



University of California
San Francisco

Interrogating cancer metabolism using hyperpolarized ^{13}C -MRS

BioE 241

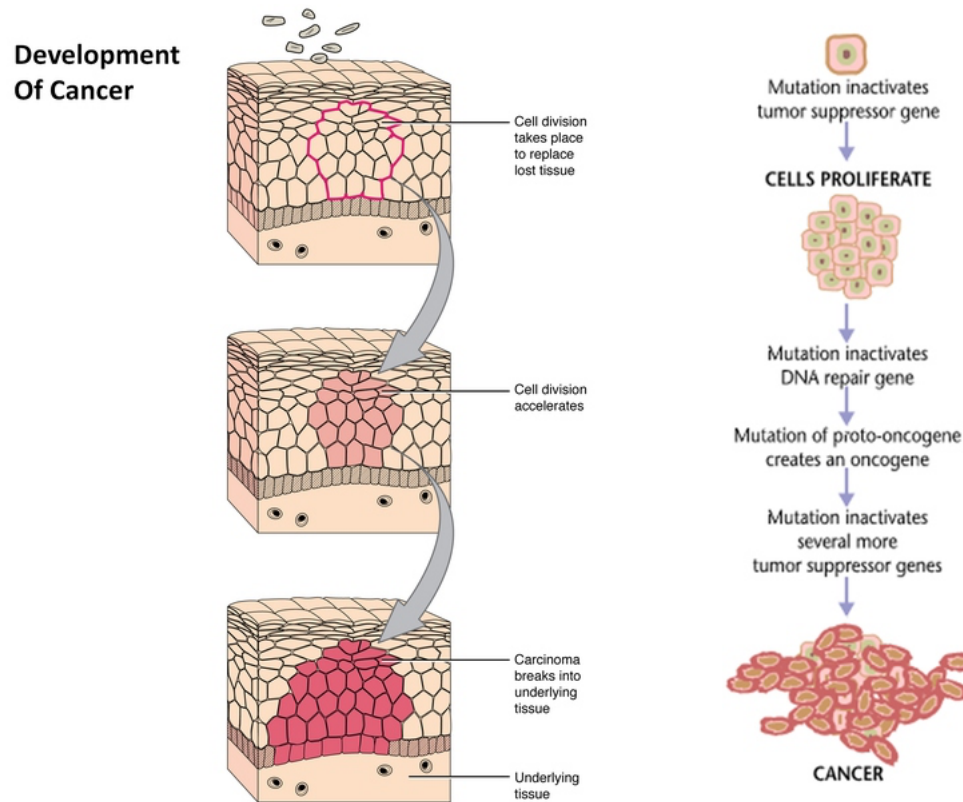
Pavithra Viswanath
Ronen Lab

3/4/19

Learning objectives

