Partnering to Advance Life Sciences

Combining high-throughput, discovery metabolomics approach with expert scientific guidance to enable biomarker discovery, deep insight into disease, treatment effect and outcome.

Metabolon is an industry leading partner of discovery metabolomics providing complete research services including expert study design, data generation, full interpretation and post study analysis tools. Metabolon has conducted more than 3000 client studies with more than 600 organizations and contributed to more than 350 peer-reviewed publications.

Study design inquiries: info@metabolon.com

metabolon.com

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Practical Applications of Metabolomics

Fundamentals in Metabolomic Technology and Application

Edward Karoly
Associate Director, Project Management
Academics
June 2, 2014

ekaroly@metabolon.com

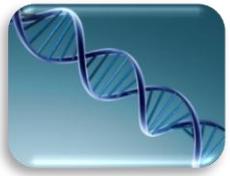
METABOLON®
SOLUTIONS BEYOND THE DATA...™

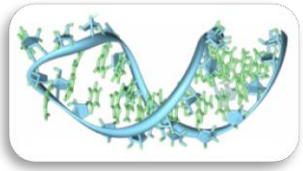
METABOLON

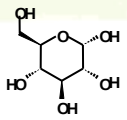
- Pioneer and leader in metabolomics:
  - 60+ issued patents
  - >350+ peer-reviewed publications
- Founded in 2003 in RTP, NC (150+ employees)
- Worldwide operations (Asia, Europe, US)
- Over 550 clients and >3000 studies
- Completed 600 client studies

Science & technology-driven company providing biological insight through global biochemical profiling

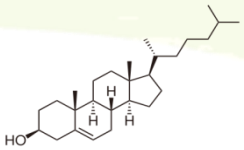
Focus on Metabolism



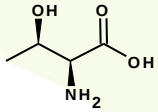




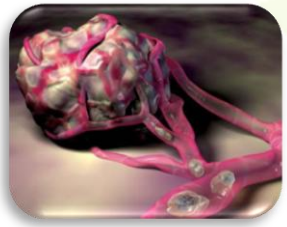
glucose



cholesterol



threonine



DNA

RNA

Proteins

Biochemicals

Mechanistic Insight into Phenotype

Biochemistry Advantages

- Any sample type
- Broad applications
- Condensed & information rich
- Bridge between genome and phenotype

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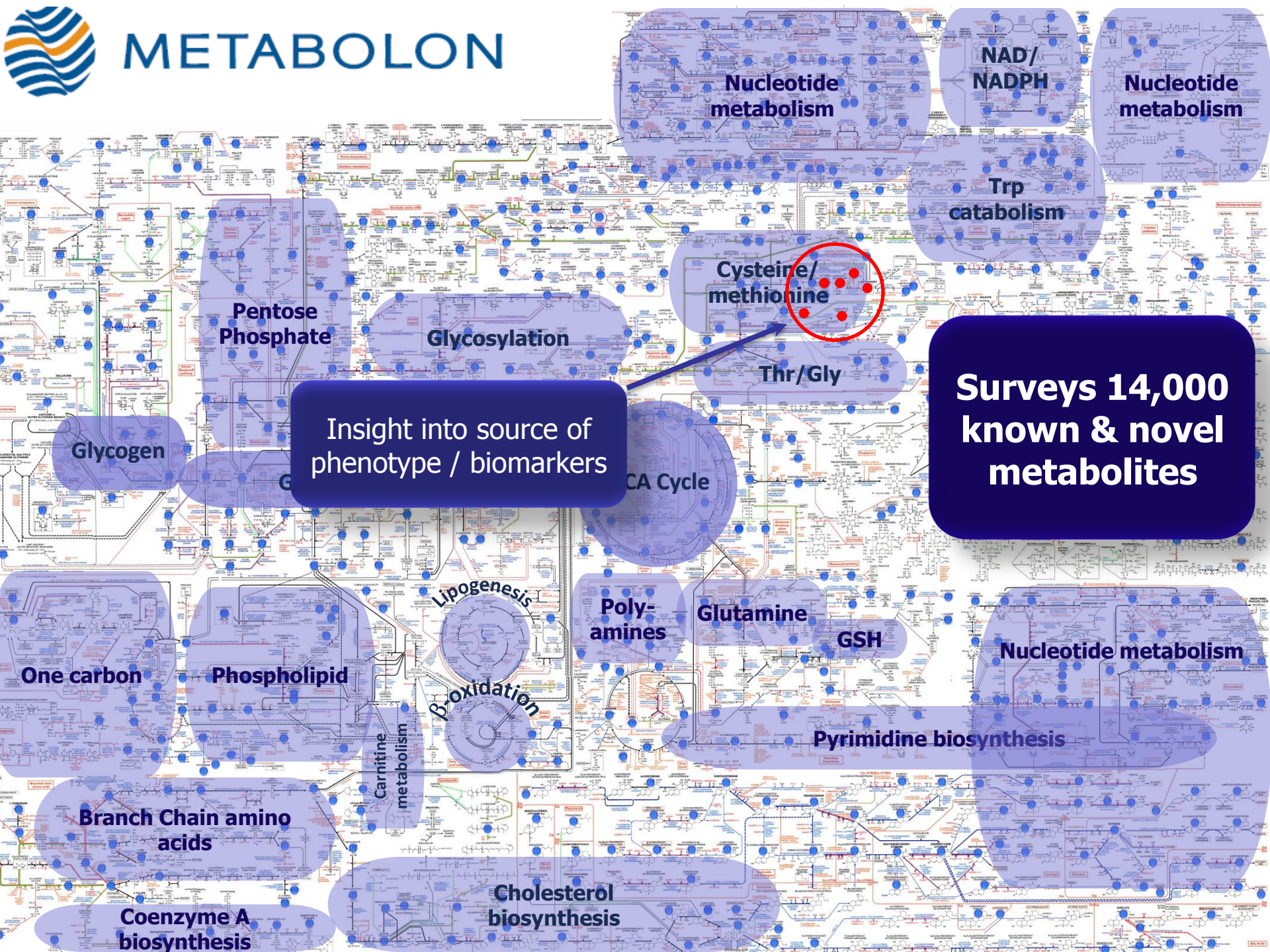
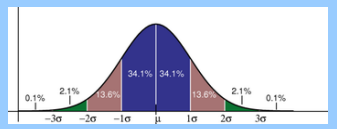

METABOLON
Nucleotide metabolism
NAD/ NADPH
Nucleotide metabolism
Trp catabolism
Cysteine/ methionine
Pentose Phosphate
Glycosylation
Thr/Gly
Surveys 14,000 known & novel metabolites
Insight into source of phenotype / biomarkers
Glycogen
CA Cycle
Lipogenesis
Poly- amines
Glutamine
GSH
Nucleotide metabolism
One carbon
Phospholipid
B-oxidation
Pyrimidine biosynthesis
Carnitine metabolism
Branch Chain amino acids
Cholesterol biosynthesis
Coenzyme A biosynthesis

