

Biomarkers at the interphase of academia and industry

COST CliniMark summerschool
Spetses
23 September 2019



Prof Alain van Gool

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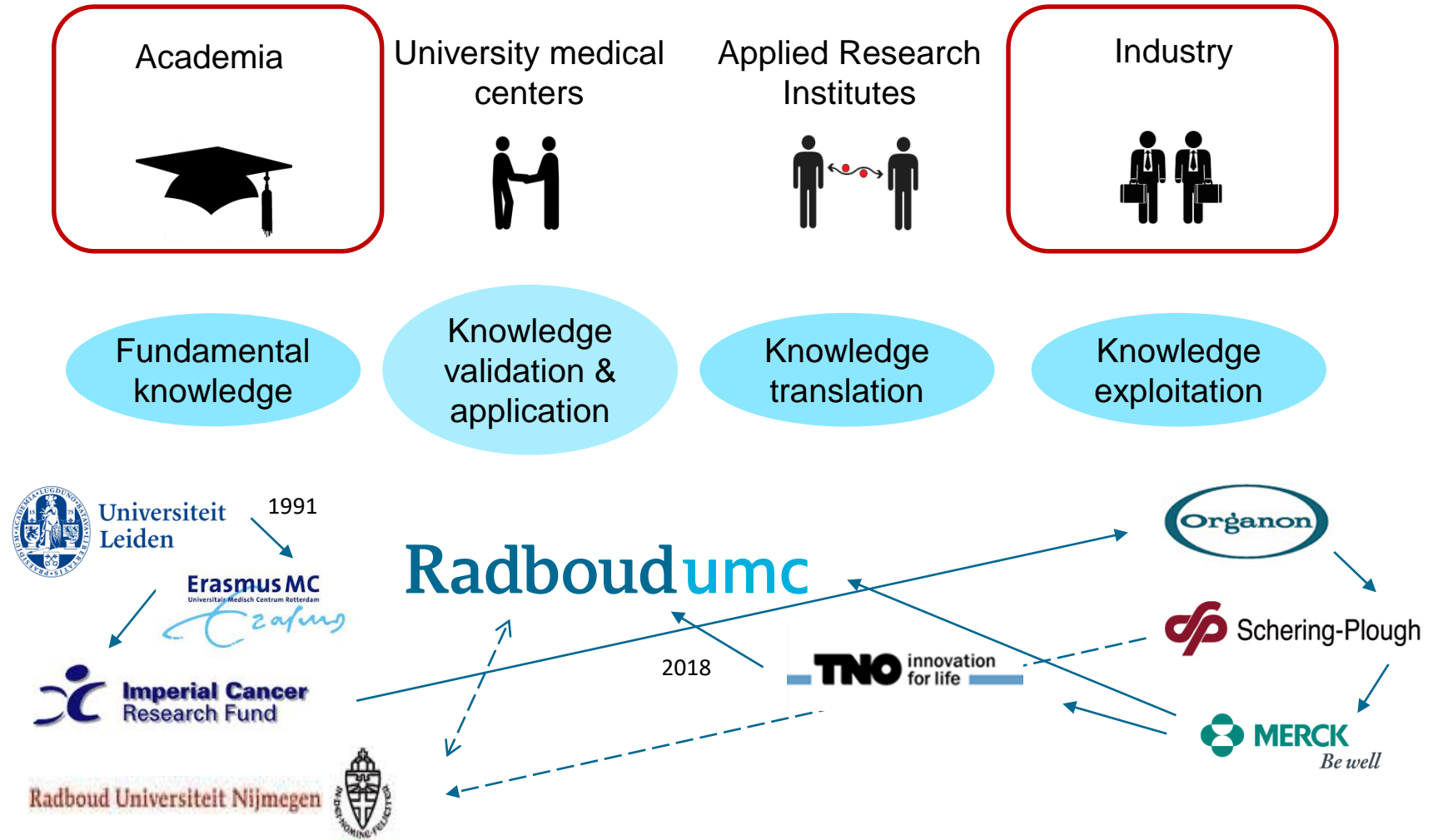
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Radboudumc
university medical center

Daily Program

	Mon, Sep 23	Tue, Sep 24	Wed, Sep 25	Thu, Sep 26	Fri, Sep 27
09:00	Arrival and Registration	Introduction to omics and Biomarkers Antonia Vlahou	Biomarkers for doping Sulev Kõks	Biomarkers in Screening for Obstructive Sleep Apnea Deborah Penque	Biomarkers for psychiatric disorders Chris Turck
09:30					
10:00		Biomarker panels by CE-MS Harald Mischak	Biomarker clinical implementation Eva Martinez-Caceres	Proteomics for anxiety disorders: mind the mitochondria Michaela Filiou	Predictive biomarkers for CVD Andreas Simm
10:30					
11:00		Coffee break	Coffee break	Coffee break	Coffee break
11:30		Quality control in biomarker research Andrea Wutte	Targeted proteomics assays for biomarkers Virginie Brun	Proteomics for biomarker discovery Michalis Aivaliotis	Biomarkers of healthy ageing Niki Chondrogianni
12:00					
12.30		Student talks 1-13	Student talks 19-31	Student talks 36-48	Student talks 49-60
13:00					
13.30					
14:00		Lunch Break	Lunch Break	Lunch Break	Lunch Break
14:30		Poster viewing	Poster viewing	Poster viewing	Poster viewing
15:00		Discussions Free time	Discussions Free time	Discussions Free time	Discussions Free time
15.30		High sensitivity immunoassays Stanislav Kuula	Analytical validation of sRAGE MRM assay Rainer Bischoff	Cultural excursion	Meet the expert Biomarker assay validation Study design, MRM data analysis
16:00					Eureka: something is rotten in the biomarker kingdom Makis Zoidakis
16:30		Student talks 14-18	Student talks 32-35		Coffee break
17:00		Coffee break	Coffee break		Epigenetics and Redox Biomarkers Alexander Bürkle
17:30		Changing the biomarker implementation paradigm Peter Groenen	Biomarkers used in clinical practice for monitoring biological drugs Begoña Oliver		Oxidative stress and biomarkers Grune Tilman
18:00					
18:30		Genomics biomarkers Lila Koumandou	Liquid biopsy preparation Chris Sutton		
19:00		Welcome Niki Chondrogianni Makis Zoidakis	Molecular diagnostics: from bench to clinic Daria Ler		
19:30		Biomarkers at the interphase of academia and industry Alain van Gool	Poster session discussions		Summing-Up Round Table
20:00					
20:30	Welcome reception	Dinner	Dinner	Dinner	Farewell reception / awards

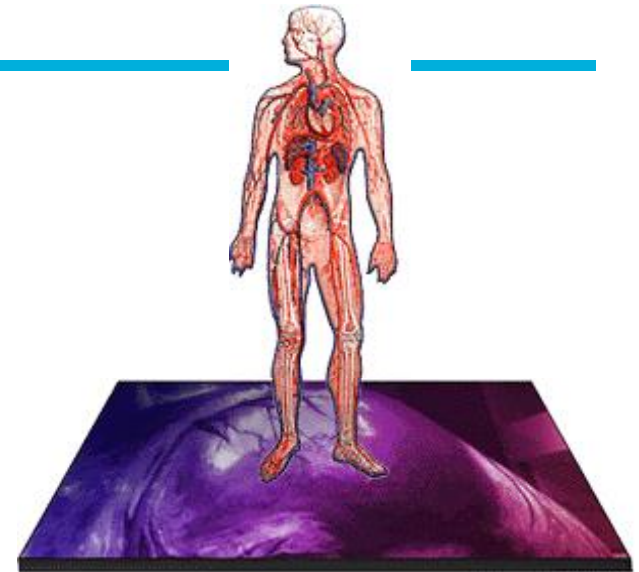
Alain's path in translational health research



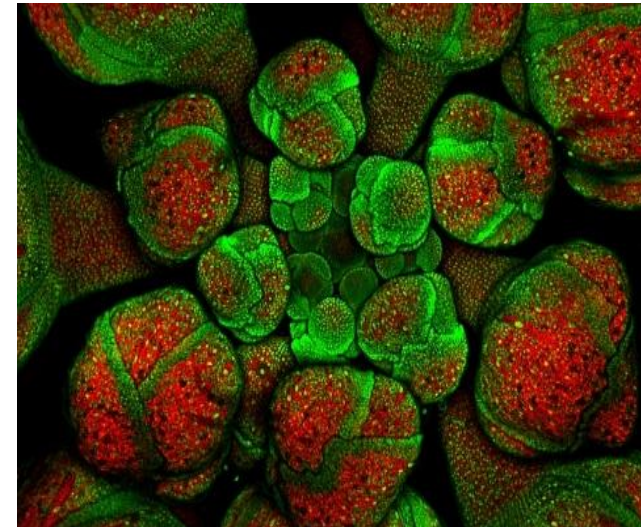
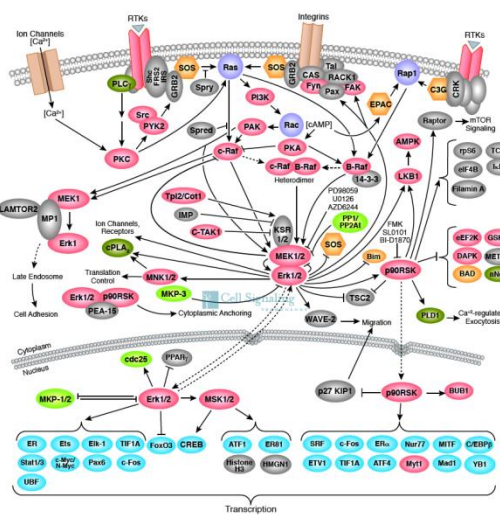
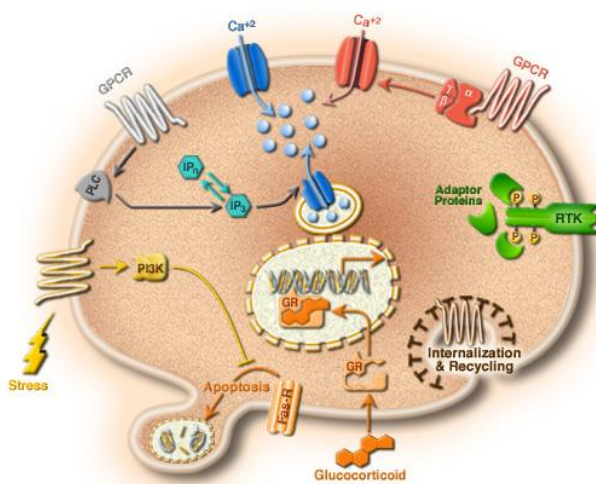
Outline

- **Biomarkers in pharmaceutical industry**
- Biomarkers in academic research and healthcare
- Biomarkers in personalized health (care)
- Translational innovation gaps
- Outlook

Crash course in molecular biology



DNA, protein, cell, tissue, system biology



A short story: B-RAF mutations and melanoma

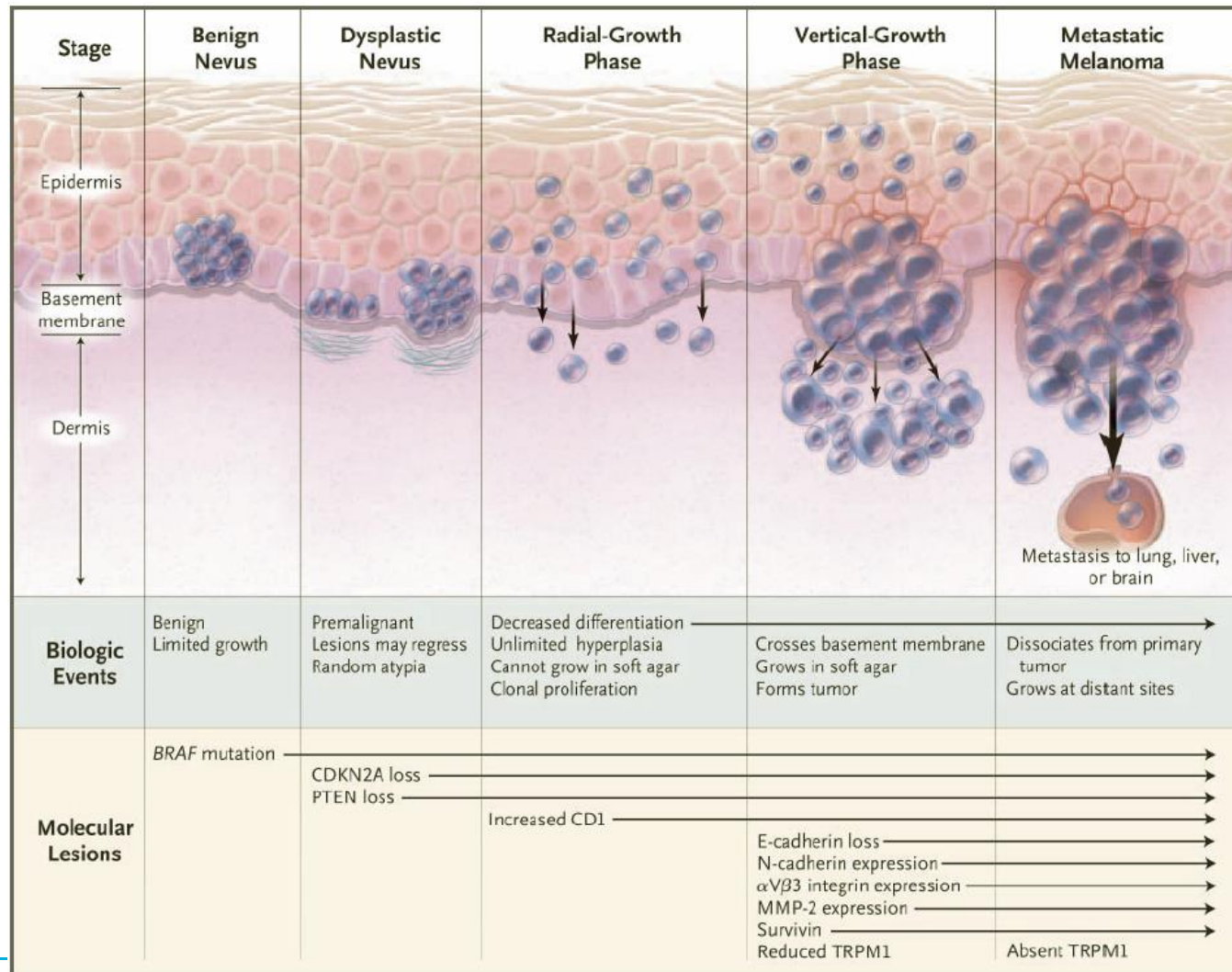
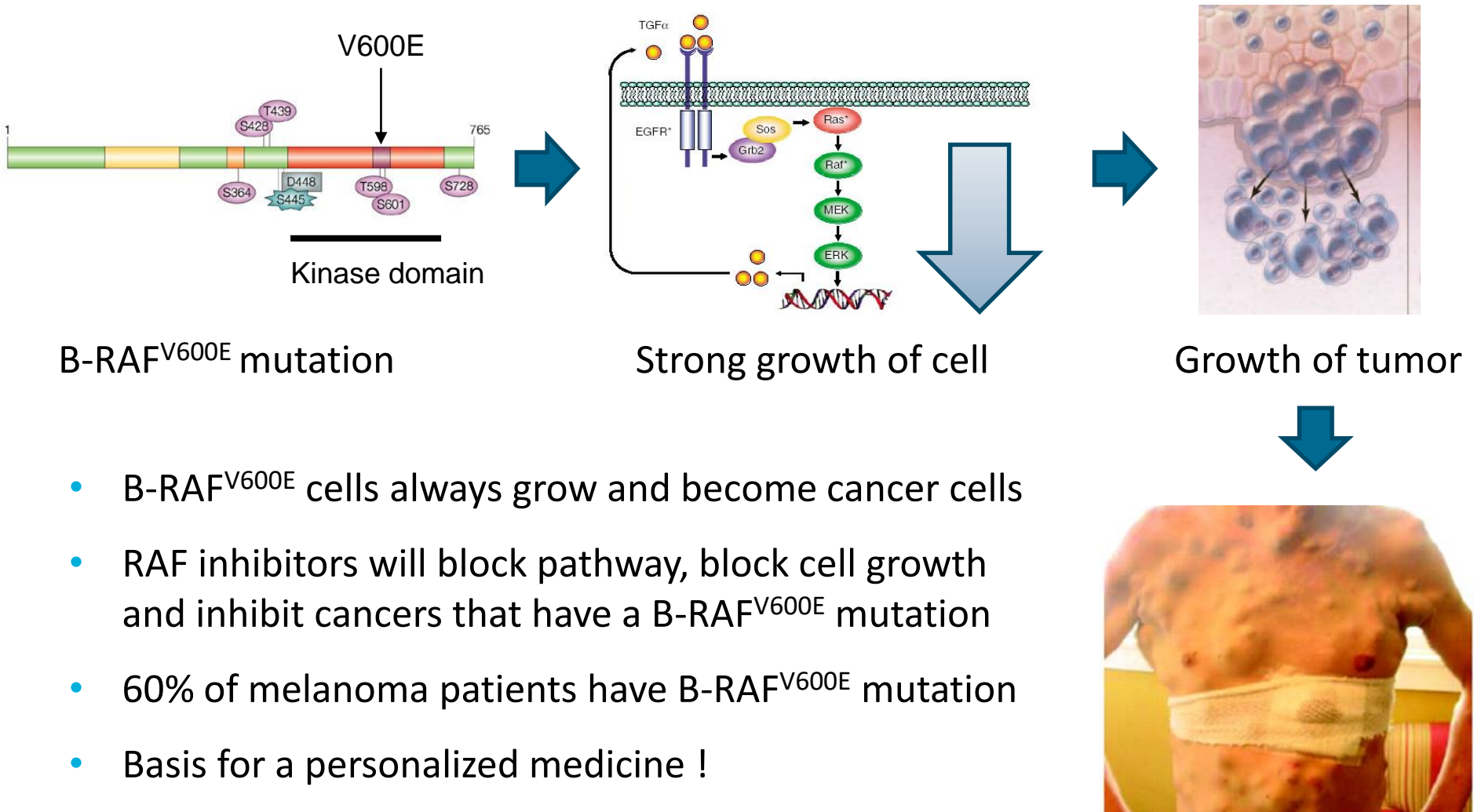


Figure 2. Biologic Events and Molecular Changes in the Progression of Melanoma.

{Miller and Mihm,
2006}

Example: Personalized medicine in melanoma



- B-RAF^{V600E} cells always grow and become cancer cells
- RAF inhibitors will block pathway, block cell growth and inhibit cancers that have a B-RAF^{V600E} mutation
- 60% of melanoma patients have B-RAF^{V600E} mutation
- Basis for a personalized medicine !

Personalized medicine in melanoma

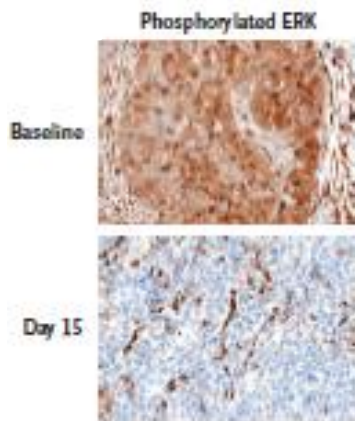
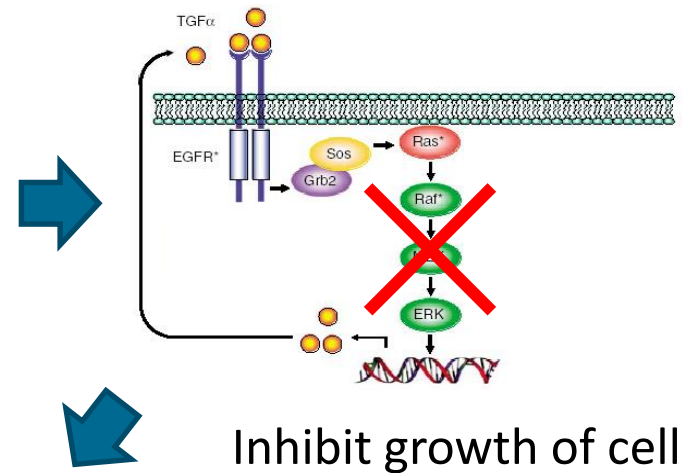
ZELBORAF[®]
(vemurafenib) tablets



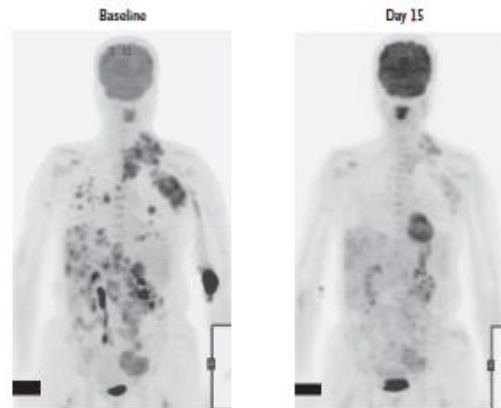
B-RAF inhibitor



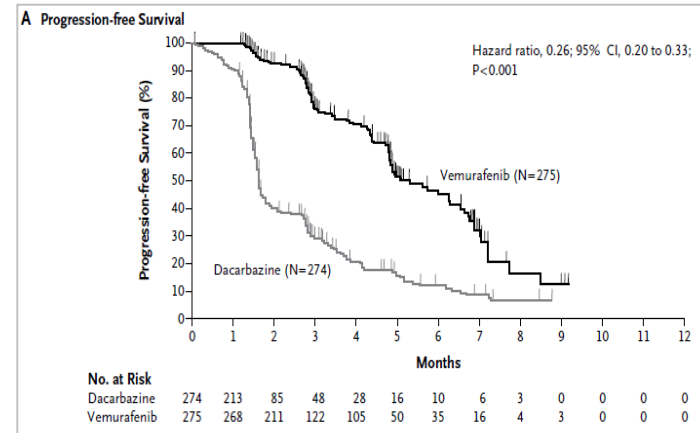
Treat patients with
B-RAF^{V600E} mutation



