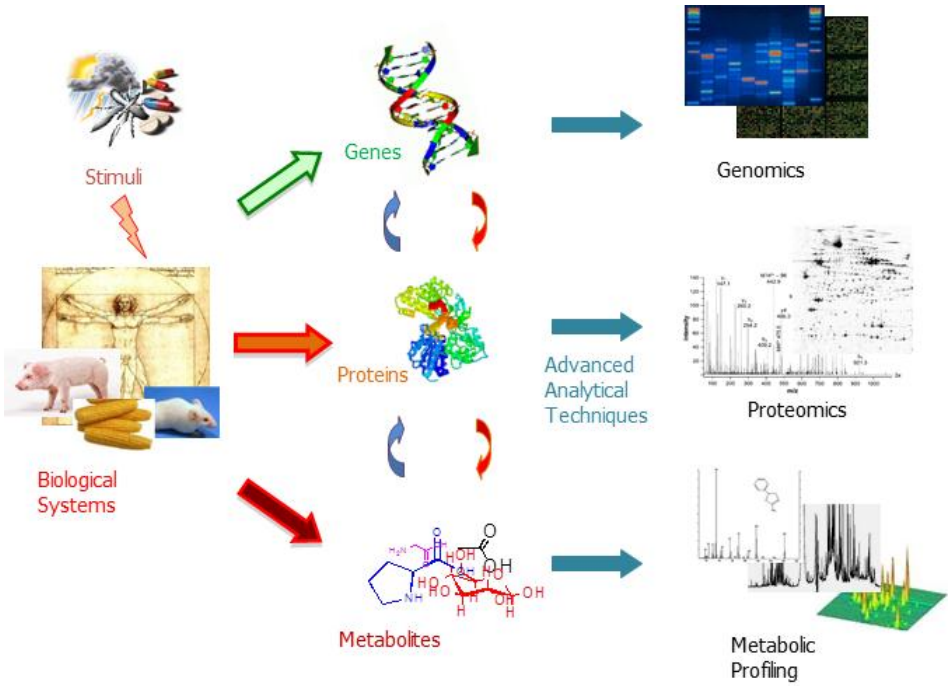
Leveraging Advanced Metabolism Knowledge to Fight Cancer

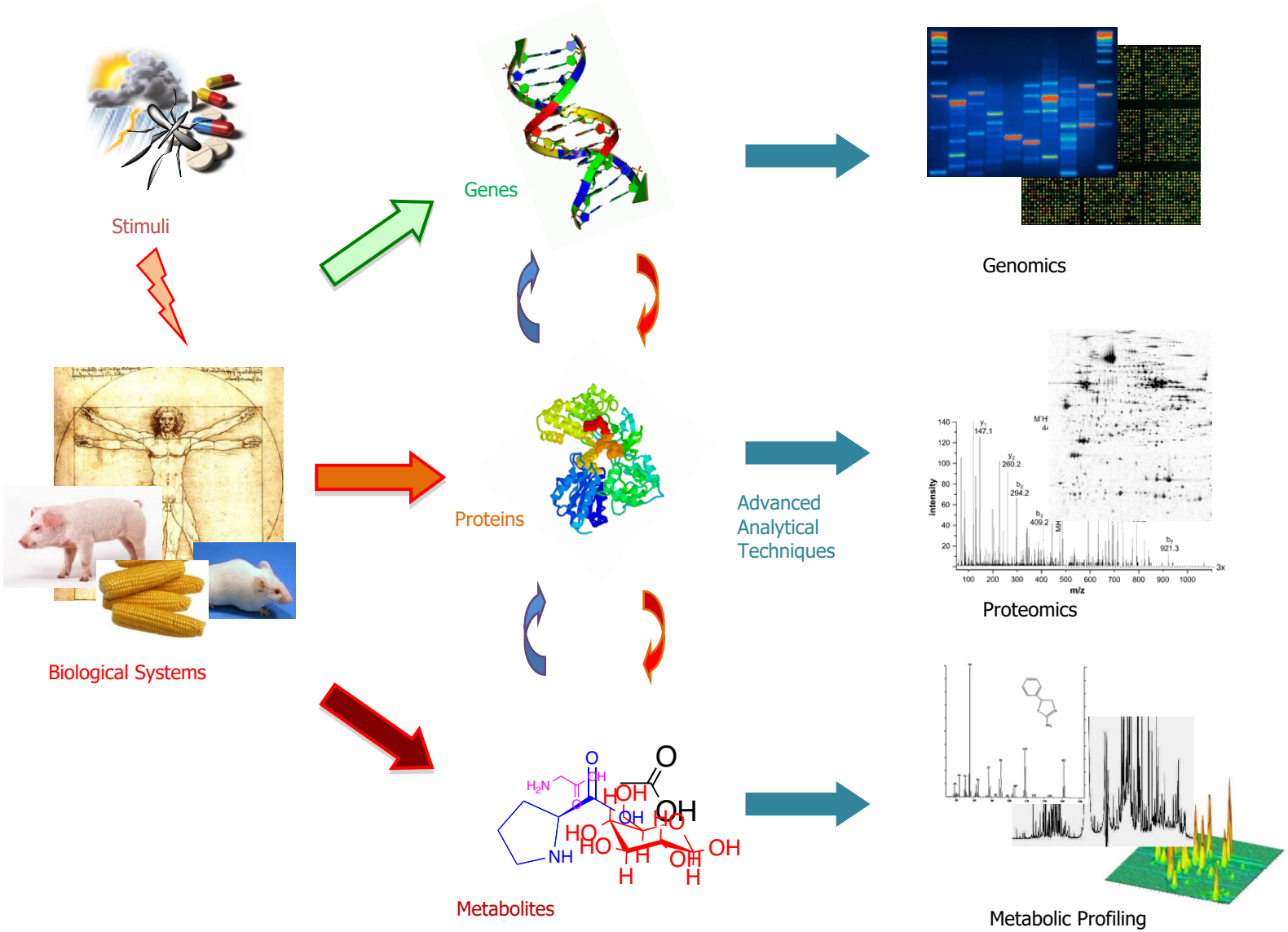
Daniel Raftery
Anesthesia and Pain Medicine
Northwest Metabolomics Research Center



UNIVERSITY of WASHINGTON

FRED HUTCHINSON CANCER RESEARCH CENTER

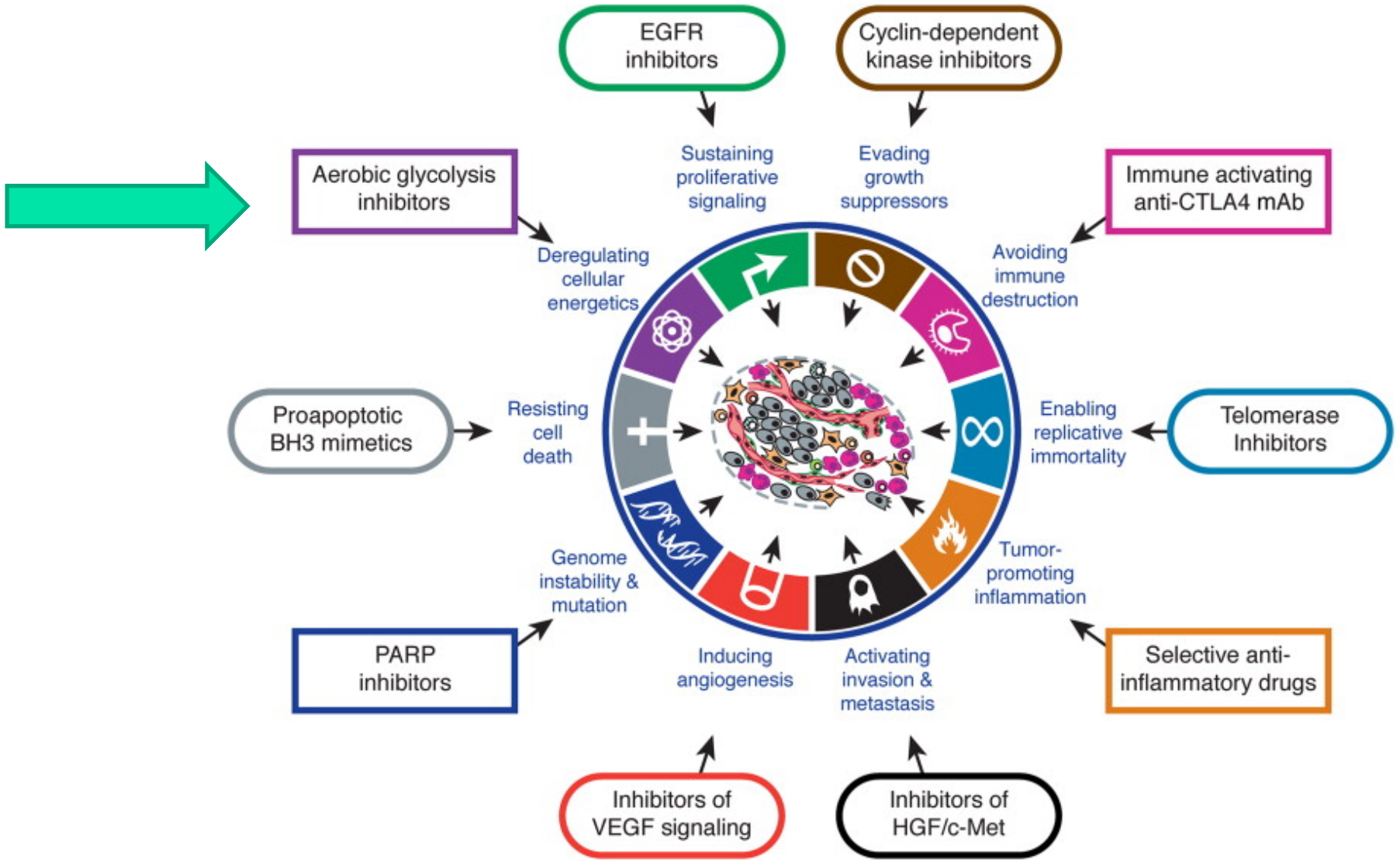
Metabolism in Context



Genotype + Environment --> Phenotype

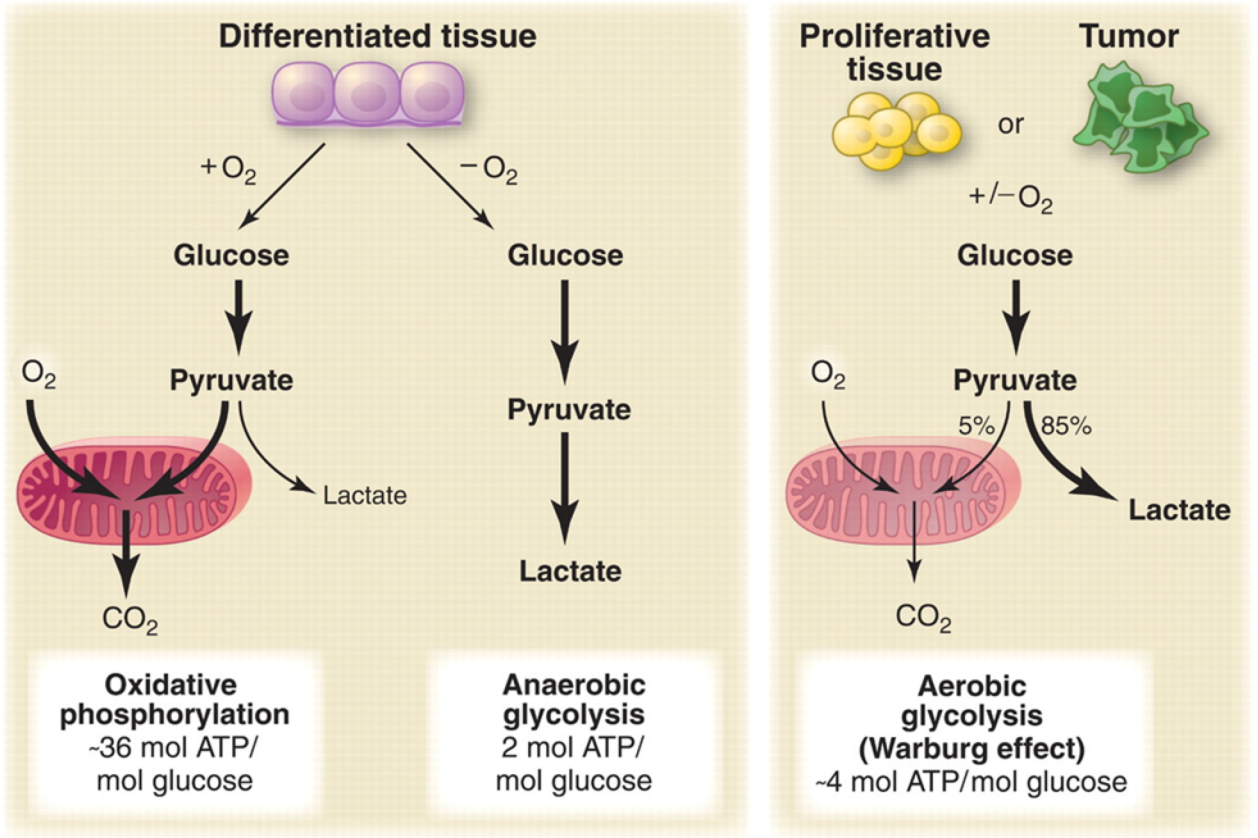
Metabolomics is the closest 'omics to phenotype