Metabolon Platform Technology



Statistical Analysis

Disease Biomarkers
Mechanistic Understanding
Drug MoA
Cellular Characteristics

Interpretation
- >3000 Studies
- Institutional Knowledge
- Expert Biochemists

Cytoscape

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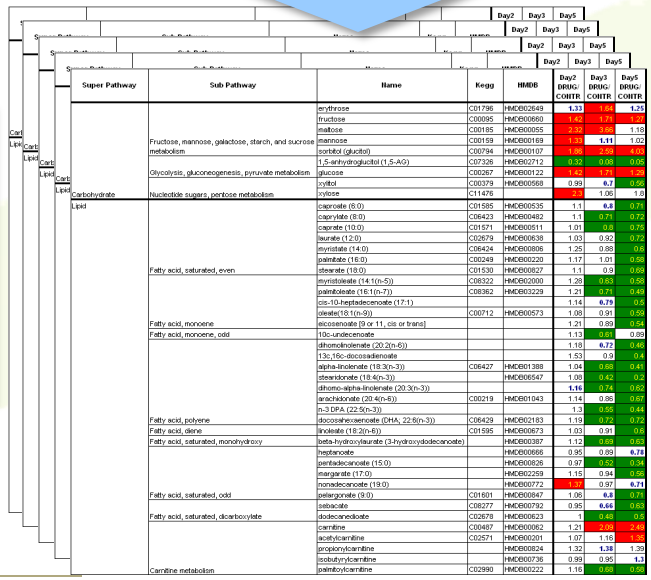
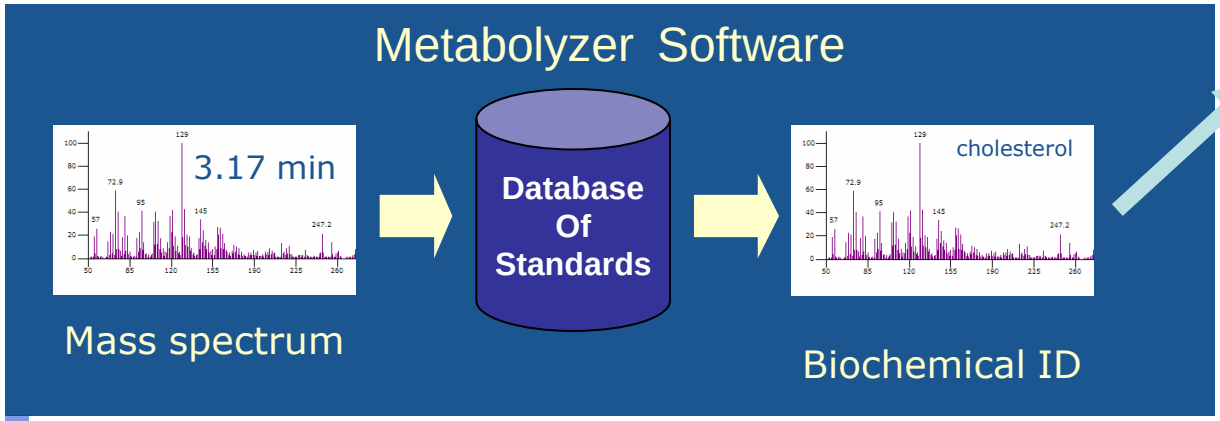
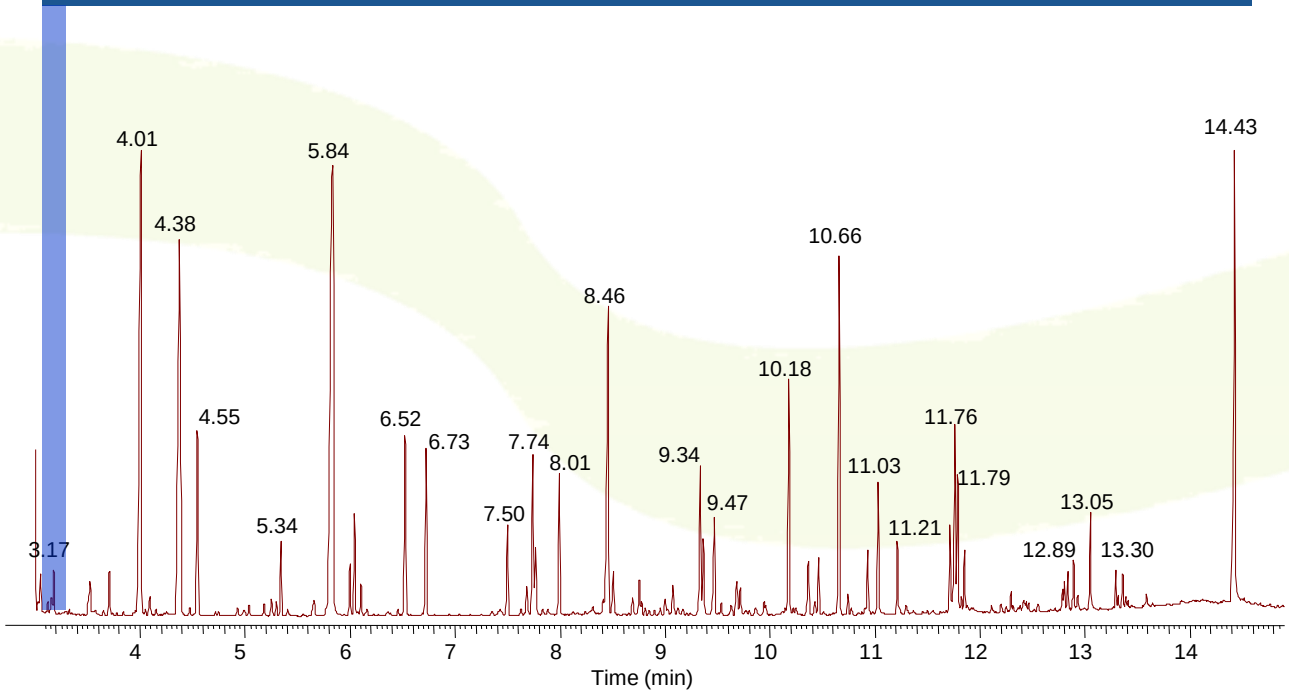


Heat Maps by Pathway

Proprietary Software: Automated Biochemical Identification



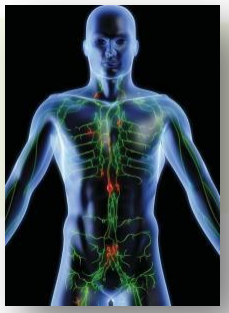
Biochemical	Amount
cholesterol	143,789



Advancing biology through understanding metabolism

Biological Question → Complex Metabolite Data → Analysis of Biochemistry → Solution

Prostate Cancer Aggressiveness



Benign

Localized

Metastatic

Hundreds of thousands ion features/study

Metabolon Software

400-1000 biochemicals/ sample

Heat Maps

Classification

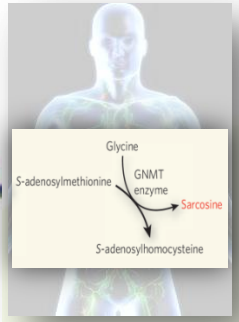
Pathway Analysis

Z-score

Individual Plots

Institutional knowledge

Sarcosine modulates invasiveness



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Compatibility & Integration With Other 'Omics Data

Recent work has re-ignited an interest in metabolism, particularly since it has shown that it can bolster genomic, transcriptomic & proteomic inquiry, serving as a key integrator of these data.

Examples of metabolomics producing clarity & focus in other 'omics data: sets

Theme	Key Finding	Reference
Brain cancer	Revealed the functional defect of mutant IDH1 enzyme and a potential marker for imaging	Dang et al., 2009; Reitman et al., 2011
Prostate cancer	Identified a pathway (sarcosine) of aggressiveness, focusing molecular biology follow-up work	Sreekumar et al., 2009; Song et al., 2011
Glycine metabolism in cancer	Provided focus within large cancer genomic database to reveal novel cancer driving pathway	Jain et al., 2012
GWAS studies	Landmark work re-awakened researchers to the value of metabolites to strengthen genomics	Suhre et al., 2011, 2014
Metabolic disease & SREBP-1 metabolism	Produced focus & potential targets for hepatic dysfunction in obesity & metabolic syndrome	Walker et al., 2011
Fungicidal drugs	Clarified & strengthened gene expression findings to reveal new antifungal targets	Belenky et al., 2013
Obesity	Provided support for a new paradigm for obesity driven by timing of feeding	Hatori et al., 2012
Metabolic disease & ER stress	Revealed that PEMT is a key player in ER stress	Fu et al., 2011
Metabolic disease - Ghrelin/GOAT	Revealed an unanticipated role for the Ghrelin/GOAT system in the regulation of bile acid metabolism	Kang et al., 2012

Translatability of Metabolites

Biochemical pathways are highly conserved and, hence, metabolites are particularly translatable across species. Biomarkers identified in preclinical models have a high likelihood of being relevant in human disease.

