

# BIOCRATES Life Sciences AG

The “Deep Phenotyping” Company

**Preclinical and Clinical Biomarker  
Discovery in Complex, Multifactorial  
Diseases**

*Applications in Prognosis, Treatment  
Prediction and System Diagnostics*

**Dr. Wulf Fischer-Knuppertz, CEO**

# BIOCRATES - The Company

## *Targeted Metabolomics*



- **2002: Founded in Innsbruck, Austria**
- **Founders: leading university professors**
- **Fully integrated mass spectrometry based platform for targeted metabolomics**
- **Biomarker candidates for several indications (CKD, T2D, Alzheimer's D., breast cancer)**
- **2006: Established analytical services lab**
- **2008: World's first metabolomics kit on market**
- **Status 2014: 1 CE-marked IVD, 6 research kits, commercially available, analytical services lab**
- **40 Employees**

# “Deep Phenotyping“

## Mass spectrometry based metabolic analysis

**BIOCRATES**  
LIFE SCIENCES  
The Deep Phenotyping Company

We provide the Phenotype to the Genotype!

**Metabolic Phenotyping**

- Targeted & Quantitative Analysis
- Standardized & Quality Controlled
- Easy to use Kits & Services

We provide **deep insights** into metabolic pathways.

## Examples for Applications in

- *Diagnosis of Disease*
- *Prognosis of Disease*
- *Prediction of Drug Response*
- *Mode of Action (MOA)*
- *Gene Function*

# Blood Test may predict Alzheimer's Disease

## Nature Medicine publication

nature  
medicine

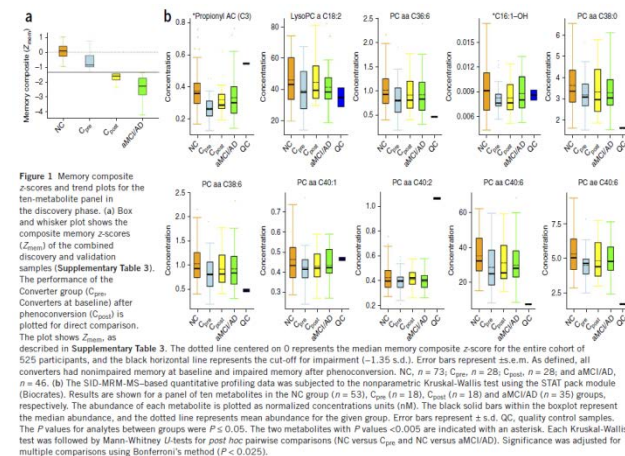
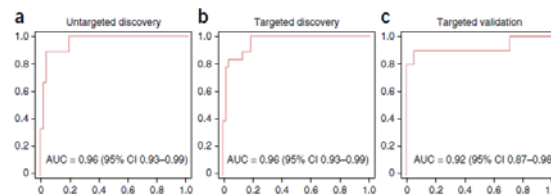
LETTERS

### Plasma phospholipids identify antecedent memory impairment in older adults

Mark Mapstone<sup>1</sup>, Amrita K Cheema<sup>2,3</sup>, Massimo S Fianadaca<sup>4,5</sup>, Xiaogang Zhong<sup>6</sup>, Timothy R Mhyre<sup>5</sup>, Linda H MacArthur<sup>5</sup>, William J Hall<sup>7</sup>, Susan G Fisher<sup>8,14</sup>, Derick R Peterson<sup>9</sup>, James M Haley<sup>10</sup>, Michael D Nazar<sup>11</sup>, Steven A Rich<sup>12</sup>, Dan J Berlau<sup>13,14</sup>, Carrie B Peltz<sup>13</sup>, Ming T Tan<sup>6</sup>, Claudia H Kawas<sup>13</sup> & Howard J Federoff<sup>4,5</sup>

Received 27 August 2013; accepted 9 January 2014; published online 9 March 2014; doi:10.1038/nm.3466

**Figure 2** ROC results for the lipidomics analyses. (a–c) Plots of ROC results from the models derived from the three phases of the lipidomics analysis. Simple logistic models using only the metabolites identified in each phase of the lipidomics analysis were developed and applied to determine the success of the models for classifying the  $C_{pm}$  and NC groups. The red line in each plot represents the AUC obtained from the discovery-phase LASSO analysis (a), the targeted analysis of the ten metabolites in the discovery phase (b) and the application of the ten-metabolite panel developed from the targeted discovery phase in the independent validation phase (c). The ROC plots represent sensitivity (i.e., true positive rate) versus 1 – specificity (i.e., false positive rate).



- Discovery and validation of a set of ten lipids from peripheral blood that **predicted phenoconversion to either amnesic mild cognitive impairment or Alzheimer's disease within a 2–3 year timeframe with over 90% accuracy.**
- This biomarker panel, reflecting cell membrane integrity, may be **sensitive to early neurodegeneration of preclinical Alzheimer's disease.**



**These results can be considered as a major step toward the NIA-AA (National Institute on Aging and Alzheimer's Association) consensus statement mandate for biomarkers of preclinical AD.**

# Diagnosis of Schizophrenia

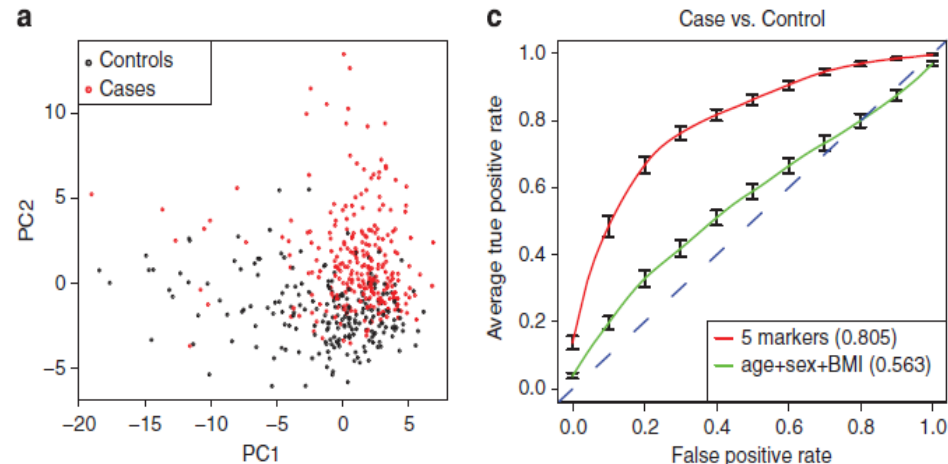
Citation: *Transl Psychiatry* (2012) 2, e149; doi:10.1038/tp.2012.76  
© 2012 Macmillan Publishers Limited All rights reserved 2158-3188/12  
www.nature.com/tp



## Schizophrenia shows a unique metabolomics signature in plasma

Y He<sup>1,2,3,10</sup>, Z Yu<sup>1,10</sup>, I Giegling<sup>4</sup>, L Xie<sup>3</sup>, AM Hartmann<sup>4</sup>, C Prehn<sup>5</sup>, J Adamski<sup>5,6</sup>, R Kahn<sup>7</sup>, Y Li<sup>2,3</sup>, T Illig<sup>1,8</sup>, R Wang-Sattler<sup>1,11</sup> and D Rujescu<sup>4,9,11</sup>

Currently, the diagnosis of schizophrenia is still merely based on interview of the person and family members. ... 5 metabolites reached 80.5% sensitivity and specificity for detection of schizophrenia ...



This study illustrated that the metabolic deviations detected in plasma may serve as potential biomarkers to aid diagnosis of schizophrenia. (ornithine, arginine, glutamine, histidine, PC ae C38:6)

# Diagnosis of Breast Cancer

*Int. J. Mol. Sci.* 2013, 14, 8047-8061; doi:10.3390/ijms14048047

Article

## Mass Spectrometry-Based Quantitative Metabolomics Revealed a Distinct Lipid Profile in Breast Cancer Patients

Yunping Qiu<sup>1,2</sup>, Bingsen Zhou<sup>3</sup>, Mingming Su<sup>4</sup>, Sarah Baxter<sup>4</sup>, Xiaojiao Zheng<sup>1</sup>, Xueqing Zhao<sup>5</sup>, Yun Yen<sup>3</sup> and Wei Jia<sup>1,2,\*</sup>

OPEN ACCESS

International Journal of  
**Molecular Sciences**

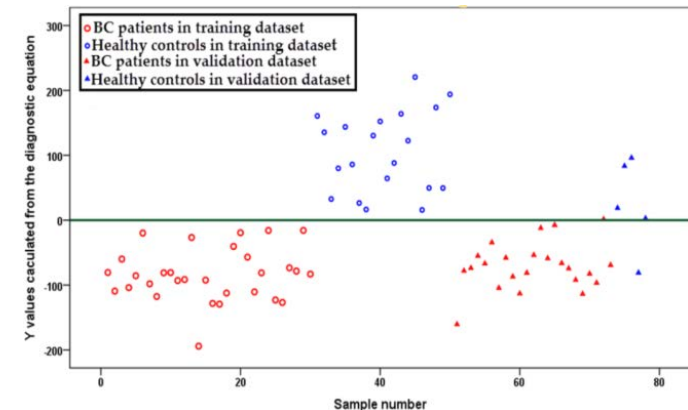
ISSN 1422-0067  
www.mdpi.com/journal/ijms

About 20% of breast cancer patients still cannot be detected with mammography.

A diagnostic equation using three metabolites (lysoPC a C16:0, PC ae C42:5 and PC aa C34:2) was established, which successfully separated breast cancer patients from healthy controls with a sensitivity of 98.1% and a specificity of 96.0 %.



based on 39 metabolites



$$y = \text{lysoPC a C16:0} \times 1.034 + \text{PC ae C42:5} \times 44.248 - \text{PC aa C34:2} \times 0.585 - 37.002$$

# Prediction of Drug Response - Cancer

MOLECULAR ONCOLOGY XXX (2012) 1–11



available at [www.sciencedirect.com](http://www.sciencedirect.com)

SciVerse ScienceDirect

[www.elsevier.com/locate/molonc](http://www.elsevier.com/locate/molonc)



## Metabolomics approach for predicting response to neoadjuvant chemotherapy for breast cancer

Siwei Wei<sup>a</sup>, Lingyan Liu<sup>b</sup>, Jian Zhang<sup>a</sup>, Jeremiah Bowers<sup>a</sup>, G.A. Nagana Gowda<sup>a</sup>, Harald Seeger<sup>c</sup>, Tanja Fehm<sup>c</sup>, Hans J. Neubauer<sup>c</sup>, Ulrich Vogel<sup>d</sup>, Susan E. Clare<sup>e</sup>, Daniel Raftery<sup>a,\*,1</sup>

A prediction model developed by combining NMR and MS derived metabolites **correctly identified 80% of the patients whose tumors did not show complete response to chemotherapy**. These results show promise for larger studies that could **result in more personalized treatment protocols for breast cancer patients**.

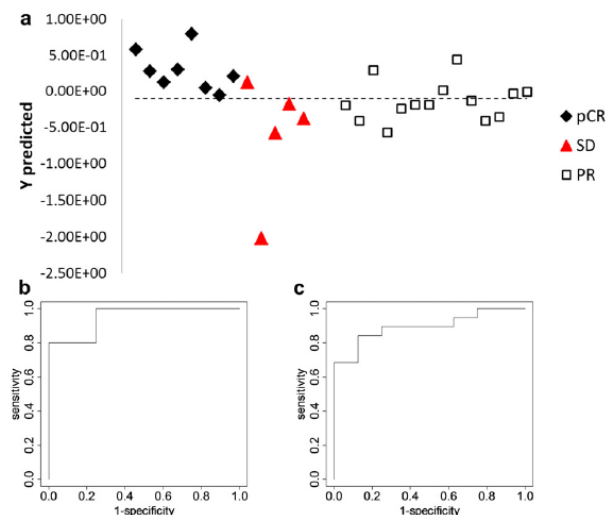


Figure 4 – (a) Prediction results for the PLS-DA model based on combining isoleucine, glutamine, and threonine detected by NMR and linolenic acid detected by LC-MS; (b) ROC curve for pCR vs. SD using the cross-validated predicted class values (AUROC = 0.95); (c) ROC curve for pCR vs. the other two groups combined using the cross-validated predicted class values (AUROC = 0.89).

