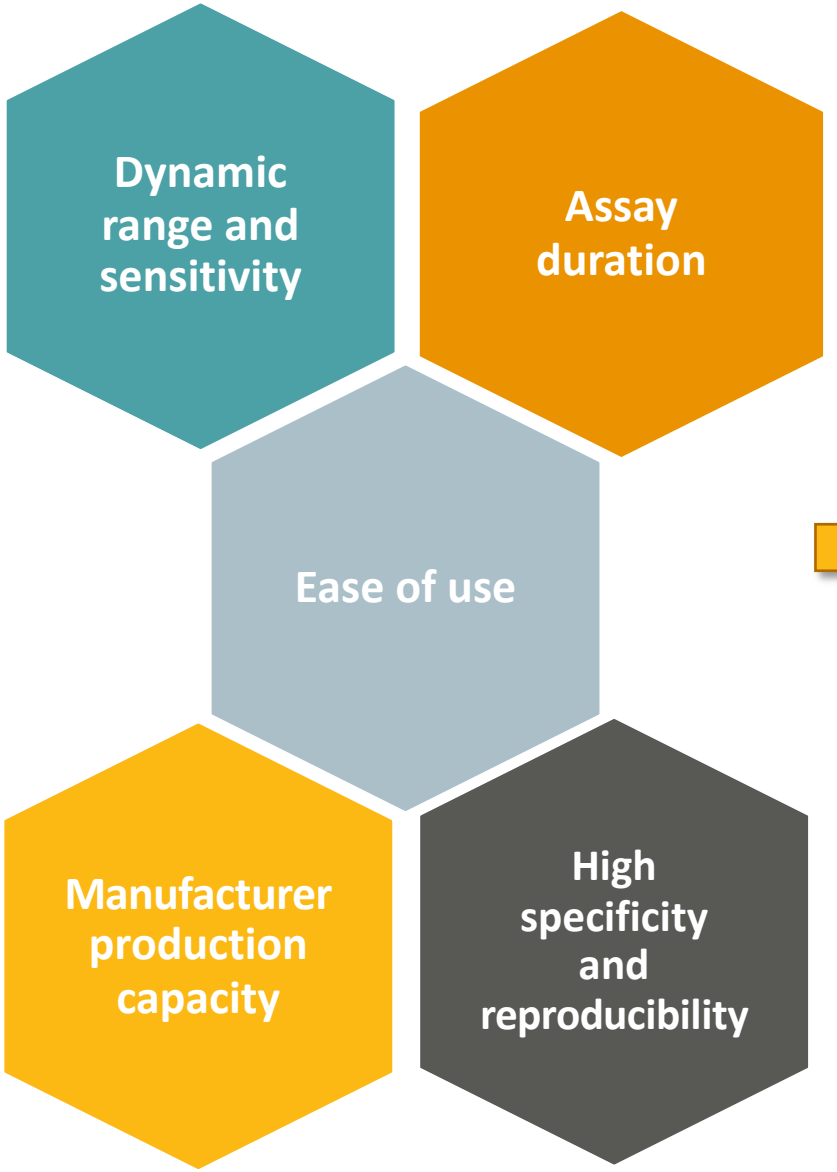
- Demonstrate safety and efficacy
- Use an **IVD** to assess BMX levels to define patients inclusion
- BMX cut off value confirmed or refined using data from previous studies

RUO Selection according to CoU

CoU

Define eligibility to treatment into PoC study by identifying patients at high risk for acute inflammatory condition

