

BIOCRATES Life Sciences AG

The “Deep Phenotyping” Company

m⁴ - Personalisierte Medizin

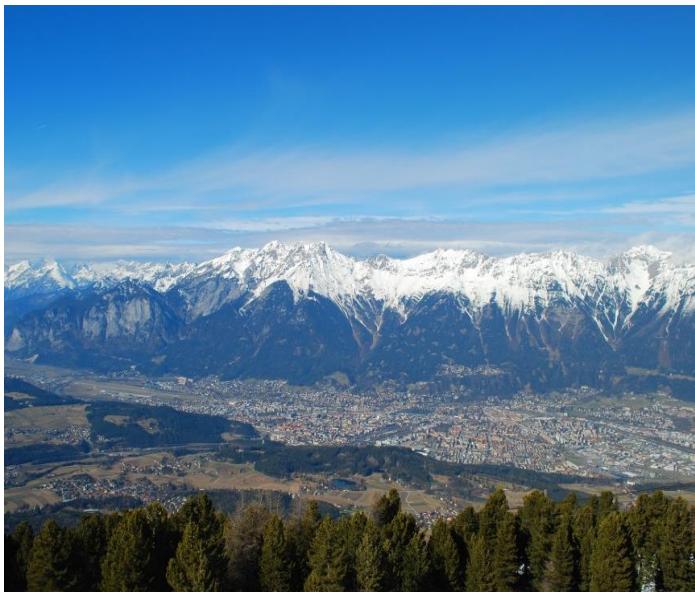


**Metabolic Phenotyping:
A Revolution in Disease Insights and
System Diagnostics?**

Dr. Wulf Fischer-Knuppertz, CEO

BIOCRATES - The Company

Targeted Metabolomics



- 2002: Founded in Innsbruck, Austria
- Founders: leading university professors (Adelbert Roscher, Hartmut Glossmann, Günther Bonn, Michael Popp)
- Fully integrated mass spectrometry based platform for targeted metabolomics
- Biomarker candidates for several indications (CKD, T2D)
- 2006: Established analytical services lab
- 2008: World's first metabolomics kit on market
- Status 2014: 1 CE-marked IVD, 5 research kits, commercially available, analytical services lab
- 40 Employees

Agenda

1. “Deep Phenotyping”
2. **Potential in Diagnosis + Prognosis + System Biology (GWAS)**
3. **System Diagnostics**
4. **Metabolites Link to Pathophysiology and to Disease**
5. Biocrates **Metabolomics** Technology and Products

“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

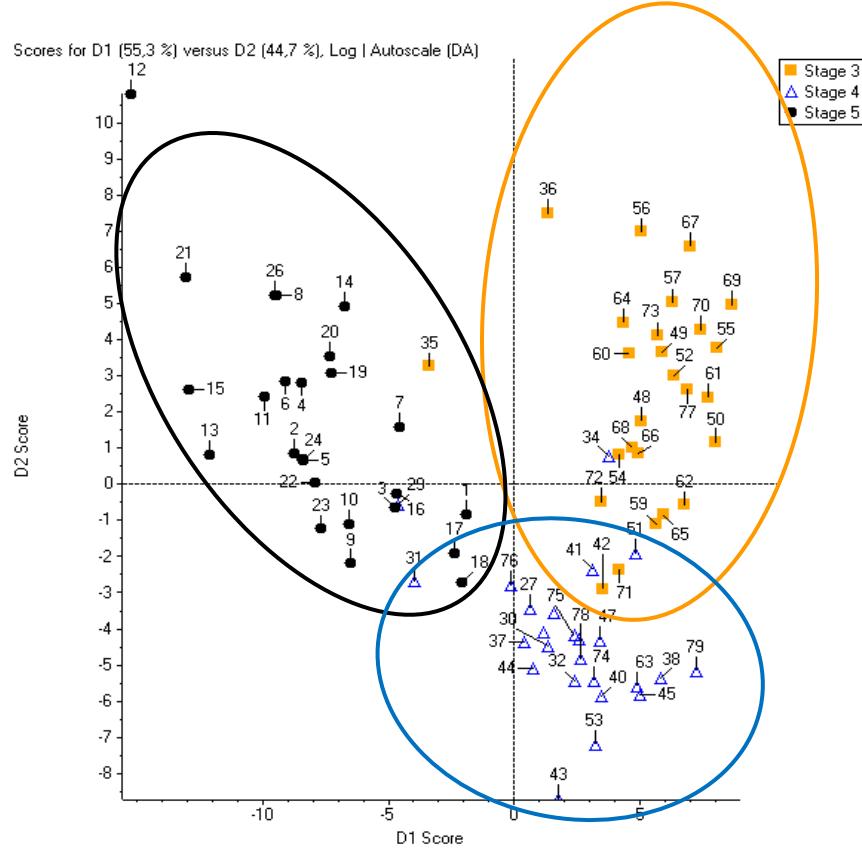
We provide
deep insights
into metabolic
pathways.

Examples for Diagnostic Applications and Prognosis/ Prediction

Individual Disease Specific Signature

Diagnosis and subclassification

Separation of stages 3 – 5 of chronic kidney disease



Lundin & Weinberger, 2010; Lundin et al., 2011;
Argilés et al., 2013; Duranton et al., 2014

Diagnosis of Breast Cancer

Article

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

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

Yunping Qiu ^{1,2}, Bingsen Zhou ³, Mingming Su ⁴, Sarah Baxter ⁴, Xiaojiao Zheng ¹, Xueqing Zhao ⁵, Yun Yen ³ and Wei Jia ^{1,2,*}

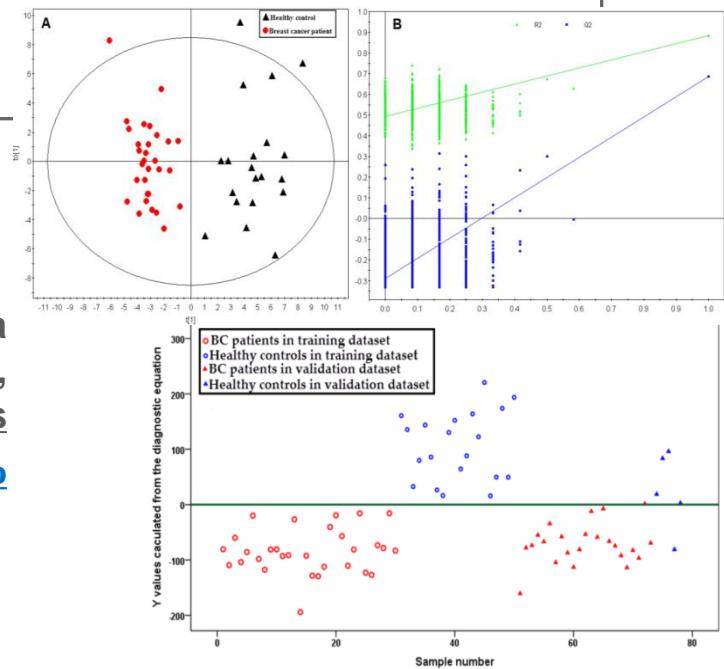
About 20% of breast cancer patients still cannot be detected with mammography.

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 %.



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

OPEN ACCESS
International Journal of
Molecular Sciences
ISSN 1422-0067
www.mdpi.com/journal/ijms



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. Vouk¹, N. Hevir¹, M. Ribič-Pucelj², G. Haarpaintner³, H. Scherb⁴, J. Osredkar⁵, G. Möller⁶, C. Prehn⁶, T. Lanišnik Rižner^{1,*}, and J. Adamski⁶

...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.