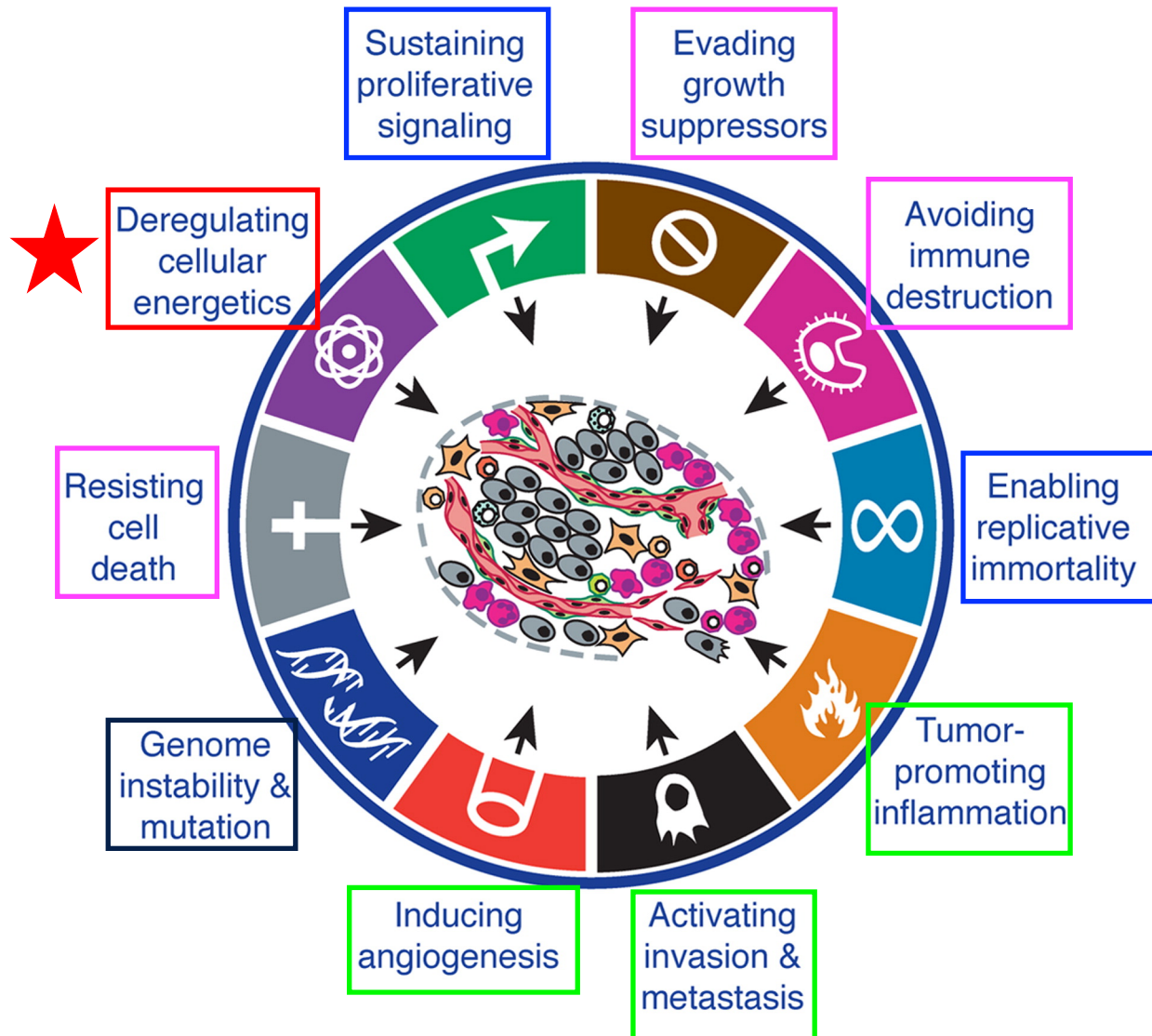
- Understand the importance various facets of metabolic reprogramming in cancer
- Learn about the various hyperpolarized ^{13}C -MRS probes and their use to monitor tumor metabolism in cell and animal models of cancer