Cells stop growing

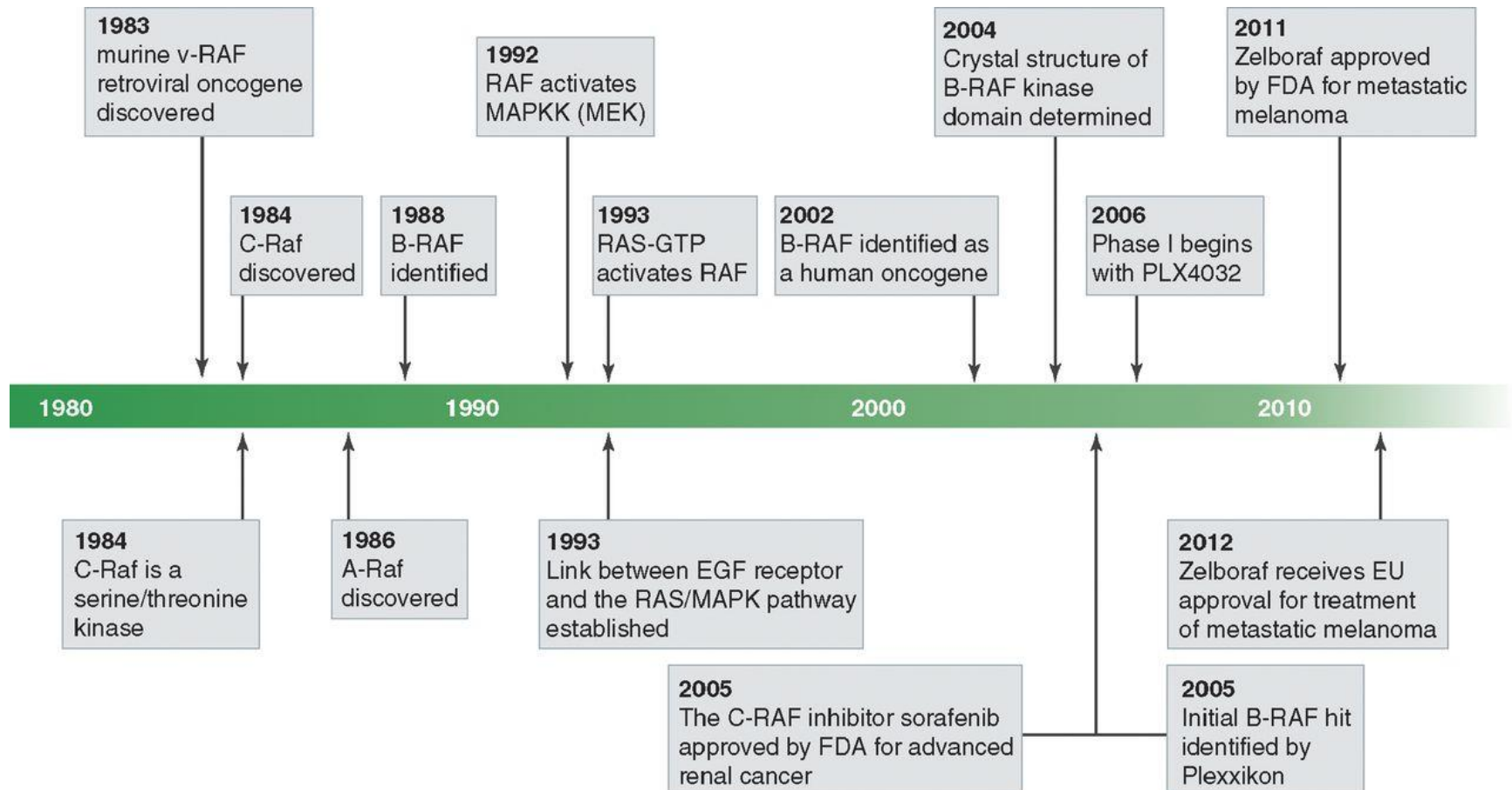


Tumors disappear



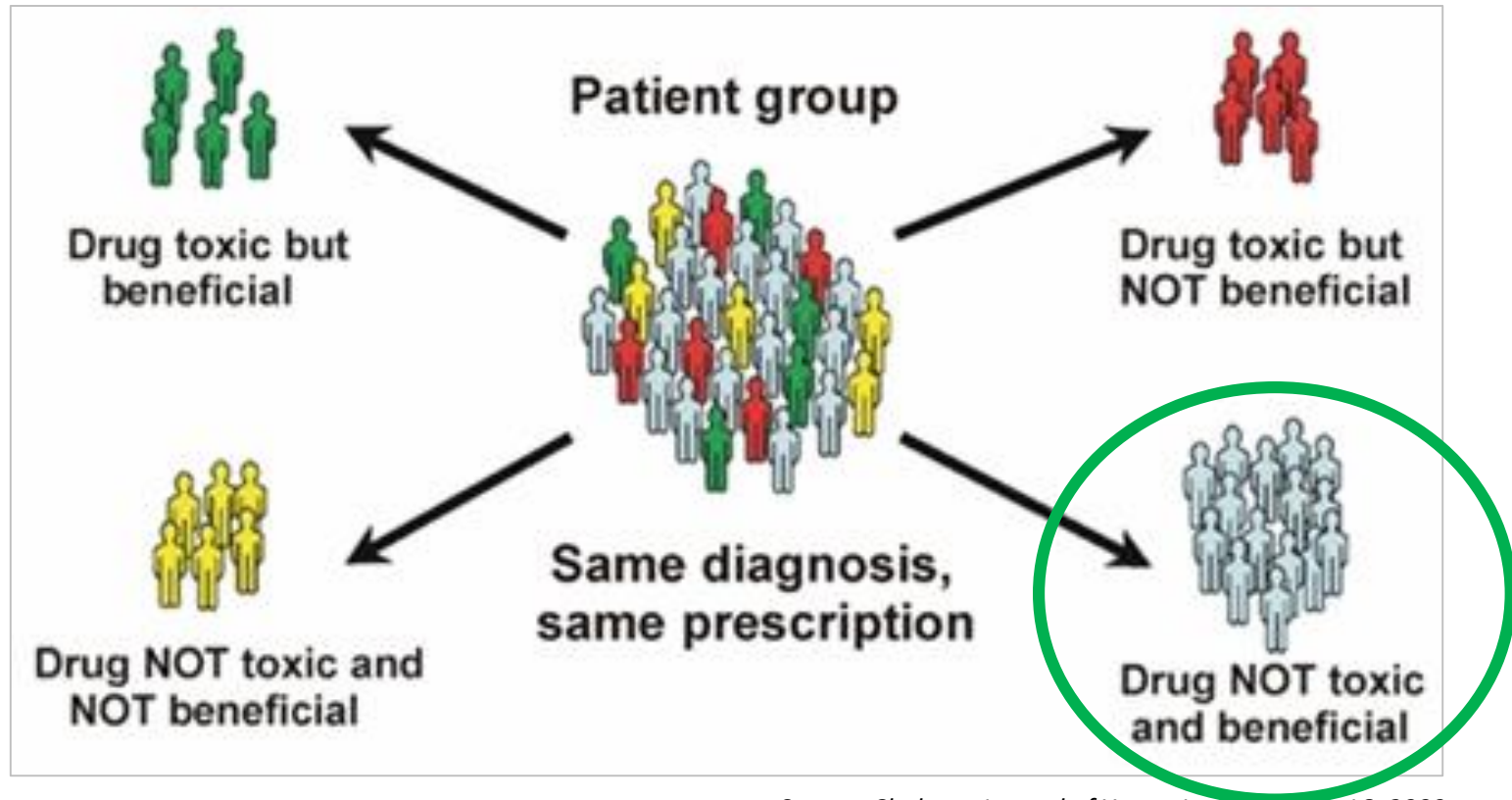
Patients live longer

Fast development of Vemurafenib (Zelboraf)



{Source: Davis M J , Schlessinger J J Cell Biol 2012}

Principle of Personalized Medicine

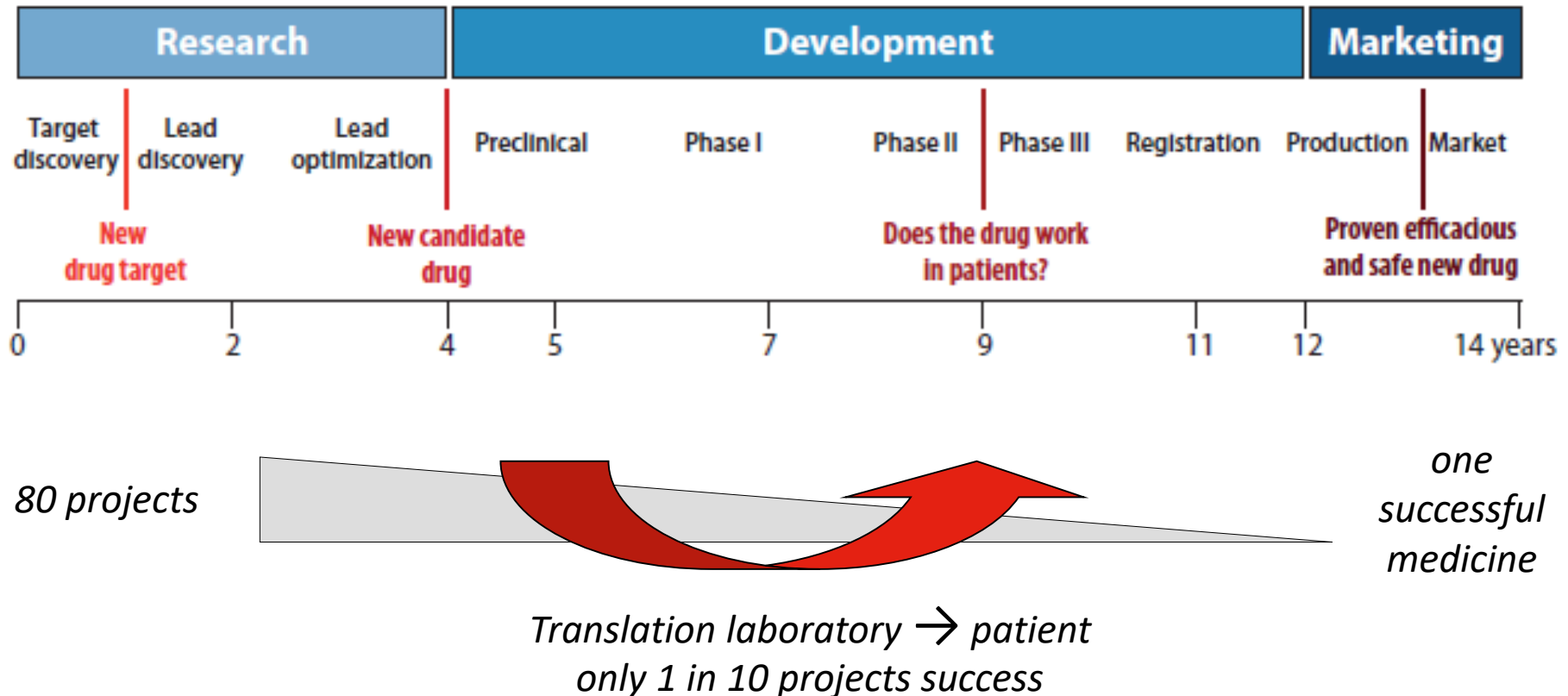


Source: Chakma, *Journal of Young Investigators*, 16, 2009

- The **right drug** for **right patient** at **right dose** at **right time**
- Molecular biomarkers as key drivers of patient selection
- = Precision medicine (USA) or Stratified medicine (UK)



The pharmaceutical R&D phases



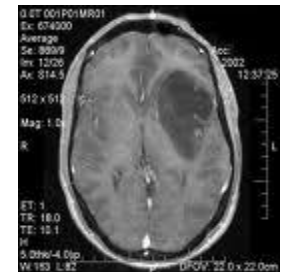
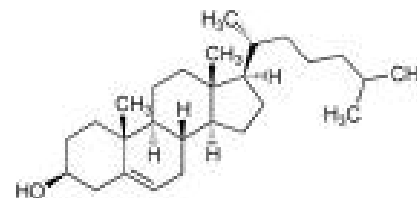
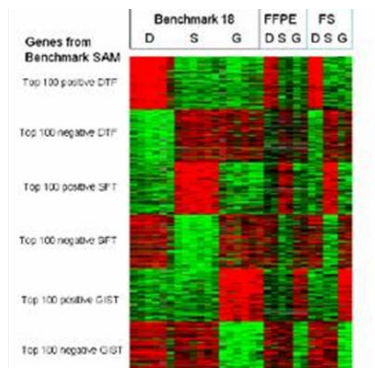
- Need to choose the most successful compound/project as early as possible.
- Improve success of **translational medicine** using **biomarkers**

Biomarkers

- › Definition: ‘a **characteristic** that is **objectively** measured and evaluated as an indicator of **normal** biological processes, **pathogenic** processes, or **pharmacologic responses** to a therapeutic intervention’

{Biomarkers definition working group, 2001 }

- › Or ‘Whatever works in adding value’
- › Molecular biomarkers provide a molecular impression of a biological system (cell, animal, human)
- › Biomarkers can be various sorts of data, or combinations thereof



Biomarker-based translational medicine in pharma

- **Exposure ?**
 - *Does the compound get to the site of action?*
- **Mechanism ?**
 - *Does the compound cause its intended pharmacological/ functional effects?*
- **Efficacy ?**
 - *Does the compound have beneficial effects on disease or clinical pathophysiology?*
- **Safety ?**
 - *What is the therapeutic window (how safe is the drug)?*
- **Responders ?**
 - *How do sources of variability in drug response in target population affect efficacy and safety?*



{van Gool et al, Drug Disc Today 2010}
{Kumar, van Gool, RSC biomarkers, 2013}

美药剂公司先灵葆雅 本地设转化医学研发中心

邹美琳 报道

美国大规模跨国药剂公司先灵葆雅 (Schering-Plough) 在本地投资开设转化医学研究中心, 进一步发展和巩固新加坡作为本区域科研与医药枢纽的地位。

先灵葆雅是美国首个在本地开展生产业务的药剂公司, 位于启奥生物医药园 (Biopolis) 的转化医学研究中心 (Translational Medicine Research Centre, 简称 TMRC), 是它在世界各地设立的第一家转化医学研究与发展中心。

生物标志 好比汽车的GPS

以生物标志为标志的转化医学研究中心设有实验室, 集中于生物标志 (biomarker) 的研究和发展, 使医学研究能更有效地转化为惠及病人的成果。

生物标志还指利用分析方式测量人体的生理反应、疾病发展过程、药物生理反应以及安全性等,



除了转化医学研究中心的实验室, 美国跨国药剂公司先灵葆雅计划今年底开设临床研究与测试部门, 投入转化医药的临床试验。

是药剂科研领域的一项关键程序。

转化医学研究中心负责人埃里克·施普伦格 (Erik Sprengers) 昨天在中心举行开幕仪式上受访

并谈到生物标志的重要性时形容, 生物标志好比一辆汽车的全球定位系统 (GPS), 有助于提早告知科研人员处于试验性阶段的药物是否能取得良

好效果。

埃里克·施普伦格透露, 市面上出现的每一种新药物, 都是经过上百次的失败试验阶段才得以成功推出, 有了生物标志技

术, 药剂科研人员便能够把宝贵的资源投入可行的研究上, 在更短时间内研发对抗各种疾病的新药物。

除了转化医学研究中

心的实验室, 先灵葆雅也计划在今年底开设临床研究与测试部门, 专门进行志愿者与病人的转化医药临床试验。临床研究部门全面投入运作后, 预计每年可进行二三十项转化医药的临床试验。

虽然不愿透露中心的总投资额, 埃里克·施普伦格说, 新加坡提供了非常好的研发与制造基础设施及环境, 这是一项长期投资项目, 也是公司在几年前定下的发展计划, 近期的环球经济放缓并不影响中心的人力资源配置和研究资金。

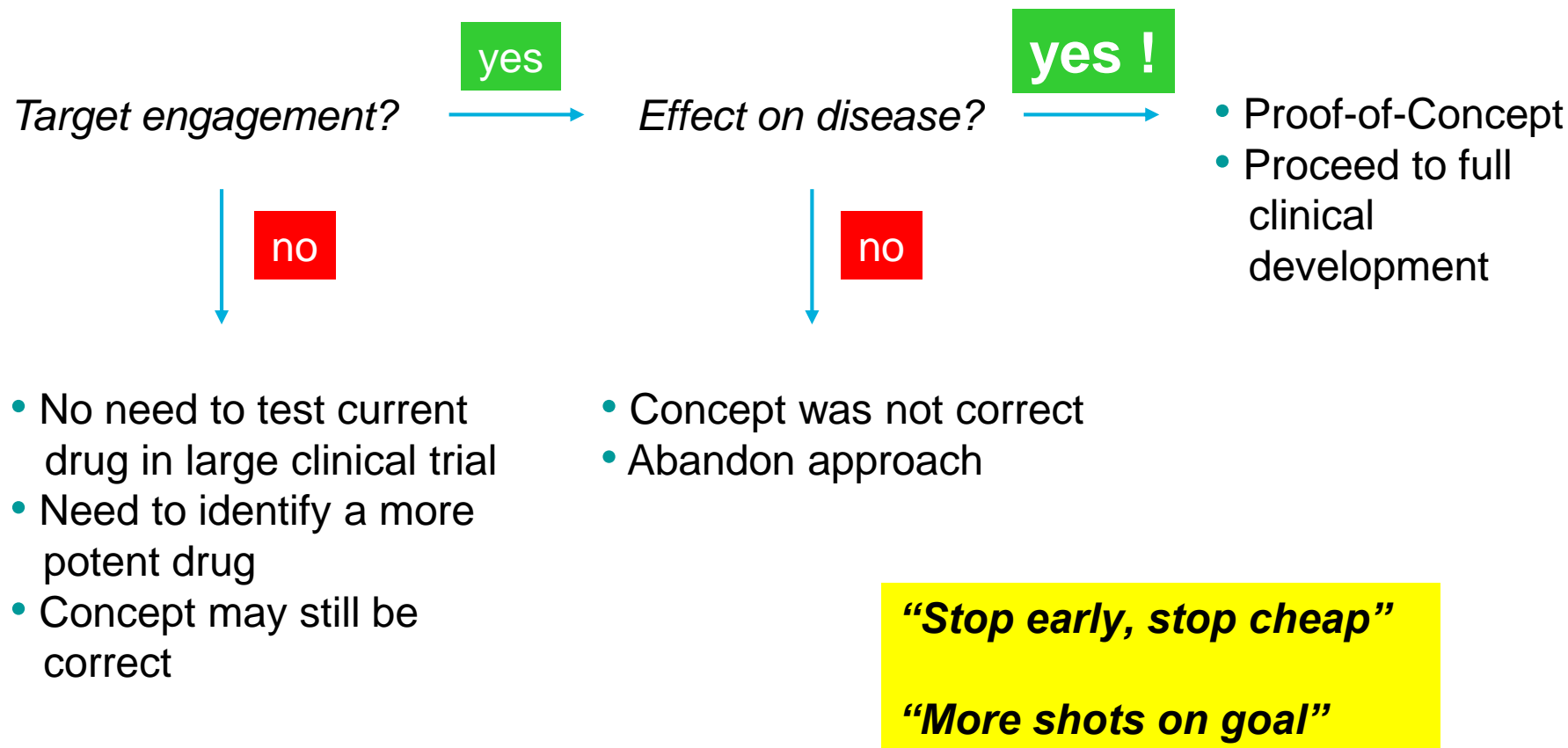
他透露, 转化医学研究中心将在未来几年把聘请人数增至超过50人, 当中75%将是本地人。

另一方面, 经济发展局日前发布的数据显示, 在2008年全年, 生物医药制造业的产值下跌6.6%。经发局局长马宣仁医生昨天出席开幕仪式后受访说, 鉴于眼前的环球经济衰退情况, 预料药剂业领域在今年的制造总产值将不会有所增长或稍微下跌。

不过, 他指出, 生物医药领域方面的新科研投资预料能够取得稳健表现, 其中一个好消息包括今年将迎来一些新设立的生物医药厂, 相信也能为新加坡人制造更多的就业机会。

Biomarker strategy: Data-driven decisions

To be made during testing of drug in preclinical and clinical disease models:



{Kumar, van Gool, RSC biomarkers, 2013}

Pharma strategy based on best practice

OUTLOOK

Lessons learned from the fate of AstraZeneca's drug pipeline: a five-dimensional framework

David Cook, Dearg Brown, Robert Alexander, Ruth March, Paul Morgan, Gemma Satterthwaite and Menelas N. Pangalos

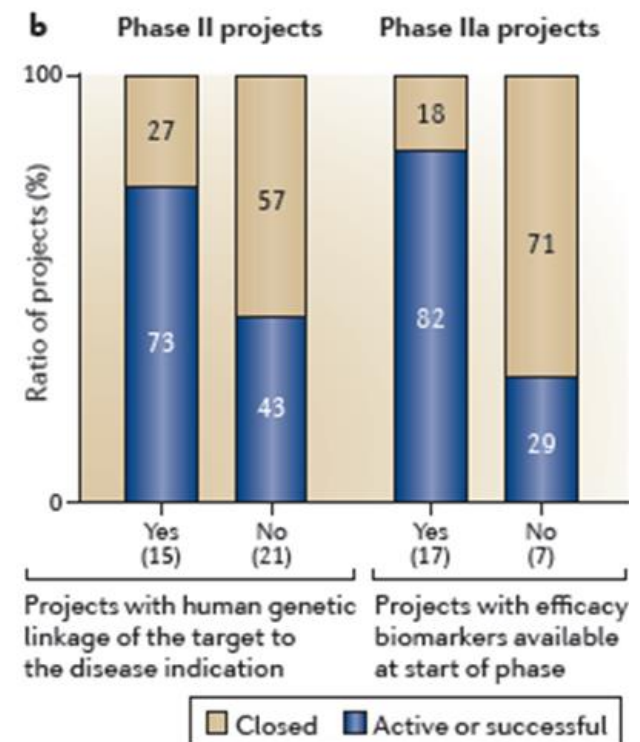
NATURE REVIEWS | DRUG DISCOVERY

VOLUME 13 | JUNE 2014 | 419



The 5R's assessment:

- Right Target
- Right Tissue
- Right Safety
- Right Patients
- Right Commercial Potential



Evaluation of success 5R strategy



PERSPECTIVES

OUTLOOK

Impact of a five-dimensional framework on R&D productivity at AstraZeneca

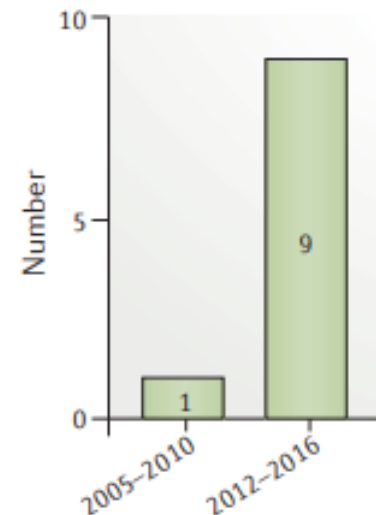
Paul Morgan, Dean G. Brown, Simon Lenné, Ulf Eriksson, Mark Fidock, Bengt Hamrén, James Matcham, Jerome Mettetal, David Michael A. Snowden and Menelas N. Pang

Abstract | In 2011, AstraZeneca embarked on a new R&D strategy with the aim to improve productivity. The company was below industry averages in 2005–2010 but was to focus decision-making on five technical dimensions: right target, right molecule, right tissue, right safety, right patient and right cost. This paper describes the progress made using this '5R' strategy and how this experience could be useful to other companies.

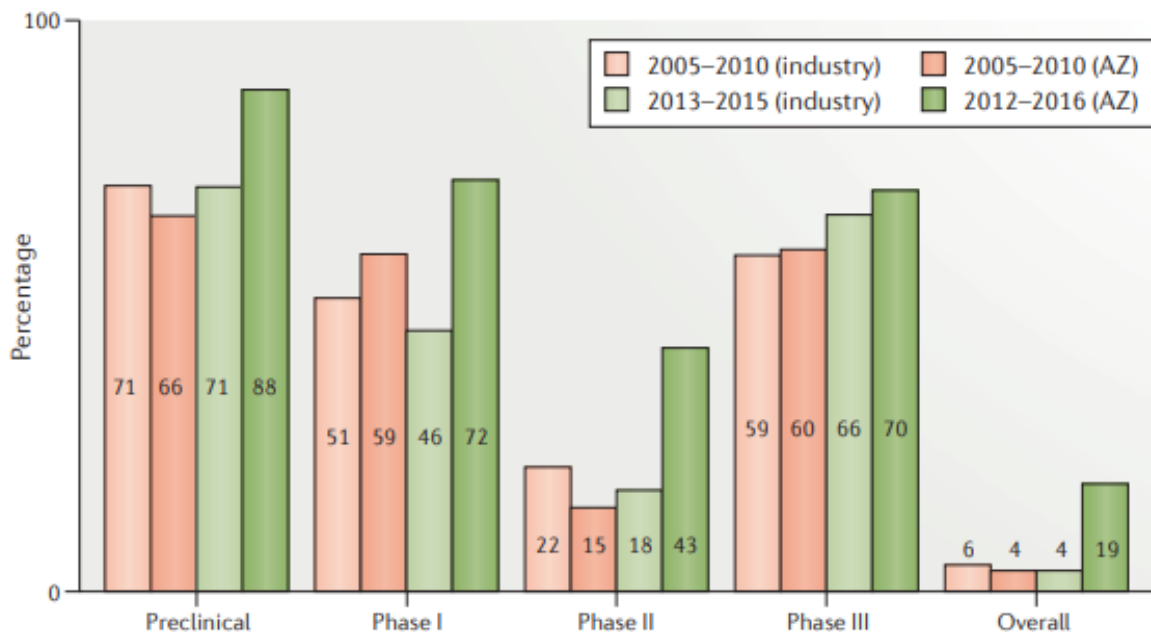
NATURE REVIEWS | DRUG DISCOVERY

candidate drug nomination to phase III completion were 4%, compared with 5% for the industry on average (based on data from the Centre for Medicines Research (CMR) International). By phase, the company was broadly in line with the CMR industry averages, but success rates

c Number of diagnostics launched



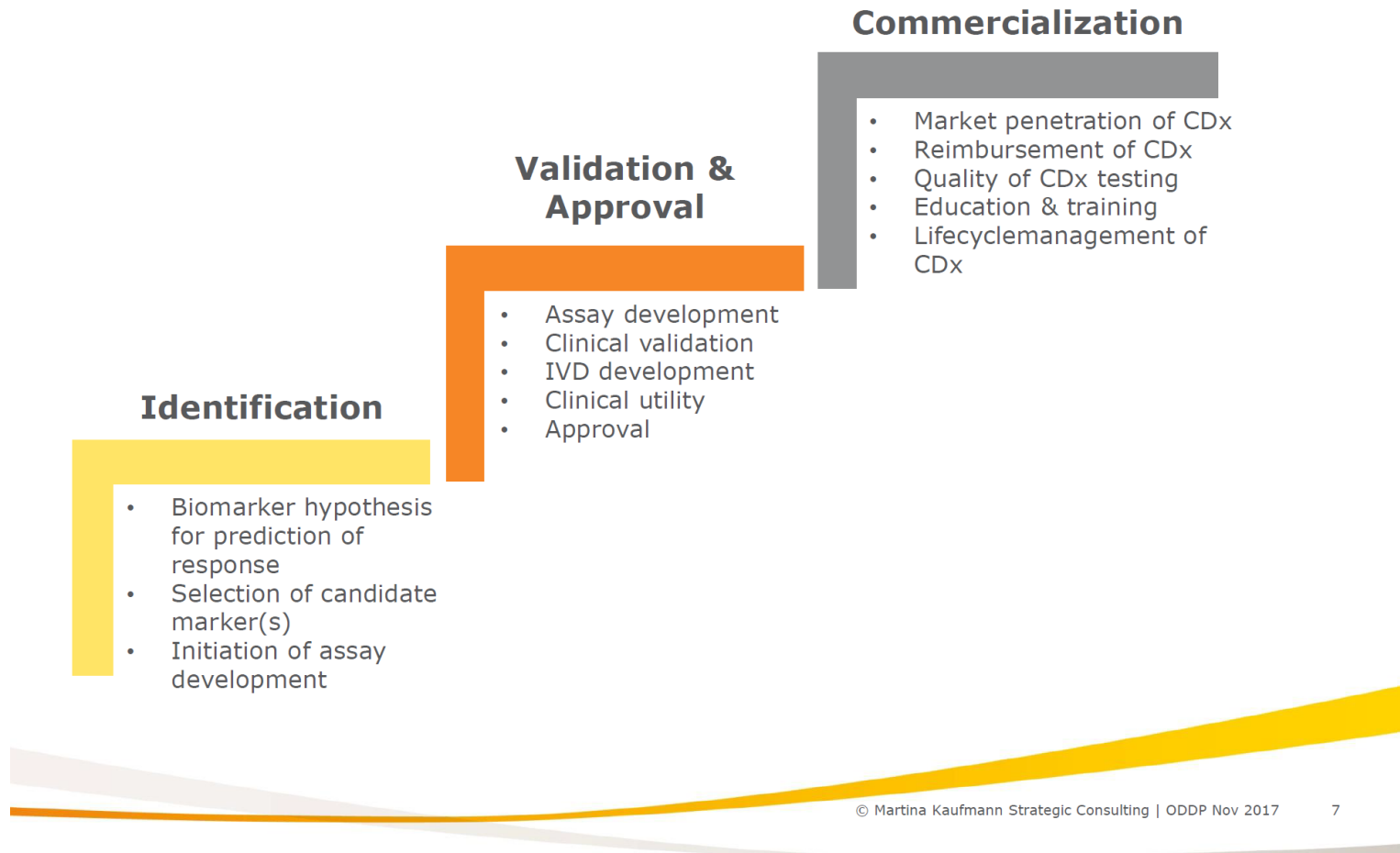
a Project success rates



Biomarker R&D pipeline



MARTINA KAUFMANN
STRATEGIC CONSULTING
Individual Solutions for Personalized Medicine



