Using ^{13}C and ^{15}N Isotopomer Metabolic Flux via Glucose and Glutamine to Understand Cancer's Metabolic Dependencies by SRM-LC-MS/MS

ASMS 2013 - Minneapolis

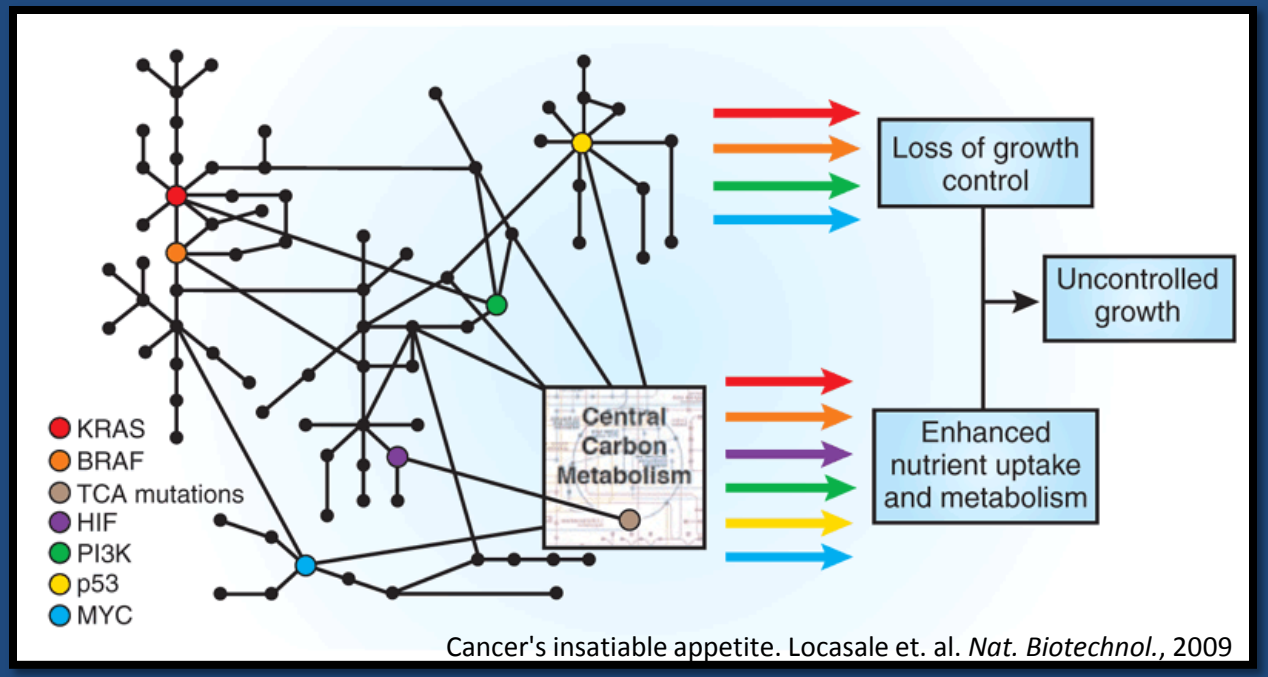
John M. Asara, Ph.D.

Beth Israel Deaconess Medical Center
Harvard Medical School
Boston, MA

Beth Israel Deaconess Medical Center

Harvard Medical School

It's more than just kinase activity and genetic defects in cancer



Cancer's insatiable appetite. Locasale et. al. *Nat. Biotechnol.*, 2009

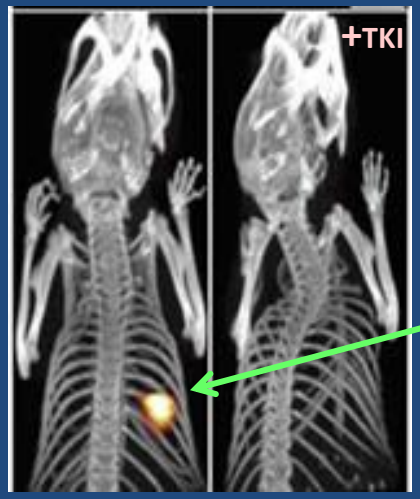
Signaling Network ⟷ ***Metabolism***

↓

Cell growth and proliferation (Cancer)

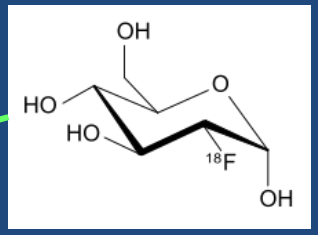
•We can use this to our advantage when tracing labeled carbon through cancers

'*Warburg Effect* states that **glucose** is taken up at a high rate and converted to lactate by cancer cells'



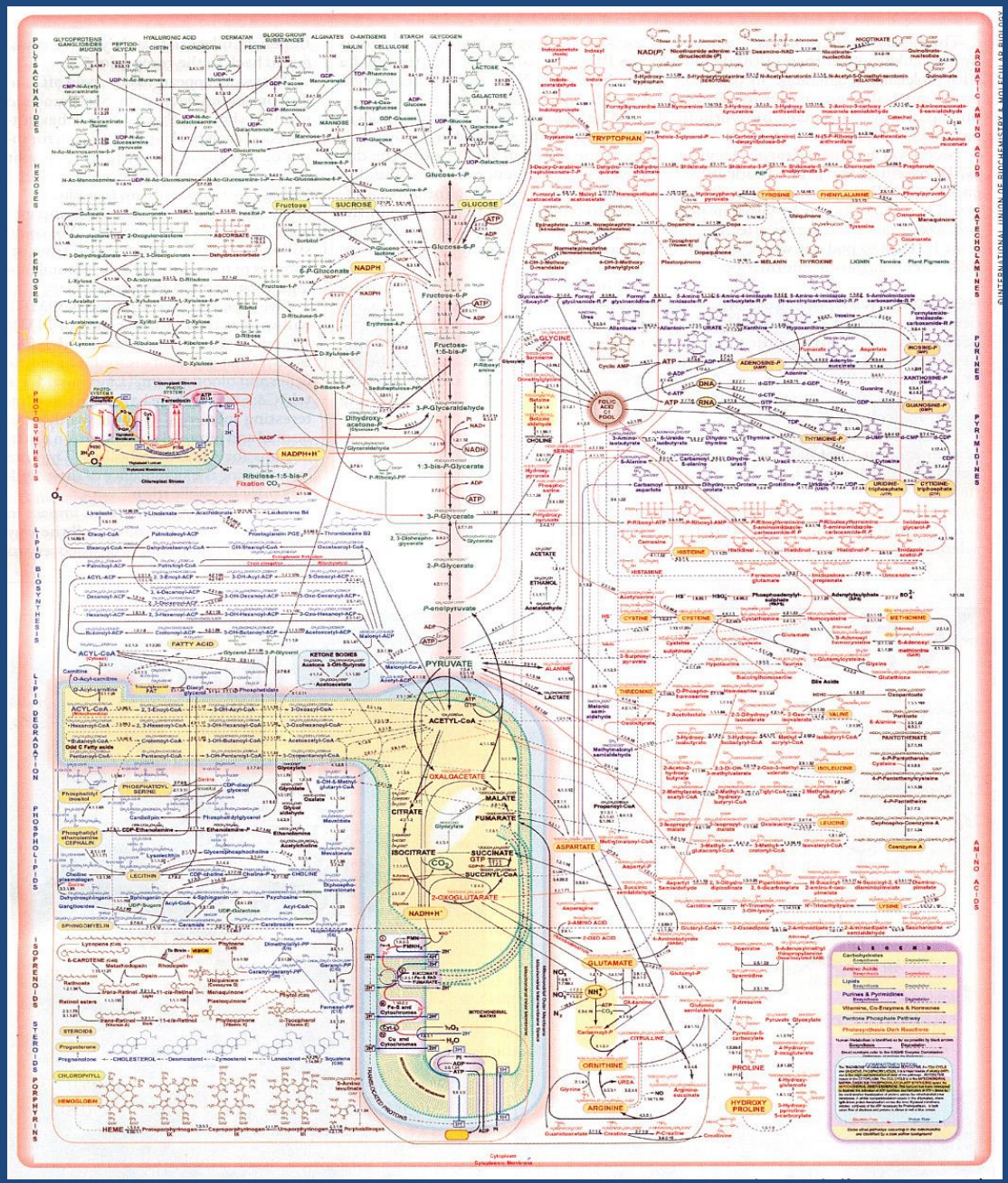
Lung tumor

FDG – PET scan (labeled glucose)