Examples of metabolomics results with striking translatability:

Theme	Key Finding	Reference
Diabetes	Markers showed exquisite translatability in IR/diabetic cats	Gall et al., 2010 & unpublished data
Sickle cell disease	Markers that define a new target in SCD are concordant in SCD patient plasma	Zhang et al., 2010
Niemann-Pick C1	Markers translated across multiple species & tracked with treatment	Porter et al., 2010
Kidney cancer	Strongly concordant signatures between humans & animal models	Kim et al., 2011; Ganti et al., 2012
Chronic lymphocytic leukemia (CLL)	Biomarkers from chronic lymphocytic leukemia (CLL) patients exhibited translatability to in vitro models	Tili et al., 2012
Fatty liver disease	Strongly concordant signatures between humans & animal models in this series of studies	Watkins et al., 2003; Puri et al., 2007, 2009; Kalhan et al., 2011; Fu et al., 2011
Atherosclerosis	Identified optimal animal models(s) for drug R&D & the pathways that translated across multiple species	Yin et al., 2012
Ovarian cancer	Markers concordant with those detected in a precisely constructed mouse model	Fong et al., 2011; Szabova et al., 2012
Testicular toxicity	Pathways in animal model that caused tox were the same as a human genetic disorder	Takei et al., 2010

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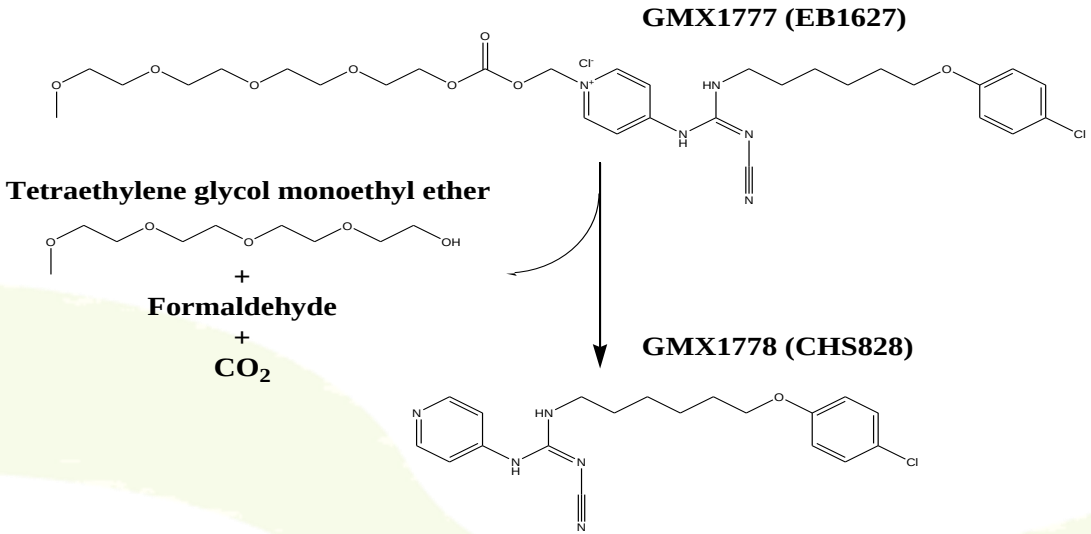
Finding a Drug's Mechanism of Action

Gemin X

GMX1777/1778 Background

- **GMX1777 is a soluble pro-drug which is rapidly converted *in vivo* to GMX1778, the active cyanoguanidinopyridine.**
- **In-licensed by GeminX from Leo Pharma in 2006 (progressed to clinical trials).**



Mechanism of action uncertain but thought to involve inhibition of NF-κB transcriptional activity.

Broad spectrum antitumor activity in several tumor types and potent *in vivo* anti-tumor activity in human xenograft models.

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Study Design and Initial Results

Experimental Details

- Dexamethasone resistant cells (multiple myeloma line IM-9)
- Dosing according to following (cultures per group):

Time / Dose	6 hr	13 hr	20 hr	27 hr
DMSO	6	6	6	6
Drug	6	6	6	6

Results of Global Biochemical Profiling

		6hr	13hr	20hr	27hr
Significantly Altered Biochemicals $p \leq 0.1$, q-value ≤ 0.2	Increased	25	38	58	45
	Decreased	2	8	7	20
	Total	27	46	65	65

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Drug Effects
Biochemicals & Pathways

Pathway	Sub Pathway	Biochemical Name	KEGG	HMDB	6 hr	13 hr	20 hr	27 hr	
Carbohydrate Metabolism	Aminosugars metabolism	N-acetylgalactosamine	C01074	HMDB00835	1.47	1.07	0.99	0.81	
		N-acetylneuraminate	C00270	HMDB00230	0.87	1.39	1.09	0.80	
		UDP-N-acetylgalactosamine	C00203	HMDB00290	1.08	1.26	0.80	0.43	
	Fructose, mannose, galactose, starch, and sucrose metabolism	fructose	C00095	HMDB00660	0.98	0.46	1.83	18.13	
		mannitol	C00392	HMDB00765	0.66	1.00	1.01	1.80	
		GDP-mannose	C00096	HMDB01163	1.00	0.70	1.67	1.55	
		sorbitol	C00794	HMDB00247	1.59	3.82	17.15	22.68	3
	Glycolysis, gluconeogenesis, pyruvate metabolism	glucose	C00031	HMDB00122	1.28	1.11	1.61	1.22	
		fructose 1-phosphate	C01094	HMDB01076	1.09	1.79	3.15	30.52	
		3-phosphoglycerate	C00597	HMDB00807	0.90	1.18	1.59	2.81	
		phosphoenolpyruvate (PEP)	C00074	HMDB00263	1.10	1.06	1.20	2.36	
		lactate	C00186	HMDB00190	1.10	0.81	2.92	2.43	
	Nucleotide sugars, pentose metabolism	arabitol	C00474	HMDB01851	1.31	1.36	1.78	1.27	
		2-deoxyribose	C01801	HMDB03224	1.09	2.01	2.89	1.68	
Lipid Metabolism	Carnitine metabolism	acetylcarnitine	C02571	HMDB00201	1.05	0.80	1.57	0.92	
	Glycerolipid metabolism	phosphoethanolamine	C00346	HMDB00224	1.72	1.75	2.68	8.00	
		choline			0.77	1.37	1.87	1.87	
		glycerol 3-phosphate (G3P)	C00093	HMDB00126	1.11	1.09	1.16	1.18	
		glycerophosphorylcholine (GPC)	C00670	HMDB00086	1.09	0.97	2.37	2.72	
	Inositol metabolism	myo-inositol	C00137	HMDB00211	1.02	1.19	1.39	1.71	
	Sterol/Steroid	cholesterol	C00187	HMDB00067	0.87	1.13	1.46	1.14	
Energy Metabolism	Krebs cycle	citrate	C00158	HMDB00094	1.02	0.64	0.51	0.27	
		fumarate	C00122	HMDB00134	1.36	1.79	3.35	2.44	2
		malate	C00149	HMDB00156	1.48	1.87	4.13	2.68	
	Oxidative phosphorylation	phosphate	C00009	HMDB01429	1.09	1.18	1.30	1.30	
Nucleotide Metabolism	Purine metabolism, (hypo)xanthine/inosine containing	hypoxanthine	C00262	HMDB00157	1.06	1.35	1.17	0.80	
		inosine			0.97	1.24	0.99	0.82	
	Purine metabolism, adenine containing	adenine	C00147	HMDB00034	1.04	1.30	1.19	2.74	
		adenosine	C00212	HMDB00050	1.37	1.53	0.72	0.81	
		nicotinamide adenine dinucleotide (NAD+)	C00003	HMDB00902	0.38	0.09	0.03	0.01	1
		adenosine 5'-monophosphate (AMP)	C00020	HMDB00045	0.97	1.08	1.02	0.59	
		adenylosuccinate	C03794	HMDB00536	0.84	1.30	9.89	15.05	
			C00212	HMDB00122	0.97	1.26	1.01	1.26	

