

1st International Electronic Conference on Metabolomics

1-30 November 2016
chaired by Dr. Peter Meikle



Clinical Metabolomics: Analytical Tool for Drug Development.

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Clinical Metabolomics: Analytical Tool for Drug Development.

POPULATION BASED PHENOMIC STRATIFICATION USING *BERG* CLINICAL TRIALS

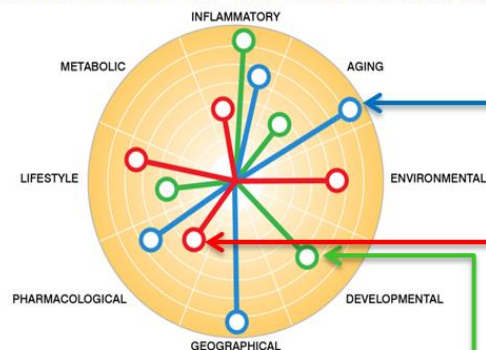
Environmental/Physiological Influence

Genome (~30,000 genes) Transcriptome (~10⁵ RNA transcripts) Proteome (Including PTM) (10⁵ proteins) Metabolome/Lipidome (1,000's Metabolites/Lipids)

PHENOME



POPULATION VARIATION/MOLECULAR SIGNATURE OF CO-MORBIDITIES

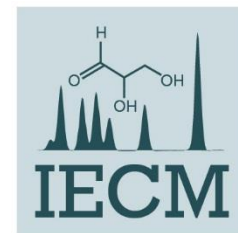


POPULATION MOLECULAR DIVERSITY



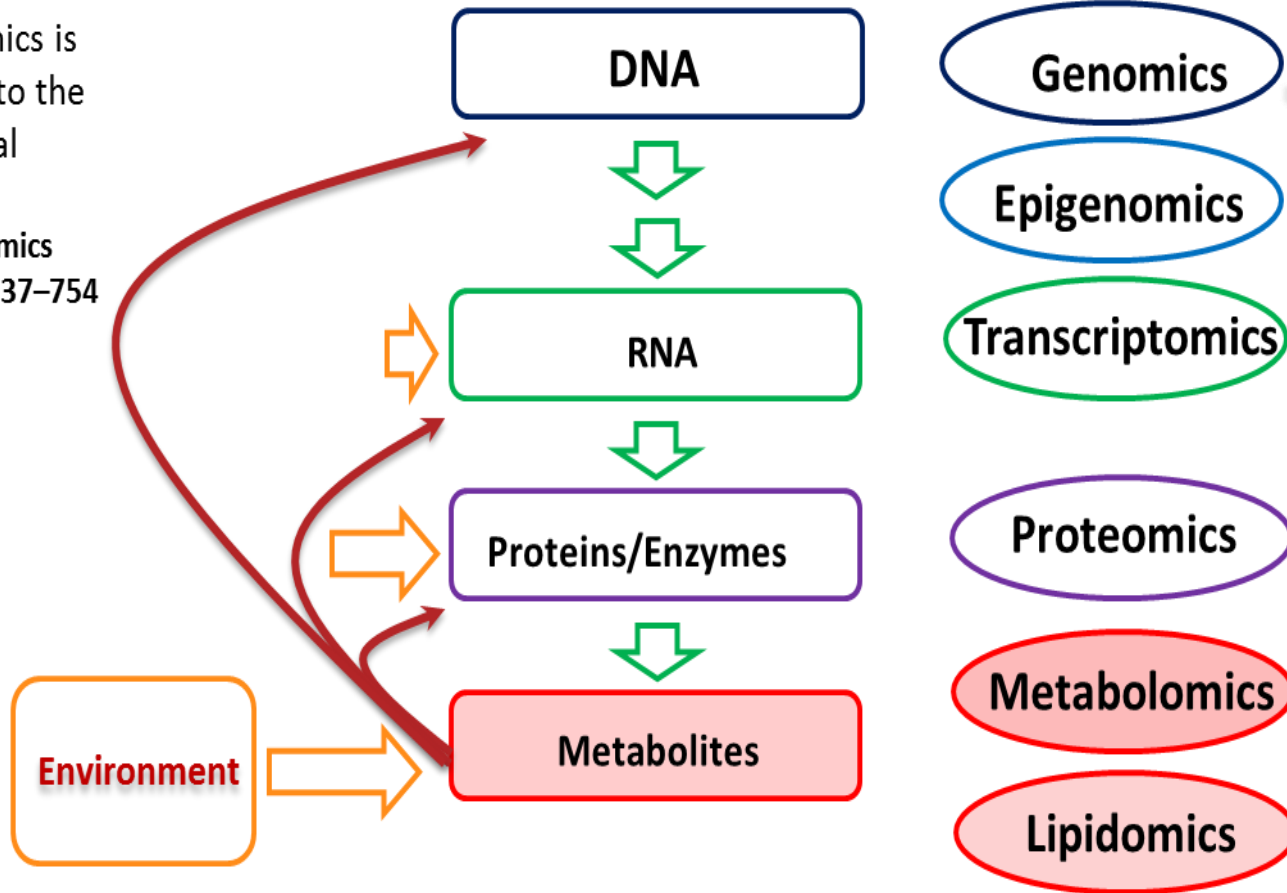
Abstract: It is recognized that altered metabolic states reports on the chronic and acute disease statuses. Decades of research have shown that metabolism is not a self-regulating network operating independently but rather heavily integrated into every cellular process and involved in organ system functions. Therefore global monitoring of metabolic processes is recommended for more comprehensive understanding of the initiation and advancement of disease. Mass spectrometry based metabolomics, in particular, demonstrates tremendous promise in delivering high throughput quantitative information on alterations in metabolism associated with disease onset/progression and response to pharmaceutical intervention. Recent advances in mass spectrometry and informatics tools have facilitated emerging in house OMICS platforms capable of translating biological output into viable therapeutic candidates and assist in stratifying patient populations. At BERG, we have implemented an industrial level high throughput metabolomics platform providing both high quality and depth of information allowing for reliable and broadest capture of the metabolome for the pre-clinical and clinical matrixes analyzed. Global metabolomics platform dedicated for theranostic and clinical studies as well as tracer metabolomics are harvested to facilitate CDx biomarkers discovery in a unique way. Highlights of the BERG's in-depth patient stratification approaches as well as biology based drugs are presented.

Keywords: Metabolomics, discovery, clinical, network, patient stratification, CDx markers.



SYSTEMS BIOLOGY

...Pure genomics is almost blind to the environmental elements...
Pharmacogenomics
(2015), 16(7), 737-754



Human Metabolomes

3100 (T3DB)

Toxins/Env. Chemicals

1000 (DrugBank)

Drug metabolites

30000 (FooDB)

Food additives/Phytochemicals

1450 (DrugBank)

Drugs

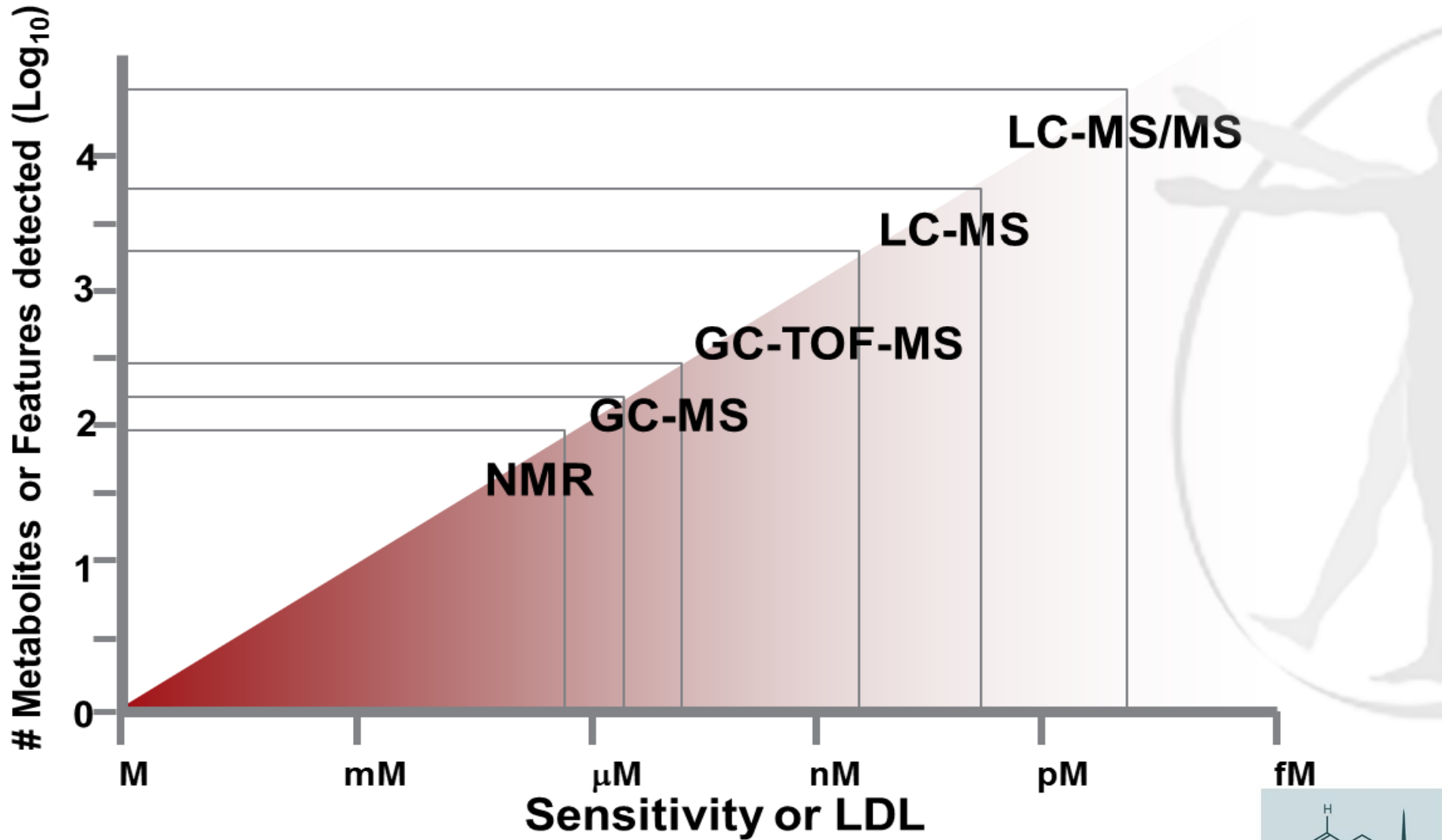
8500 (HMDB)