Engelman *et. al.*, *Nat. Medicine*, 2008

Map of the Human Metabolome

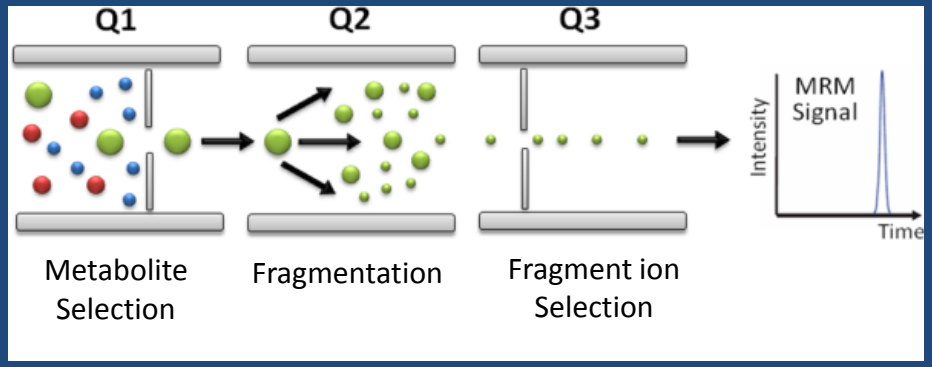


Platform for Targeted Endogenous Polar Metabolite Profiling

+/- switching

Amide XBridge
HILIC - 1 column
4.6mm x 15cm
pH=9.0, NH_4^+
400 µL/min

Selected Reaction Monitoring (SRM) ~300 transitions (258 unique metabolites -^{12}C & ^{13}C)

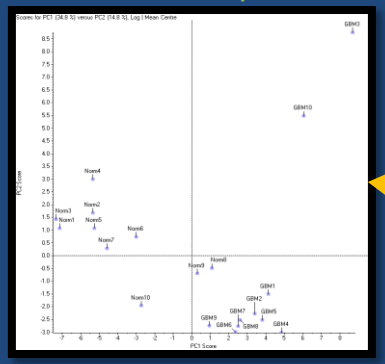


Extract metabolites with **80% methanol**
From cells, tumor tissue, fluids, etc.

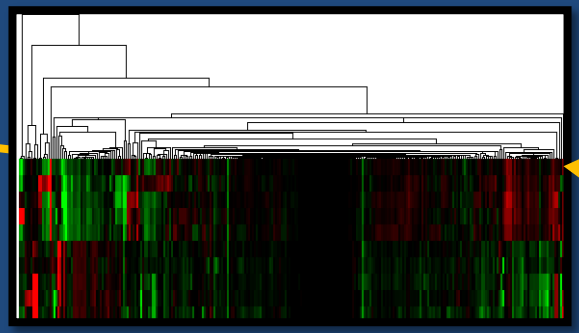
Cancer cells

Mean R^2 = 0.978
Mean CV= 0.12
FWHH = ~9 seconds
Cycle time = 1.67 sec
3-4 msec dwell
10-14 points per peaks

PCA analysis
MarkerView
Metaboanalyst.ca



Clustering (MatLab, Metaboanalyst.ca)
KEGG pathway mapping



MultiQuant v2.0 Peak Area integration software

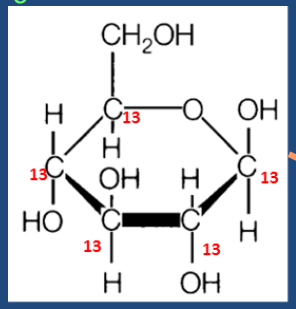
Sample Name	avg DMSO	avg BEZ	avg BKM	avg U0126
3-phosphoglycerate	1330385.962	871663.9395	1038599.88	943607.0569
3-phospho-serine	152487.2158	83097.6478	73986.5328	31000.0000
D.glyceraldehdye.3-phosphate	254477.5084	293208.1613	209570.2472	194280.8415
dihydroxy.acetone-phosphate	357217.2808	274197.6204	227350.7244	210859.1435
fructose.1,6-bisphosphate	1059370.361	808511.4636	1082381.874	682001.4833
fructose.6-phosphate	1471332.891	1019002.062	1046811.137	1054938.996
glucose.1-phosphate	761216.5713	605856.8664	815435.949	811274.948
glucose.6-phosphate	955670.737	704956.8497	635987.2986	741371.4211
hexose-phosphate	19302214.34	14375529.9	20558058.05	16548067.87
lactate	95442398.42	94957148.33	103044596.9	101662913.9
phosphoenolpyruvate	258107.2535	217613.2952	323347.9553	382350.6681

Yuan, et. al., *Nature Protocols*, 2012.

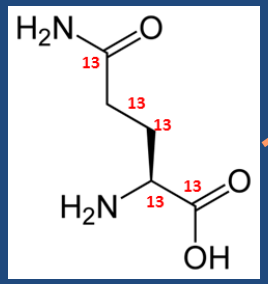
Steady-State Metabolic Flux Analysis: measure the destination of the labeled carbon atoms through glycolysis and related pathways

"SILAC" version for metabolomics

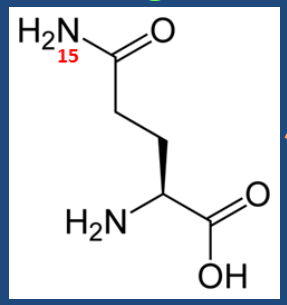
$^{13}C_6$-labeled glucose



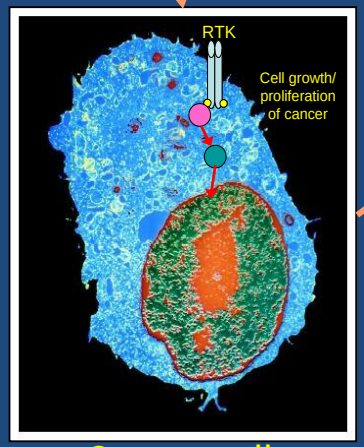
$^{13}C_5$-labeled glutamine



^{15}N-labeled glutamine



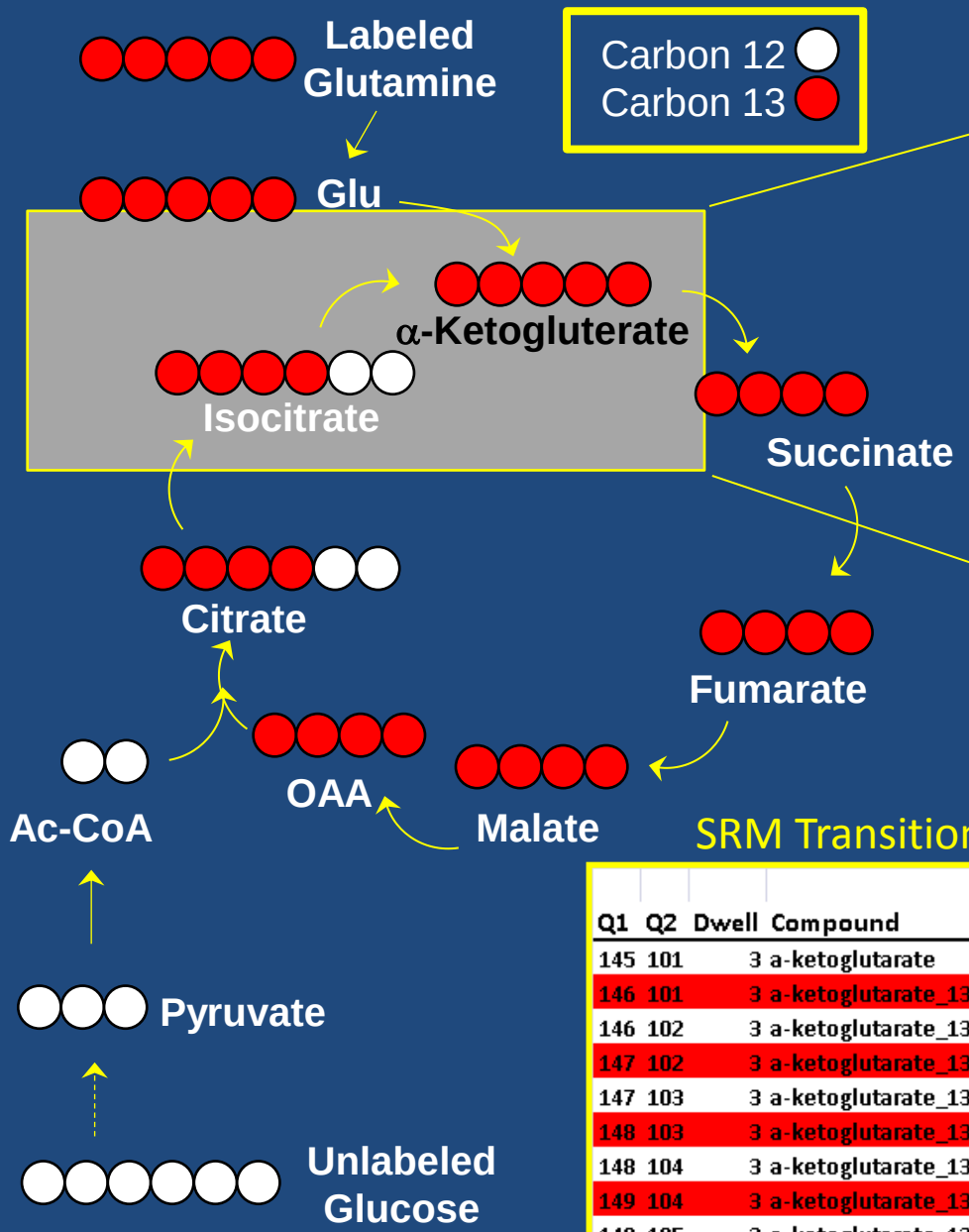
Cambridge Isotope Laboratories (CIL)