Cancer is a complex, multi-step process

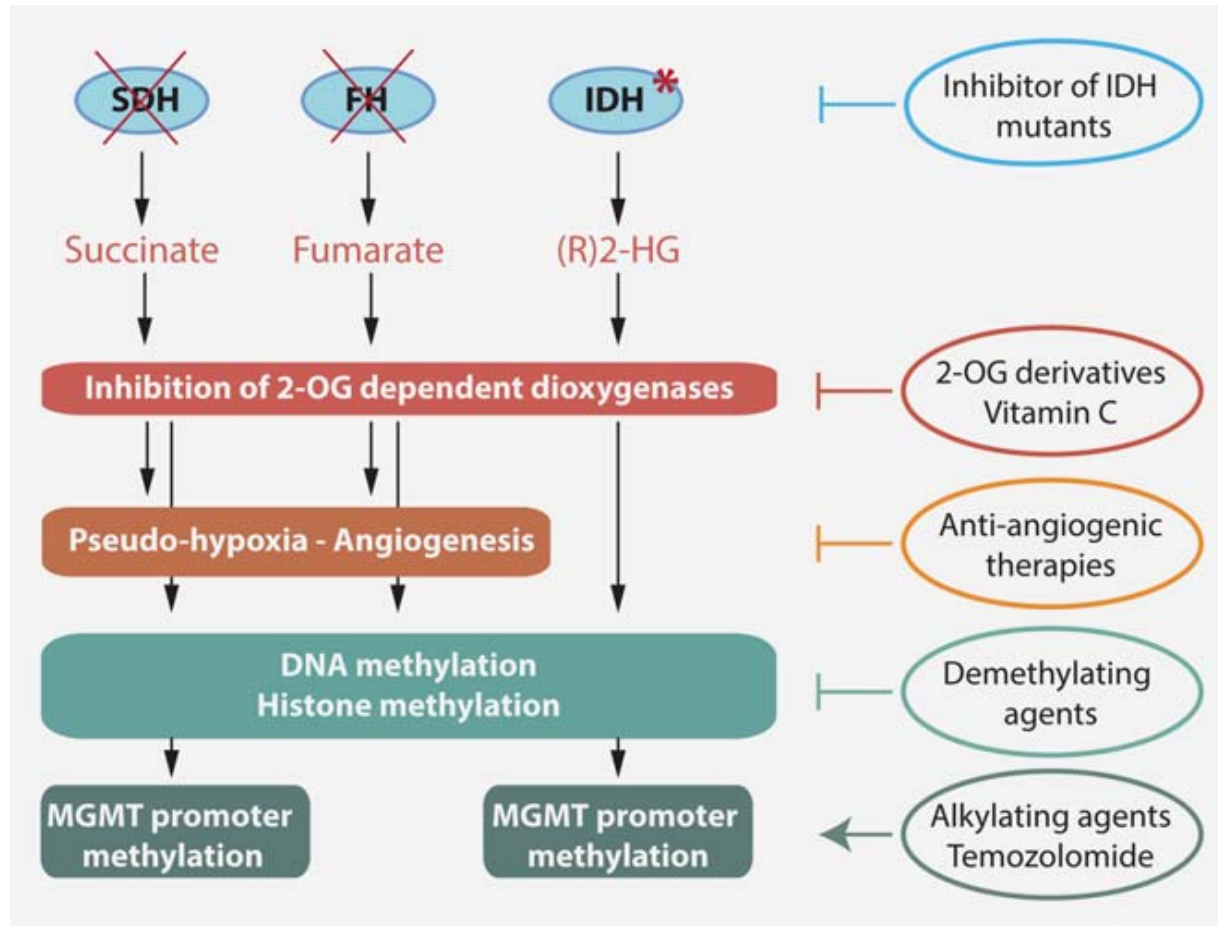


Tumorigenesis is a multistep process involving genetic alterations that drive the progressive transformation of normal human cells into highly malignant cells