Altered Metabolism is a Recent (Re)-Addition to the Hallmarks of Cancer



D. Hanahan and R. Weinberg

Altered Metabolism in Cancer: Warburg Effect



Glutamine found to replace missing energy

Thompson et al, Science 2009

How To Study Cancer Metabolism: Metabolomics

- **Analysis of small molecules in bio-systems**

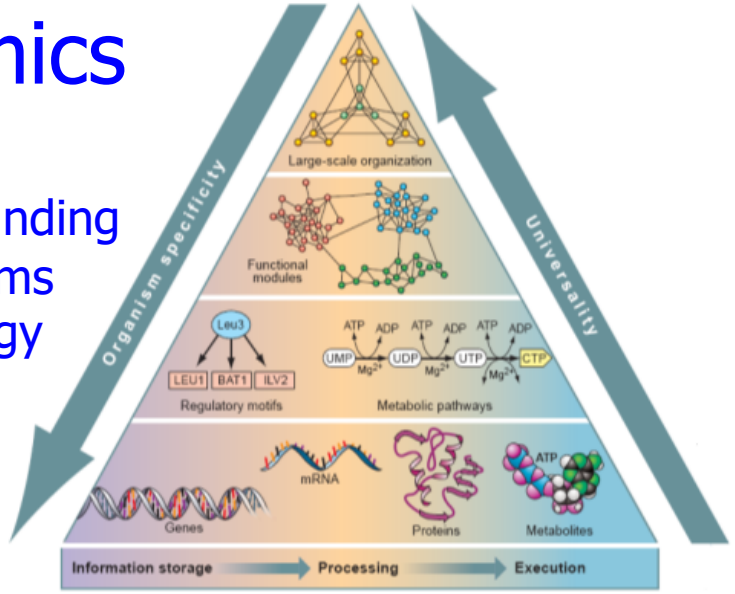
  ~20,000 aq + 200,000+ lipids

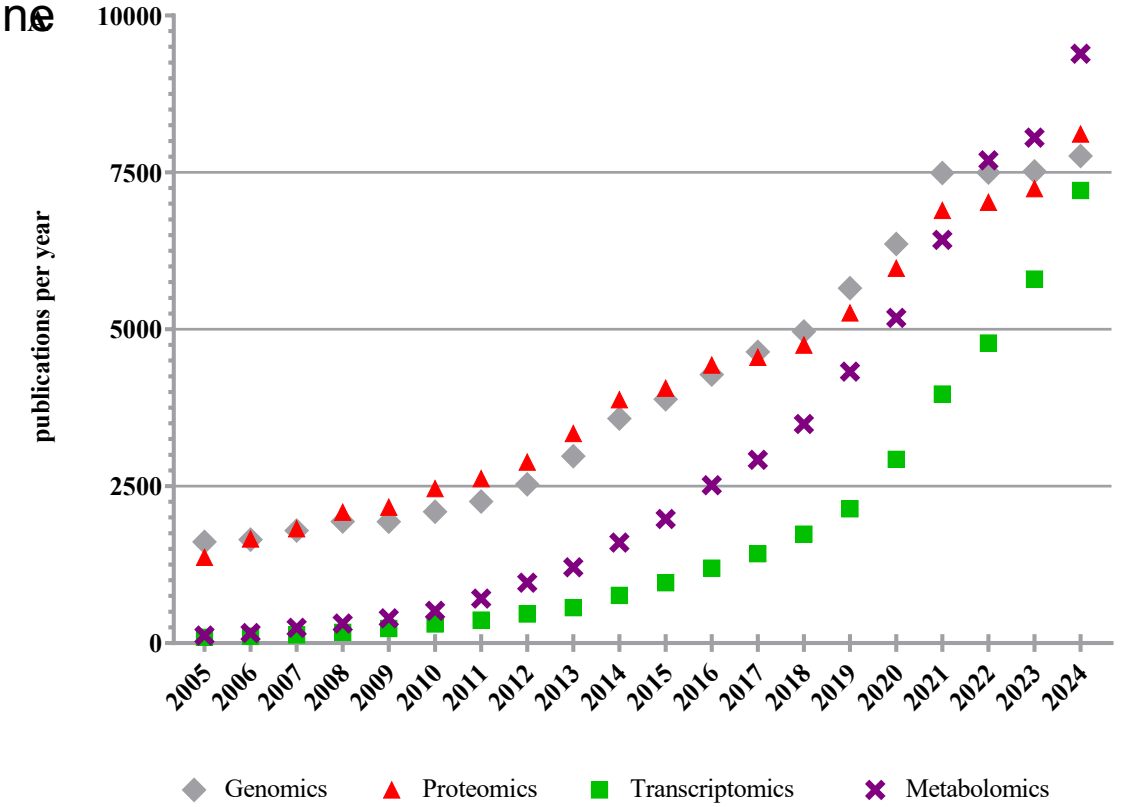
  Endogenous + Exogenous metabolites

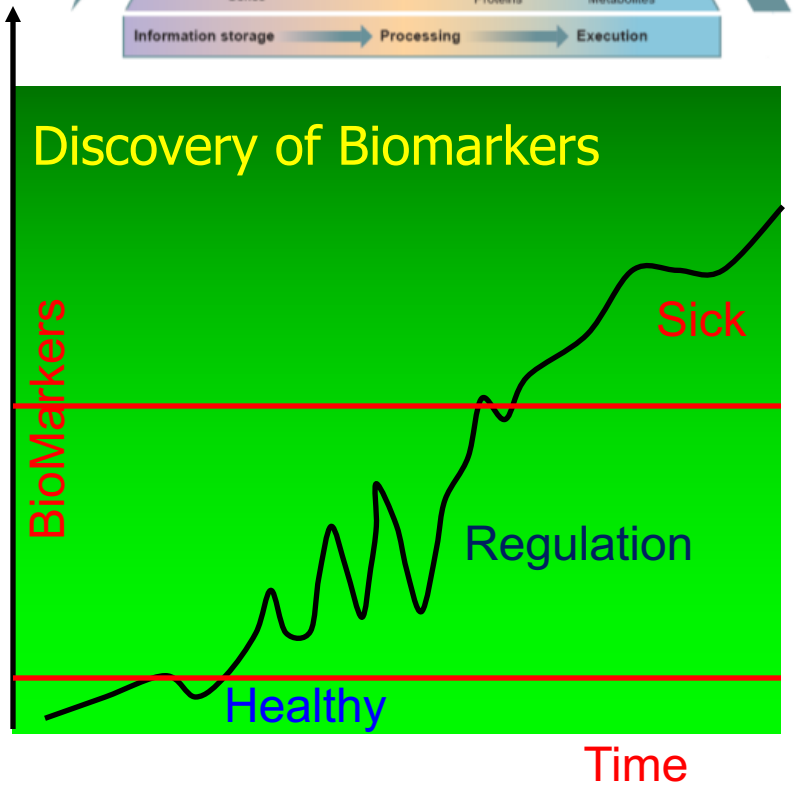
- **Applications in Metabolomics**

  Disease Diagnostics
  Personalized Medicine
  Food and Nutrition
  Cellular Metabolism
  Drug Discovery
  Toxicology
  Sys Bio Research

Understanding Systems Biology







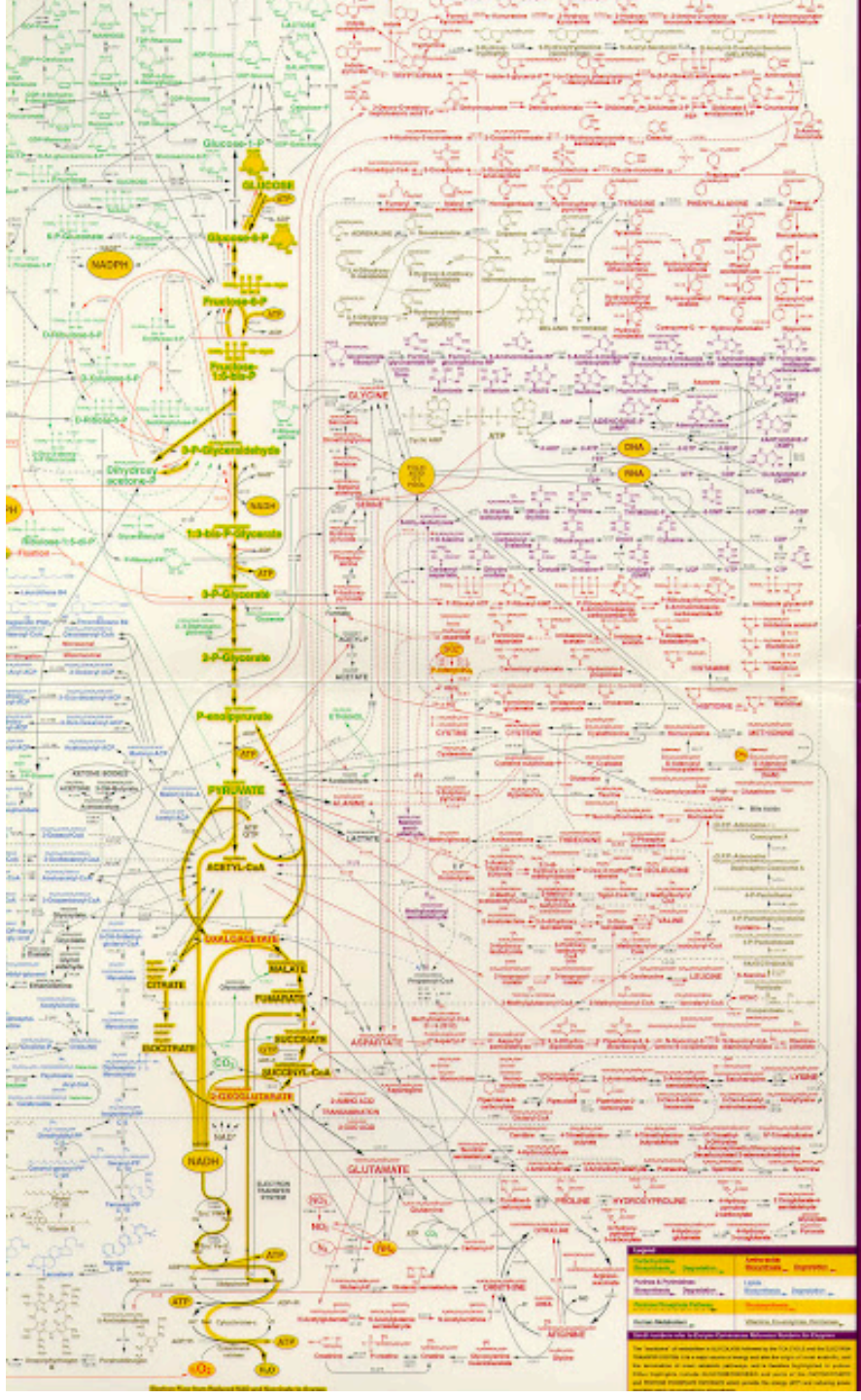
Brief History

- 2000 BC Chinese/Greek apocryphal story of ants
- 1800-1900: Identification of various metabolites
- 1930 – 50's Metabolite pathways identified
- 1950 -1960's: MS and NMR development
- 1960's: First "metabolomics" studies
- 1970's: LC and chemometrics development
- 1980's: LC-MS and high field NMR development
- 1998-99: Metabonomics and metabolomics coined
- 2000's: Development of statistical methods and databases
- Field is expanding rapidly (>1000 papers/year)

Metabolism

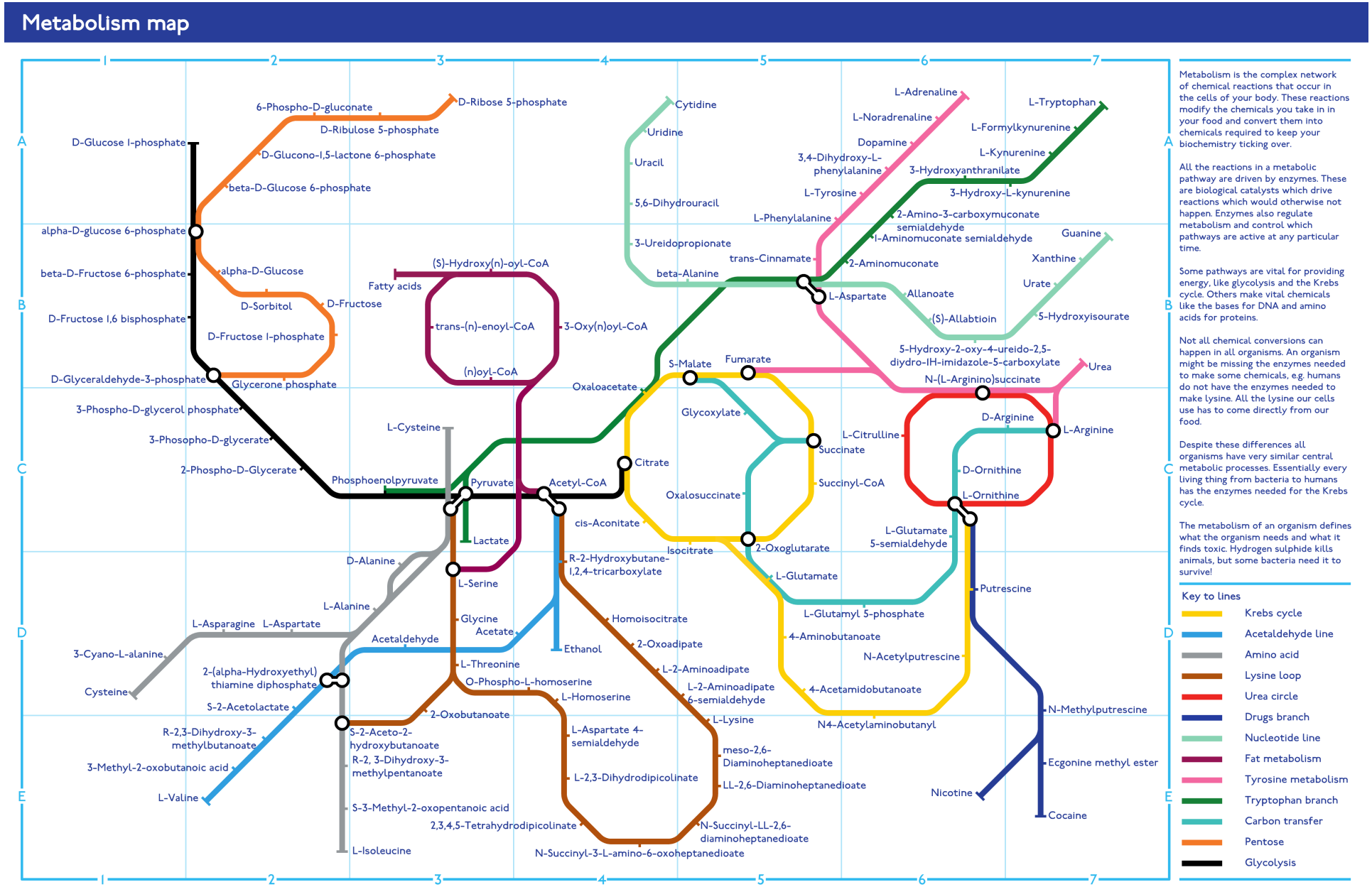
Is:

- Complex
- Interconnected
- Influenced by genetics & environment (food, stresses including illness)
- Affects upstream biology (gene expression, epigenetics, protein function)



Metabolic Map

Metabolic Maps

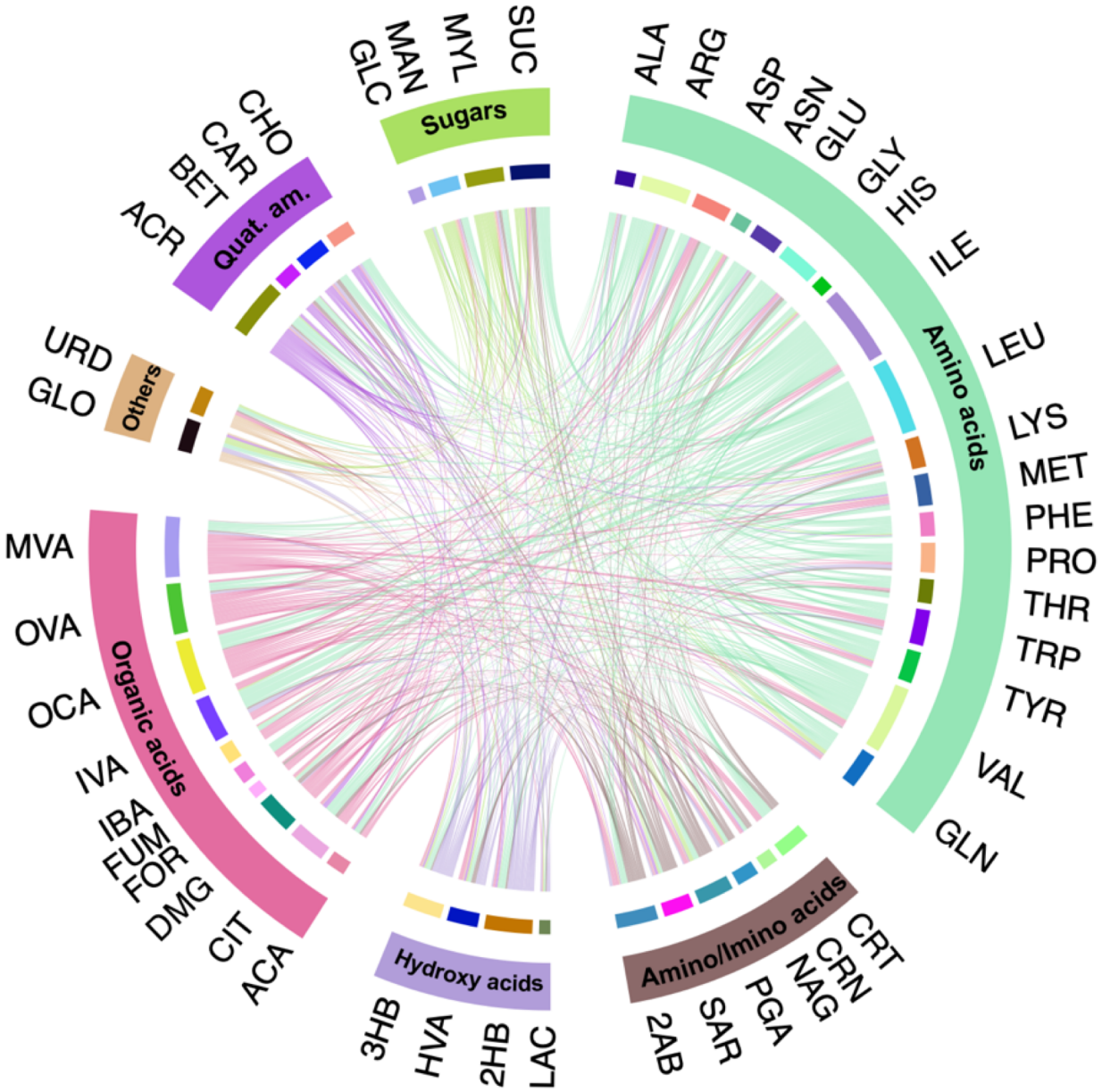


Networks of Metabolites from Modeling

From recent modeling studies we've performed, we found metabolites are often connected to a broad range of metabolite classes and pathways.

These are connected through the metabolite-metabolite correlations.

Indicates broad range of metabolite networks beyond canonical (KEGG) pathways are connected biologically among the metabolites.



Li et al, PNAS 2024

Metabolomics and Public Health

Growing number of diseases now associated with altered metabolism:

Cancers, CVD, Diabetes, Alzheimer's, TB, Hepatitis, etc.