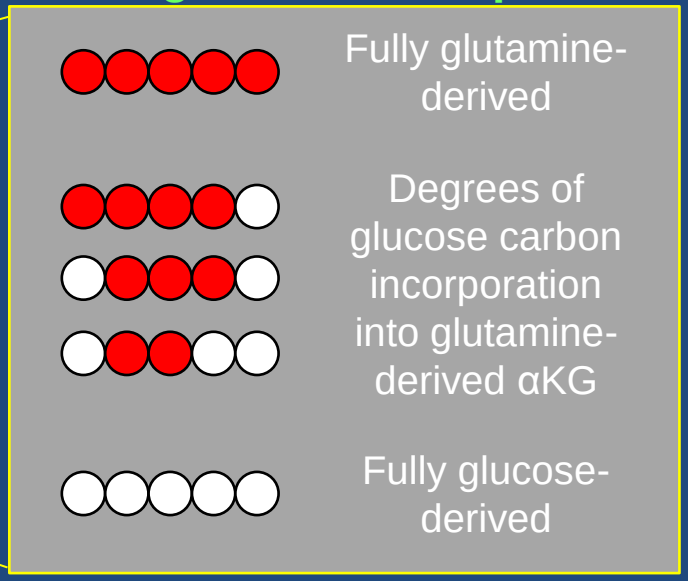
Cancer cell

Casperflights.com

Tracking Glutamine Metabolism by Isotopomer Steady-State Flux



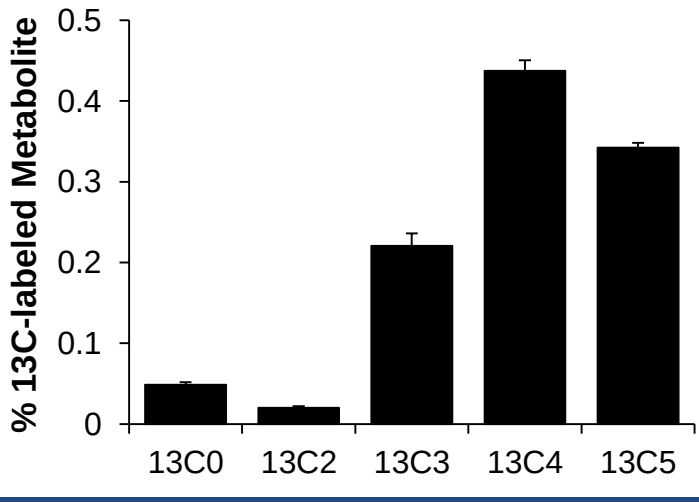
α-Ketogluterate Isotopomers



SRM Transitions

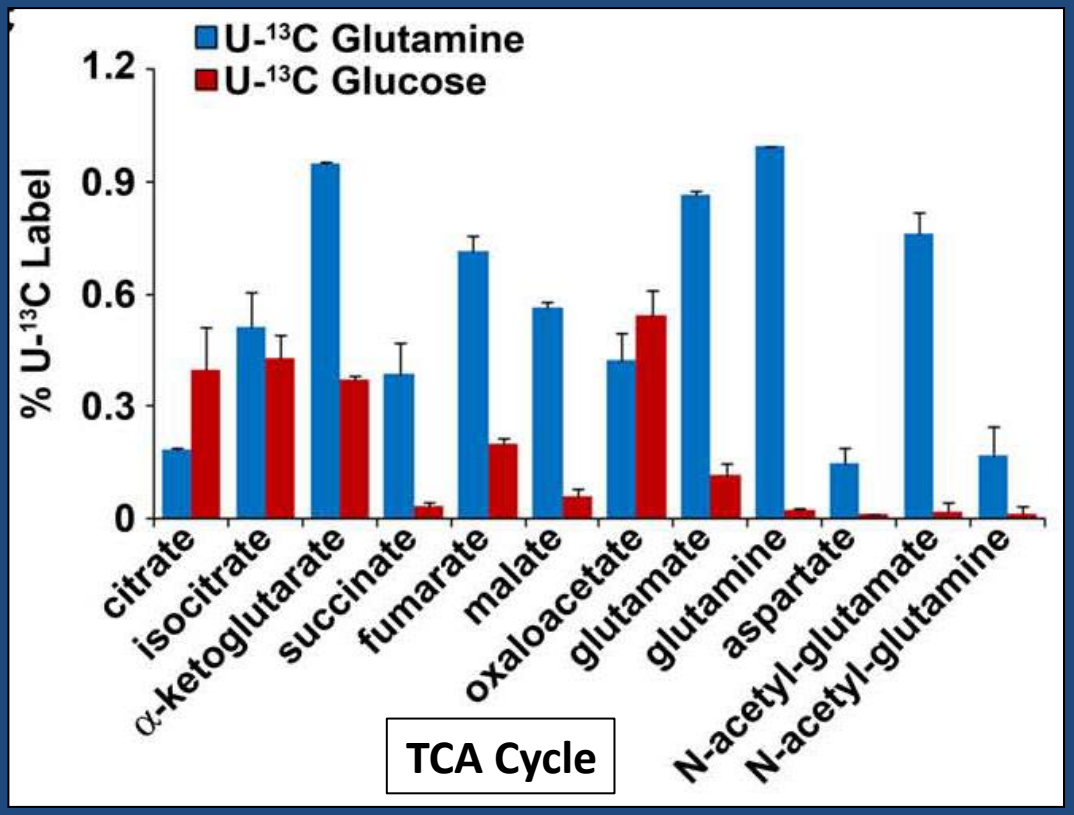
Q1	Q2	Dwell	Compound	CE
145	101		3 a-ketoglutarate	-12
146	101		3 a-ketoglutarate_13C1(2)	-12
146	102		3 a-ketoglutarate_13C1	-12
147	102		3 a-ketoglutarate_13C2(2)	-12
147	103		3 a-ketoglutarate_13C2	-12
148	103		3 a-ketoglutarate_13C3(2)	-12
148	104		3 a-ketoglutarate_13C3	-12
149	104		3 a-ketoglutarate_13C4(2)	-12
149	105		3 a-ketoglutarate_13C4	-12
150	105		3 a-ketoglutarate_13Q	-12