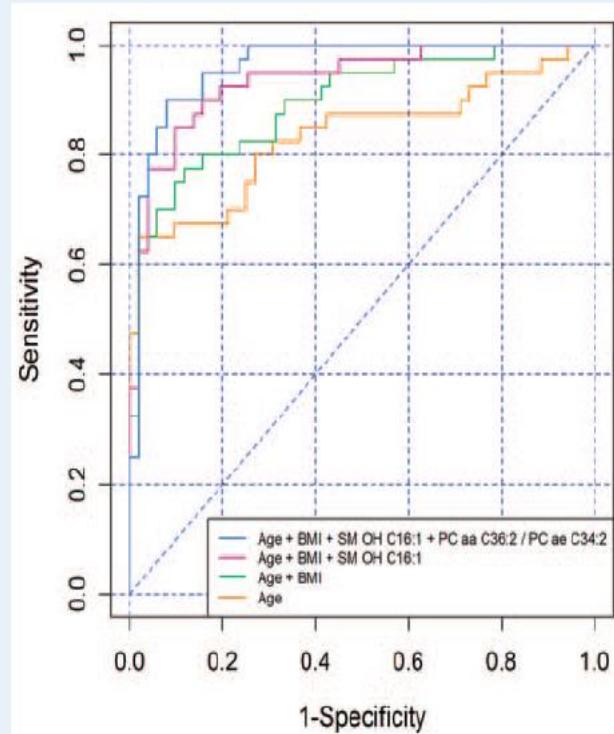


Figure 4 ROC curve. ROC curve shows improving effects of successive addition of separate variables to the model for differentiation between endometriosis patients and healthy controls.

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

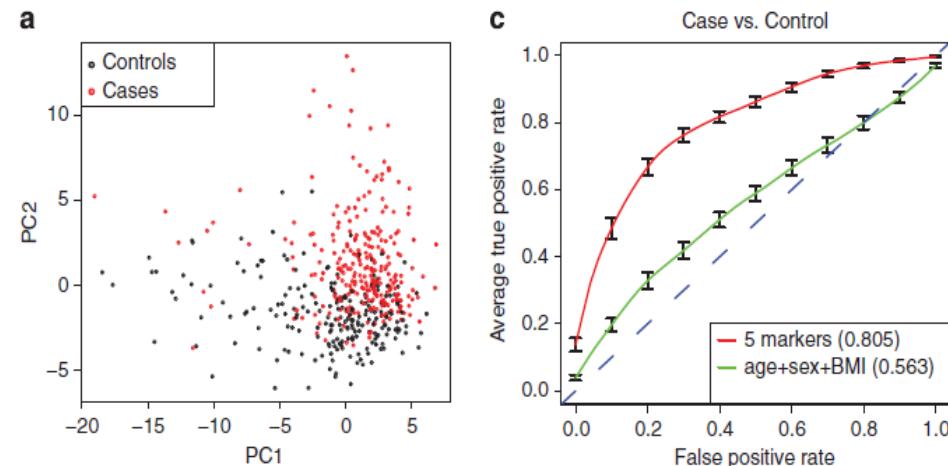


www.nature.com/tp

Schizophrenia shows a unique metabolomics signature in plasma

Y He^{1,2,3,10}, Z Yu^{1,10}, I Giegling⁴, L Xie³, AM Hartmann⁴, C Prehn⁵, J Adamski^{5,6}, R Kahn⁷, Y Li^{2,3}, T Illig^{1,8}, R Wang-Sattler^{1,11}
 and D Rujescu^{4,9,11}

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)

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,¹ Norbert Stefan,² Zhonghao Yu,³ Kristin Mühlenbruch,⁴ Dagmar Drogan,¹ Hans-Georg Joost,⁵ Andreas Fritzsche,² Hans-Ulrich Häring,² Martin Hrabé de Angelis,⁶ Annette Peters,⁷ Michael Roden,^{8,9} Cornelia Prehn,⁶ Rui Wang-Sattler,³ Thomas Illig,^{3,10} Matthias B. Schulze,⁴ Jerzy Adamski,⁶ Heiner Boeing,¹ and Tobias Pischon^{1,11}

Received 22 April 2012 and accepted 22 July 2012.

diabetes.diabetesjournals.org

nature
medicine

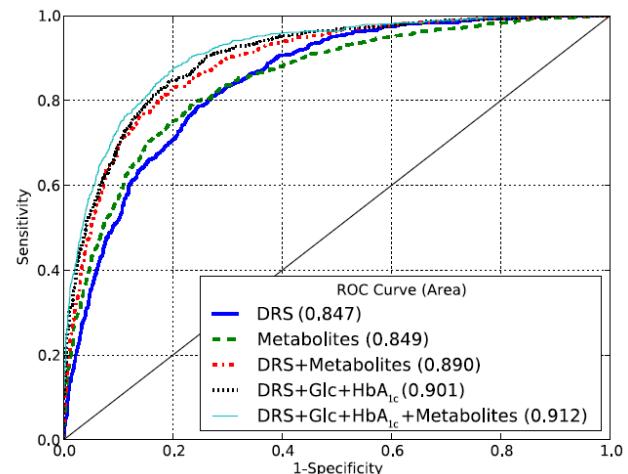
ARTICLES

Metabolite profiles and the risk of developing diabetes

Thomas J Wang^{1–3}, Martin G Larson^{3,4}, Ramachandran S Vasan^{3,5}, Susan Cheng^{2,3,6}, Eugene P Rhee^{1,7,8}, Elizabeth McCabe^{2,3}, Gregory D Lewis^{1,2,8}, Caroline S Fox^{3,9,10}, Paul F Jacques¹¹, Céline Fernandez¹², Christopher J O'Donnell^{2,3,8}, Stephen A Carr⁸, Vamsi K Mootha^{8,13,14}, Jose C Florez^{8,13}, Amanda Souza⁸, Olle Melander¹⁵, Clary B Clish⁸ & Robert E Gerszten^{1,2,8}

Received 7 April 2010; accepted 19 January 2011; published online 20 March 2011; doi:10.1038/nm.2307

The metabolites significantly improved T2D prediction compared with established risk factors.



Prognosis of Drug Response

MOLECULAR ONCOLOGY XXX (2012) 1–11



available at www.sciencedirect.com
SciVerse ScienceDirect

www.elsevier.com/locate/molonc

Molecular Oncology

Metabolomics approach for predicting response to neoadjuvant chemotherapy for breast cancer

Siwei Wei^a, Lingyan Liu^b, Jian Zhang^a, Jeremiah Bowers^a, G.A. Nagana Gowda^a, Harald Seeger^c, Tanja Fehm^c, Hans J. Neubauer^c, Ulrich Vogel^d, Susan E. Clare^e, Daniel Raftery^{a,*}

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.

