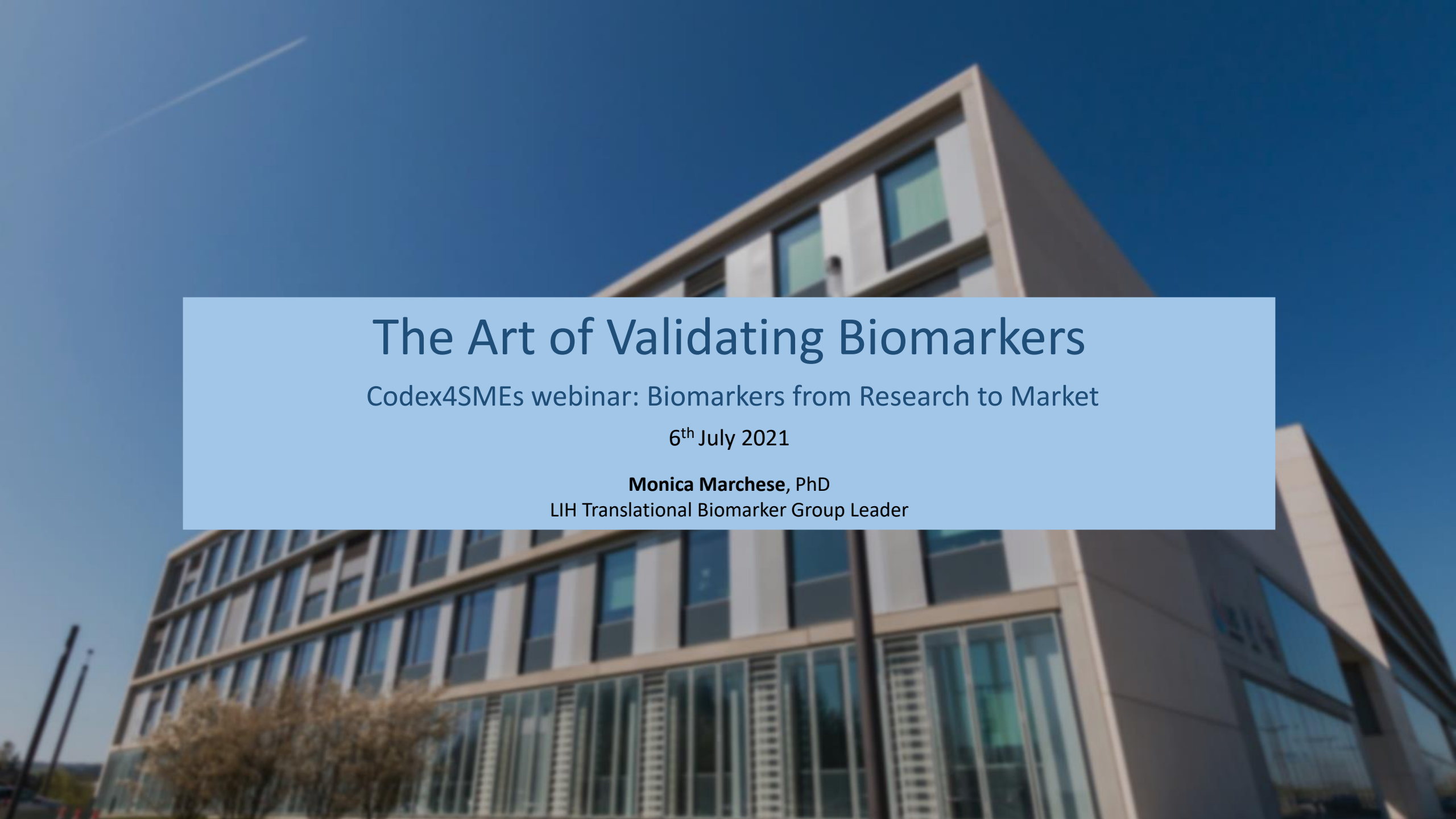


The Art of Validating Biomarkers

Codex4SMEs webinar: Biomarkers from Research to Market

6th July 2021

Monica Marchese, PhD
LIH Translational Biomarker Group Leader

What is a biomarker?

A biomarker is an objectively measurable characteristic that relates to normal biological processes, pathogenic processes, anatomic measurements, or pharmacological responses to therapeutic intervention.

[BEST: Biomarkers, EndpointS and other Tools—2016].