The Field of Metabolomics is Focused on:

Foods and nutrition – effect on health/disease
Microbiome studies – Effect on health
Microbial studies: Infectious disease
Environmental studies: Exposome
Precision/Personalized Medicine
And More

Plus,
Drug discovery efforts in pharma
Fundamental Systems Biology

Important Cancer Related Metabolomic Findings

nature Vol 457|12 February 2009|doi:10.1038/nature07762

LETTERS

Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression

Arun Sreekumar[1,2,3,4], Laila M. Poisson[5]*, Thekkelnaycke M. Rajendiran[1,3]*, Amjad P. Khan[1,3]*, Qi Cao[1,3], Jindan Yu[1,3], Bharathi Laxman[1,3], Rohit Mehra[1,3], Robert J. Lonigro[1,4], Yong Li[1,3], Mukesh K. Nyati[4,6], Aarif Ahsan[6], Shanker Kalyana-Sundaram[1,3], Bo Han[1,3], Xuhong Cao[1,3], Jaeman Byun[7], Gilbert S. Omenn[2,7,8], Debashis Ghosh[4,5,11], Subramaniam Pennathur[2,4,7], Danny C. Alexander[12], Alvin Berger[12], Jeffrey R. Shuster[12], John T. Wei[4,9], Sooryanarayana Varambally[1,3,4], Christopher Beecher[1,2,3] & Arul M. Chinnaiyan[1,2,3,4,9,10]

Sarcosine found as a strong tissue marker of PC aggressiveness.

Vol 462|10 December 2009|doi:10.1038/nature08617 nature

ARTICLES

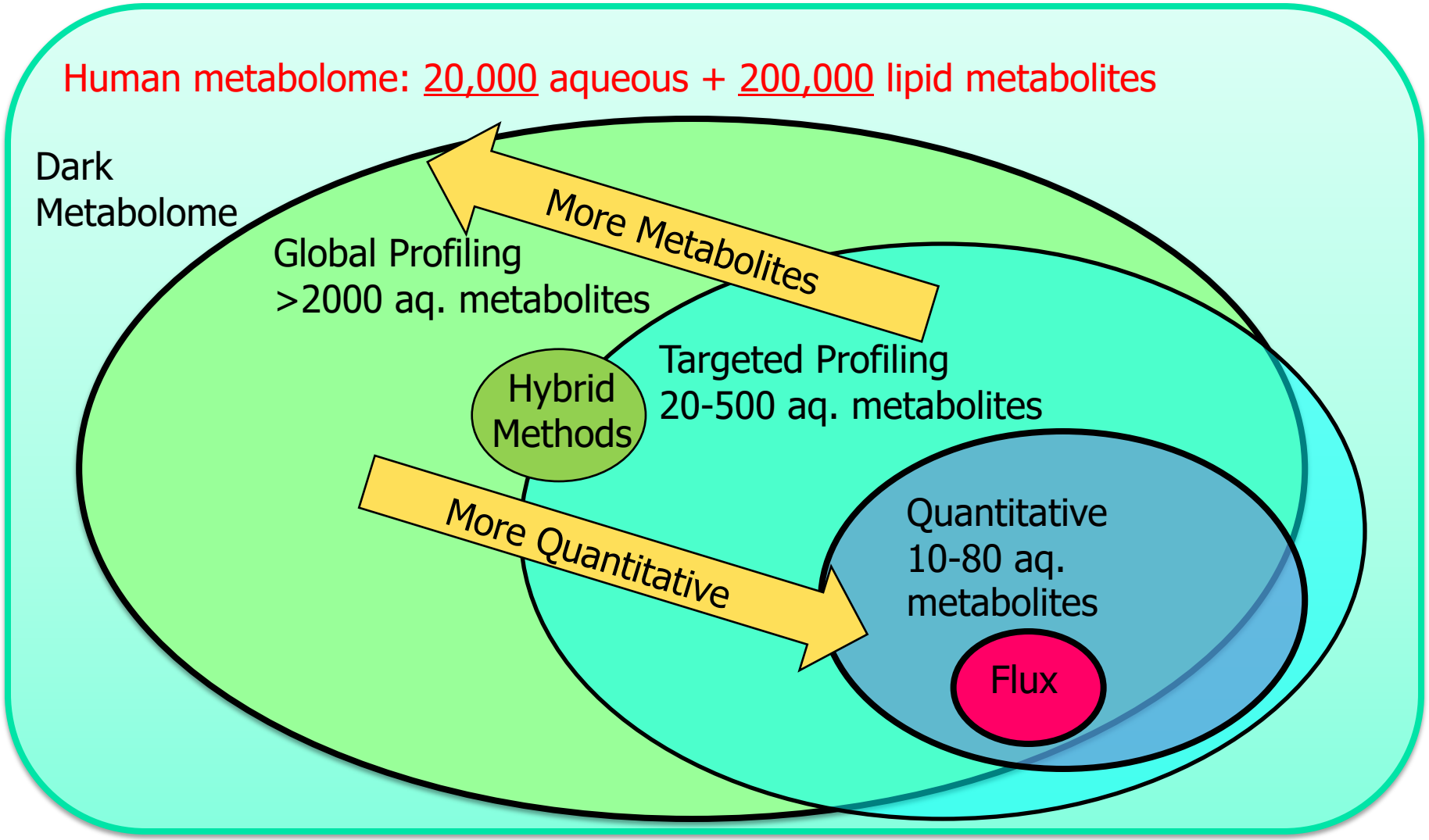
Cancer-associated IDH1 mutations produce 2-hydroxyglutarate

Lenny Dang[1], David W. White[1], Stefan Gross[1], Bryson D. Bennett[2], Mark A. Bittinger[1], Edward M. Driggers[1], Valeria R. Fantin[1], Hyun Gyung Jang[1], Shengfang Jin[1], Marie C. Keenan[1], Kevin M. Marks[1], Robert M. Prins[3], Patrick S. Ward[4], Katharine E. Yen[1], Linda M. Liau[3], Joshua D. Rabinowitz[2], Lewis C. Cantley[5], Craig B. Thompson[4], Matthew G. Vander Heiden[1]† & Shinsan M. Su[1]

New findings link genetic defect with metabolic up-regulation of metabolite linked with brain cancer.

The Metabolome and Its Measure

Metabolome = small molecules <1000 molecular weight



No Universal Detector for Metabolomics

UNIVERSITY of WASHINGTON Search

NW-MRC Northwest Metabolomics Research Center

HOME | SERVICES | GETTING STARTED | RESEARCH | INSTRUMENTATION | USEFUL LINKS

Metabolomics Capabilities

- Targeted Metabolomics
- Untargeted Metabolomics
- Unknown identification
- Bioinformatics Expertise
- New Assay Development
- Metabolic Flux Analysis
- Validation Studies

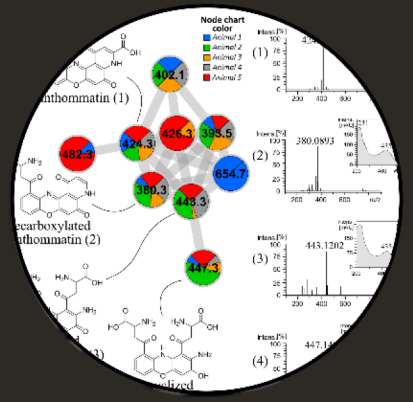
Northwest Metabolomics Research Center
nwmetabolomics.org

UNTARGETED ANALYSIS

Quantitative analysis of known metabolites within biological pathways

DATA PROCESSING

Global profiling or qualitative analysis of biological matrices



METABOLITE IDENTIFICATION

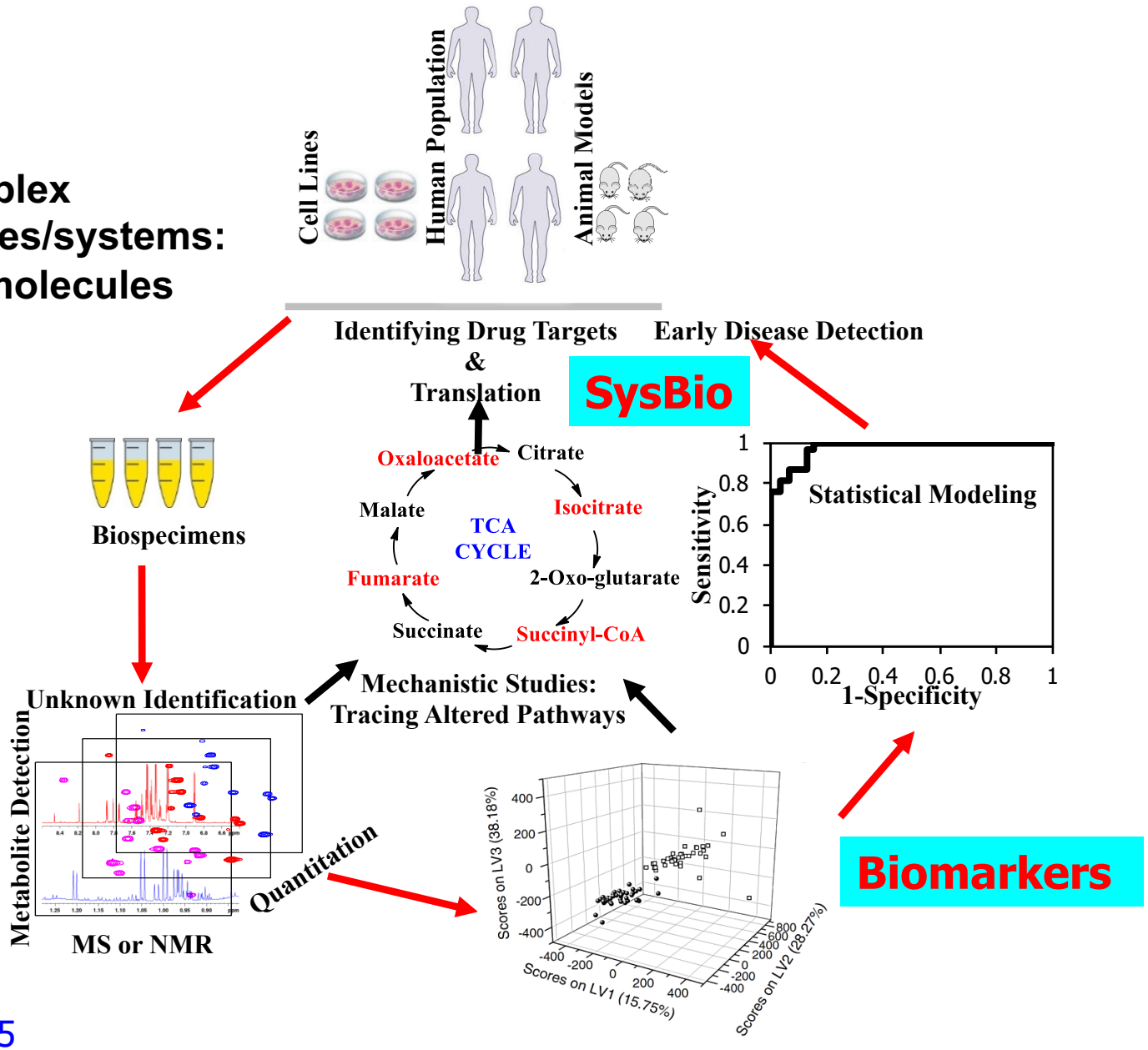
Network-based metabolite annotation by MS2 spectra, NMR

TARGETED ANALYSIS

Quantitative validation of biomarker candidates

Metabolomics Methods and Applications

Analysis of complex biological samples/systems: 1000's of small molecules

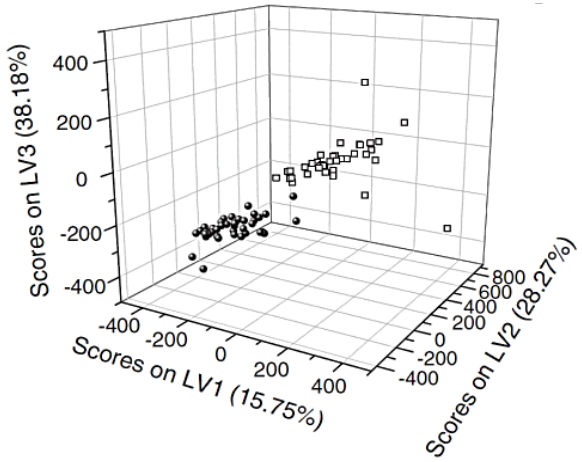


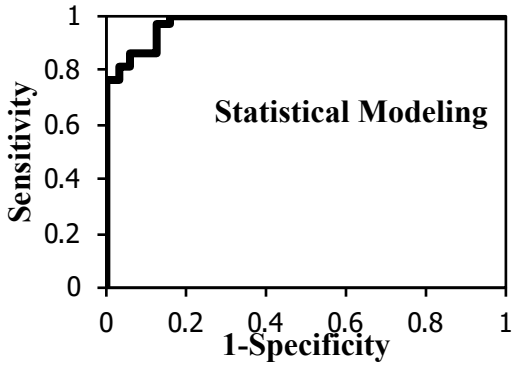
Gowda & Raftery
J. Magn. Reson. 2015

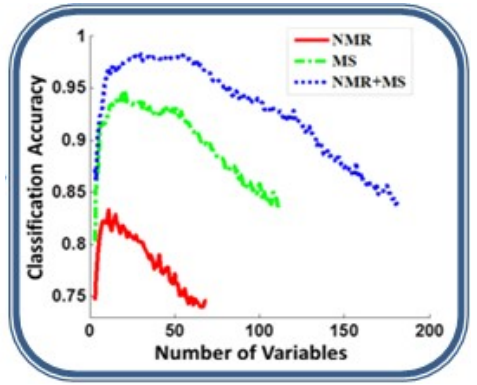
Bioinformatic Analysis

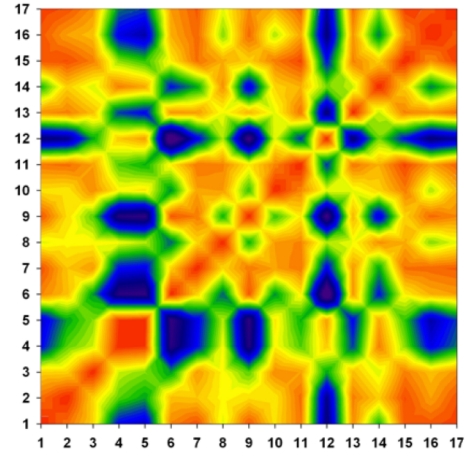
Broad range of analyses performed on metabolomics data for

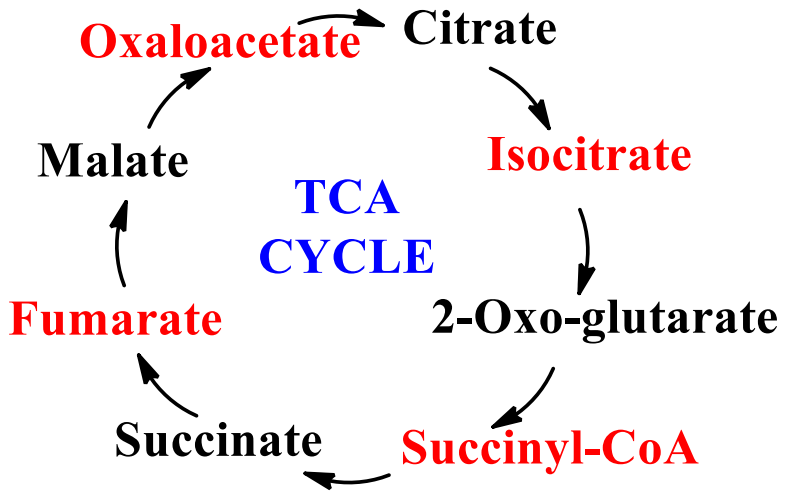
- **Statistical analysis**
- **Biomarker discovery**
- **Metabolic target identification**
- **Pathway analysis**
- **Biological interpretation**