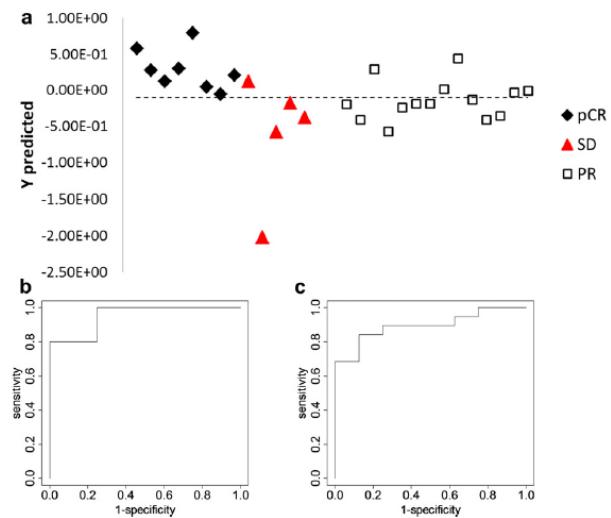


Figure 4 – (a) Prediction results for the PLS-DA model based on combining isoleucine, glutamine, and threonine detected by NMR and linolenic acid detected by LC–MS; (b) ROC curve for pCR vs. SD using the cross-validated predicted class values (AUROC = 0.95); (c) ROC curve for pCR vs. the other two groups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

Prognosis of Drug Response

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,¹ Alexandra Gruber,² Benedikt Giessrigl,³ Georg Krupitza,³ Thomas Szekeres,⁴ and Denise Sonntag²

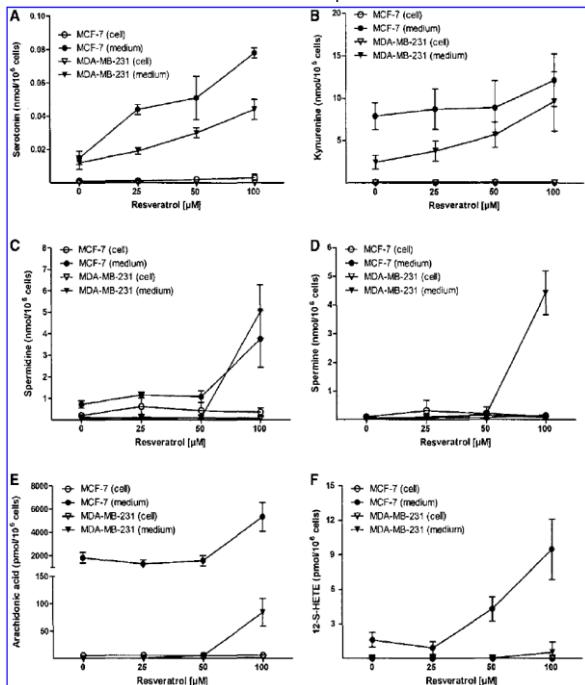


FIG. 1. Induction of serotonin (A), kynureine (B), spermidine (C), spemine (D), arachidonic acid (E), and 12S-HETE (F) in the human breast cancer cell lines MCF-7 and MDA-MB-231 after incubation with resveratrol (0–100 μM) for 72 h. Data represent the mean ± SD of triplicate determinations.

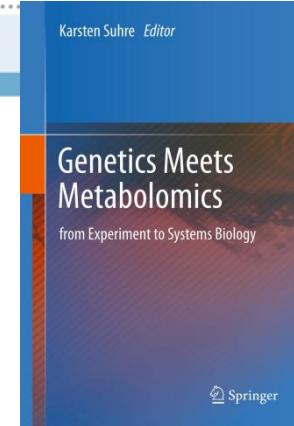
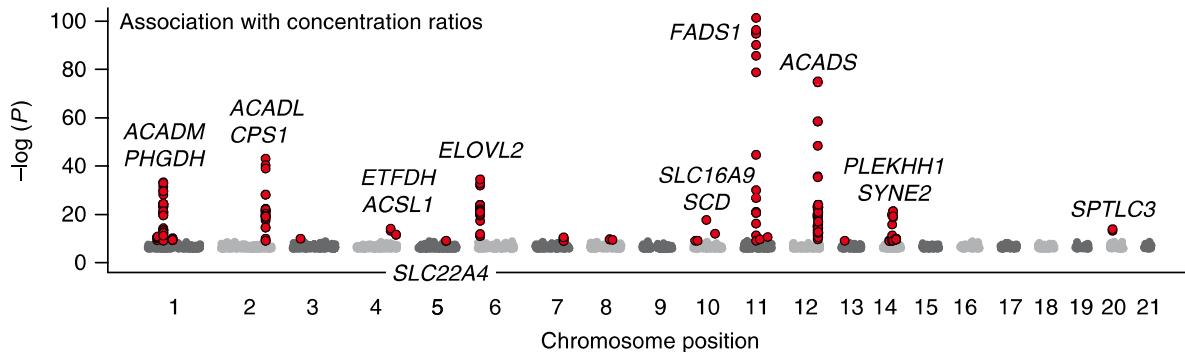
Resveratrol is a naturally occurring anticancer compound present in grapes and wine with antiproliferative properties against breast cancer cells and xenografts.

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

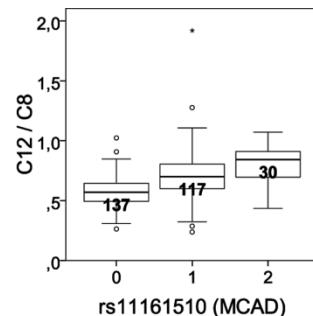
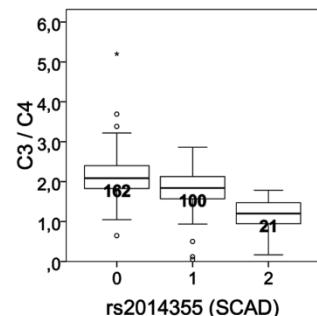
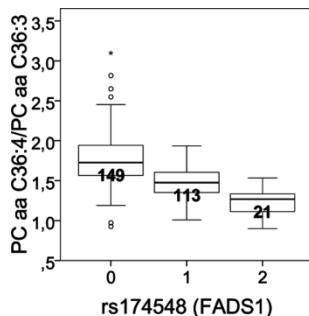
! G2P, Tox, Cell Culture, Cancer !

The Dawning of System Biology

Combination of genomics and metabolomics



- Improved statistical power when applying metabolite ratios as phenotype for GWAS
 - Genome-wide significances even in **relatively small cohorts (284 subjects)**
 - Fantastic associations **in standard-sized studies (p-values down to 10-179)**
- High percentage of variance explained (up to 36 % for phospholipid metabolism)
- More meaningful hits in genes for enzymes and transporters (**functional confirmation of genotype**)
- Clear **intermediary phenotypes of heterozygotes**



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

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, 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