Biomarkers: past, present and future

- 6,000 years ago, Arab, Hindu and Chinese civilizations reported sweet tasting urine in patients displaying symptoms of what was later identified as diabetes

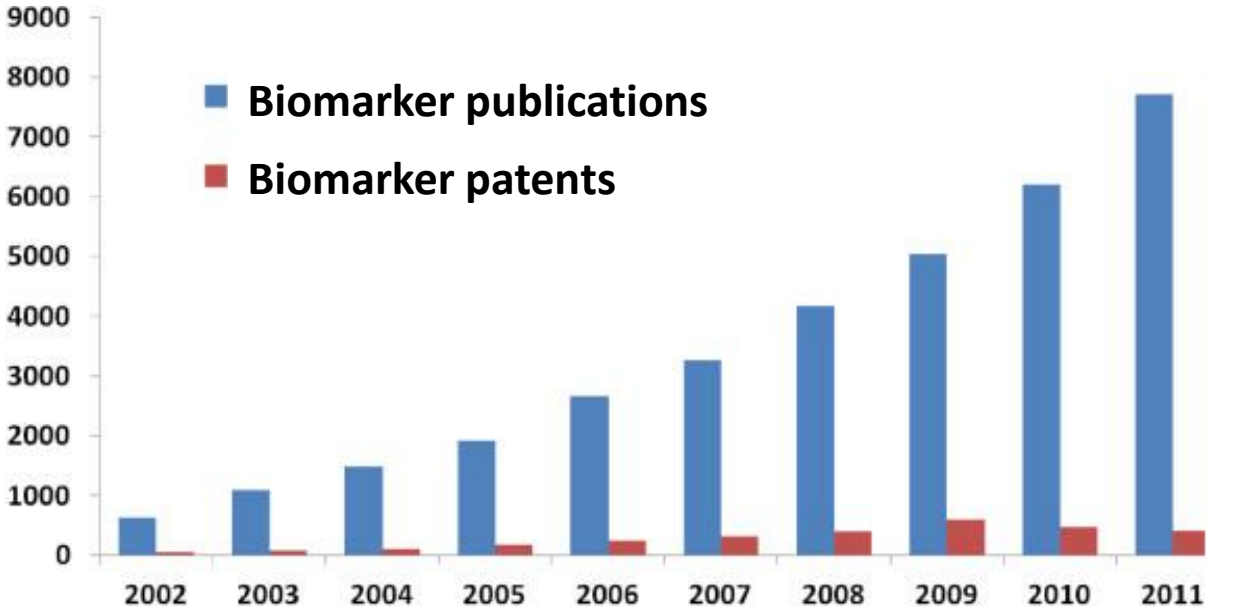
- During the Middle Ages, uroscopy reached new levels of diagnostic dominance when nearly every condition or disease was identified with different features of urine

- Matthew Dobson, in the 18th century, confirmed that the sweetness in the urine was caused by sugar, thus linking forever high glucose levels in blood and urine together with diabetes into one clinical diagnostic paradigm.
  [Armstrong JJ, Kidney Int., 2007]

- Nowadays, new high-throughput technologies have exploded the potential of biomarker research
- Increased computer processing power has fueled the development of methodological strategies based on artificial intelligence (AI)
- Knowledge extraction from big data is generating a new paradigm for AI-guided biomarker discovery and therapy
- Biomarker discovery is aimed beyond simple disease associations, to the exploration of more complex and holistic representations of disease, targeting incidence, progression or stratification

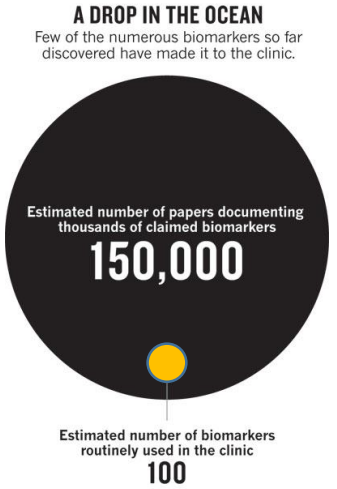
However…

…there is a gap between BM discovery and BM translation



Year	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Biomarkers Publikation	625	1087	1478	1920	2661	3270	4168	5033	6208	7720
Biomarker Patents	50	74	95	180	238	320	396	593	471	407