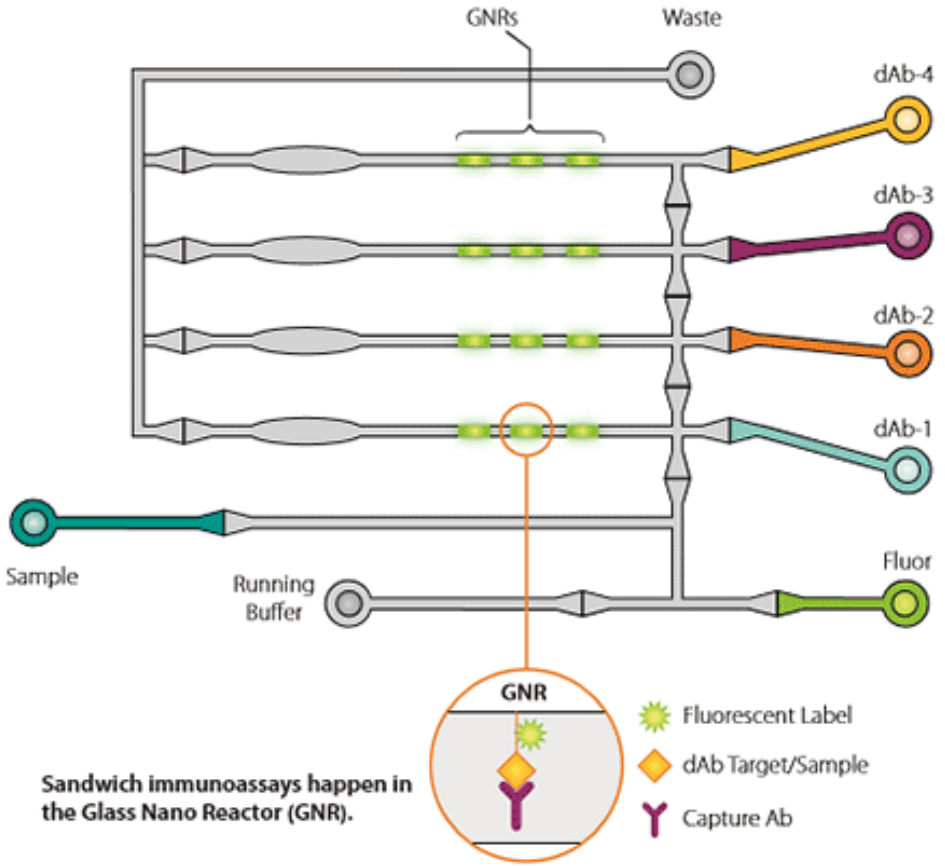
Assay will be developed and validated at Sobi then transferred to clinical sites.



5 different kits were tested

Ella platform: automated immunoassay workflow

1. Sample is routed through microfluidic channels.
2. Capture antibody captures target analyte.
3. Stringent wash removes unbound analyte.
4. Detection antibody migrates through microfluidic channel.
5. Stringent wash removes unbound detection antibody.
6. Scan GNRs.