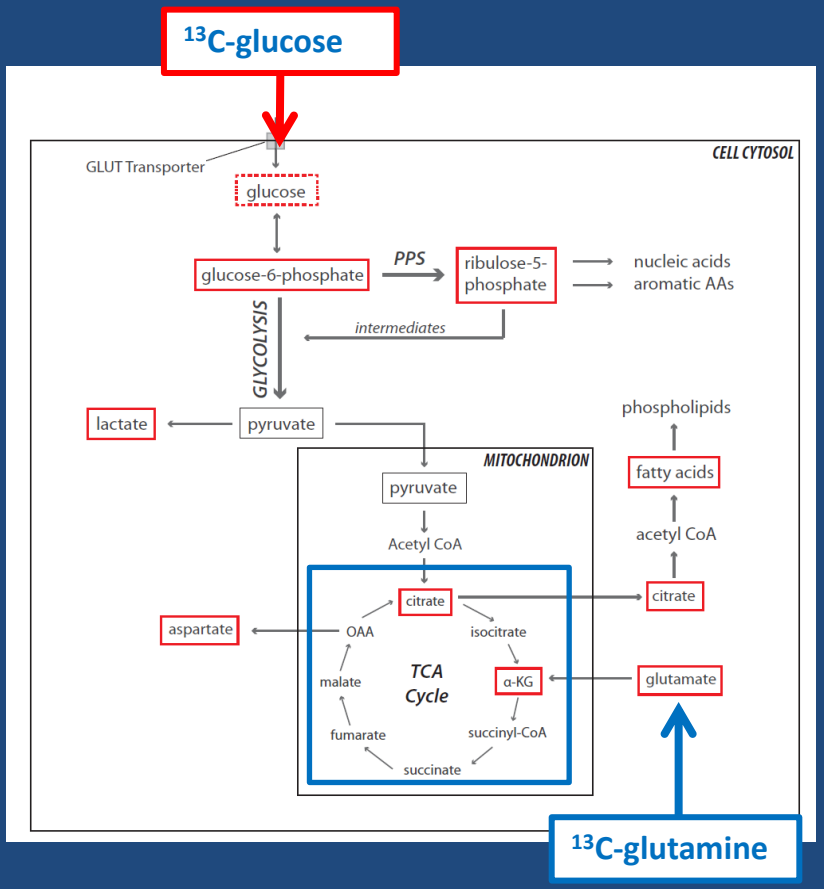
α-KG Isotopomer Analysis in Gln-addicted Pancreatic Cancer



Costas Lyssiotis

Example of ^{13}C Labeling in Cells Showing that Glutamine Predominantly Fuels the TCA Cycle



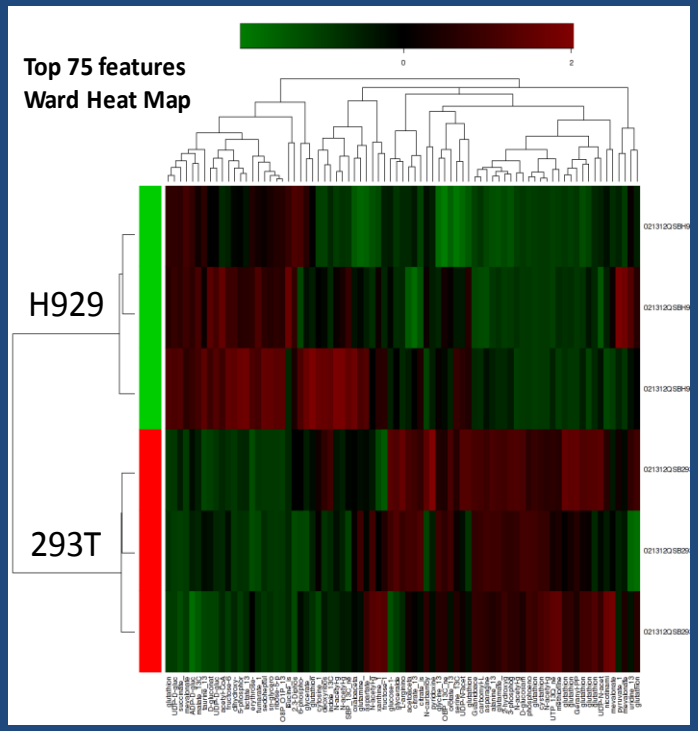


^{13}C Glutamine and ^{13}C Glucose Labeling in H929 Multiple Myeloma cells

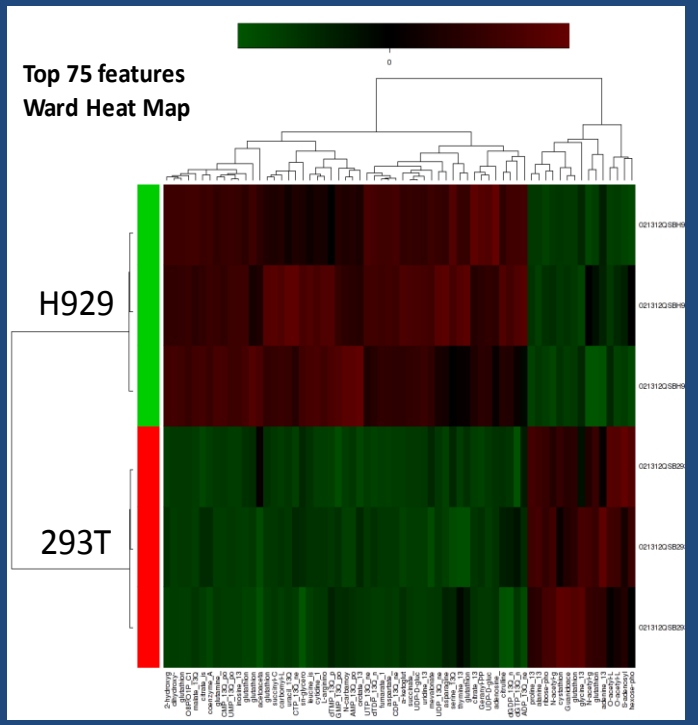
Multiple Myeloma: a cancer of the plasma cells in bone marrow

- Hypercalcemia (**bone loss**)
- Lesions / **Fractures**
- Abnormal blood levels
- Low immunity (excess monoclonal IgG)