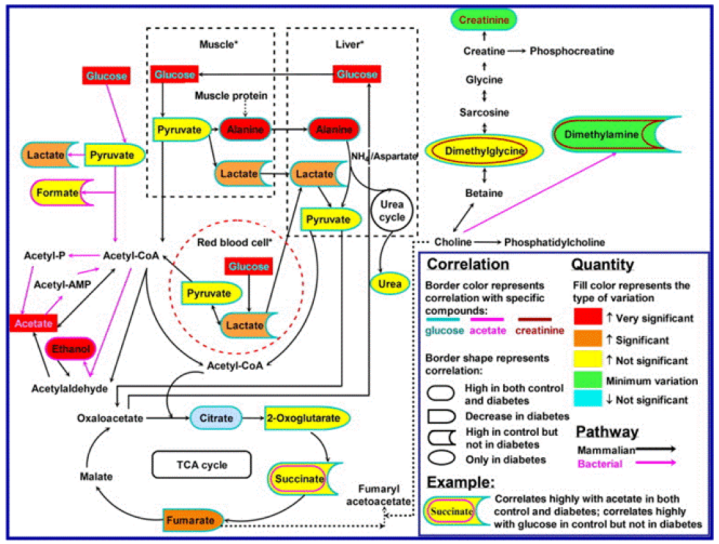


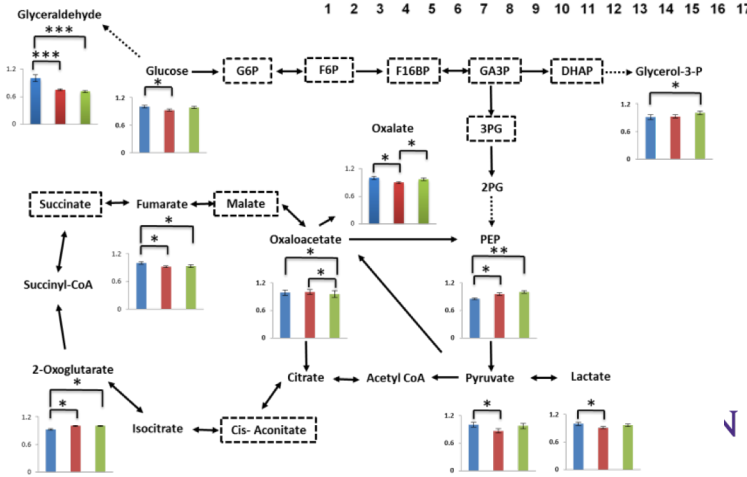






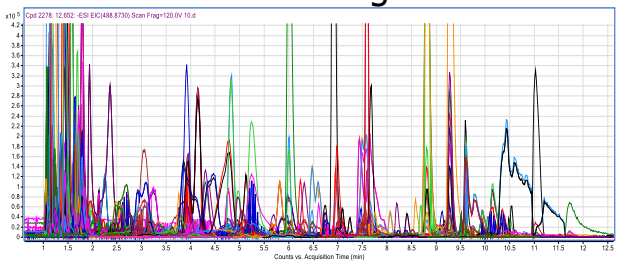






Typical Metabolomics Data Analysis Workflow

Global Profiling Data

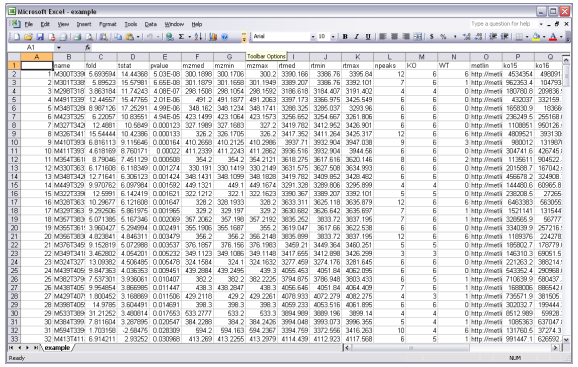


MS or NMR data

1,000,000 data points

Instrument manufacturer or 3rd party software

2,000 - 5,000 features

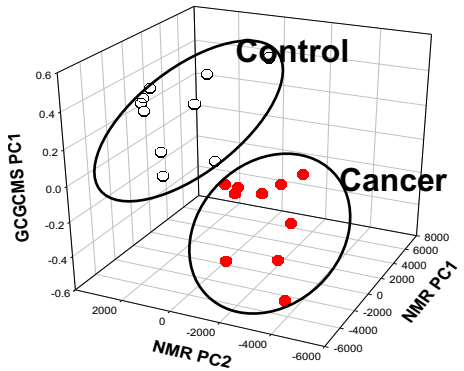


Library of compound spectra

400 - 600 Identified metabolites

Statistical methods: Feature selection

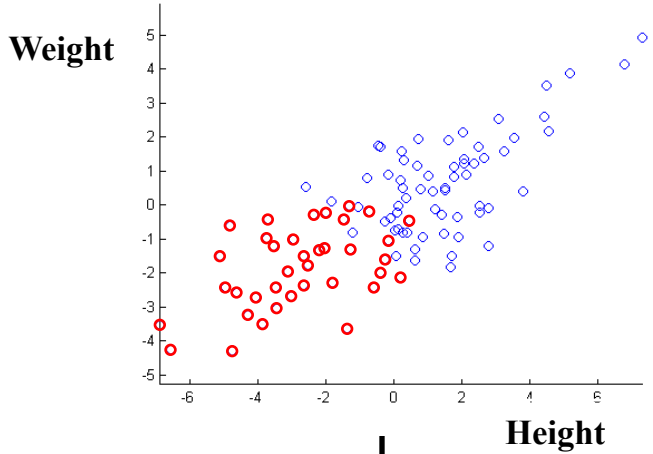
10-50 statistically different metabolites

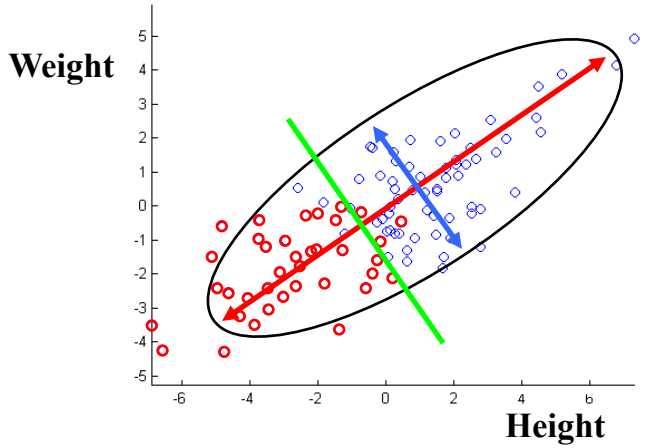


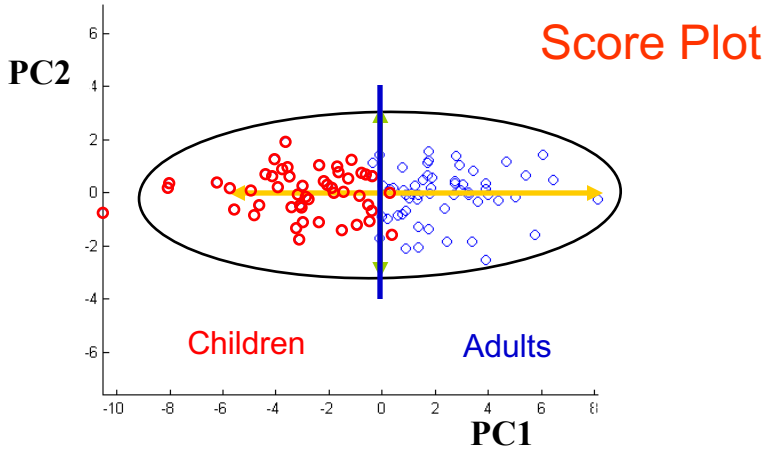
Statistical methods: Model building and testing

Statistical model for validation

Simple Example of Machine Learning: Principal Component Analysis (PCA)





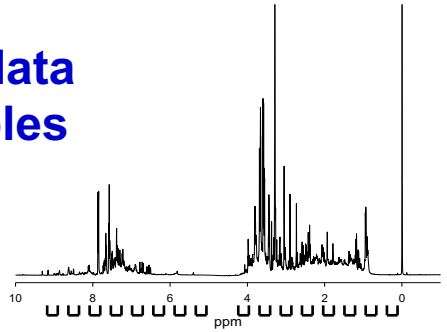


Score Plot

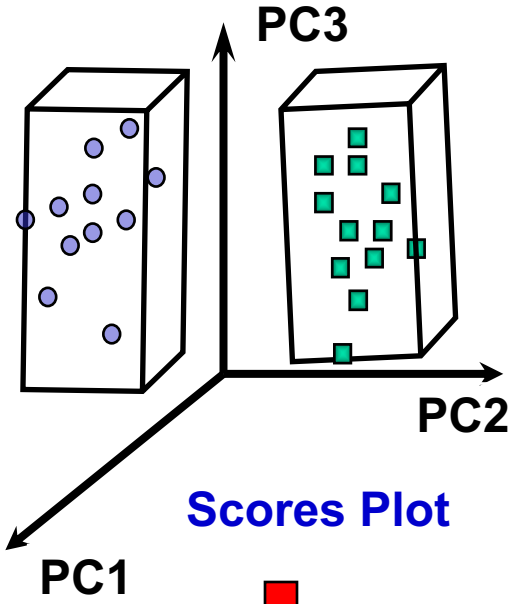
- Starting point in multivariate analysis
- The goal of PCA is to find the direction of greatest variability
- The scores plots allow one to classify clusters
- The corresponding loadings plots show how they are classified

PCA Procedure for Metabolomics

High resolution data from many samples

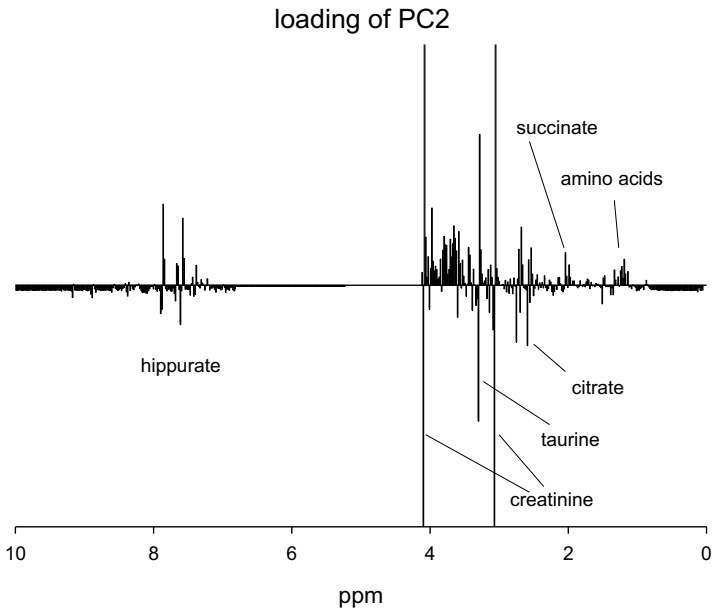


PCA scores



Scores Plot

PCA loadings

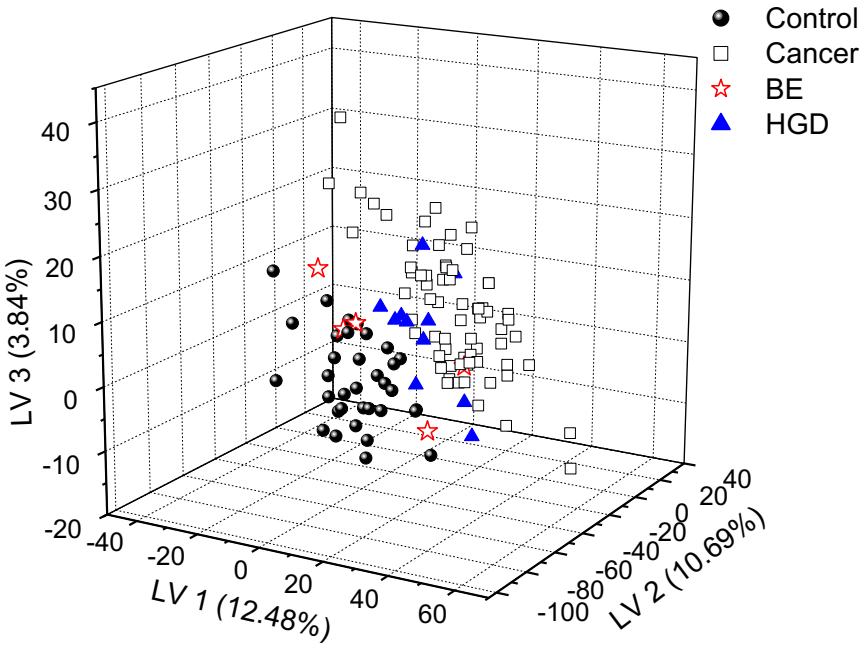


information on biomarkers, metabolic pathways, systems biology, disease mechanisms

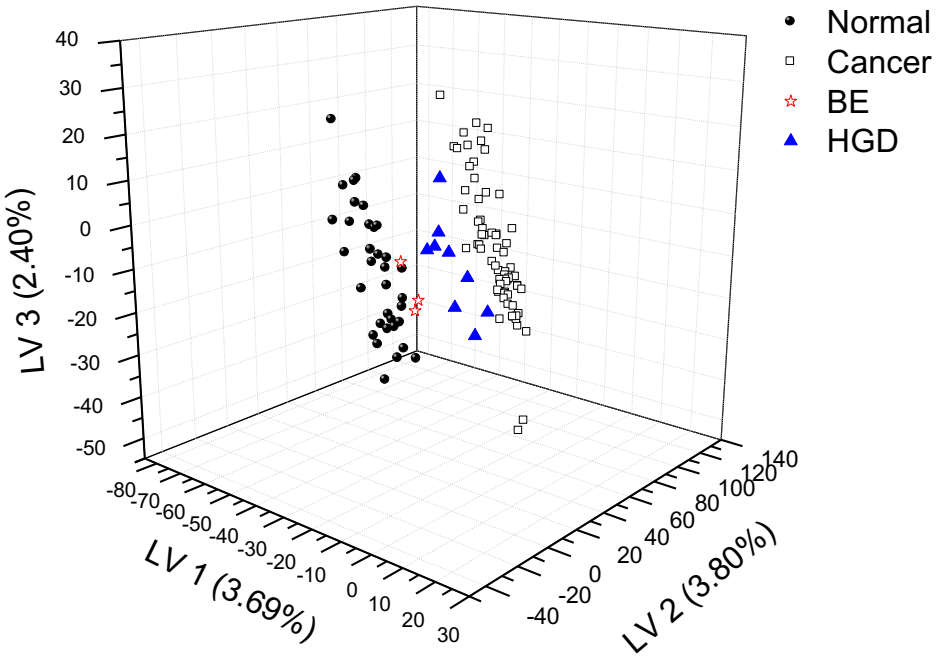
Global Metabolomics of Esophageal Cancer

Analysis of serum samples from patients with EC, at risk patients and healthy controls

PLS-DA score plot for the whole NMR spectra



PLS-DA score plot for the whole LC-MS spectra



However, the clinically relevant comparison, BE vs EC, is harder to distinguish.