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

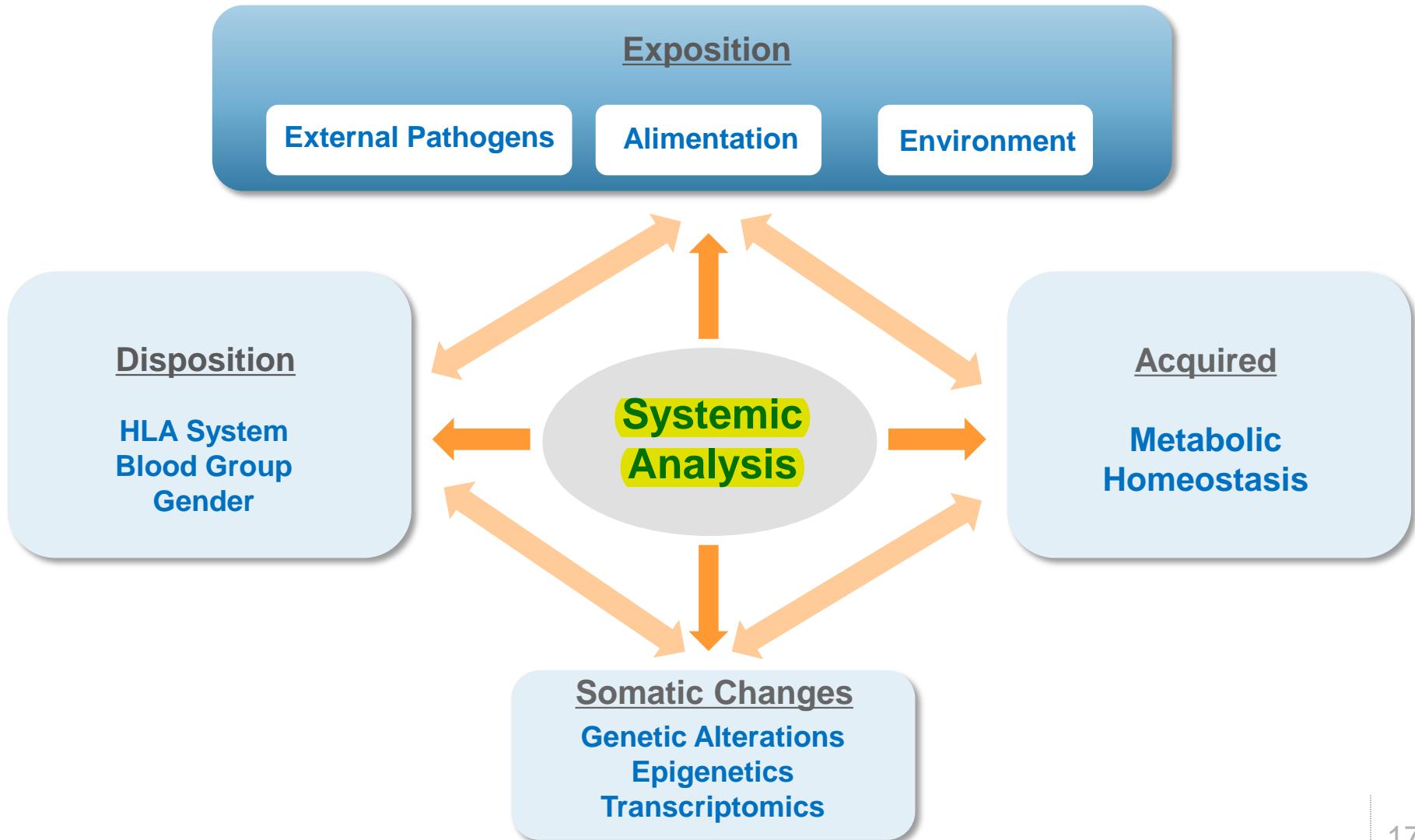
Pathways/Signaling

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

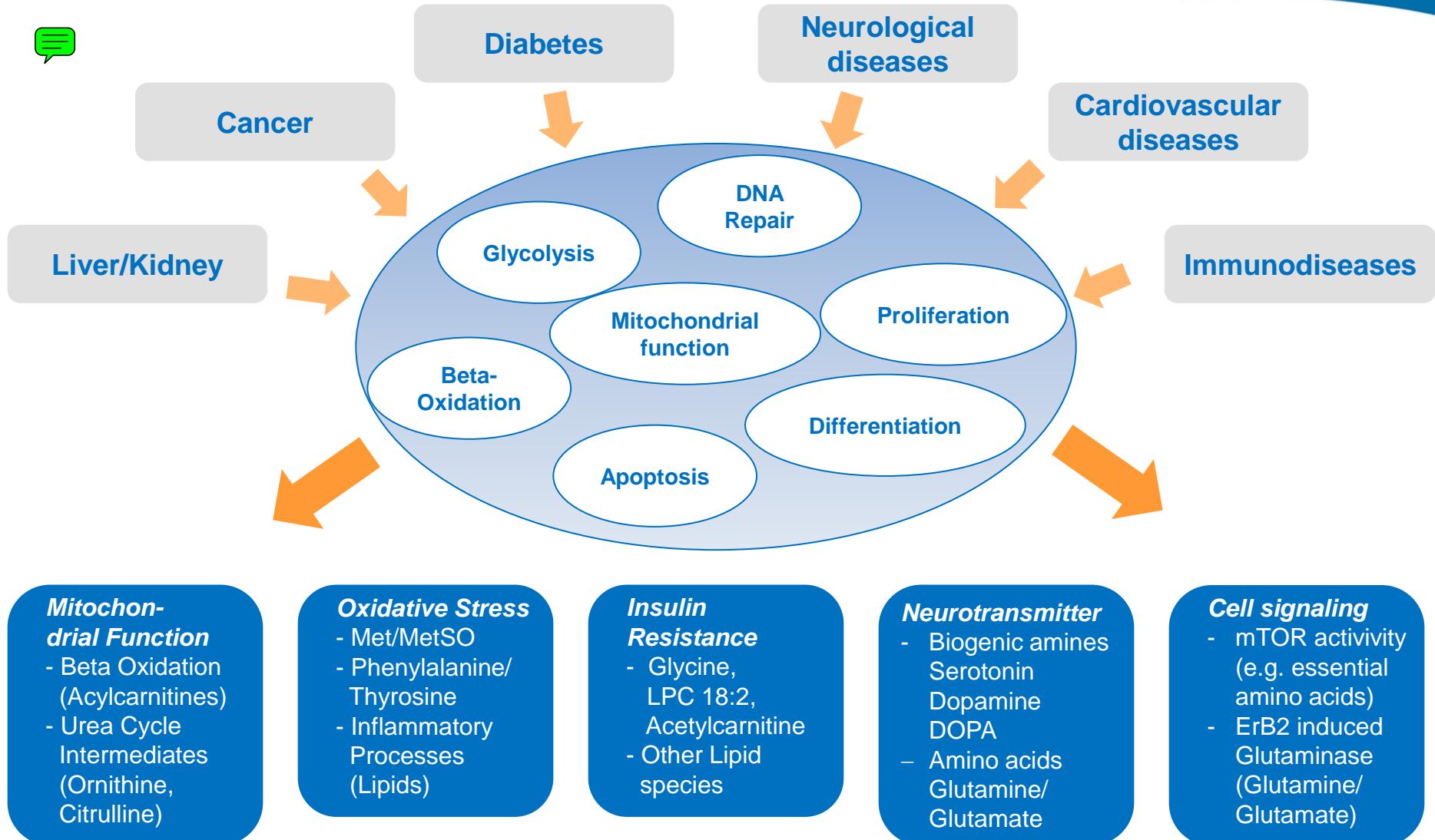
(some with signaling functions)