Hallmarks of cancer



IDH, FH and SDH mutations- paradigm shift in the role of metabolism in cancer

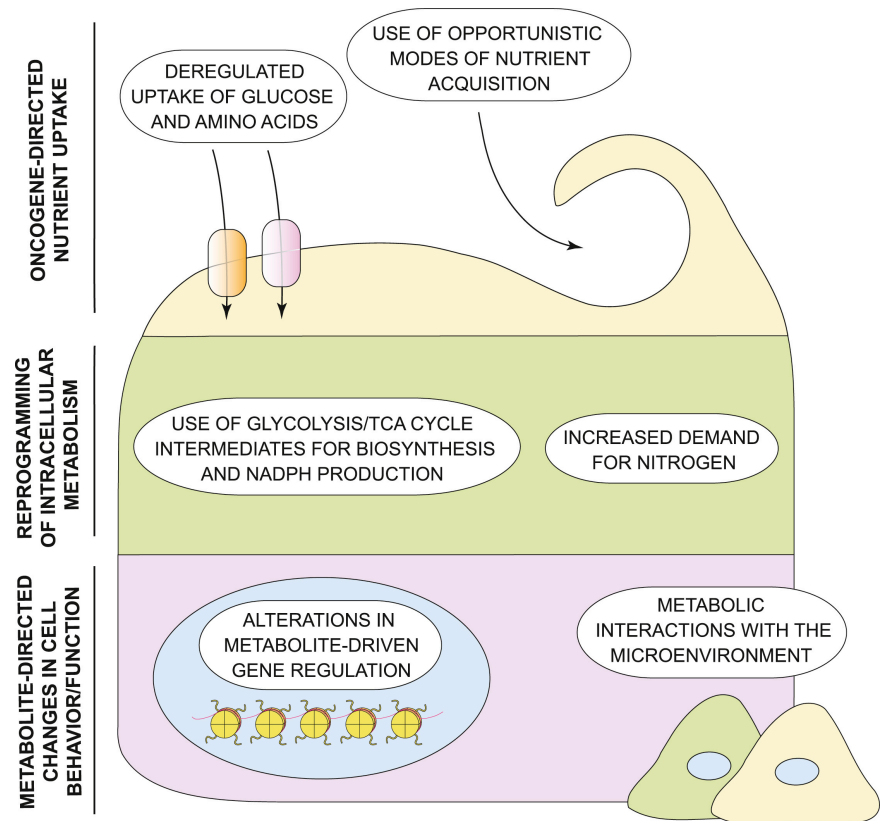


Mutations in metabolic enzyme drives cancer

Metabolic reprogramming is central to tumorigenesis

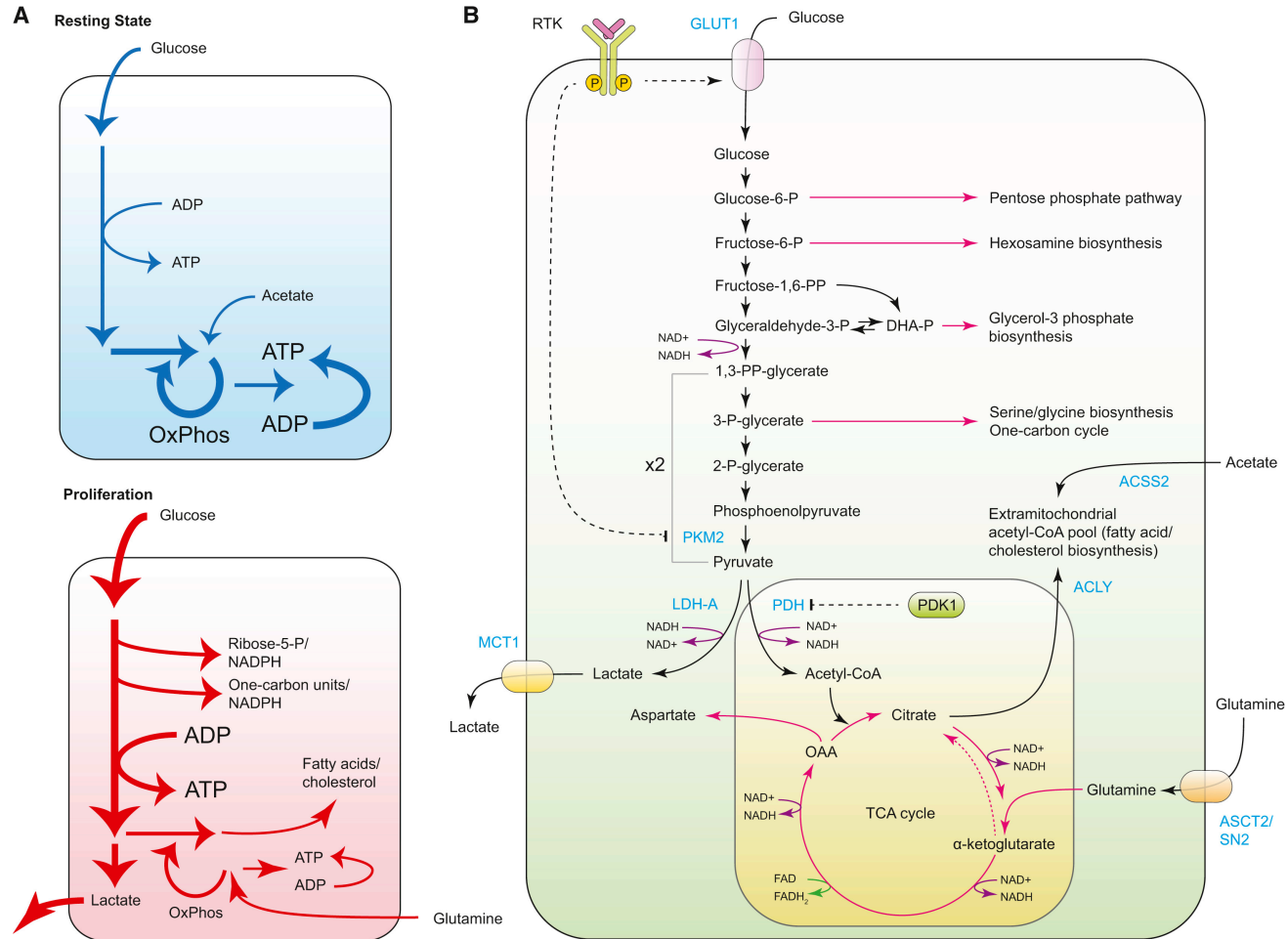
Three layers of metabolic reprogramming:

- gain access to conventional and unconventional nutrient sources
- build new biomass to sustain deregulated proliferation
- exploit the ability of select metabolites to affect cell fate of both tumor cells as well as variety of normal cell types within the tumor microenvironment.