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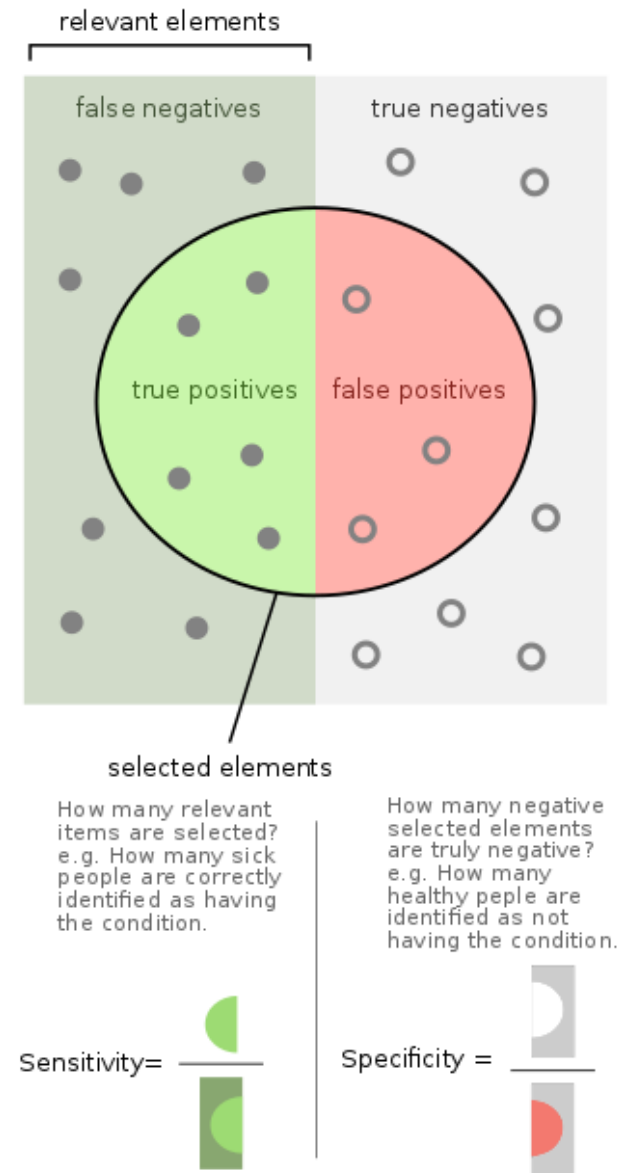
Biomarker discovery

Biological

- Definition what is needed (Context of Use), a.o. Specificity, Selectivity
- Design discovery study well
 - Objectives to address (clinical) need
 - End-user of outcome
 - Biological, sample analytical, data analytical path
 - Practical issues to consider (eg time)
 - Power calculations
 - Output with clear biological / mechanistic rationale
 - Path for (clinical) validation
- Confirmation in independent study
- Publish in peer-reviewed journals

Biomarker specificity/selectivity

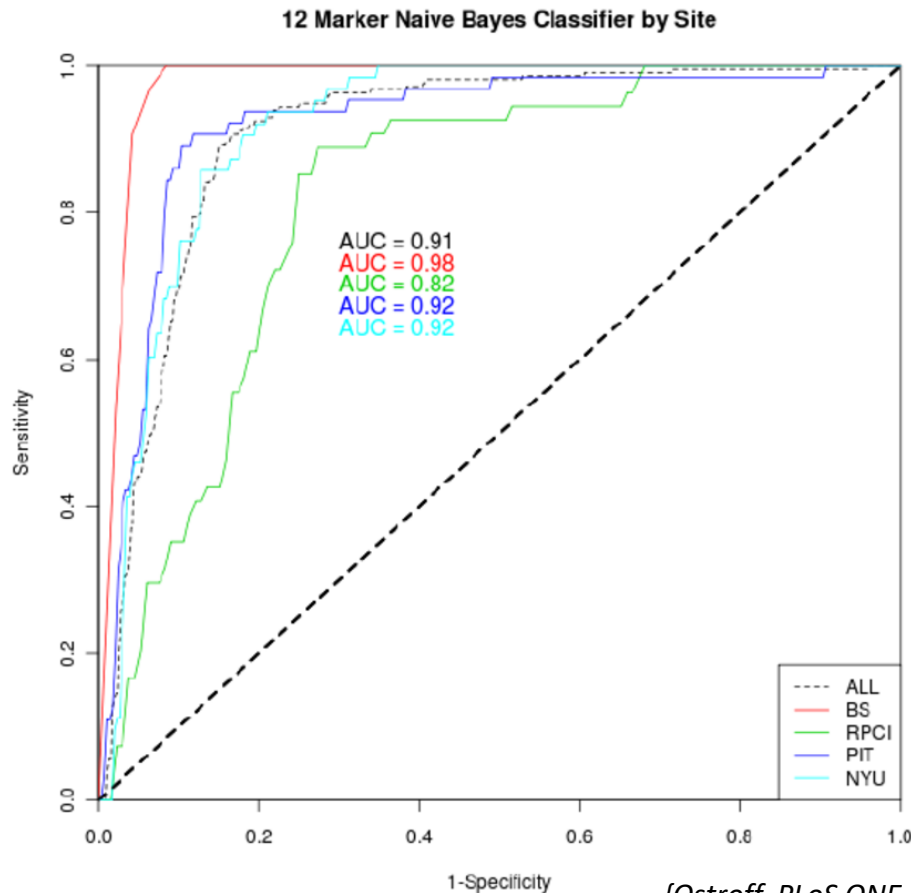
- **Sensitivity** (also called the **true positive rate**, the **recall**, or **probability of detection**[1] in some fields) measures the proportion of actual positives that are correctly identified as such (e.g., the percentage of sick people who are correctly identified as having the condition).
- **Specificity** (also called the **true negative rate**) measures the proportion of actual negatives that are correctly identified as such (e.g., the percentage of healthy people who are correctly identified as not having the condition).



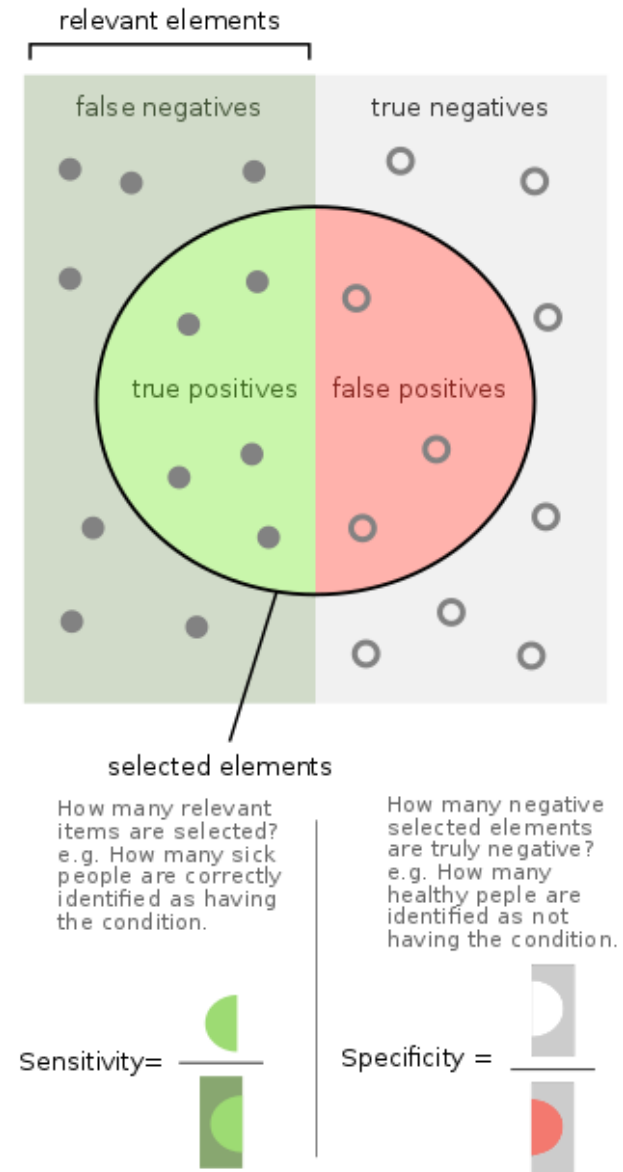
{Revolve.com}

Biomarker specificity/selectivity

Receiver-operator curves (ROC)



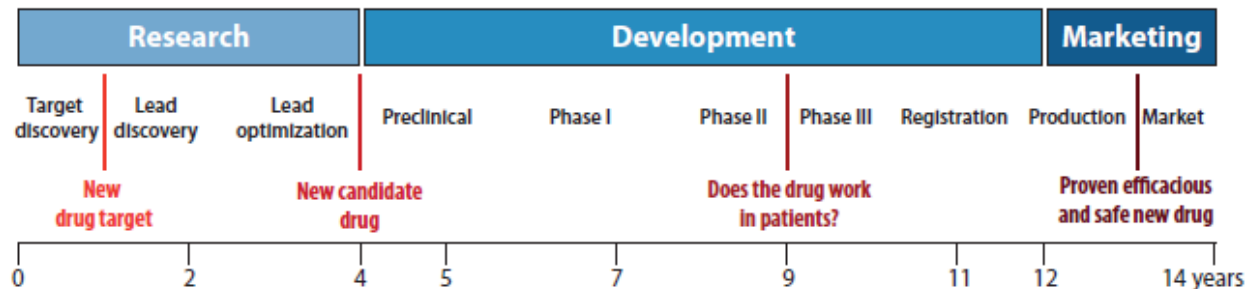
{Ostroff, PLoS ONE, 2010}



Biomarker fit-for-purpose robustness

Stage	#samples	#replicates	CV	costs	throughput
Exploratory	low	high	high	high	low
Probably valid	medium	medium	medium	medium	medium
Valid	high	low	low	low	high

- Different robustness needed in early vs late drug development
- However, most assays should preferably yield quantitative data.



Biomarker validation

Technical

- Format
- Reagent source
- Reagent stability
- Equipment
- Signal/noise
- Dilution effects
- Linearity
- Recovery
- Matrix effects
- Sample processing
- Calibration curve
- Assay variability
- Operator variability
- Upscaling
- Specificity
- Selectivity
- Reproducibility
- Carry-over
- LOD
- LLOQ
- %CV

Guidance for Industry

Bioanalytical Method Validation

Source: FDA guidance document 2001



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

21 July 2011
EMA/CHMP/EWP/192217/2009
Committee for Medicinal Products for Human Use (CHMP)

Guideline on bioanalytical method validation

university medical center

Source: EMA guidance document 2011

Biomarker qualification

Clinical

- Confirmation of discovery finding
- In independent samples
- By independent investigators
- By multiple labs
- Same assay or different assay
- Same or different calibrators
- Shared controls
- Effect size similar
- Clinical acceptance
- Publish in peer-reviewed journals
- Adequate assay robustness for planned application
- Adoption by enduser
- Health Technology Assessment

Biomarker development to clinical assay

- Health Technology Assessment (cost-benefit)
- Development and production of prototype test
- Stringent analytical validation
- Retest to ensure clinical utility (specificity/selectivity)
- Marketing
- Acceptance in field
 - Clinicians
 - Patient organisations
 - Regulatory agencies
 - Insurance providers

Outline

- Biomarkers in pharmaceutical industry
- **Biomarkers in academic research and healthcare**
- Biomarkers in personalized health (care)
- Translational innovation gaps
- Outlook

A short story:

Personalized healthcare in rare metabolic diseases

Normal Dutch parents

Son Brian, 2002, low birth weight, lactic acidosis, hypoglycaemia

Intellectual disability, movement disorder, epilepsy

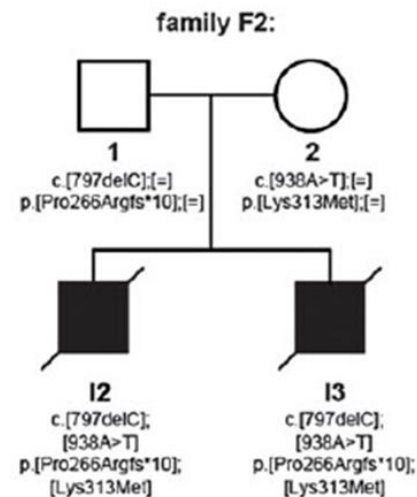
† age 3,5 yr (respiratory failure)

Son Joel, 2009, same clinical phenotype

† age 1,5 yr (epilepsy)

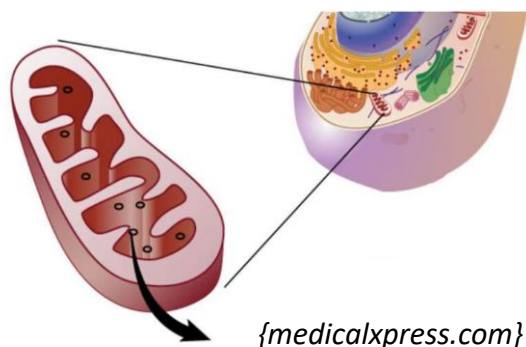
Clinical phenotype:

Suspicion of mitochondrial dysfunction

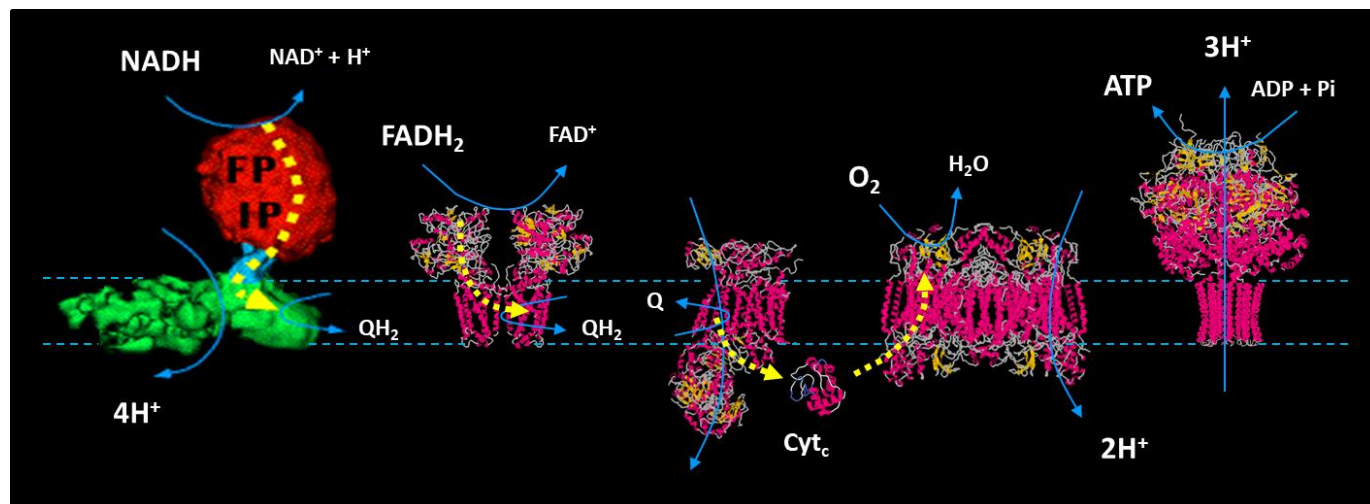


{Wortmann et al, Human Biology 2017}

Lab tests



- ATP production ↓, Creatine phosphate production ↓
- But OXPHOS enzyme complex I-V normal
- Candidate gene sequencing: no variant
- Mechanism of disease?
- In 2010: Whole Exome Sequencing - WARS2 mutations



	NADH:ubiquinone oxidoreductase (complex I)	Succinate dehydrogenase (complex II)	Ubiquinone-cytochrome c oxidoreductase (complex III)	Cytochrome c oxidase (complex IV)	F ₁ /F ₀ -ATP synthase (complex V)
subunits (genes)	44	4	11	13	13
mtDNA	7	0	1	3	2
nDNA	37	4	10	10	15

{Rodenburg, Biochim
Biophys Acta, 2016}

New mechanism of disease

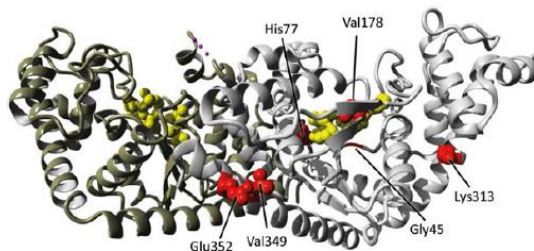
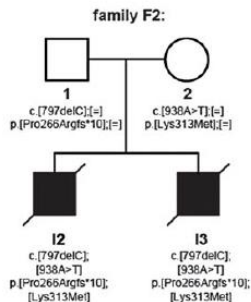
Received: 19 July 2017 | Revised: 7 September 2017 | Accepted: 10 September 2017
DOI: 10.1002/humu.23340

RESEARCH ARTICLE

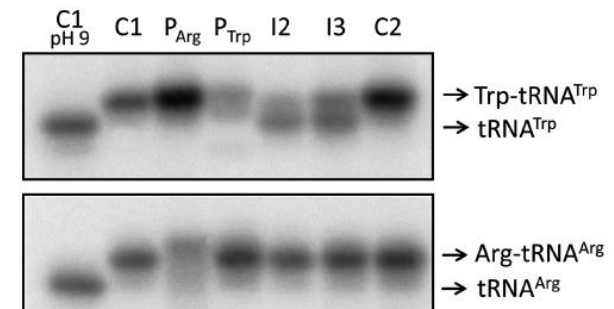
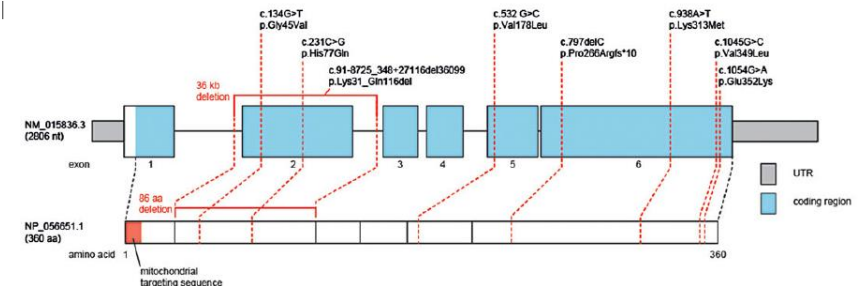


Biallelic variants in WARS2 encoding mitochondrial tryptophanyl-tRNA synthase in six individuals with mitochondrial encephalopathy

Saskia B. Wortmann^{1,2,3*} | Sharita Timal^{4,5*} | Hanka Venselaar⁶ |
Liesbeth T. Wintjes⁴ | Robert Kopajtich² | René G. Feichtinger¹ | Carla Onnekink^{7,8} |
Mareike Mühlmeister⁴ | Ulrich Brandt⁴ | Jan A. Smeitink⁴ | Joris A. Veltman^{9,10} |
Wolfgang Sperl¹ | Dirk Lefeber⁵ | Ger Puijn^{7,8} | Vesna Stojanovic^{11,12} |
Peter Freisinger¹³ | Francjan v Spronsen¹⁴ | Terry GJ Derks¹⁴ |
Hermine E. Veenstra-Kno1¹⁵ | Johannes A Mayr¹ | Agnes Rötig¹⁶ |
Mark Tarnopolsky¹⁷ | Holger Prokisch^{2,3*} | Richard J. Rodenburg^{4*}



- WARS2 is mtDNA-coded tryptophanyl-tRNA synthases
- Novel mutation causes instability of WARS2 protein
- Less charging of Trp-tRNA^{Trp}
- New prenatal genetic test !