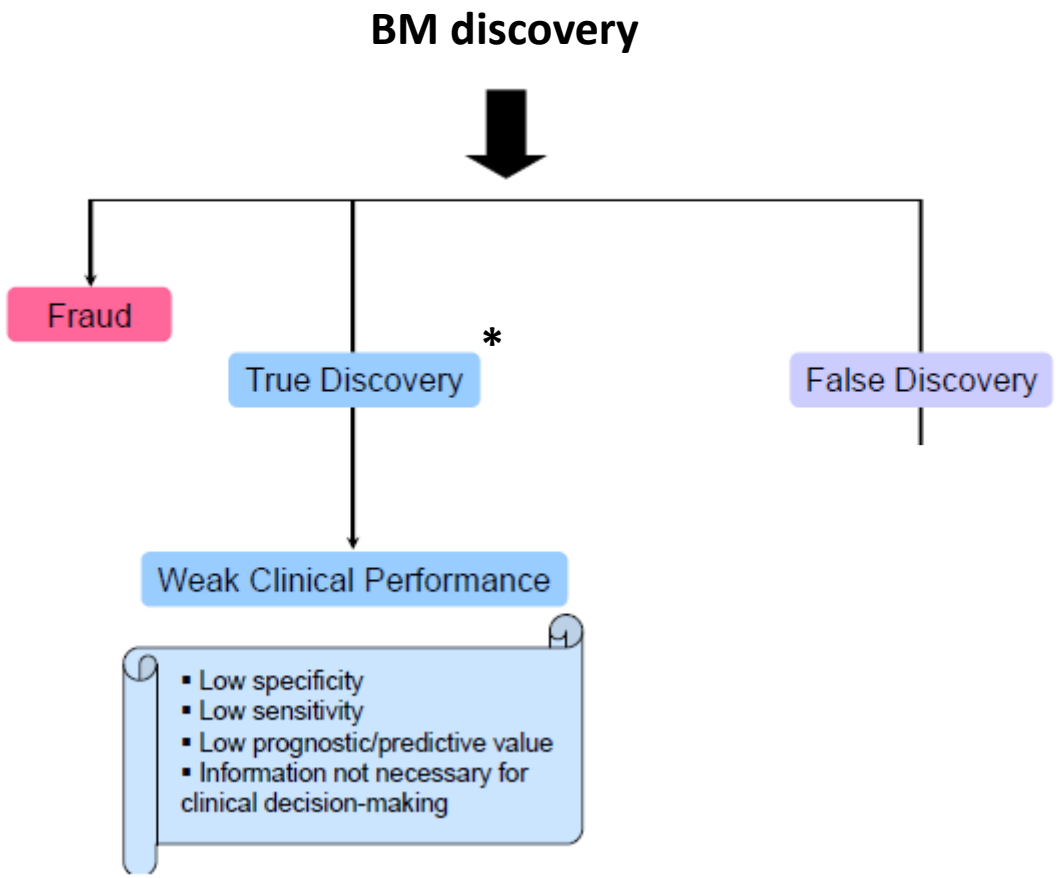
(Drucker E. and Krapfenbauer K., EPMA J, 2013)



(Poste G., Nature, 2011)

(List of Cleared or Approved Companion Diagnostic Devices, 2020)

Why do so many biomarkers fail to reach the clinic?