13C-Glucose



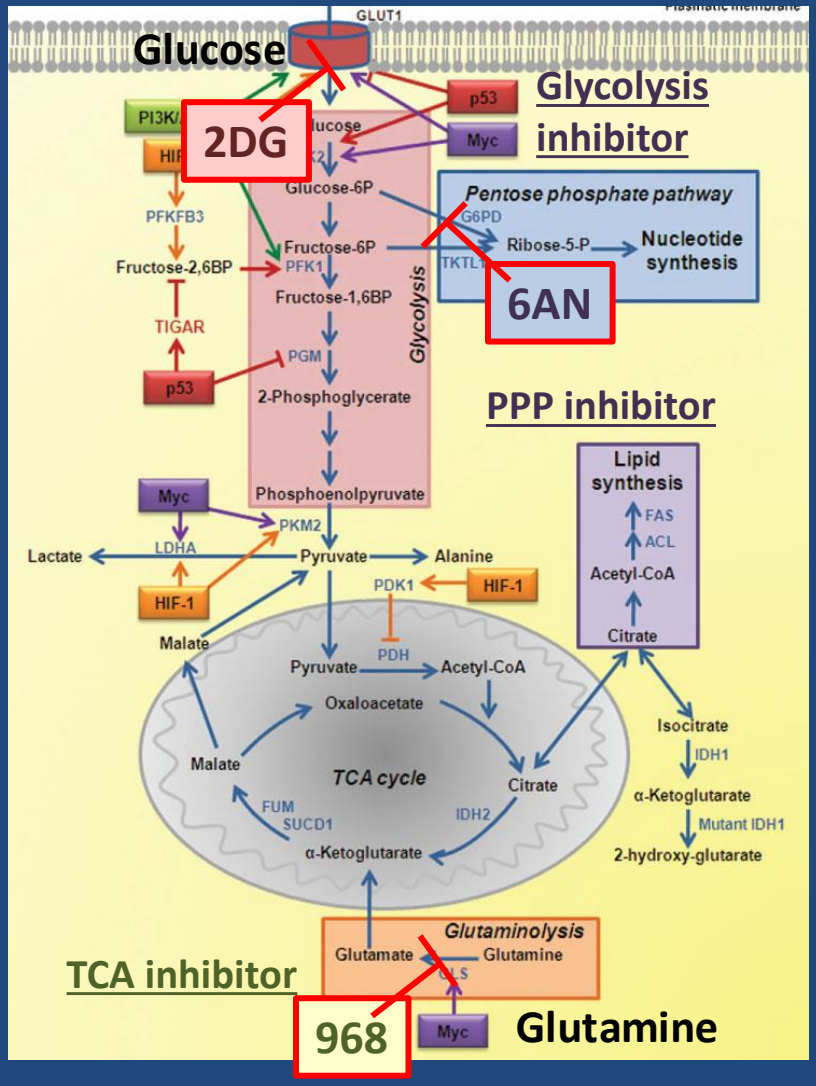
13C-Glutamine

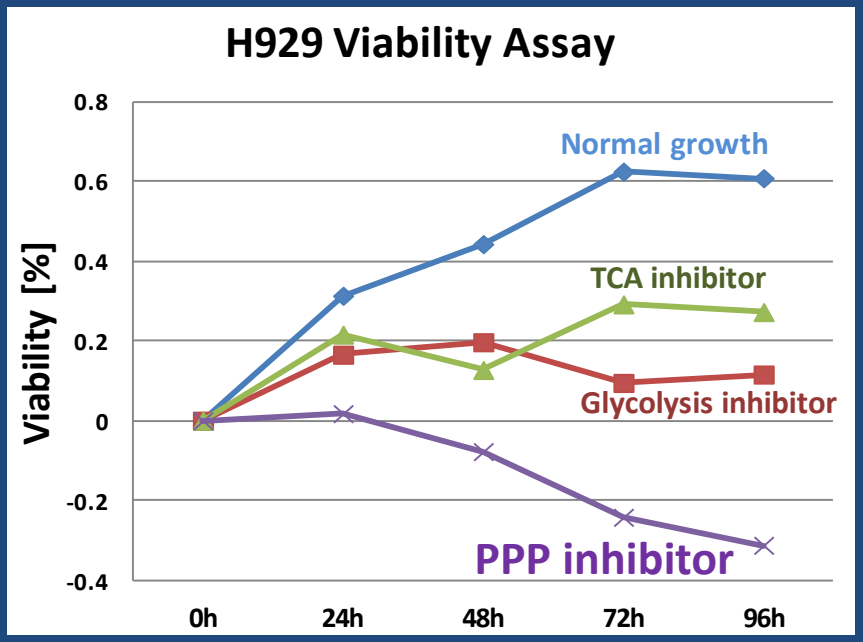


Multiple Myeloma

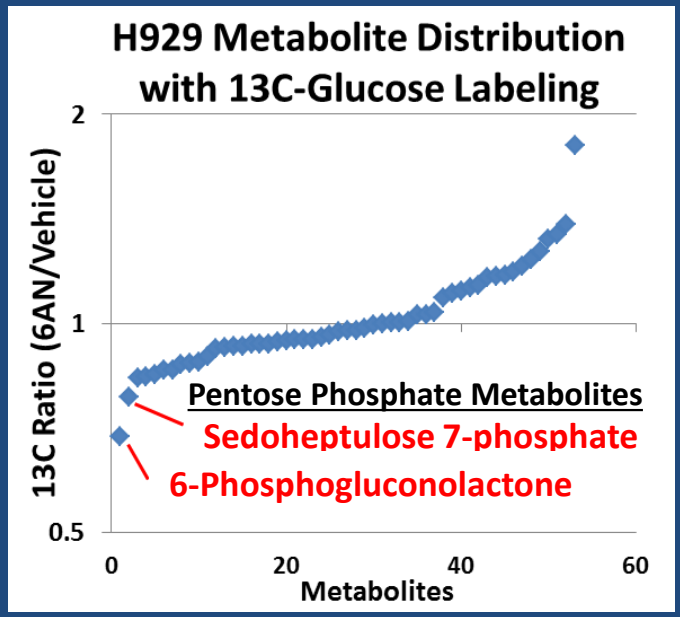
Control

H929 Multiple Myeloma Cells Treated with Metabolic Inhibitors and Targets Verified with ^{13}C Glucose Tracing



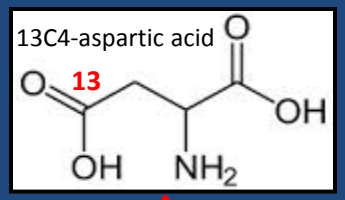


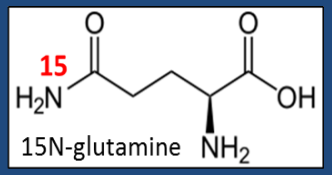
13C-Tracing for Target Validation



- H929 MM cells are more highly dependent upon glucose than glutamine for growth

The TSC1/TSC2 – mTOR Pathway is a Metabolic Switch for Cell Growth and Proliferation





TSC1/TSC2

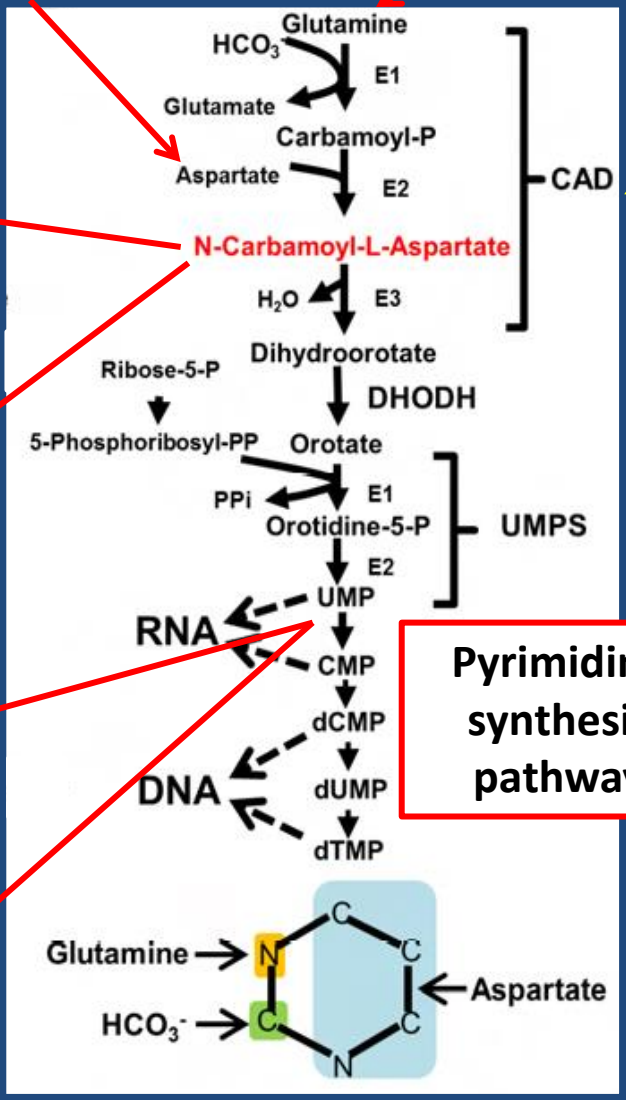
mTORC1

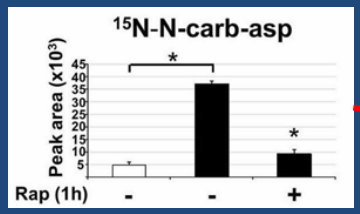
S1859 PO_4

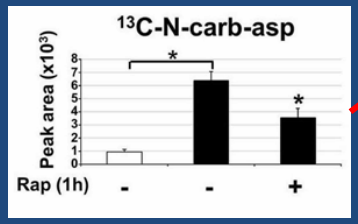
S6K1

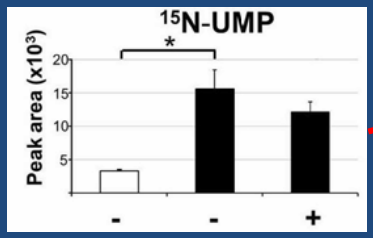
Rapamycin

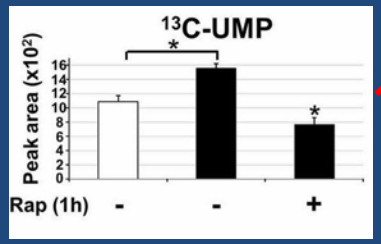
Growth and Proliferation





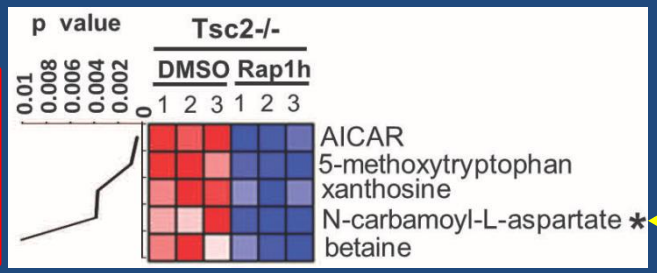






Pyrimidine synthesis pathway

Unlabeled Polar Profiling
(selected from 225 metabolites)



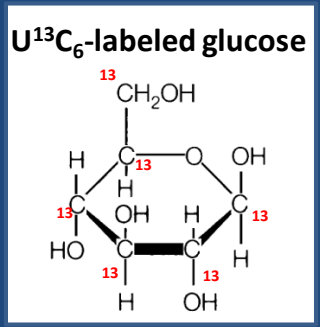
Ben-Sahra et. al., *Science*, 2013

In Vivo Labeling of ^{13}C-Glucose

Two methods of Injecting ^{13}C into mice: Which method works best?