{Wortmann et al, Human Biology 2017}

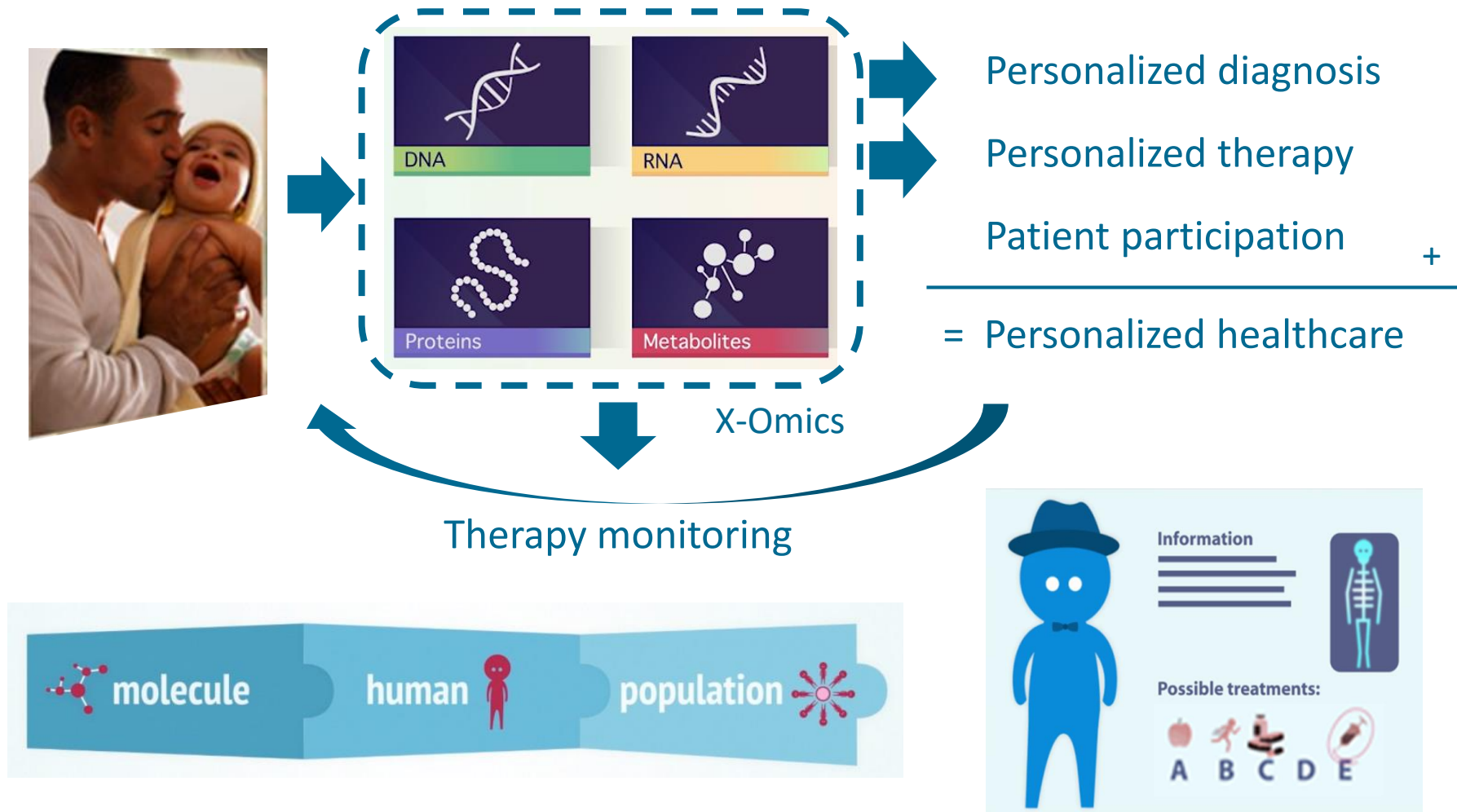
Meet & greet @ Translational Metabolic Laboratory



Lessons learned

- Technology innovation is driving impact in personalized healthcare
- Crucial to combine different molecular views to understand human health and disease (X-omics)
- Fast translational of biomarker research to implementation in academic clinical laboratories

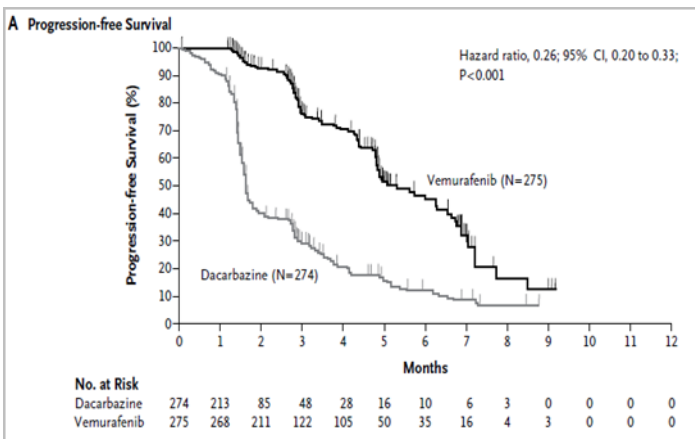
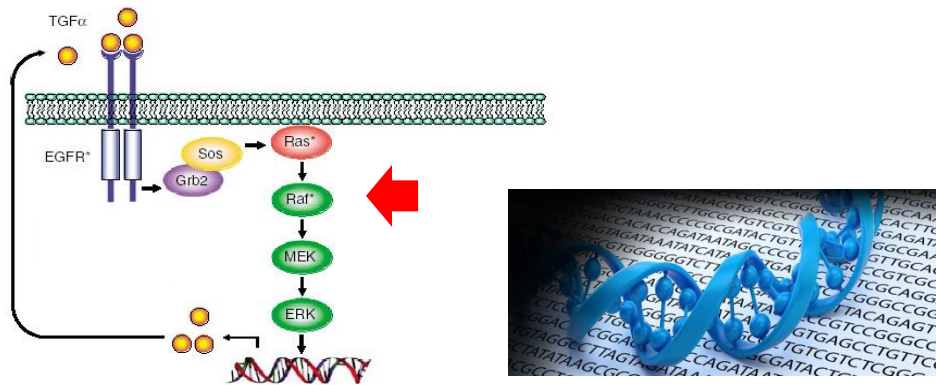
Role of molecular biomarkers in Personalized Healthcare



Genomic impact in Personalized Health(care)

Personalized medicine:

B-RAF^{V600E} drugs for melanoma

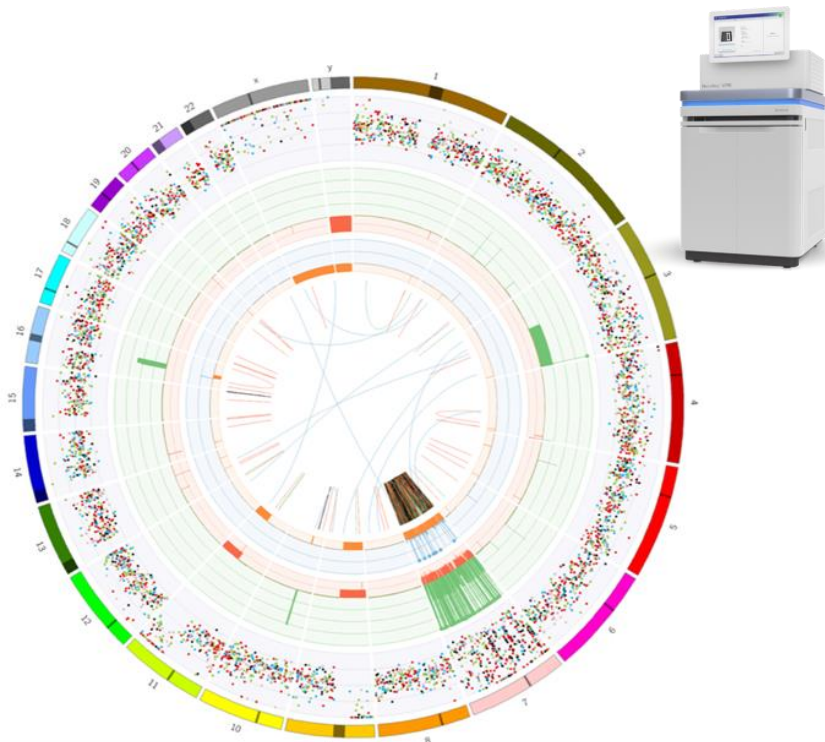


Personalized health:

BRCA-driven preventive surgery

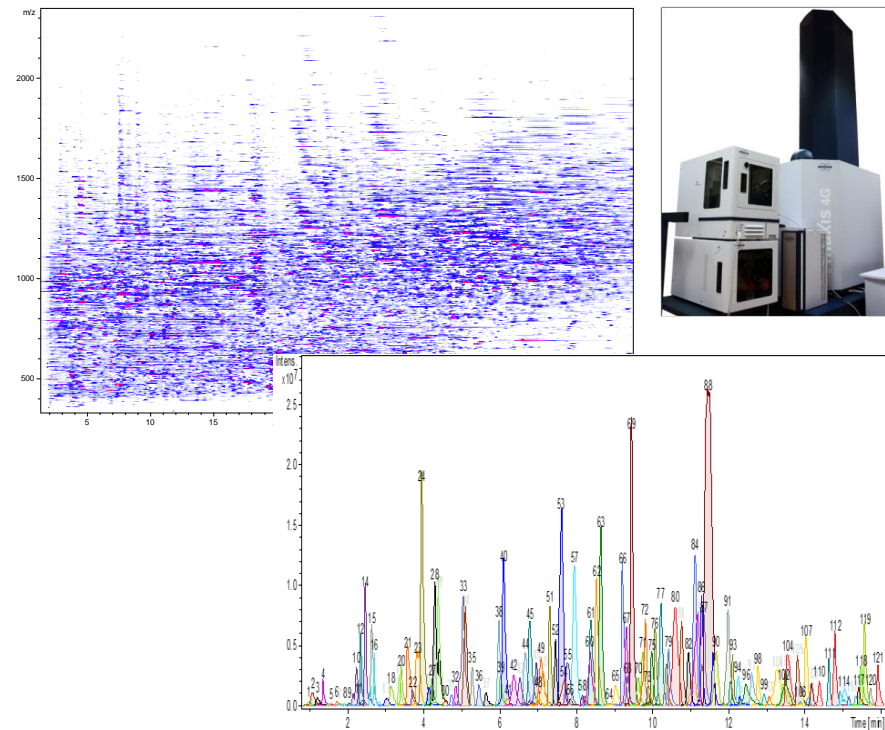


The power of omics in diagnostics



Genomics

Testing 3.000.000.000 DNA bases
in 1 assay



Proteomics, glycomics, metabolomics

Testing of 10.000-50.000 proteins and
metabolites in 1 assay

The power of omics in diagnostics

- Higher diagnostic yield
- Contextualisation of change

**Single
biomarker**

↑ increase
↓ decrease

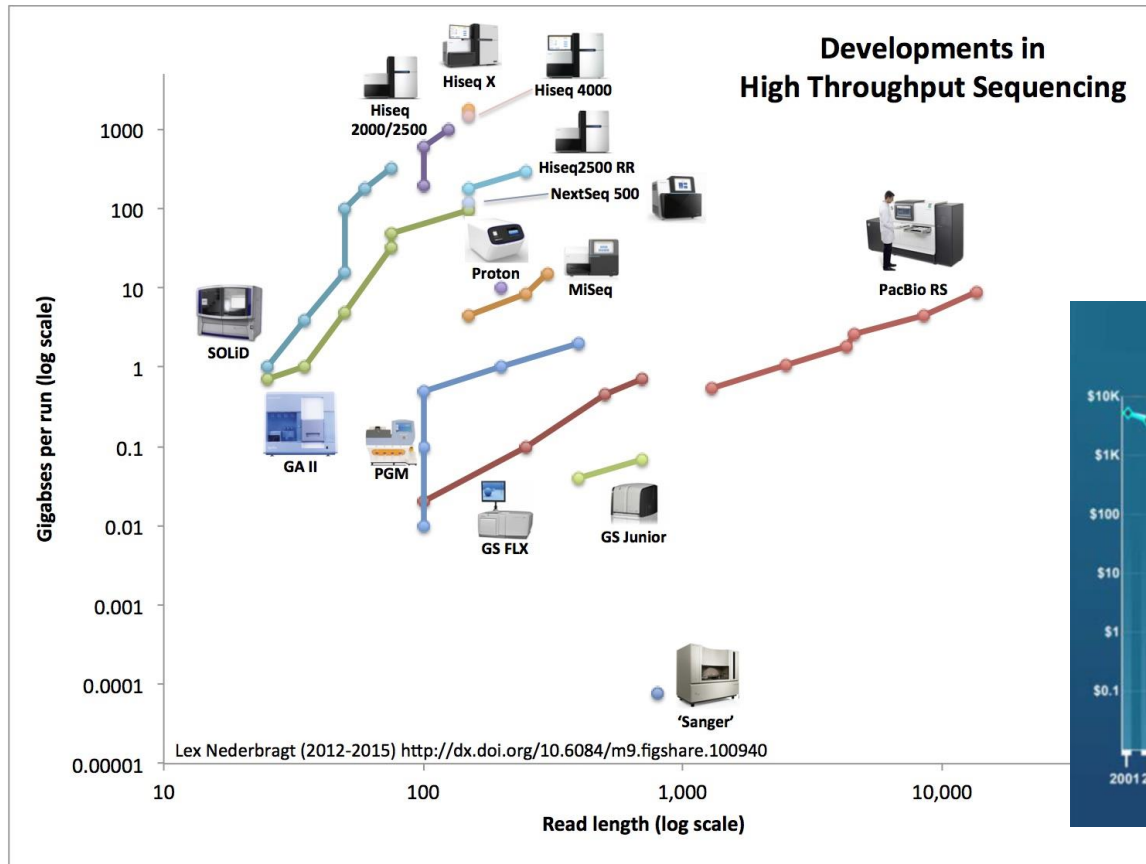
Patient 1

**Omics
panel**

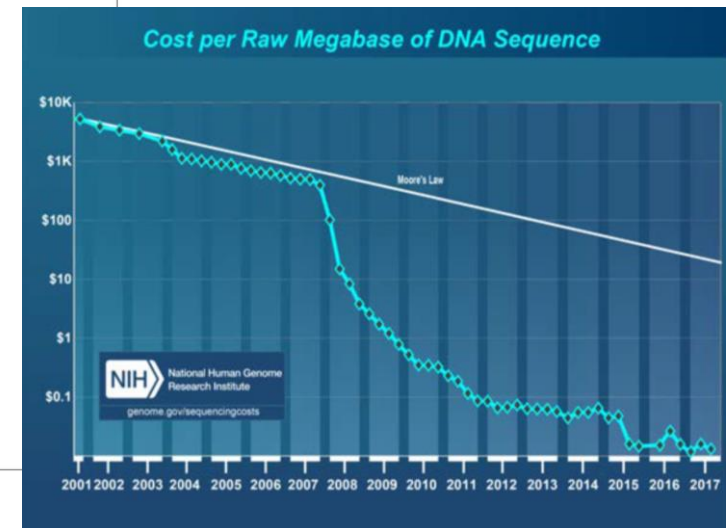
Patient 2

Genomics developments

Developments in High Throughput Sequencing



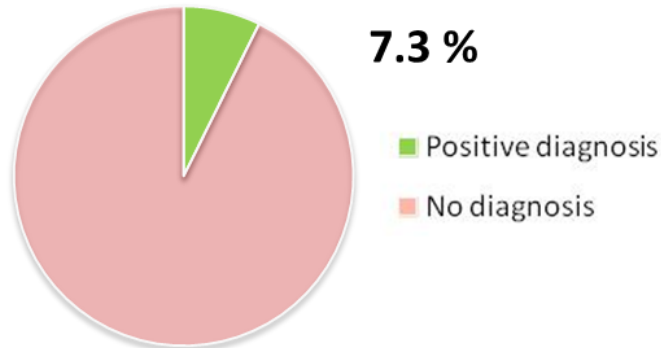
Cost per Raw Megabase of DNA Sequence



More and longer reads

at lower costs

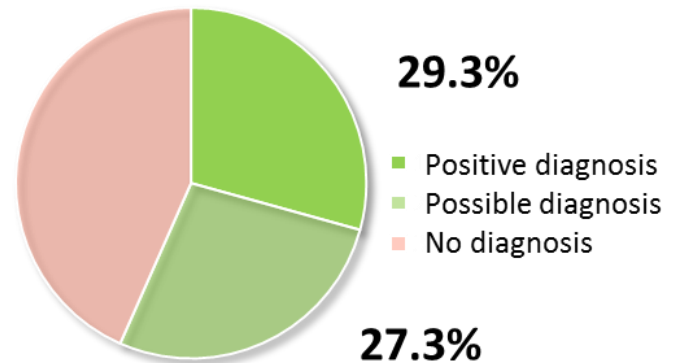
Diagnostic progress by Whole Exome Sequencing



Sanger sequencing

Gene-by-gene

5.4 tests / patient (1-28)



Whole Exome Sequencing

All genes at once

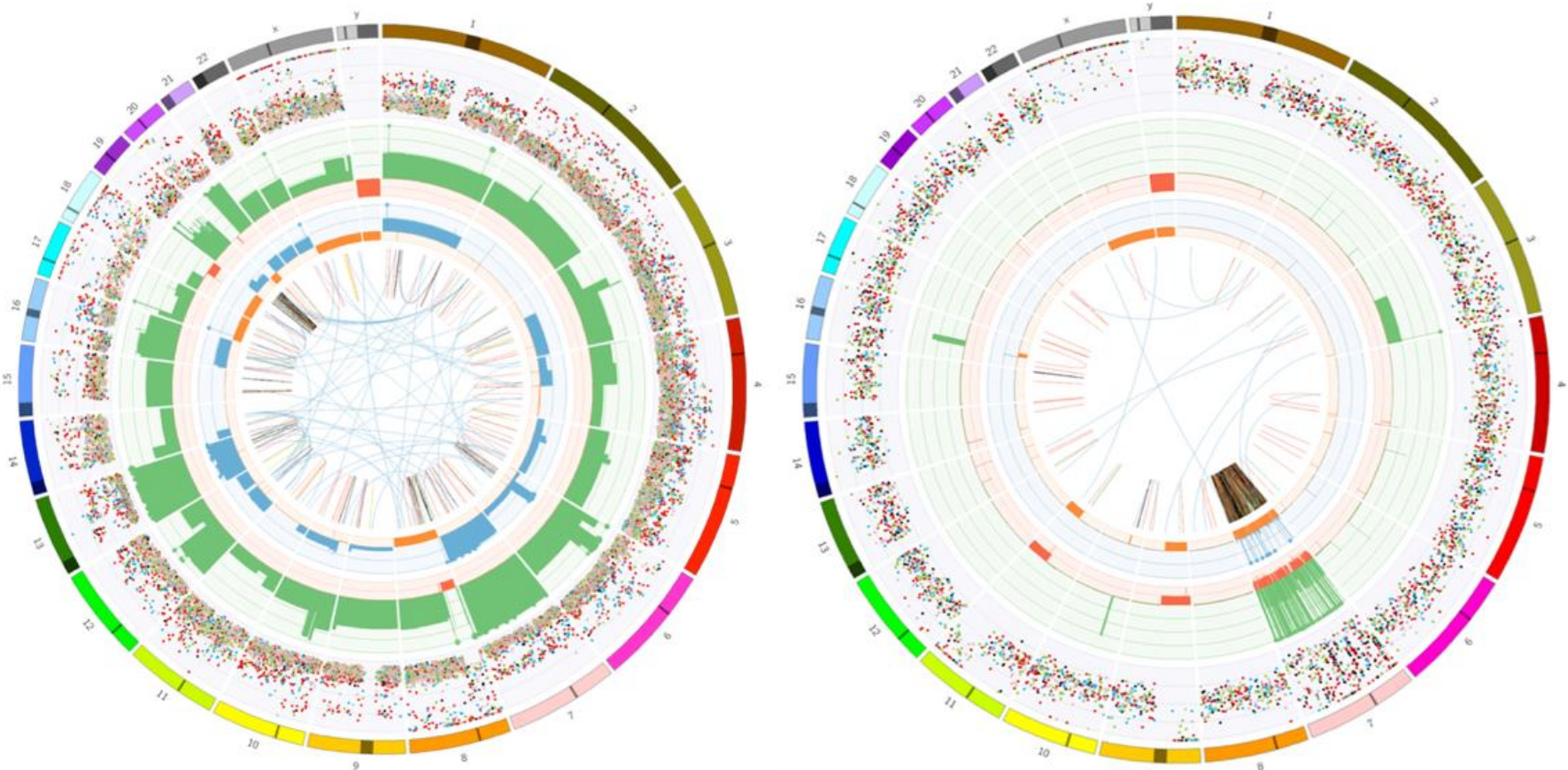
1 test / patient

Retrospective analysis of Intellectual Disability cohort (n=150)

Human Genetics Nijmegen (Lisenka Vissers, Marcel Nelen, Han Brunner et al)



Next: Whole Genome Sequencing



Circus plots of Whole Genome Sequences of two metastatic cancer patients

Source: Edwin Cuppen, Hartwig Medical Foundation

Interpretation of genetic variants becomes the issue

Guidelines American College of Medical Genetics and Genomics (ACMG)

	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very Strong
Population Data	MAF is too high for disorder <i>BA1/BS1</i> OR observation in controls inconsistent with disease penetrance <i>BS2</i>			Absent in population databases <i>PM2</i>	Prevalence in affecteds statistically increased over controls <i>PS4</i>	
Computational And Predictive Data		Multiple lines of computational evidence suggest no impact on gene /gene product <i>BP4</i> Missense in gene where only truncating cause disease <i>BP1</i> Silent variant with non predicted splice impact <i>BP3</i>	Multiple lines of computational evidence support a deleterious effect on the gene /gene product <i>PP3</i>	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before <i>PM5</i> Protein length	Same amino acid change as an established pathogenic variant <i>PS1</i>	Predicted null variant in a gene where LOF is a known mechanism of disease <i>PM1</i>
Functional Data	Well-established functional studies no effect <i>BS3</i>			Well-established functional studies show a deleterious effect <i>PS3</i>		
Segregation Data	Non-segregation with disease <i>PP2</i>		Co-segregation with disease in multiple affected family members <i>PP1</i>	Increased segregation data		
De novo Data				<i>De novo</i> (without paternity & maternity confirmed) <i>PM6</i>	<i>De novo</i> (paternity & maternity confirmed) <i>PS2</i>	
Allelic Data		Observed in <i>trans</i> with a dominant variant <i>BP2</i> Observed in <i>cis</i> with a pathogenic variant <i>BP2</i>		For recessive disorders, detected in <i>trans</i> with a pathogenic variant <i>PM3</i>		
Other Database		Reputable source w/out shared data = benign <i>BP6</i>	Reputable source = pathogenic <i>PP5</i>			
Other Data		Found in case with an alternate cause <i>BP5</i>	Patient's phenotype or FH highly specific for gene <i>PP4</i>			

“Functional studies can be a powerful tool in support of pathogenicity”

Variant classification:

1. Benign
2. Likely benign
3. Uncertain significance
4. Likely pathogenic
5. Pathogenic

More sequence data = more unknown variants

Richards et al., Genet Med. (2015)17:405-24

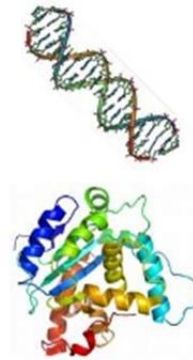
Complexity in protein biology

Truncation
Phosphorylation
Acetylation
Ubiquitination
N-Glycosylation
...