# Diagnosis of Endometriosis

Hum. Reprod. Advance Access published August 1, 2012  
Human Reproduction, Vol.0, No.0 pp. 1–11, 2012  
doi:10.1093/humrep/des152

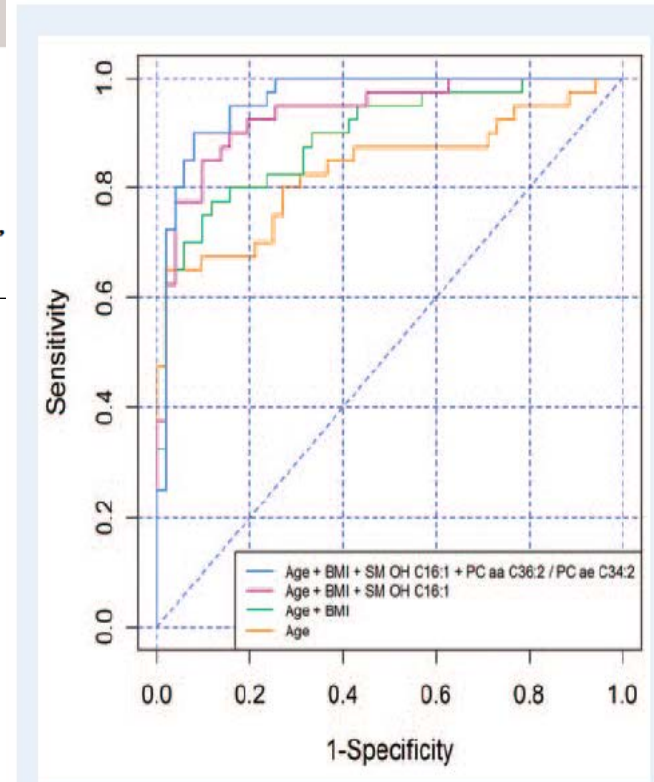
human reproduction ORIGINAL ARTICLE *Gynaecology*

## Discovery of phosphatidylcholines and sphingomyelins as biomarkers for ovarian endometriosis

K. Vouk<sup>1</sup>, N. Hevir<sup>1</sup>, M. Ribič-Pucelj<sup>2</sup>, G. Haarpaintner<sup>3</sup>, H. Scherb<sup>4</sup>, J. Osredkar<sup>5</sup>, G. Möller<sup>6</sup>, C. Prehn<sup>6</sup>, T. Lanišnik Rižner<sup>1,\*</sup>, and J. Adamski<sup>6</sup>

...resulted in a sensitivity of 90.0%, a specificity of 84.3% and a ratio of the positive likelihood ratio to the negative likelihood ratio of 48.3.

Our results suggest that endometriosis is associated with elevated levels of **sphingomyelins** and **phosphatidylcholines**, which might contribute to the **suppression of apoptosis** and affect lipid-associated signaling pathways.



**Figure 4** ROC curve. ROC curve shows improving effects of successive addition of separate variables to the model for differentiation between endometriosis patients and healthy controls.

# Prognosis of Risk of T2D

ORIGINAL ARTICLE

## Identification of Serum Metabolites Associated With Risk of Type 2 Diabetes Using a Targeted Metabolomic Approach

Anna Floegel,<sup>1</sup> Norbert Stefan,<sup>2</sup> Zhonghao Yu,<sup>3</sup> Kristin Mühlenbruch,<sup>4</sup> Dagmar Drohan,<sup>1</sup> Hans-Georg Joost,<sup>5</sup> Andreas Fritsche,<sup>2</sup> Hans-Ulrich Häring,<sup>2</sup> Martin Hrabě de Angelis,<sup>6</sup> Annette Peters,<sup>7</sup> Michael Roden,<sup>8,9</sup> Cornelia Prehn,<sup>6</sup> Rui Wang-Sattler,<sup>3</sup> Thomas Illig,<sup>3,10</sup> Matthias B. Schulze,<sup>4</sup> Jerzy Adamski,<sup>6</sup> Heiner Boeing,<sup>1</sup> and Tobias Pischon<sup>1,11</sup>

Received 22 April 2012 and accepted 22 July 2012.

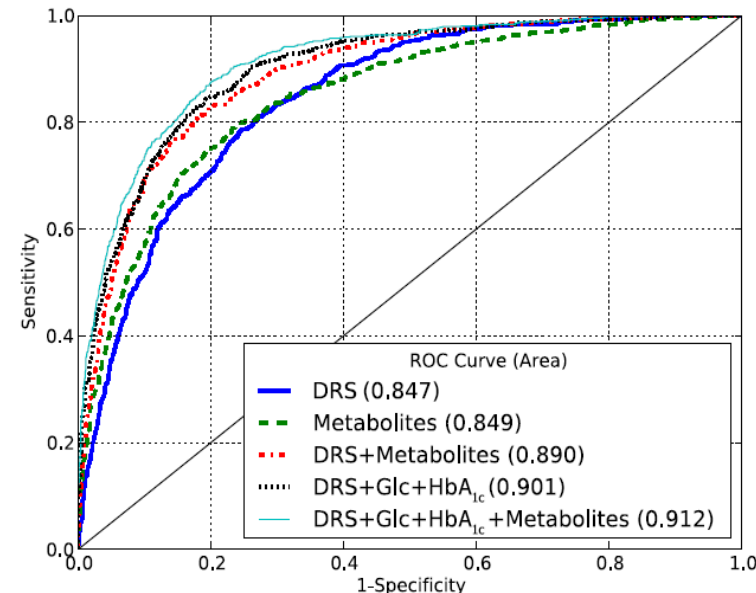
diabetes.diabetesjournals.org

- EPIC Potsdam study (identification of metabolites)
- KORA study (risk prediction, replication)
- Tübingen Family study (compared with established risk factors insuline sensitivity/secretion)



**14 metabolites improved T2D prediction compared with established risk factors.**

- fasting plasma glucose
- glycated hemoglobin A1c

(Hexose, Amino acids, PCs, lysoPCs)



# Pharmacokinetics and Pharmacodynamics Cancer

**2013 NCRI Cancer Conference**  
3 - 6 November 2013  
The BT Convention Centre Liverpool UK  
[conference.ncri.org.uk](http://conference.ncri.org.uk)

**Identification of plasma metabolite changes following phosphatidylinositol-3-kinase (PI3K) inhibition with GDC-0941, a potent and selective pan-Class I inhibitor of PI3K: preclinical discovery followed by clinical qualification in a Phase I clinical trial.**

Joo Ern Ang<sup>1,2</sup>, Rupinder Pandher<sup>1</sup>, Yasmin Asad<sup>1</sup>, Alan Henley<sup>1</sup>, Melanie Valenti<sup>1</sup>, Gary Box<sup>1</sup>, Alexis de haven Brandon<sup>1</sup>, Richard Baird<sup>1</sup>, Lori Friedman<sup>3</sup>, Mika Derynck<sup>3</sup>, Suzanne Eccles<sup>1</sup>, Stan Kaye<sup>1,2</sup>, Paul Workman<sup>1</sup>, Johann de Bono<sup>1,2</sup>, Florence Raynaud<sup>1,2</sup>

<sup>1</sup>Cancer Research UK Cancer Therapeutics Unit, The Institute of Cancer Research, Sutton, Surrey, UK, <sup>2</sup>Drug Development Unit, The Royal Marsden NHS Foundation Trust, Sutton, Surrey, UK, <sup>3</sup>Genentech Inc, South San Francisco, California, USA

A mass spectrometry-based metabolomic platform was used to identify novel pharmacodynamic plasma metabolite biomarkers of systemic modulation of PI3K signaling.

- This study provides the **first link between systemic modulation of the PI3K pathway and changes in plasma metabolites** which may have utility as **minimally invasive pharmacodynamic biomarkers**.
- The findings provide **additional support for the association of insulin resistance with branched-chain amino acids and related metabolites following PI3K inhibition**.
- These data suggest that plasma **metabolomic is a valid strategy to monitor the pharmacodynamic development of novel anticancer agents**.

# Prediction of Drug Response - Cancer

OMICS A Journal of Integrative Biology  
Volume 15, Numbers 1 and 2, 2011  
© Mary Ann Liebert, Inc.  
DOI: 10.1089/omi.2010.0114

## Metabolomic Analysis of Resveratrol-Induced Effects in the Human Breast Cancer Cell Lines MCF-7 and MDA-MB-231

Walter Jäger,<sup>1</sup> Alexandra Gruber,<sup>2</sup> Benedikt Giessrigl,<sup>3</sup> Georg Krupitza,<sup>3</sup> Thomas Szekeres,<sup>4</sup> and Denise Sonntag<sup>2</sup>

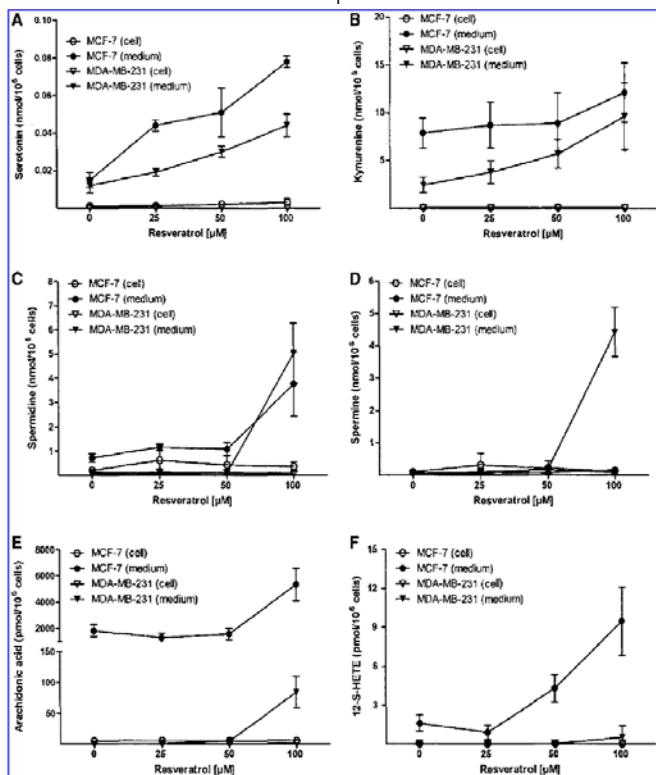


FIG. 1. Induction of serotonin (A), kynurenine (B), spermidine (C), spermine (D), arachidonic acid (E), and 12S-HETE (F) in the human breast cancer cell lines MCF-7 and MDA-MB-231 after incubation with resveratrol (0–100 μM) for 72 h. Data represent the mean ± SD of triplicate determinations.

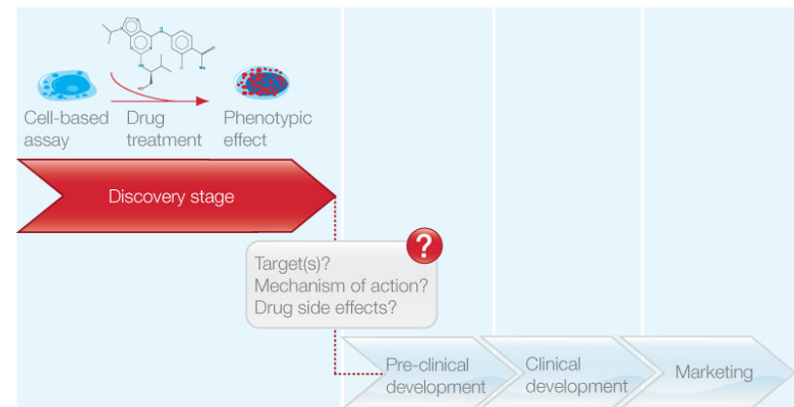
**Resveratrol** is a naturally occurring anticancer compound present in grapes and wine with antiproliferative properties against breast cancer cells and xenografts.