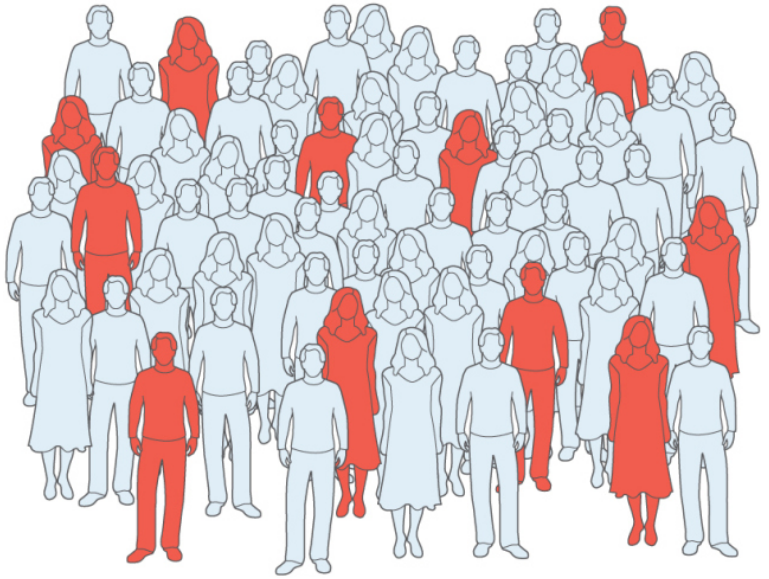
Zhang J, *et al.* J Thorac Cardiovasc Surg., 2011
Zhang J, *et al.* PlosOne, 2012.
Buas et al. Metabolomics, 2017

Diagnostic Development Using Metabolomics

- Diagnostic biomarkers typically
  - have excellent accuracy, >90%
  - odds ratios of ~100
  - Used to identify disease in 1 patient

Colorectal Cancer (CRC) and Early Diagnosis

- No.3 leading cancer type in the US.
- No.3 cause of cancer death in the US.
- Five-year Relative Survival Rates:
  - Local: 90%
  - Regional: 70%
  - Distant: 12%



Picture source: AGAJournals.org

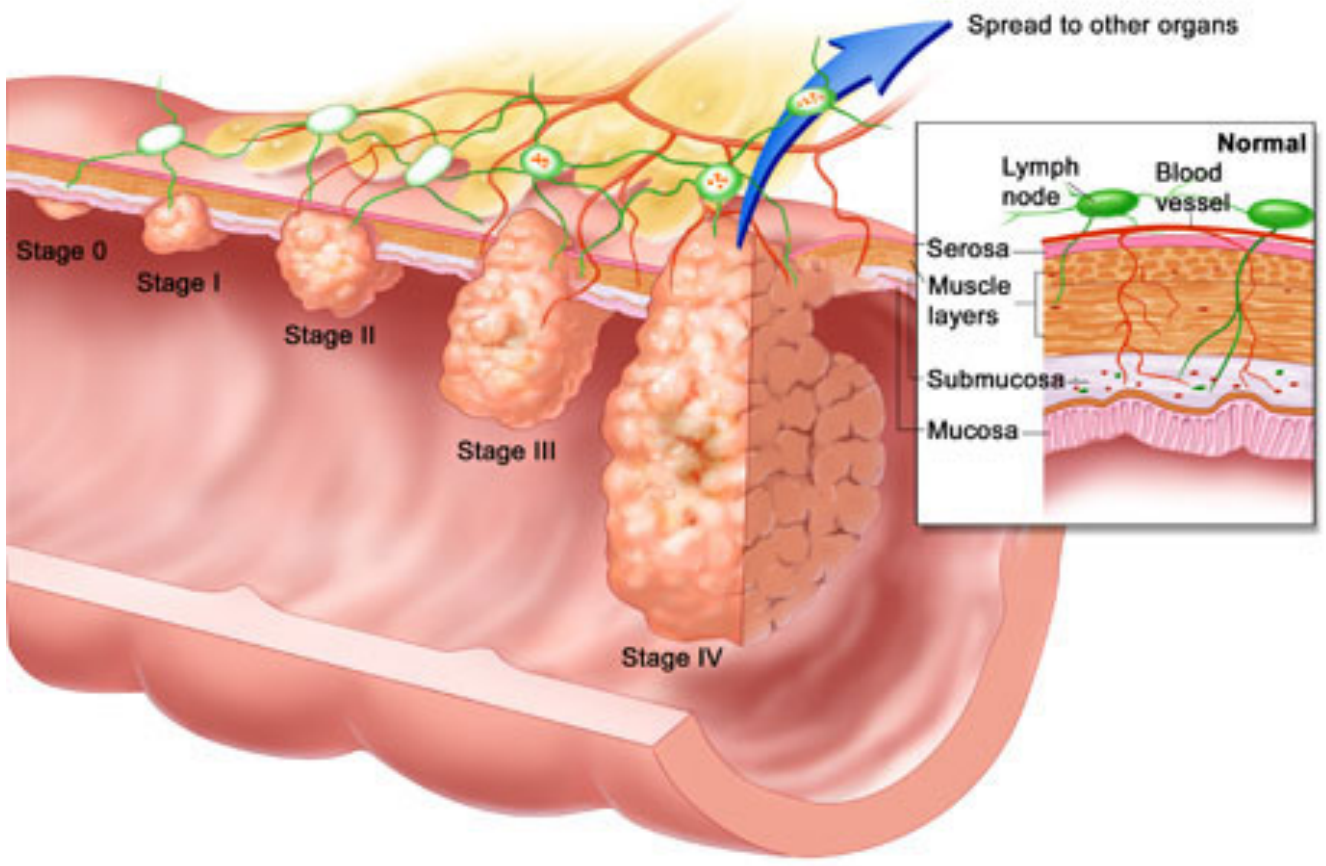
- Early detection gives more therapy options and saves lives

American Cancer Society, Surveillance Research

Colon Cancer Development

CC can develop for 10-20 years before polyps convert to cancer.

<u>Risk factors:</u>
Age
Race
Gender
Smoking
Diet
Diabetes
Other cancers
Industrial Countries



Classical Screening Tests

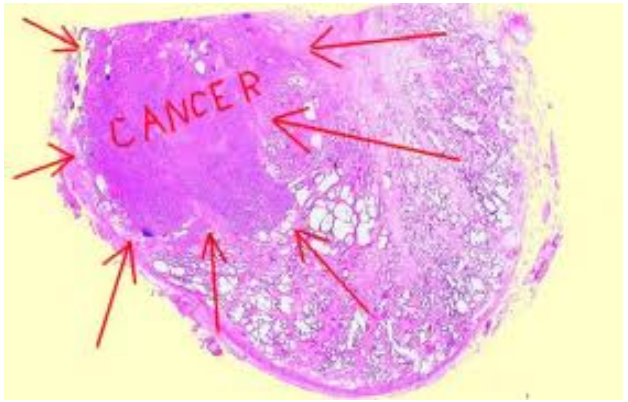
Colonoscopy

Blackdoctor.org

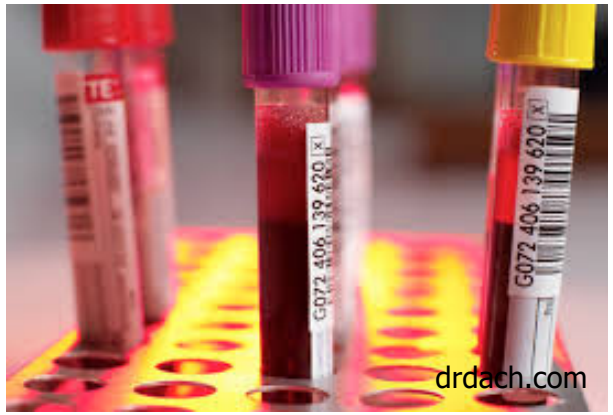
Stool Test

Nytimes.com

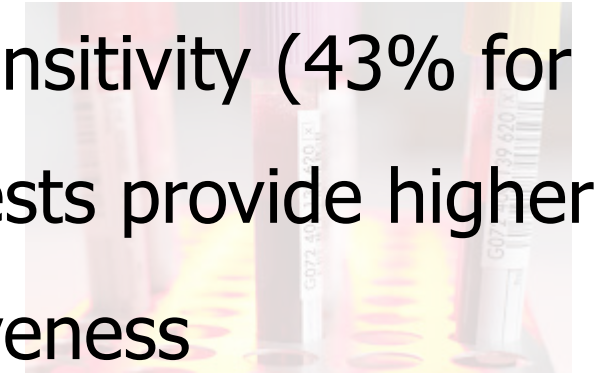
Biopsy

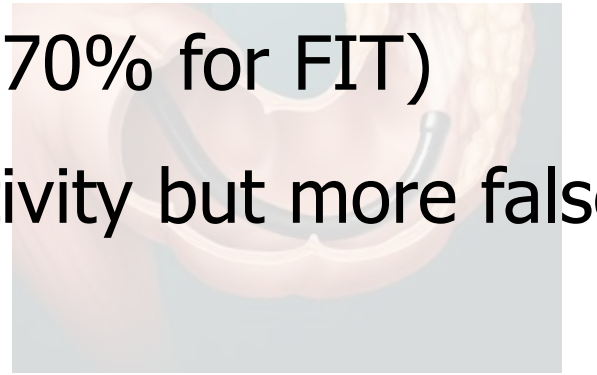


Blood test?

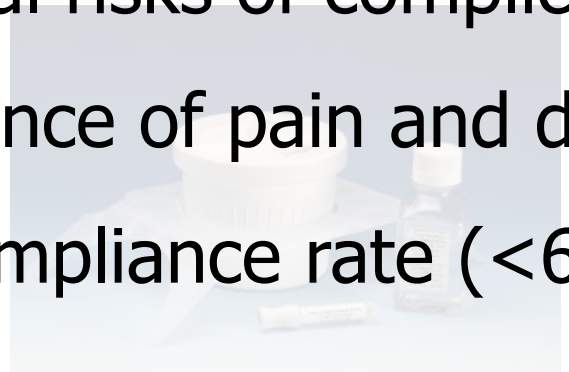


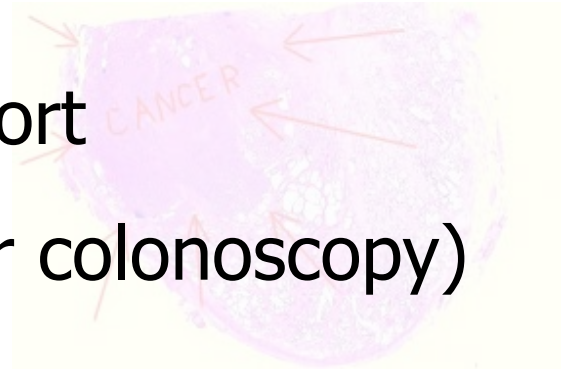
Drawbacks





- Low sensitivity (43% for FOBT, 70% for FIT)
- New tests provide higher sensitivity but more false positives
- Invasiveness
- Potential risks of complications
- Experience of pain and discomfort
- Low compliance rate (<60% for colonoscopy)





Study Information

		Total n=234	CRC n=66	Polyps n=76	Healthy Control n=92
Age	Median	58	56	57
	Min	27	37	18
	Max	88	86	80
Gender	Male	30	37	45
	Female	36	39	47
Cancer stage	Stage I/II	21	–	–
	Stage III	17	–	–
	Stage IV	28	–	–
Diagnosis	Colon Cancer	39	–	–
	Rectal Cancer	27	–	–

- 114 metabolites detected by targeted LC-MS
- Clinical info: age, gender, BMI, smoking, alcohol, diagnosis

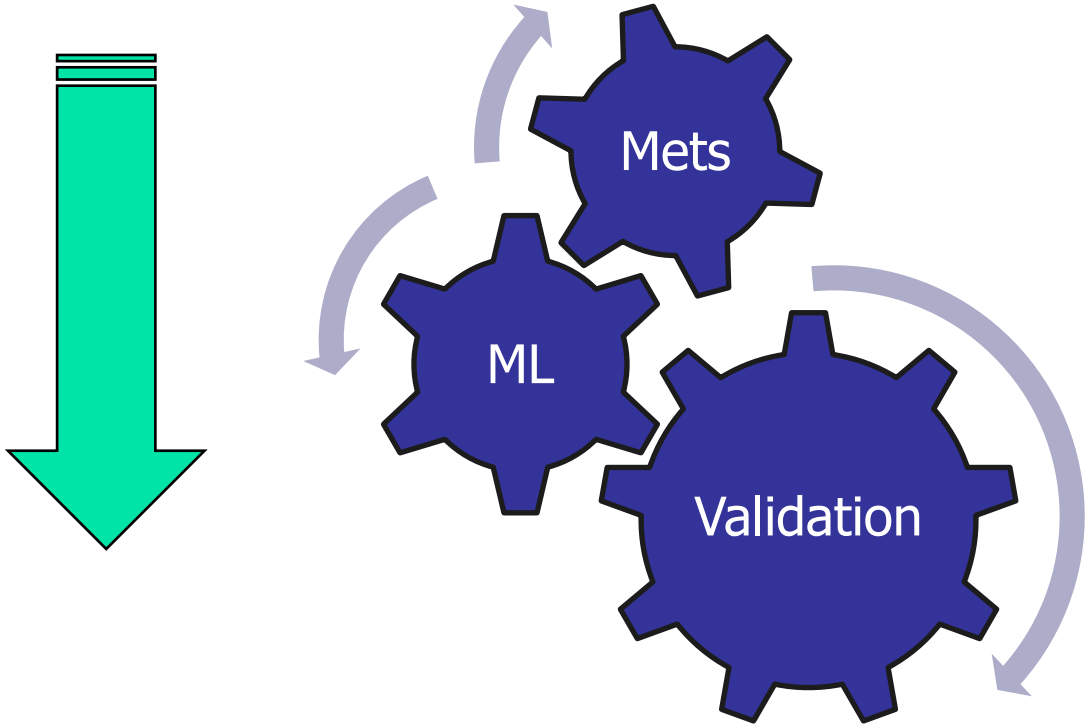
Zhu et al., J. Prot. Res.

Single Metabolite Performance

Metabolites	AUROC	Std. Error	95% Confidence Interval		Sensitivity	Specificity	Accuracy
			Lower Bound	Upper Bound			
Histidine	0.719	0.040	0.640	0.798	0.924	0.467	0.658
Glyceraldehyde	0.702	0.042	0.619	0.785	0.742	0.641	0.686
Glycochenodeoxycholate	0.688	0.042	0.605	0.770	0.879	0.435	0.620
Hyppuric Acid	0.684	0.044	0.597	0.771	0.591	0.794	0.709
Methionine	0.680	0.043	0.596	0.764	0.667	0.630	0.646
Lysine	0.680	0.043	0.595	0.764	0.530	0.794	0.684
Linolenic Acid	0.668	0.044	0.581	0.755	0.439	0.880	0.696
Glycocholate	0.665	0.043	0.580	0.749	0.742	0.565	0.703
Glutamic acid	0.660	0.044	0.574	0.746	0.606	0.707	0.665
N-AcetylGlycine	0.657	0.044	0.570	0.744	0.788	0.511	0.623
2'-Deoxyuridine	0.656	0.044	0.571	0.742	0.576	0.685	0.639
Allantoin	0.653	0.043	0.568	0.739	0.606	0.663	0.639
Glutamine	0.652	0.044	0.566	0.739	0.546	0.707	0.639
Aspartic Acid	0.649	0.046	0.559	0.739	0.439	0.859	0.684
Dimethylglycine	0.649	0.044	0.562	0.736	0.606	0.663	0.639
Maleic Acid)	0.649	0.045	0.560	0.737	0.606	0.707	0.665
Hydroxyproline/Aminolevulinate	0.647	0.044	0.561	0.733	0.682	0.587	0.627
Adenylosuccinate	0.642	0.045	0.553	0.731	0.439	0.815	0.658
Malonic Acid/3HBA	0.637	0.048	0.542	0.731	0.546	0.815	0.703

Multi-Metabolite Approach

When single metabolites don't work, combine them:



Results:
Using 12 metabolites + clinical variables: Accuracy = 93%
Promising, but not fantastic.
Performance typically degrades in validation process.

Cologard Stool Test

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Multitarget Stool DNA Testing for Colorectal-Cancer Screening

Thomas F. Imperiale, M.D., David F. Ransohoff, M.D., Steven H. Itzkowitz, M.D., Theodore R. Levin, M.D., Philip Lavin, Ph.D., Graham P. Lidgard, Ph.D., David A. Ahlquist, M.D., and Barry M. Berger, M.D.

Uses BMP4, NDRG4, KRAS gene panel
+ FIT for human hemoglobin.
94% accuracy

10,000 patient trial (300 CRC patients)
$100M, FDA approved
Now covered by insurance

Diagnostic vs Risk Biomarkers

- Diagnostic biomarkers typically
  - have excellent accuracy, >90%
  - odds ratios of ~100
  - Used to identify disease in 1 patient
- Risk biomarkers are used
  - At population level
  - Odds ratios are typically 2-6 or so
  - Used to affect behavior at a population level

Nutrition and Physical Activity Assessment Study

Women's Health Initiative (WHI)

Goals:

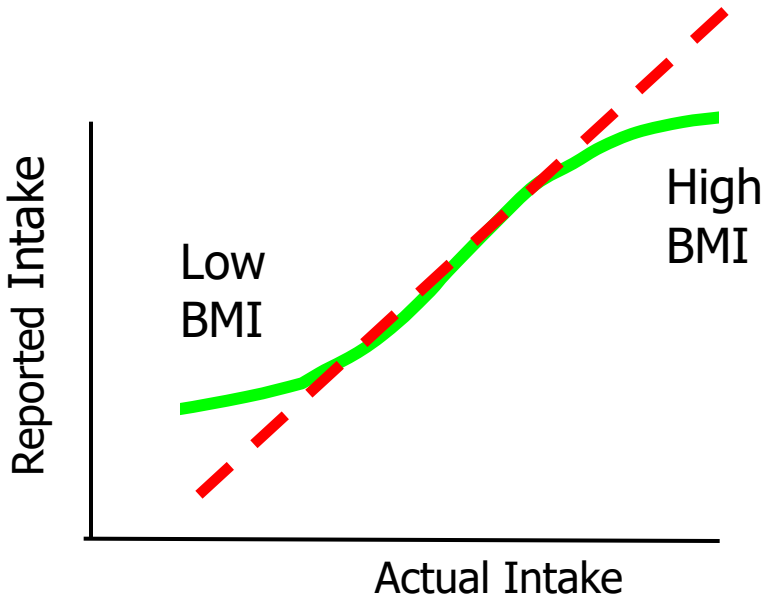
1) Identify potential biomarkers of macro and micro-nutrients
2) Use these biomarkers to correct FFQs
3) Improve disease risk prediction based on improved dietary information

NPAAS Feeding Study	WHI Extension Study	WHI nested case-control
153 subjects	450 subjects	1506 subjects
Blood, 24 hr urine, spot urine	Blood, 24 hr urine	Blood, spot urine
Develop cross validated biomarkers of nutrients using metabolomics data.	Calculate intake values for each nutrition variable Develop calibration equations for each nutrition variable Use FFQ 4-day food record and 24-hour dietary recall.	753 controls 753 who developed CRC or BC Calculate nutrition related disease risk for CRC and BC without use of FFQ.