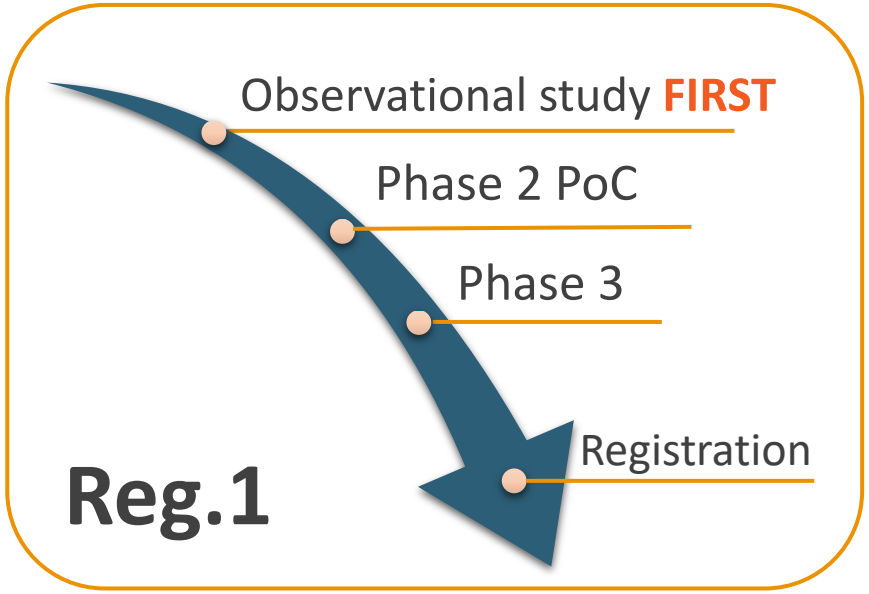


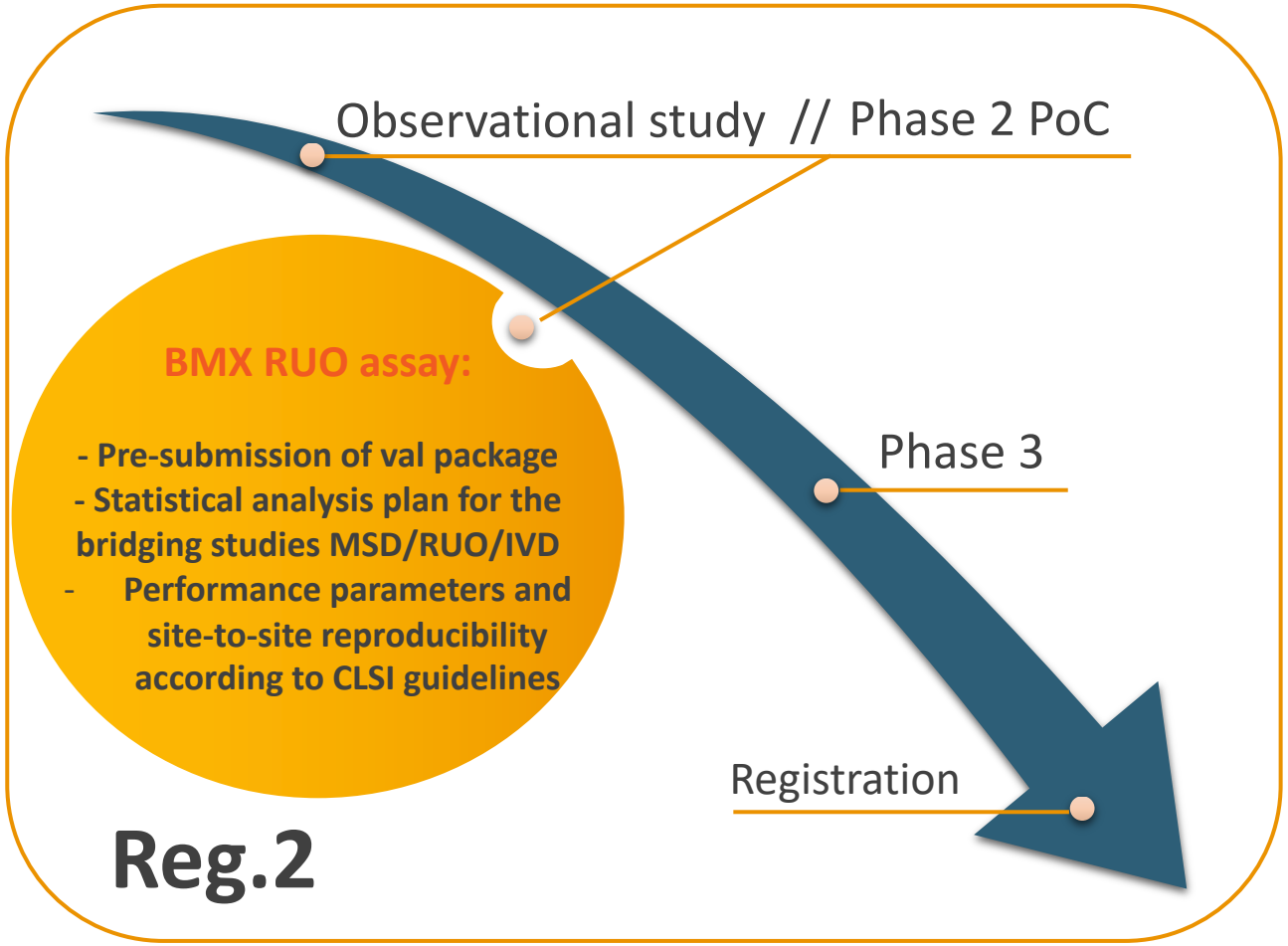
Scan barcode

Add samples and wash buffer

Run cartridge

Regulatory authorities feedback





Different options for Clinical Development Strategy

Option	How?	Pros	Cons
1- Conservative approach	Use IVD to include patients with BMX > threshold	• IVD validated according to CLSI guidelines • Pre-submission reg authorities • Only 1 bridging (MSD to IVD)	• 14 months to get IVD ready
2- No cohort enrichment	No use of BMX as inclusion criteria, treat all patients with the drug	• No need to measure BMX on sites • Allows to collect PK data to determine the dosing • Start Phase 3 when IVD ready	• Need of 600 patients minimum to see efficacy: recruitment time too long • Ethical concerns • Economical concern • Not aligned authorities consultation, need to change protocol • No additional information on BMX threshold
3- Investigator Initiated Trial	SOBI AG is not the Sponsor of the trial, but the site's investigator	• Use RUO assay internally validated • Do not consider recommendation to run observational study first	• Limited number of centers • Legal concern: ownership of data and data review/ access? • Quality of the data? • Bridging MSD to RUO + RUO to IVD for Phase 3
4- Go outside of reg 1 and reg 2 countries	Go in other countries	• Use RUO assay internally validated • Launch in other countries in Phase 3 with IVD	• Sites number are limited • Samples logistics • Bridging MSD to RUO + RUO to IVD for Phase 3 • Timelines/ feasibility to assess