Metabolomic analysis elucidated several small molecules as **markers for the response of breast cancer cells to Resveratrol.**

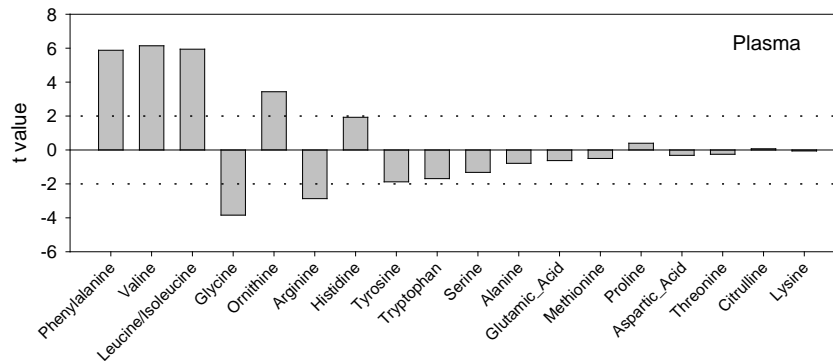
# Mode of Action Screening (MOA)

## In drug development

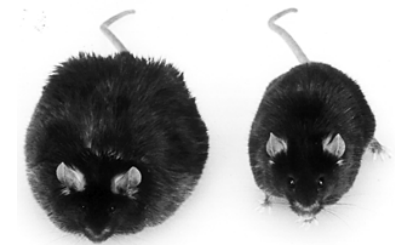
**Mode of action** information characterizes the interaction of a compound with its target to understand *how the compound interacts with the target and will modulate its activity*.



## → MOA Study on T2D mouse model

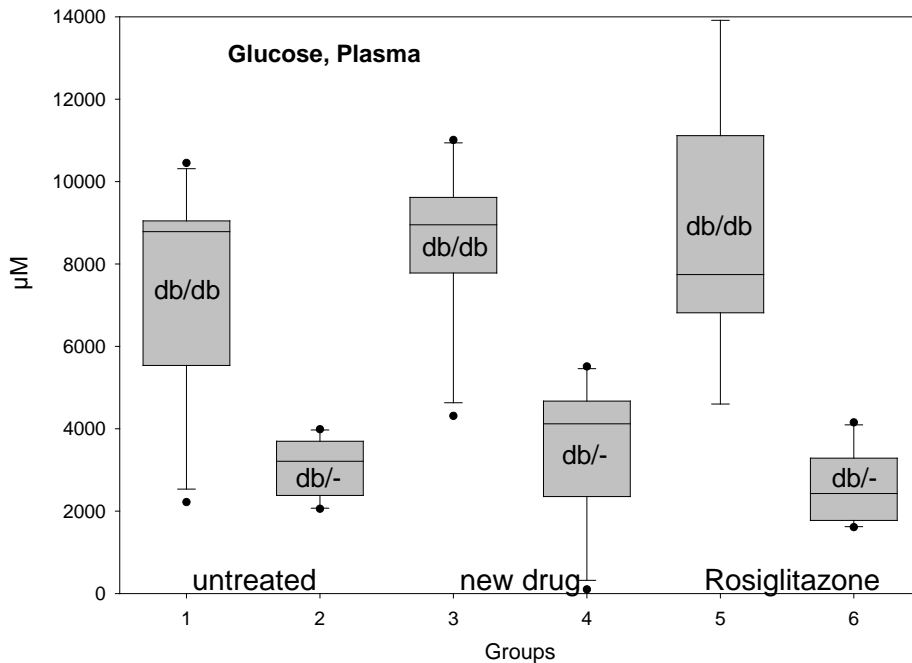


**Amino acids  
in plasma  
db/db versus control**

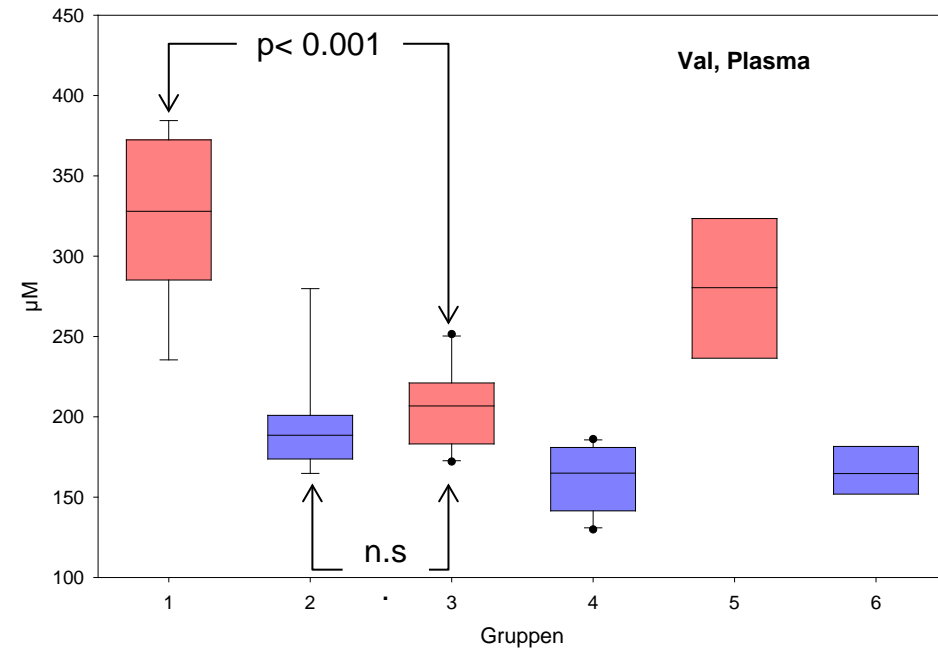


# Higher Sensitivity in preclinical PD

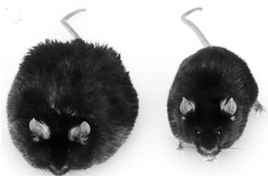
## Early selection of anti-diabetic drug candidates



Neither Rosiglitazone nor drug candidate improve hyperglycemia in db/db mice



Drug candidate but not Rosiglitazone improves short-term metabolic control to level of healthy controls



*Altmaier et al., 2008; Weinberger, 2008*

# GWAS: The Dawning of System Biology

## Combination genomics and metabolomics in Nature Genetics

LETTERS

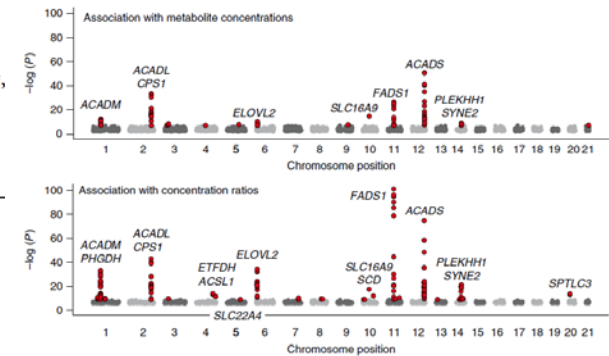
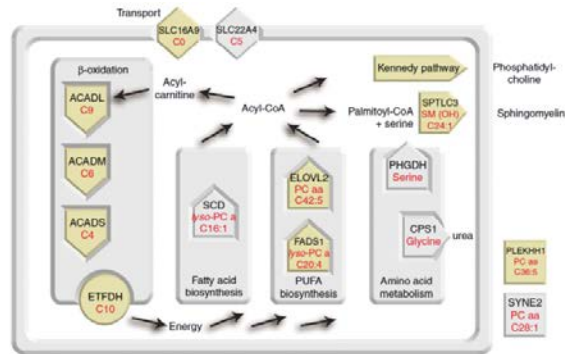
nature  
genetics

### A genome-wide perspective of genetic variation in human metabolism

Thomas Illig<sup>1,13</sup>, Christian Gieger<sup>1,13</sup>, Guangju Zhai<sup>2</sup>, Werner Römisch-Margl<sup>3</sup>, Rui Wang-Sattler<sup>1</sup>, Cornelia Prehn<sup>4</sup>, Elisabeth Altmaier<sup>3,5</sup>, Gabi Kastenmüller<sup>3</sup>, Bernet S Kato<sup>2</sup>, Hans-Werner Mewes<sup>3,6</sup>, Thomas Meitinger<sup>7,8</sup>, Martin Hrabé de Angelis<sup>4,9</sup>, Florian Kronenberg<sup>10</sup>, Nicole Soranzo<sup>2,11</sup>, H-Erich Wichmann<sup>1,12</sup>, Tim D Spector<sup>2</sup>, Jerzy Adamski<sup>4,9</sup> & Karsten Suhre<sup>3,5</sup>

Received 22 May; accepted 10 November; published online 27 December 2009; doi:10.1038/ng.407

**Figure 2** A systemic view of genetic variation in human metabolism, as identified in this study. Eight of nine replicated genetic polymorphisms (beige) and also four of five suggestive loci (gray) are located in or near genes encoding enzymes that are central to the different processes in human lipid metabolism, including  $\beta$ -oxidation (*ACADS*, *ACADM* and *ACADL*), polyunsaturated fatty acid biosynthesis (*FADS1* and *ELOVL2*), fatty acid synthesis (*SCD*), breakdown of fats and proteins to energy (*ETFDH*) and biosynthesis of phospholipids (*SPTLC3*). Two SNPs are located in or near genes encoding carrier proteins (*SLC22A4* and *SLC16A9*), and two SNPs involve enzymes that are related to amino acid metabolism (*PHGDH* and *CPS1*). Only for two genetic variants does the attribution of a metabolic function remain elusive (*PLEKHH1* and *SYNE2*). For each locus, the most strongly associating single metabolite is indicated in red.



**Figure 1** Manhattan plot of the strength of association with metabolite concentrations (above, data points with  $P < 10^{-7}$  are plotted in red) and concentration ratios (below, data points with  $P < 10^{-9}$  are plotted in red), based on association with 1,029 samples (step 1 of discovery stage). For each SNP, only the metabolic trait with the lowest  $P$  value of association is shown; thus, multiple dots indicate that several SNPs support the association at that locus.

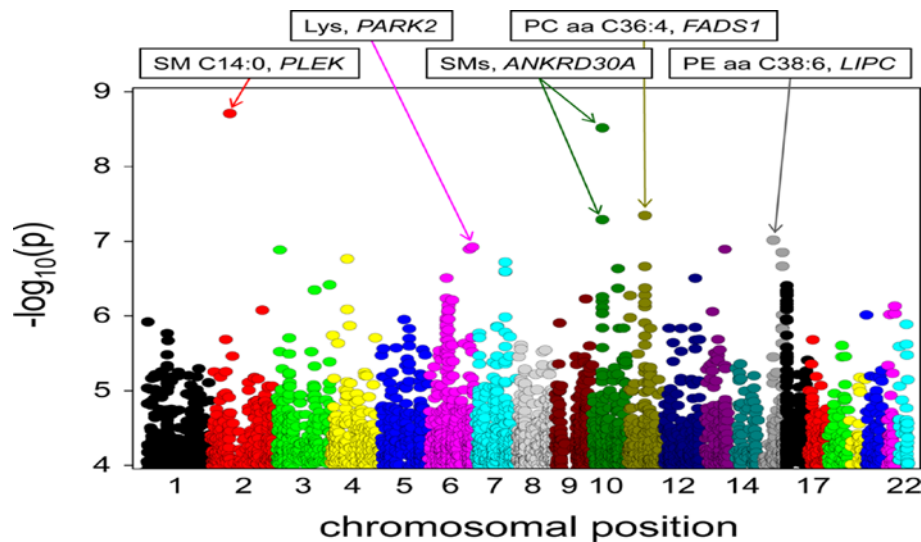
Serum metabolite concentrations provide a direct readout of biological processes in the human body, and they are associated with disorders such as cardiovascular and metabolic diseases.

The use of metabolite concentration ratios as proxies for enzymatic reaction rates reduced the variance and yielded robust statistical associations with  $P$  values ranging from  $3 \times 10^{-24}$  to  $6.5 \times 10^{-179}$ .

# GWAS: The Dawning of System Biology

## Combination of genomics and metabolomics

- First combination of **GWAS** and metabolomics data published in 2008
- Collaboration Helmholtz and Biocrates on diabetes-enriched **KORA** cohort
- SNPs association with differences in metabolic homeostasis



Gieger et al., 2008  
Illig et al., 2010  
Adamski, 2012  
Suhre & Gieger, 2012  
Adamski & Suhre, 2013  
...

**FADS1 (SNP in fatty acid desaturase 1)  
associated with PC aa C 36:4**

**even better PC aa C36:4 / PC aa C36:3!**

- Improved statistical power when applying metabolite ratios as phenotype for GWAS
  - Genome-wide significances even in **relatively small cohorts (284 subjects)**
  - Phantastic associations **in standard-sized studies (p-values down to 10<sup>-179</sup>)**
- More meaningful hits in genes for enzymes and transporters (**functional confirmation of genotype**)
- **Replication study (Illig et al. Nature Genetics) with UK Twin study**

# Cohort Studies/ Molecular Epidemiology

## *Enhanced by multiple levels of comparability*

Standardized Metabolic Phenotyping provides levels of comparability for molecular epidemiology projects, enhancing throughput, output and quality.