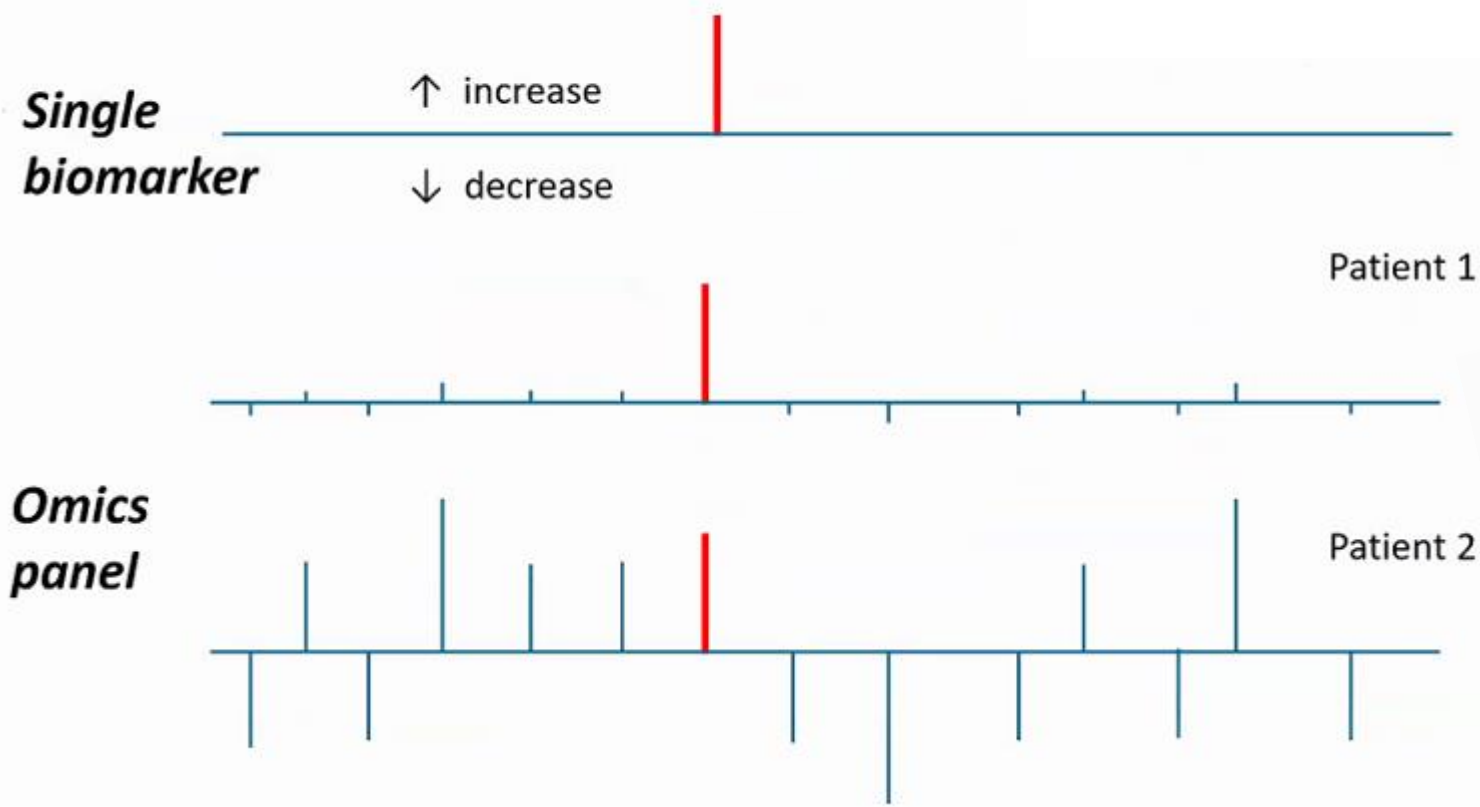


* Statistically significant discovery data but poor or no clinical usefulness
  - **p53** and breast cancer prognosis:
    1,500 ca. papers mostly confirming its weak prognostic value
  - **KIM-1** and **NGAL** for kidney disease:
    sensitive enough but affected by inflammatory status

Unreliable BM data translate into irreproducible science

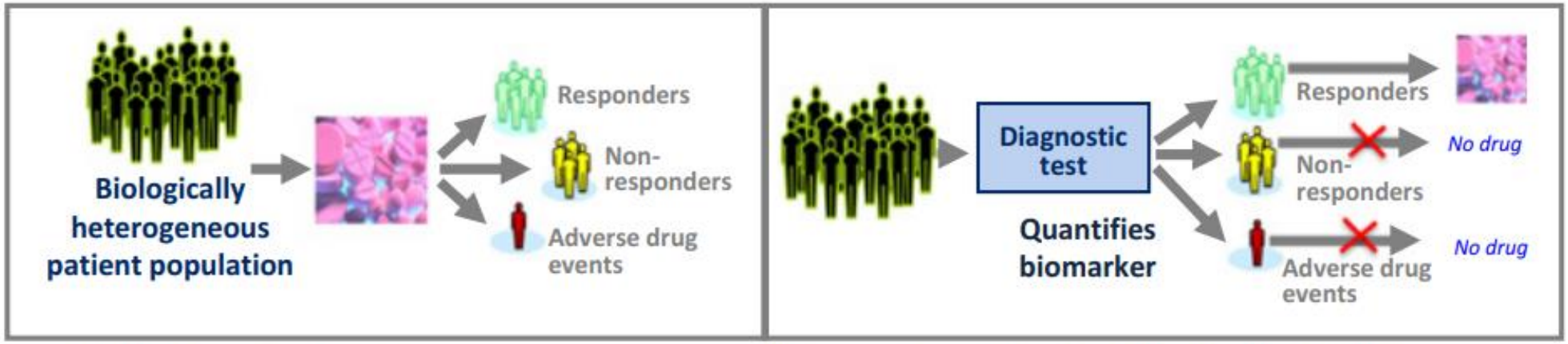
Biomarkers in personalized medicine

- Biomarkers have progressed from ‘simple’ physico-chemical tests to complex frameworks paving the way for the transformation of personalized and value-based healthcare
- To understand human health and disease is crucial to combine different molecular views [X-omics, Alain Van Gool, 2021)