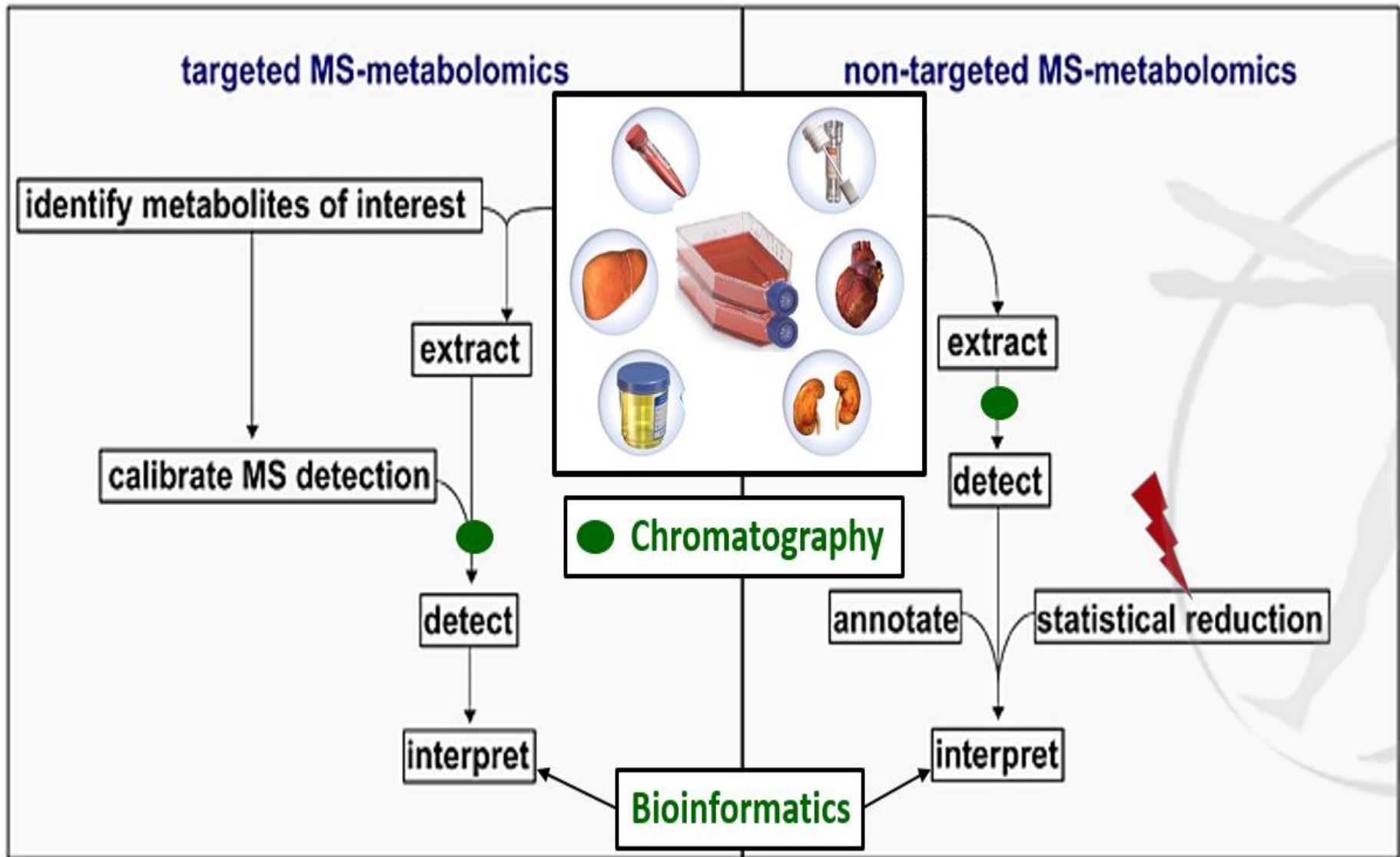
Endogenous metabolites



Gut Microbiome

TECHNOLOGY & SENSITIVITY





$$\frac{\text{variables}}{\text{samples}} =$$

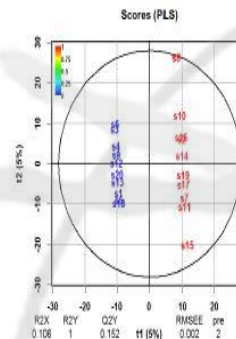
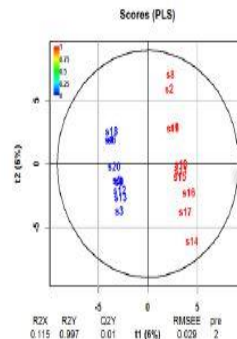
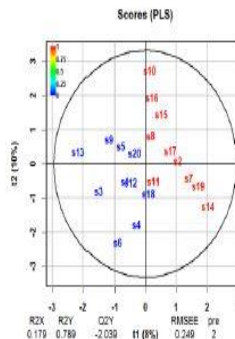
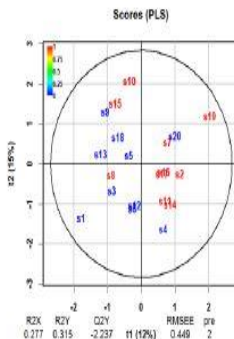
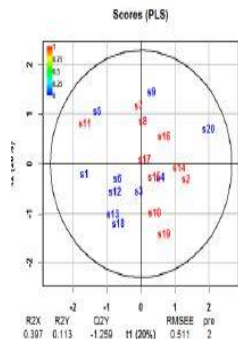
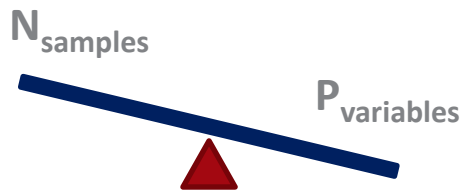
0.2

0.5

1

10

100



Metabolomics (2015) 11:9-26
DOI 10.1007/s11306-014-0707-1

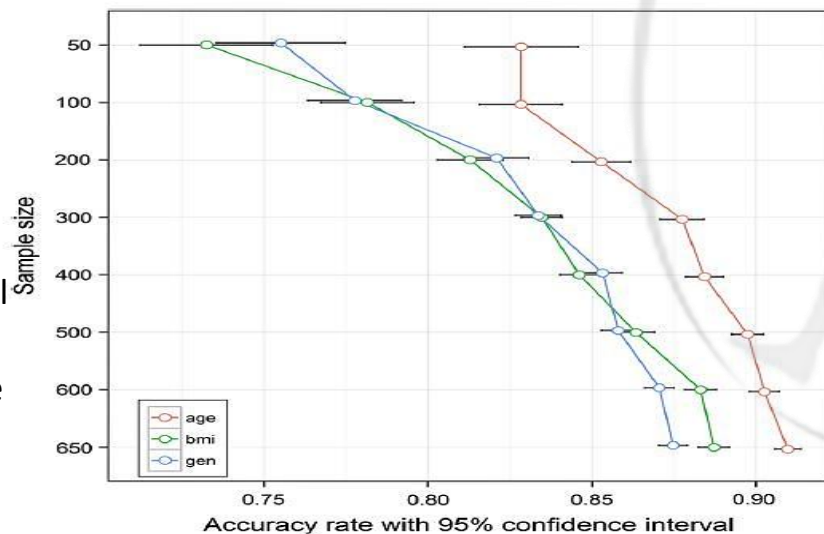
ORIGINAL ARTICLE

Molecular phenotyping of a UK population: defining the human serum metabolome

Warwick B. Dunn · Wanchang Lin · David Broadhurst · Paul Begley · Marie Brown · Eva Zelena · Andrew A. Vaughan · Antony Halsall · Nadine Harding · Joshua D. Knowles · Sue Francis-McIntyre · Andy Tseng ·

..... It is becoming increasingly evident that many biological studies are underpowered with regard to their ability to come to a robust and statistically significant and justifiable biological conclusion

Szymanska E., Saccenti E., Smilde A. and Westerhuis J. (2012). *Metabolomics*, 8:3-16



Metabolic Biomarkers

Disease	Metabolite
Anemia	Folic Acid (folates)
	Vitamin B12
Bone Diseases	Vitamin D, 1,25 Dihydroxy
Cardiac Markers	CyclicAMP
	Homocysteine
CNS Diseases	5-Hydroxy indole acetic acid
	Dihydroxyphenyl acetic acid
	Homovanillic acid
Diabetes	Free Fatty Acids (FFA)
	Glucose
Endocrinology	Testosterone
	Cortisol
Gastroenterology	Serotonin
Infectious Diseases	N/A
Inflammation/Immunity	CyclicAMP
	Cortisol
	Prostaglandin E2
Lipid Metabolism	Cholesterol
	TG
Nephrology	Creatinine
Oncology	N/A
Thyroid Markers	Total Thyroxin (T4)
	Liothyronine



Inherited Metabolic Diseases

Argininemia
Argininosuccinic Acid Lyase deficiency
Beta ketothiolase deficiency
Carnitine cycle disorders
Carnitine palmitoyl transferase 1 deficiency (CPT-1)
Carnitine translocase deficiency
Citrullinemia
Fatty Acid Oxidation defects
Galactosemia
Glutaric acidemia types 1 and 2
Glutathione synthetase deficiency
Homocystinuria
Hypermethioninemia
Hyperprolinemia
Isovaleric acidemia
Long chain hydroxyl acyl CoA dehydrogenase deficiency (LCHAD)
Lysosomal Storage Diseases
Maple syrup urine disease
Malonic aciduria
Medium chain acyl CoA dehydrogenase deficiency (MCAD)
Metabolic acidosis
3-Methylcrotonyl CoA carboxylase deficiency
Methylmalonic acidemia
Multiple Acyl CoA dehydrogenase deficiency
Organic acidemias
Ornithine carbamoylase deficiency
Phenylketonuria
Propionic acidemia
Short chain acyl CoA dehydrogenase deficiency (SCAD)
Trimethylaminuria
Tyrosinemia
Urea cycle defects

Phenylalanine as a Biomarker

Phenylketonuria

Human Serum

20 - 400 folds Up



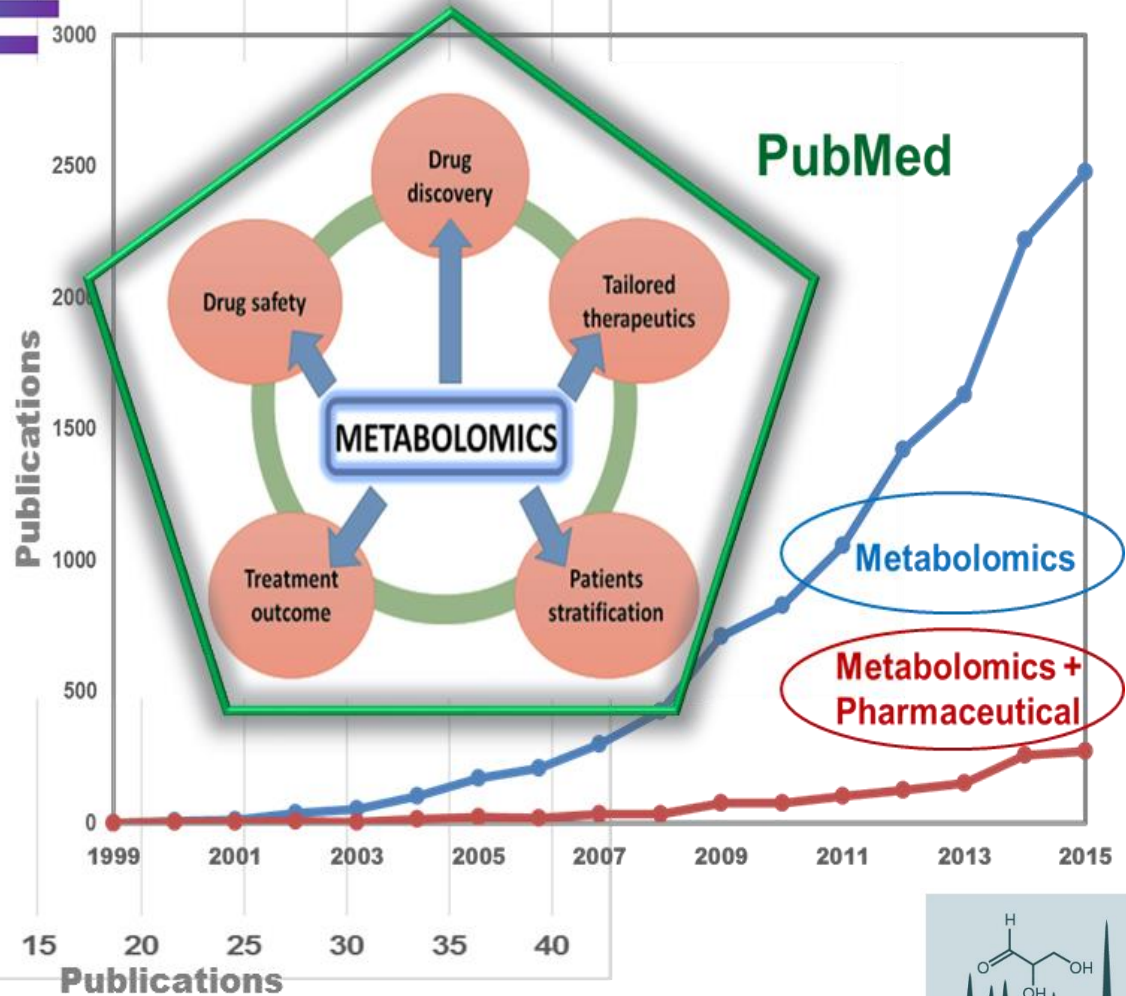
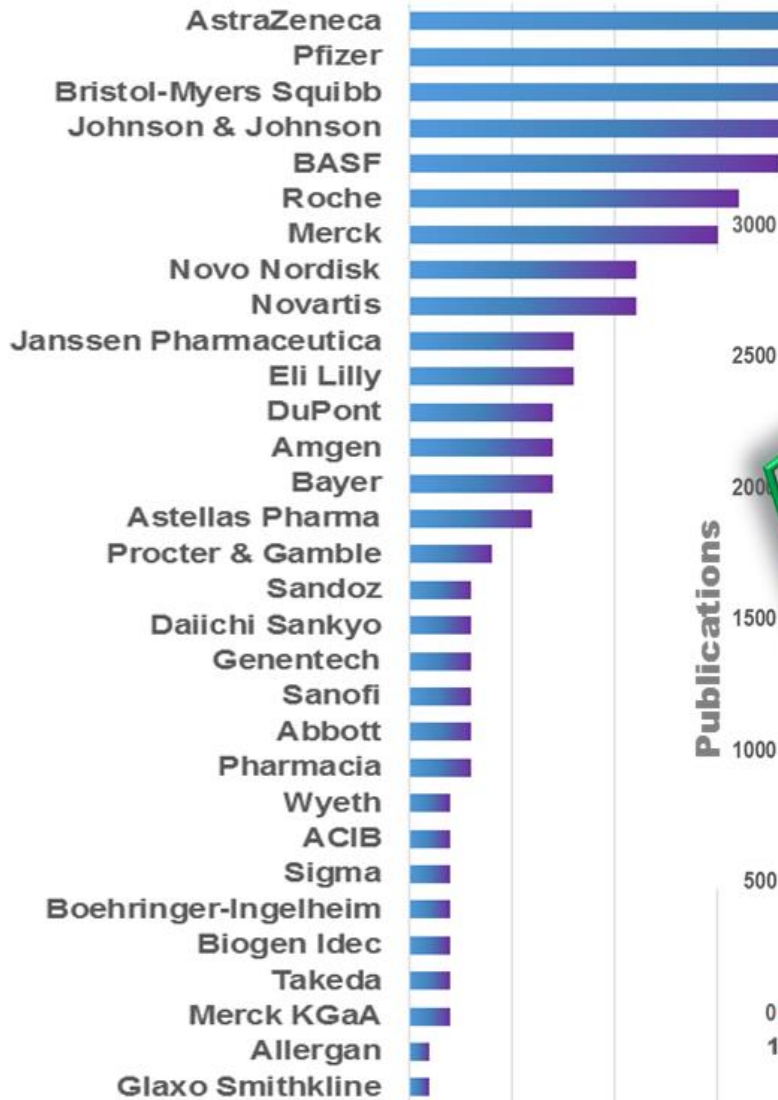
Metabolomics articles in PubMed reporting on Phenylalanine alterations