U-$^{13}C_6$ Glucose Intraperitoneal (IP) Injection experiments:

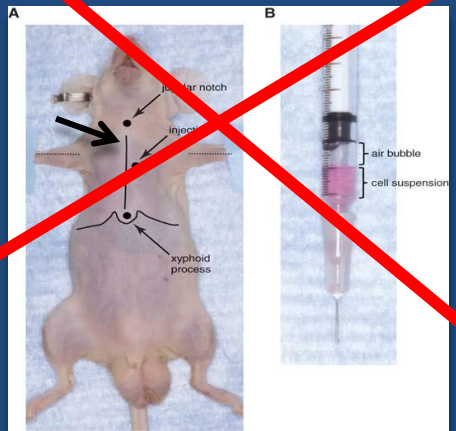
^{13}C-glucose solution (2g/kg; fasted O/N). 30-60 min later, the mice were sac'd and tumor and other organs were harvested.



Extract metabolites for LC-MS/MS

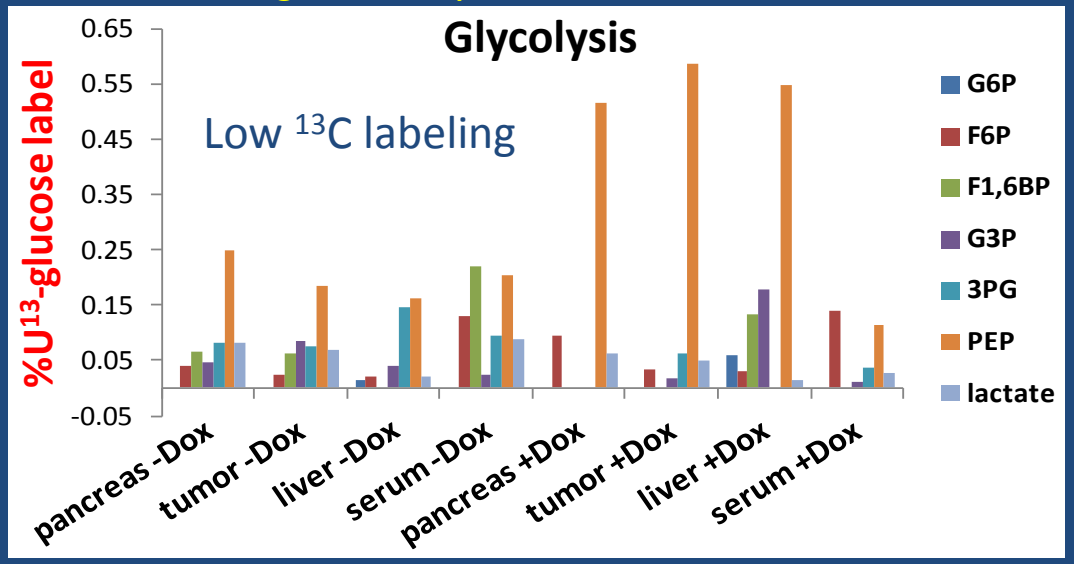
U-^{13}C Glucose Jugular Bolus : Infusion experiments:

400mg/kg ^{13}C glucose in 0.2 mL saline : 12 mg/kg/min at 150 µL/hr for 1.5 hr. tumor, liver and serum were harvested. ***Mice died after 30 min.! – No longer do this.***



Tracking blood glucose and early sacrifice improves ^{13}C labeling

2 hr sacrifice, *low glucose uptake*, no blood monitor

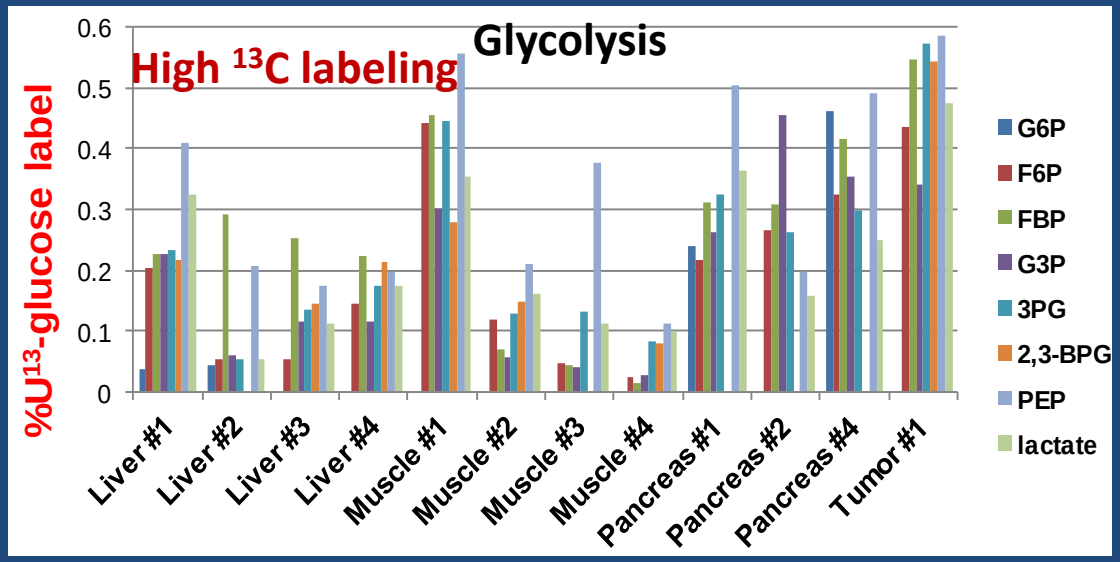


In Vivo Labeling of mice

Test blood glucose/sac mice

LC-MS/MS (SRM)

30-60 min sacrifice, monitor blood glucose
(low level=*high glucose uptake*)

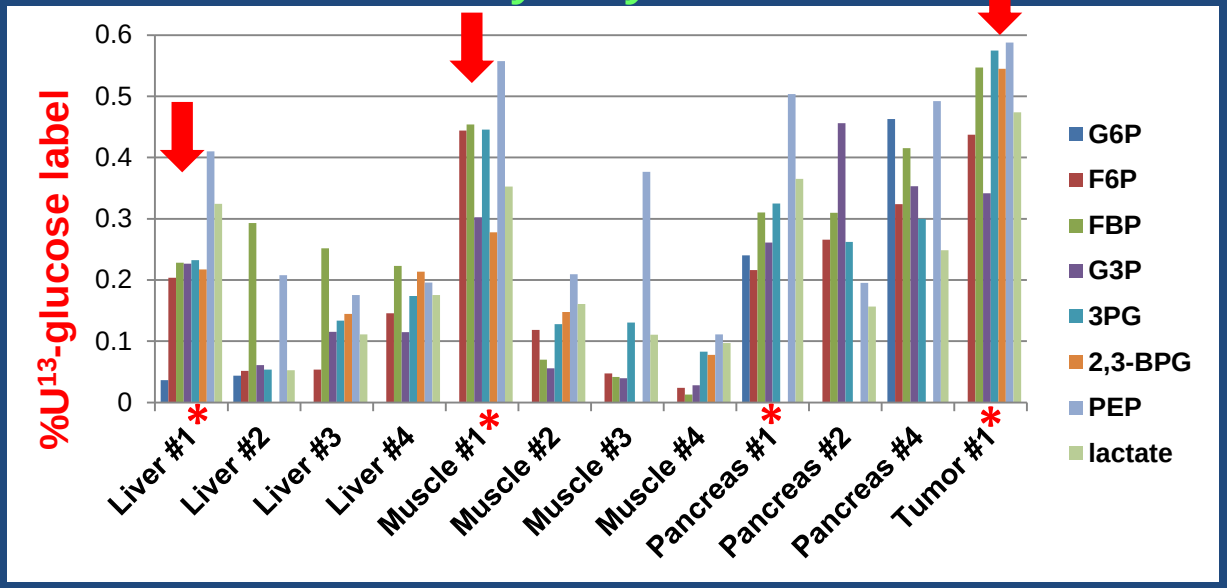


Use of Glucose Tolerance Test to Monitor Optimal Sac time of mice

Mouse	Sick	Tumor	Dox	Weight (g)	Sac'd (min)	Blood [Glucose] (mg/dL)
#1	Yes	Yes, huge	No	29	30	113
#2	No	No	No	34	60	315
#3	No	No	Yes	44	60	212
#4	No	Yes, very small	Yes	29	30	193