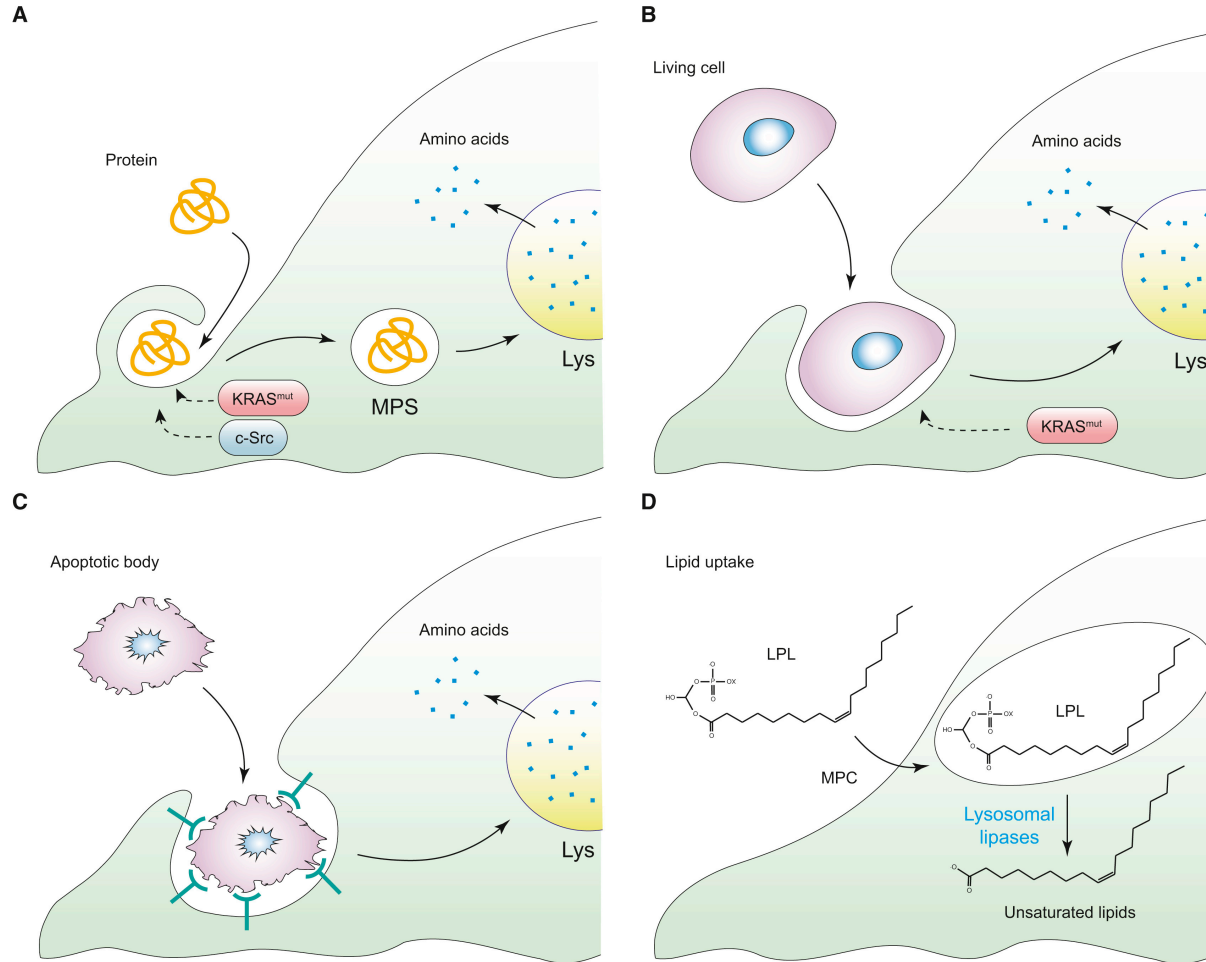
Pavlova et al., Cell Metabolism, 23, 27-47, 2016