→ *Eventually leading to more successful transfer of epidemiological findings to patient care.*

**Level 1:** Reproducible, quantitative measurements over long intervalls (follow up determinations in cohorts)



**Level 2:** Comparable data sets between platforms allowing pooling of data between remote working groups and analytics at multiple centers



**Level 3:** Retrospective re-evaluation and discovery of previously overlooked relationships published by other researchers



# European Consortia on Brain, CVD and CKD (FP7)

## *Biocrates is the partner for Metabolic Phenotyping*

**sysVASC is an EU funded research project to identify molecular targets for vascular disease treatment**

The collaborative research project sysVASC aims to identify the causative events in cardiovascular diseases in order to predict novel therapeutic targets. The sysVASC consortium includes 17 partners from 10 countries including: 5 SMEs, 4 renowned universities, 4 leading research centres and 3 prominent academic clinical centres.

**BiomarCaRE** stands for **Biomarker for Cardiovascular Risk Assessment in Europe**. It is a research project co-funded under the the European Unions' [Seventh Framework Programme](#).

### Europe tackles Chronic Kidney Disease with Systems Biology

**SysKid**, a large-scale integrating European research project, aims at understanding chronic kidney disease in the context of diabetes and hypertension.

**SysKid** – which stands for »Systems Biology towards Novel Chronic Kidney Disease Diagnosis and Treatment« – will pave the way for progress in prevention, new diagnostic strategies and treatment options for declining kidney function, which affects millions of patients suffering from diabetes and hypertension.

**Brainage:** Impact of Prenatal Stress on brain ageing fetal programming, undernutrition and stress  
Mission: Slowing brain ageing and susceptibility to age-associated diseases: Early programming of pathways and genes and late interventional targets.

# Metabolic Phenotyping

## *Applications overview acc. to literature (see Biocrates homepage)*

- **Basic Systems Biology Research**

- **Disease related Biomarker Discovery**

Diabetes, chronic kidney disease, cancer, sepsis, CNS disorders (Alzheimer's disease, Schizophrenia,...), cardiovascular diseases, inflammatory diseases, endocrine disorders...

- **Health and Pharmaceuticals**

Drug development  
Personalized medicine  
Translational medicine  
Epidemiology

- **Nutrition and Lifestyle Research**

Assessment of vitamin status  
Evaluation of diet and nutrition related effects on health  
Analyze effects of different lifestyles

- **Bioprocess Optimization**

Metabolic characterization of production cell lines  
Media optimization

# What is New - Different ?

## Rationale of Biocrates enabled “System Diagnostics”

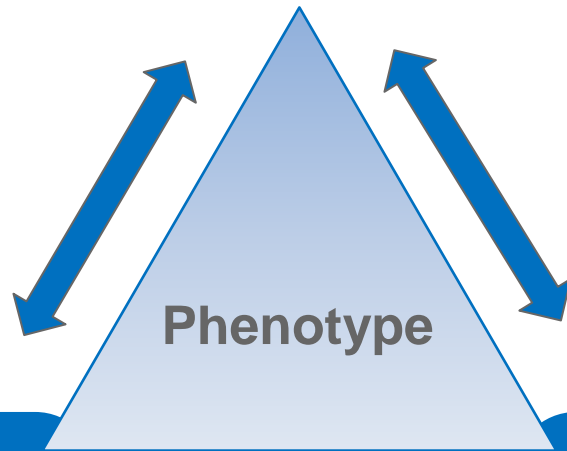
# Metabolic Phenotyping

## From single biomarker to metabolic signature

### *Diseases / Disorders*

*Personalized  
Medicine*

*Metabolic  
Endpoints*



**Phenotype**

#### **Use**

- Early diagnosis
- Disease Staging
- Drug response prediction
- Treatment efficacy
- Toxicology markers



***Metabolic  
Signatures***

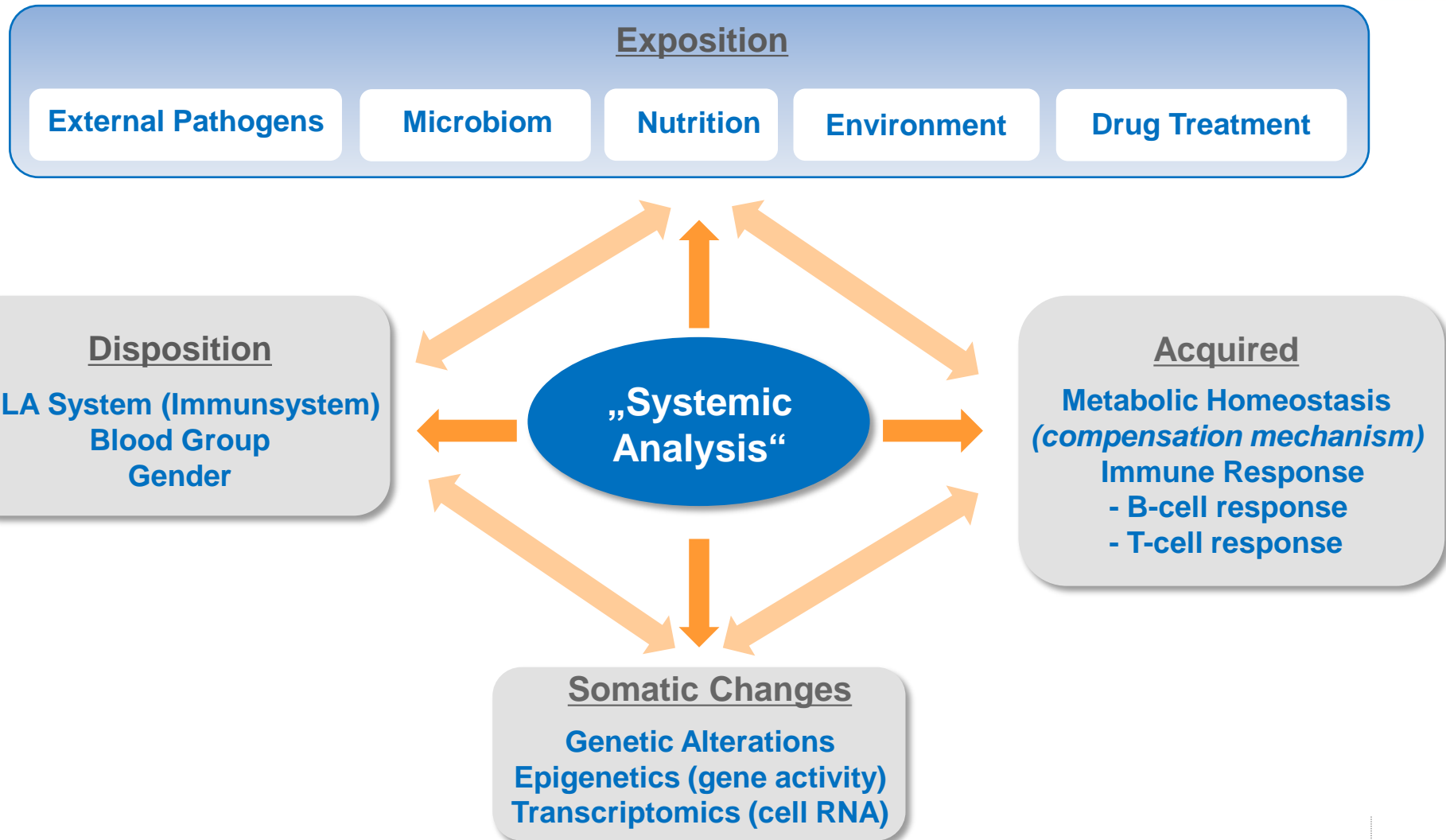
#### ***Pathways/Signaling***

- Amino Acid
- Fatty Acid
- Lipids
- Energy Metabolism
- Biogenic Amines

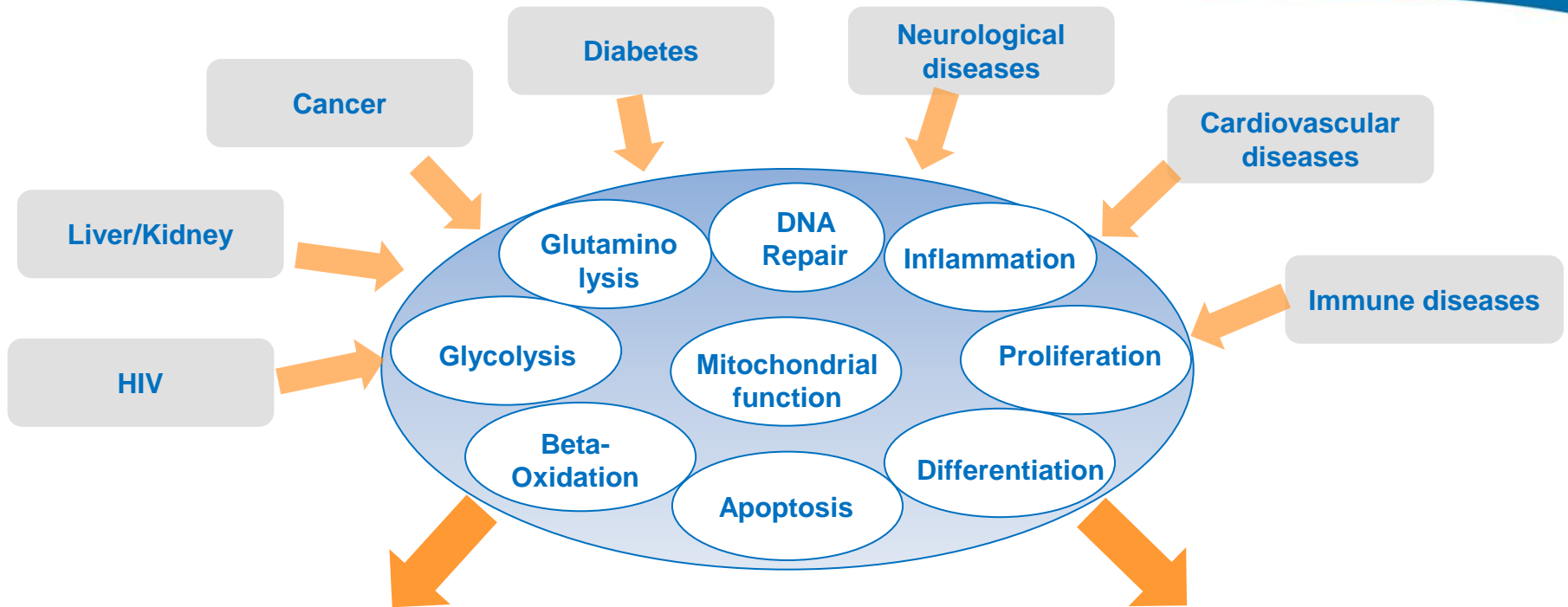
(some with signaling functions)

# Comprehensive Individual System Analysis

## Surrogate marker for individual phenotype expression



# Phenotype reveals Pathophysiological Mechanism and Disease Specific Markers



## Mitochondrial Function

- Beta Oxidation (Acylcarnitines)
- Urea Cycle Intermediates (Ornithine, Citrulline)