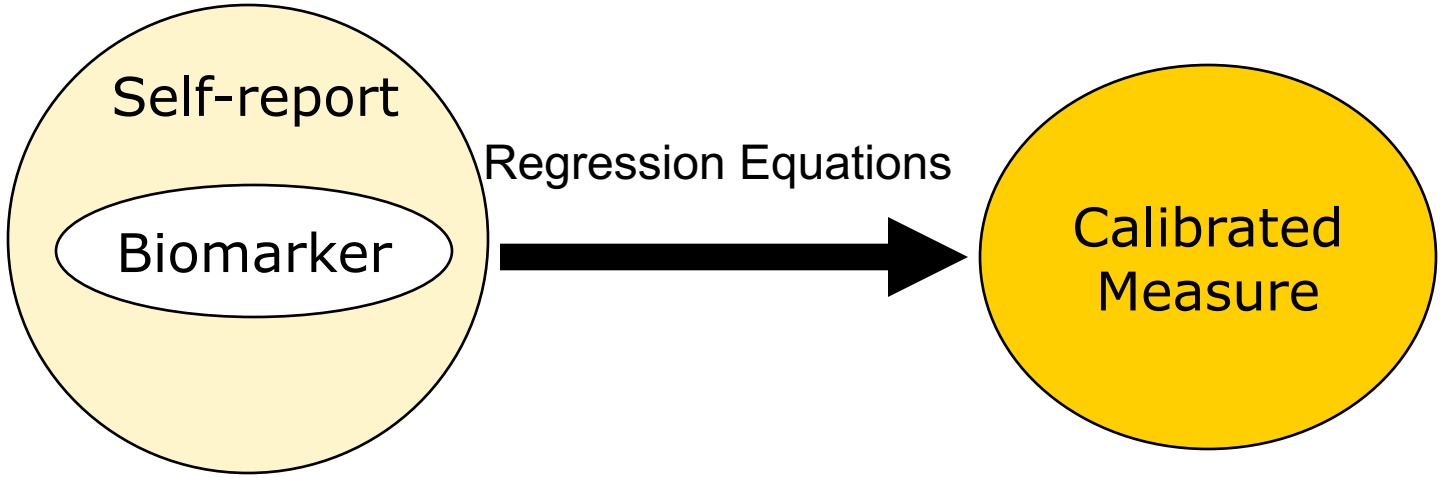
Metabolomics:	Blood	Urine
	Targeted LC-MS	NMR
	Lipidomics	GC-MS

Biomarker-Calibration Approach

Problem:
Food frequency data are very not reliable



Solution:
Calibration



The biomarkers are measured in a representative subset, then can be extrapolated to larger datasets

Metabolomics Data and Analysis

153 study samples
19 blinded duplicate samples used to test reproducibility

Sample	Platform	Features (#)	Metabolites (#ID'd)	<20% Missing	Ave. CV (%, BD)
Serum	Lipidomics		1070	664	5.5
Serum	LC-MS/MS		303	155	7.2
Urine (24-hr)	GC-MS	285		138	31.3
Urine (spot)	GC-MS	285		135	31.3
Urine (24 hr)	NMR		57	57	4.0
Urine (spot)	NMR		57	57	1.2

Statistical Analysis
QC normalization
Log transformation
80/20 split for training/testing
Regression analysis using LASSO
Penalty parameter determined using 5-fold CV of training set
Regression model built to test correlation: outcome vs predicted

Results: Correlation of Metabolites and Intake

CV-R^2	CV-R^2	CV-R^2 w/DLW	correlation
Protein (%E)	36.3%	45.0%	0.67
Protein (g/d)		52.0%	0.72
Carbohydrate (%E)	37.3%	37.0%	0.61
Carbohydrate (kcal/d)		55.9%	0.75
Energy (kcal/d)		55.5%	0.74

- Multiplatform approach allows broader metabolome coverage and a comparison of data but is complicated to put together.
- Improved results when personal characteristics and DLW/UN included.
- Habitual diets are most realistic, as they don't perturb the gut microbiome as much. But they also limit the study unless efforts are made to find participants with widely different diets.
- Urine>blood and blood+urine for carbohydrate measures. But DLW is still very important in the model.

Zheng et al., Eur J. Nutr. 2021

Metabolite Based Disease Risk Modeling

Based on calibration equations, metabolite biomarkers were then extrapolated into case-control set (~1500 samples with outcomes data) to identify disease/diet associations and disease risk.

NPAAS Feeding Study 153 subjects	WHI Extension Study 450 subjects	WHI nested case-control 1506 subjects
Develop metabolite biomarkers for: Animal protein Plant protein, Carbohydrates Dietary fiber	Calibrate FFQs using metabolite biomarkers as intake measures	Use calibrated FFQs to predict disease risk and compare to outcomes data. Cancer risk CVD risk Diabetes risk

Biomarker-Calibrated Macronutrient Intake and Chronic Disease Risk among Postmenopausal Women

	Biomarker Calibrated Risk (Hazard Ratios) for 20% Increase in:				
Outcome (Participants)	Protein Density	Total Protein Density	Carbohydrate Density	Fiber Density	Fat Density
Breast Cancer (5,311)	1.03	0.92	**0.84**	0.97	**1.16**
Colon Cancer (1,101)	**1.28**	**0.59**	0.93	0.99	**1.26**
Heart Disease (2,869)	**1.20**	**0.75**	0.90	**0.80**	**1.13**
T2 Diabetes (12,145)	1.03	1.11	**0.74**	**0.93**	**1.19**

Animal protein ↑ Risk CC, HD ↑
Carbohydrate ↑ Risk BC, T2D ↓
Fat ↑ Risk BC, CC, HD, T2D ↑

Plant protein ↑ Risk ↓
Fiber ↑ Risk HD and T2D ↓

Analyses included total energy intake, in Women's Health Initiative cohorts (***n*= 81,894**) of postmenopausal U.S. women enrolled during 1993-1998 at 40 U.S. clinical centers and followed through February 2020.

Prentice et al., *J Nutr*, 2021, 2023

Targeting Cancer-Altered Metabolism

Cancer metabolism: a therapeutic perspective

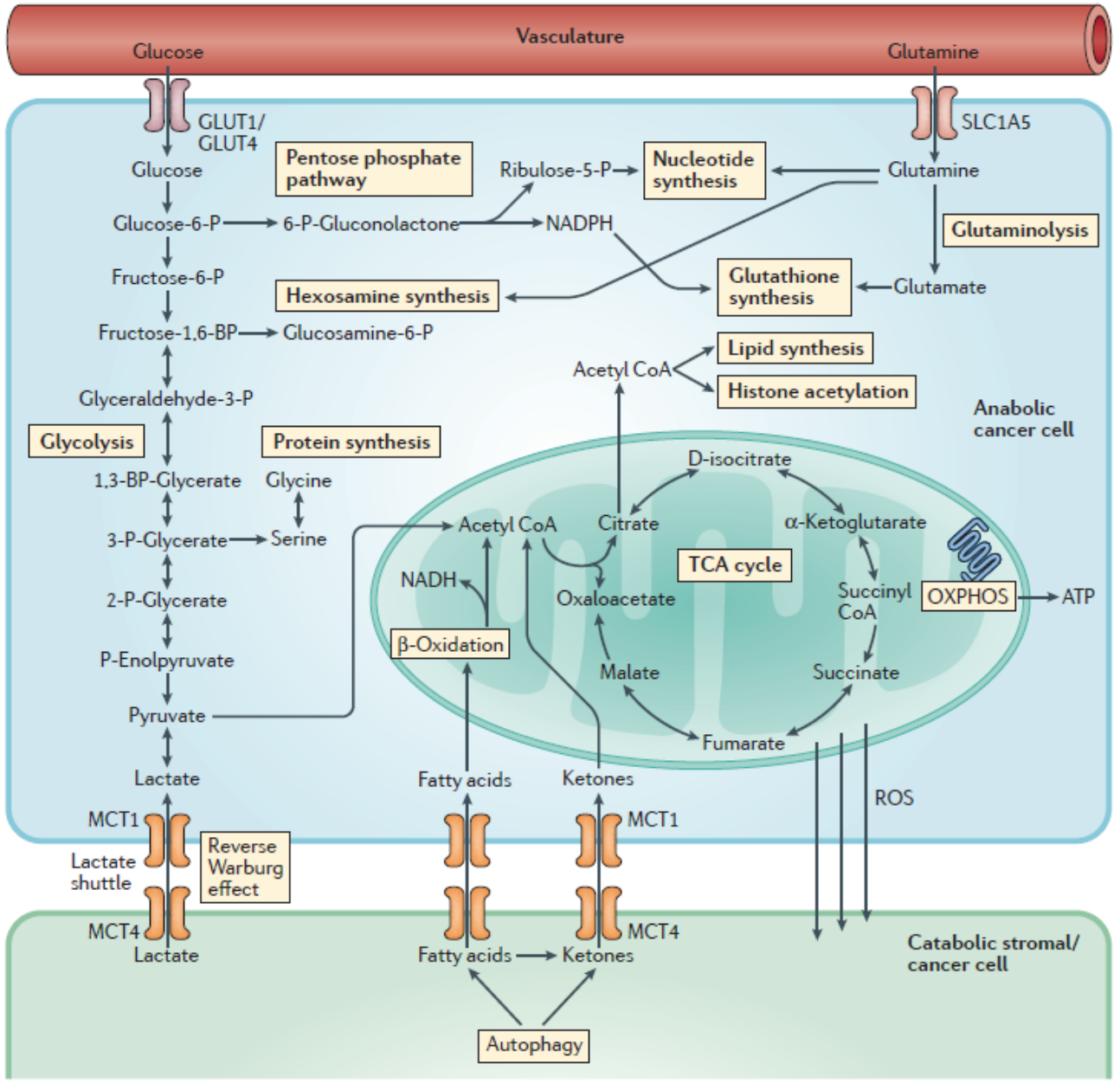
Ubaldo E. Martinez-Outschoorn[1], Maria Peiris-Pagès[2,3]*, Richard G. Pestell[1], Federica Sotgia[2–4]* and Michael P. Lisanti[2,3]**

Nature Reviews: Clinical Oncology 14, 11 (2017)

Tumors have high uptake of nutrients to to generate high levels of ATP and biosynthesis to support progression.

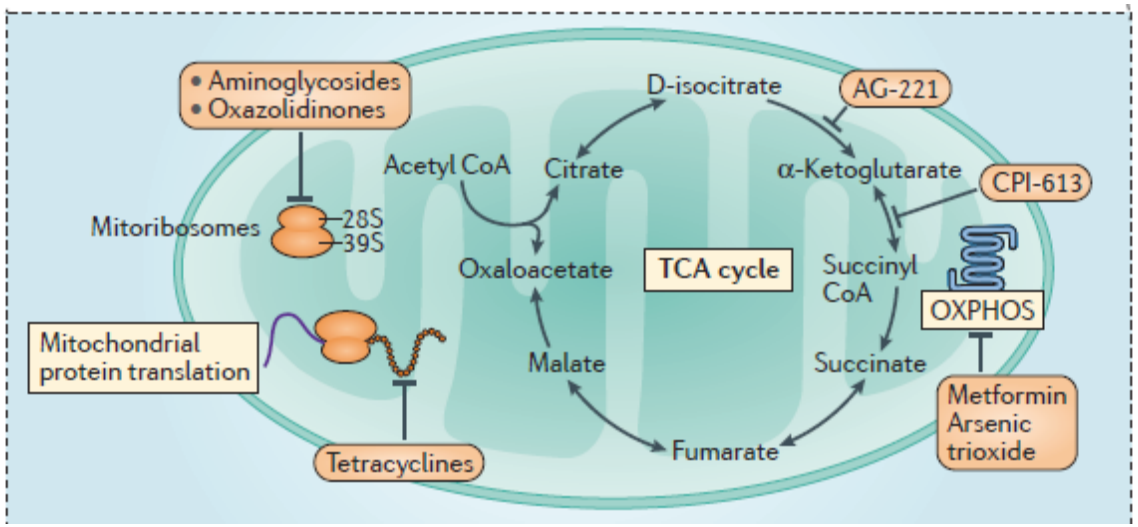
Targeting glycolysis and mitochondrial metabolism as well as other substrates should be effective

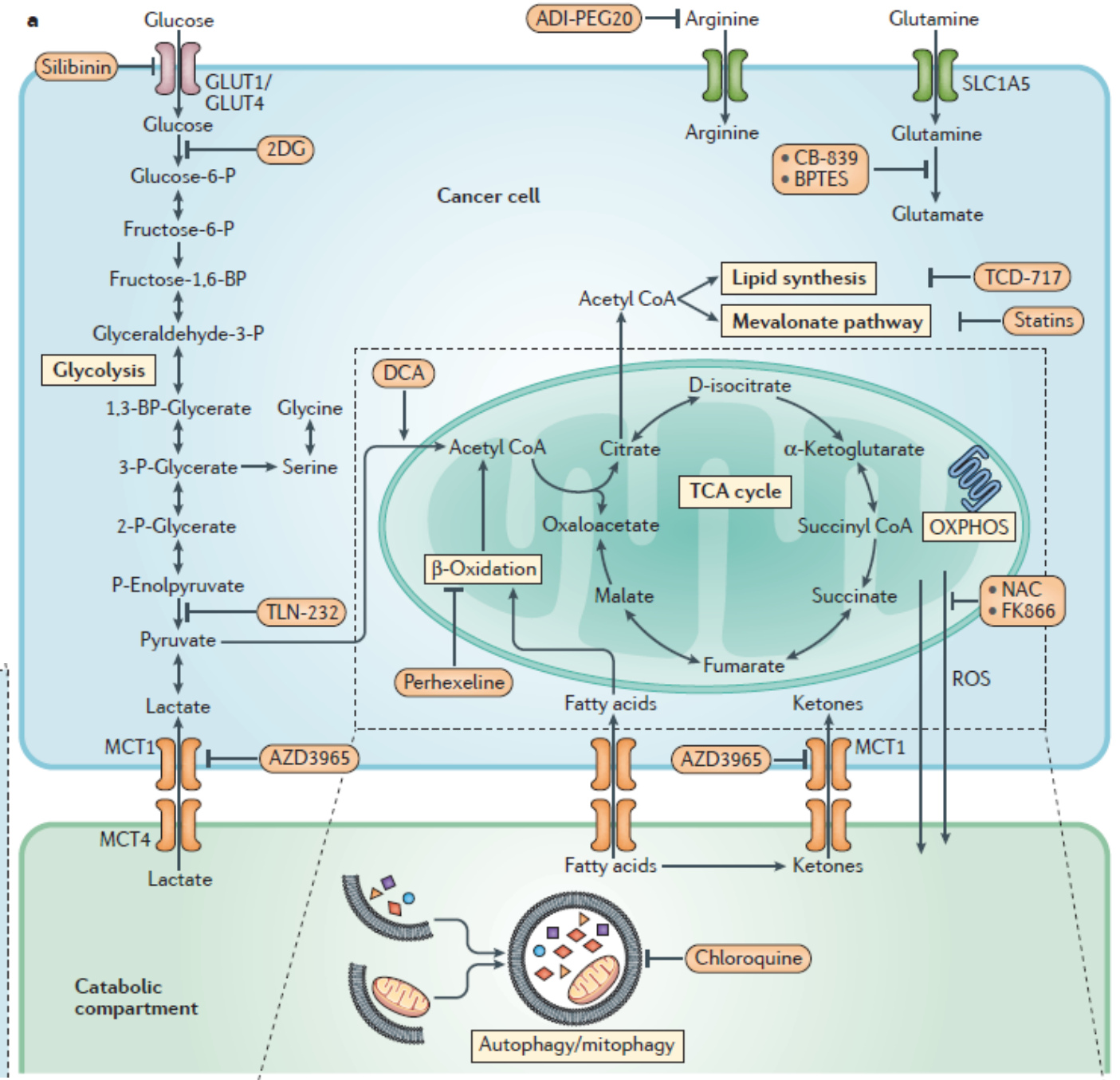
Details on metabolic levels and fluxes will be key to evaluate metabolic approaches.