Comprehensive Individual System Analysis

Surrogate marker for individual phenotype expression

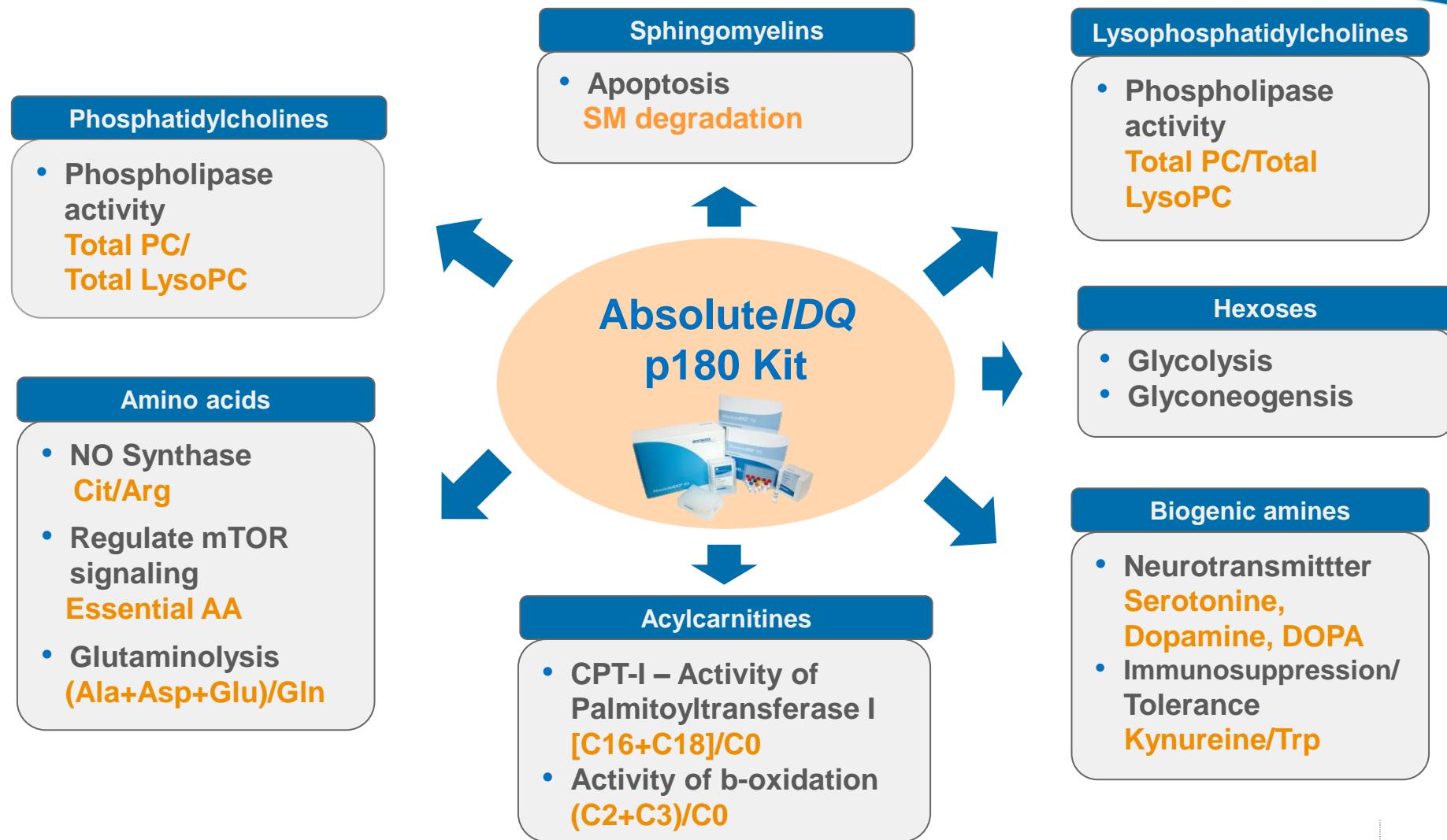


Phenotype reveals Pathophysiological Mechanism and Disease Specific Markers



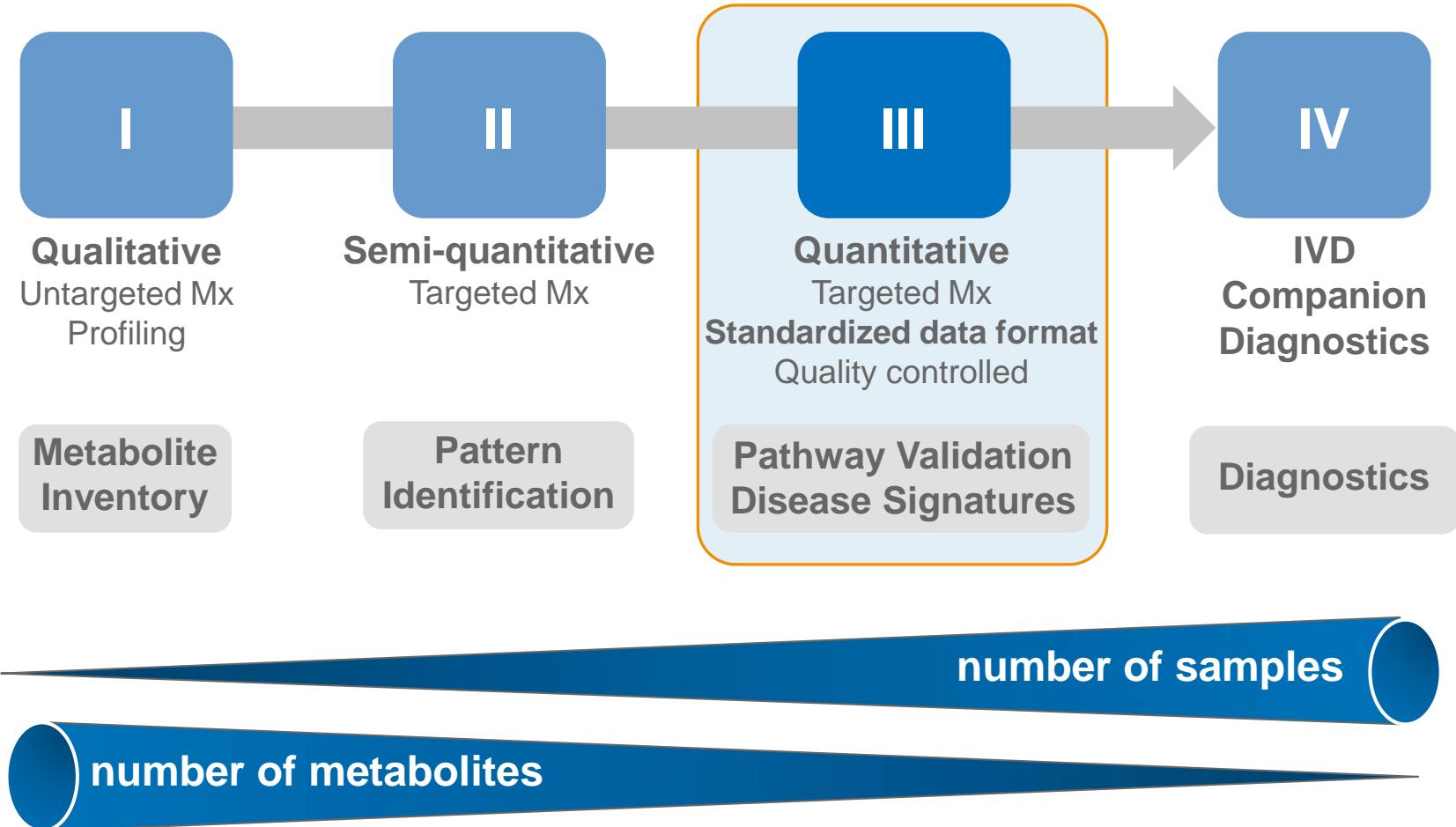
Technology and Products

Analyte Groups covered by AbsoluteIDQ p180 Kit



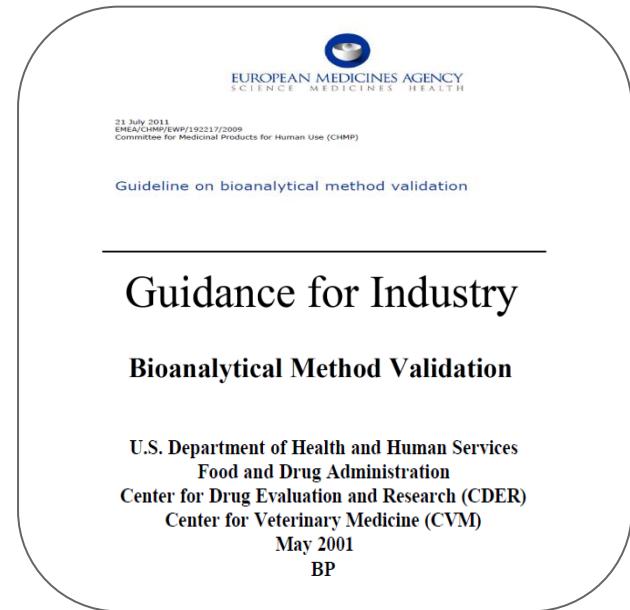
Evolution of Metabolomics (Mx) Research

Biocrates provides targeted, standardized analytic

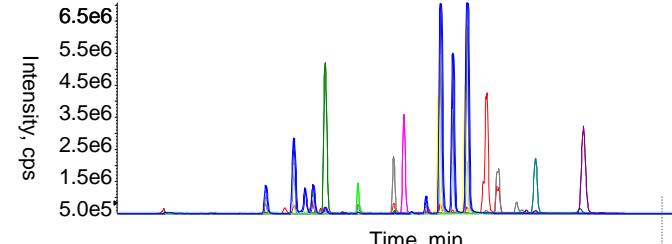
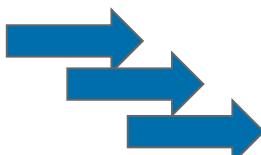
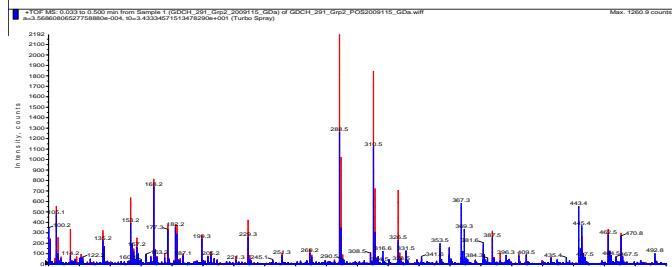


Metabolomics by Biocrates Kits

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



Broad Analytical Panel

> 210 Analytes in Kits, > 630 Analytes in Services Lab

Metabolite Classes	Metabolites	Contract Research	p150 Kit	p180 Kit	MetaDis Kit	SteroIDQ Kit	Stero17 Kit	Vitamin D
Acylcarnitines	40 - 41	x	x	x	x			
Amino acids	14 - 21	x	x	x	x			
Hexose	1	x	x	x	x			
Sphingolipids	15	x	x	x	x			
Glycerophospholipids	90 - 92	x	x	x	x			
Biogenic amines	14 - 19	x		x	x			
Steroid hormones	16-17	x				x	x	
Neurotransmitters	9	x						
Bile acids	17	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						