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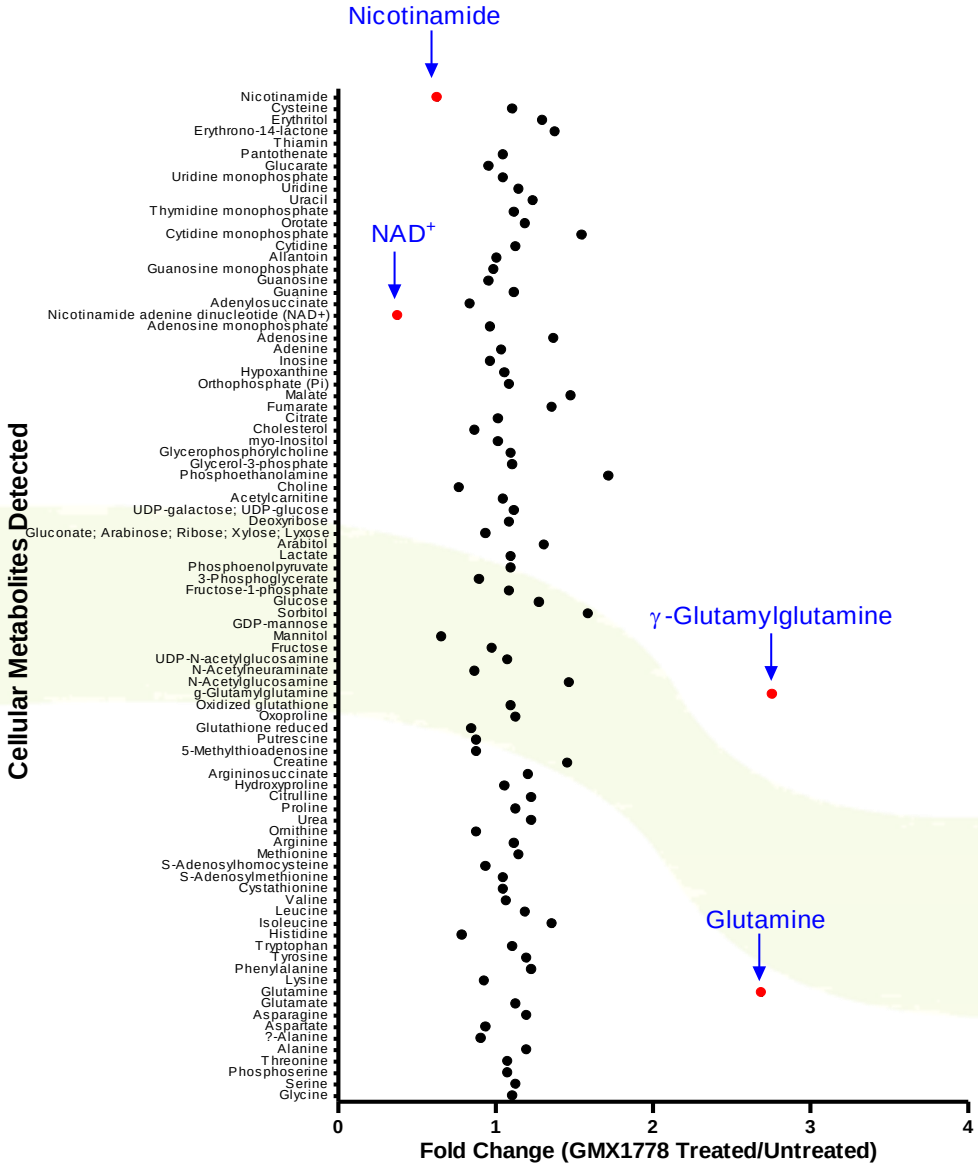
Changes in Global Metabolic Profile
Six hours after GMX1778 Treatment



Four biochemicals showed greatest change after 6 hours of drug treatment:

Largest Increase
- Glutamine (2.7 fold)
- γ-Glutamylglutamine (2.8 fold)

Largest Decrease
- Nicotinamide (0.7 fold)
- NAD+ (0.38 fold)

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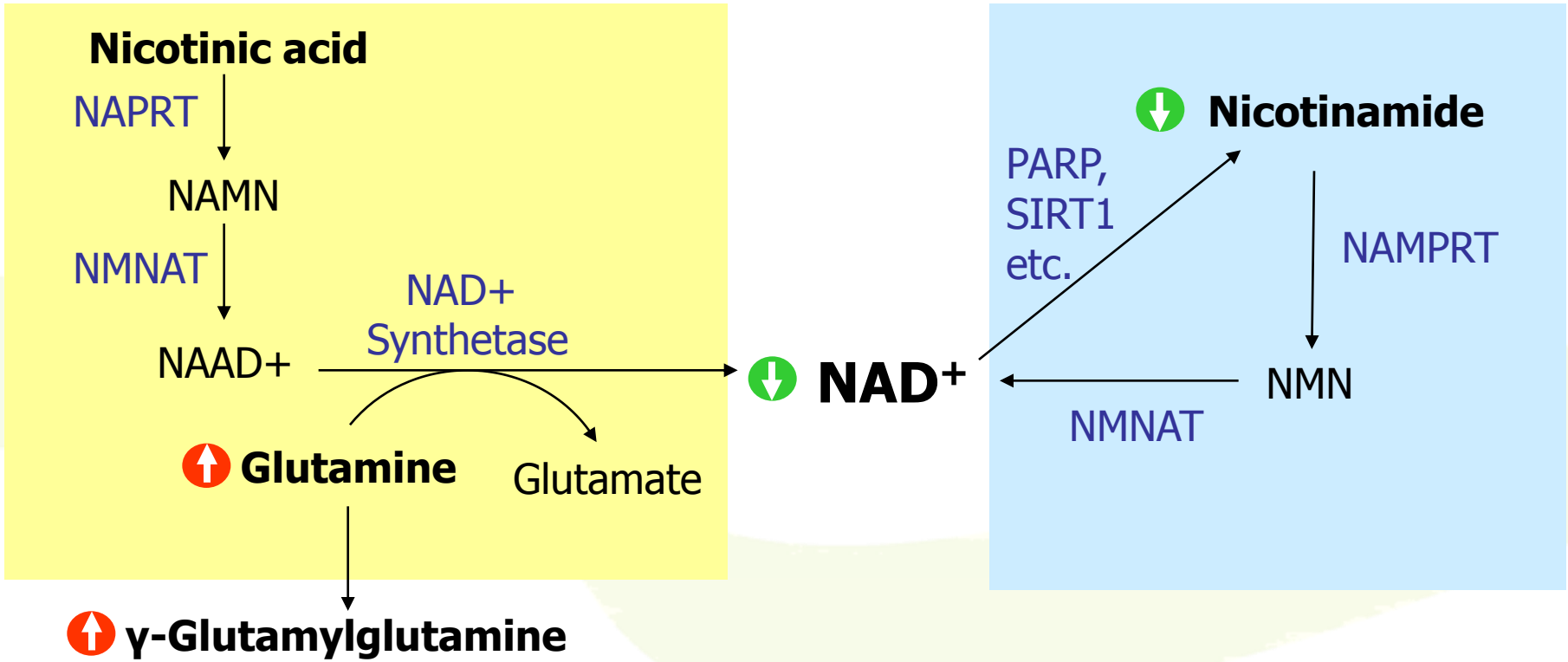
NAD+ Biosynthesis
Two Pathways



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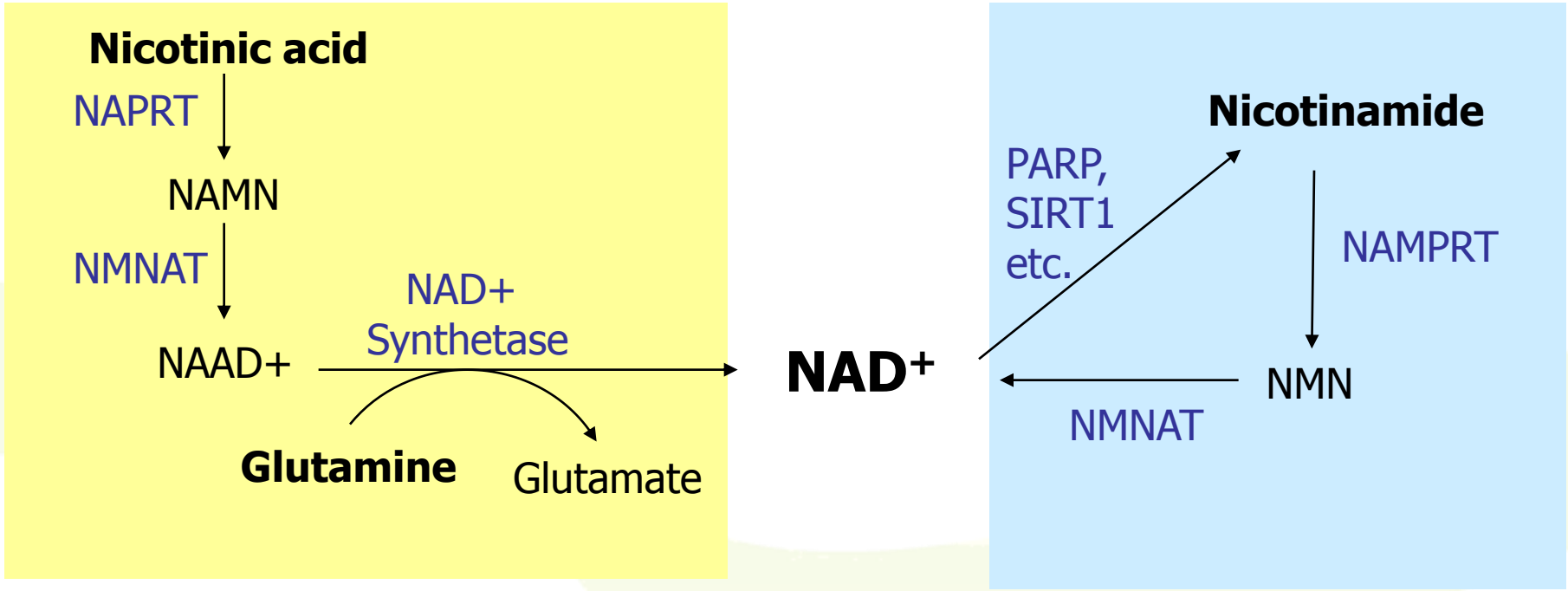
Cell Rescue Experiments