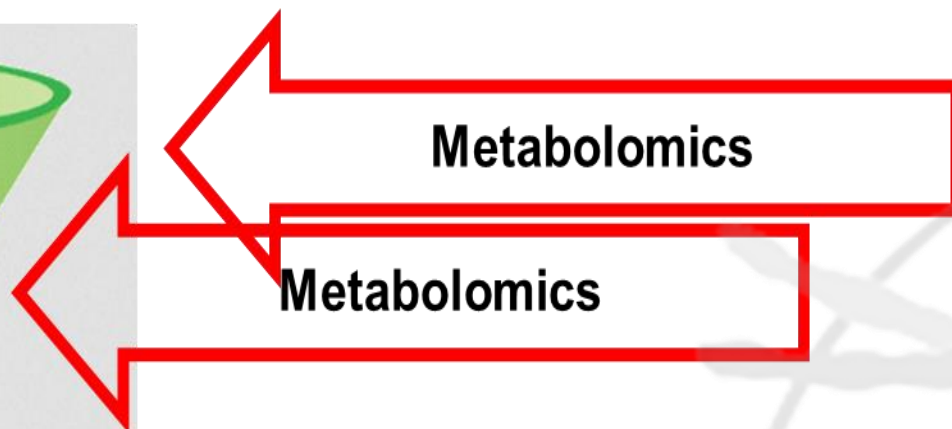
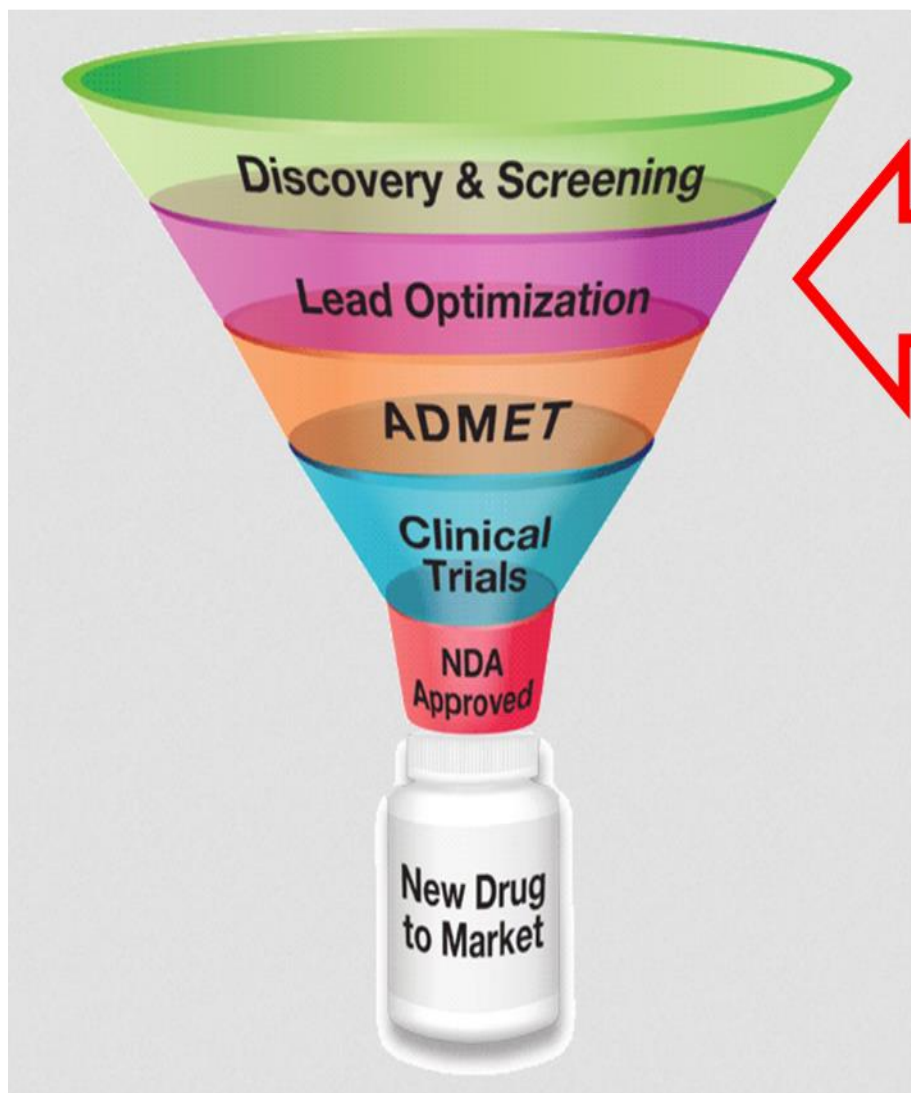
Condition	Subject	Sample	Changes	Year
Hepatocellular carcinoma	Human	Serum	Up	2015
Severe Sepsis and Septic Shock	Human	Urine	Down	2015
Asthma	Human	Serum	Up	2015
Rheumatoid arthritis	Human	Serum	Up	2015
Bladder cancer	Human	Serum, urine, tumor	Up	2015
Chronic Kidney Disease	Human	Serum	Up	2015
Hepatobiliary cancer	Human	Serum	Up	2015
Depression	Human	Plasma	Down	2015
Hepatocellular carcinoma	Human	Serum	Up	2015
Physical training	Human	Urine	Up	2015
Heart failure	Human	Plasma	Alteration	2015
Renal cell cancer	Human	Urine	Up	2015
Cardiovascular event risk	Human	Plasma	Up	2015
Alzheimer's disease	Human	Plasma	Up	2014
Type-2 diabetes mellitus	Human	Plasma	Up	2015

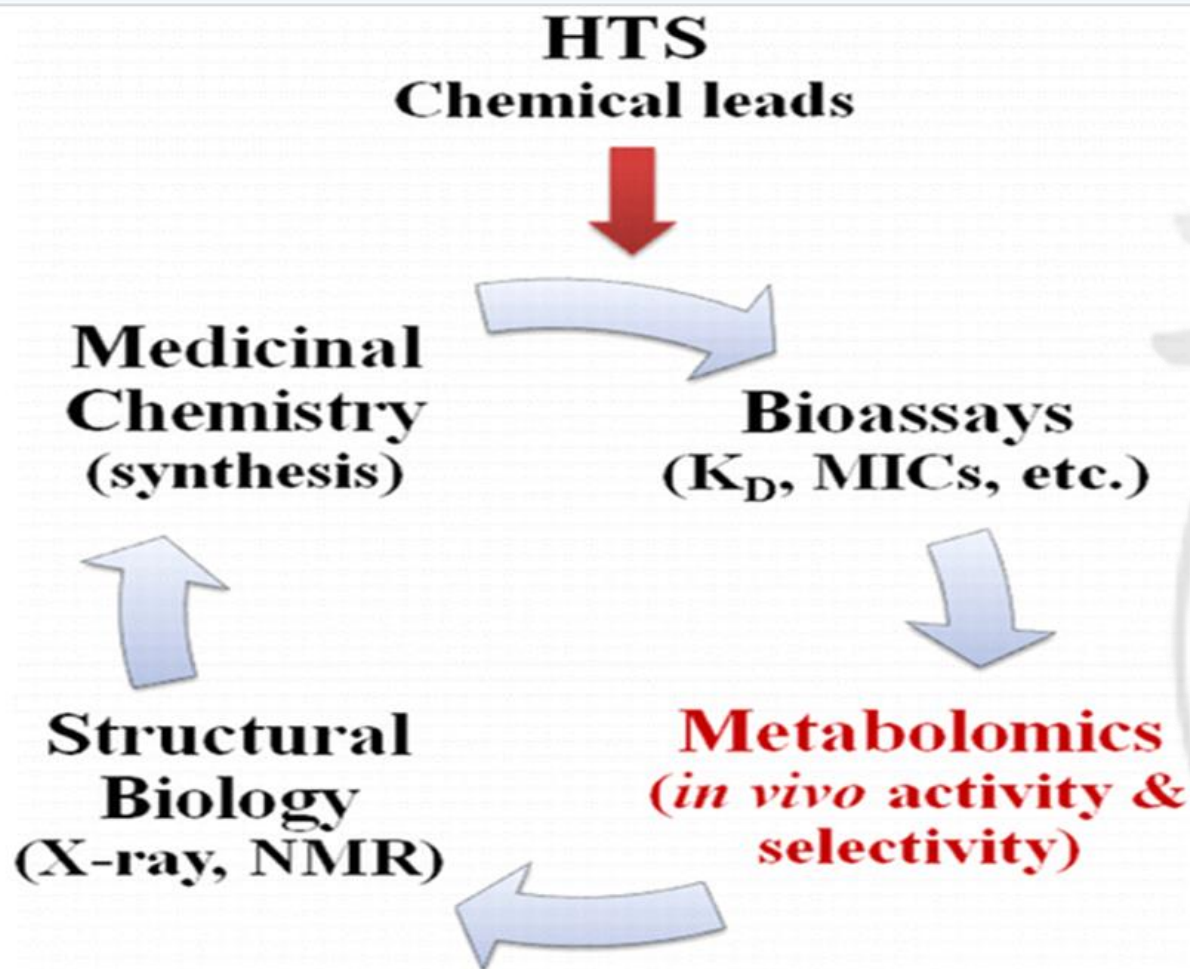
Current Challenges

- Lack of analytical validation for measuring biomarkers and often a lack of reliable evidence about their performance
- Lack of a common vocabulary and taxonomy for biomarkers
- Inadequate scientific information on the causes, biochemical pathways, and natural histories of many diseases, making identification of disease-specific biomarkers difficult
- Lack of public access to existing research and information on potential biomarkers
- Lack of generally-accepted evidentiary standards for qualifying new biomarkers for particular contexts of use

PubMed

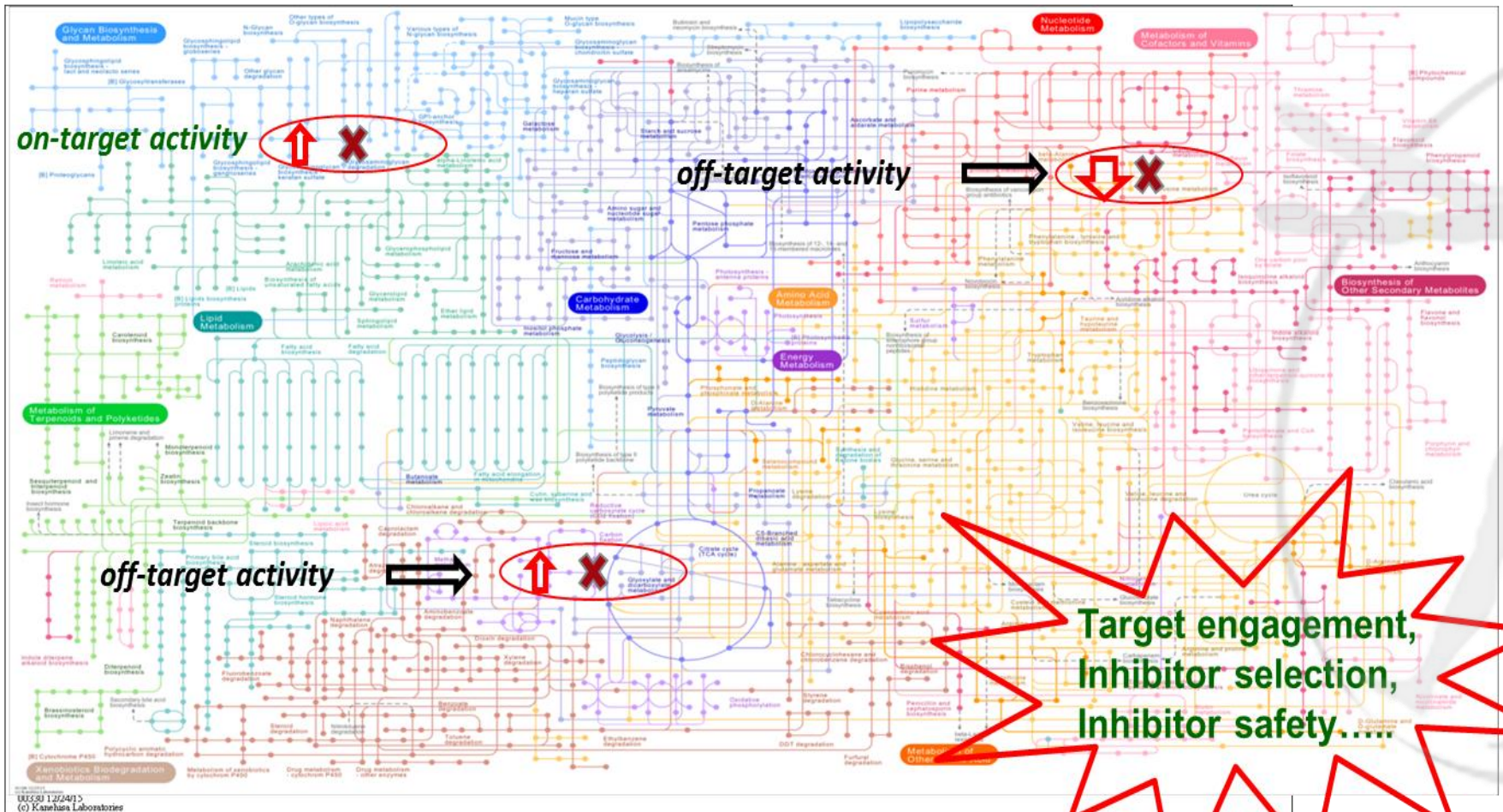






Robert Powers: The Current State of Drug Discovery and a Potential Role for NMR Metabolomics, *J. Med. Chem.*, 2014, 57 (14), pp 5860–5870

