METABOLON®

GLOBAL METABOLOMICS

INNOVATIVE TECHNOLOGY FOR BIOLOGICAL INSIGHT

Unlocking Biology with Metabolomics

Surveying metabolites is crucial to unlocking biology because almost every factor impacting the phenoytpe—from genetics and the microbiota, to disease and drug exposure—exerts influence by altering metabolite levels.

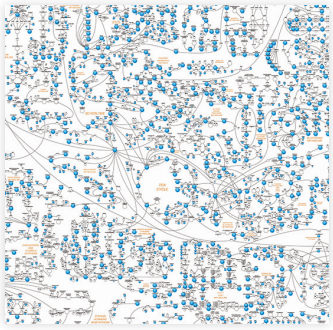
By identifying, quantifying, tracking and mapping all of the metabolites of a system under study, metabolomics can provide unique biological insight and empower biomarker discovery.

While many laboratories have metabolite profiling or analytical chemistry capabilities, comprehensive metabolomics technologies are extremely rare. This is because accurate and unbiased metabolite identification across the entire metabolome presents sizable challenges that very few laboratories are equipped to handle.

A Metabolomics Solution: Precision Metabolomics™

With more than 15 years of experience and continuous innovation, Metabolon has overcome the challenges of whole-metabolome profiling and created the industry's most advanced metabolomics technology, Precision Metabolomics.

Powering Precision Metabolomics is our global metabolomics platform, which is an ideal tool for gaining biological insight and discovering biomarkers.



The Metabolome is comprised of thousands of chemically and structurally diverse metabolites.

Global Metabolomics offers the most comprehensive screening of this vast space.

Our global metabolomics technology effectively functions as an automated "metabolic sequencer" that unlocks critical information contained within the metabolome. It can quickly and accurately identify and quantitate upwards of 1,000 metabolites with less than 5% process variability and is compatible with almost any sample type.

This exceptional data is then imported into our pathway analysis environment for intuitive and insightful interpretation. This combination of data represents a unique system for creating knowledge across all areas of life sciences.

THE METABOLON ADVANTAGE

The Data

- Coverage Depth
  - 1,000+ metabolites/ sample*
  - Supported by vast library of standards
- Quality
  - Accurate, Tier 1 IDs
  - Median RSD of <5%

Institutional Knowledge

- 15+ years of experience
- 7,000+ studies
- 1,000+ publications
- Pathway analysis & informatics drive expert interpretation

Speed & Capacity

* The number of metabolites detected will vary based on service offering and sample type/quantity.

GLOBAL METABOLOMICS PLATFORM

METABOLITE COVERAGE AND METHODS

Metabolite Coverage

Global Metabolomics provides the industry's broadest class coverage from a single sample.

Around 1,000 metabolites across diverse classes can be measured* from 100 µL of plasma/serum, 50-100 mg of tissue, or a 50-100 µL cell pellet with approximately 5% CVs.

* The metabolites detected from the above classes can vary based on the type of sample and the abundance levels in those samples.

Amino Acid Metabolism	Cofactor & Vitamin Metabolism	Nucleotide Metabolism	Microbiome Metabolism
Amino Acid catabolism Bioactive intermediates & trace amines Glutathione metabolism Inflammatory mediators Microbiome metabolism Polyamines/ornithine metabolism Urea Cycle	Ascorbate metabolism CoA metabolism FAD metabolism Folate metabolism NAD/NADP metabolism PLP metabolism SAM metabolism Many other cofactors and vitamins (tocopherol, B12, Biotin)	Degradation of nucleotides Deoxyribonucleotides DNA damage FAD metabolism Modified nucleotides Nucleotide Coenzymes Purine and pyrimidine *de novo* synthesis Purine and pyrimidine salvage synthesis Ribose metabolism	2° Bile acids Aromatic amino acids Energy Choline/carnitine Xenobiotics Fatty acids/short chain & medium chain Vitamins Polyamines
Carbohydrate Metabolism	**Energy Metabolism**	**Lipid Metabolism**	**Novel Metabolites**
Gluconeogenesis Glucose metabolism Glycogen metabolism Glycosylation pathways Metabolism of other carbon sources Metabolism of sugars (fructose, galactose) Polyol metabolism Pyruvate metabolism	Acyl-carnitines Beta-oxidation Creatine metabolism FAD metabolism Glycolysis Mitochondrial function Pentose phosphate pathway	Bile acids Bioactive lipids Cholesterol Fatty acids Sphingosine Inflammatory mediators Lysolipids Sterols Oxidized lipids (COX, LOX)	Novel drug metabolites Novel xenobiotics Novel microbiota metabolites Novel by-products of non-canonical host metabolism