DNA

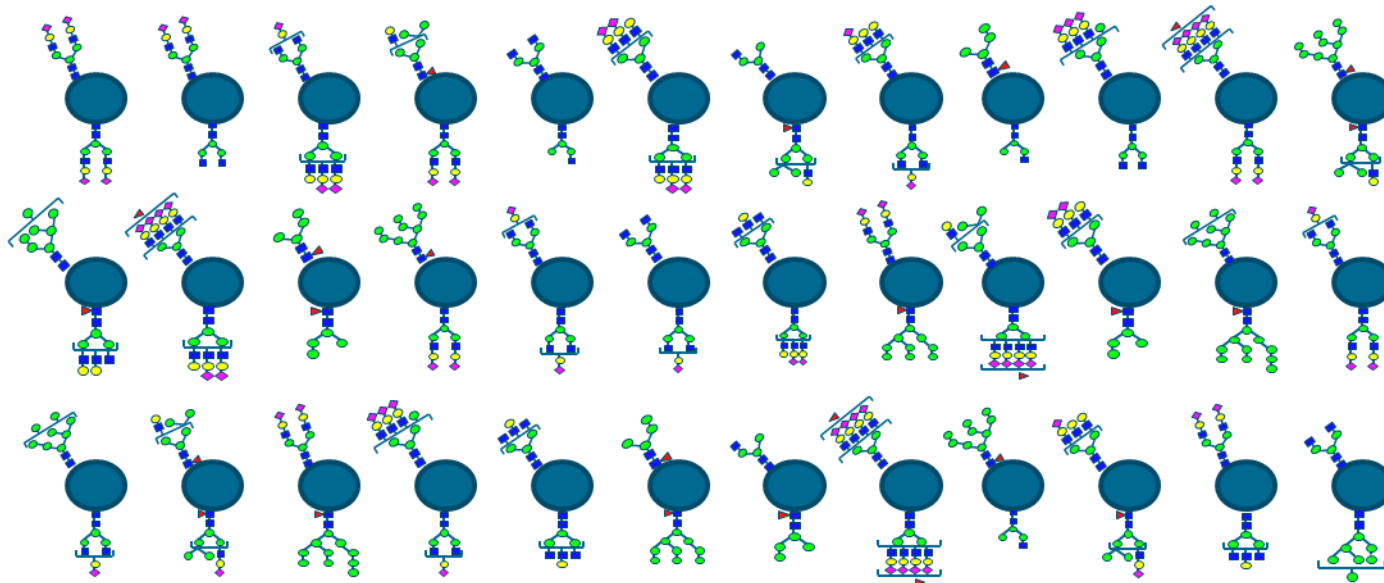


Protein



21.000 genes

1.000.000 -2.000.000
protein forms

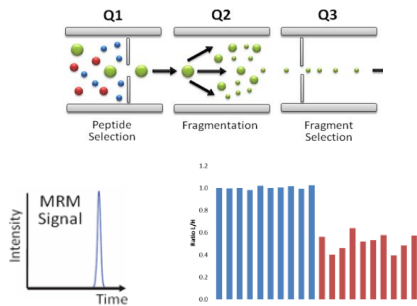


Our functional Omics platforms

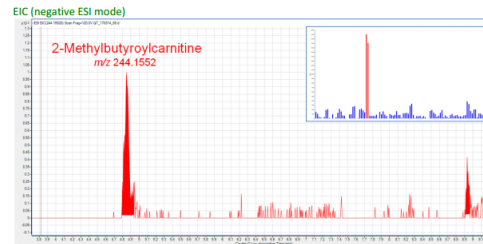
Research

Biomarkers

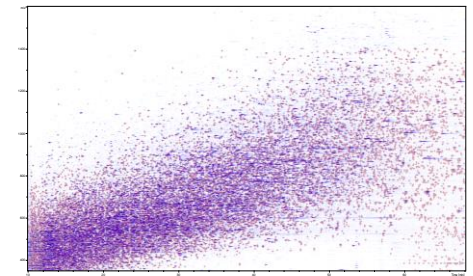
Diagnostics



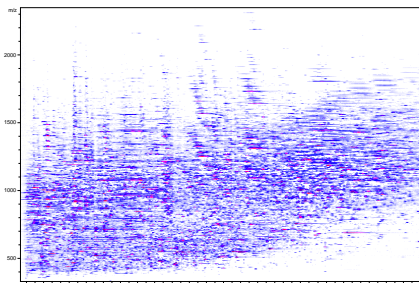
Targeted analyses



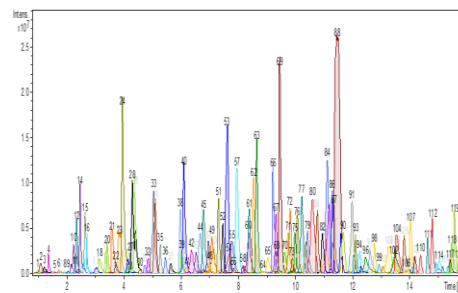
Metabolomics



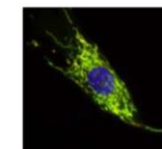
Proteomics



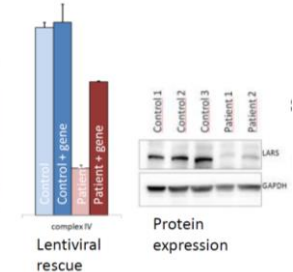
Glycomics/glycoproteomics



Top-down proteomics



Subcellular localization

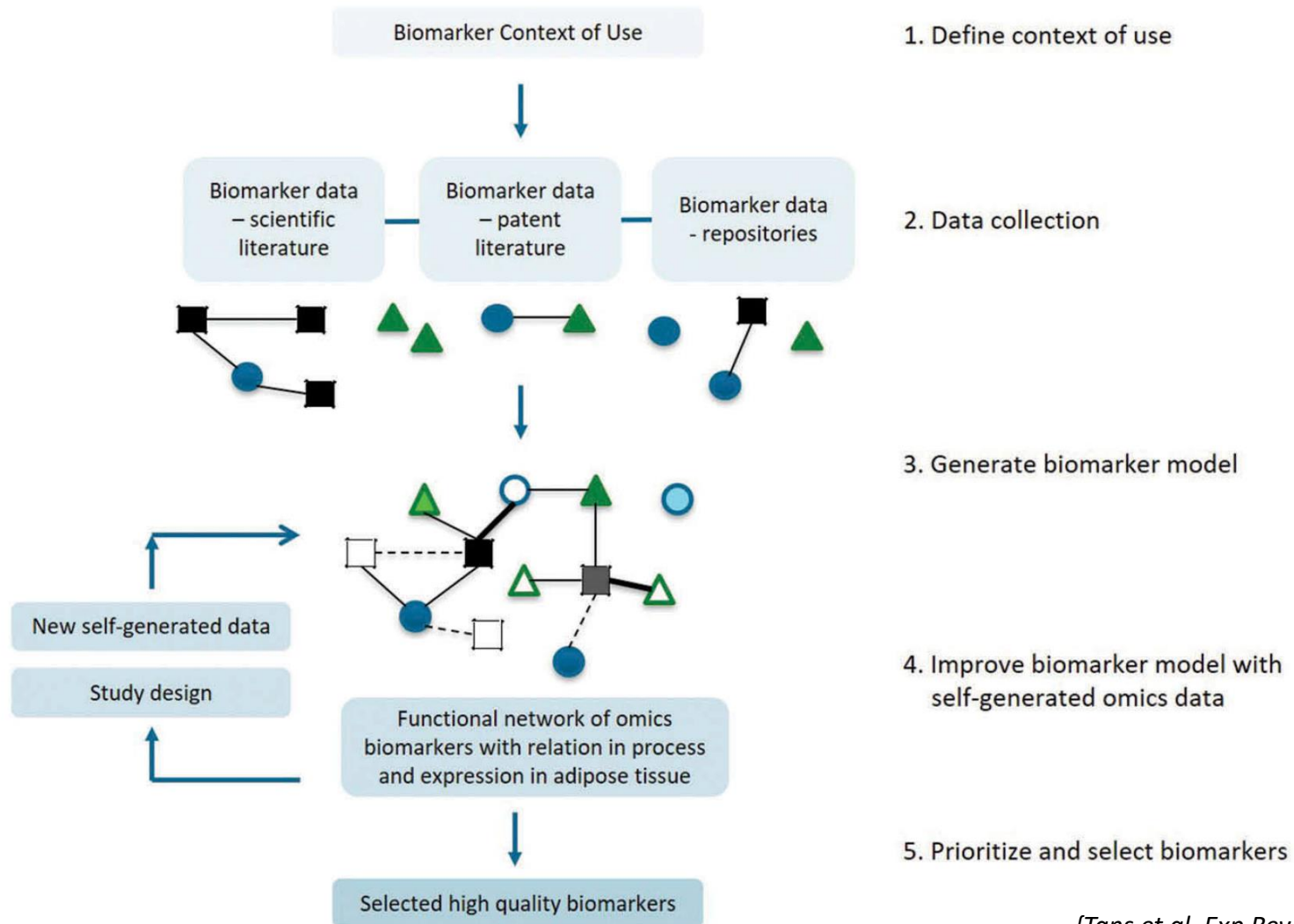


Functional genomics

Translational Metabolic Laboratory

(www.youtube.com/watch?v=yhLbuXOH7rg)

Use Omics in Biomarker R&D



{Tans et al, Exp Rev Proteomics 2018}

Precision medicine in genetic-metabolic disease – current

Personalized diagnosis

New disease mechanisms

Personalized therapies

Genomics (WES)



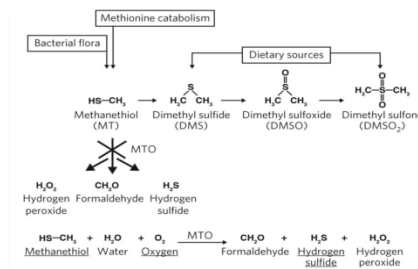
Metabolomics



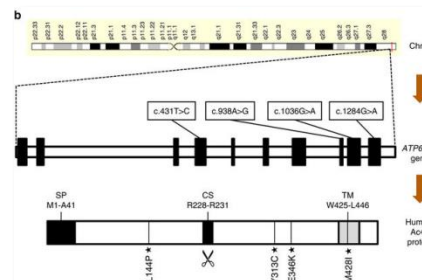
Glycomics

@Translational Metabolic Laboratory

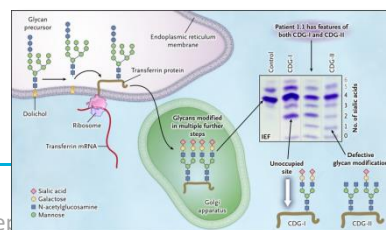
Nature Genetics 2018



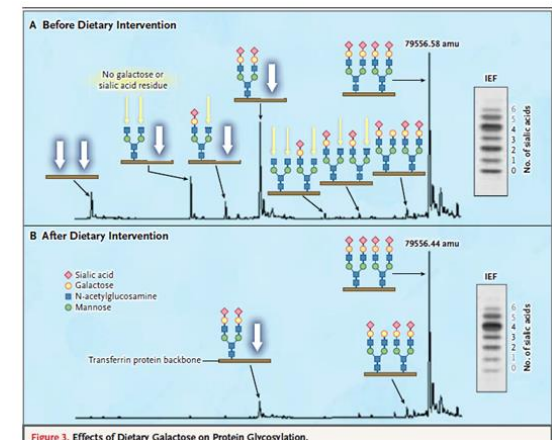
Nature 2016



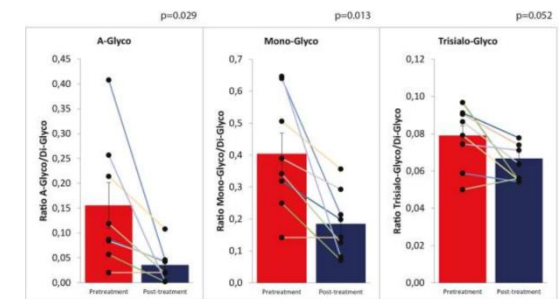
NEJM 2014



NEJM 2014



Genet Med 2017



Precision medicine in genetic-metabolic disease - future

Personalized diagnosis

New disease mechanisms

New personalized therapies

Genomics
(WGS)



Deep Learning
Artificial Intelligence
System biology



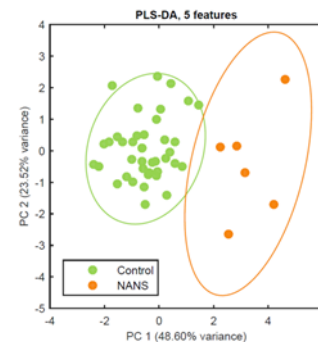
Metabolomics



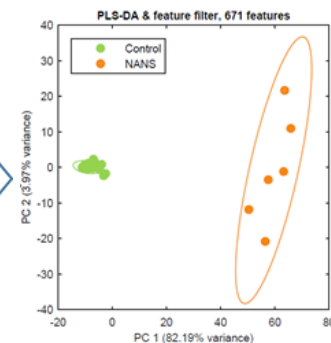
Glycomics



Glycoproteomics

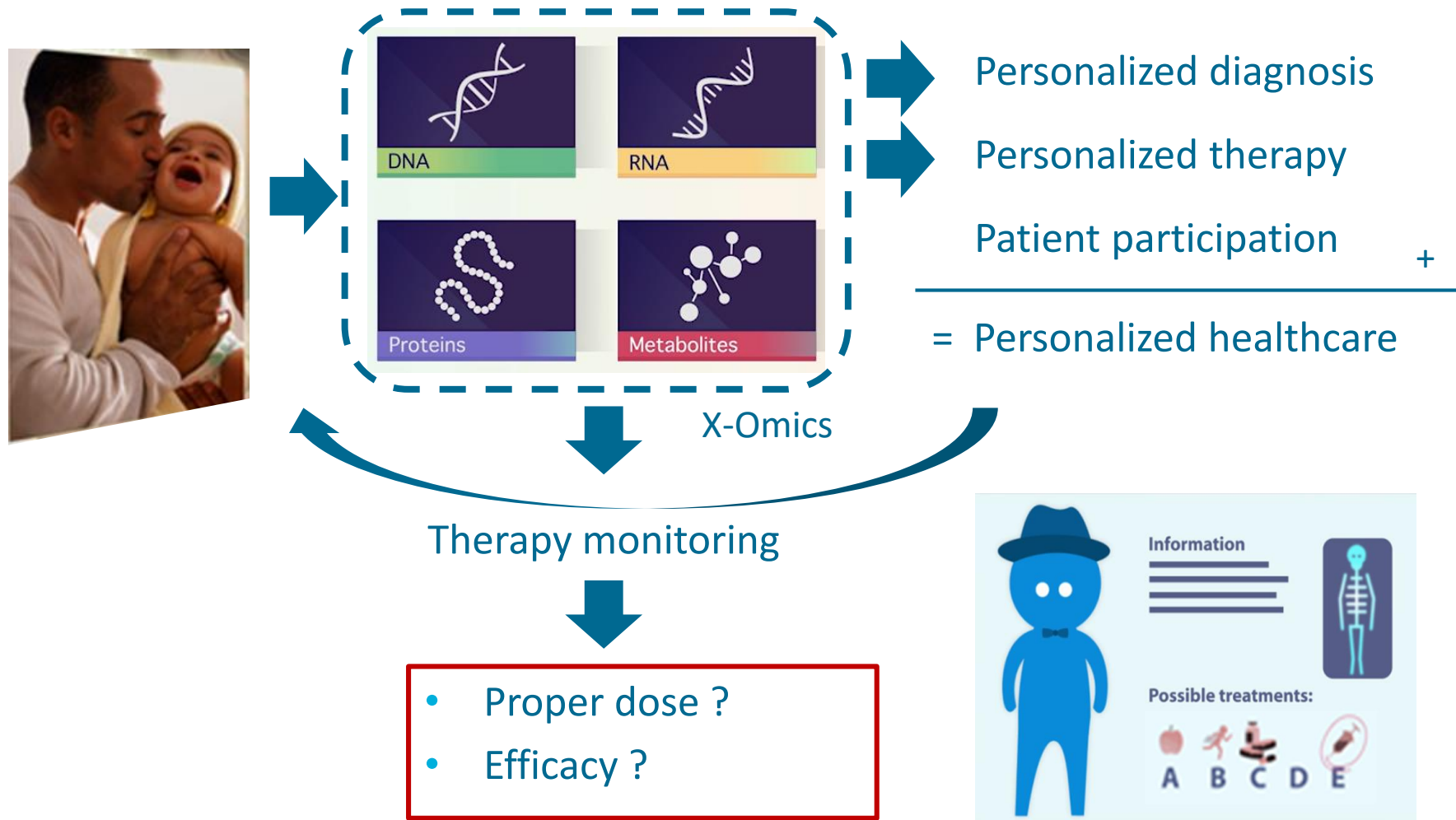


Nature Genetics 2016



Pilot 2017

Role of molecular biomarkers in Personalized Healthcare



Therapeutic drug monitoring

Therapeutic drug monitoring (TDM) is the clinical practice of measuring **drugs concentration** and **patient's response** at designated intervals thereby optimizing individual dosage regimens.

Benefits of TDM:

Patients:

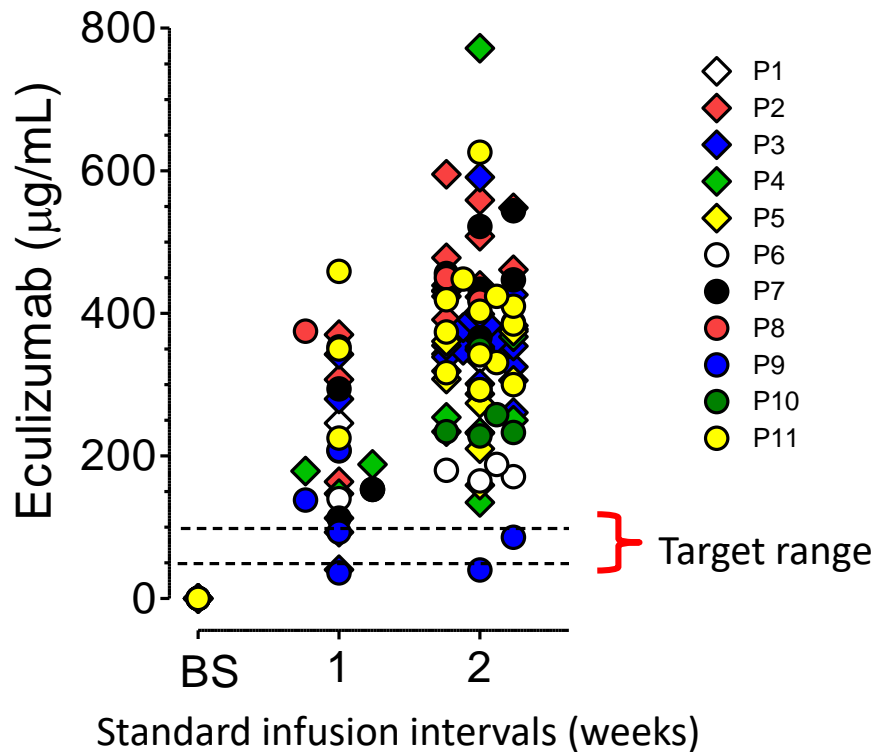
- Prevents underdosing thus **ensures efficiency**

- Prevents overdosing and unnecessary **side effects**

Payers:

- Reduces health care-related **costs**

Optimisation of Soliris treatment in atypical Hemolytic Uremic syndrome



Standard infusion intervals (weeks)

{Volokhina et al., Clin Pharmacol Ther, 2017}

- Solaris (Eculizumab) is very expensive drug
- Life-long infusions
- Frequent overdosing
- Room for improvement !



{Elena Volokhina, Bert van den Heuvel}

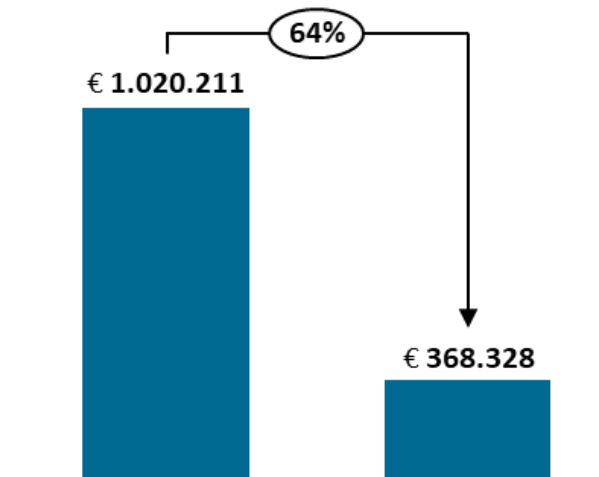
Cost-reductions



CUREiHUS (n=37)

Period [1-1-2016 – 01-03-2019]	Standard treatment	Cure-i-HUS
Total	€ 37.747.822	€ 13.628.141
Per Patient	€ 1.020.211	€ 368.328
Total reduction	€ 24.119.681	
Reduction per patiënt	€ 651.883	
Reduction per year	€ 7.616.741	

Radboudumc n= 22
 VUmc n= 6
 ErasmusMC n= 5
 UMCG n= 4



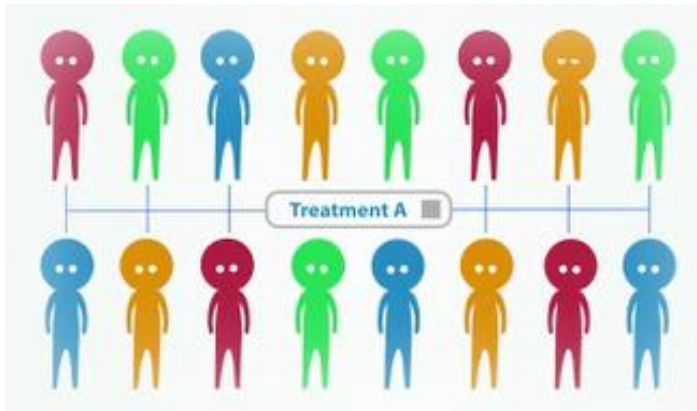
By:

- Adjustment dosing
- Reduce treatment after recovery

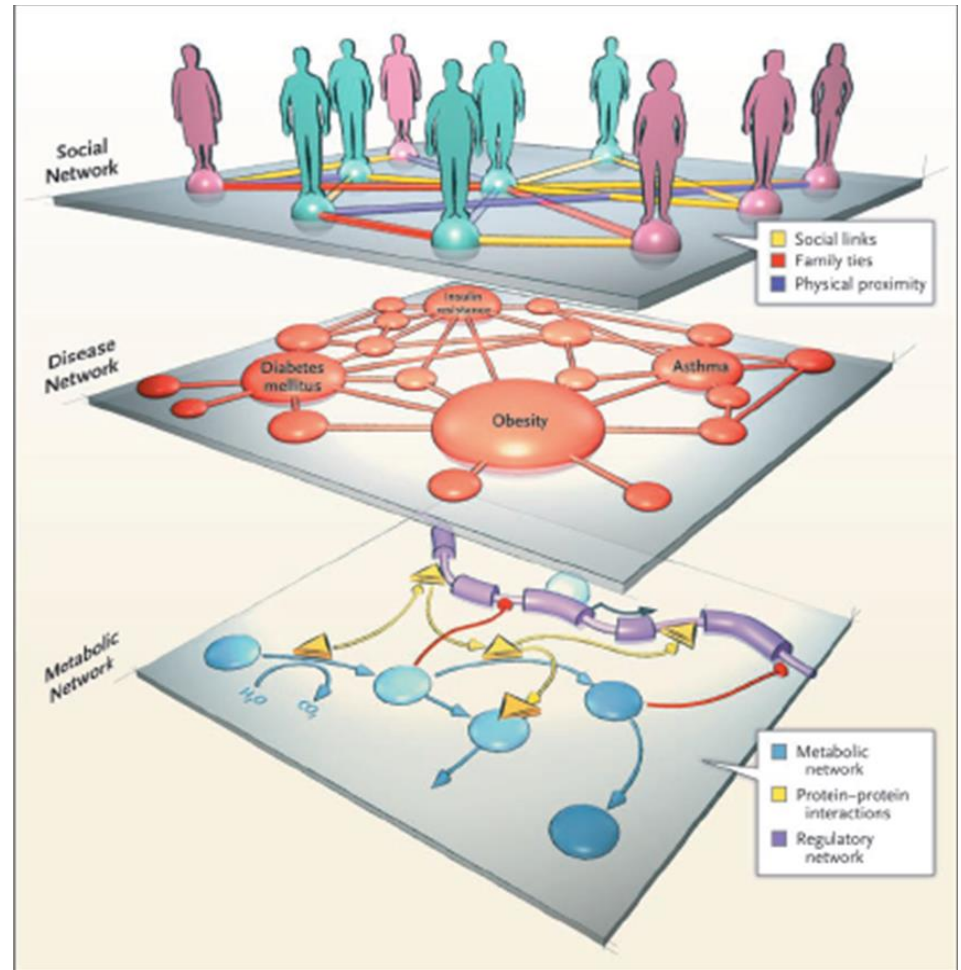
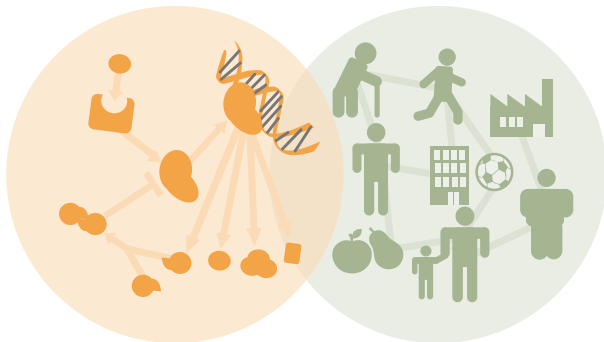
Outline

- Biomarkers in pharmaceutical industry
- Biomarkers in academic research and healthcare
- **Biomarkers in personalized health (care)**
- Translational innovation gaps
- Outlook

Moving to personalized health(care)



- People are more than linear pathways
- Different systems and networks
- Different risk factors
- Different preferences



{Source: Barabási 2007 NEJM 357; 4}

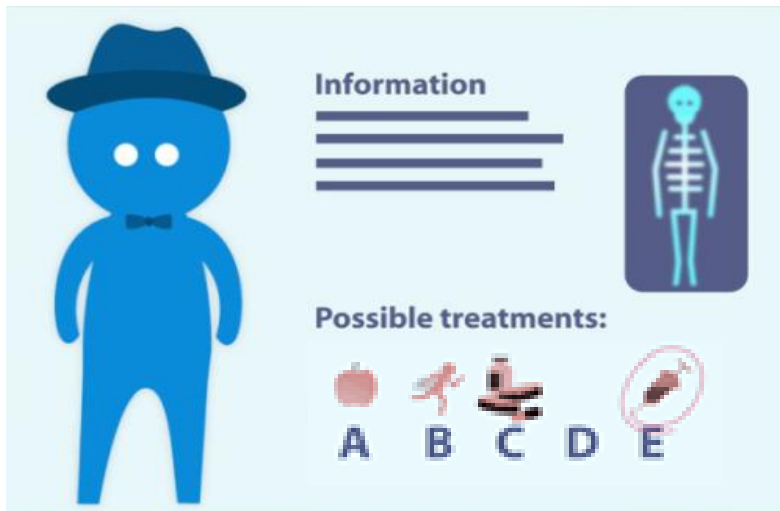
3 key aspects of personalized health(care)

'I want to stay healthy. If not, how do I get healthy?'

1. What to measure?
2. How much can it change?
3. What should be the follow-up for me?

We need a personalized data-driven GPS for health

- Monitor on background
- Alert when you are at risk
- Advice what to do
- Powered by biomarkers



A changing world: We are getting digital !!



Exponential developments

- digital biomarkers

54





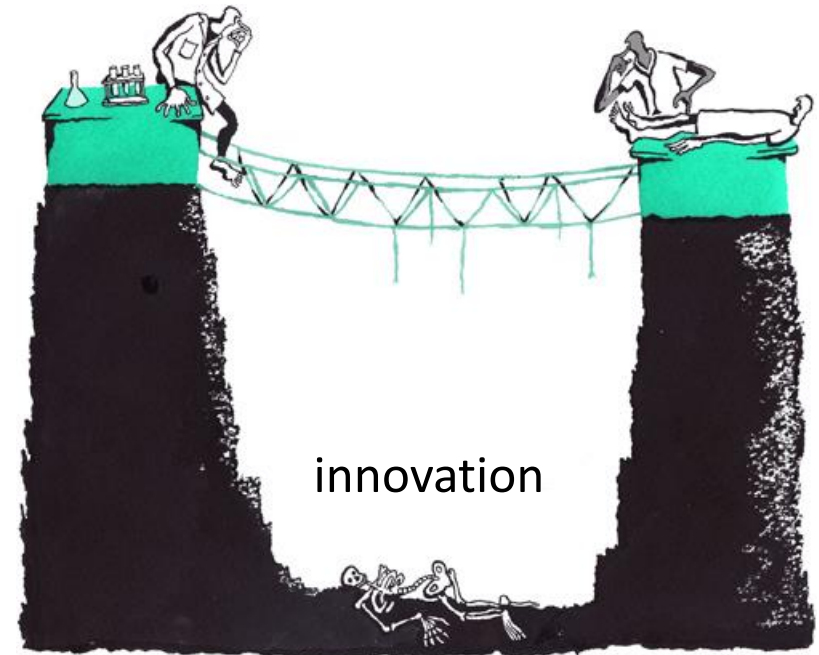
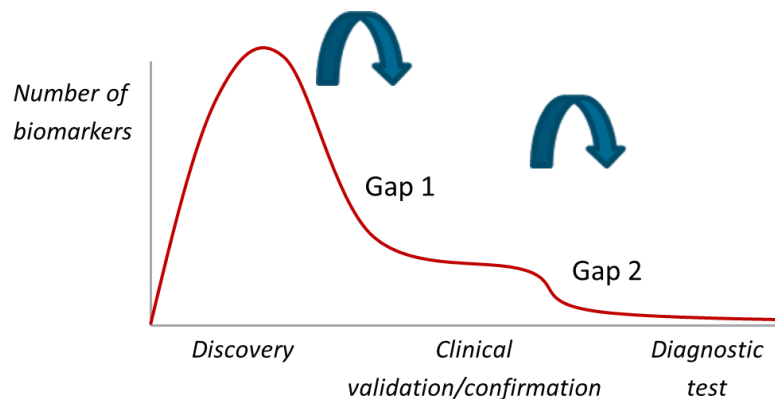
Outline

- Biomarkers in pharmaceutical industry
- Biomarkers in academic research and healthcare
- Biomarkers in personalized health (care)
- **Translational innovation gaps**
- Outlook



However ... 3 translational innovation gaps !