Many Drugs Already Exist to Target Metabolism

Question is how to effectively evaluate these and their combinations





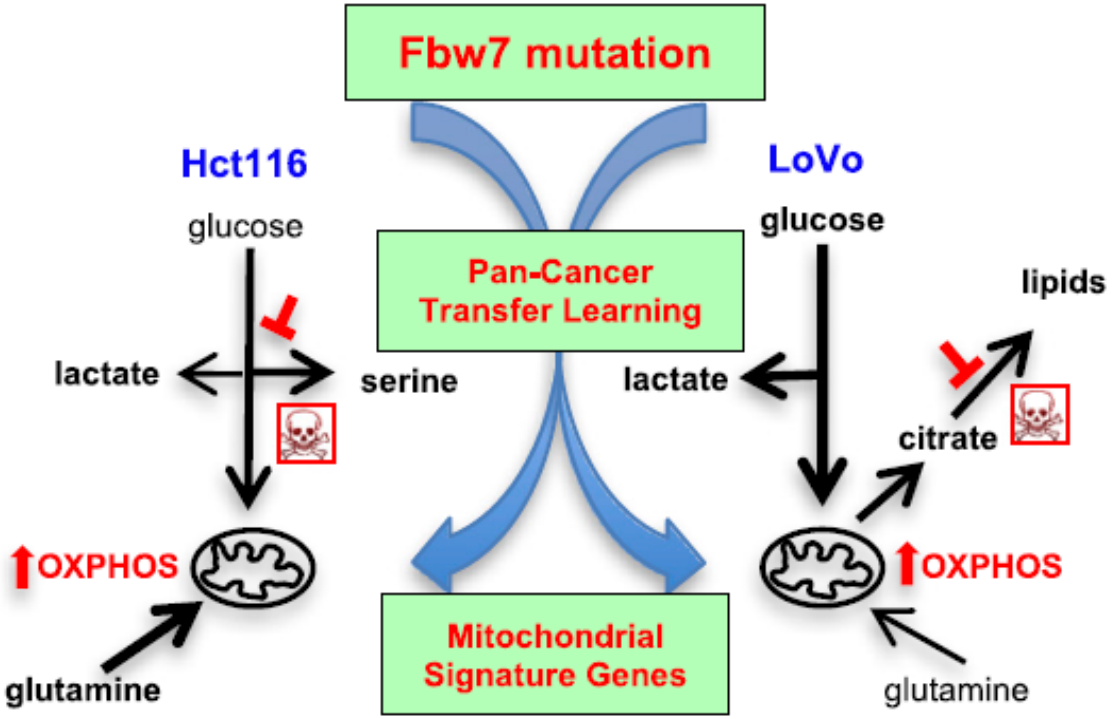
Targeting Altered Metabolism in Colon Cancer

Altered metabolism in cancer has a long history dating back to 1936 experiments by Otto Warburg.

Over the past decade interest has grown with the possibility of developing metabolite focused therapies using known inhibitors as a start.

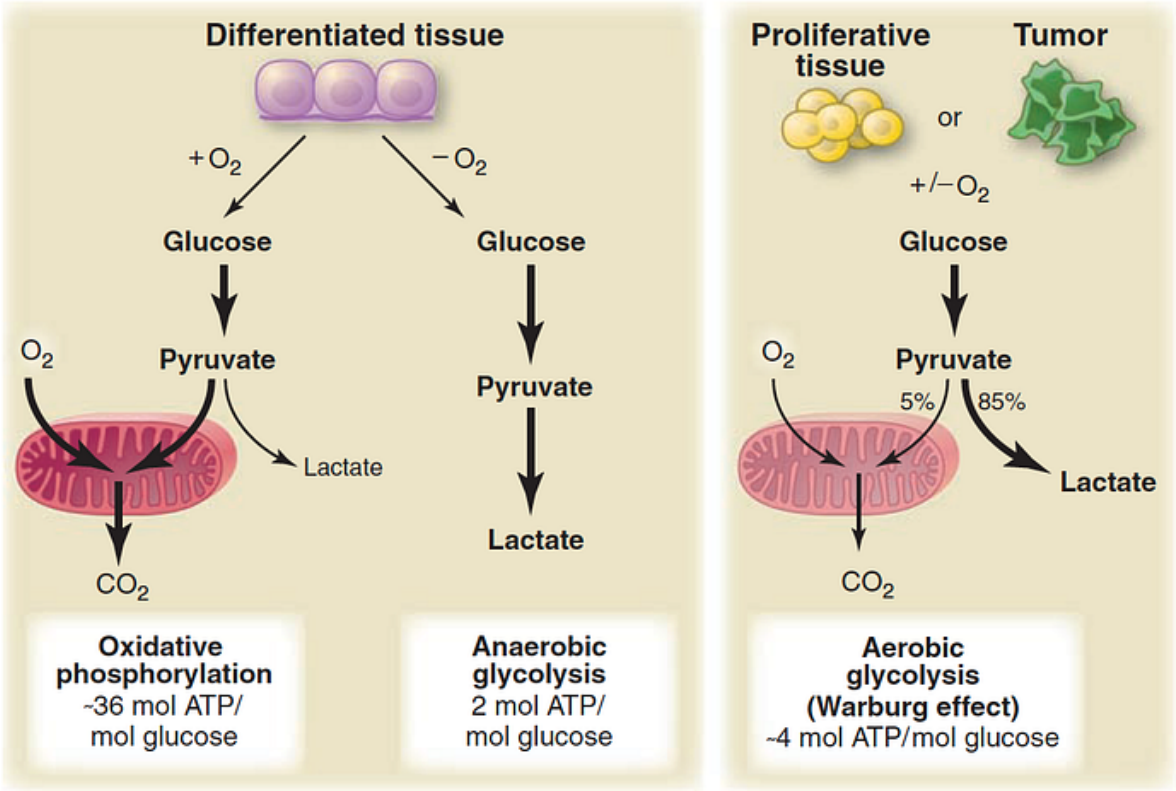
Here we investigated two colon cancer lines and found quite different metabolic alterations.

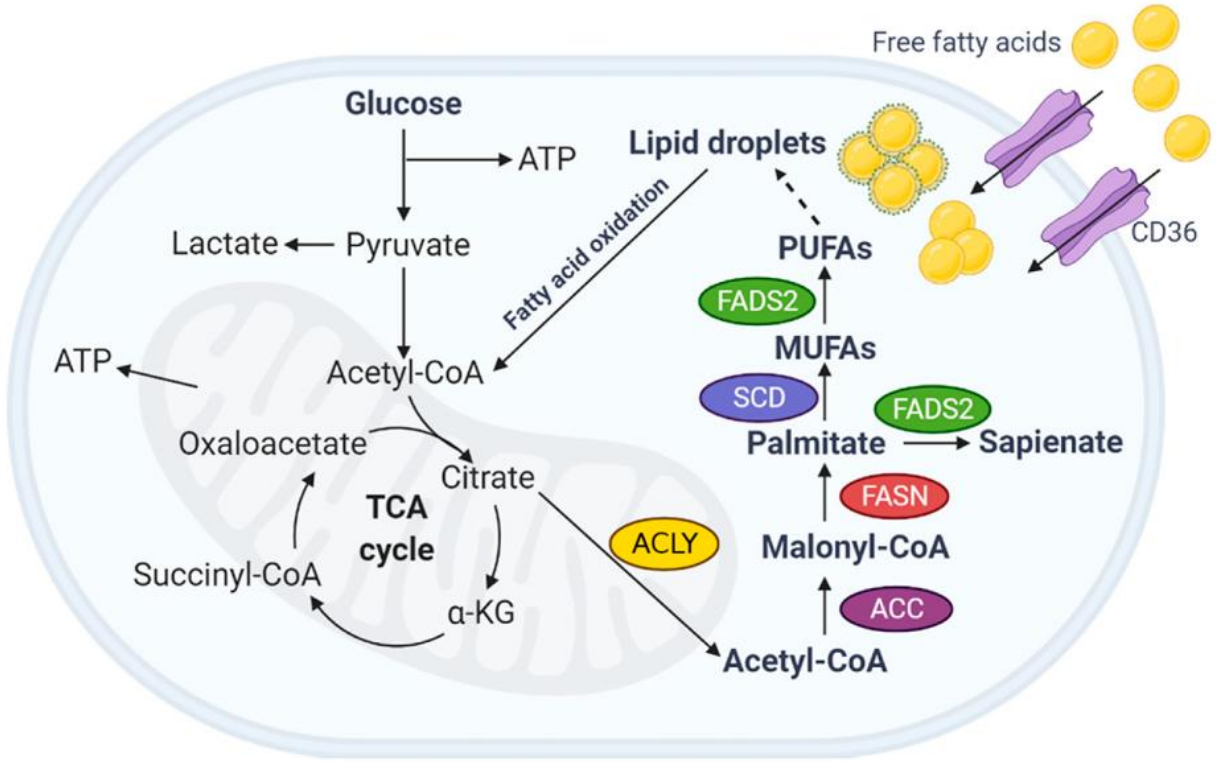
Isotope tracer studies identified key pathways and suggested possible inhibitor strategies.



Davis et al., PNAS 2018

Mechanism-Based Therapy Combinations

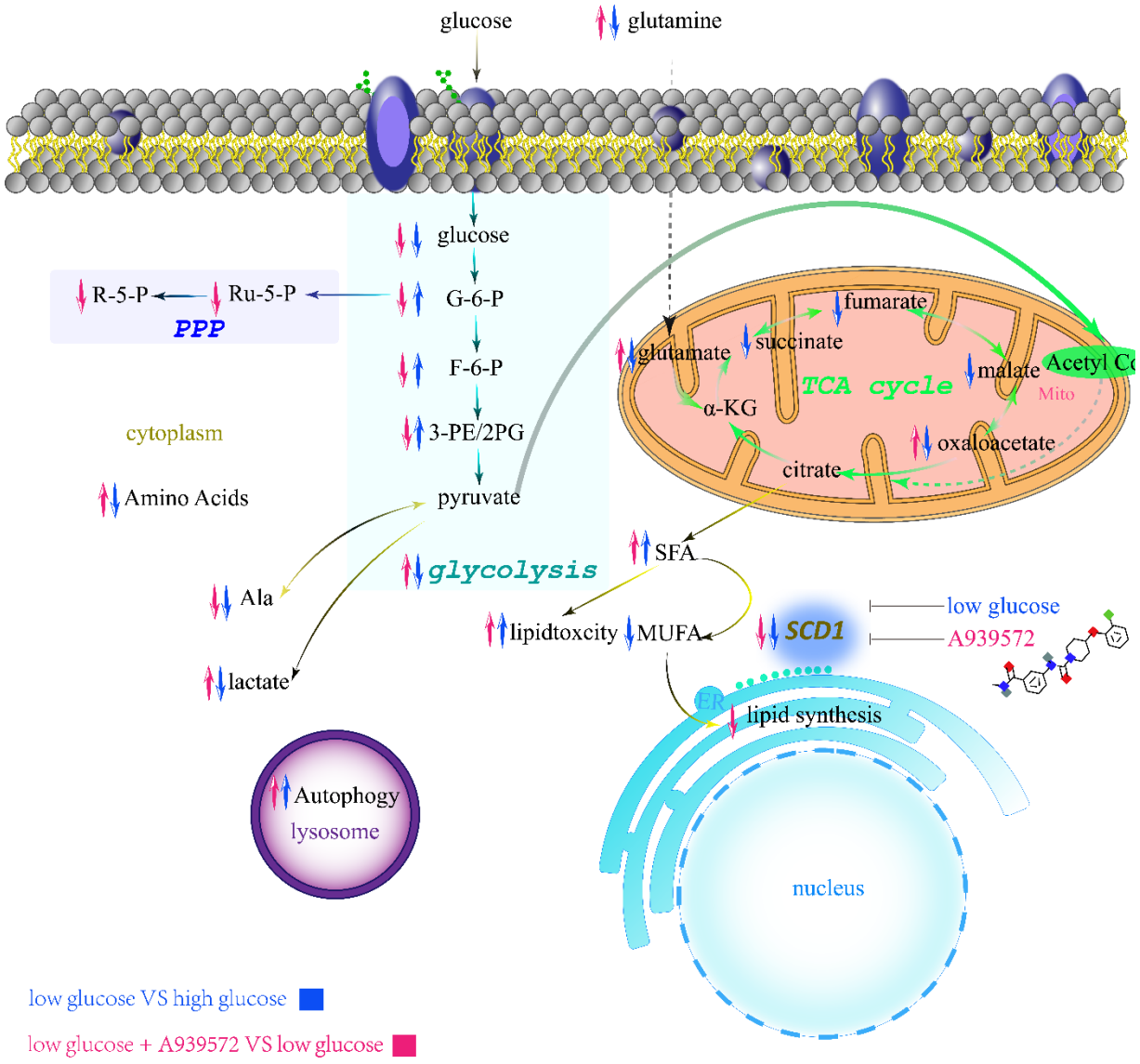




Cancer cells prioritize glycolysis over oxidative phosphorylation, even with oxygen.

Altered fatty acid metabolism is a hallmark of many cancer types, which can be targeted.

Results



- ✓ A synergistic reduction in cancer cell viability when glucose deprivation and fatty acid inhibition were combined.
- ✓ The accumulation of cytotoxic saturated fatty acids were observed.
- ✓ Additional changes in cellular metabolism and lipid composition may initiate cell death responses.

Metabolomics. 2024

Anti-BACH1 + Metformin Combination Therapy

LETTER

https://doi.org/10.1038/s41586-019-1005-x

Effective breast cancer combination therapy targeting BACH1 and mitochondrial metabolism

Jiyoung Lee[1], Ali E. Yesilkanal[1], Joseph P. Wynne[1], Casey Frankenberger[1], Juan Liu[2], Jielin Yan[1], Mohamad Elbaz[1], Daniel C. Rabe[1], Felicia D. Rustandy[1], Payal Tiwari[1], Elizabeth A. Grossman[3,4,5], Peter C. Hart[6], Christie Kang[6], Sydney M. Sanderson[2], Jorge Andrade[7], Daniel K. Nomura[3,4,5], Marcelo G. Bonini[6,8], Jason W. Locasale[2] & Marsha Rich Rosner[1]*

- TNBCs overexpress BACH1, heme-binding transcription factor target mitochondrial metabolism.
- BACH1 decreases glucose utilization and affects ETC gene expression.
- Addition of metformin, a diabetes drug, that also targets ETC, suppressing tumor growth.