Methods

Global Metabolomics TECHNOLOGY

Sample →

Advanced mLIMS for Systematic Sample Management
Achieve low-variability, high-traceability, capacity and turn-around

Accurate Data Capture & Rigorous Processing
Generate broad metabolome coverage and accurate IDs with low process variability

Extensive Quality Controls
Ensure accurate data with complete traceability of all process steps

→ **Data Interpretation**

Precision Metabolomics combines multiple mass spectrometry methods and a proprietary LIMS system with the industry's largest reference library of authenticated metabolite standards and a suite of patented informatics and quality-control software. This allows us to automatically and rapidly identify and quantitate metabolites. A team of chemical spectral analysts then performs final quality-control using proprietary software tools. These features collectively allow Precision Metabolomics to overcome the "signal-to-noise" challenges that plague many metabolomics platforms. The result is the rapid production of the highest quality data possible.

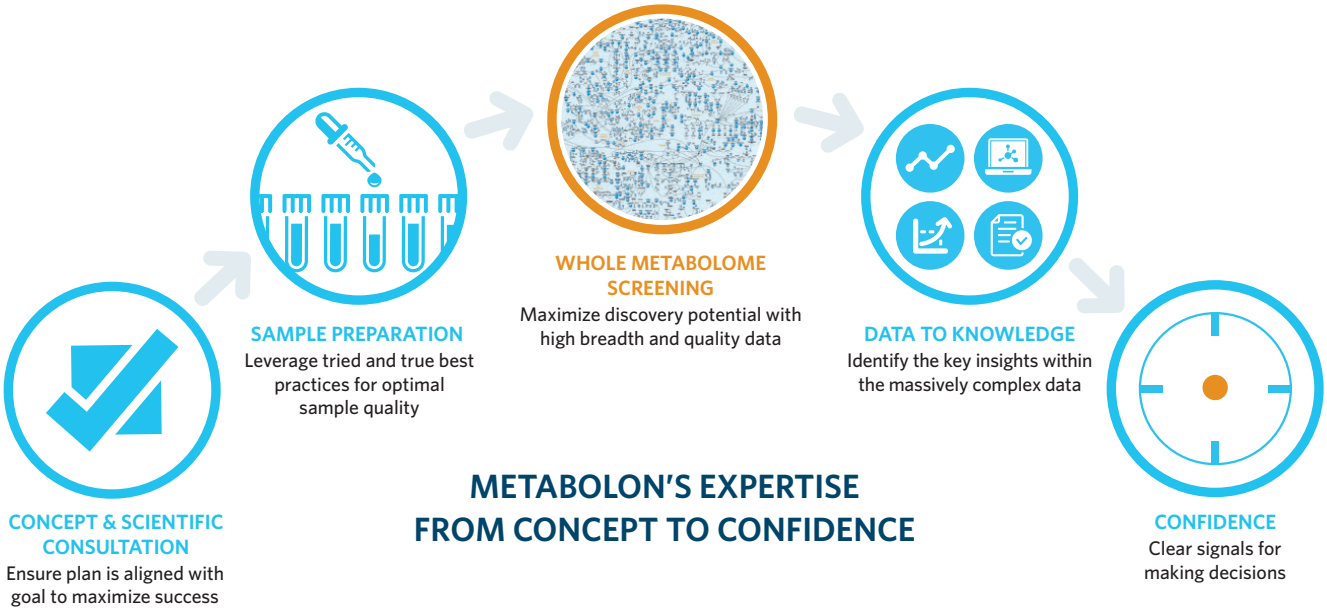
Supporting References

- A. M. Evans, B. R. Bridgewater et al., Metabolomics **4**, 2153-0769 (2014).
- A. Evans, M. Mitchell et al., Metabolomics: Open Access, (2012).
- C. D. DeHaven, A. Evans et al., INTECH Open Access Publisher (2012).
- C. D. Dehaven, A. M. Evans et al., J Cheminform **2**, (2010).
- A. M. Evans, C. D. DeHaven et al., Analytical Chemistry **81**, 6656-6667 (2009).

OUR PROCESS

AN EXPERIENCED PARTNER, FACILITATING YOUR SUCCESS

We are committed to helping you harness the power of metabolomics to achieve your research goals. We've designed our process to be a complete solution that produces actionable knowledge and drives research forward. The entire process is supported by a team of Ph.D.-level biochemists, biologists and bio-informaticists with decades of experience.