1. Research to research
2. Research to diagnostics
3. Research to society



{See www.slideshare.net/alainvangool}

1. Research to research

PERSPECTIVE

The Economics of Reproducibility in Preclinical Research

Leonard P. Freedman^{1*}, Iain M. Cockburn², Timothy S. Simcoe^{2,3}

¹ Global Biological Standards Institute, Washington, D.C., United States of America, ² Boston University School of Management, Boston, Massachusetts, United States of America, ³ Council of Economic Advisers, Washington, D.C., United States of America

{Freedman et al, PLOS Biology, 2015}

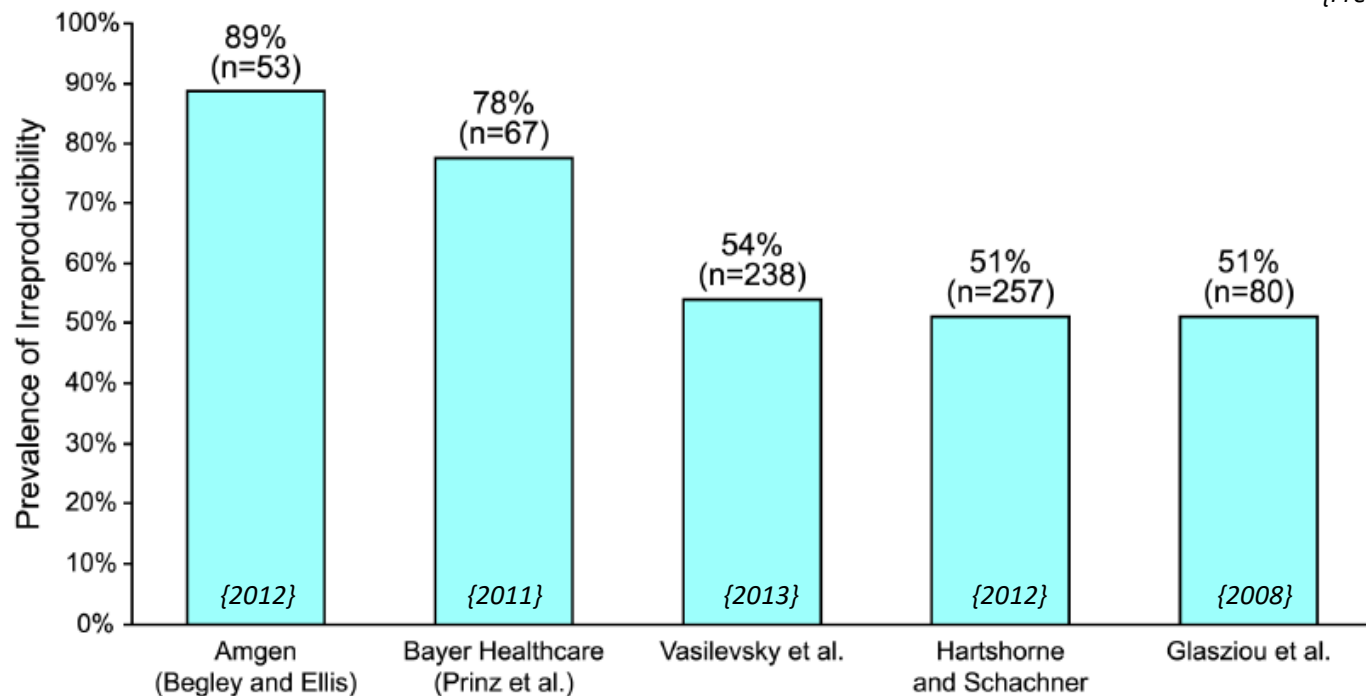
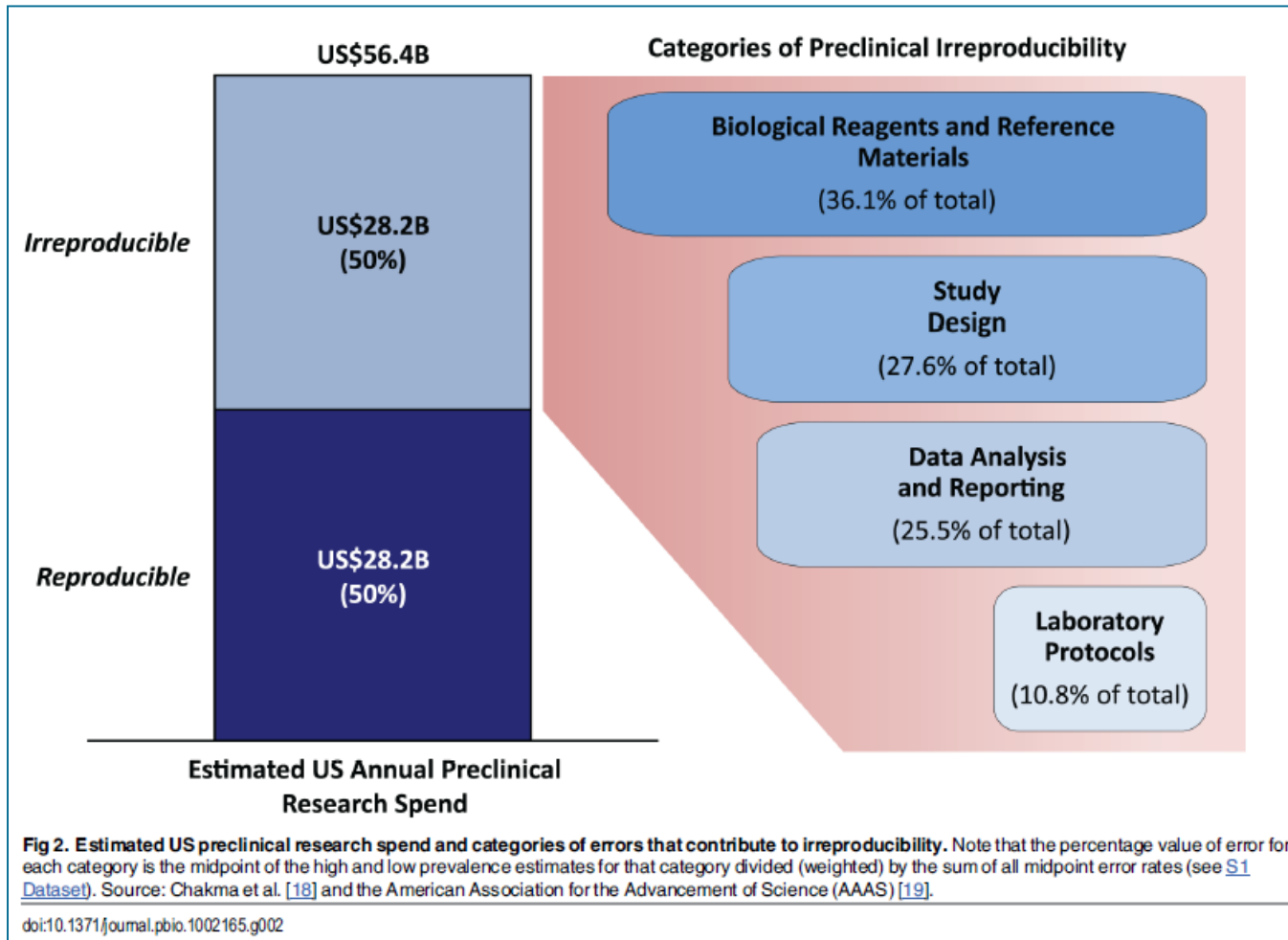


Fig 1. Studies reporting the prevalence of irreproducibility. Source: Begley and Ellis [6], Prinz et al. [7], Vasilevsky [8], Hartshorne and Schachner [5], and Glasziou et al. [9].

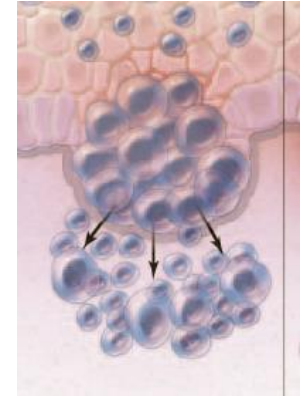
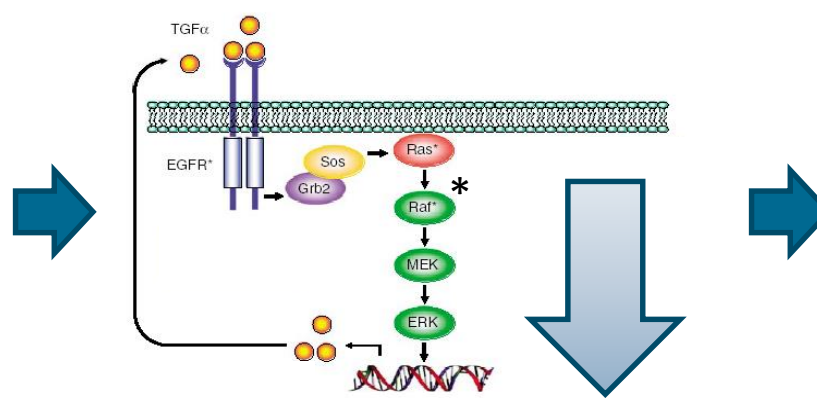
doi:10.1371/journal.pbio.1002165.g001

Categories of errors leading to irreproducibility



{Freedman et al,
PLOS Biology, 2015}

A short story: Personalized medicine in melanoma



B-RAF^{V600E} mutation

Strong growth of cell

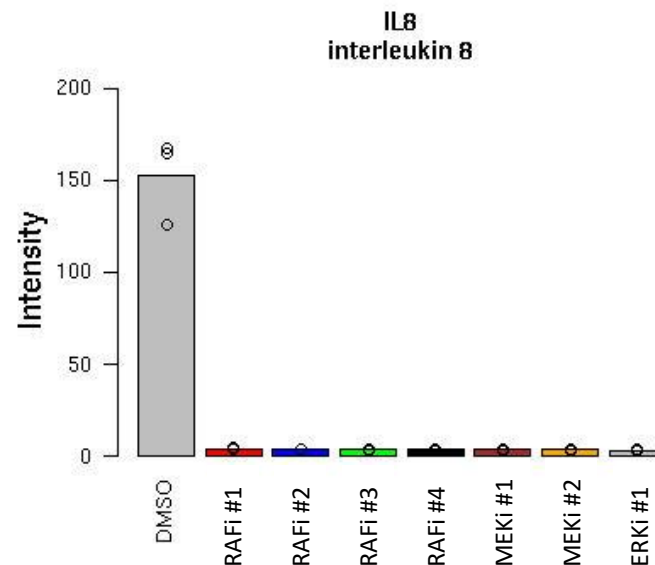
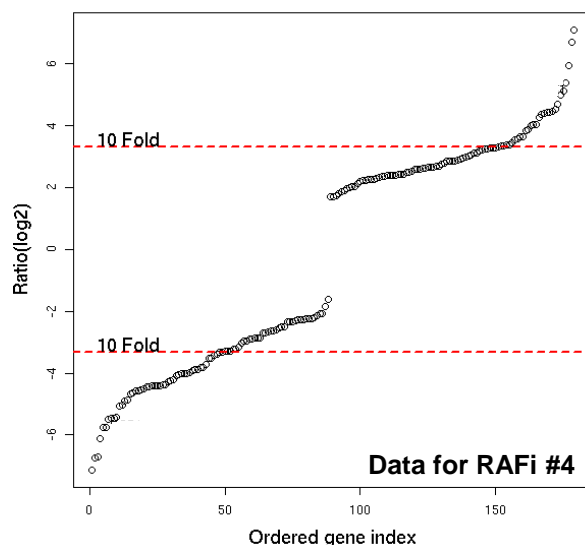
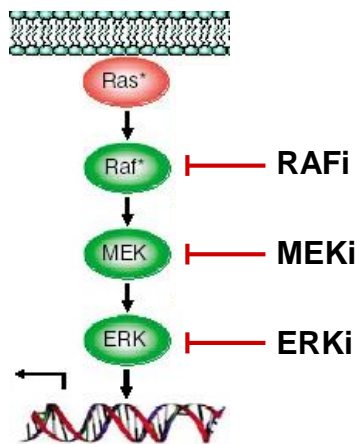
Growth of tumor

- B-RAF^{V600E} cells always grow and become cancer cells
- RAF inhibitors will block pathway, block cell growth and inhibit cancers that have a B-RAF^{V600E} mutation
- 60% of melanoma patients have B-RAF^{V600E} mutation
- Basis for a personalized medicine !



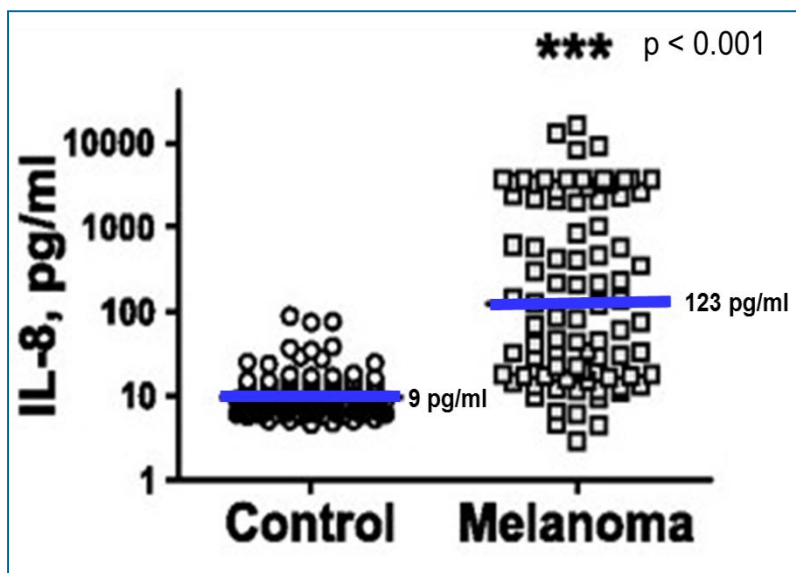
Biomarkers to support clinical development

- Within Schering-Plough 4 Lead Optimisation programs in ERK pathway (2009)
- Need for blood-based biomarker that indicated downstream effects of drugs:
 - Inhibition ERK pathway (pharmacodynamic)
 - Tumor inhibition (efficacy)
- Extensive transcriptomics profiling: **IL-8** as promising candidate biomarker



Validation study to confirm IL-8 in melanoma

Literature



Objectives:

- Confirm elevated IL-8 in melanoma
- Develop IL-8 assays for clinical use

Cancer Therapy: Clinical

Multiplex Analysis of Serum Cytokines in Melanoma Patients Treated with Interferon- α 2b

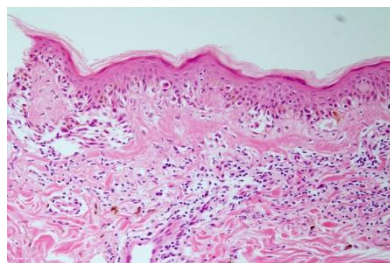
Zoya R. Yurkovetsky,^{1,2} John M. Kirkwood,^{1,2} Howard D. Edington,^{1,3} Adele M. Marrangoni,¹ Lyudmila Velikokhatnaya,¹ Matthew T. Winans,¹ Elieser Gorelik,^{1,4,5} and Anna E. Lokshin^{1,2}

{Yurkovetsky, et al. Clin Cancer Res, 2007}

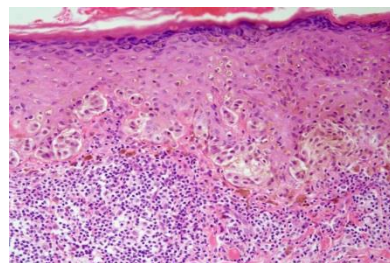
Validation study to confirm IL-8 in melanoma

59 melanoma samples (tumor tissue (ffpe) + matching serum & plasma, stage I-IV, from two independent biobanks) + 40 healthy serum & plasma samples

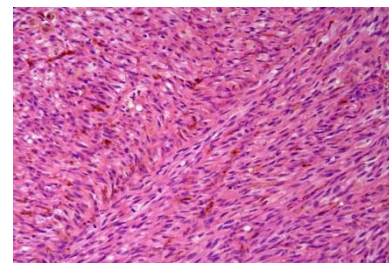
1. **Genetic analysis** for BRAF^{V600E/D} mutation in genomic DNA from **tissue** **OK**
2. **IL-8 mRNA analysis** in **tissue** samples by *in situ* hybridisation using bDNA probes (multiplexing with 12 ERK pathway response transcripts) **OK**
3. **IL-8 protein analysis** in **tissue** samples by immunohistochemistry (in parallel with 4 other ERK pathway response proteins, Ki67, Tunnel) **OK**
4. **IL-8 protein analysis** in **matching plasma and serum** by IL-8 immunoassay (3 formats: ELISA, Luminex, Mesoscale; singleplex and multiplex) **?**



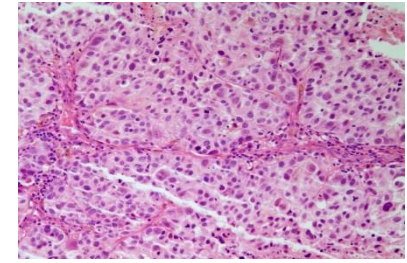
Stage 1



Stage 2



Stage 3

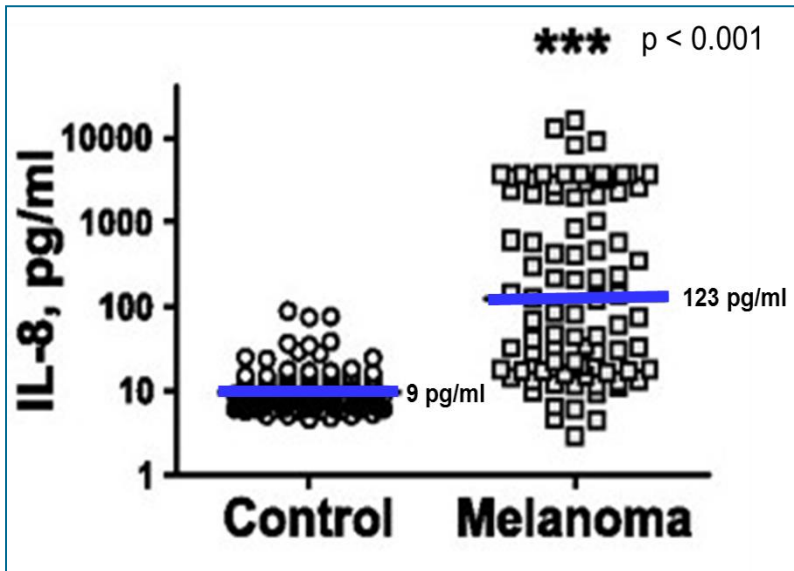


Stage 4

H&E staining; 20x

Validation study to confirm IL-8 in melanoma

Literature



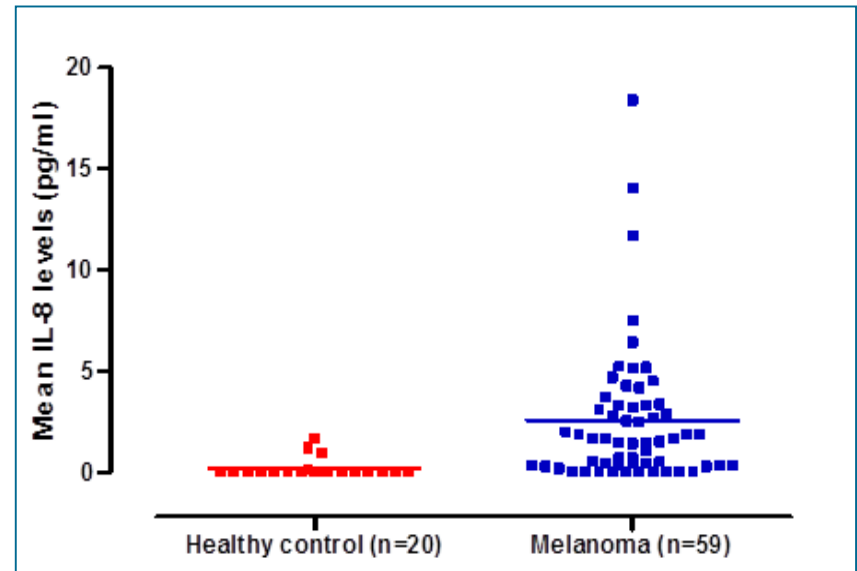
Cancer Therapy: Clinical

Multiplex Analysis of Serum Cytokines in Melanoma Patients Treated with Interferon- α 2b

Zoya R. Yurkovetsky,^{1,2} John M. Kirkwood,^{1,2} Howard D. Edington,^{1,3} Adele M. Marrangoni,¹ Lyudmila Velikokhatnaya,¹ Matthew T. Winans,¹ Elieser Gorelik,^{1,4,5} and Anna E. Lokshin^{1,2}

{Yurkovetsky, et al. Clin Cancer Res, 2007}

Own data

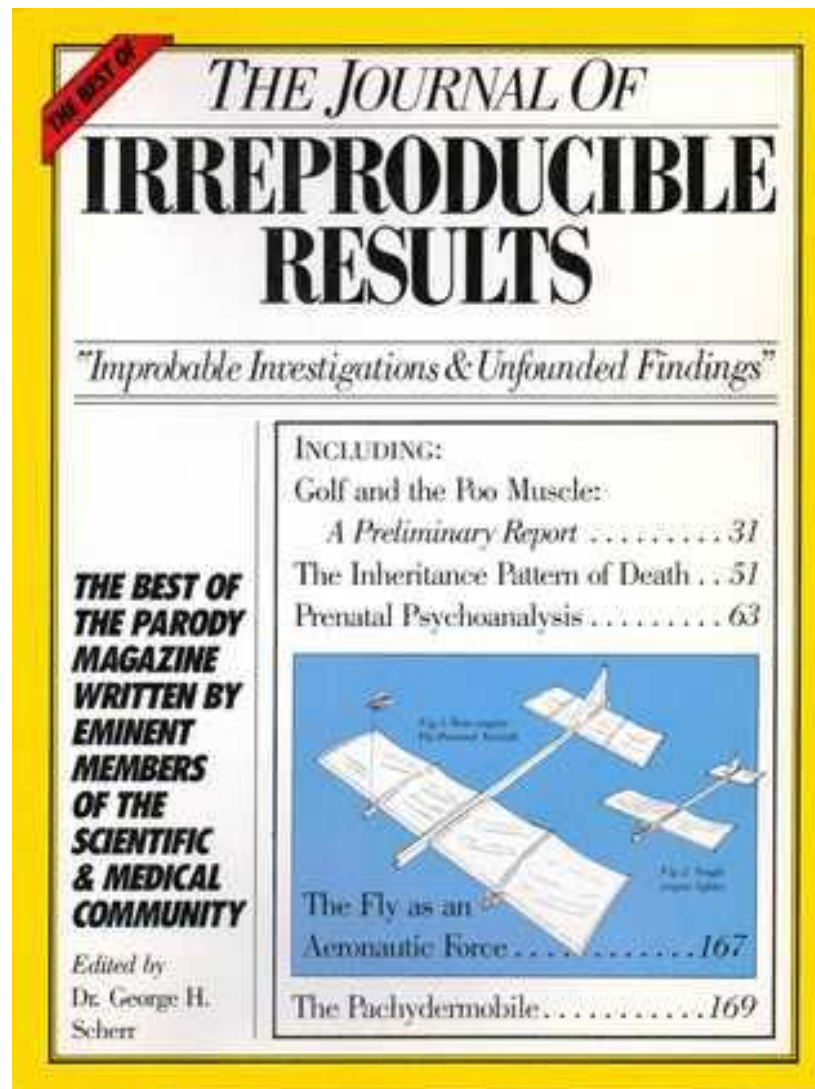


{Unpublished, 2010}

(6 months, 4 fte, USD 1.000.000)

Cause?