- **Large tumors take up glucose at a rapid rate, lowering blood glucose levels**

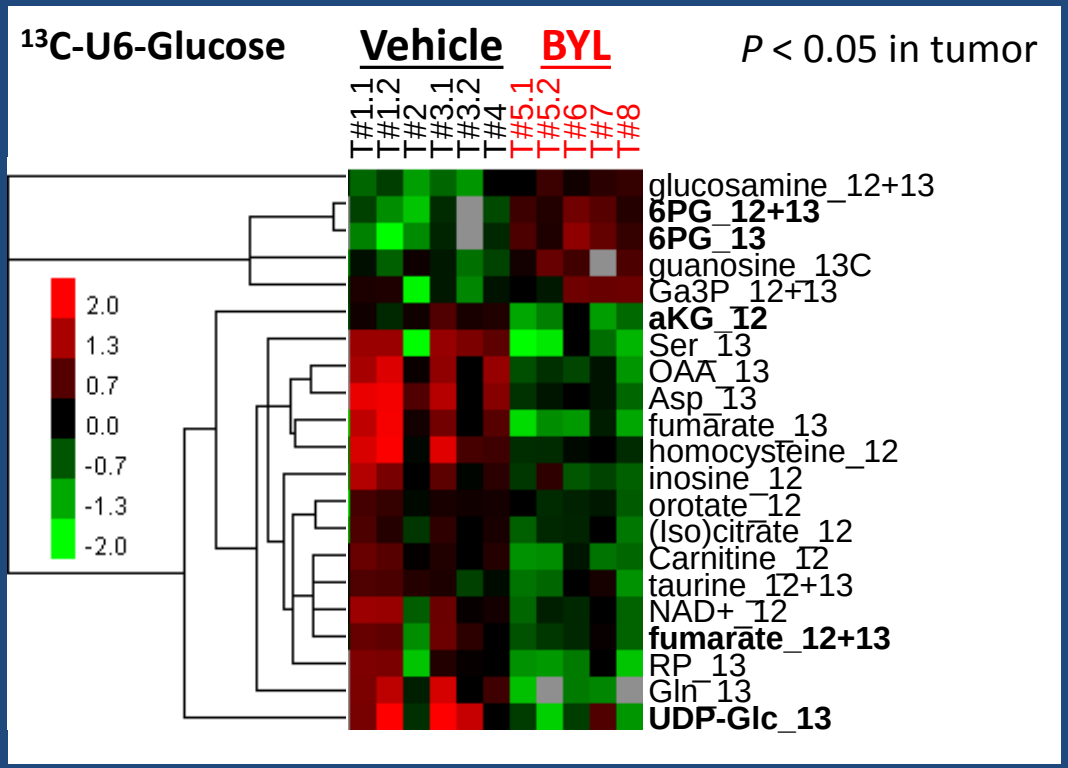
Glycolysis



Labeling with ^{13}C can be achieved to ~50-60% incorporation

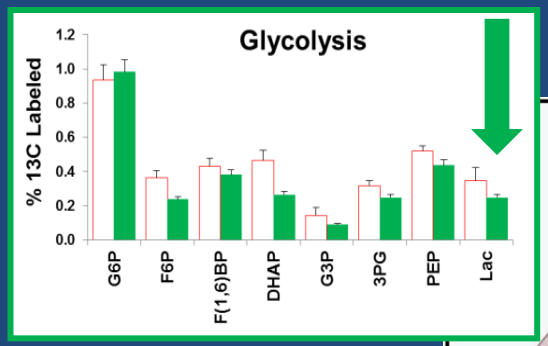
Significantly ($P < 0.05$) differentially regulated metabolites in *labeled tumors* upon PI3K Inhibitor treatment

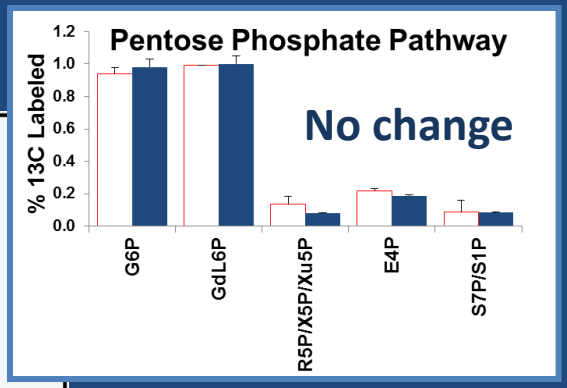
In Vivo Labeling of mice

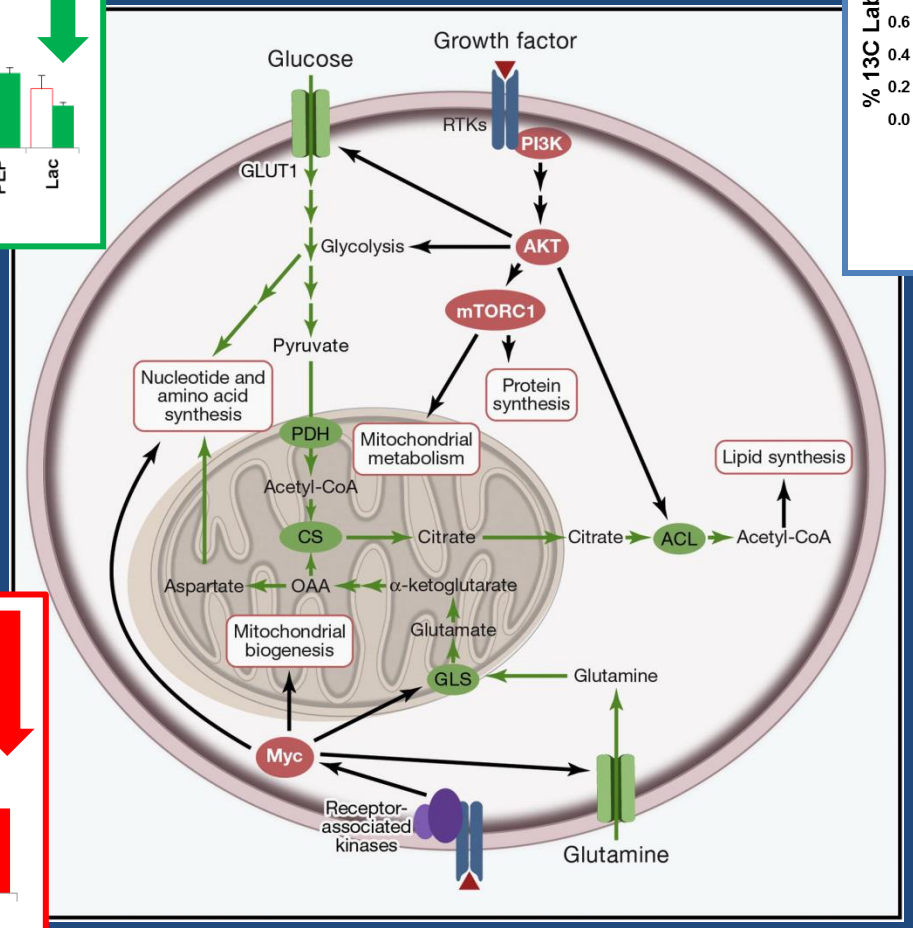


***In Vivo* Labeled Mice Can Reveal Regulated Metabolic Pathways**

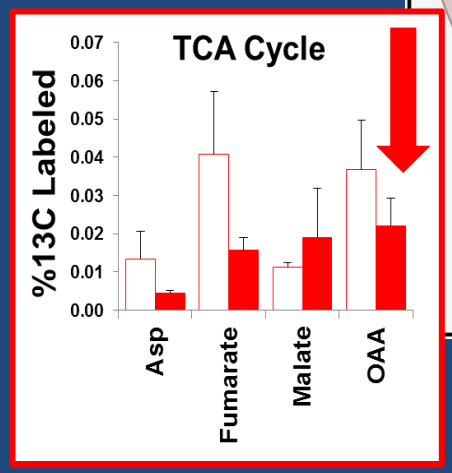
PI3K Inhibitor BYL Abrogates Flux Through Central Metabolism in ^{13}C-Glucose Labeled Mouse Tumors