Scientific Consultation

The value of metabolomics is heightened through our collaborative process. Our team of accomplished Ph.D. scientists works with you through every step of the process to ensure that the results are both meaningful and tractable.

Whole Metabolome Screening

Our innovative technology provides the deepest coverage of the metabolome of any published platform in the industry.

Data to Knowledge

Data are an essential component to metabolomic discoveries, but interpretation of the data is the final barrier to discovery. Our expertise and institutional knowledge clear this barrier through interpretation services led by our Ph.D. scientists and access to bioinformatics tools that enable you to explore the data.

From our initial consultation to the delivery of results, the entire process of working with Metabolon is designed to empower your informed decision making so that you can move forward with confidence.

DELIVERABLES

CONVERT DATA TO KNOWLEDGE WITH METABOLOMICS

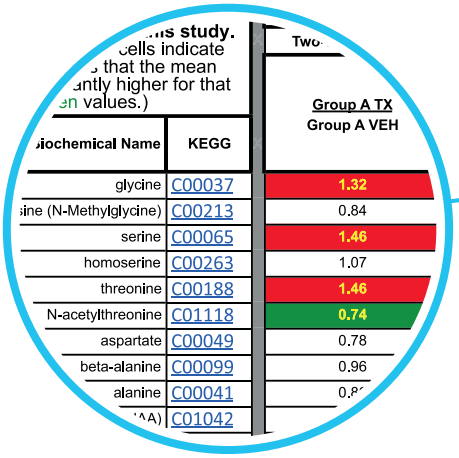
Our commitment to ensuring success includes deliverables that facilitate your understanding and put your metabolomics results into action. The following three elements form the core of our deliverables.

1 Quality Controlled Data

Metabolites detected in each sample and their abundance levels are compiled. Discovery and success are maximized by accurately identifying and quantitating up to 1,000 metabolites in a laboratory that operates under the principles of a Quality Management System.

2 Statistics – Heat Maps, Plots & Visualizations

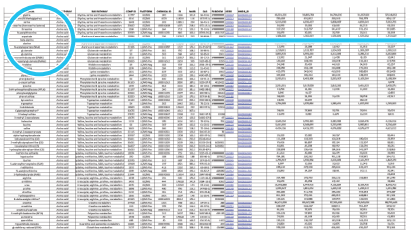
Mean abundances for each metabolite within each "group" are calculated and statistics are performed. Data exploration is empowered with heat maps and plots organized into meticulously-curated metabolic pathways. Access, share, visualize and analyze the data in the MetaboLync® Client Portal.



3 Data Interpretation & Reporting

Statistics are analyzed within the context of the biology. Key insights and a path forward are revealed via our highly accomplished team of Ph.D. scientists and unique in-house bioinformatics tools.

Excel file

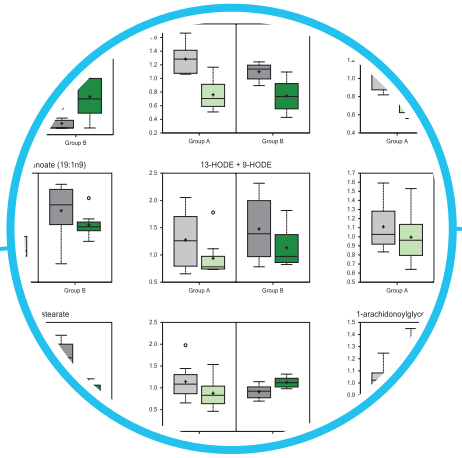


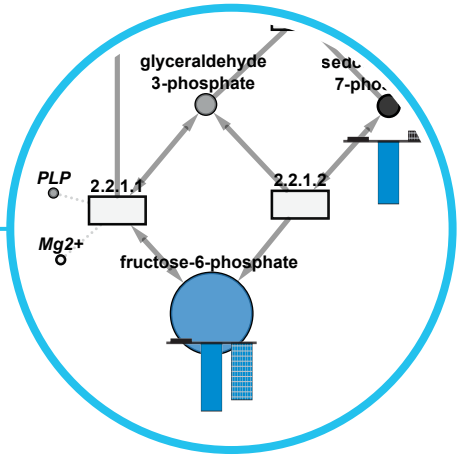
	100.
	VEH
	Group A
BIOCHEMICAL	
glycine	40,591,220
sarcosine (N-Methylglycine)	759,459
serine	5,913,698
homoserine (homoserine lactone)	56,990
threonine	317,292
N-acetylthreonine	34,470
aspartate	1,389,600
beta-alanine	316,803
alanine	63,057,472
N-acetylaspartate (NAA)	12,975
glutamate	1,218,001
glutamine	3,803,92
minobutyrate (GABA)	15
lutamate (NAAG)	

Excel file, plus:

Heat map and plot worksheets

Access to visualization tools within MetaboLync™





Written report and PowerPoint slides (or figures in report)

TABLE OF CONTENTS

DATA INTERPRETATION

A FINAL REPORT DELIVERS KEY INSIGHTS AND A PATH FORWARD

FINAL REPORT

Evaluation of a first-in-class Arginase inhibitor as single agent and in combination with an immune check-point inhibitor.