## Oxidative Stress

- Met/MetSO
- Phenylalanine/Thyrosine
- Inflammatory Processes Lipids PG

## Insulin Resistance

- Glycine, LPC 18:2, Acetylcarnitine
- Other Lipid species

## Neurotransmitter

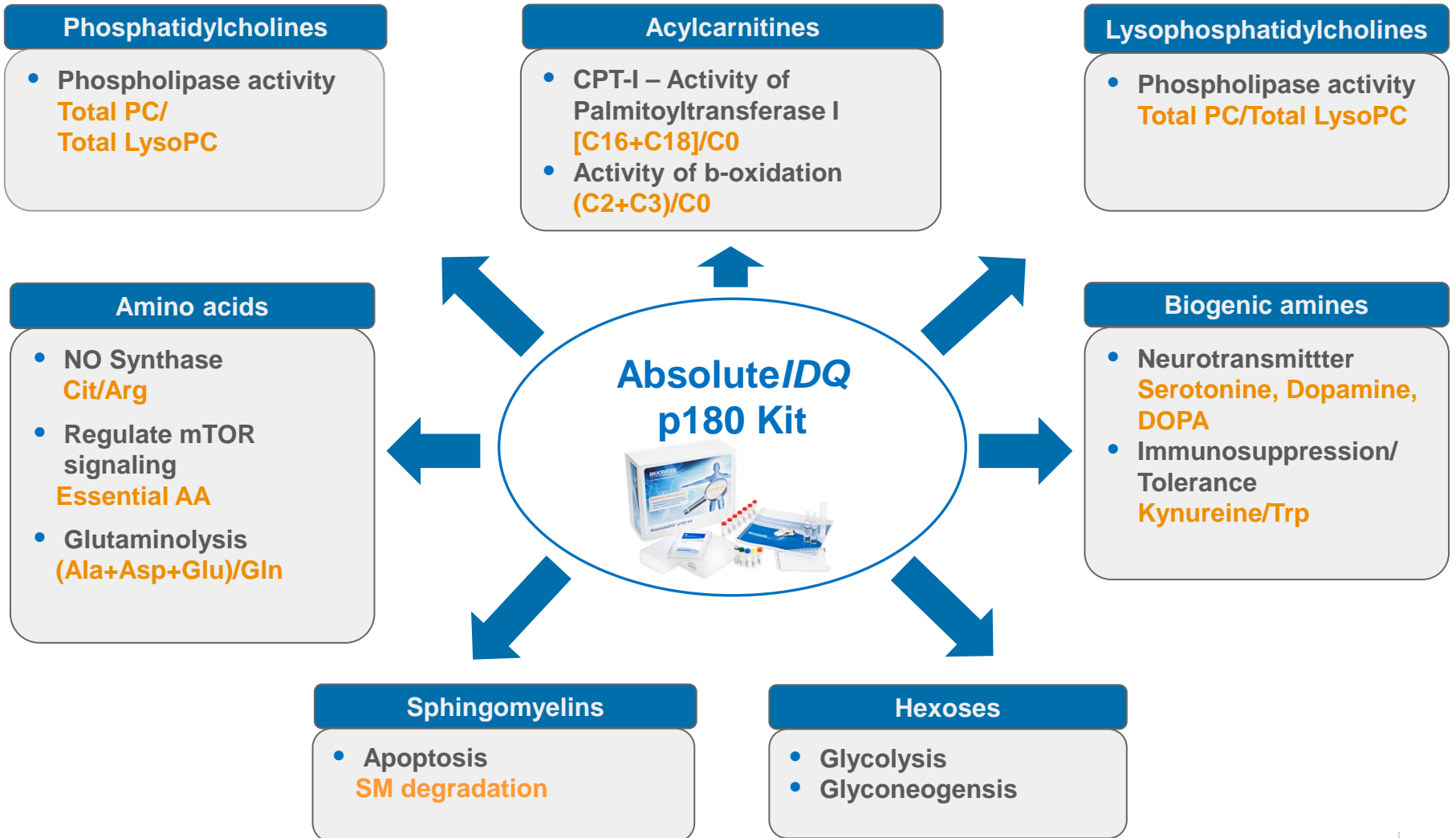
- Biogenic amines Serotonin Dopamine DOPA
- Amino acids Glutamine/ Glutamate

## Cell signaling

- mTOR activity (e.g. essential amino acids)
- ErbB2 induced Glutaminase Glutamine/Glutamate

# Analyte Groups covered by Absolute/IDQ p180 Kit

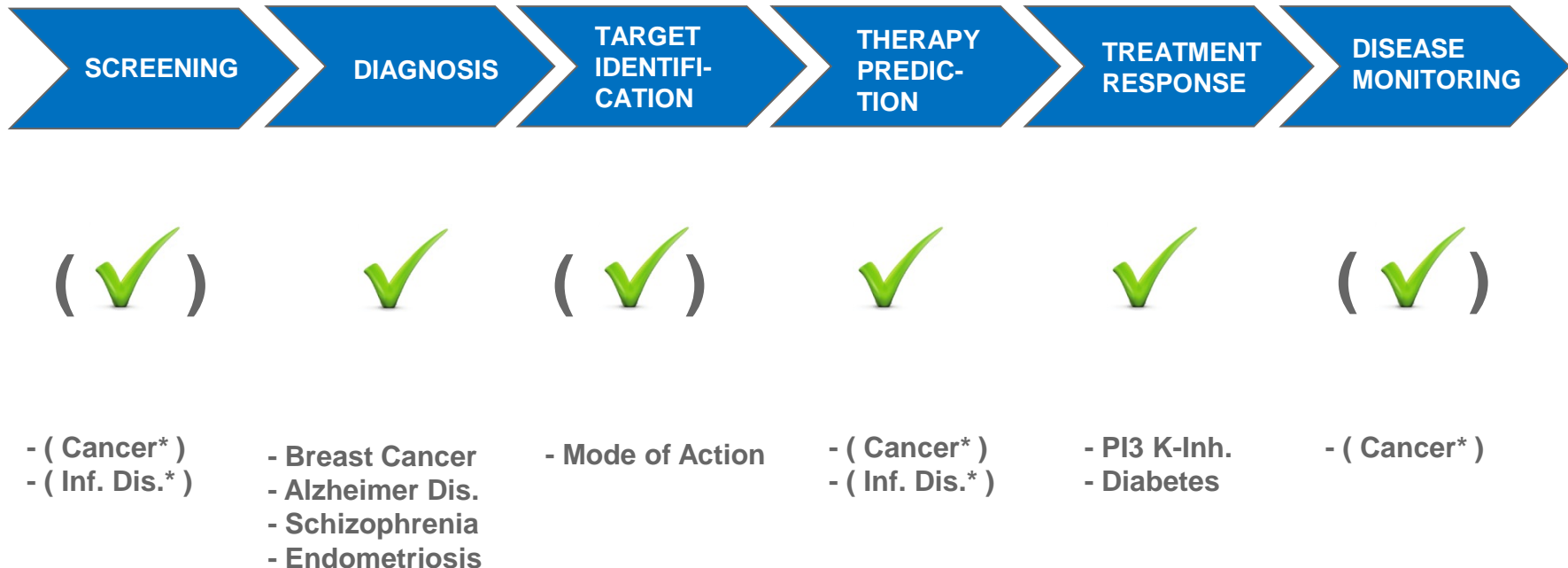
## Pathophysiological pathways covered by standard Kit



# Potential of Metabolic Phenotyping

## Biomarker along the disease pathway

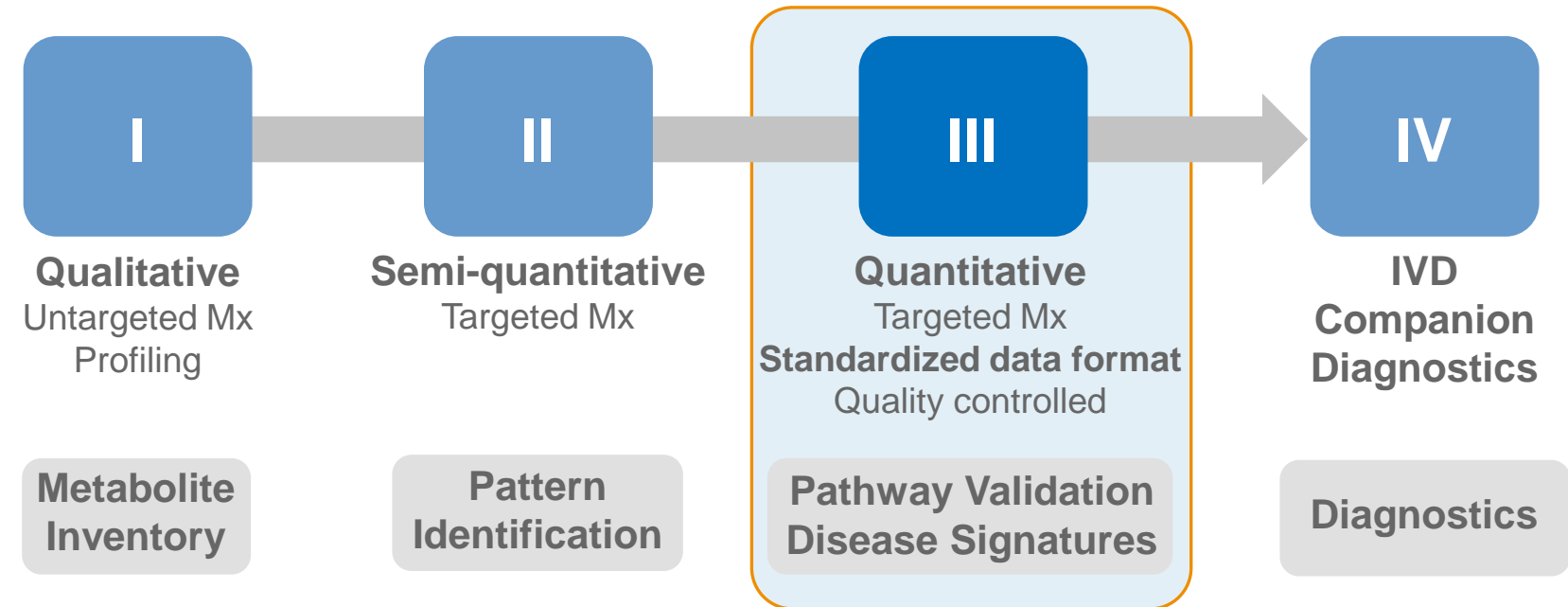
Metabolic Phenotyping provides value along the whole disease pathway  
- Growing with the number of publications



\* Unpublished data

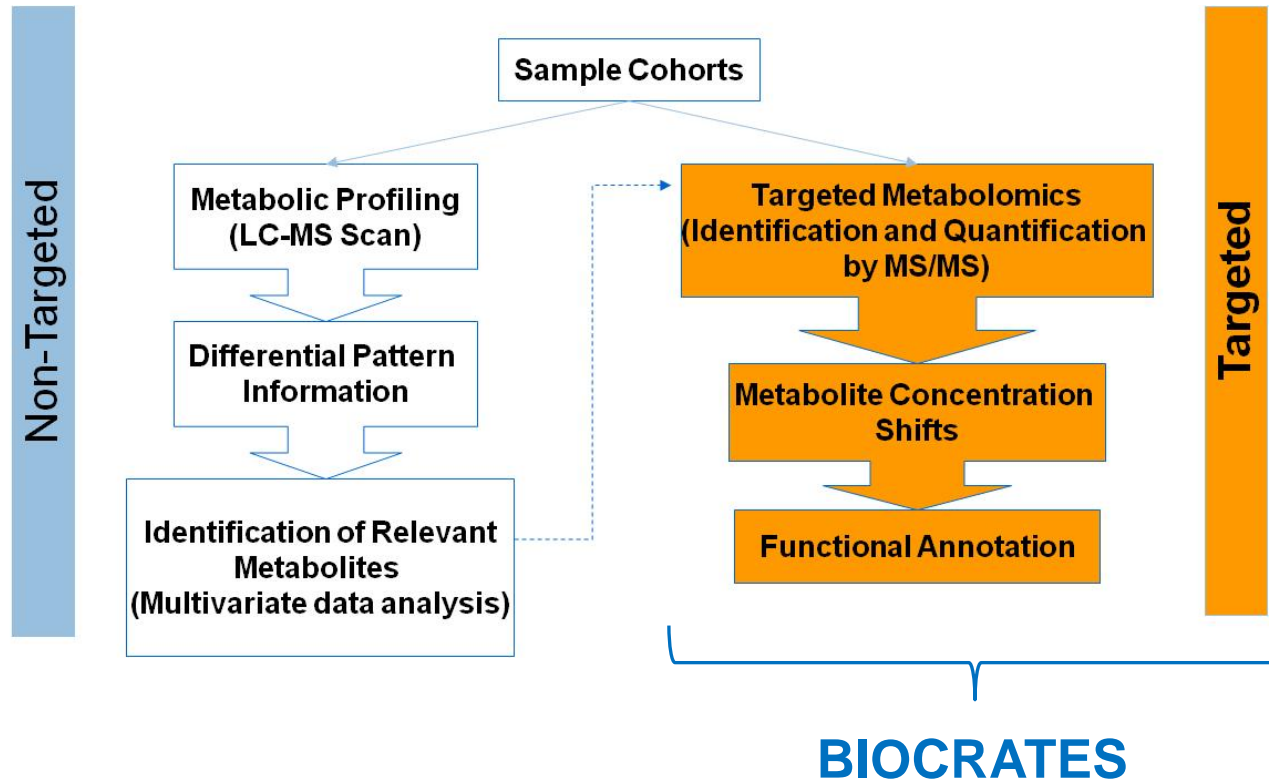
# Evolution of Metabolomics (Mx) Research