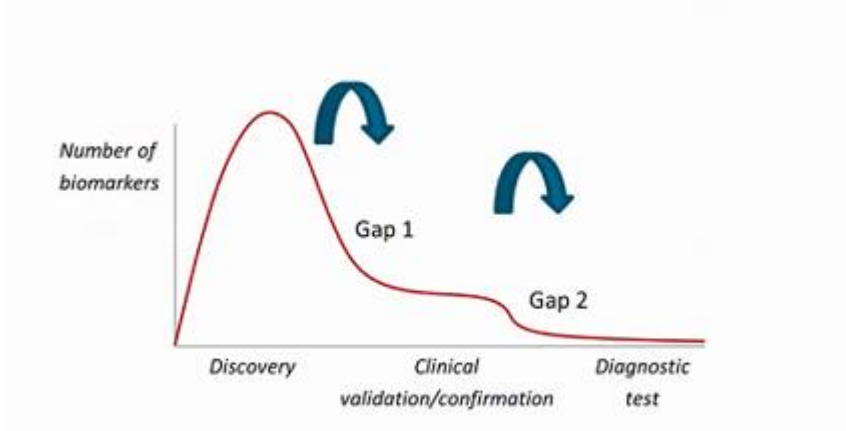


(Modified from Alain Van Gool‘s presentation at the EATRIS Summer School in PM, 2021)

Not validated biomarkers will fail PM



(Landeck et al., Exp Dermatol, 2016)



(Modified from Alain Van Gool 's presentation at the EATRIS Summer School in PM, 2021)

Validation of a biomarker is a necessary component to deliver high-quality, reliable and reproducible research data necessary for the effective use of biomarkers.
[Hunter et al., Curr Drug Targets, 2010]

What is ‘Biomarker Validation’?

Recommended guidelines

- General Guidance for Fit-for-purpose Biomarker Validation [19]
- Best Practices for Biospecimen Resources, NCI, NIH [64]
- List of Cleared or Approved Companion Diagnostic Devices, FDA [165]
- Regulations of General Biological Products Standards, FDA [121]
- Guidance for Gene Expression Profiling Platforms, FDA [117]
- Standards for Next Generation Sequencing [168, 169]
- Principles of Analytical Validation for Immunohistochemical Assays [167]
- Guidelines for Validation of Cell Based Fluorescence Assays [170]

Clinical Chemistry
892–902 (2013)

CLSI documents

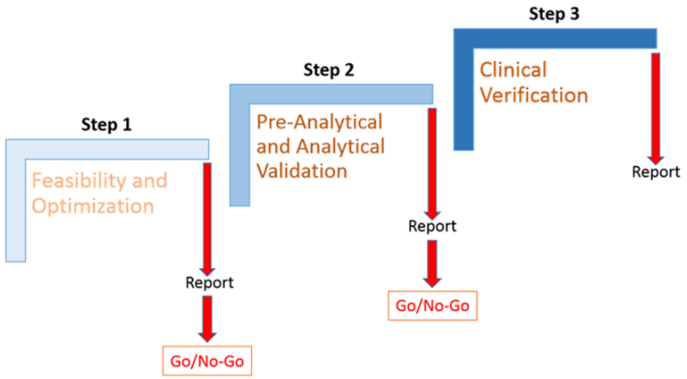
- Guidelines for Evaluation of Qualitative Test Performance [181]
- Guidelines for Evaluation of Precision Performance of Clinical Chemistry Devices [180]
- Guidelines for Verification of Precision and Estimation of Bias [166]