Deregulated nutrient uptake is a common metabolic hallmark



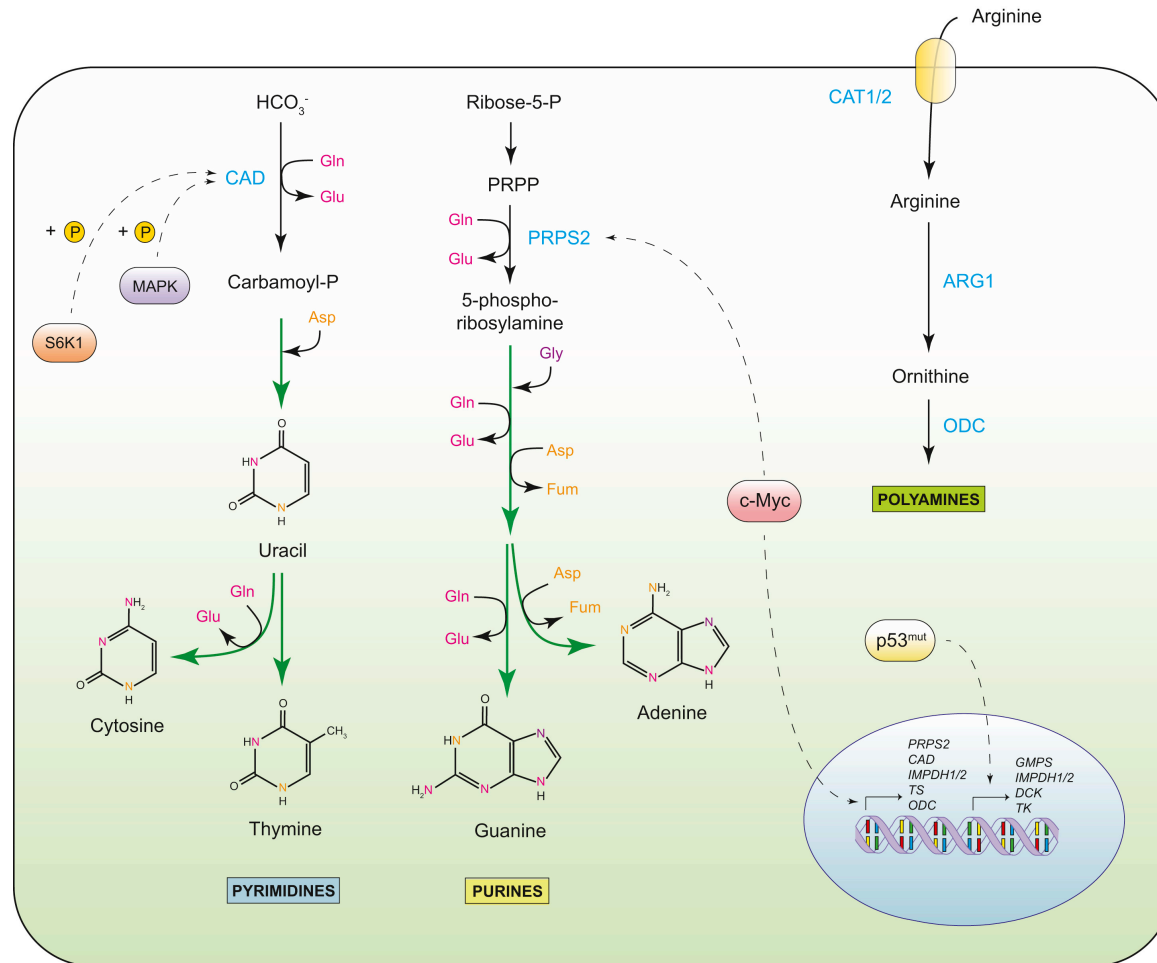
Glucose and glutamine are the two principal nutrients- they are the source of carbon intermediates and reducing equivalents for biosynthesis