ELLA RUO assay validation according to CoU

Calibration curve

- Comparison between fresh versus printed calibration curve

Accuracy and Precision

- Intra and inter
- Endogenous QCs
- 60 sets of each level
- 30 runs
- 4 operators
- 15 days
- 4 lots of cartridge

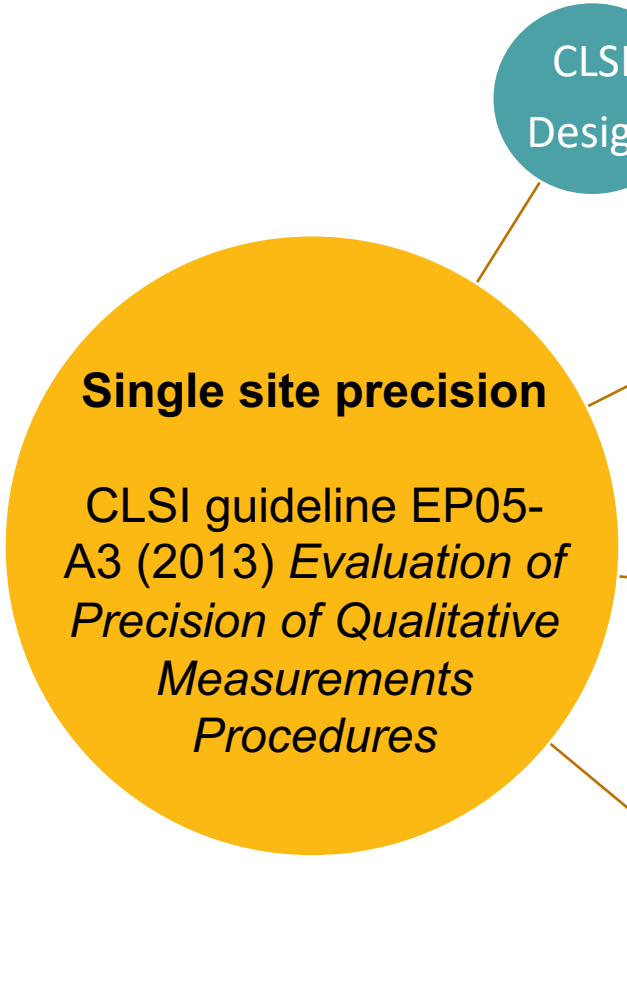
Subsequent parameters

- HV Baseline
- Parallelism
- Whole Sample imprecision
- Hemolysis/ Lipemia
- Drug interference
- Specificity
- Centrifugation impact
- Hook effect

Stability

- Short term:
  - Stable up to 24h at Ambient temp and 4°C
  - Up to 5 F/T
- Long term:
  - Up to 6 months at -20°C
  - Up to 11 months at -80°C

ELLA RUO assay validation according to CoU



- 20x2x2
- Each sample analysed on 20 days, 2 runs/day, 2 replicates/run

- 15x2x2
- Based on preliminary statistical evaluation from development

- Followed guideline
- ANOVA mixed model analysis of variance

- %CV = 4.0% between days, 5.2% between runs (within day), and 6.0% for the residuals (operators, lots and others)
- **Global variability (all parameters) 8.9%**
- 30 runs, 4 different operators and 4 lots of cartridges

ELLA Bridging with MSD

Estimate the potential bias between the methods

Evaluate how to translate the cut-off value of BMX

Classification Concordance: does the classification of samples (above or below BMX cut-off) agree between the two methods?