A. Add Nicotinic Acid

B. Add Nicotinamide



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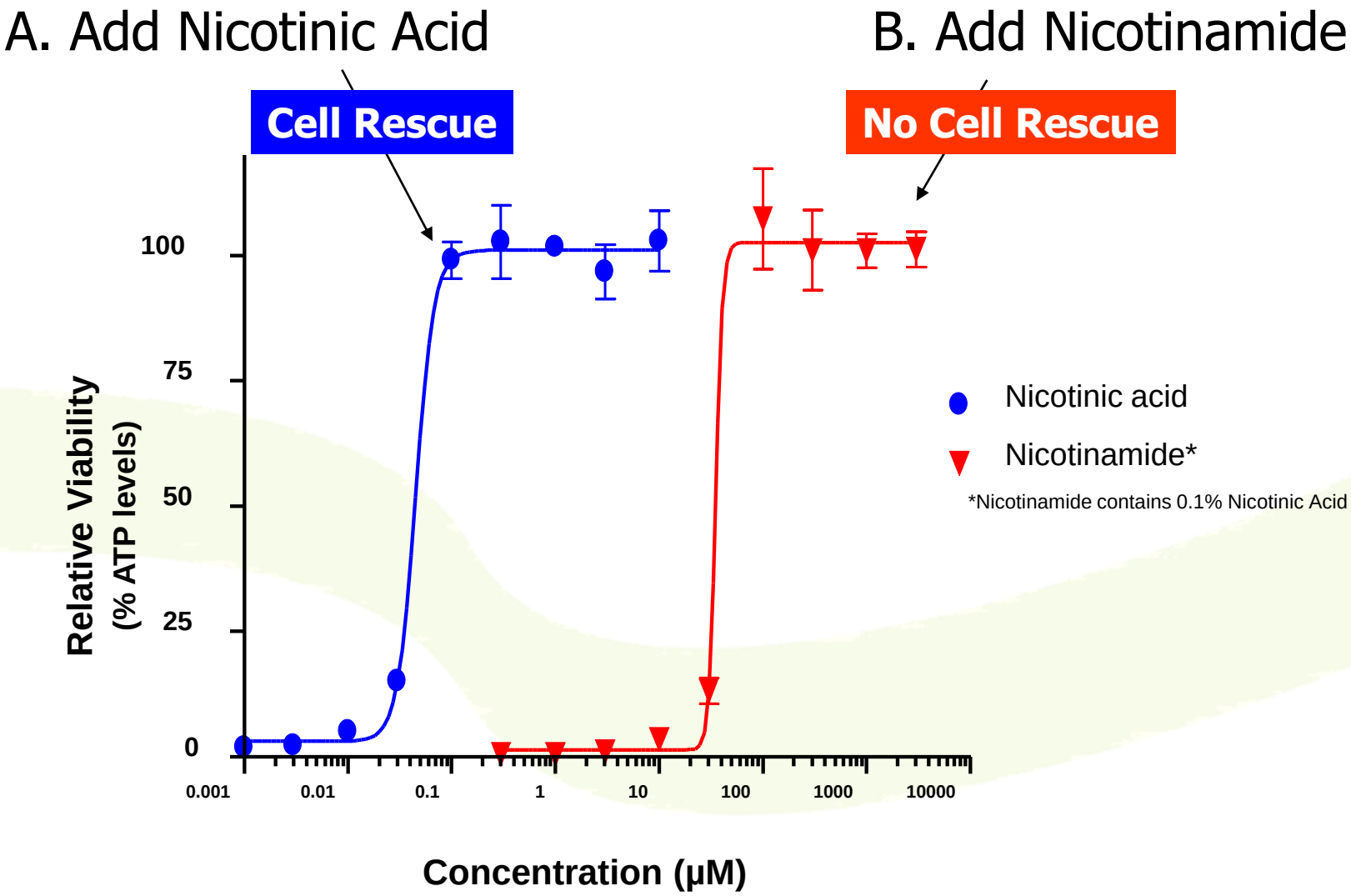
Cell Rescue Experiments



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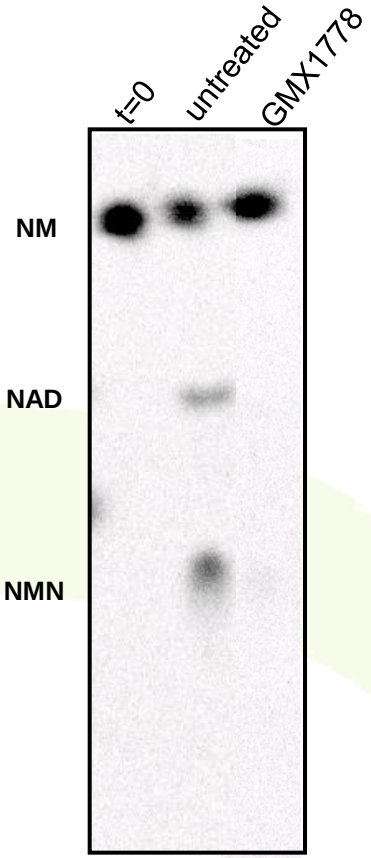
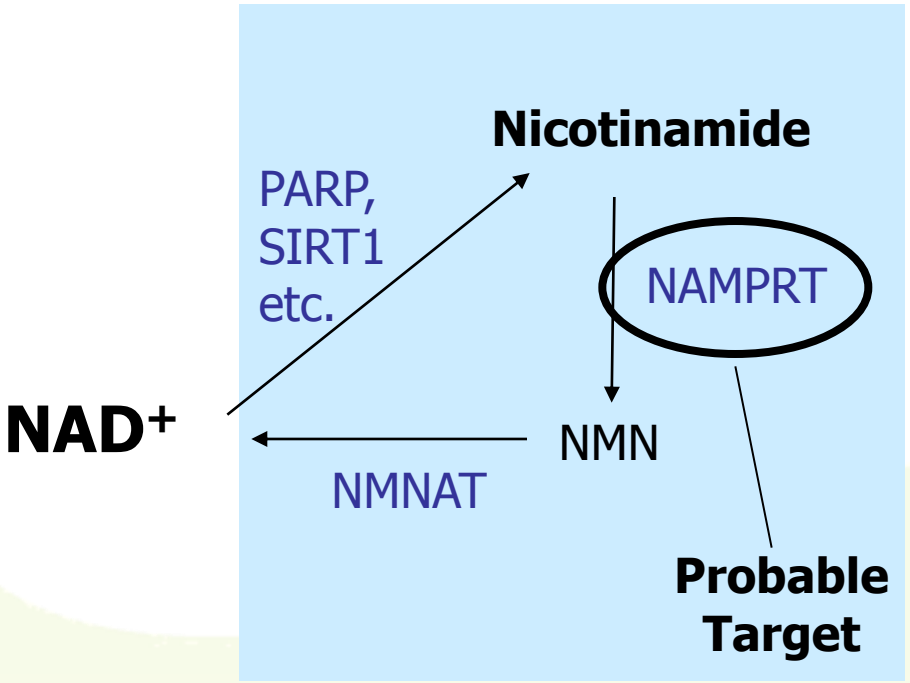
Drug Inhibits NAD+ Synthesis from Nicotinamide