Ward & Thompson, *Cancer Cell*, 2012



In Vivo Labeling of mice

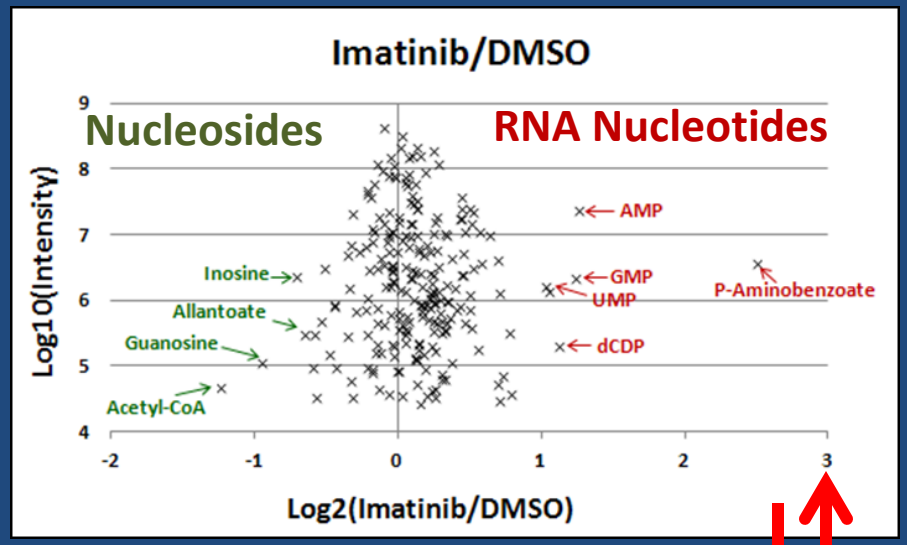
Vehicle
PI3K Inhibitor

Glycolysis and TCA Cycle Are Affected by PI3K inhibition

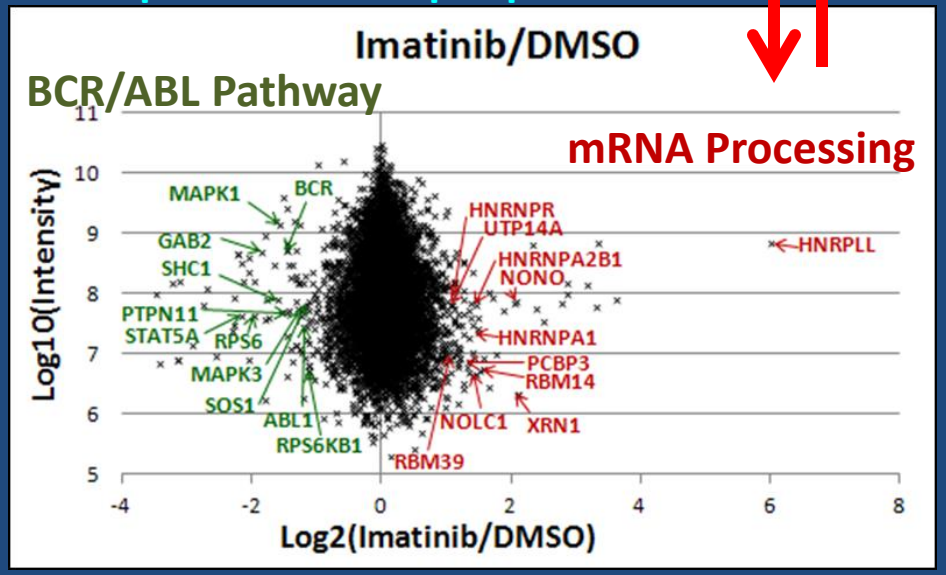
Costas Lyssiotis, Gerburg Wulf

H929 Cells Respond to Kinase Inhibition through Nucleotide Accumulation – Metabolome and Phosphoproteome Talking…..

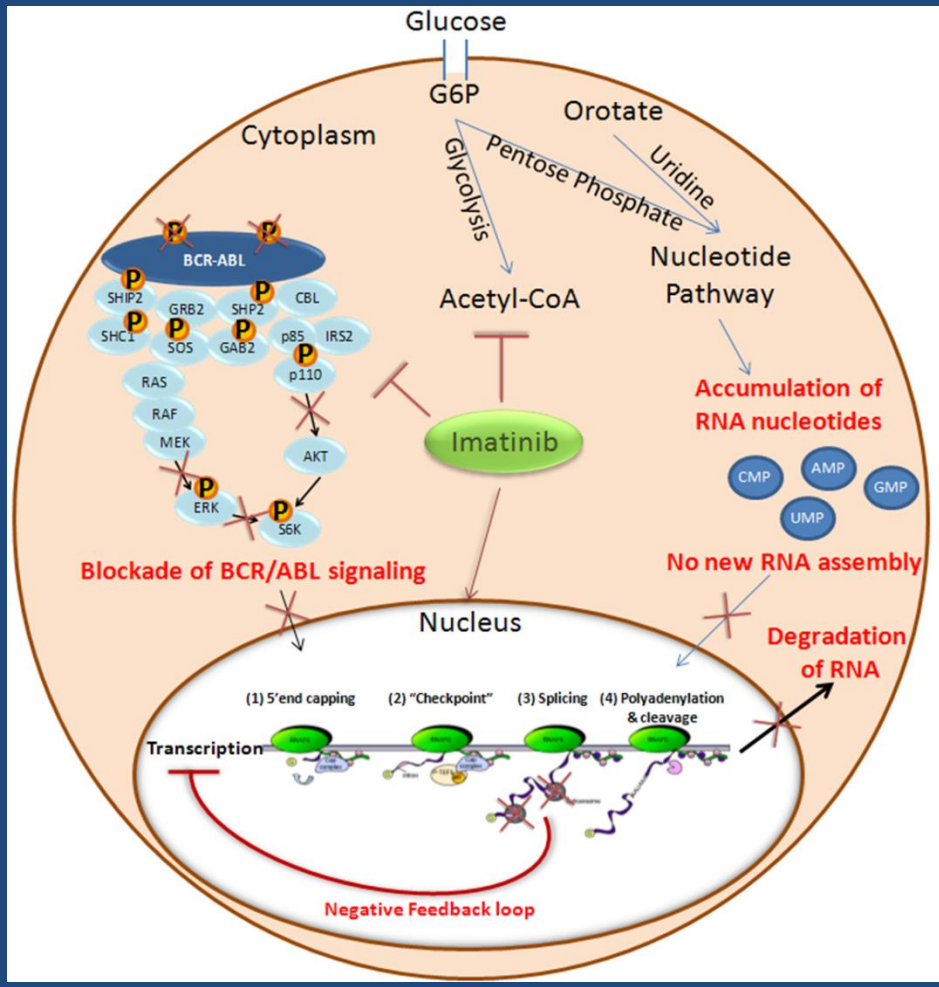
Polar Metabolomics



Triple SILAC Phosphoproteomics



Biological Model for H929



ThP17 Poster #296, Susanne Breitkopf

Summary

- Targeted MS can be used successfully track the fate of ^{13}C or ^{15}N labeled molecules in cells and *in vivo*

- Tracking the activated or altered metabolic pathways in cancers is important for recognizing cancer cell types that have a growth survival benefit

- Understanding these is important for determining how we can intervene to block these pathways altered metabolic pathways with "smart drugs"

- Cross-talk between proteomic and metabolic pathways will be important for understanding cancer cell progression

Acknowledgements

Beth Israel Deaconess Medical Center / Harvard Medical School

Costas Lyssiotis – ^{13}C labeling/SRM methods
Susanne Breitkopf – ^{13}C labeling/mass spec analysis
Min Yuan – mass spec analysis
Gary Bellinger –*in vivo* mouse experiments
Issam Ben-Sahra – ^{13}C and ^{15}N labeling/SRM methods
Ashish Juvekar – *in vivo* mouse experiments
Gerburg Wulf
Lewis Cantley
Brendan Manning

Funding

National Institutes of Health
Dana-Farber Harvard Cancer Center
BIDMC