Design and statistical analysis based on the CLSI guideline EP09c *Measurement Procedure Comparison and Bias Estimation Using Patient Samples*

60 individual sera measured 5 times, by 5 analysts on 5 days

20 samples close to BMX cut off, rest covered the range

ELLA and MSD Correlation assessment

- Statistical analysis was performed according to CLSI guideline
- Shows linear relationship
- MSD equivalent cutoff of 300 pg/mL on the Ella™ is 1065 pg/mL
- 90% of samples (18/20) were in concordance of classification between the 2 methods
- Samples not in concordance were within + 11% of the MSD cut off
- 99.9% chance that a sample with observed Ella™ concentration >1200 pg/mL will be classified as positive if the same sample is measured again

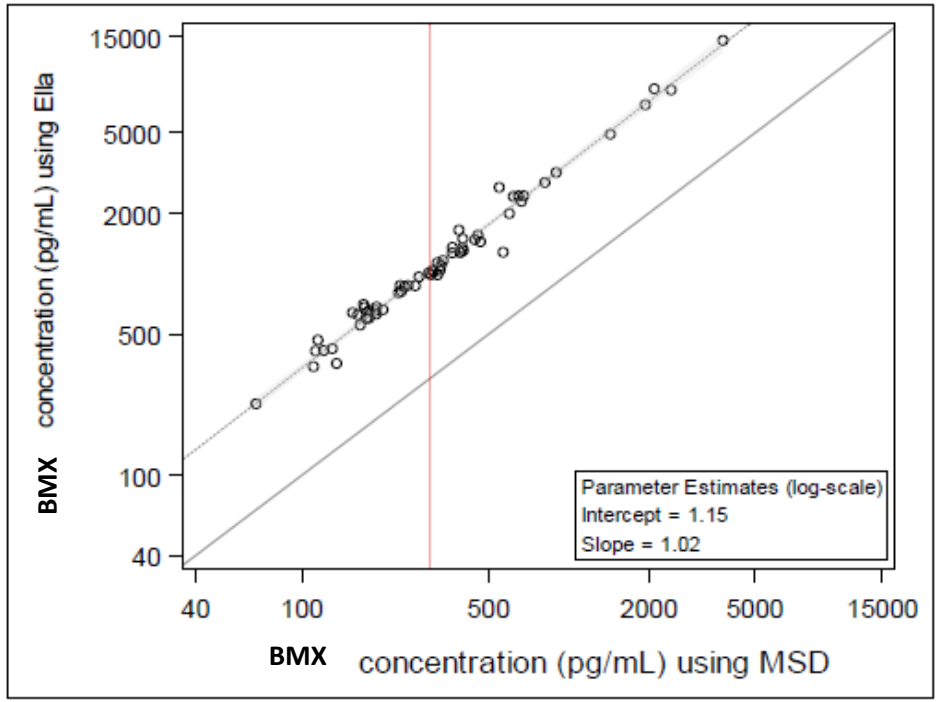


Figure 1. Scatter plot of normalized MSD concentration vs Ella™ concentration visualized on logarithmic scale together with fitted Deming regression line (dashed line) with 95% CI band.