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RESULTS AND BIOLOGICAL INTERPRETATION

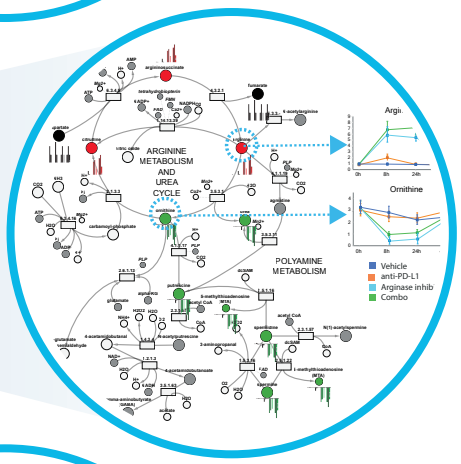
A first-in-class Arginase inhibitor was assessed as a single agent and in combination with an immune check-point inhibitor in a BRAF(V600E)-mutant syngeneic tumor graft mouse model. Tumor tissue was isolated from mice sacrificed at baseline, 8, 24, and 48 hours. Specimens were flash frozen and shipped to Metabolon for metabolomics analysis with the DiscoveryHD4™ global metabolomics platform and the Complex Lipid Panel. Statistically significant, metabolic alterations were identified for both single agents and the combination. These changes were mapped to associated pathways and then integrated into plausible implications to the target biology, tumor biology, and whole-animal physiology.

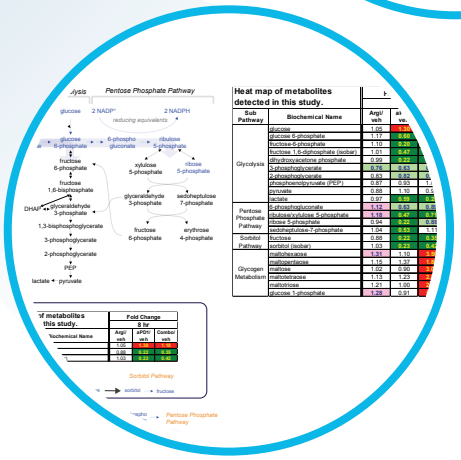
This analysis provides the major findings listed below and detailed within the body of this report.
Key findings from the screening, statistics and interpretation of over 1,600 metabolites are:

- Arginase inhibition resulted in clear target engagement as indicated by elevations in arginine and citrulline and reductions in downstream metabolites and pathways (e.g. ornithine and polyamines, respectively).
- Check-point inhibition caused a reduction in glucose-dependent metabolic activity – glycolysis and the pentose phosphate pathway (PPP). In addition to these pathways being regarded as important for tumor growth, the PPP is also important for the recycling of oxidized glutathione. This latter point may be a key to the synergistic activity of the combination.
- Synergistic activity of the combination treatment is posited to occur by a corollary effect of the arginase inhibitor inducing oxidative stress via NOS induction of NO (peroxynitrates, etc.) In the context of checkpoint inhibition, tumors can't combat this elevated oxidative stress due to the reduction in pentose phosphate pathway-dependent recycling of oxidized glutathione.
- Overall, very few additional pathways appeared altered outside of those that map to expected or plausible MoA or efficacy related signals.

Key Results

Interpretation





Interpretation

METABOLYNC ANALYSIS TOOLS

CUSTOM-BUILT TOOLS FOR TRANSFORMING DATA TO KNOWLEDGE

The MetaboLync® Client Portal, hosted in the cloud, allows Metabolon clients to access, share, visualize and analyze their metabolomics results.