Metabolomics Impact on Metabolic Perturbations Discovery

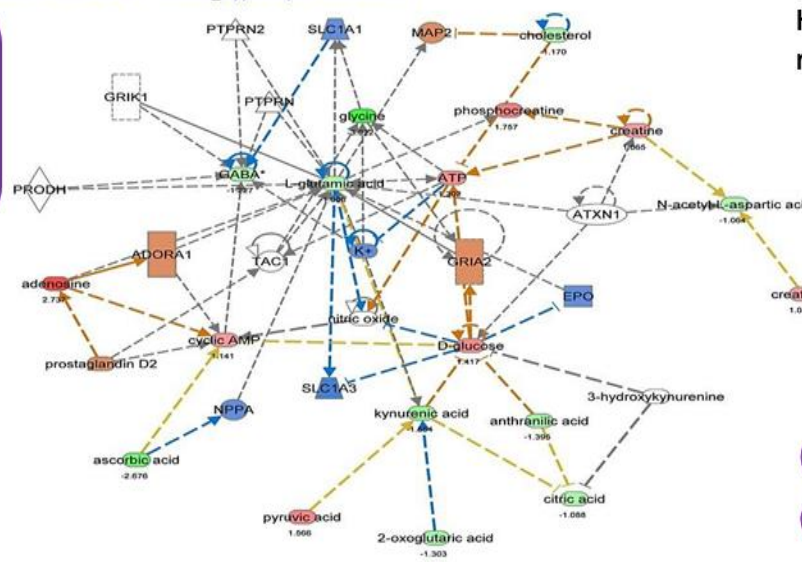


Metabolomics approaches to tackling MOA in preclinical animal study.

8th INTERNATIONAL MEETING ON METABOTROPIC GLUTAMATE RECEPTORS Taormina, Sicily-Italy
September, 2014

LY3020371: In vivo characterization of a novel mGlu2/3 receptor antagonist.
J.M. Witkin, C. Overshiner, X. Li, G. Gilmour, J. Li, L. Rorick-Kehn, K. Rasmussen, B. Johnson, SN Mitchell, K.G. Phillips, K.A. Wafford, D.L. McKinzie, A. Nikolayev, V.V. Tolstikov, M-S Kuo, P.L. Ornstein, C.H. Mitch, R. Li, S.C. Smith, X-S Wang, B.A. Heinz, D. Allen, S. Swanson and J.A. Monn (USA and UK)

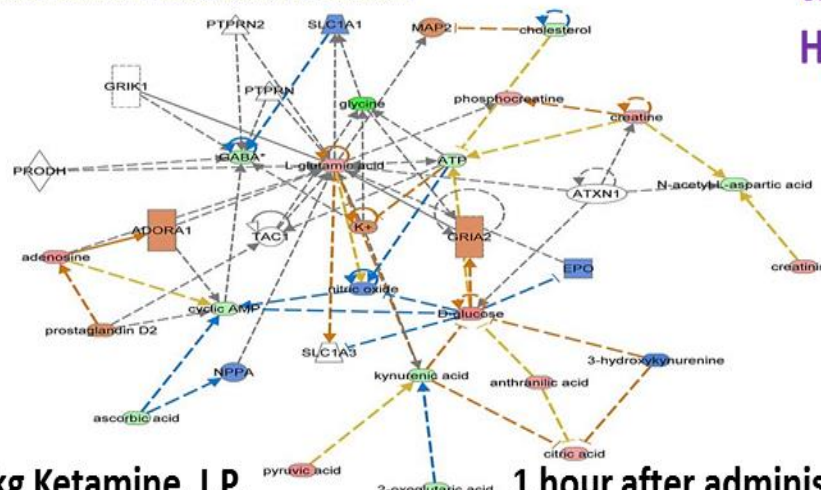
Network 2 : Observation 1 : mGlu23_Hippocampus : Observation 1



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10 mg/kg LY3020371, I.P. 1 hour after administration

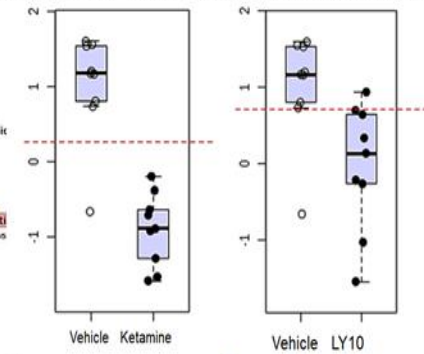
Network 2 : Observation 1 : mGlu23_Hippocampus : Observation 2



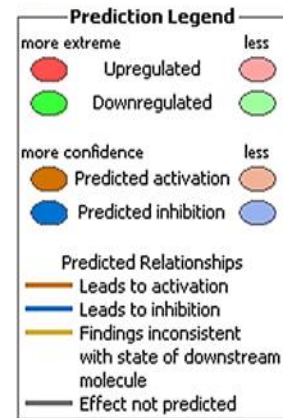
10 mg/kg Ketamine, I.P.

1 hour after administration

Hydroxyisocaproic acid/ nicotinamide ratio in CSF 1 hour after administration

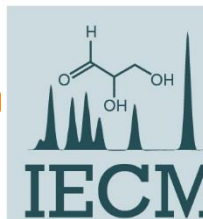


Common pathways - GRIA2(mGlu2/3) and ADORA1 – are predicted to be activated in Hippocampus.

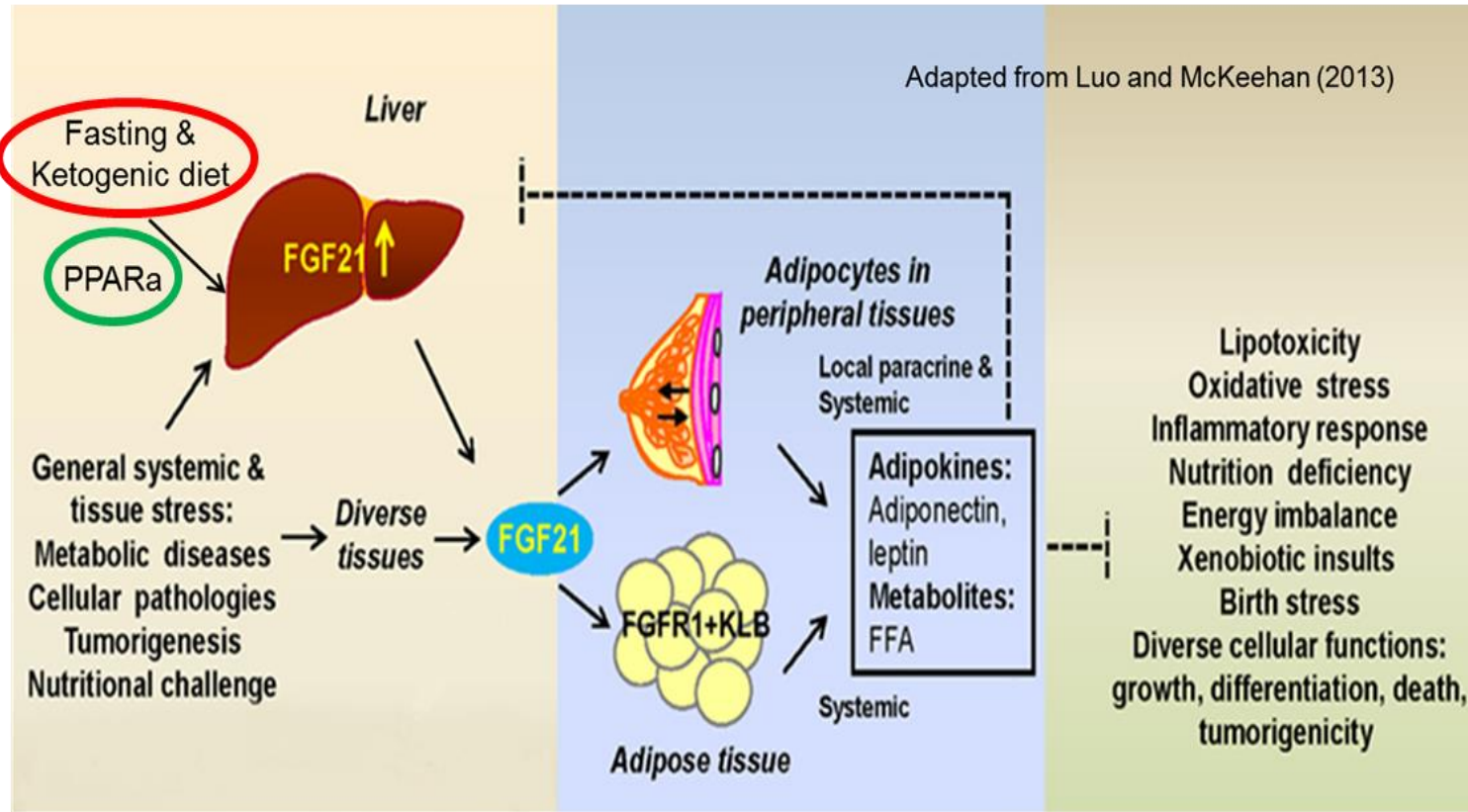


Ketamine has been tested in treatment-resistant bipolar disorder, major depressive disorder, and people in a suicidal crisis in emergency rooms. *Wikipedia*

Lilly



ANIMAL STUDY: FGF21 DIETARY INDUCTION

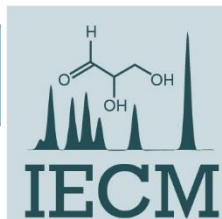


**Liver
Metabolism
and
Nonalcoholic
Liver Disease.
Keystone
Symposia on
Molecular and
Cellular
Biology
March 22-27,
2015 Whistler,
BC, Canada**

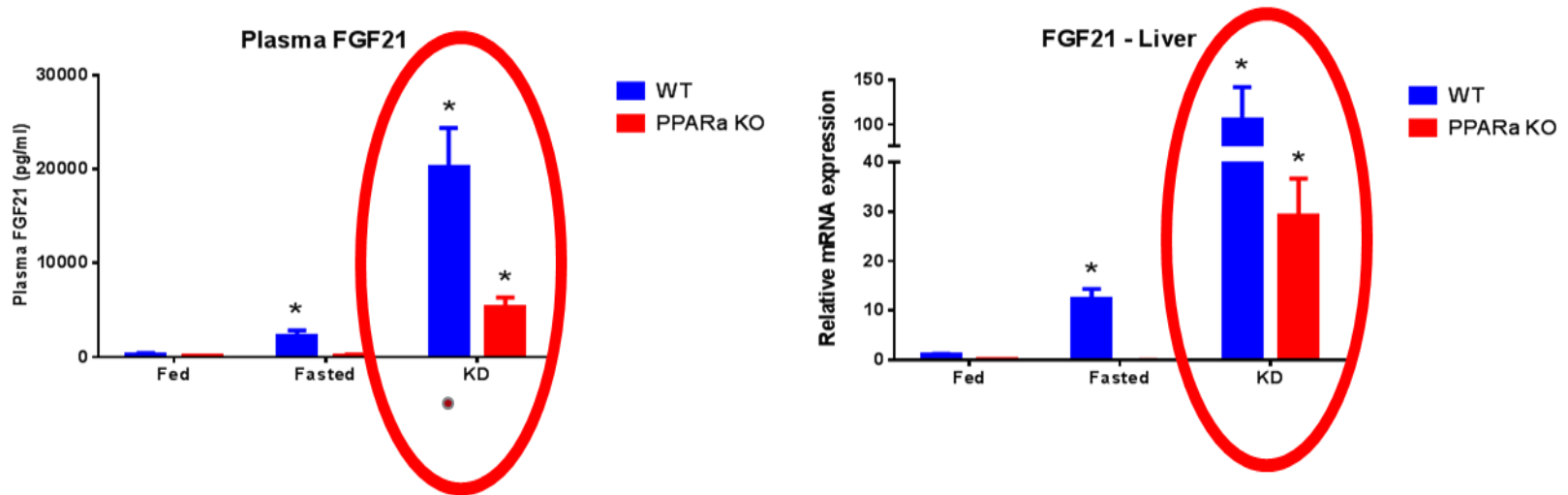
In mice FGF21 is strongly induced in liver by prolonged fasting via PPAR-alpha and in turn induces the transcriptional coactivator PGC-1 α and stimulates hepatic gluconeogenesis, fatty acid oxidation, and ketogenesis. *Wiki*