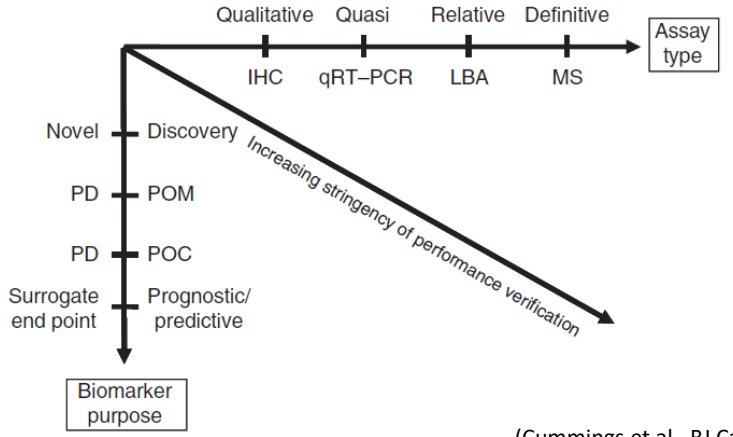
- Guidelines for Quality Assurance for Immunohistochemistry [145]
- Guidelines for Performance of Single Cell Immune Response Assays [69]
- Guidelines for Enumeration of Immunologically Defined Cell Populations by Flow Cytometry [182]

n to comply with the most recent

The LIH/IBBL approach to biomarker validation

- BM pre-analytical validation assesses those parameters **that might undermine** BM robustness all along those steps preceding the actual measurement of the biomarker in the sample
- BM analytical validation (the method used to measure the BM) aims at demonstrating that the method is **reliable for the intended application taking into account the context** (basic research, pre-clinical and clinical), **affordable/sustainable for business-critical decision making** (user-friendly instrument versus high throughput platform) and **fitting a long-term translational vision** (saliva-based kits versus more invasive procedures)
- The stringency of the experimental proof required to achieve the validation of a BM correlates with the BM 'position' between research tool and clinical end point (fit-for-purpose)



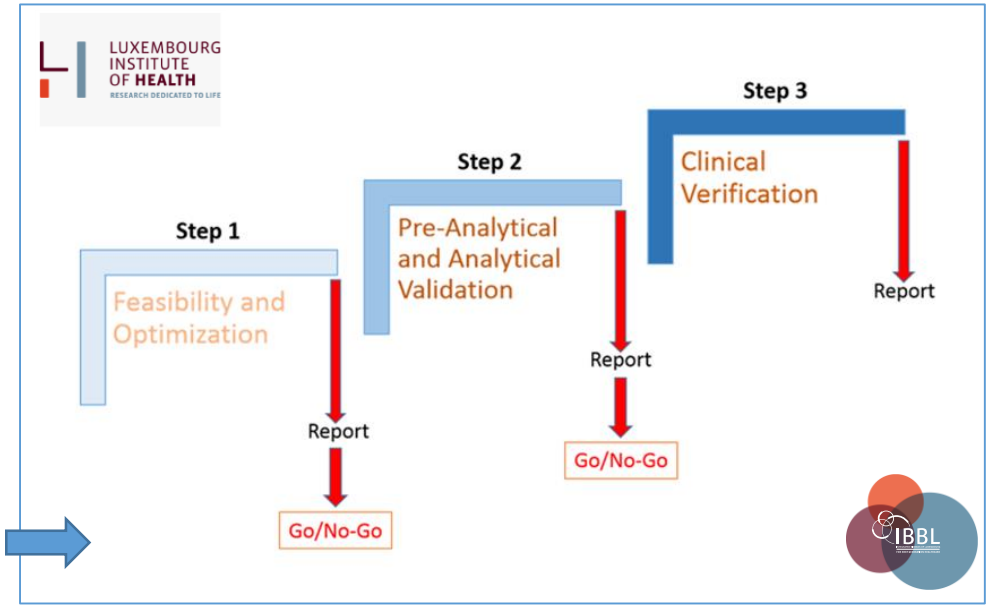


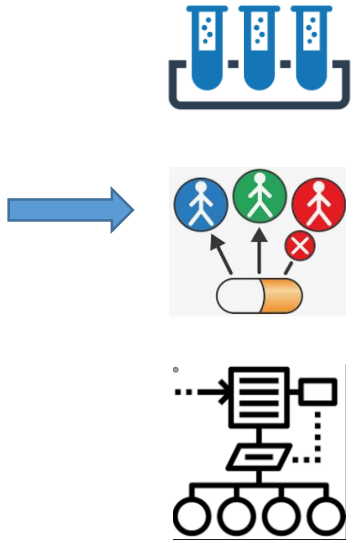
(Cummings et al., BJ Cancer, 2010)

Looking at the bigger picture

From the SME's perspective

POC -> patent -> publications





BM Translational Potential

The biomarker validation process

The Pre-Analytical and Analytical Validation (Step 1 and 2)