^{14}[C] Nicotinamide in HeLa Cells





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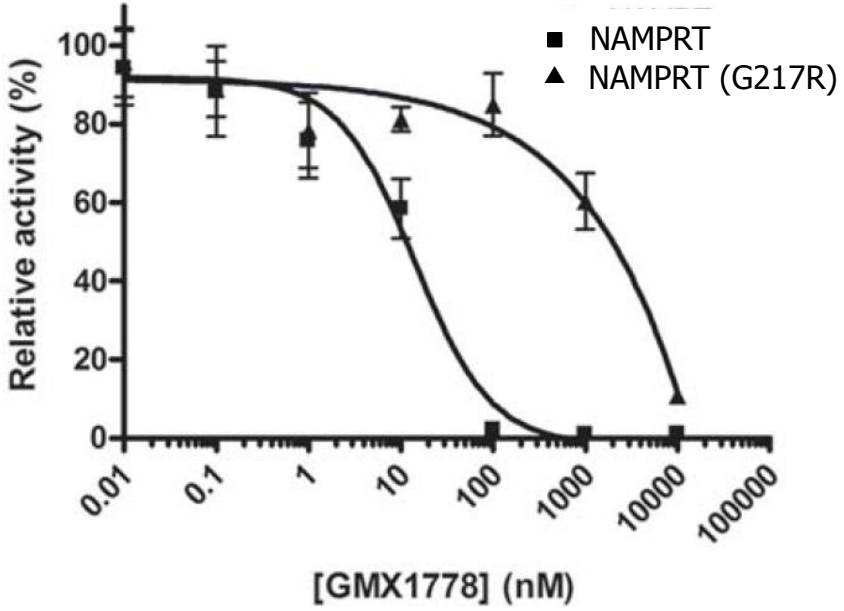
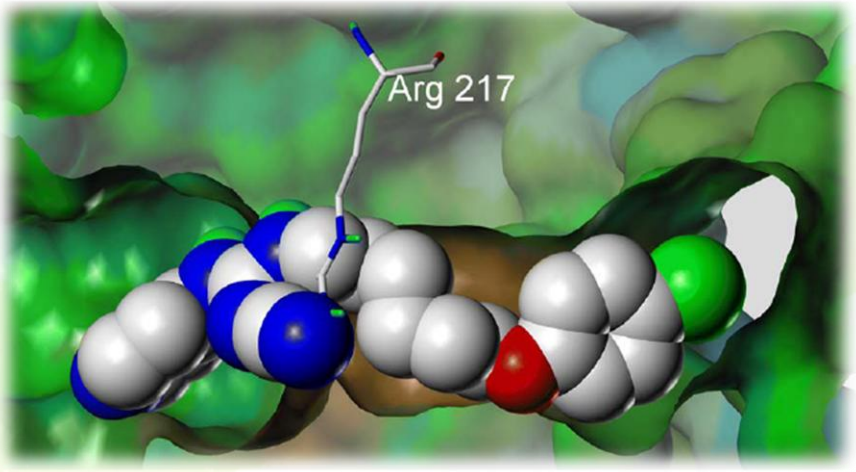
NAMPRT Confirmed as Drug Target

Compound	Ki (nM) Recombinant NAMPRT	IC_{50} (nM) Cell viability assay (72h)	
		IM-9 (Multiple myeloma)	HCT-116 (Colon)
GMX1778	2.60	3.33 ± 0.66	2.30 ± 0.64

Drug resistant enzyme





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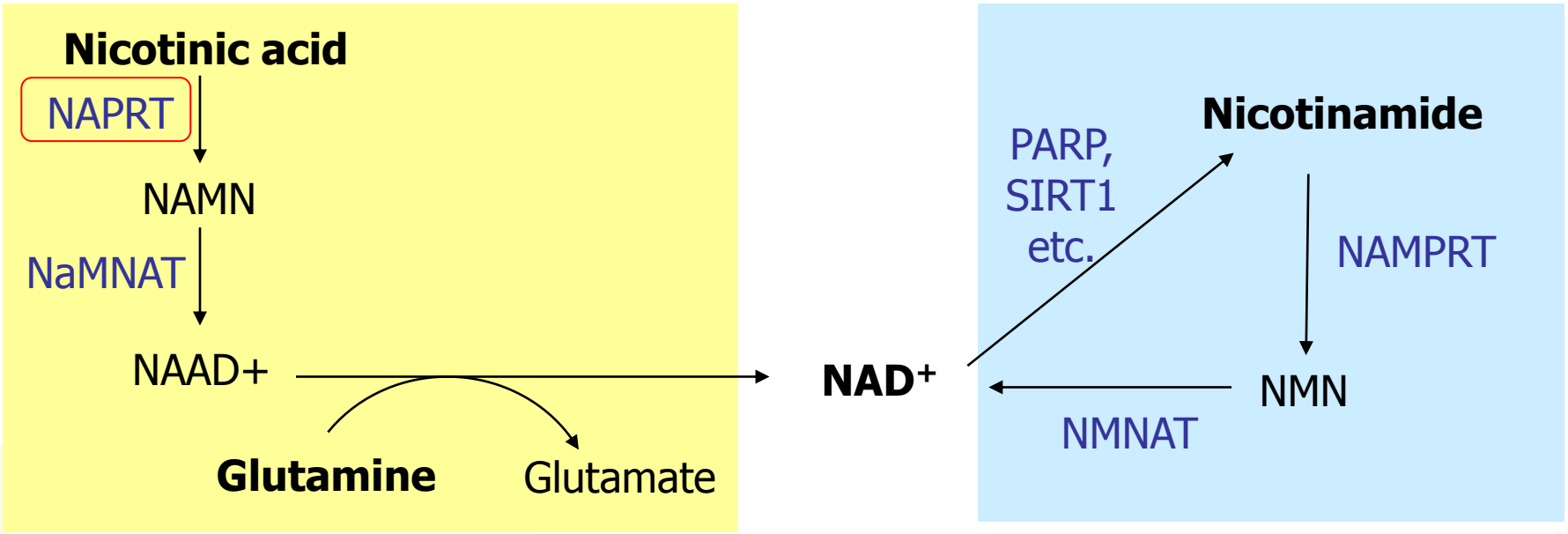
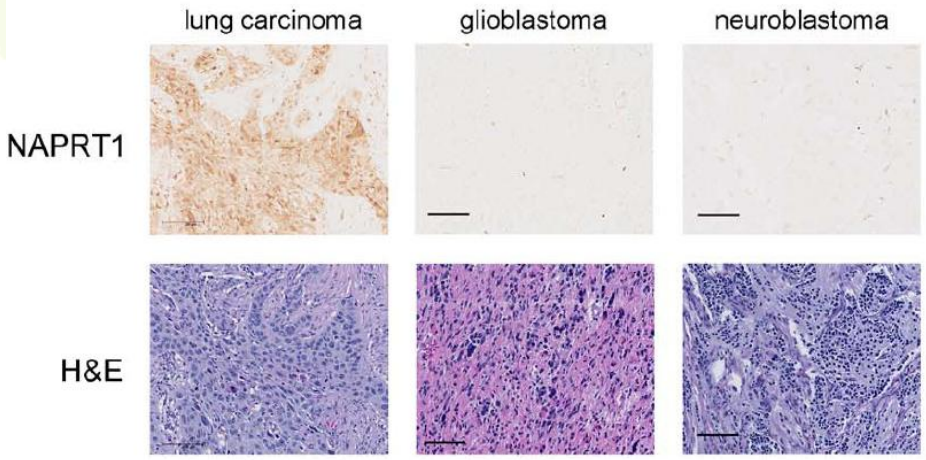
NAD^+ Synthesis – Cancer Cells





Glioblastomas, neuroblastomas and sarcomas deficient in NAPRT

Thus, co-administration of NA expands the therapeutic window of GMX1777.