Fibroblast Growth Factor 21 is an Emerging Metabolic Regulator

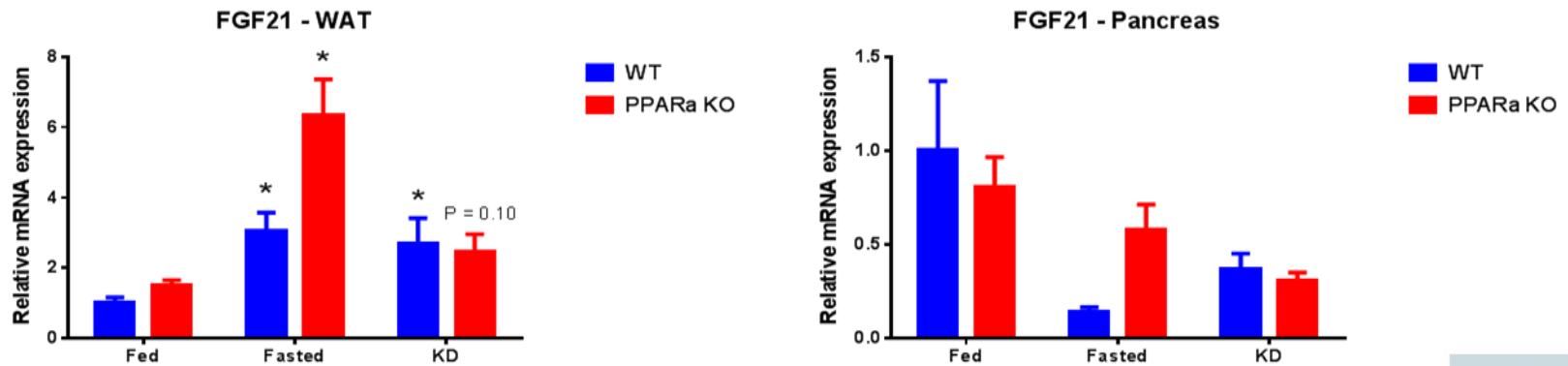
Lilly



Fasting and ketogenic diet induce different FGF21 responses in WT and PPAR alpha KO mice



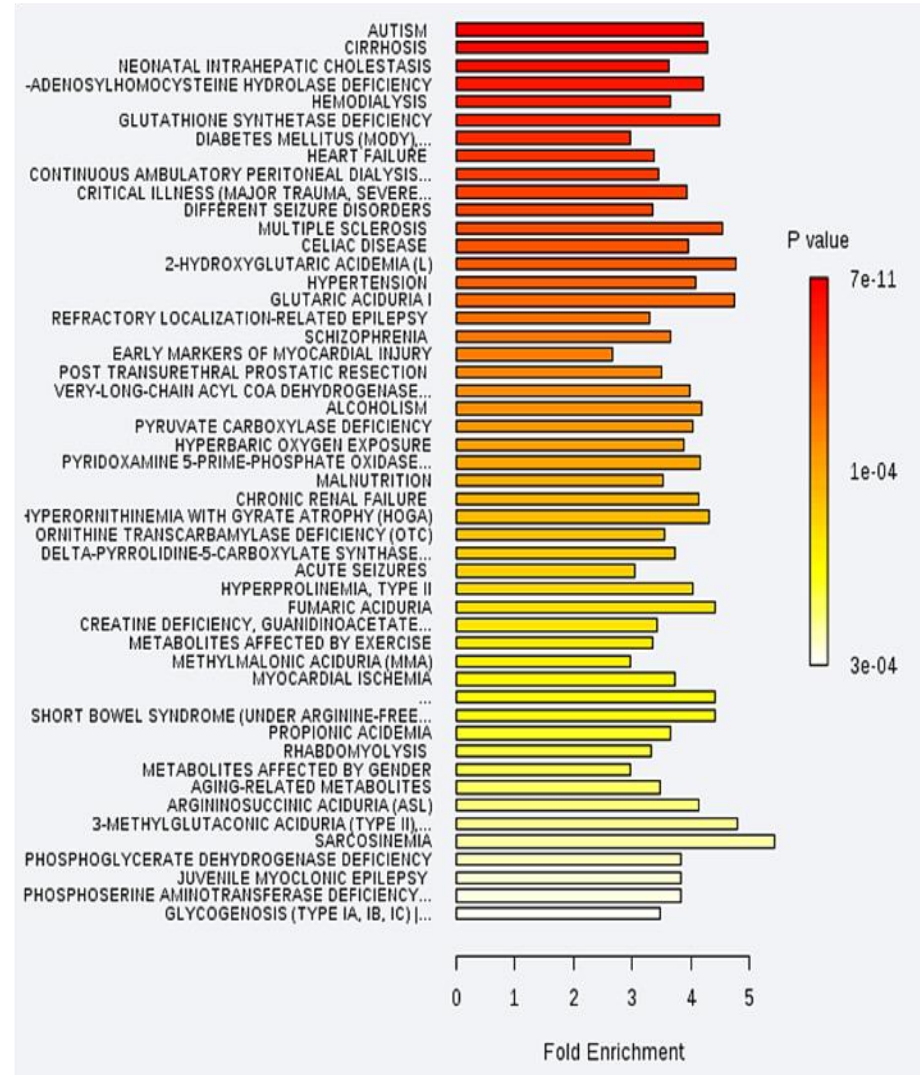
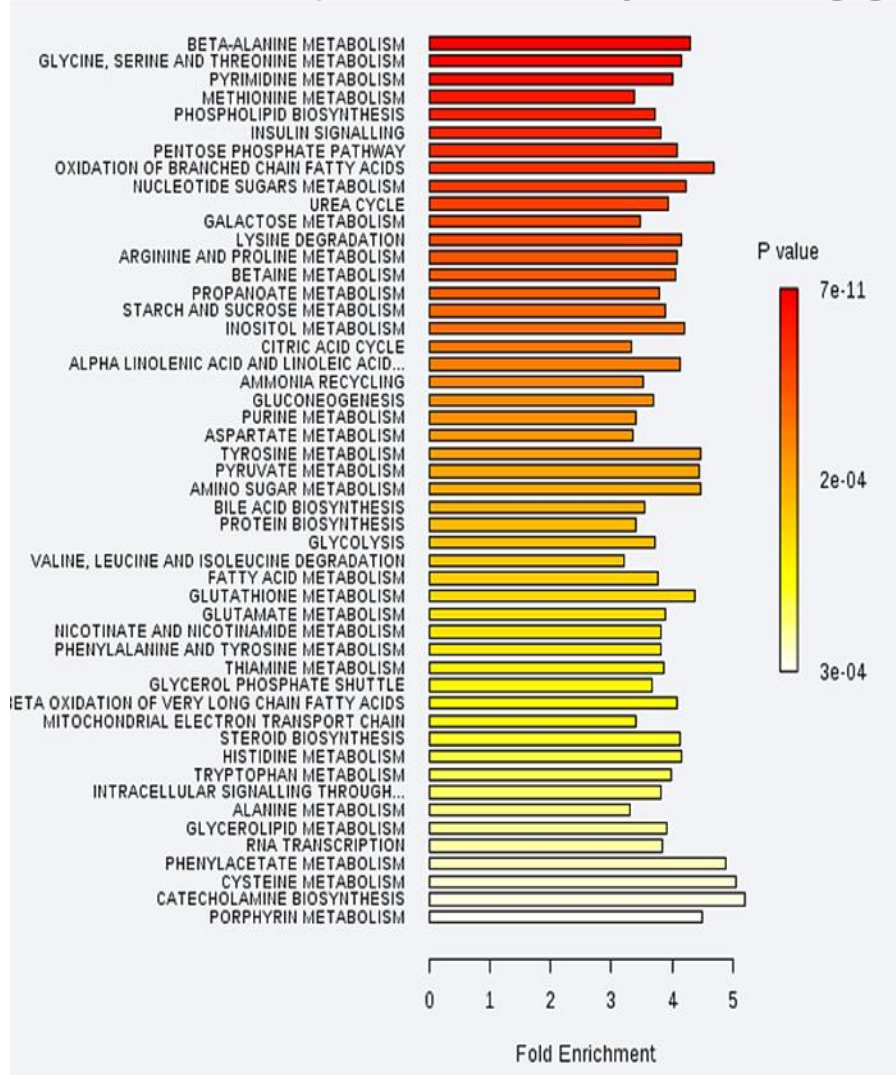
Treatment - 24hrs – acute response



Plasma FGF21 levels correlate with liver expression changes.

Lilly

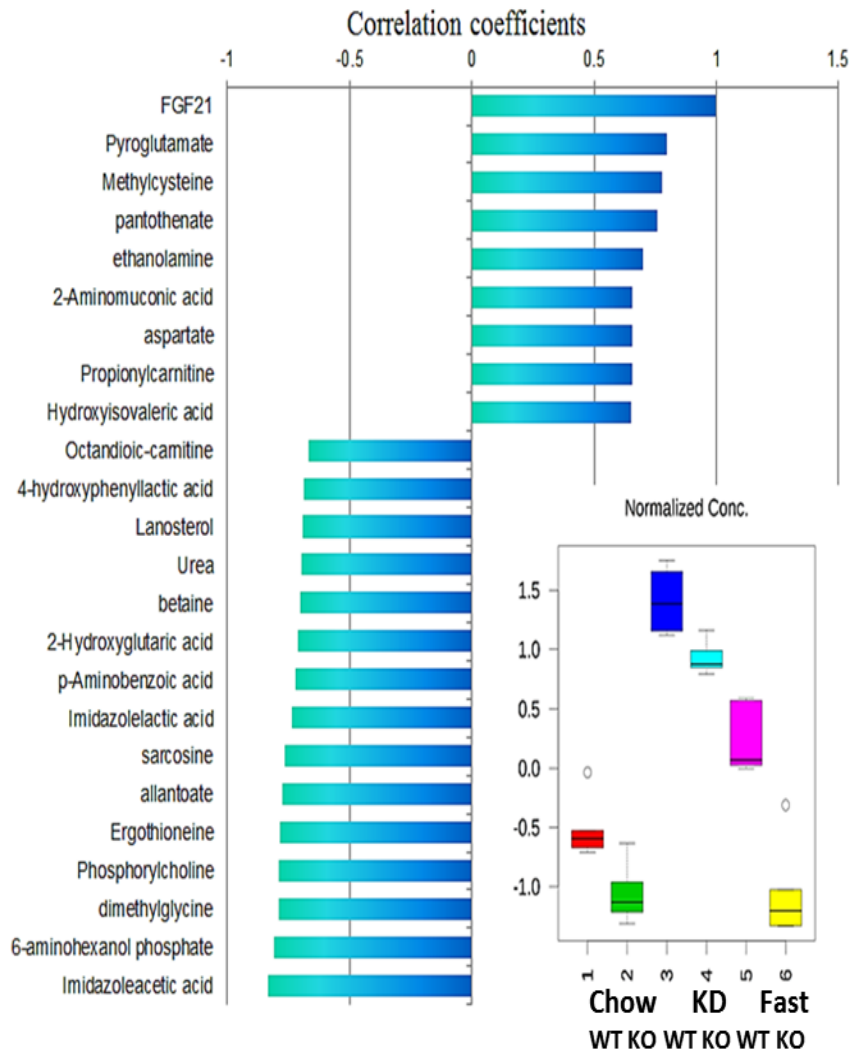
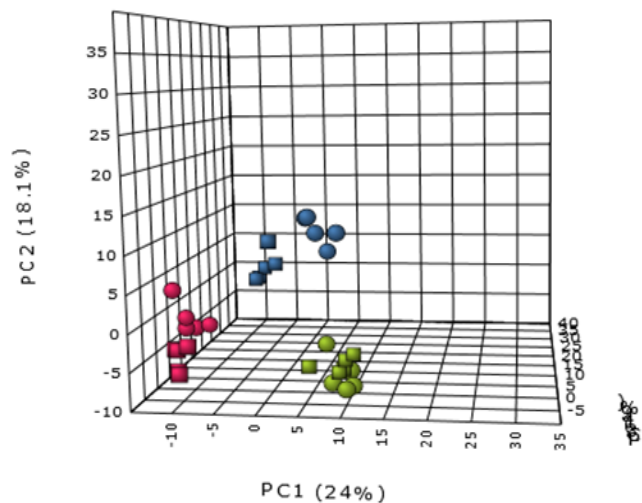
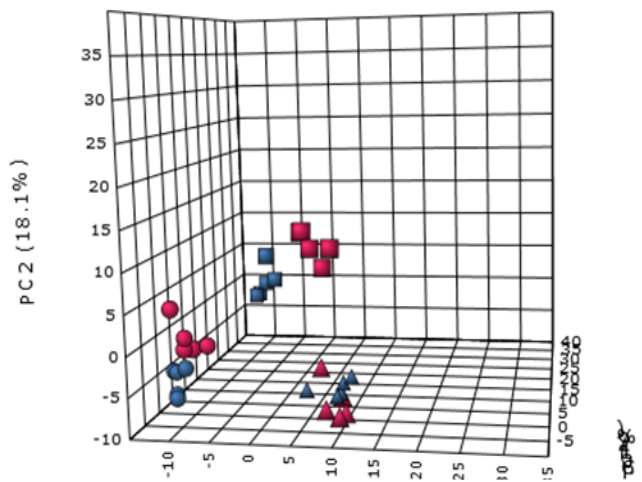
Liver samples were analyzed using global metabolomics - > 300 metabolites