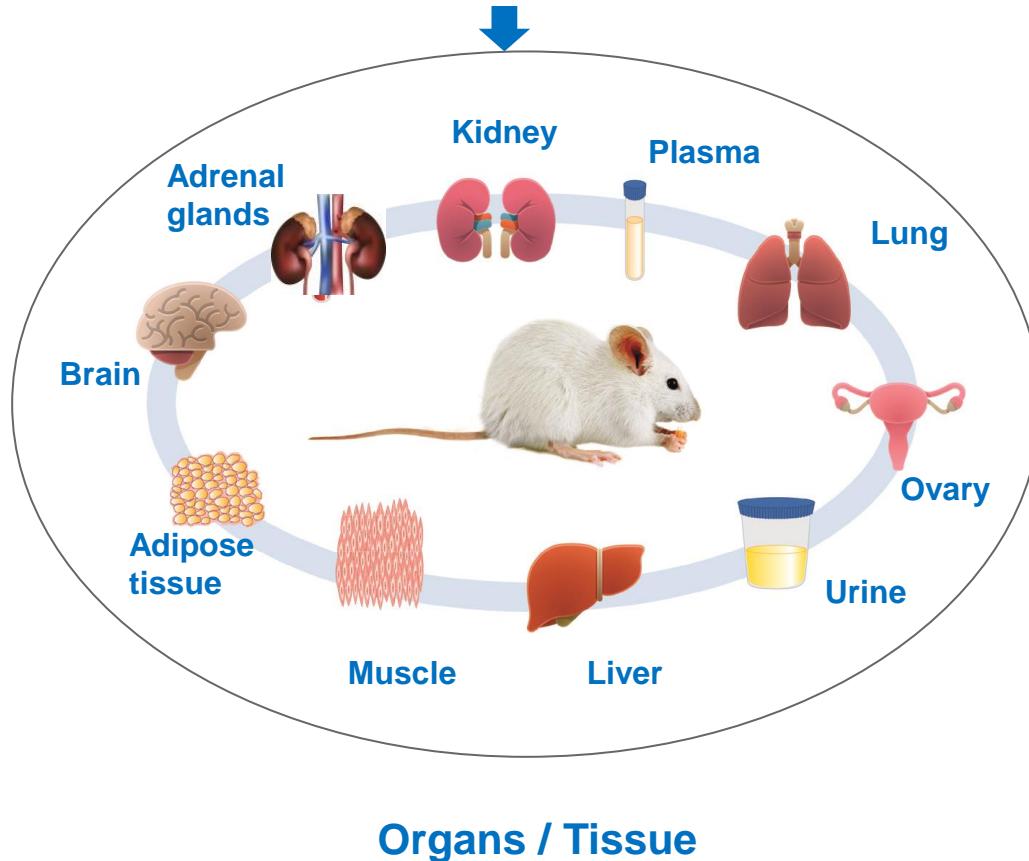
Matrices/ Species

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

BIOCRATES Kits & Services



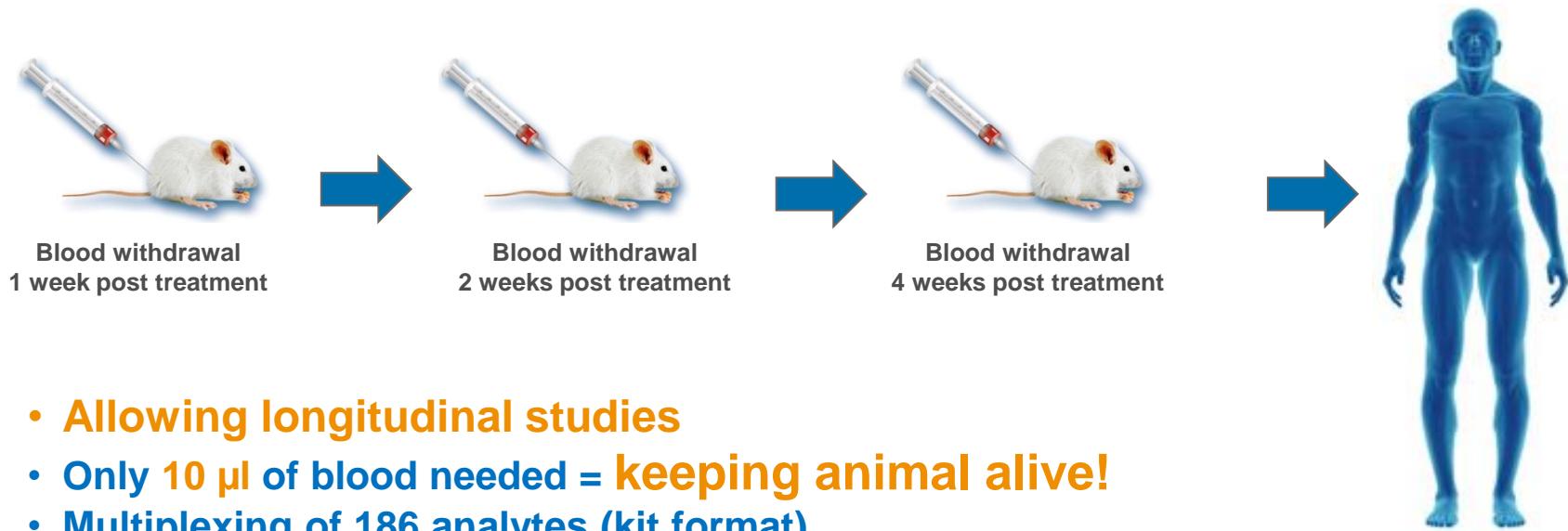
Species

- 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

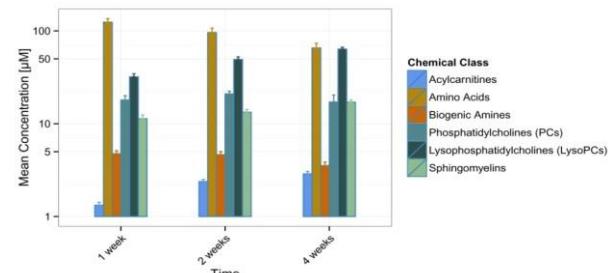
From Preclinical to Clinical Phenotyping

- Following disease progression in animal model over time



- Allowing longitudinal studies
- Only 10 µl of blood needed = keeping animal alive!
- Multiplexing of 186 analytes (kit format)
- Reproducible results
- Standardized & quality controlled & easy to use solutions
- Different genes – same metabolites

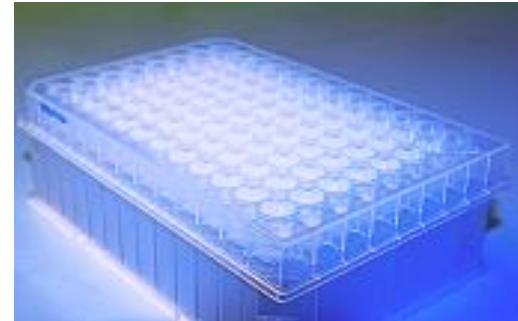
Assays validated for human plasma
Applicable to many biological materials



AbsoluteIDQ® p180 Kit



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