Cancer cells use opportunistic modes of nutrition



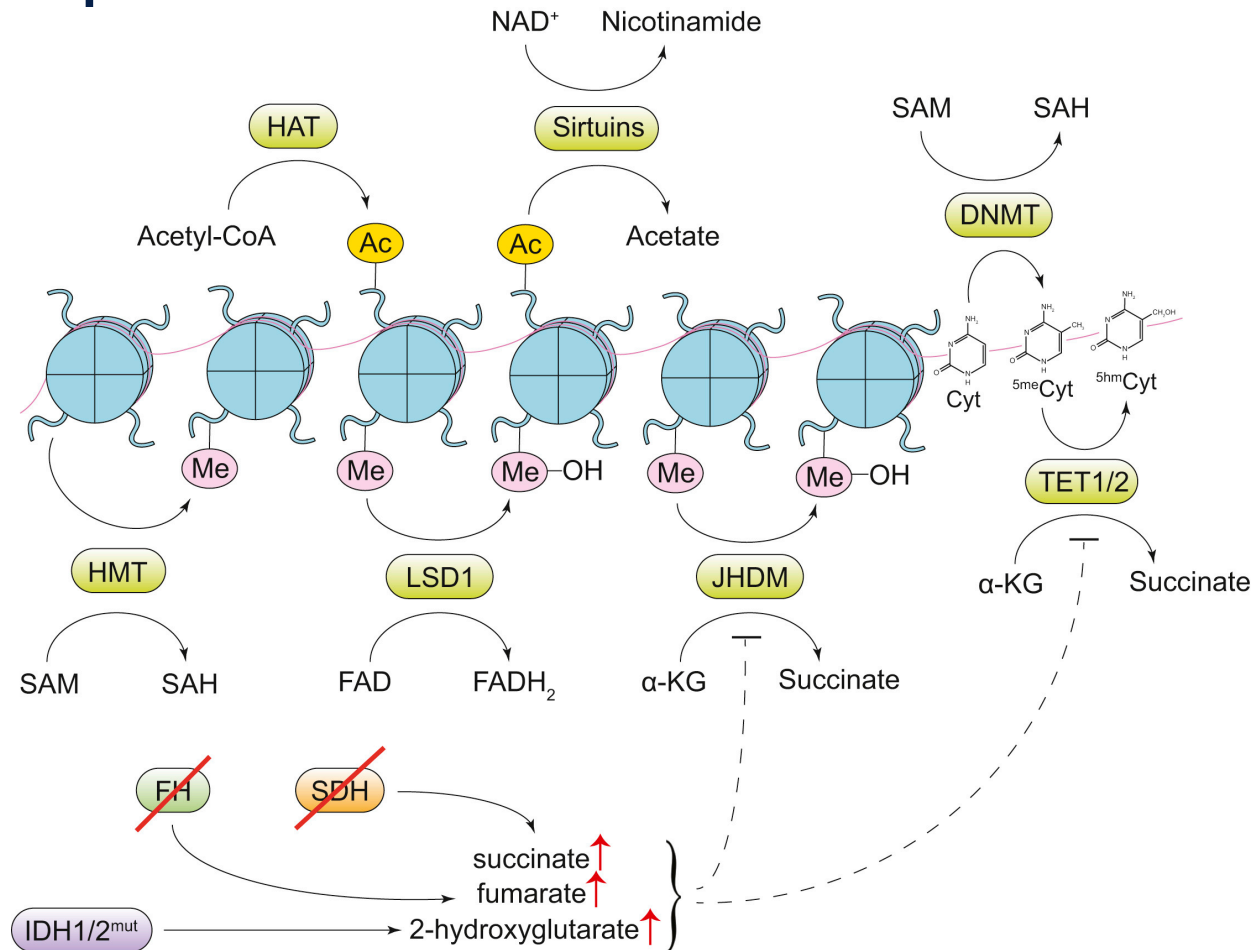
Macropinocytosis, entosis, phagocytosis of apoptotic bodies and extracellular lipid uptake are all means of taking in extra nutrients

Cancer cells have an increased demand for nitrogen



Glutamine is the principal nitrogen donor in cancer cells

Metabolite levels directly control tumor gene expression



Metabolic networks are not passive recipients of growth signals. They directly alter tumor genetic and epigenetic status

Warburg effect- the most famous hallmark

THE METABOLISM OF TUMORS IN THE BODY.

BY OTTO WARBURG, FRANZ WIND, AND ERWIN NEGELEIN.

(From the Kaiser Wilhelm Institut für Biologie, Berlin-Dahlem, Germany.)

(Received for publication, April 29, 1926.)

In this contribution we discuss the question of whether tumor cells in living animals can be killed off through lack of energy, and the related question of how the tumors are supplied with oxygen and glucose in the body.

We assume it is understood that tumor cells obtain the energy required for their existence in two ways: by respiration and by fermentation. In respiration they burn organic materials to carbon dioxide and water; in fermentation they split glucose to lactic acid. All tumors so far tested behave fundamentally alike. There is no essential difference between the cancer cells of transplanted rat tumors and spontaneous tumors, sarcoma and carcinoma cells, and the tar carcinoma, and Rous sarcoma produced by filtrate.

The fermentation of tumors was first found with cut pieces of tumor *in vitro*.¹ C. and G. Cori² demonstrated it in living animals as well. They determined the glucose and lactic acid in the axillary veins of hens having in one wing a Rous sarcoma, and found in 100 cc. of blood 23 mg. less glucose and 16 mg. more lactic acid on the tumor side than on the normal side. A corresponding experiment with a human fore-arm tumor showed in 100 cc. of blood 12 mg. less glucose and 9 mg. more lactic acid on the tumor side.