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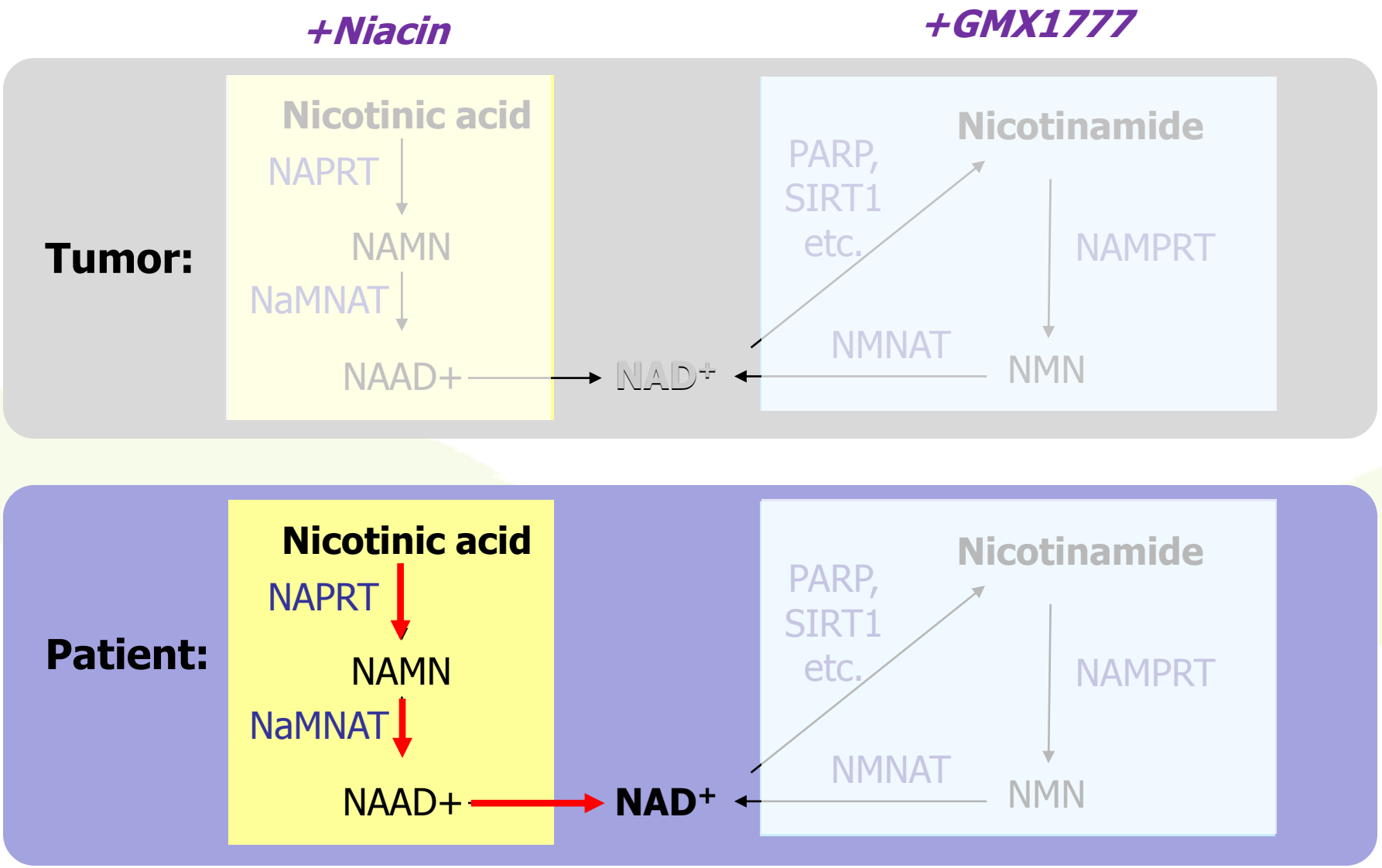
NAD^+ Synthesis – Cancer Cells



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Summary of GMX1777 Study

- The primary mechanism of the drug action was proposed through understanding global metabolic changes
- Simple, yet powerful validating experiments were performed to conclusively show NamPRT as the drug target
- Understanding the metabolism also aided in elucidating new clinical development paths forward

METABOLON

Acknowledgements

GeminX

- Anne Roulston
- Mark Watson

Metabolon

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- Matt Mitchell

Mark Watson,1*† Anne Roulston,1† Laurent Bélec,1 Xavier Billot,1 Richard Marcellus,1 Dominique Bédard,1 Cynthia Bernier,1 Stéphane Branchaud,1 Helen Chan,1 Kenza Dairi,1 Karine Gilbert,1 Daniel Goulet,1 Michel-Olivier Gratton,1 Henady Isakau,1 Anne Jang,1 Abdelkrim Khadir,1 Elizabeth Koch,1 Manon Lavoie,1 Michael Lawless,1 Mai Nguyen,2 Denis Paquette,1 Émilie Turcotte,1 Alvin Berger,3‡ Mathew Mitchell,3 Gordon C. Shore1,2 and Pierre Beauparlant1, **The Small Molecule GMX1778 is a Potent Inhibitor of NAD+ Biosynthesis: Strategy for Enhanced Therapy in NAPRT1-Deficient Tumors, MCB (2009) Nov;29(21):5872-88**

Beauparlant, Pierre; Bedard, Dominique; Bernier, Cynthia; Chan, Helen; Gilbert, Karine; Goulet, Daniel; Gratton, Michel-Olivier; Lavoie, Manon; Roulston, Anne; Turcotte, Emilie; Watson, Mark. **Preclinical development of the nicotinamide phosphoribosyl transferase inhibitor prodrug GMX1777. Anti-Cancer Drugs (2009), 20(5), 346-354.**

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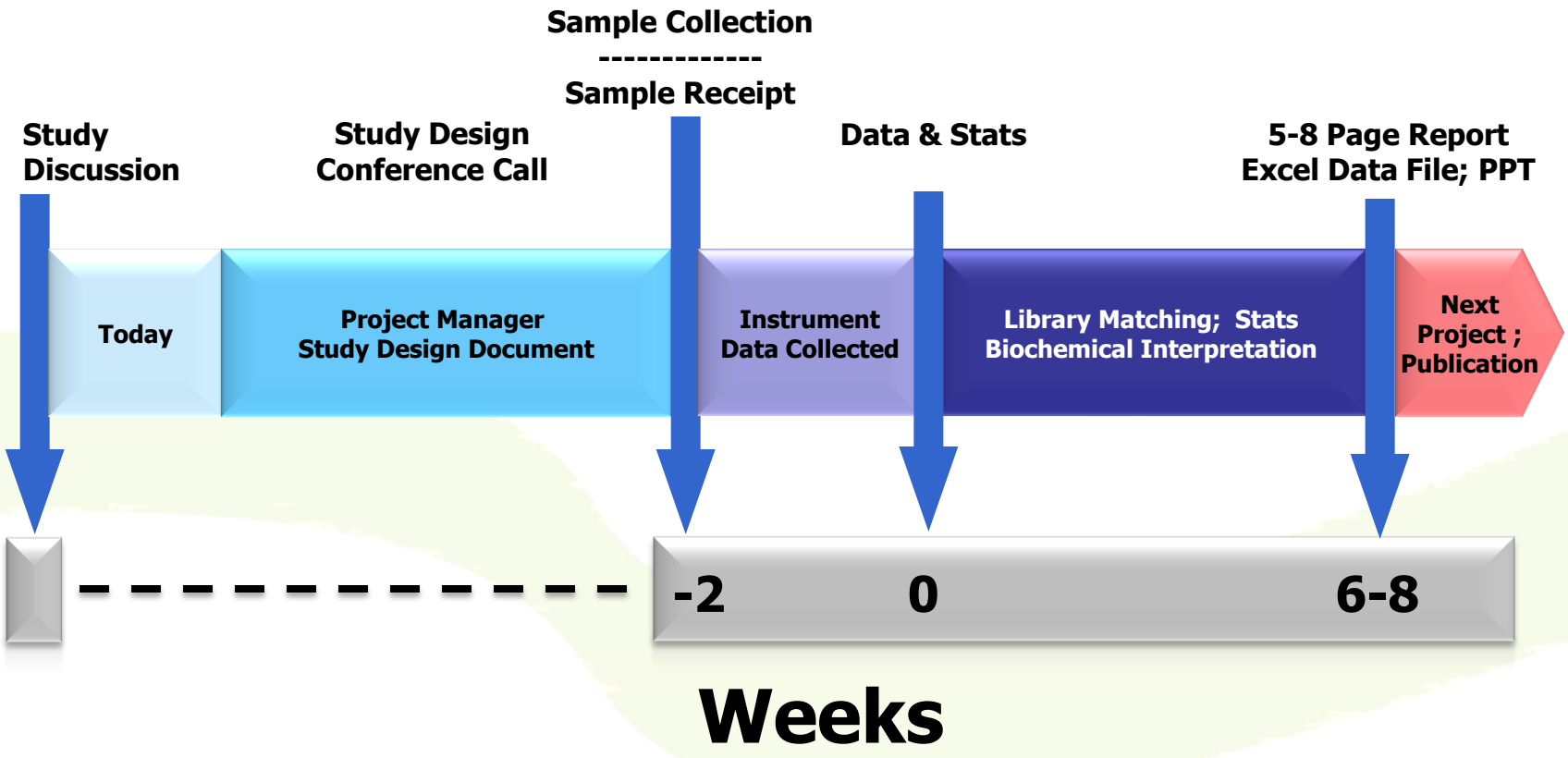
Pathway of an mView Project