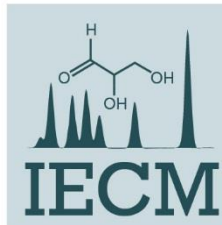
Metabolite Enrichment Analysis is a way to identify biologically meaningful patterns that are significantly enriched in metabolomic data *Lilly*

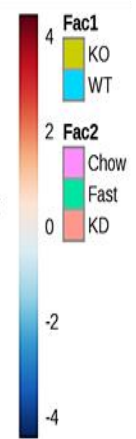
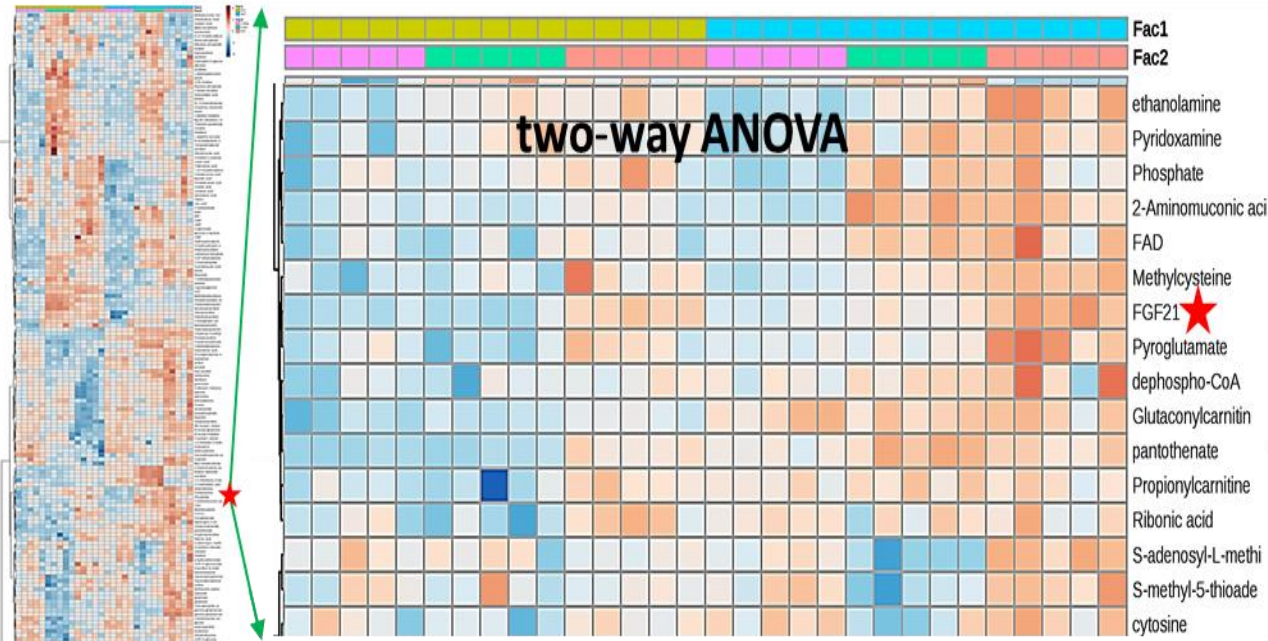


two-way ANOVA

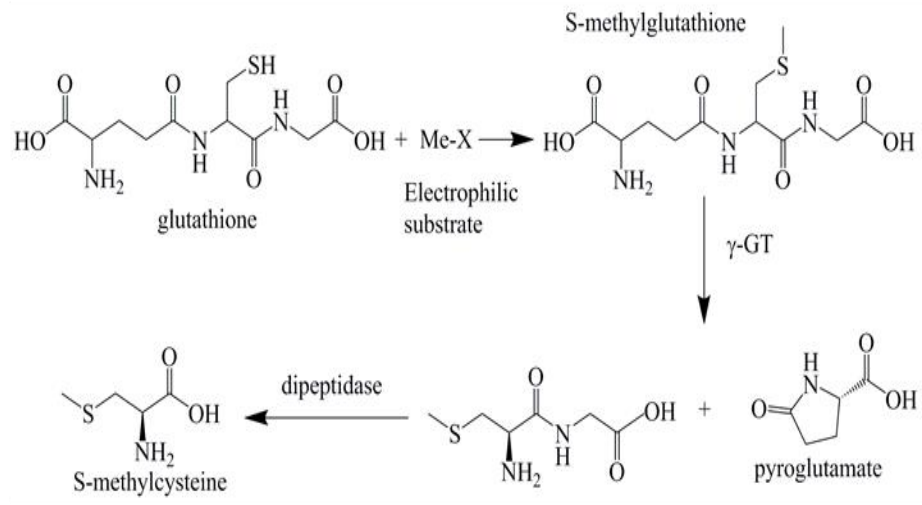


Correlation analysis performed against a given pattern – FGF21





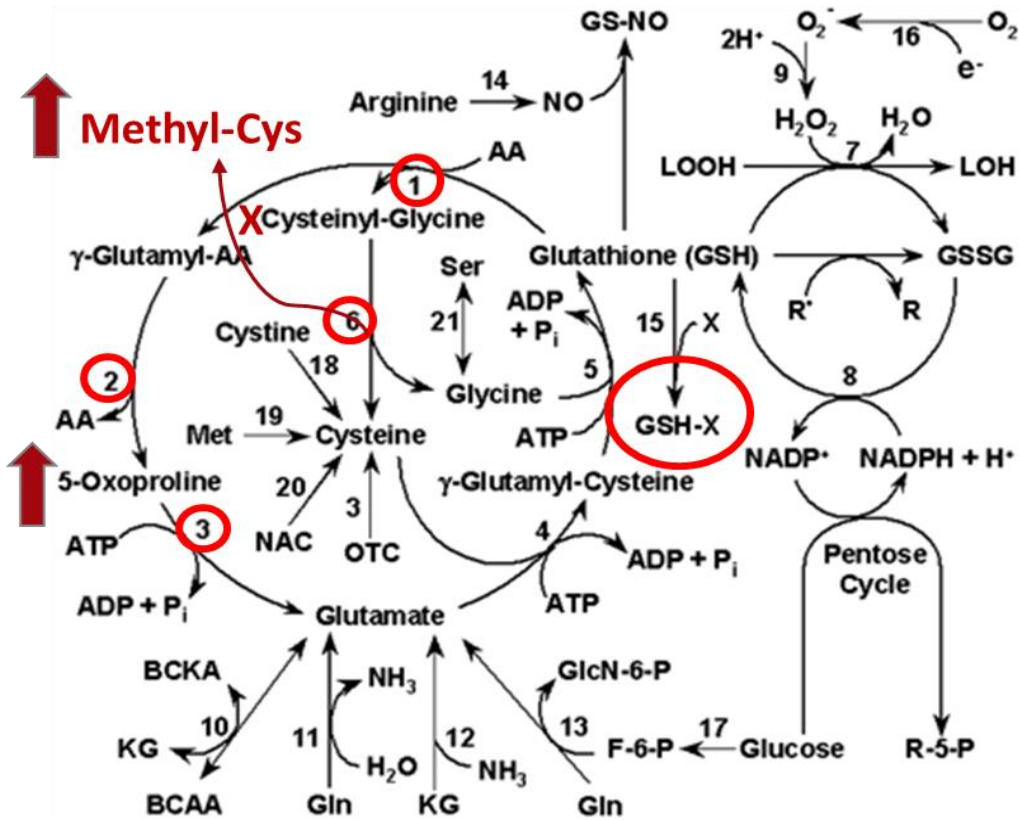
•S-methylcysteine (SMC) is formed after exposure to monohalomethanes in rodents as well as in humans. SMC is a minor amino acid naturally excreted in human urine, a protective agent against oxidative stress and a biotransformation product of methyl bromide. - Neurotoxicology. **2004** Sep; 25(5):817-23. - Biomed. Chromatogr. **2011**; 25: 330–343



Glutathione-mercapturic pathway.
 γ -GT - Gamma-glutamyltransferase,
dipeptidase – hepatic cysteinylglycine
 S-conjugate dipeptidase

GSH reacts with various electrophiles, physiological metabolites, and xenobiotics to form mercapturates. These reactions are initiated by glutathione-S-transferase.





Glutathione Metabolism and Its Implications for Health.

J. Nutr. 2004, 134(3), 489-92

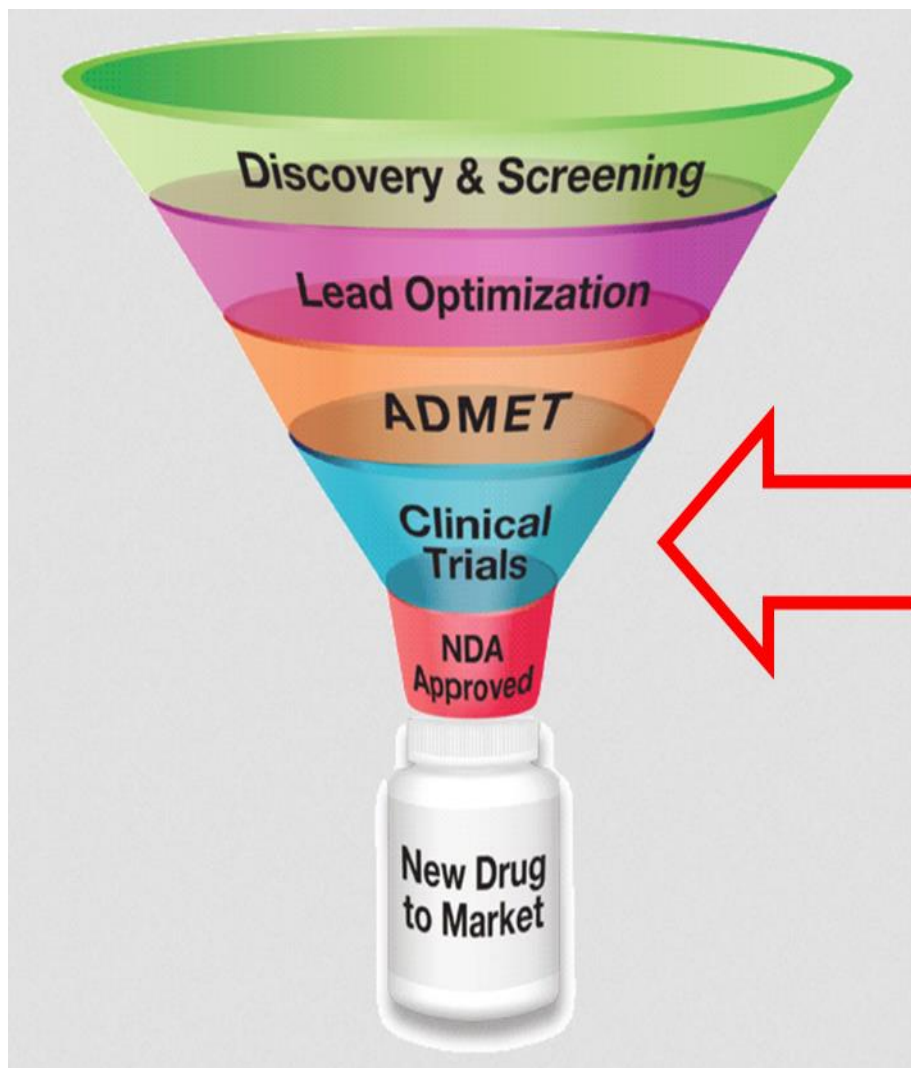
- 1) γ -glutamyl transpeptidase
- 6) dipeptidase
- 2) γ -glutamyl cyclotransferase
- 3) 5-oxoprolinase

X - Methyl

Case summary

1. A ketogenic diet dramatically increases and sustains endogenous FGF21 production in wild-type and PPAR α KO mice indicating PPAR α -independent regulation of FGF21.
2. A cluster of metabolites associated with FGF21 production in PPAR α KO mice was identified and suggests this increase is associated with increased metabolic stress in the liver.
3. Pattern correlation and two way ANOVA analyses suggest significant changes to the transmethylation pathway and glutathione metabolism in response to acute dietary challenges.

Lilly

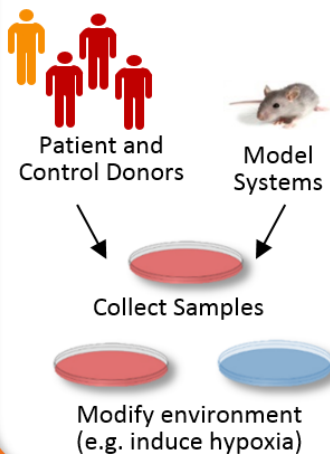


BERG's Interrogative Biology™ Platform

BERG models, interrogates, and analyzes disease biology at a systems level to agnostically identify actionable targets.

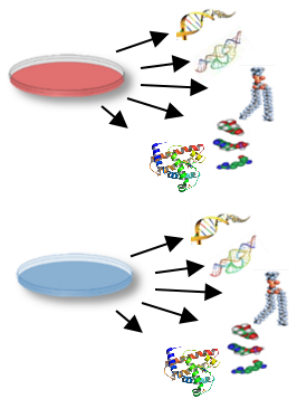
Interrogate Biological System

Can be applied to patient samples or model systems.



Harvest Information

Isolate, analyze and quantify DNA, RNA, proteins, lipids, and metabolites.