*Biocrates provides targeted, standardized analytics*



number of samples

number of metabolites

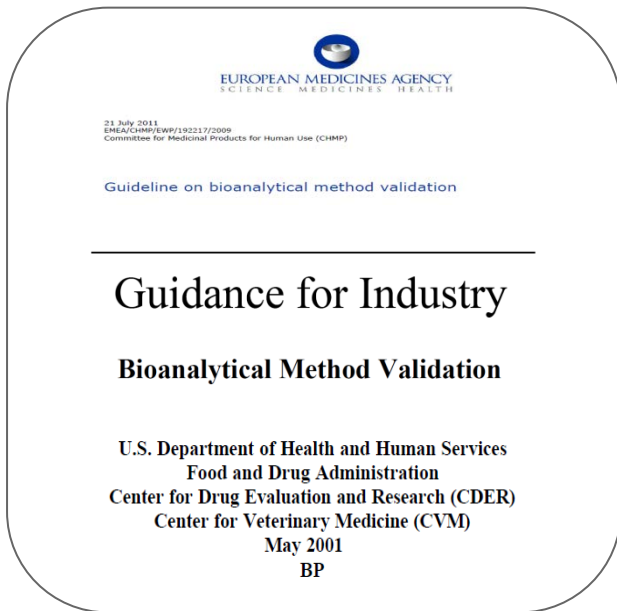


- > 10 years experience in method development for quantitative targeted metabolomics
- Preannotation of high number of metabolites for most important pathways
- Direct functional annotation of metabolite concentration shifts

# Metabolomics by BIOCRATES

*Save jump-start to establish a metabolomics lab*

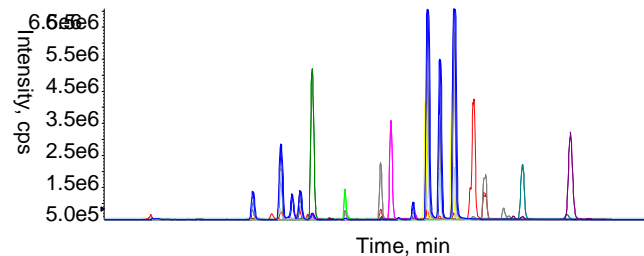
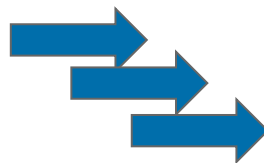
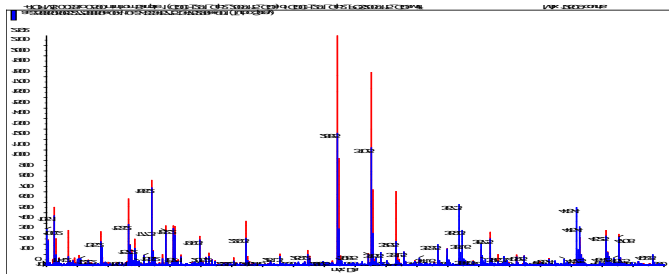
- **Standardization** in sample and data analysis
- Standardized read-out
- **Accuracy** of metabolite concentration!
- **Precision** of analysis!
- **Quality controlled** analysis!
- Inter-laboratory **comparison!**
- **Ready to use** on your MS lab in 1 day!
- Validated considering **EMA and FDA** guidance



Metabolite  
Inventory

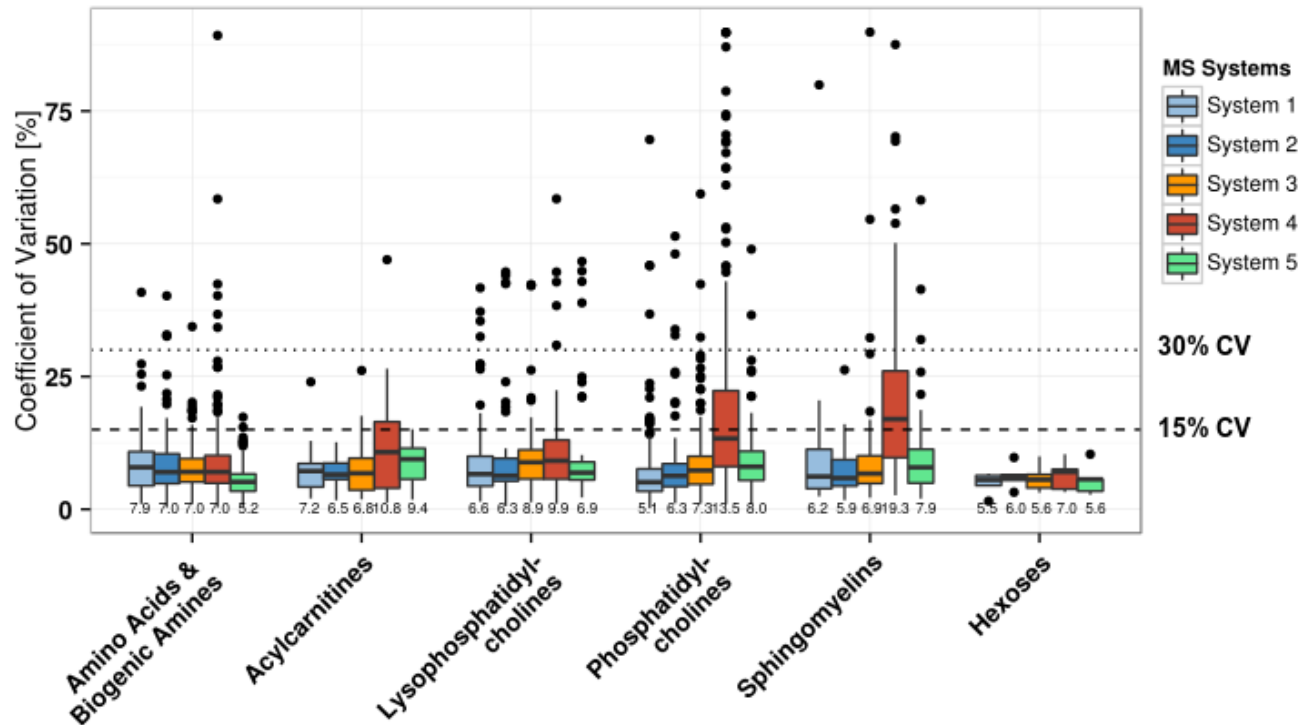
Pattern  
Identification

Pathway Validation  
Disease Signatures



# Absolute/IDQ<sup>®</sup> p180 Kit – 186 Analytes

## Analytical performance in 'Internal Ring Trial'



- Measurements of spiked reference plasma in **3-6 replicates**, values <LLOQ were excluded
- **LC-MS/MS** metabolite classes achieve **<15% CV (FDA, EMA guideline)** on all instruments
- **FIA-MS/MS** metabolite classes achieve **<15% CV (FDA, EMA guideline)** except System 4 <30%
- Assumptions for higher CV on System 4: more suppression effects, isotope effects

# Broad Analytical Panel

> 220 analytes in kits, > 630 analytes in service lab

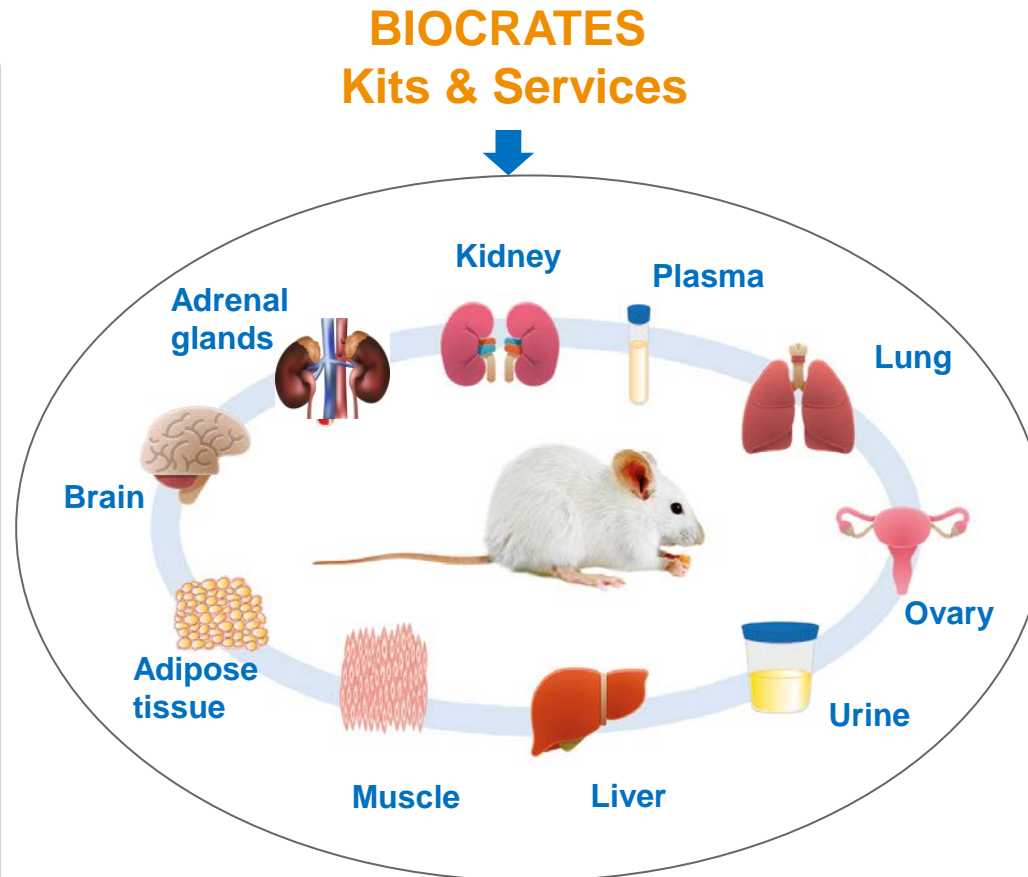
| Metabolite Classes              | Metabolites | Contract Research | p150 Kit | p180 Kit | Stero/DQ Kit | Stero17 Kit | Vitamin D Kit | Bile Acids Kit |
|---------------------------------|-------------|-------------------|----------|----------|--------------|-------------|---------------|----------------|
| Acylcarnitines                  | 40 - 41     | x                 | x        | x        |              |             |               |                |
| Amino acids                     | 14 - 21     | x                 | x        | x        |              |             |               |                |
| Hexose                          | 1           | x                 | x        | x        |              |             |               |                |
| Sphingolipids                   | 15          | x                 | x        | x        |              |             |               |                |
| Glycerophospholipids            | 90 - 92     | x                 | x        | x        |              |             |               |                |
| Biogenic amines                 | 14 - 19     | x                 |          | x        |              |             |               |                |
| Steroid hormones                | 16-17       | x                 |          |          | x            | x           |               |                |
| Neurotransmitters               | 9           | x                 |          |          |              |             |               |                |
| Bile acids                      | 16-19       | x                 |          |          |              |             |               | x              |
| Eicosanoids                     | 17          | x                 |          |          |              |             |               |                |
| Fatty acids                     | 62          | x                 |          |          |              |             |               |                |
| Intermediates energy metabolism | 15          | x                 |          |          |              |             |               |                |
| Oxysterols                      | 16          | x                 |          |          |              |             |               |                |
| Phospholipids and ceramides     | 331         | x                 |          |          |              |             |               |                |
| Vitamins                        | 12          | x                 |          |          |              |             |               |                |
| Vitamin D                       | 2           | x                 |          |          |              |             | x             |                |
| Fat-soluble vitamins            | 4           | x                 |          |          |              |             |               |                |
| Oxidative stress                | 6           | x                 |          |          |              |             |               |                |

# Matrices/ Species

Wide array of matrices/ species can be measured

## Matrices

- Plasma, serum (only 10 µL)
- Dried blood spots
- Cell culture medium
- Tissue
- Tumour tissue:
- Lung lavage (BALF)
- Skin samples
- Blister liquid / skins
- Feces
- Follicular fluid
- Milk
- Urine
- CSF
- Saliva
- Cells
- Cell culture supplements