Share negative outcomes

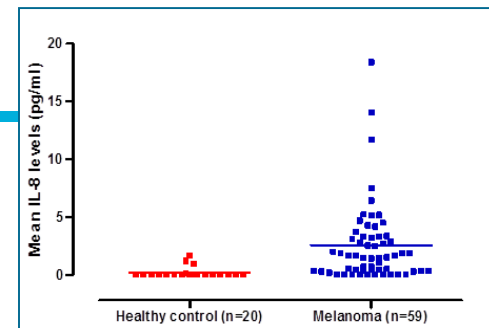
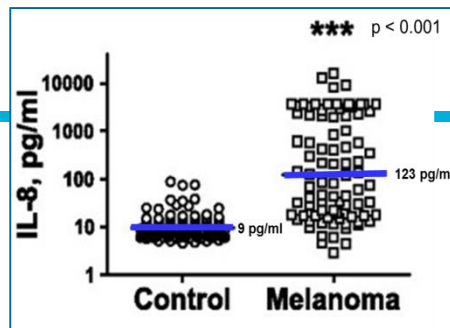


Lessons learned?

Particularly for this case:

1. Know sample history
 - IL-8 protein appeared sensitive to freeze-thawing
2. Know all relevant information from the source (patient)
 - Tumor load may be too low for our patients
3. Do these type of expensive validation studies together !
 - Share burden, increase power, ensure better data

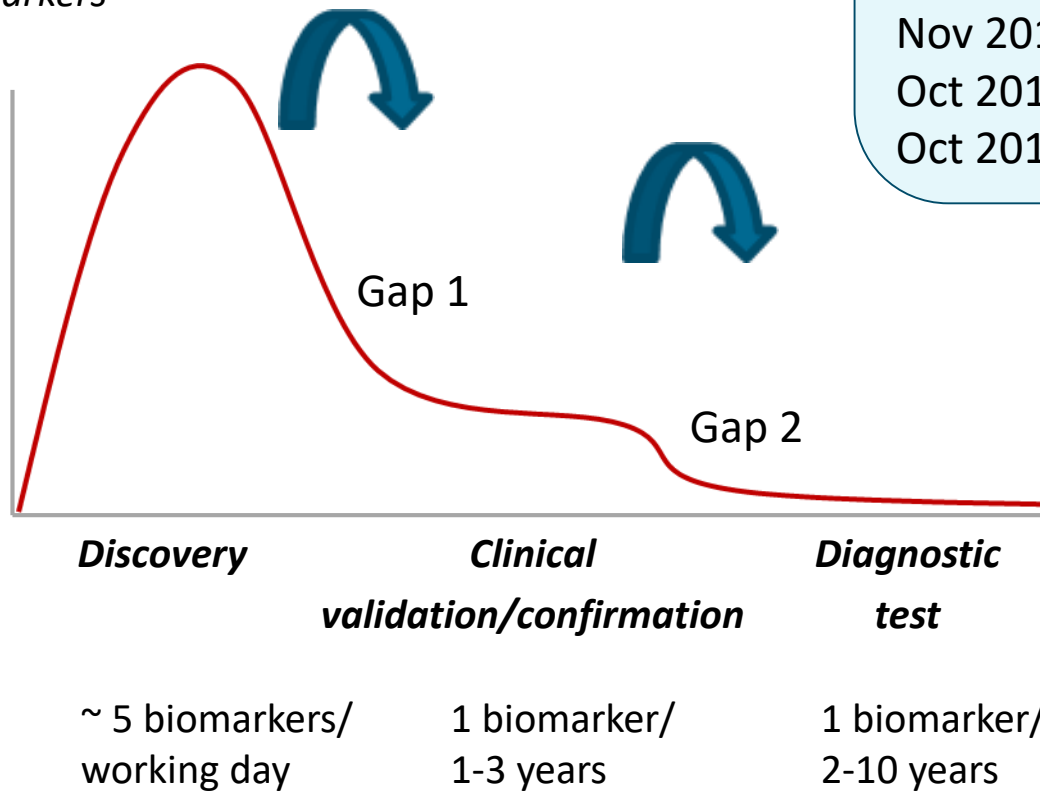
**If we want to innovate clinical molecular biomarkers,
we need to increase quality, not quantity of our research.**



{Stephan Nierkens, UMCUtrecht}

2. Research to diagnostics

Number of
biomarkers



Discovered biomarkers in prostate cancer

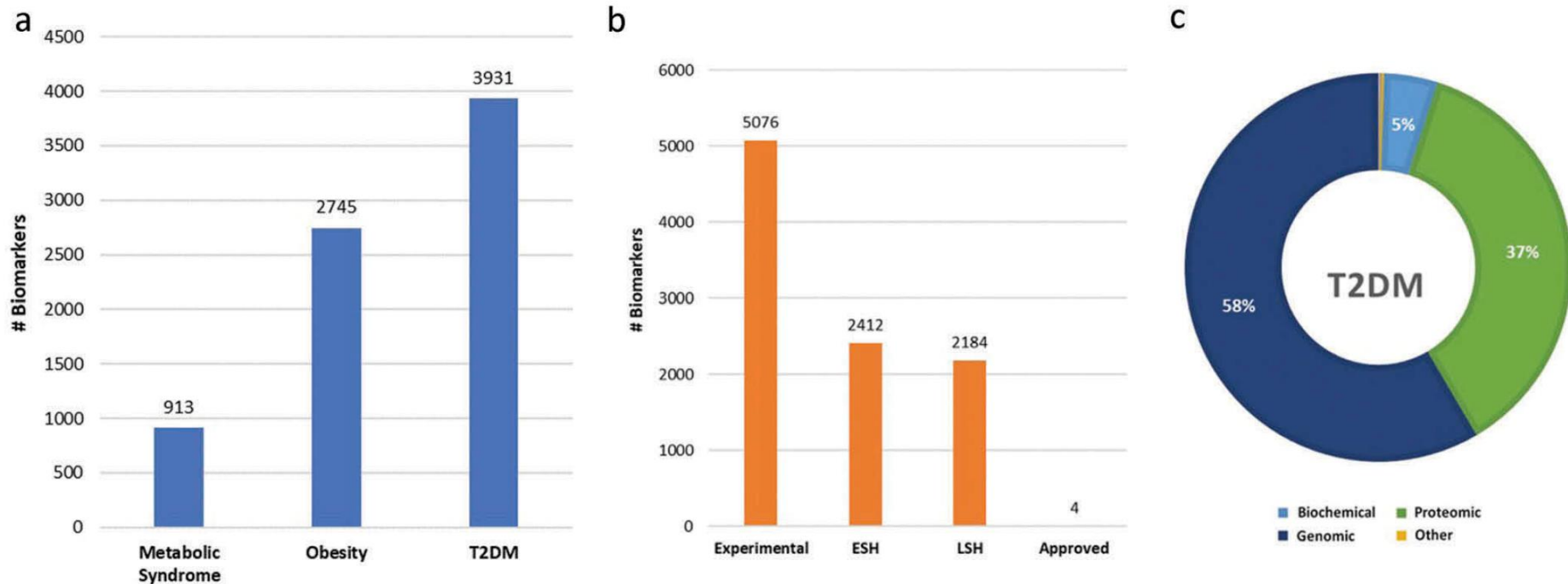
May 2011:	2,231 biomarkers
Nov 2012:	6,562 biomarkers
Oct 2013:	8,358 biomarkers
Nov 2014:	10,350 biomarkers
Oct 2015:	11,856 biomarkers
Nov 2016:	14,481 biomarkers
Oct 2017:	15,463 biomarkers
Oct 2018:	16,480 biomarkers

22 Sept 2019:

386 Pharmacogenomic
biomarkers in drug
labeling (all drugs)



Biomarkers in type-2 diabetes melitis



EXPERT REVIEW OF PROTEOMICS
2019, VOL. 16, NO. 2, 105–115
<https://doi.org/10.1080/14789450.2018.1551134>

Taylor & Francis
Taylor & Francis Group

Check for updates

REVIEW

The future of protein biomarker research in type 2 diabetes mellitus

Roel Tans^a, Lars Verschuren^b, Hans J. C. T. Wessels^a, Stephan J. L. Bakker^c, Cees J. Tack^d, Jolein Gloerich^a and Alain J. van Gool^a

^aTranslational Metabolic Laboratory, Department of Laboratory Medicine and Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, The Netherlands; ^bDepartment of Microbiology and Systems Biology, TNO, Zeist, The Netherlands; ^cDepartment of Internal Medicine, University Medical Centre Groningen, University of Groningen, Groningen, The Netherlands; ^dDepartment of Internal Medicine and Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, The Netherlands

{Tans et al, Exp Rev Proteomics 2018}

Good example of multi-laboratory biomarker validation



Alzheimer's & Dementia 7 (2011) 386–395

Alzheimer's
&
Dementia

The Alzheimer's Association external quality control program for cerebrospinal fluid biomarkers

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3 biomarkers:

- Aβ42
- T-Tau
- P-Tau

Good example of multi-laboratory biomarker validation

Possible sources of variability between CSF studies

Source	Cause	Solution
Preanalytical	Subject selection	Harmonization of clinical procedures
	Diagnostic criteria	
	Intersubject variability	Knowledge of the issues
	Biologic	
	Circadian rhythms	
	CSF collection	
	Lumbar puncture	Standardization
	Binding to catheter tubing	Determine empirically
	Binding to collection tube	
	Binding to storage tube	
Analytical	Hemolysis	Analytic index
	Sensitivity	Monitor
	Specificity	
	Calibration	
	Instrument	Perform maintenance
	Pipetting	
	Analyst	
	Competency	Train
	Familiarization with the method	
	Forward/reverse pipetting	Standardize
Postanalytical	Reagent handling	
	Data handling	Standardize
	Analyzing	
	single/duplicates	
	Decisions for rejecting data	
	Type of curve fitting used	
	Software for data calculation	
	Documentation	
	Poor test procedure instructions	Standardize
	Minimal method optimization	Identify "Best Practices" and set standards
Kit manufacturing	Reagents	
	Source of reference standard	
	Buffer-based system	
	Lot-lot variability	
	Vendor-vendor variability	
	No quality controls	

Potential sources of variability

Participating laboratories

City	Country	Lab type (Clinical/Research/Industry)	Principal investigator
Aarhus	Denmark	Clinical	Aase Handberg
Amsterdam	Netherlands	Clinical/Research	Marinus A. Blankestein
Athens	Greece	Clinical/Research	Elisabeth Kapaki
Austin	USA	Industry	William Nowatzke
Baltimore	USA	Clinical/Research	Marilyn Albert
Barcelona	Spain	Clinical/Research	Albert Lladó
Beerse	Belgium	Industry	Marc Mercken
Bonn	Germany	Clinical/Research	Monika Heneka
Brescia	Italy	Clinical/Research	Roberta Frisoni
Charlestown	USA	Research	Robert Hyman
Erlangen	Germany	Clinical/Research	Thomas Skjott
Frankfurt	Germany	Clinical/Research	Michael Frotscher
Geneva	Switzerland	Clinical/Research	Manfred Windisch
Gramat	France	Research	Kaj Blennow
Göteborg	Sweden	Clinical/Research	Anders Skjott
Göttingen	Germany	Clinical/Research	Annette Spreer
Heidelberg	Germany	Clinical/Research	Johannes Schröder
Innsbruck	Austria	Clinical/Research	Christian Humpel
Kuopio	Finland	Clinical/Research	Hilkka Soininen
La Jolla	USA	Research	Robert Rissman
Melbourne	Australia	Clinical/Research	Douglas Galasko
Milano	Italy	Clinical/Research	Colin Masters
Nijmegen	Netherlands	Clinical/Research	Daniela Galimberti
Oslo	Norway	Clinical/Research	Marcel Verbeek
Perth	Australia	Clinical/Research	Anders Skjott
Perugia	Italy	Clinical/Research	Ralph Martins
Philadelphia	USA	Clinical/Research	Lucilla Parnetti
Rochester	USA	Research	Leslie M. Shaw
Rome	Italy	Research	John Q. Trojanowski
South San Francisco	USA	Clinical/Research	Ronald C. Petersen
Sao Paulo	Brazil	Research	Alessandro Stefani
Seattle	USA	Industry	Daniel Kidd
Sendai	Japan	Clinical/Research	Wagner Gattaz
St. Louis	USA	Clinical/Research	Thomas Montine
Staten Island	USA	Clinical/Research	Hiroyuki Arai
Stockholm	Sweden	Clinical/Research	Anne M. Fagan
Szeged	Hungary	Clinical/Research	David M. Holtzman
Tokyo	Japan	Clinical/Research	Khalid Iqbal
Umeå	Sweden	Clinical/Research	Gunilla Dahlfors
Uppsala	Sweden	Clinical/Research	Carole M. Verhoeven

Participating laboratories

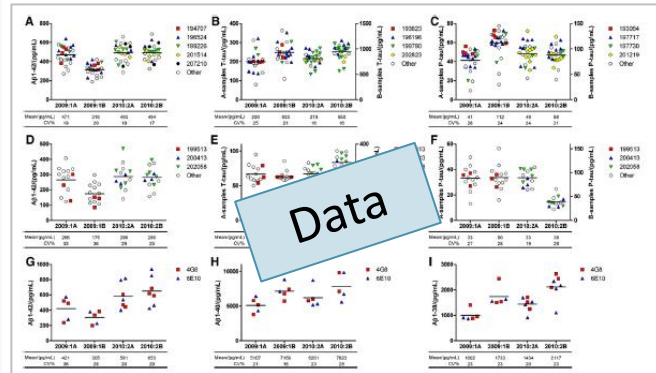


Fig. 1. Results for enzyme-linked immunosorbent assay (ELISA; A-C), xMAP (D-F), and Meso Scale Discovery (G-I) from rounds 1 (1A and 1B) and 2 (2A and 2B). Panels B, C, E, and F have secondary y-axes owing to large differences between samples. Different symbols indicate different kit batches for ELISA and xMAP, and different assays for Meso Scale Discovery (BE10, blue triangle; 4G8, red square).

Supplementary Table 3

Factors contributing to the total variability^a

Technology	Biomarker	Ranking of factors contributing to the total variability ^b
ELISA	Aβ42	1. Between-laboratory and within-laboratory (equal contributions) 2. Between-lot
	T-tau	1. Within-laboratory 2. Between-laboratory
	P-tau	1. Between-laboratory 2. Within-laboratory
Luminex	Aβ42	1. Within-laboratory 2. Between-laboratory
	T-tau	1. Within-laboratory 2. Between-laboratory
	P-tau	1. Within-laboratory 2. Between-laboratory

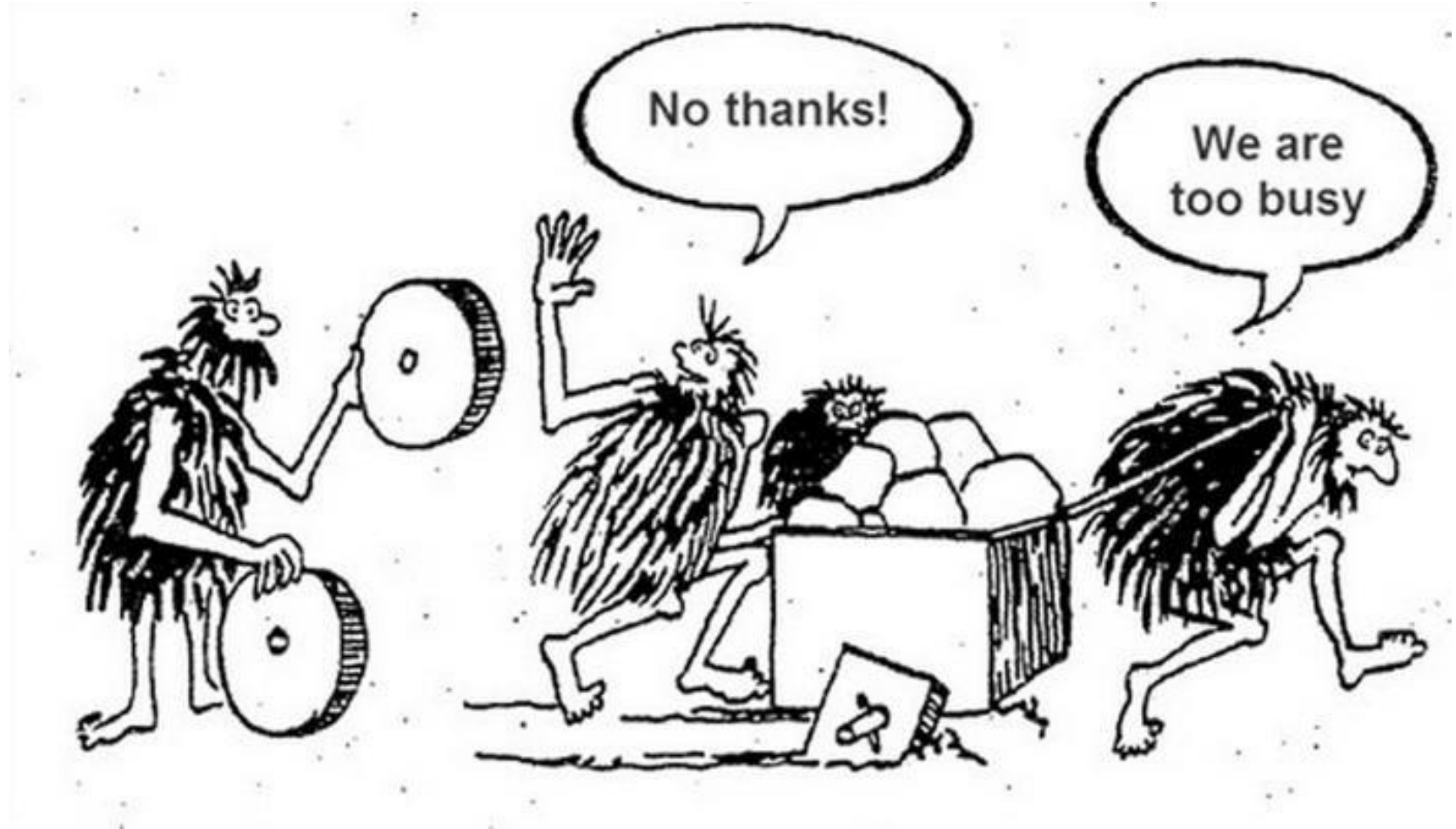
^a Variability estimated using variance component analysis.

^b 1 indicates the most contribution and 3 the least contribution to the total variability. Ranking should be interpreted with caution due to few data points.

Conclusions

Adoption of best biomarker practice ???

Choice for biomarker scientists: discover or confirm?



Publication bias

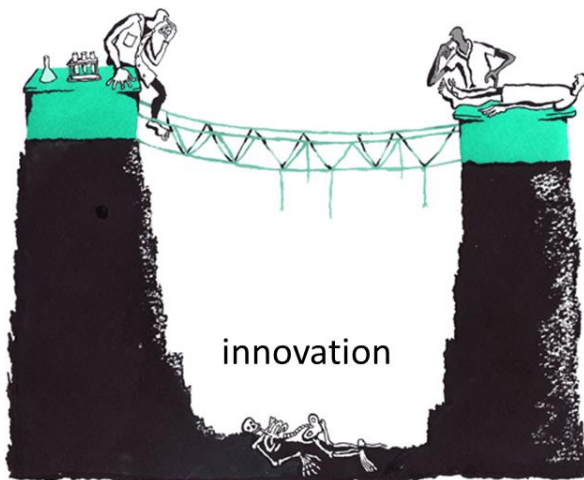
Lost in the citation valley

Gerard Pasterkamp, Imo Hoefer & Berent Prakken

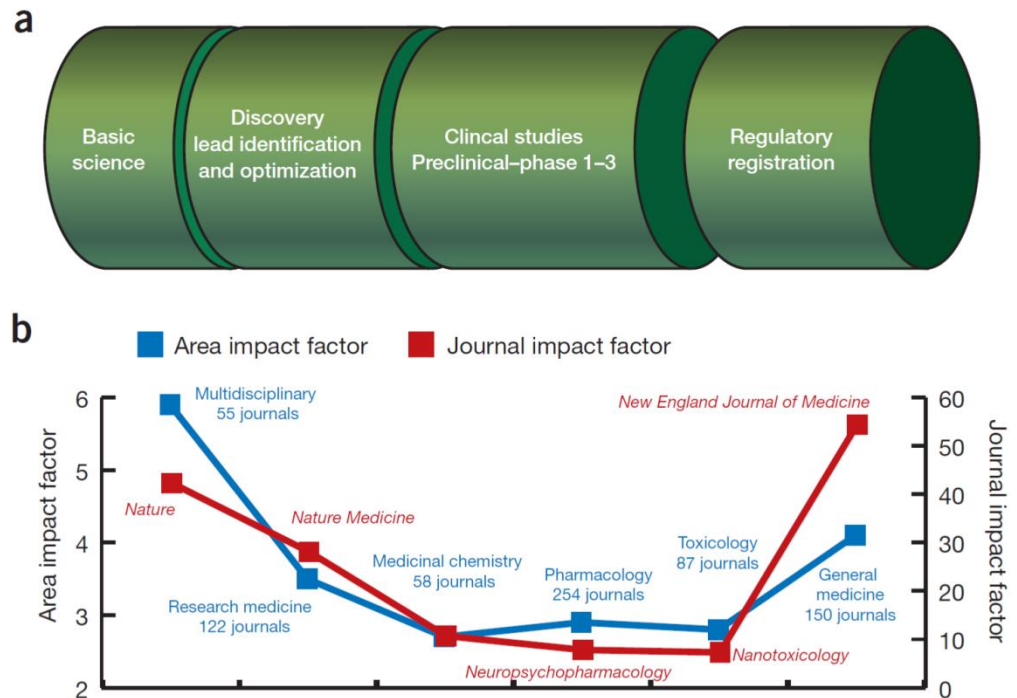
Changing the academic reward system to improve translational medicine should start by moving away from a myopic focus on publications.

1016

VOLUME 34 NUMBER 10 OCTOBER 2016 NATURE BIOTECHNOLOGY



research fields accrue citations at different rates. The 'valley of death' is a 'valley of impact' Publications in high-impact journals and



3. Research to society



- Up to 1.000.000 signals per analysis



- Point-of-care analysis of few biomarkers

News

Phone app that can detect heart attack a week out considered by NHS



The smartphone app tracks changes in tone of voice to warn of heart attacks CREDIT: ALAMY



By Henry Bodkin

9 JUNE 2016 • 12:20AM

A smartphone app that can tell if users are in danger of having a heart attack by the tone of their voice is being considered for use by the NHS.

Clinical trials of the software showed it accurately predicted admission to hospital for people with congestive cardiac failure one week before they were taken gravely ill.

The app is one of a wealth of gadgets and systems under review by the health service with the aim of revolutionising personalised healthcare.

“Post-traumatic Test Syndrome” ?

theranos



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23andMe

Bloedwaardentest.nl

Time for quality, not quantity !

eatris

European infrastructure
for translational medicine



lygature

pioneering medicine.
together.