	Definitive quantitative (Mass-spec)	Relative quantitative (ELISA)	Quasi-quantitative (Flow-cytometry)	Qualitative (IHC)
BM robustness(*)	X	X	X	X
Sample stability	X	X	X	X
Precision (**)	X	X	X	
LOD/LOQ	X	X	X	
Linearity	X	X		
Parallelism	X	X		
Trueness	X	X		
Spike and recovery	X	X		

(*) : pre/post-centrifugation delays, freeze-thaw cycles, collection container type, matrix type, etc.
(**) : inter-lot and inter-operator

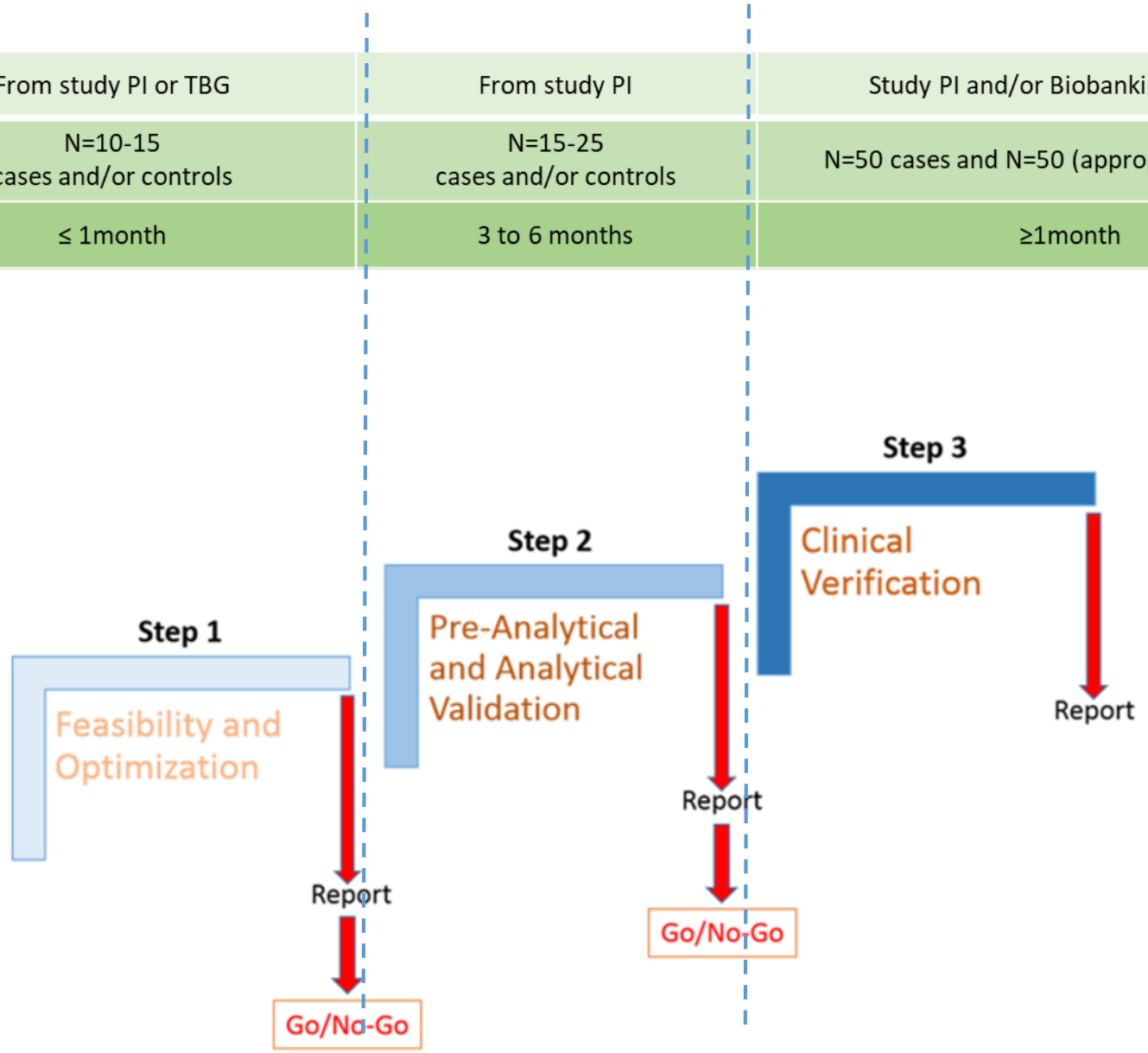
The Clinical Verification (Step 3) => small scale Clinical Validation

Assessment of the Clinical Specificity and Sensitivity:

- ❑ Measurement of the candidate BM/s on samples from the same cohort (not tested during the proof-of concept) and other sets of samples from different cohorts
- ❑ Evaluation of clinical sensitivity and specificity via ROC analysis

Samples and timelines

Sample source	From study PI or TBG	From study PI	Study PI and/or Biobanking network
Sample input	N=10-15 cases and/or controls	N=15-25 cases and/or controls	N=50 cases and N=50 (appropriate) controls
Timelines	≤ 1month	3 to 6 months	≥1month



What the LIH TBG can offer

1. **BM (technical) validation** (partial or full-fledged) for pre-existing BMs, or newly developed, to:
    - assess their robustness and fitness-for-purpose
    - extend the validation scope to another population 'type' and/or disease/physiological (ex.: aging) status
    - evaluate their potential as surrogate endpoints, to explore new therapies, and for patient stratification

2. **BM consultation services**: preparation of a BM dossier to assess:
    - pre-analytical factors that might generate BM artifactual data-> tube/container type, sample processing delays, etc.
    - method fitness-for-purpose according to CLSI guidelines and IBBL expertise
    - retrospective assessment of previously collected biospecimens

3. **Support BM discovery studies** thanks to the insight and experience gained so far in BMV

Case studies from the Codex4SMEs experience

Case 1:

SME already distributing and ELISA on the market for condition A, wanting to extend the validation scope to condition B.

Step 1: ✔

Step 2: ✔

Step 3: on-going

Case 2:

SME presenting a BM just out of POC, requiring a thorough investigation on pre-analytical factors

Step 1: ✔

Step 2: ✖ ✖

Case 3:

SME wanting to:

- Verify various pre-analytical conditions
- Confirm findings obtained from a high-throughput platfom into a RT-qPCR system (platform bridging)

Step 1: on-going

Step 2: on-going

Case 4:

SME studying a cirtical cellular checkpoint by means of a new quantitative platform (tweaking pre-analytics and analytical method)

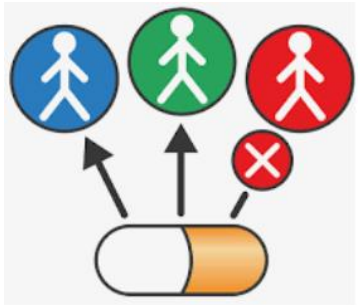
Step 1: ✔

Step 2: on-going

Is biomarker validation mandatory?

April 5th 2017:

- the Regulation (EU) 2017/745 on medical devices (MDR)
- the Regulation (EU) 2017/746 on in vitro diagnostic (IVDR)



CLASS C

- ❑ Scientific Validity
- ❑ Analytical Performance **including** the determination of pre-analytical specimen features and traceability of values assigned to calibrators and/or control materials
- ❑ Clinical Performance

Pre-analytical processes in medical diagnostics: New regulatory requirements and standards

Georges Dagher[a], Karl-Friedrich Becker[b], Serena Bonin[c], Carole Foy[d], Stefania Gelmini[e], Mikael Kubista[f], Penelope Kungl[l], Uwe Oelmueller[g], Helen Parkes[d], Pamela Pinzani[e], Peter Riegman[h], Ulrike Schröder[i], Cornelia Stumptner[l], Paola Turano[j], Robert Sjöback[f], Andrea Wutte[k], Kurt Zatloukal[l,*]

New BIOTECHNOLOGY 52 (2019) 121–125

The Translational Biomarker Group

Monica Marchese, PhD

- orked for more than 15 years in the field of BM research for auto-immune and neurodegenerative diseases
- Expertise in automation, translation of bench assays into automated workflows
- Expertise in BM technical validation to promote their valorisation in CDx or laboratory assays

Estelle Henry

- TBG Technician (also IBBL EH&S Officer)
- Specialized in the protein field (ELISA, WB, capillary WB, etc.) with expertise cell culture and molecular biology
- Large expertise in clinical sample handling and processing
- Development of Validation Plans, set up of common, improved or newly developed analytical methods

Johanna Trouet

- TBG Assistant Scientist
- Genomic and cell-free nucleic acids extraction from various matrices, qualification and analysis
- q(RT)PCR, ddPCR, NGS, miRNA profiling,with expertise in cell culture and immunoassays
- Large expertise in clinical sample handling and processing
- Development of Validation Plans, set up of common, improved or newly developed analytical methods

Interreg North-West Europe Codex4SMEs

Thank you for your attention

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