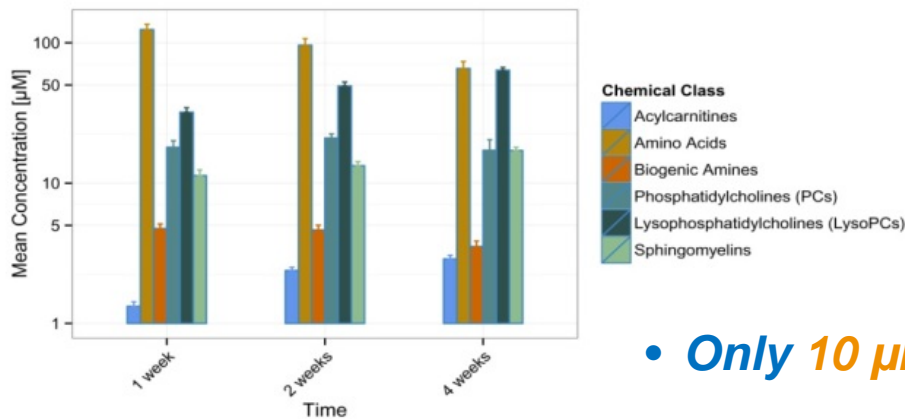
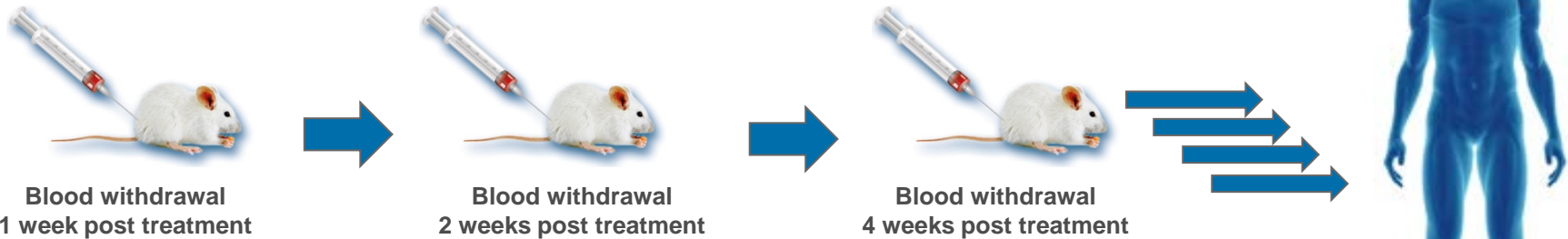
## Species

- Human
- Mouse
- Rat
- Monkey
- Cow
- Sheep
- Pig
- Dog
- Chicken
- Horse
- Rabbit
- Zebrafish
- Chinese hamster
- *C. elegans*
- Soy
- Yeast: *Pichia pastoris*
- *E. coli*

# Translational Research - From Mouse To Man

## *From preclinical to clinical Phenotyping*

- Following disease progression in animal model over time



- **Only 10 µl of blood needed = keeping animal alive!**
- **Allowing longitudinal studies**
- **Different genetic - same metabolites**

# Biocrates Research/ CE products with unique breadth

## Steroid Kits add tremendous value

### Absolute/IDQ® p180 Kit

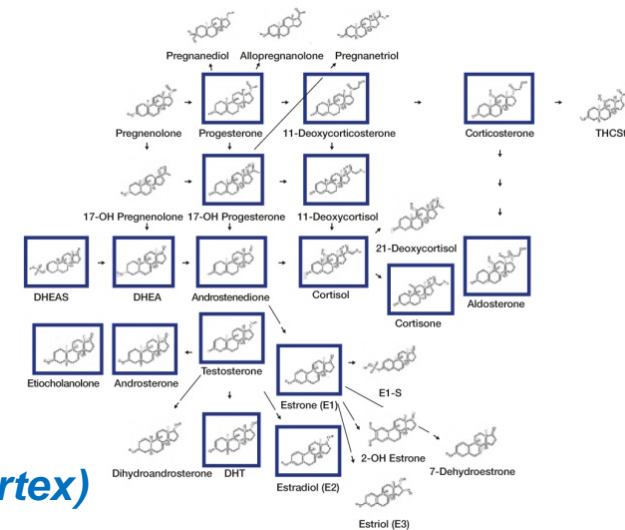
- Acylcarnitines
- Glycerophospho- and sphingolipids
- Hexose
- Amino acids (LC-MS/MS)
- Biogenic amines (LC-MS/MS)



186 analytes

### Absolute/IDQ® Stero 17 Kit/ Stero/IDQ Kit (CE-IVD)

- Mineralocorticoids
- Glucocorticoids
- Sex Steroids



- *Cardio-vascular diseases*
- *Fertility, PCOS*
- *Inborn metabolism disorders (NBS)*
- *Hormon-dependent tumors (adrenal cortex)*
- *Adrenopausal effects*
- *Cushing's syndrome*
- ...



# NEW: Biocrates® Bile Acids Kit\*

## Standardized Bile Acids Phenotyping in mouse and man

### Analysis of Endogenous Bile Acids in Blood

- 16 human specific bile acids
- 19 mouse specific bile acids
- From only 10µL of human plasma/ serum or mouse plasma
- Ready to use components for LC/MS/MS based assay
- All major MS-platforms (AB Sciex™, Waters®, Thermo™) with ESI ion source
- HPLC and UHPLC
- Robust, fast, simple, reliable sample preparation on 96-well patented filter plate
- Validated in accordance with EMA guidelines

| No | Analyte    | Name   | Internal Standard | Human plasma/ serum | Mouse plasma | Calibration range (LLOQ – LLOQ n µM) |
|----|------------|--|-------------------|---------------------|--------------|--------------------------------------|
| 1  | CA         | Cholic acid                                  | d5-CA             | ✓                   | ✓            | 0.03 – 75                            |
| 2  | CDCA       | Chenodeoxycholic acid                        | d5-CDCA           | ✓                   | ✓            | 0.02 – 30                            |
| 3  | DCA        | Deoxycholic acid                             | d5-CDCA           | ✓                   | ✓            | 0.02 – 10                            |
| 4  | GCA        | Glycocholic acid                             | d5-GCA            | ✓                   | ✓            | 0.03 – 75                            |
| 5  | GCDCA      | Glycochenodeoxycholic acid                   | d4-GLCA           | ✓                   |              | 0.02 – 20                            |
| 6  | GDCA       | Glycodeoxycholic acid                        | d4-GLCA           | ✓                   | ✓            | 0.01 – 10                            |
| 7  | GLCA       | Glycolithocholic acid                        | d4-GLCA           | ✓                   | ✓            | 0.01 – 5                             |
| 8  | GUDCA      | Glyoursodeoxycholic acid                     | d4-GUDCA          | ✓                   | ✓            | 0.01 – 10                            |
| 9  | HDCA       | Hyodeoxycholic acid                          | d4-HDCA(b)        |                     | ✓            | 0.01 – 5                             |
| 10 | LCA        | Lithocholic acid                             | d4-LCA            | ✓                   | ✓            | 0.01 – 5                             |
| 11 | MCA(a)     | Alpha-Muricholic acid                        | d5-CA             |                     | ✓            | 0.01 – 5                             |
| 12 | MCA(b)     | Beta-Muricholic acid                         | d5-CA             |                     | ✓            | 0.01 – 10                            |
| 13 | MCA(o)     | Omega-Muricholic acid                        | d5-CA             |                     | ✓            | 0.01 – 5                             |
| 14 | TCA        | Taurocholic acid                             | d5-TCA            | ✓                   | ✓            | 0.02 – 50                            |
| 15 | TCDC       | Taurochenodeoxycholic acid                   | d5-TCDC           | ✓                   | ✓            | 0.01 – 20                            |
| 16 | TDCA       | Taurodeoxycholic acid                        | d5-TCDC           | ✓                   | ✓            | 0.01 – 10                            |
| 17 | TLCA       | Taurolithocholic acid                        | d4-GLCA           | ✓                   | ✓            | 0.01 – 5                             |
| 18 | TMCA (a+b) | Taurumuricholic acid (sum of alpha and beta) | d5-TUDCA          | ✓                   | ✓**          | 0.01 – 10                            |
| 19 | TUDCA      | Tauroursodeoxycholic acid                    | d5-TUDCA          | ✓                   | ✓*           | 0.01 – 15                            |
| 20 | UDCA       | Ursodeoxycholic acid                         | d4-HDCA(b)        | ✓                   | ✓            | 0.02 – 30                            |

- \* Partial coelution with THDCA under UHPLC conditions, quantifiable only under HPLC conditions  
 \*\* semi-quantitative for Waters Xevo™ TQ MS  
 ✓ Generally present at very low concentrations (close to or < LLOQ) in healthy samples  
 ✓ Generally present concentrations well above LLOQ in healthy samples

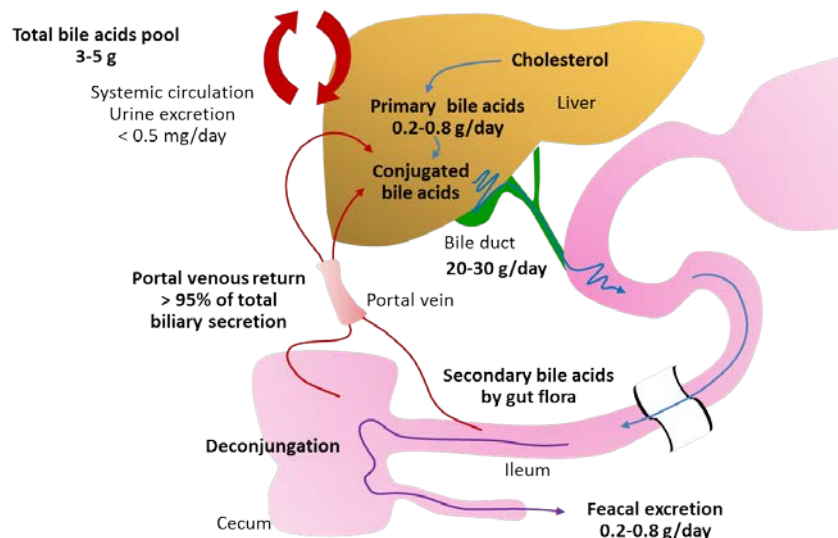


\* For Research Use only (RUO)

# Bile Acids – Biological Functions

## *From main physiological function to bile-acid-mediated signaling*

- Main physiologic function: form micelles to emulsify fats  $\Rightarrow$  help in digestion and absorption of fats and fat-soluble vitamins
- Catabolism of cholesterol, support elimination of catabolites from liver
- Signaling molecules: via nuclear farnesoid receptor (FXR) and G-protein coupled membrane receptor (TGR5)  $\Rightarrow$  important role in chronic disorders, sepsis or drug metabolism (Cytochrom P450 superfamily)
- Mediators (via secondary bile acids levels) of the gut microbiome status



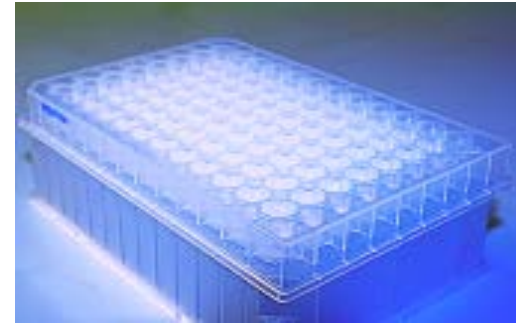
**Figure: Enterohepatic circulation of bile acids**

# Absolute/IDQ<sup>®</sup> p180 Kit

*Highly standardized workflow for 82 tests in 30 hrs.*



96 well plate format



## Key-Facts:

- 186 metabolites per sample
- 82 samples per plate

➔ 22.080 MS/MS signals

- 4 h sample preparation time (2 hours hands-on)
- 24 h sample analysis run time
- 2 hours data analysis time (automatized)