Develop Interactions Networks

Apply proprietary Bayesian algorithms to map interactions between components and reveal differences.



Bayesian artificial intelligence

Healthy vs Disease

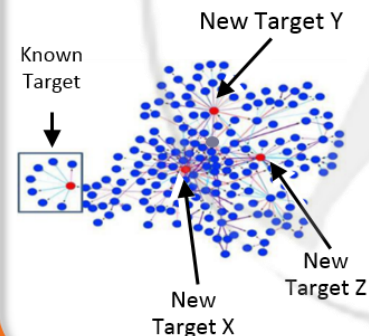


Untreated vs Treated




Identify Leads

Unique nodes and interactions can be drug targets or used to diagnose distinct biological states.

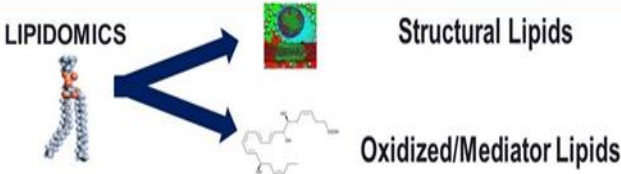


TECHNOLOGY: INTEGRATED PHENOME ASSESSMENT

FUNCTIONAL LIPIDOMICS



LIPIDOMICS



Structural Lipids
Oxidized/Mediator Lipids

High Resolution MS/MS^{ALL} Strategy Capable of Quantifying up to 2500+ Structural Lipid Molecular Species (30+) lipid classes and 110+ Oxidized Lipid Species

EPIMETABOLOMICS




METABOLOMICS




Targeted Metabolites
Untargeted Metabolites
Volatile Metabolites

High Throughput Metabolomics Workflow Integrates Targeted MRM Strategy detecting 400+ metabolites along with High Resolution Untargeted Discovery and GC MS Analysis of Volatile Metabolites

FUNCTIONAL PROTEOMICS



PROTEOMICS



Multiplexed Isotopically Labelled
PTM Proteomics

Biofluid Analysis Undergoes TOP-14 Depletion and TMT10 Isotopic Labelling for Robust Quantitative Analysis Across Batches Using a Deep Coverage Strategy on a Thermo QE+ MS System

MOA, real-time monitoring

Stable isotope tracers and mass isotopomer analysis



Targeted **SIRM** Non-targeted

In House Protocols

POPULATION BASED PHENOMIC STRATIFICATION USING *BERG* CLINICAL TRIALS

Environmental/Physiological Influence

Genome
(~30,000 genes)

Transcriptome
(~10⁵ RNA transcripts)

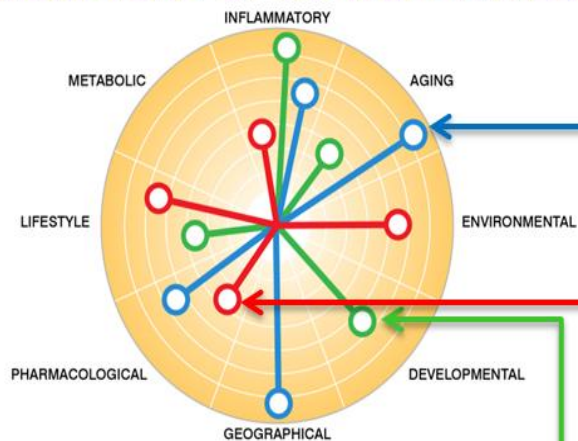
Proteome (Including PTM)
(10⁵ proteins)

Metabolome/Lipidome
(1,000's Metabolites/Lipids)

PHENOME



POPULATION VARIATION/MOLECULAR SIGNATURE OF CO-MORBIDITIES



POPULATION MOLECULAR DIVERSITY





COMPANY

CLINICAL

RESEARCH

CONTACT

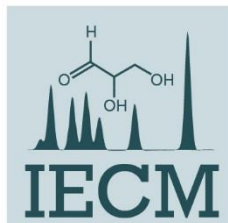
CLINICAL/PENDING TRIALS

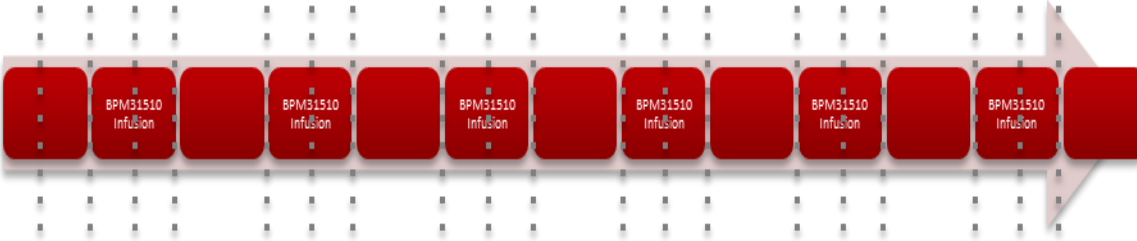
Clinical Programs

Therapeutics

Oncology	Discovery	Target Validation	Pre-Clinical	IND	Phase I	Phase II	Phase III
Topical 31510: Skin Cancer							
31510-IV: Solid Tumors							
31510-IV: Chemotherapy Co-treatment							

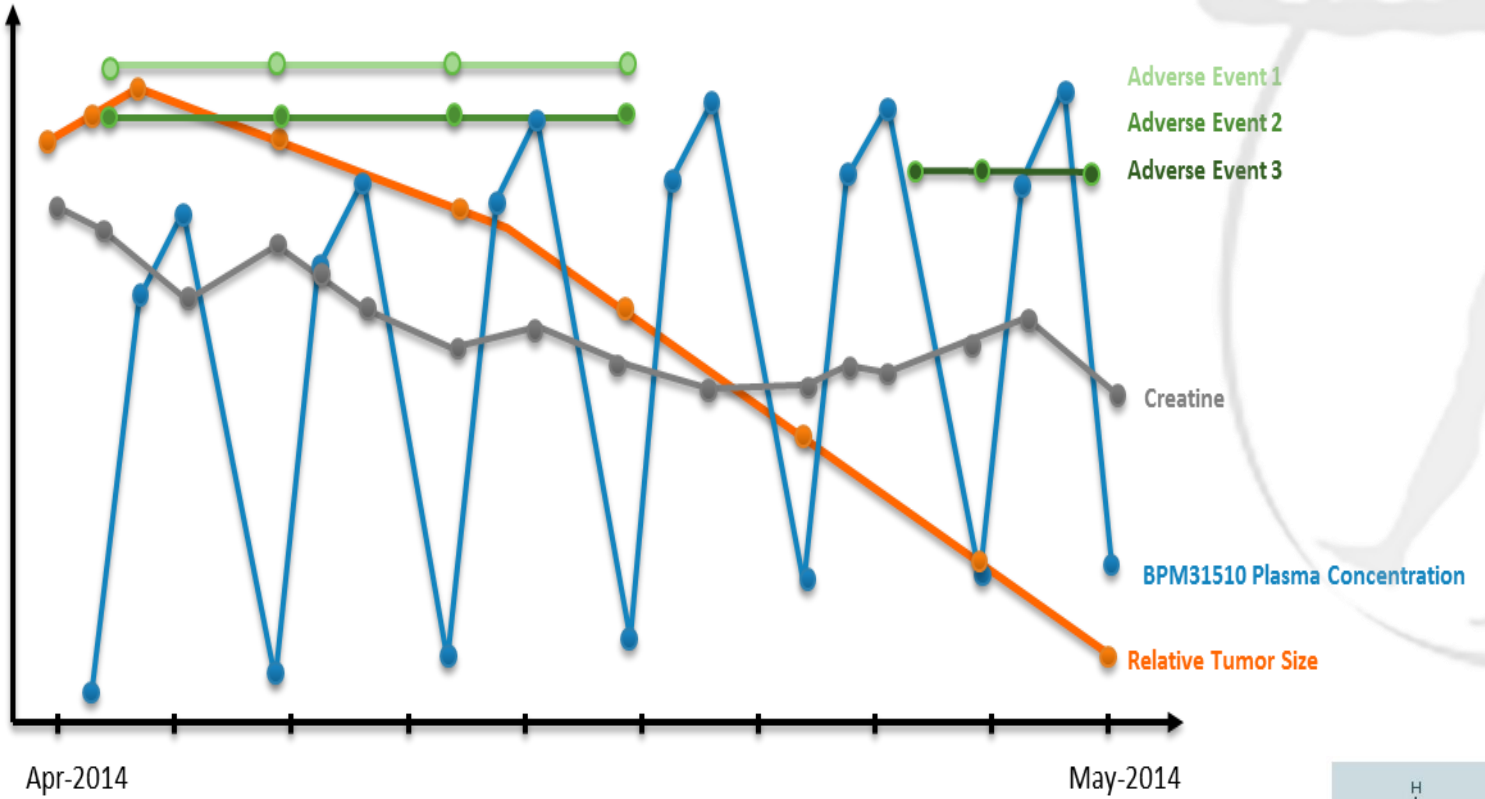
This is an open label trial evaluating BPM 31510 as a single agent in patients with advanced refractory solid tumors. This is a dose-finding trial currently on-going at the following clinical sites: Weill Cornell Medical College, MD Anderson Cancer Center, and Palo Alto Medical Center.





Clinical Information

- Age: ●●
- Gender: ●●●●●
- Race: ●●
- Tumor: ●●●
- Previous Treatments:
-
-
-
-
-
-
- ...



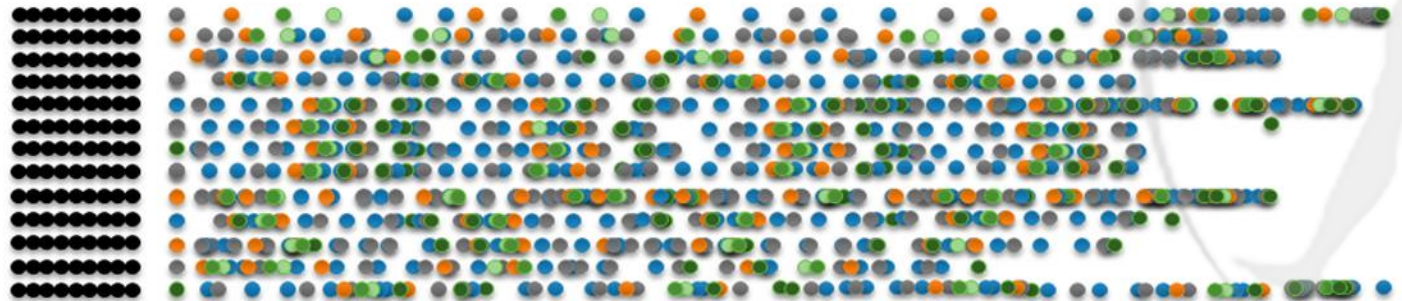
PRE-PROCESSED PATIENT PROFILE



Clinical
Information

Longitudinal trial
outputs

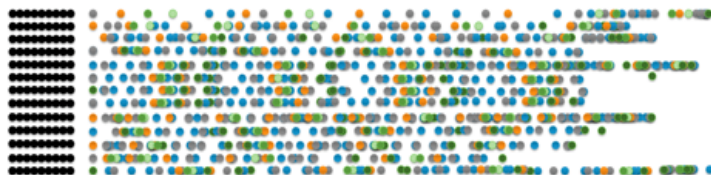
PROCESSED PATIENT PROFILE



...