Off-label Drug and Supplement Combination

www.impactjournals.com/oncotarget/ Oncotarget, 2017, Vol. 8, (No. 40), pp: 67269-67286

Research Paper

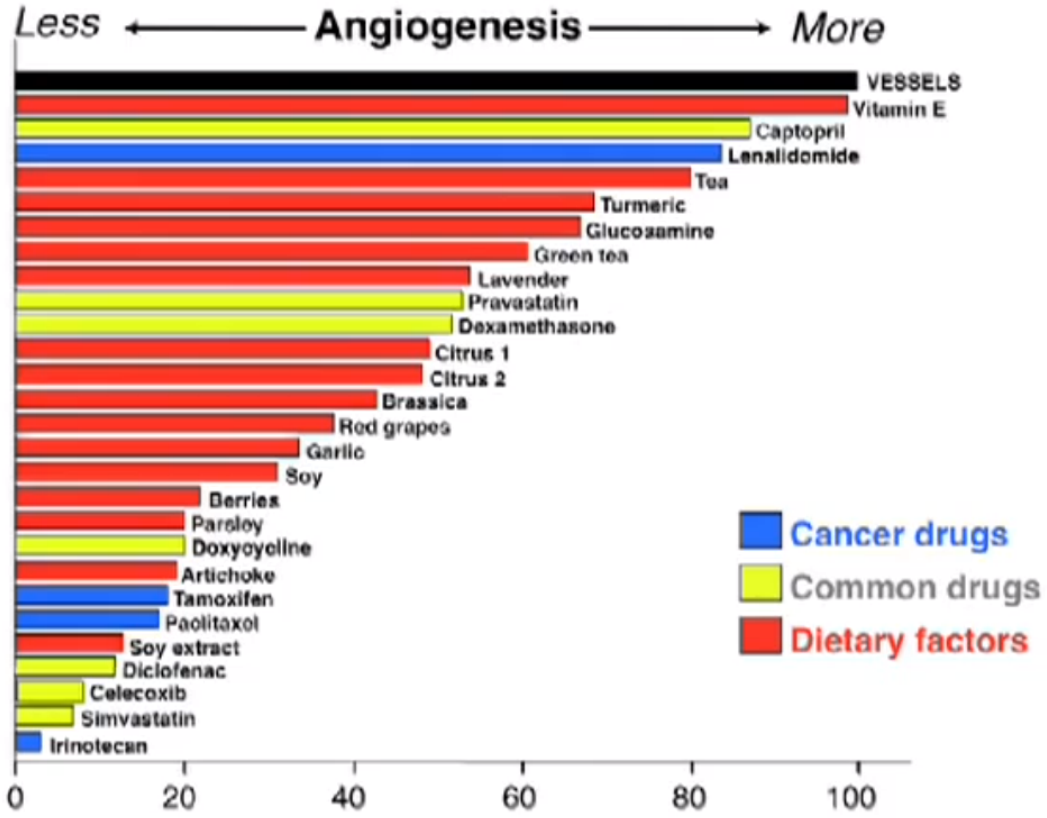
Vitamin C and Doxycycline: A synthetic lethal combination therapy targeting metabolic flexibility in cancer stem cells (CSCs)

Ernestina Marianna De Francesco[1,2], Gloria Bonuccelli[3], Marcello Maggiolini[1], Federica Sotgia[3] and Michael P. Lisanti[3]

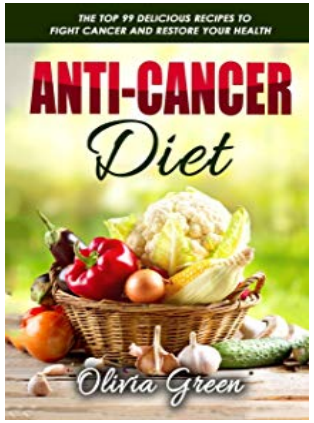
- Combination of an antibiotic and Vit C to target mitochondria
- Studied cancer stem cells that are often difficult to kill using chemotherapy
- Combinations were effective in reducing the number of cancer cell clusters

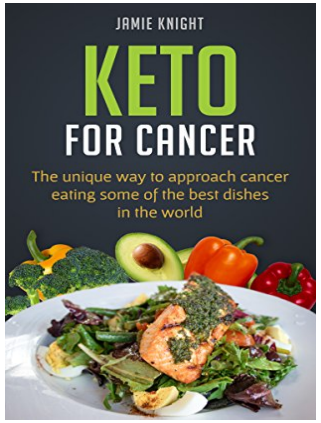
Cell Metabolism and Cancer

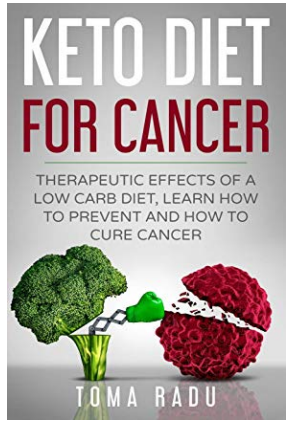
Lots of interest in the use of anticancer compounds and diets to "modulate" the metabolism and "improve" chemotherapy

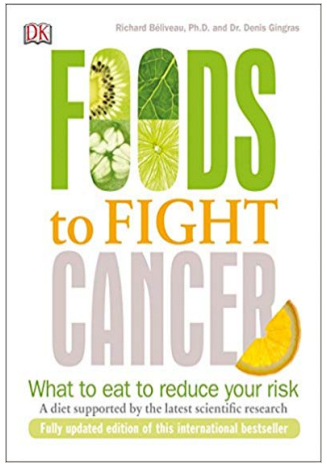


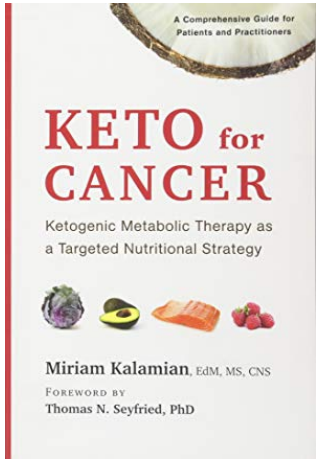
William Li: Can we eat to starve cancer?
Video on TED.com

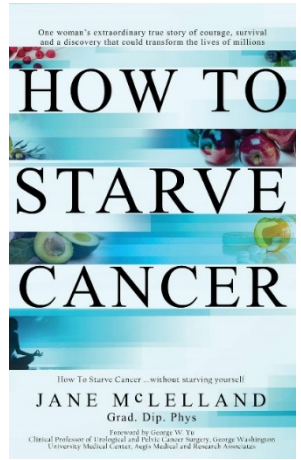




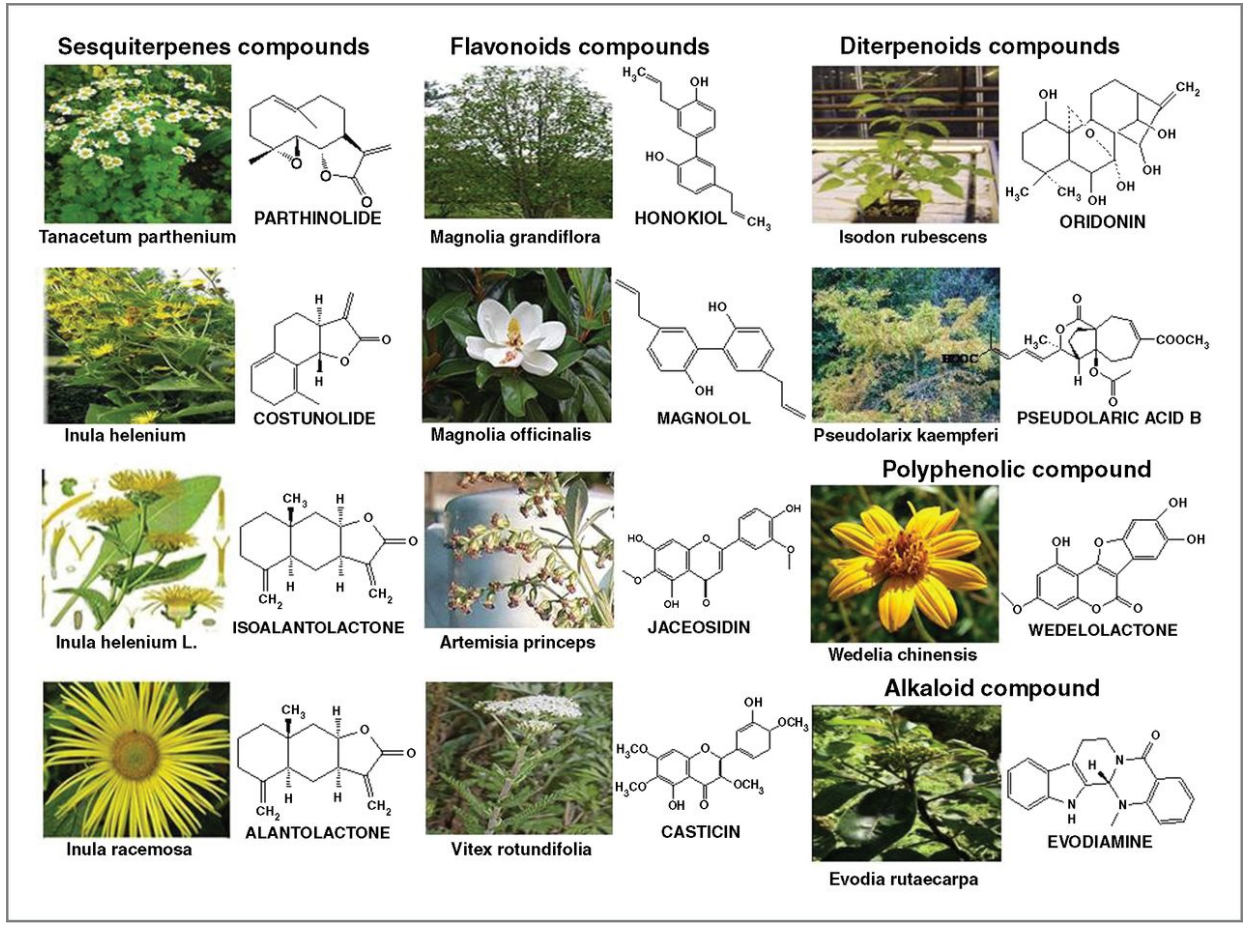


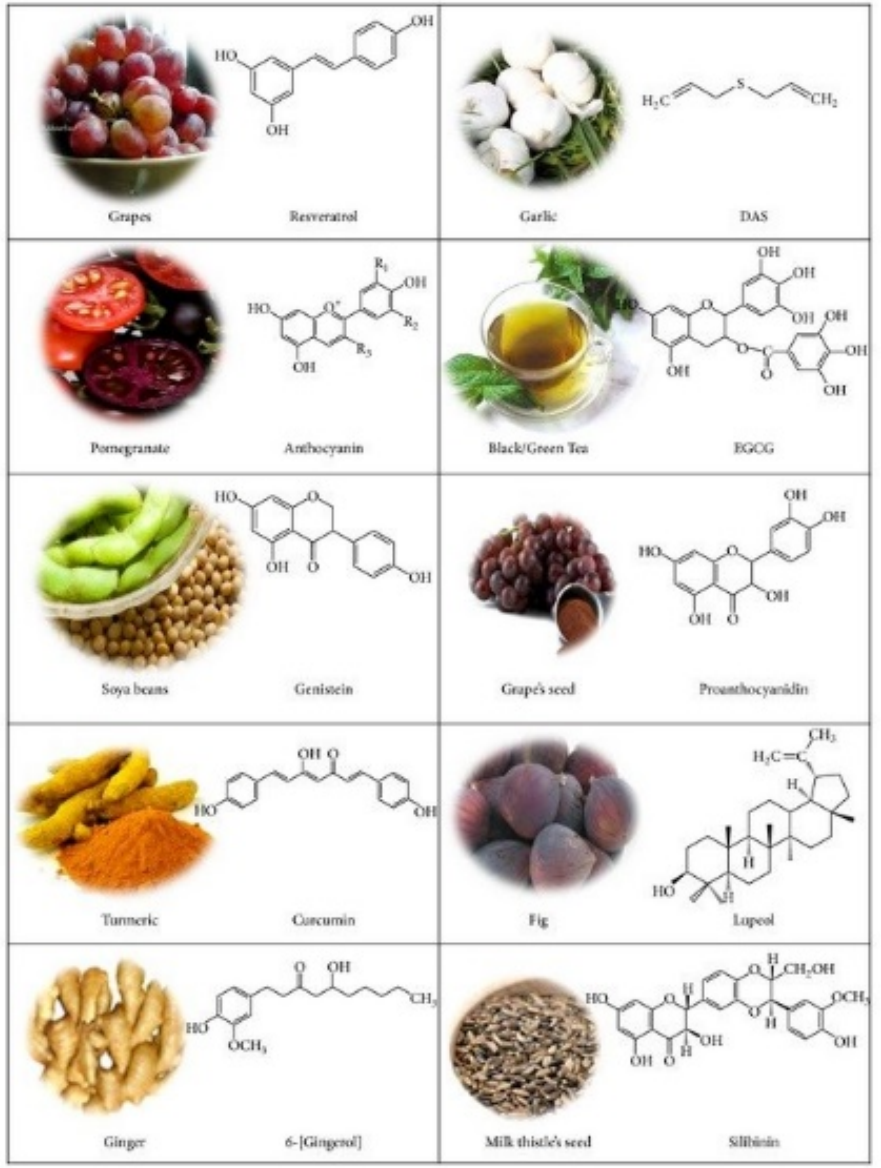






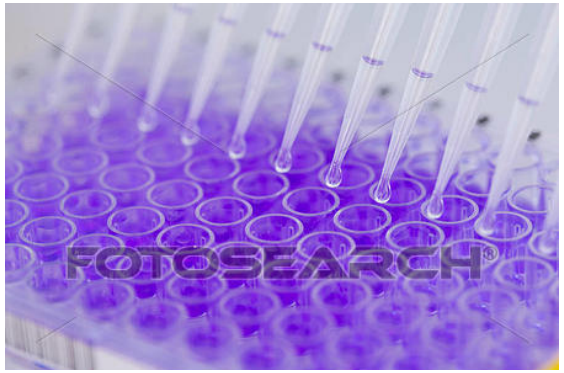
Wide Variety of Natural Products with Anticancer Potential





Singh et al., *Biomed Res int*, **2014**
Millimouno, et al. *Cancer Prev Res,* **2014**

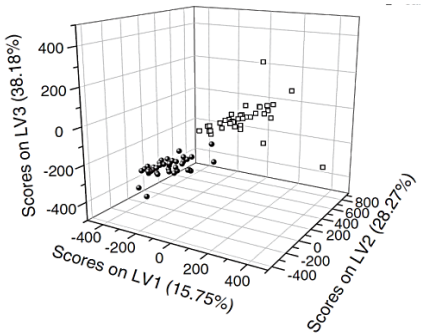
Experimental Plan



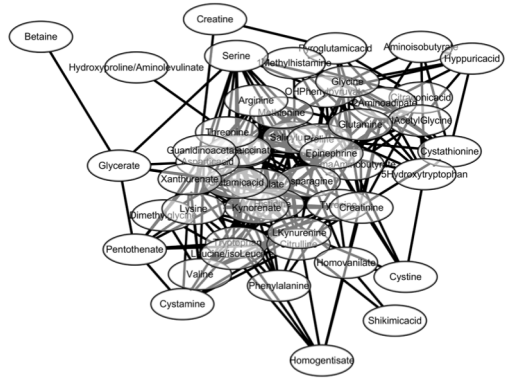
Cell samples + inhibitors/dietary compounds

Metabolomics of levels and "fluxes"

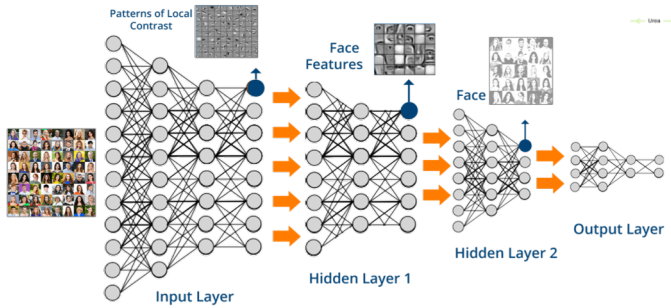
Analysis:



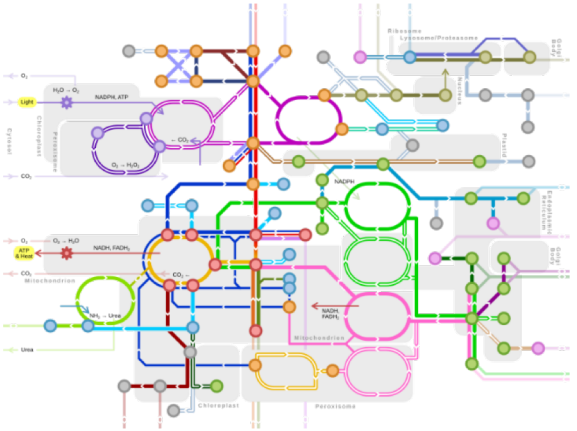
Clustering:



AI:



Modeling:



Identification of promising drug targets

Conclusions and Perspectives

- New tools in metabolomics are providing improved methods for identifying changes in metabolism
- A number of studies are pointing to detectable altered metabolism in cancer and other diseases, plus aging, etc….
- Biomarker discovery and validation are key to the development of new diagnostic tests
- Still many challenges lie ahead including understanding confounding factors and basic mechanisms
- Identifying metabolic risk factors, such as dietary intakes can benefit human health at the population level
- And identifying metabolic vulnerabilities in cancer cells can lead to novel therapeutics, including combination therapies.
- Advances in new metabolomics tools promises new discoveries in metabolism, which hopefully will lead to better diagnostics and treatments

Acknowledgements

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Marian Neuhouser (FHCC)
Jiyang Dong (Wuhan)

NIGMS
NORC, CCSG, CTMR, SDBC, Shock

Northwest Metabolomics Research Center
https://nwmetabolomics.org

Receiver Operating Characteristics Analysis

Sensitivity vs Specificity

Sensitivity: correct identification of people who have the disease

100 patients:
90 correct
Sensitivity = 90/100
=90%

Specificity: correct identification of people who do not have the disease

100 healthy:
70 correct
Specificity = 70/100
=70%

ROC curve