➔ 30 h in total for 96 tests

**1 MS/MS instrument → 6 kits / week (24/7) → ~ 500 samples / week**

# Plate Design and Assay Information

## AbsolutelDQ® p180 Kit

|   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|----|----|----|
| A | ○ | ● | ● | ● | ● | ● | ● | ● | ● | ●  | ●  | ●  |
| B | ● | ● | ● | ● | ● | ● | ● | ● | ● | ●  | ●  | ●  |
| C | ● | ● | ● | ● | ● | ● | ● | ● | ● | ●  | ●  | ●  |
| D | ● | ● | ● | ● | ● | ● | ● | ● | ● | ●  | ●  | ●  |
| E | ● | ● | ● | ● | ● | ● | ● | ● | ● | ●  | ●  | ●  |
| F | ● | ● | ● | ● | ● | ● | ● | ● | ● | ●  | ●  | ●  |
| G | ● | ● | ● | ● | ● | ● | ● | ● | ● | ●  | ●  | ●  |
| H | ● | ● | ● | ● | ● | ● | ● | ● | ● | ●  | ●  | ●  |

- 1 Blank ○
- 3 Zero Samples ●
- 7 Point Calibration (LC-MS/MS) ●
- Internal Calibrator in each well (FIA-MS/MS)
- 3 Quality Controls (low, medium, high)
  - 
  - 
  -
- 82 Samples ●

| Metabolites                          | Quantification                     |
|--------------------------------------|------------------------------------|
| Acylcarnitines                       | quantitative/<br>semi-quantitative |
| Glycerophospho-<br>and sphingolipids | semi-quantitative                  |
| Hexose                               | quantitative                       |
| Amino acids                          | quantitative                       |
| Biogenic amines                      | quantitative                       |

## Technical Validation (automated)

- check blank & zero samples criteria
- check internal standards
- check calibration criteria
- check sample criteria (LOD, ULOQ)



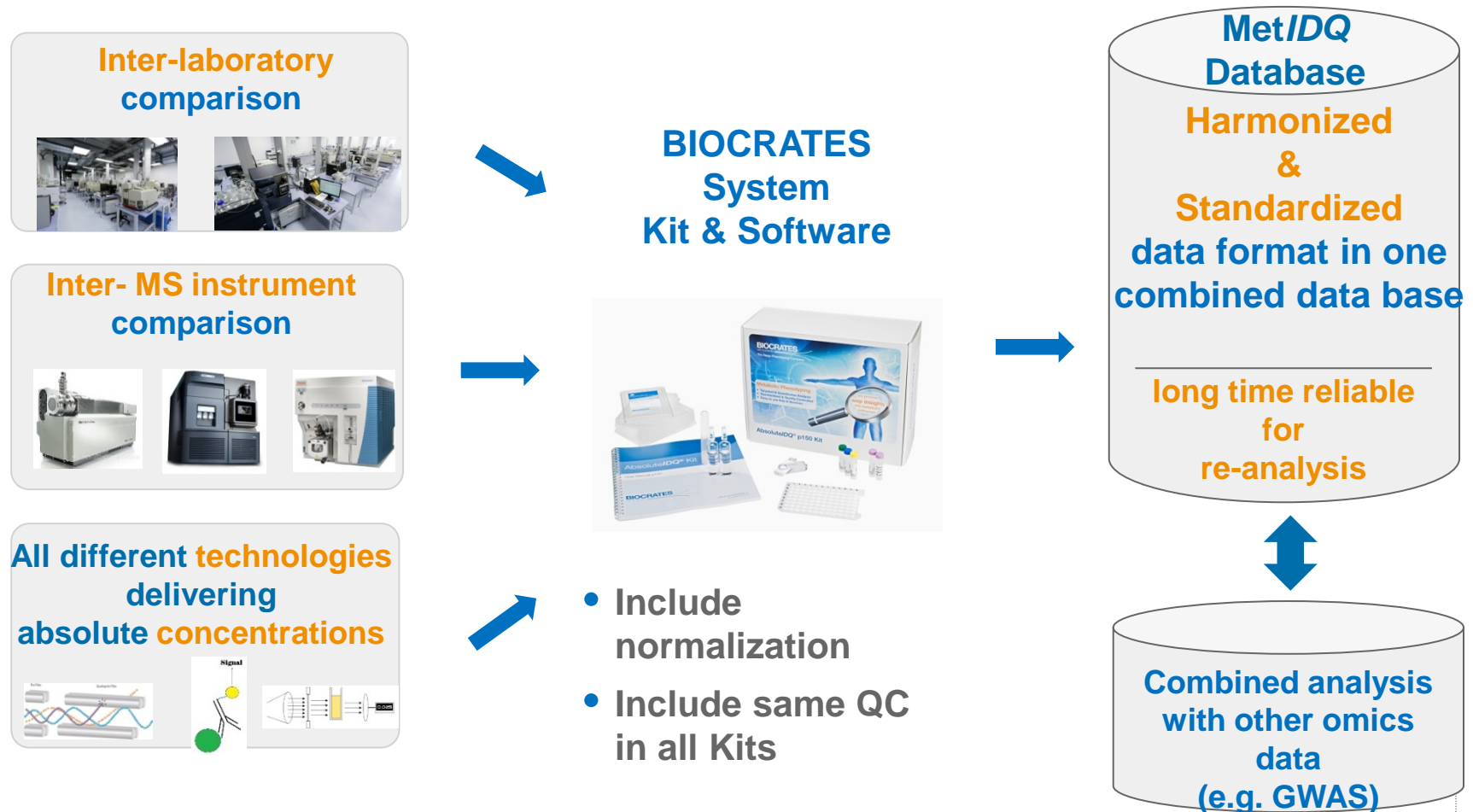
Analytical approval of the plate

**Result: 1 Excel sheet**

# Biocrates System

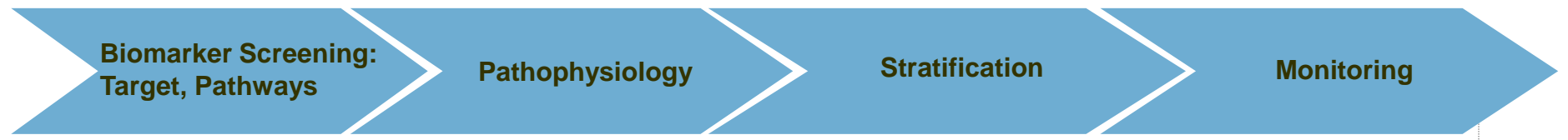
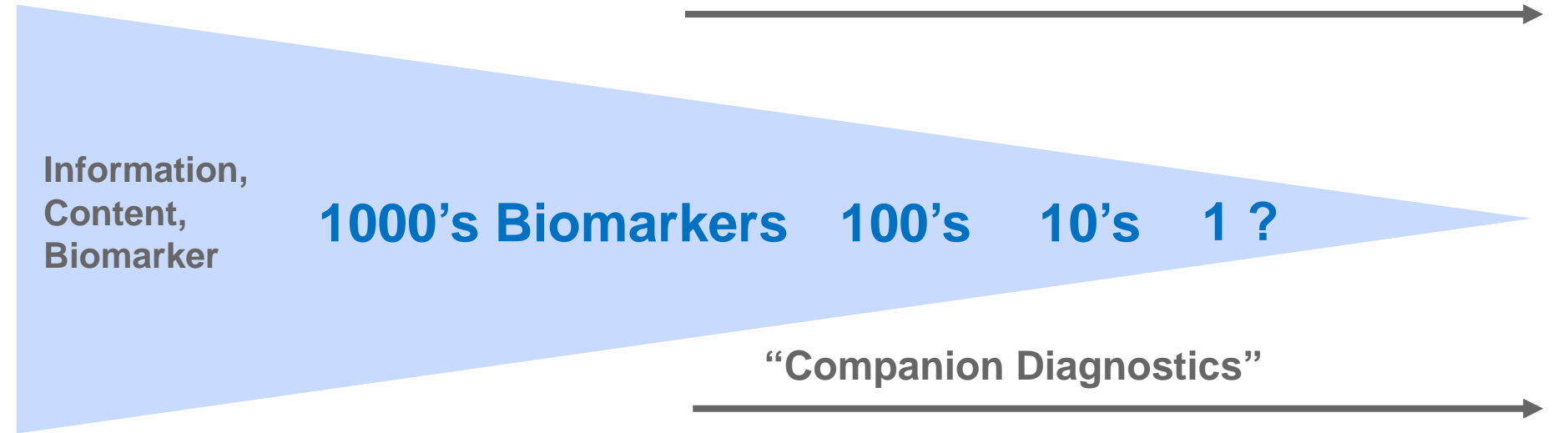
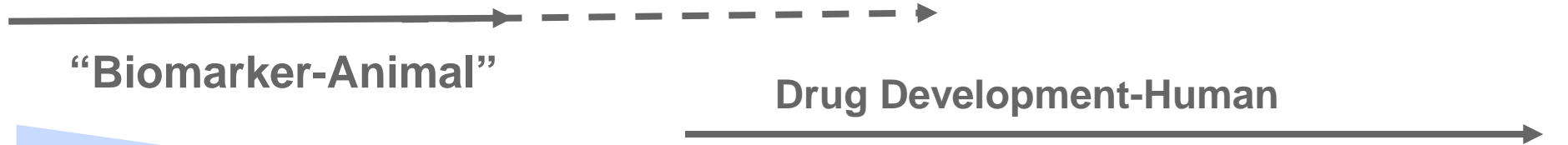
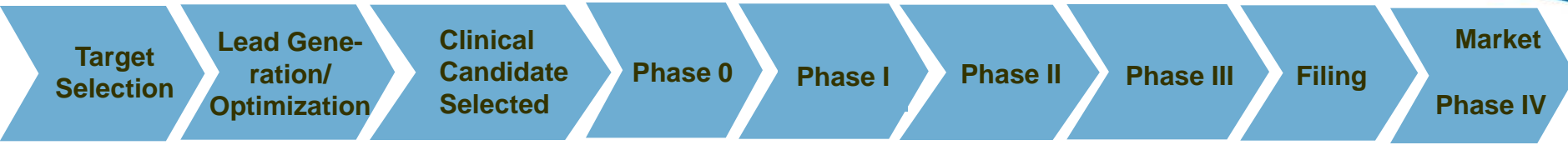
## Data standardization by kit and software

Today MS data can not be pooled lab-lab or longitudinal



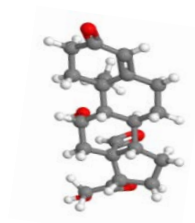
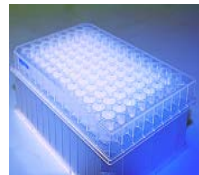
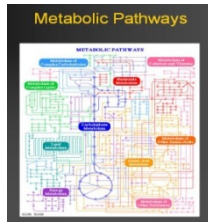
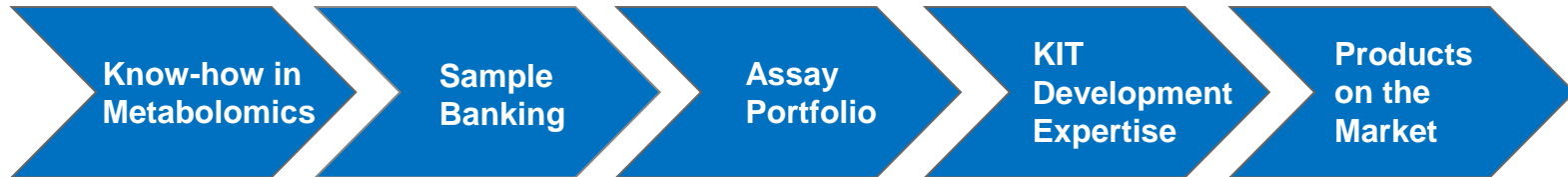
# Drug Development Process

## Biocrates can cover the whole process

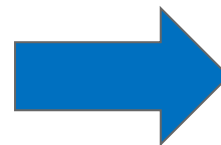


# BIOCRATES - Matching of Knowledge

## *From biomarker to kit*



**Contract Research**  
**Biomarker/ Signatures**



**Kit**  
**Disorders, Pathways**

- Spearhead to recognize new customer demands
- Indication / metabolites relationships

**→ Translation into new Kits and IT-tools**

# A Voice from the Medical Community



Dr. Edwin Scott- Asemota (Nigeria):  
**...is this Voodoo medicine?**



**...or the Next Generation Technology in IVD „System Diagnostics“?!!**

# Thank you!

## *From Genotype to Phenotype*

**BIOCRATES**  
LIFE SCIENCES  
The Deep Phenotyping Company

We provide the  
**Phenotype** to the Genotype