Deliverables and Data Analysis Tools

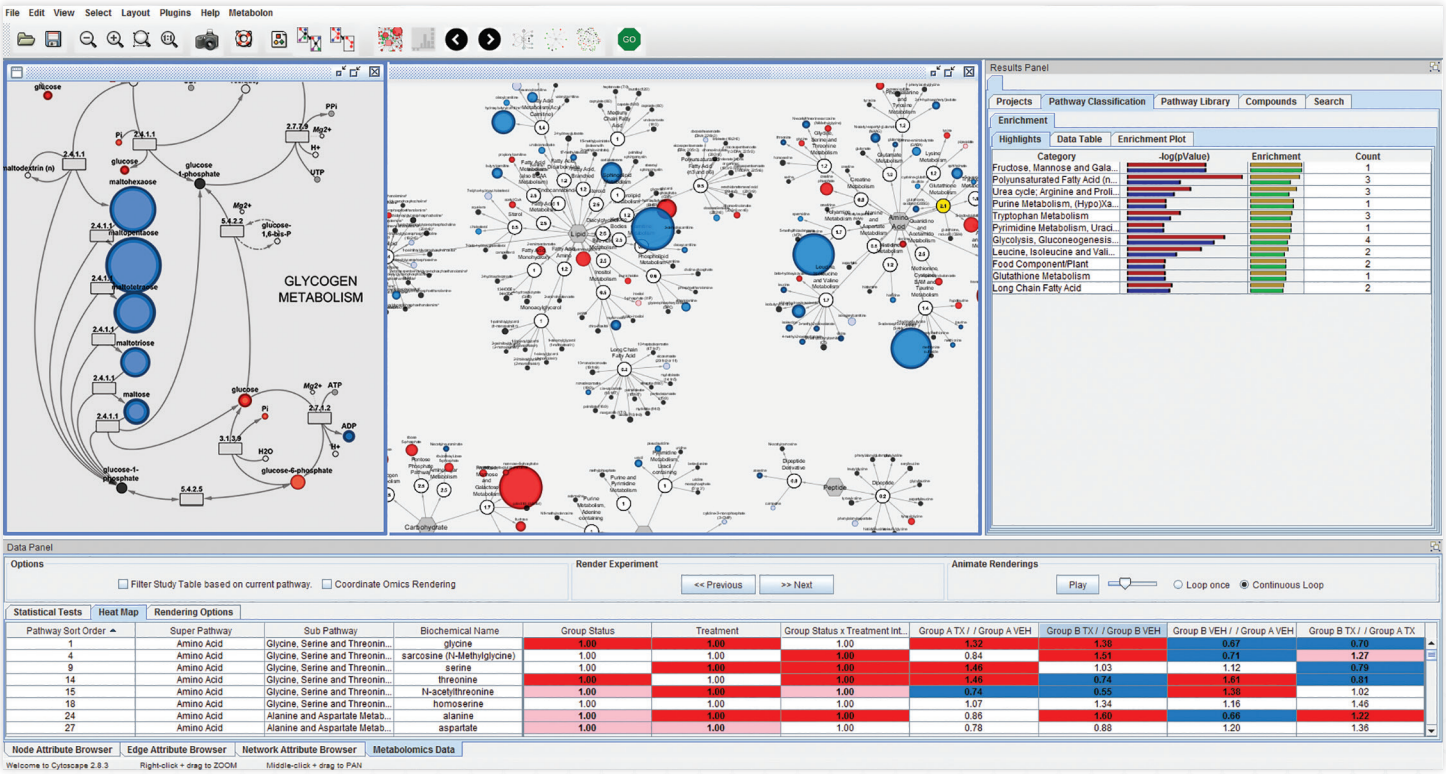
The MetaboLync Portal allows users to download and share deliverables, such as data files, reports and slide decks. It also provides access to a suite of data visualization and analysis tools that help transform data into actionable knowledge.

These include the heat map tool, which allows users to manipulate and compare statistical data, the pathway enrichment tool, which helps identify targets of interest, and the Pathway Visualizations Tool—Metabolon's custom-built Cytoscape plugin, which brings all data together for comprehensive, in-depth analysis.

Pathway Visualizations

The Pathway Visualizations plugin imports study data to create a multi-functional data display that incorporates heat maps, charts, pathway enrichment data, and an annotated biochemical "Network View" for each experiment. The Network View is an interactive visual rendering of the data within the associated metabolic pathways.

Results can also be viewed in the context of the whole metabolome by opening the curated biochemical Pathway Map. The Pathway Map is an interactive visual rendering of all charted biochemical pathways. This provides a more holistic picture of your metabolomics study results and empowers deeper understanding of biological significance.



MetaboLync Pathway Visualizations provides a richly annotated map of the metabolome from which you can extract knowledge, download publication-quality images, and share findings with your colleagues.

METABOLON®

Where **knowing** comes to **life**™

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