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Project Workflow

Today

Study Director consultation

Study design document

Suggested Sample Sizes by Tangible Outcome

	Bioreactor	Traditional cell culture	Small animals	Large animals	Human Studies
Optimal	6 - 8	8 - 10	10 - 15	12 - 20	>50
Rigorous	4 - 6	6 - 8	8 - 10	10 - 12	40 - 50
Good/acceptable	3 - 4	4 - 6	6 - 8	8 - 10	25 - 40
Acceptable but potentially noisy	N/A	3 - 4	4 - 6	6 - 8	20 - 25
Not likely to return powerful statistical results	N/A	N/A	3	4 - 5	<20

METABOLON

Project Workflow

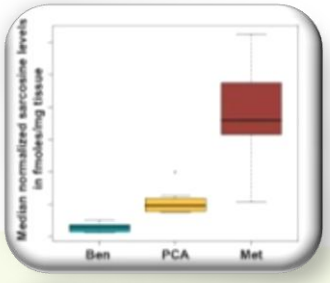
Today → Study Director consultation → Study design document → Initial data (1) month from platform

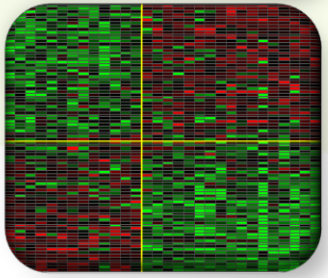
BIOCHEMICAL NAME	FOLD CHANGE (CONTROL CELLS)					
	D4 / D0	D7 / D0	D9 / D0	D11 / D0	D14 / D0	D14 / D11
glycine	0.98	0.77	0.80	0.54	0.38	0.71
serine	0.95	0.55	0.80	0.93	1.37	1.47
homoserine (homoserine lactone)	4.08	11.51	13.39	13.81	9.88	0.72
threonine	1.91	2.48	2.42	2.25	1.85	0.82
N-acetylthreonine	1.12	1.04	1.24	1.08	1.48	1.37
allo-threonine	1.18	1.71	1.77	1.60	2.14	1.33
alanine	2.26	0.76	0.44	0.63	0.39	0.62
beta-alanine	1.97	1.55	2.19	2.99	2.78	0.93
aspartate	0.15	0.08	0.12	0.07	0.08	1.20
asparagine	0.26	0.05	0.07	0.05	0.05	0.84
glutamate	0.58	0.65	0.60	0.63	0.48	0.76

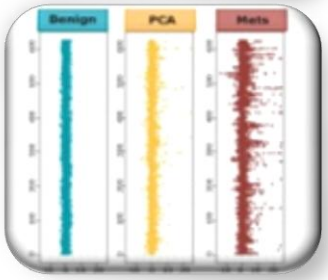
METABOLON

Project Workflow

Today → Study Director consultation / Study design document → Initial data (1) month from platform → Data / report discussion with customer



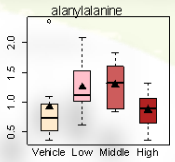


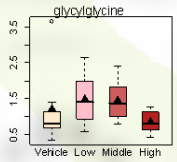


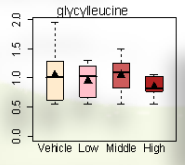
Dipeptides and Pyroglutamine

Exhibiting strong dose response curves, the dipeptides alanylalanine, glycylglycine are detected in the hypothalamus (Fig. 8). Either these are increased due to a general increase in dipeptides or they are specifically increased due to production or catabolism of peptides/proteins with these motifs. The first point is refuted since another dipeptide (Gly-Leu) detected in this study fails to exhibit this same pattern. Possibly, the increase in alanylalanine and glycylglycine may be reflective of something more specific?

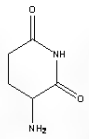
The brain is rich in neuropeptides and the processing of signal sequences and their catabolism is paramount for timely dosing of these molecules. Although these two dipeptides are far too generic to draw any firm conclusions, the possibility that they are from specific neuropeptides should be considered. For example, a delta sleep-inducing peptide (DSIP) is related to the topic described above (sleep) has a GG motif (Trp-Ala-Gly-Gly-Asp-Ala-Ser-Gly-Glu). Orexin is a neuropeptide involved in sleep and wakefulness and it has an Ala-Ala motif. Also pyroglutamine could be a product of proteolysis from a specific peptide (note, it is not simply a product of Gln since Gln does not change at all, Fig. 8).

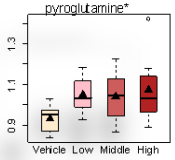


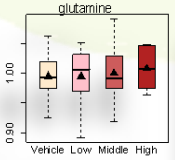




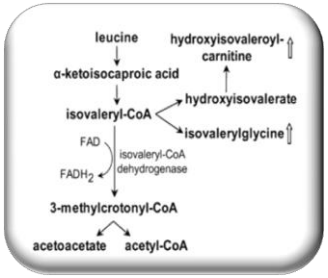
- Gly-Gly and Ala-Ala increased at LD and MD
- Trend not detected with all dipeptides

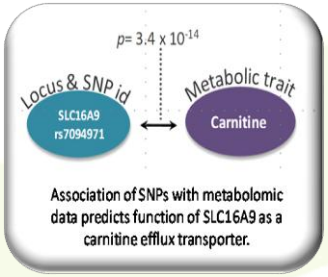


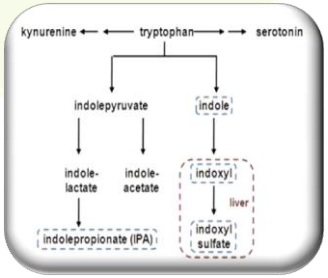




- PyroGln may be derived from breakdown of specific protein
- Trend does not appear to simply correlate with Gln levels







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Project Workflow

Today → Study Director consultation / Study design document → Initial data (1) month from platform → Data / report discussion with customer → Next project / publication

Visit www.metabolon.com

- GWAS (Serum Biochemicals and SNP Associations)
- Role of Adenosine Signaling in Sickle Cell Disease
- Metabolic Impact of IDH1 Mutations
- Metabolon's Technology Paper
- Prostate Cancer Biomarker Discovery
- Discovery of Novel Anticancer Drug Mechanism
- Drug Toxicity Studies with Mitsubishi and Teva
- Biomarkers of Depression
- Biomarkers of Dental Disease
- Human Metabolome Paper on Aging
- NASH Biomarker Discovery Paper
- Preterm Labor Biomarkers
- Prediction of Liver Dysfunction Biomarkers with Choline Diet
- Mechanism of Toxicity with EGME

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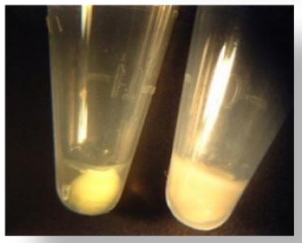
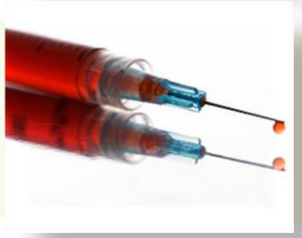
Elements of Successful Study Design

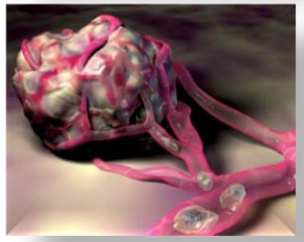
Study Material



Cell-Based Studies
50-100µl
packed cell pellet



Biological Fluids
100-200µl
plasma, urine



Tissues
50-100mg

Statistical Power

	Recommended Samples per Group
Cell lines	4-7
Small animal	6-10
Large animals	8-15
Human	25-40

Sample quantity and group size impact study results

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