Phenotyping Research Products

AbsoluteIDQ® p180 Kit

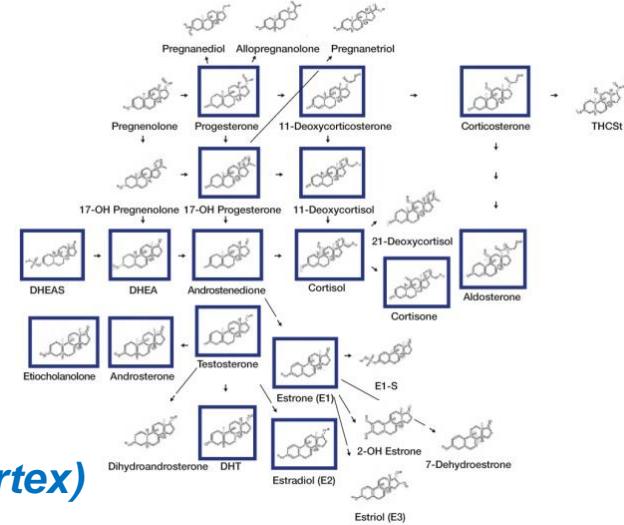
- Acylcarnitines
- Glycerophospho- and sphingolipids
- Hexose
- Amino acids (LC-MS/MS)
- Biogenic amines (LC-MS/MS)



186 analytes

AbsoluteIDQ® Stero 17 Kit

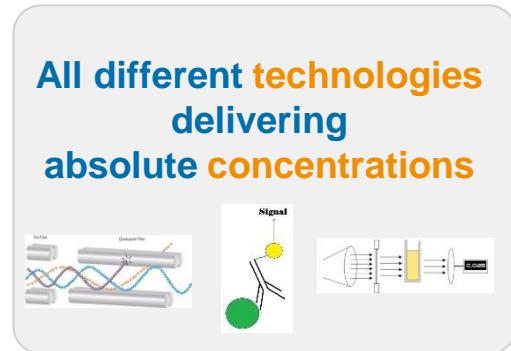
- Mineralocorticoids
- Glucocorticoids
- Sex Steroids



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

Standardization of Data Formats

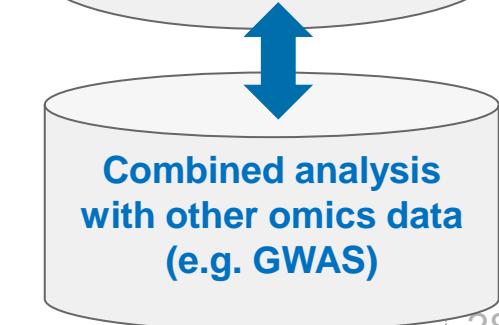
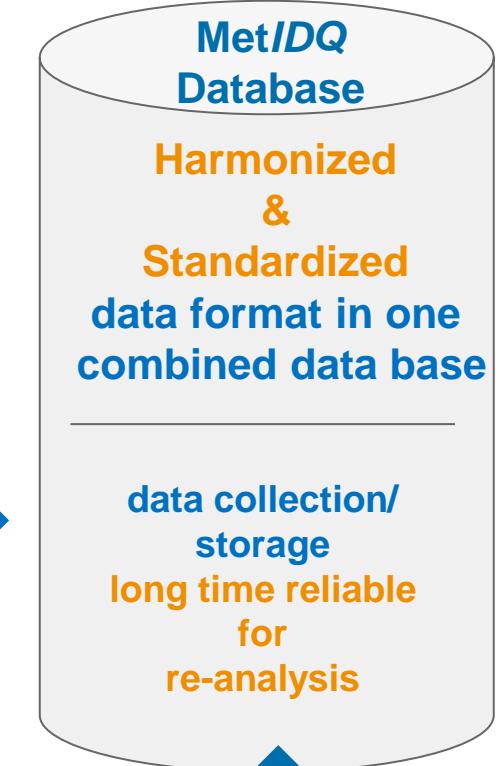
Multidimensional Compilation of Data for Analysis



BIOCRATES Kits & Software



- Include normalization
- Include quality control with same QC



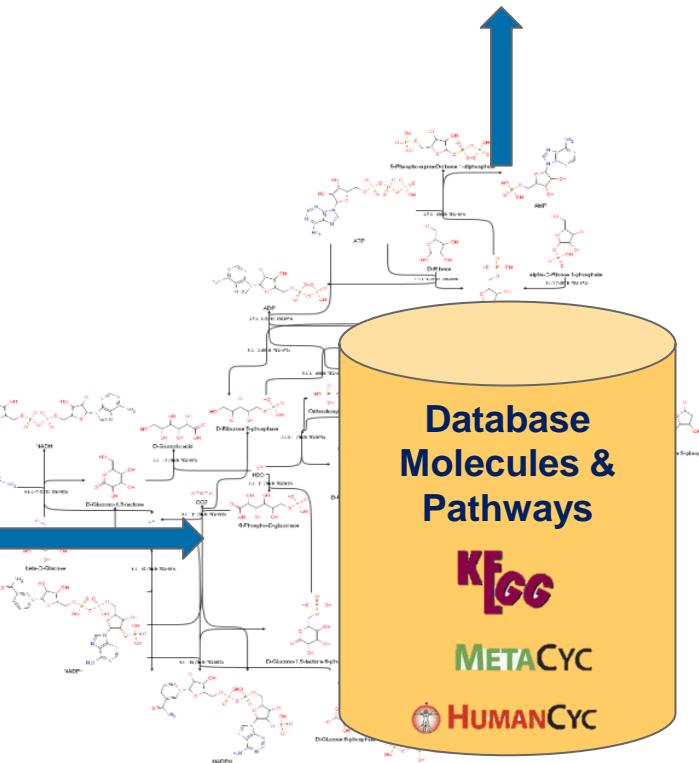
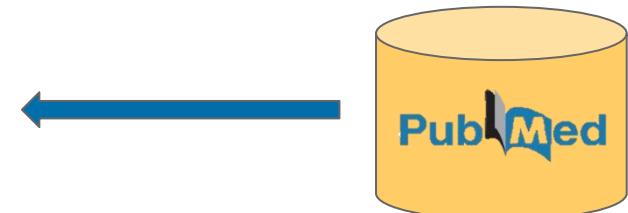
Key Know How in Kit & Data Analysis