ELLA Cross-Validation in clinical sites

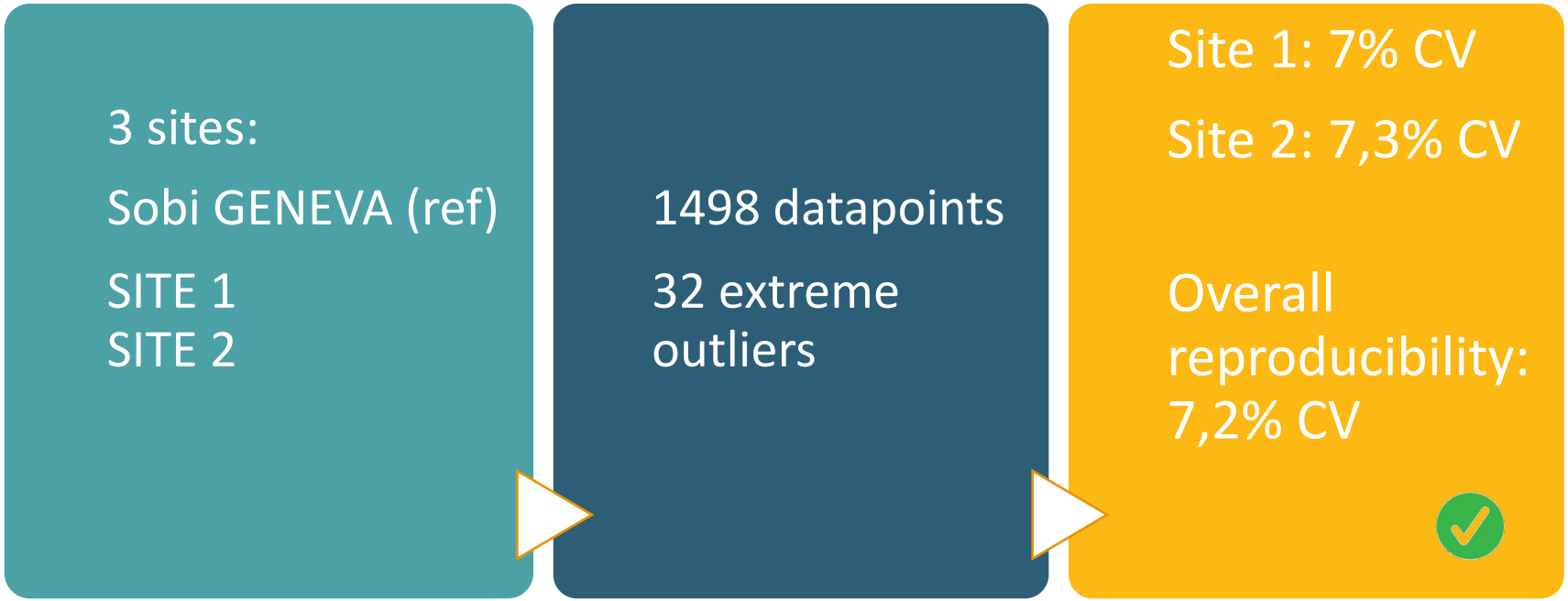
Demonstrate the agreement and the performance of the Ella method across each clinical site versus Sobi AG Laboratory

Design and statistical analysis based on the CLSI guideline EP05-A3 "*Evaluation of Precision of Quantitative Measurements Procedures*"

20 individual sera sent from Sobi to site
Analysed on 5 days in 5 replicates each day (5X5)
= 25 datapoints per sample

Criteria: precision at site ≤ 20%.
At least 80% of samples to meet the precision criteria

ELLA Cross-validation results



Conclusion

CoU

"Define eligibility to treatment according to BMX levels post-standard of care intervention, allow fast TAT for prompt inclusion decision "

CoU lean towards Ella platform in development phase

Regulatory

Regulatories imposed to validate a RUO as an IVD for a Phase 2 PoC

But the safety risk associated was very low (antibody drug used in other indications)

Validation was conducted in accordance of CoU and applied parts of CLSI guidelines with robust design and well documented statistics

Outcome

Ella validation package was well accepted by other countries authorities

Challenging to implement Ella at sites and required many resources

Facing these difficulties together with low recruitment rate, it was decided to put on hold Phase 2 and to wait for IVD to be ready to conduct Phase 2

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What could have gone wrong if PK guideline was followed and no CoU applied?

Keeping the already established MSD assay would have prevented a «fast and easy of use» assay implementation, sites would have likely declined as too challenging/ assay transfer would have failed / treatment decision delayed and efficacy impacted

We would not have reached the targeted precision with MSD assay. This important point was discussed with clinical stakeholders and was key for sites enrollement

Regulatories asked for validation according to CLSI guidelines, since assay reproducibility was key. Following PK guidelines would have prevented running the single and multi-sites precision with the right samples, the right design and the right statistics

Would have failed homogenous patients inclusion, making data interpretation impossible

Questions for round table discussion

- Wasn’t it risky to jeopardize trust with regulatories by going to other countries?
- Don’t you think cut off value should have been reinforced with more clinical data rather than bioanalytical data?
- Why was it challenging to implement Ella assay at clinical sites? Which issues did you face?
- What is the principle of a printed calibration curve?
- Was the CoU clearly defined since the beginning? Which stakeholders were involved?
- Where was the CoU documented?