BERG AI CLINICAL INFORMATION SYSTEM

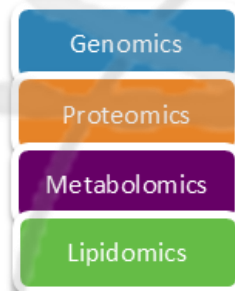
Laboratory and Clinical



bAicis[™]



Multiomic

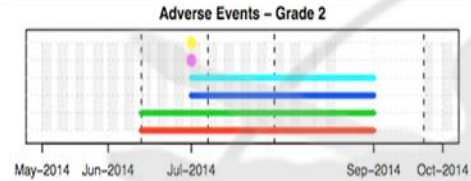
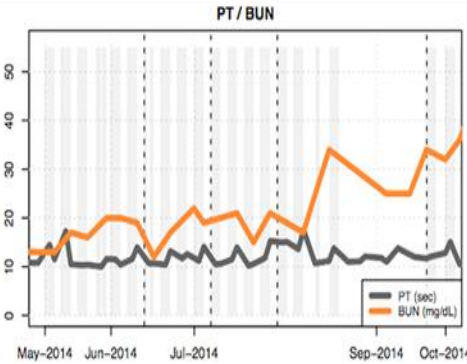
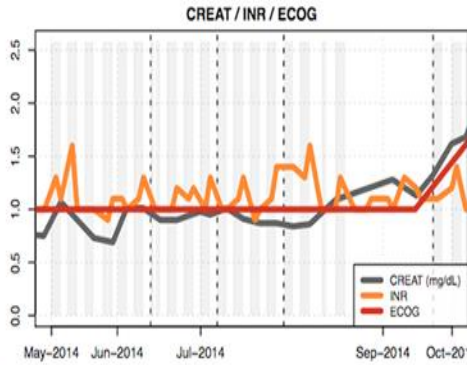
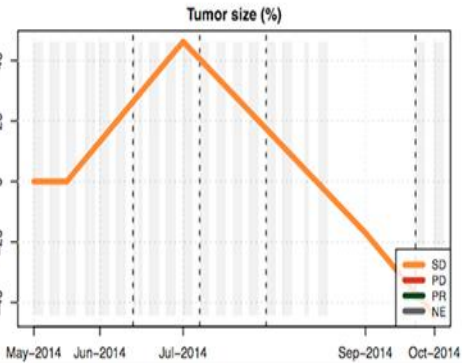


Longitudinal Molecular and Clinical Profile



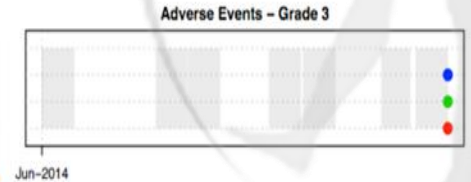
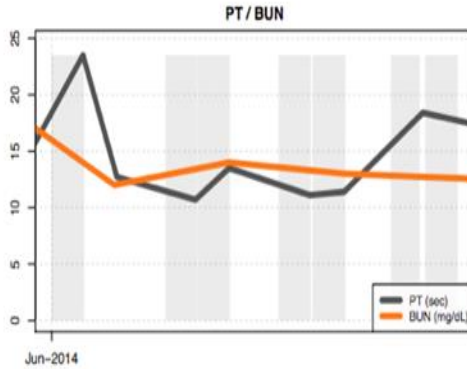
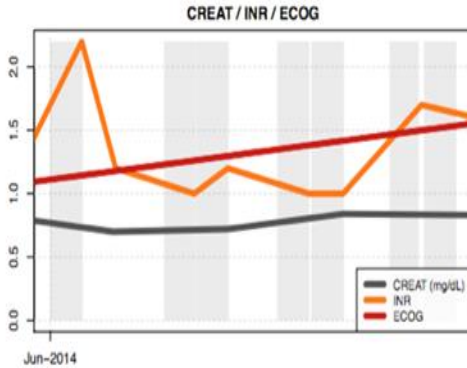
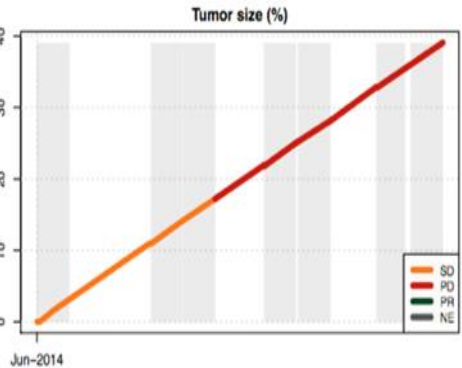
PATIENT DASHBOARD

Responder



- General system disorders.NEC
- General disorders and administration site conditions
- Tissue enzyme analyses.NEC
- Enzyme investigations.NEC
- Liver function analyses
- Hepatobiliary investigations

Non-Responder

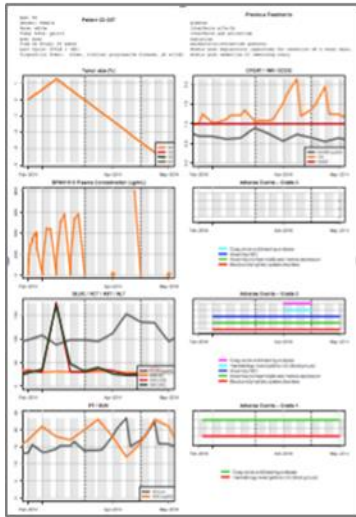


- Coagulation and bleeding analyses
- Gastrointestinal signs and symptoms.NEC
- Gastrointestinal signs and symptoms
- Gastrointestinal disorders

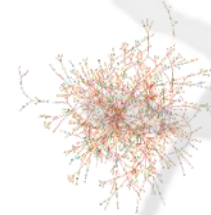
BERG AI CLINICAL INFORMATION SYSTEM

Longitudinal Molecular and Clinical Profile

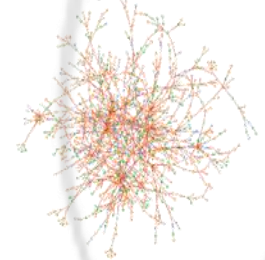
Patient Dashboard



Responsive to Treatment



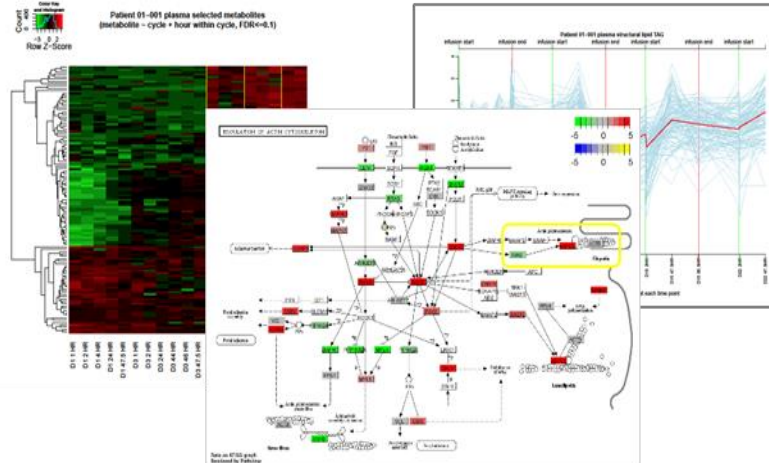
Refractory to Treatment



Adverse Events Grade ≥ 3



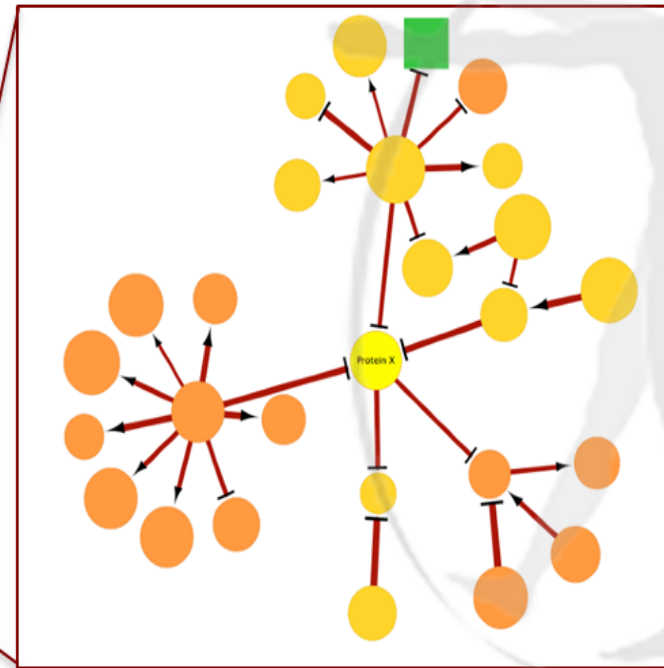
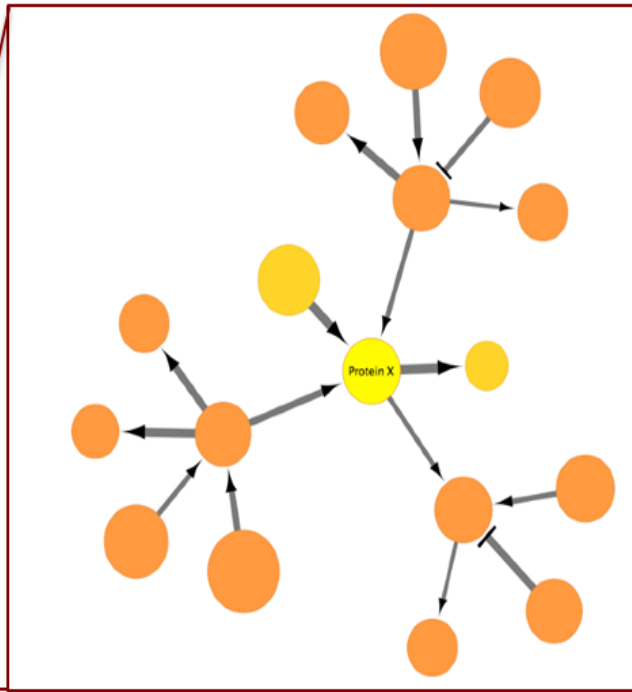
Statistical Analysis



Companion Diagnostics

Responsive to Treatment

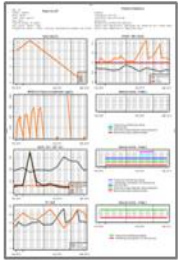
Refractory to Treatment



Potential markers selection

BERG AI CLINICAL INFORMATION SYSTEM

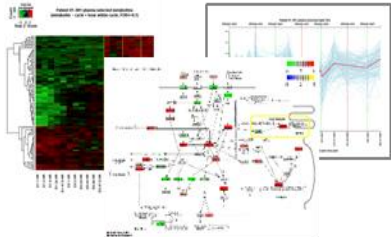
Patient Dashboard



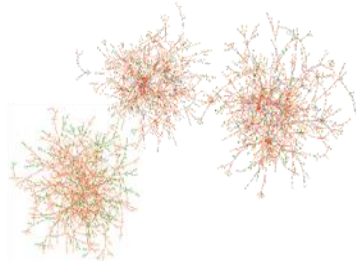
Longitudinal Molecular and Clinical Profile



Statistical Analysis



Cause-and-effect Networks



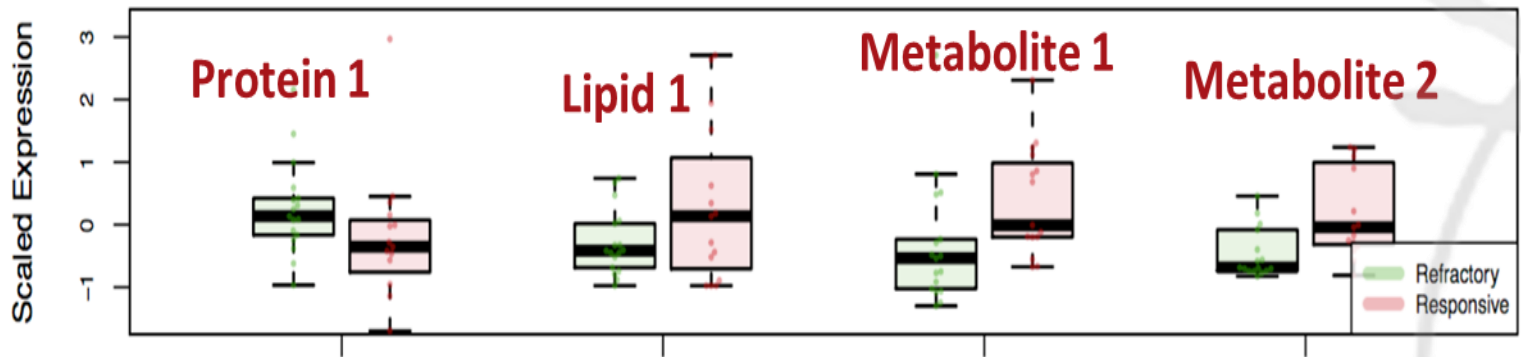
bAIcis™

**Candidate
CDx markers**

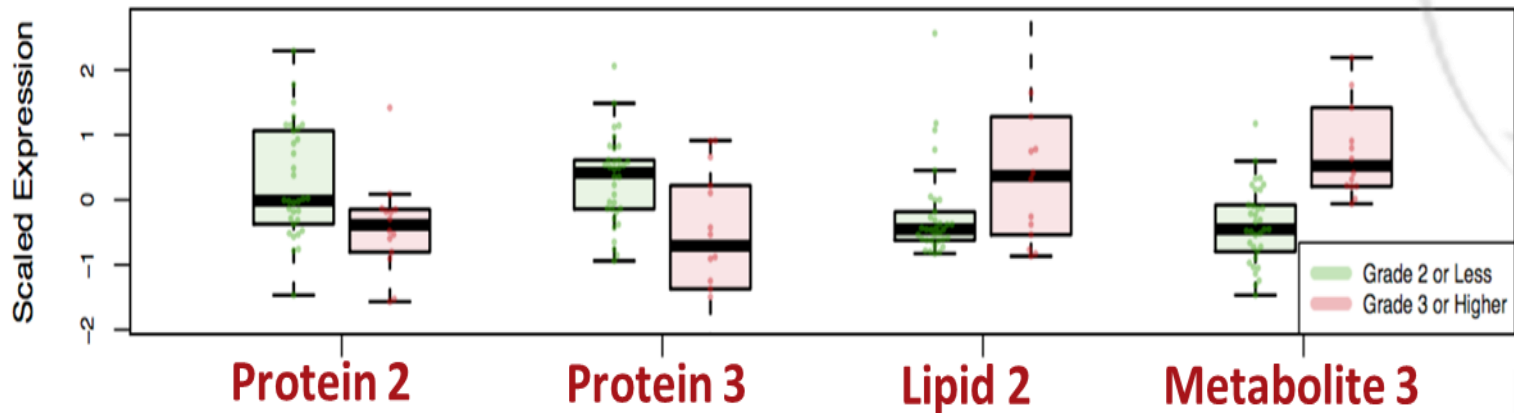
Patient Stratification

PRECISION MEDICINE - PATIENT STRATIFICATION

Candidate CDx markers
measured before treatment



Candidate CDx markers
measured before treatment



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