Integration of Run Data and External Data

→ Link metabolite signatures to pathways and to diseases

Figure: Number of publications in pubmed (last 10 years): pathways/ diseases

Dataset	Result Matrix	Total	Cancer	Diabetes	Liver disease
KEGG 60.1	Steroid degradation	144819	18531	7646	5021
KEGG 60.1	Steroid biosynthesis	96256	14267	5221	3835
KEGG 60.1	Fatty acid metabolism	80039	8583	7902	5340
KEGG 60.1	Fatty acid biosynthesis	69301	8107	7068	4812
HumanCyc 16.1	cholesterol biosynthesis I	62631	2816	7131	3690
KEGG 60.1	Steroid hormone biosynthesis	59491	9475	3077	1281
HumanCyc 16.1	tyrosine degradation I	57715	18025	1935	1245
KEGG 60.1	Tyrosine metabolism	57449	17915	1927	1240

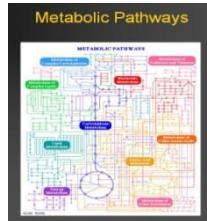
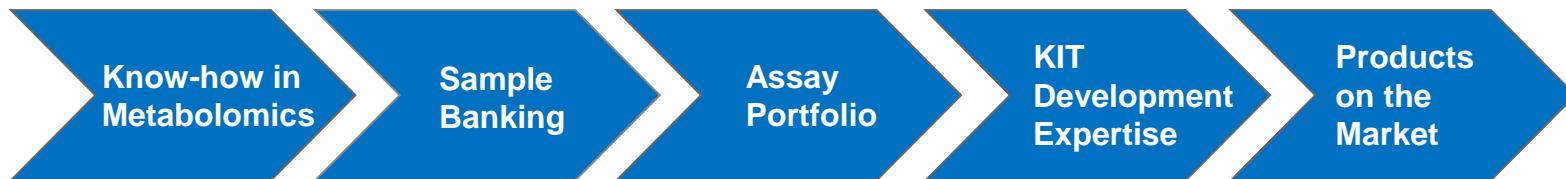


Metabolites of AbsoluteIDQ p180 Kit can be found in approx. 200 pathways



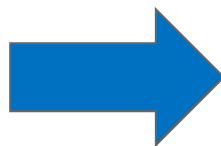
BIOCRATES - Matching of Knowledge

Fully Integrated Center of Competency



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

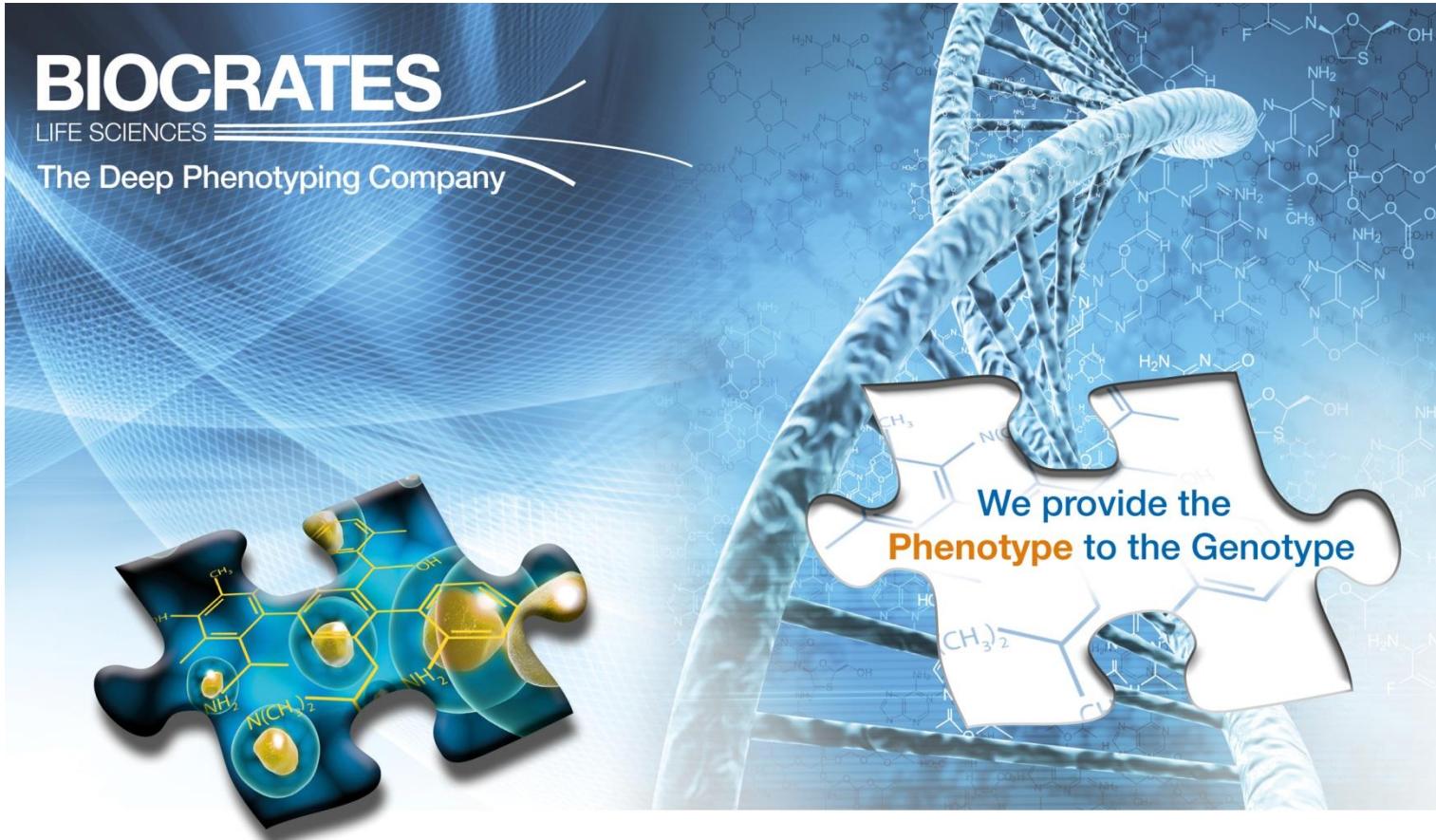


Dr. Edwin Scott- Asemota (Nigeria):
...is this Voodoo medicine?



...or the Next Generation Technology in System Diagnostics?

Thank you!
From Genotype to Phenotype



wulf.fischer-knuppertz@biocrates.com