COMMENT

Bridging the translational innovation gap through good biomarker practice

Alain J. van Gool¹, Florence Bietrix², Eric Caldenhoven³, Kurt Zatloukal⁴, Andreas Scherer⁵, Jan-Eric Litton⁶, Gerrit Meijer⁷, Niklas Blomberg⁸, Andy Smith⁸, Barend Mons⁹, Jaap Heringa¹⁰, Wim-Jan Koot³, Martin J. Smit¹¹, Marian Hajduch¹², Ton Rijnders³ and Anton Ussi²

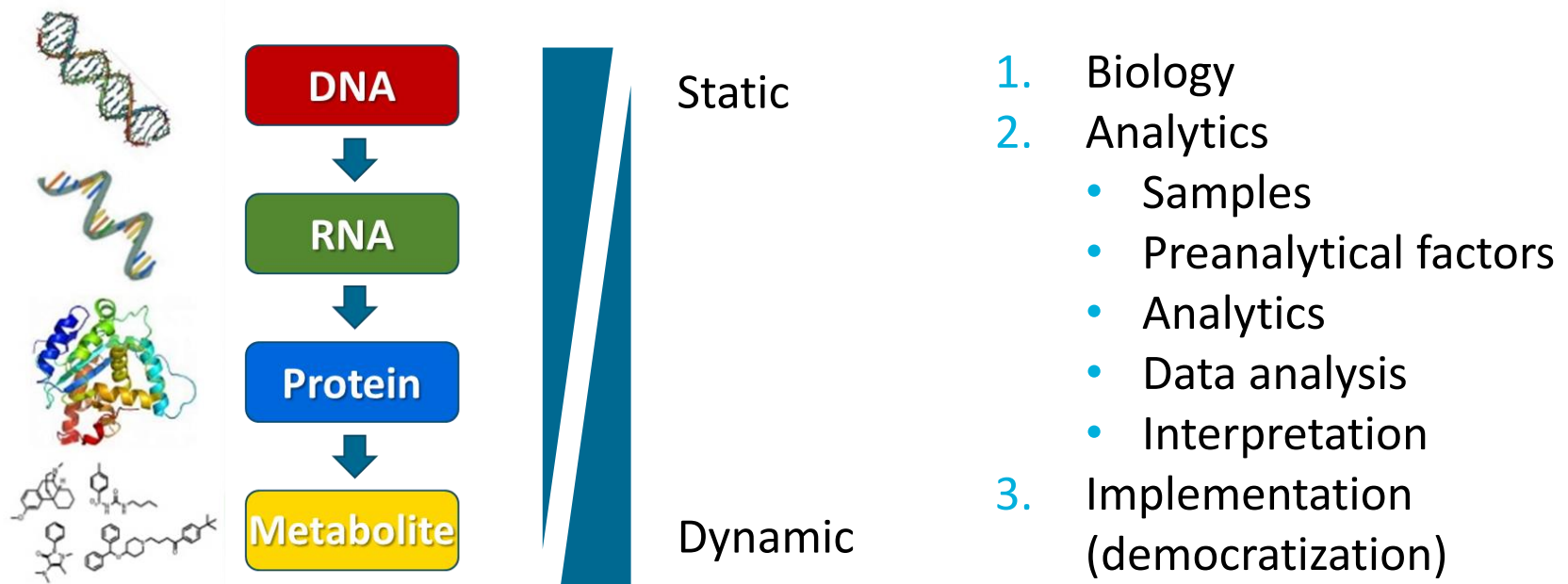
Few biomarkers progress from discovery to become validated tools or diagnostics. To bridge this gap, three European biomedical research infrastructures — EATRIS-ERIC (focused on translational medicine), BBMRI-ERIC (focused on biobanking) and ELIXIR (focused on data sharing) — are paving the way to developing and sharing best practices for biomarker validation.

{van Gool et al, Nature Reviews Drug Discovery, Apr 2017}

COST action CA16113
<http://clinimark.eu>

High need to bring molecular biomarkers to higher level

- Technologies: Quality, harmonised, standardised, cheaper, higher throughput
- Translation: Clinical interpretation and regulatory acceptance
- Genomics is advanced
- Proteomics, metabolomics (and other omics) much less so

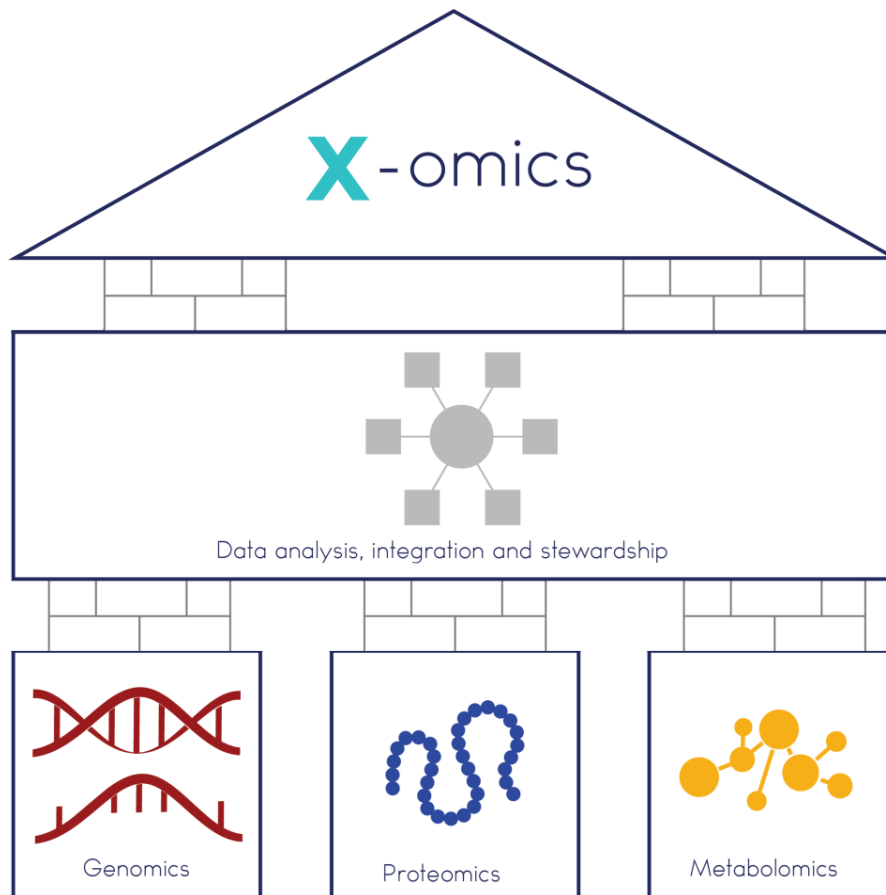




<http://clanimark.eu>



X-omics research infrastructure



Outreach

- Helpdesk
- Training
- Community
- Demonstrators
 - Cell
 - Individual
 - Population

X-omics approach

- Data analysis
- Data integration
- Data stewardship
- Study design
- Sample handling

Pushing omics technologies

- Resolution, sensitivity, coverage
- Harmonisation, standardisation, data FAIR-at-source





X-omics Community

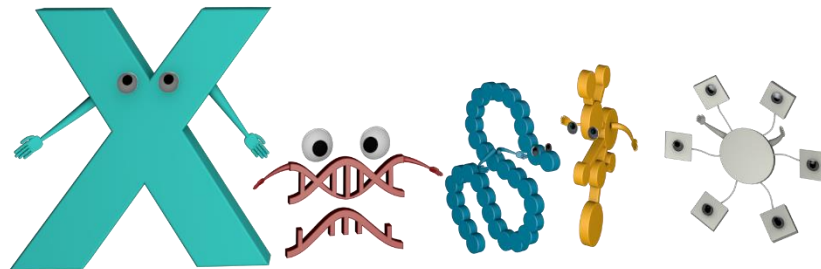
To connect researchers from all over the world,
interested in X-omics research approaches

- LinkedIn

*Connect
Discuss
Advise
Inform
Share*

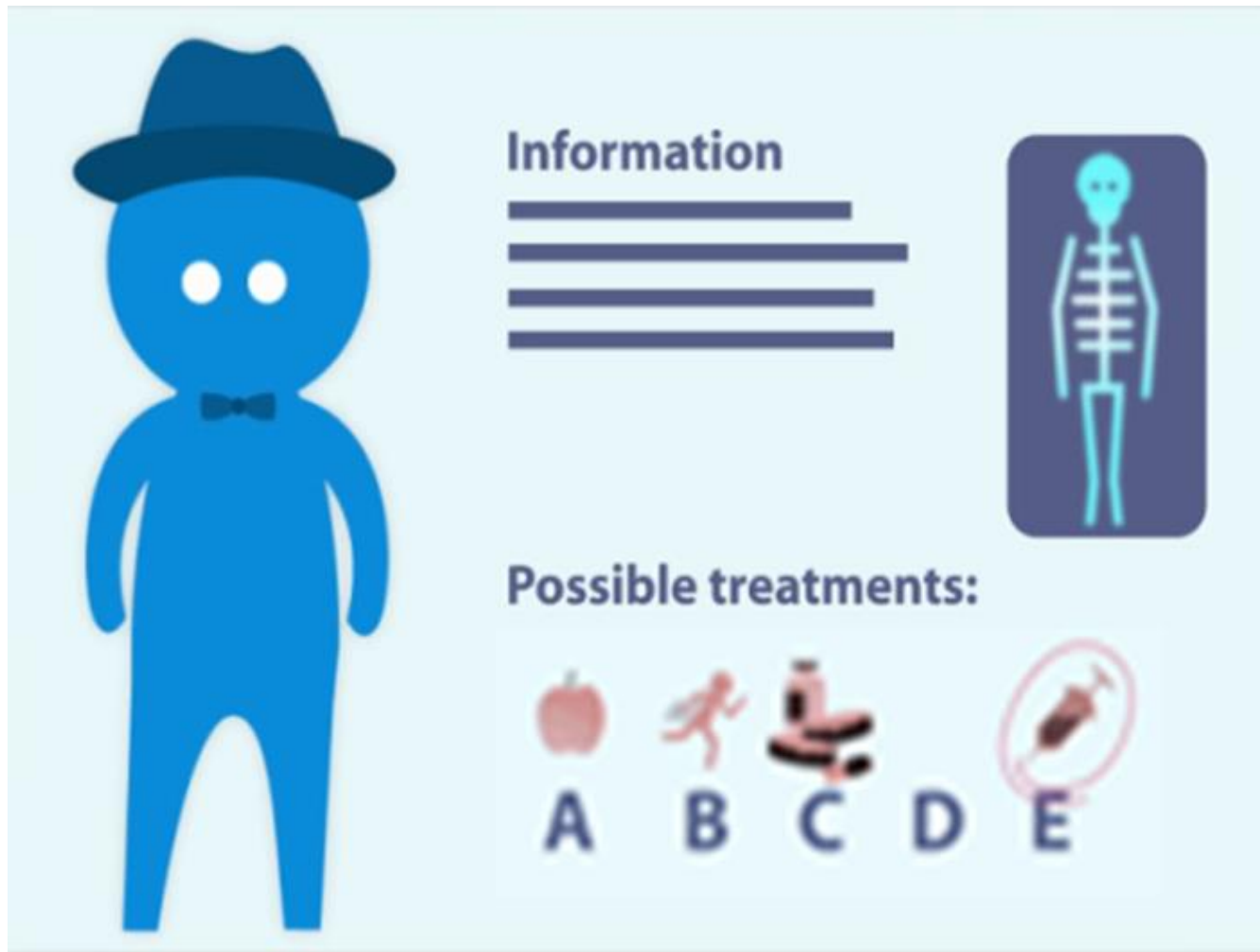
*Publications
Best practices
Protocols
Trainings
Conferences*

Find us on LinkedIn: X-omics community /
<https://www.linkedin.com/groups/13739398/>



Outline

- Biomarkers in pharmaceutical industry
- Biomarkers in academic research and healthcare
- Biomarkers in personalized health (care)
- Translational innovation gaps
- **Outlook**



Critical component in biomarker use: Data

www.nature.com/scientificdata

SCIENTIFIC DATA

OPEN **Comment: The FAIR Guiding Principles for scientific data management and stewardship**

SUBJECT CATEGORIES

- » Research data
- » Publication characteristics

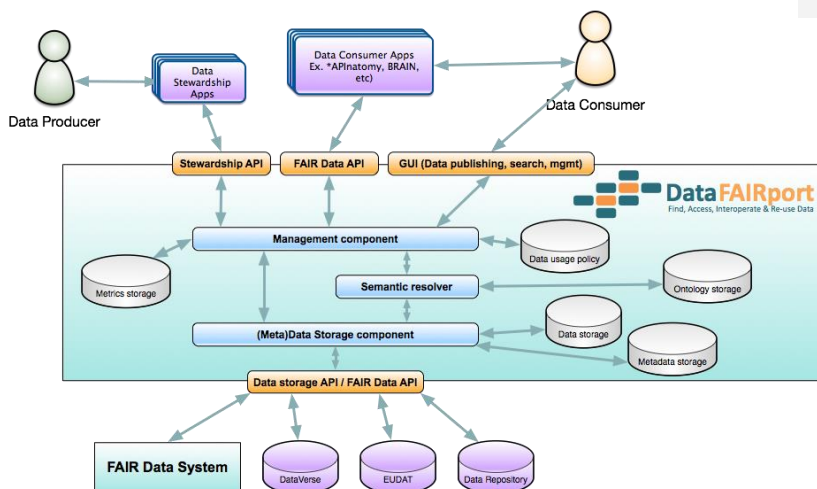
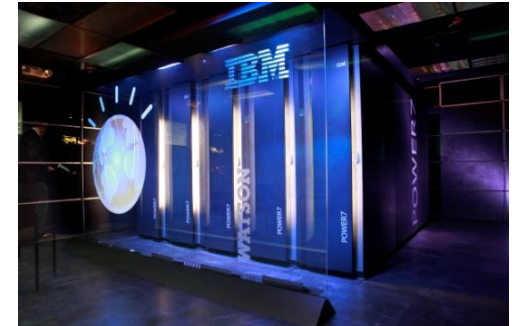
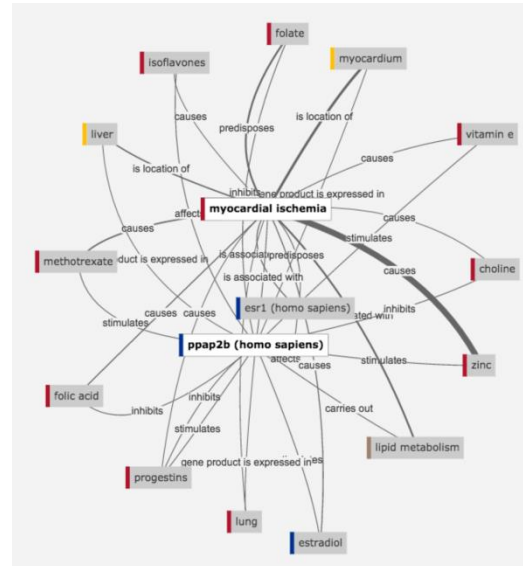
Mark D. Wilkinson *et al.*[#]

{Wilkinson *et al.*,
Nature Scientific Data, 2016}

- Data capture
- Data stewardship (FAIR)

- Findable
- Accessible
- Interoperable
- Reusable

Exponential developments in data sciences



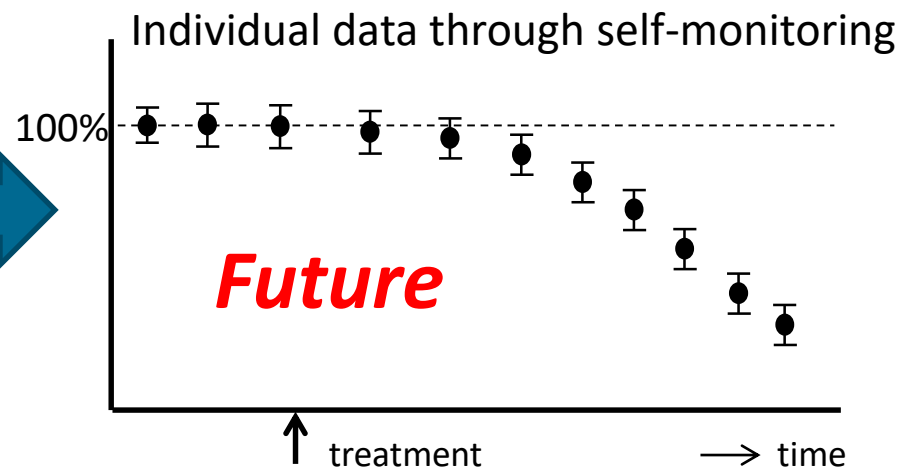
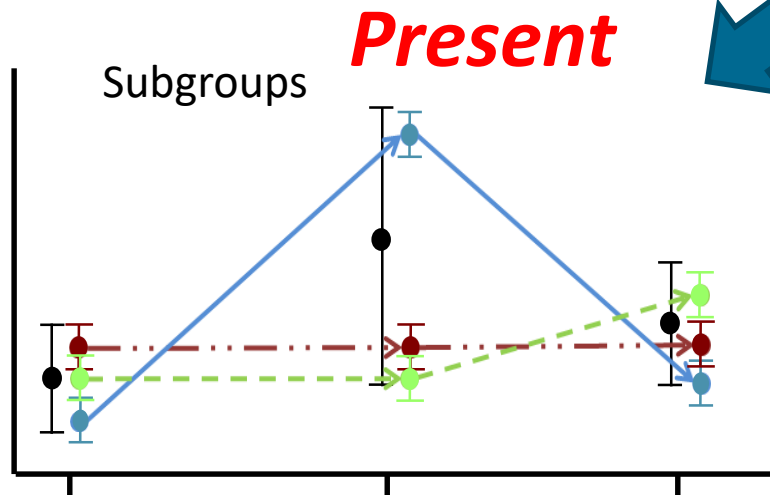
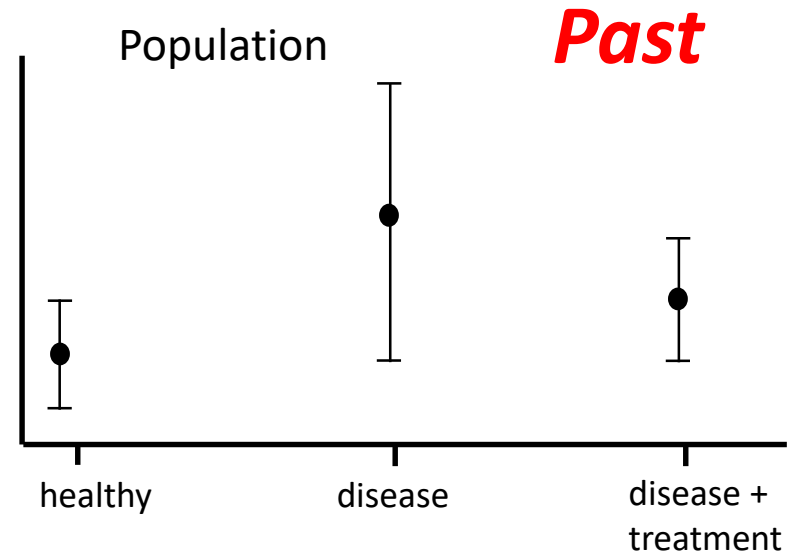
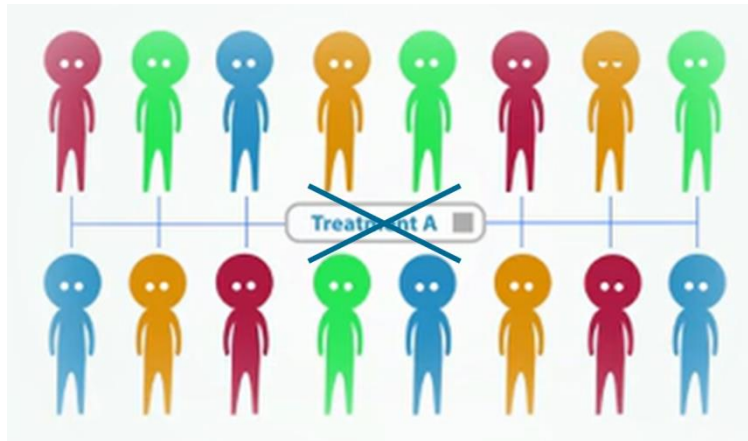
Research

JAMA | **Original Investigation**

Diagnostic Assessment of Deep Learning Algorithms for Detection of Lymph Node Metastases in Women With Breast Cancer

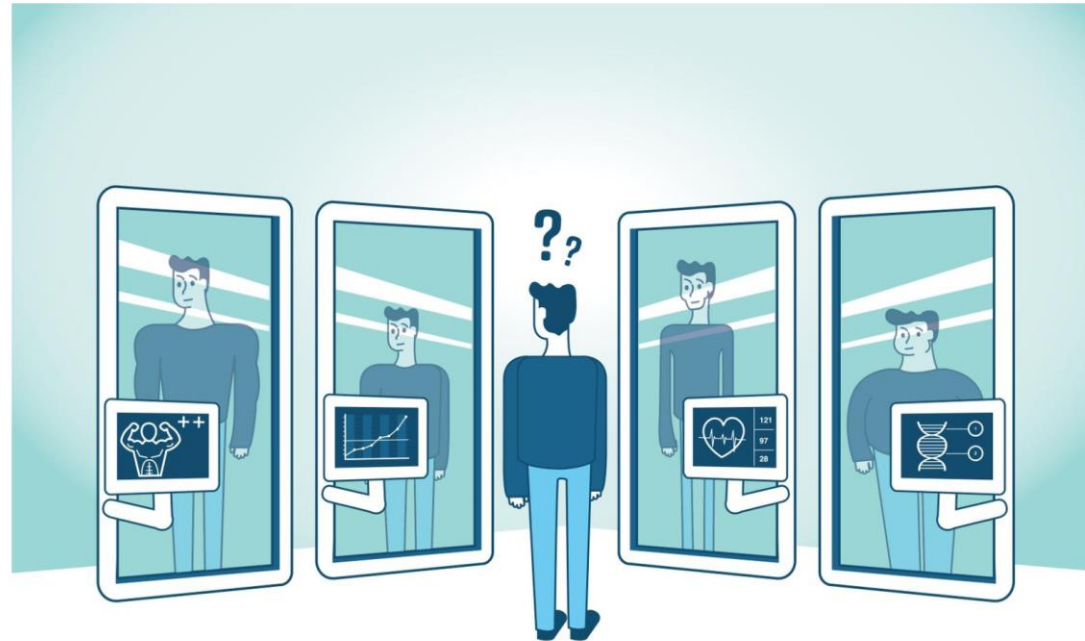
Babak Ehteshami Bejnordi, MS; Mitko Veta, PhD; Paul Johannes van Diest, MD, PhD; Bram van Ginneken, PhD; Nico Karssemeijer, PhD; Geert Litjens, PhD; Jeroen A. W. M. van der Laak, PhD; and the CAMELYON16 Consortium

Enabling personalized health data monitoring?



There is no single one reflection of health

- ***Funhouse mirror effect***
- Multiple sources of your data
 - Multiple Omics
 - Clinical chemistry
 - Electronic Patient Dossier
 - Wearables
 - Digital biomarkers
 - Commercial health tests
 - Social media
 - Surrounding
- Each are a skewed image of you
- How to deal with all of this for your personal health(care)?



{Mira Vegter, Hub Zwart, Alain van Gool: submitted}

Outline

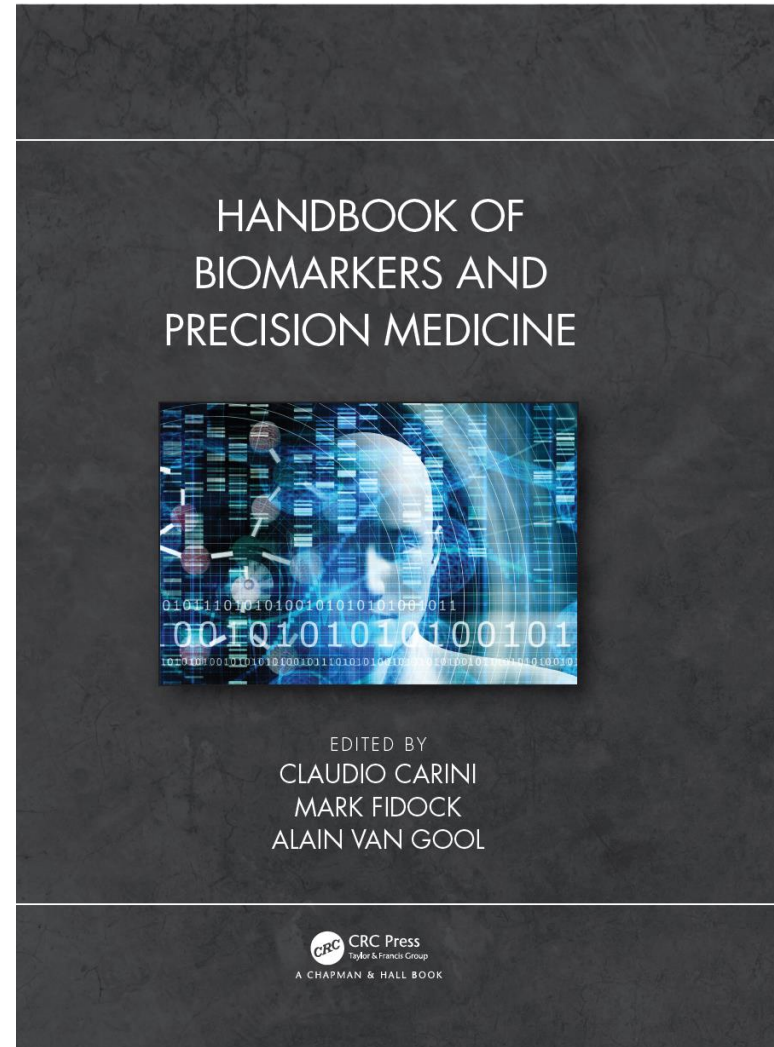
- Biomarkers in pharmaceutical industry
- Biomarkers in academic research and healthcare
- Biomarkers in personalized health (care)
- Translational innovation gaps
- Outlook

Handbook of Biomarkers and Precision Medicine

70 manuscripts from experts in pharma, diagnostics, clinic, technology

1. What is a biomarker and their role in drug development?
2. Biomarkers in preclinical sciences
3. Biomarkers in translational sciences
4. Biomarker-informed clinical trials
5. The road ahead in precision medicine
6. Lessons from the past and pioneers of the future
7. Emerging technologies
8. The next frontiers in therapeutic target areas
9. Lessons learned and what's next?

Available @on line webshops (eg. Amazon)



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X-omics.nl

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