In experiments on the nourishment of tumors through the blood stream, we, like Cori, determined the glucose and lactic acid in tumor veins. Our procedure differed from Cori's in that we compared tumor veins and arteries, *not* tumor veins and corresponding normal veins. Our differences were greater than Cori's because we went closer to

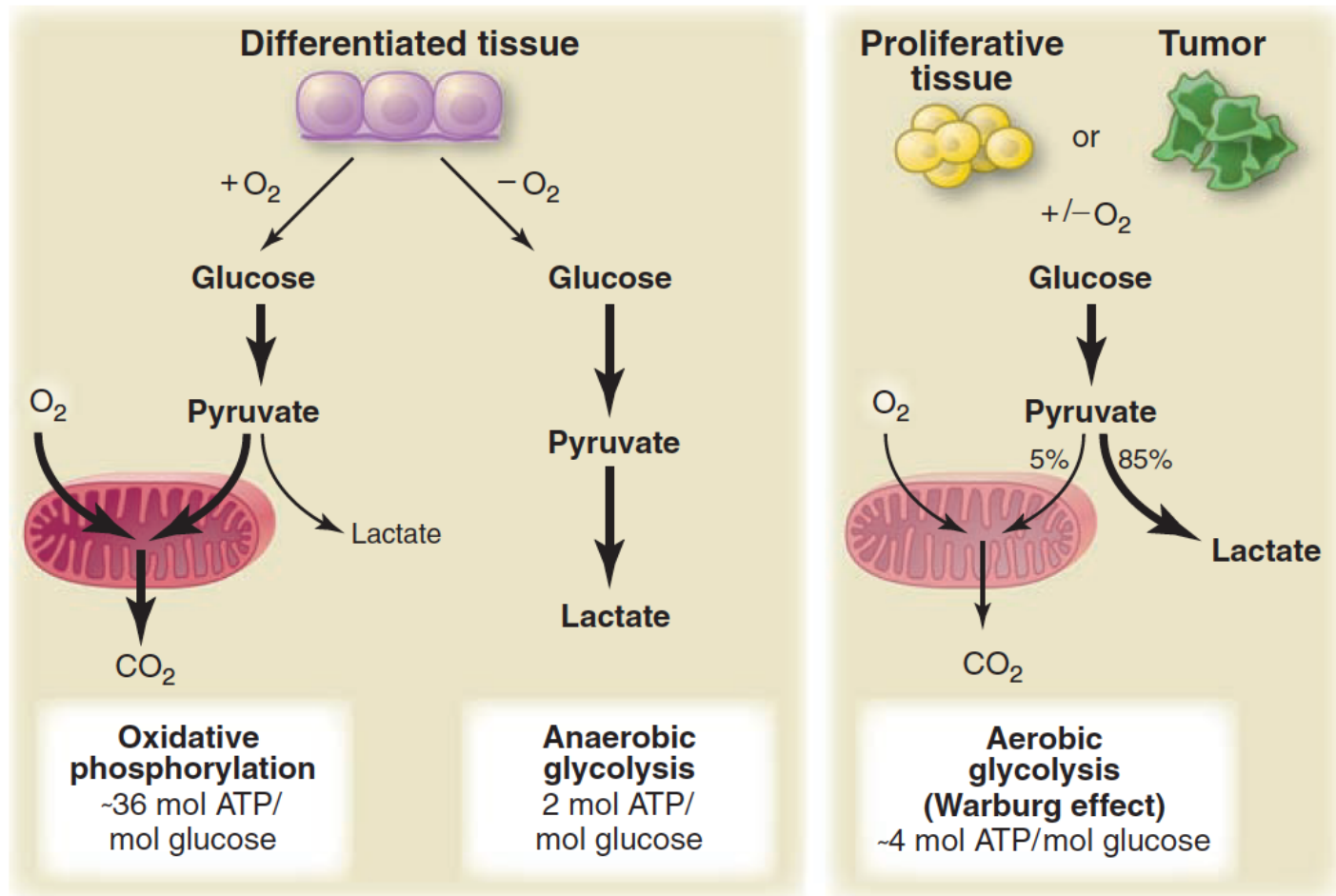
¹ Warburg, O., *Biochem. Z.*, 1923, cxlii, 317. Warburg, O., Posener, K., and Negelein, E., *Biochem. Z.*, 1924, clii, 309. Negelein, E., *Biochem. Z.*, 1925, cxviii, 121; 1925, cx, 307.

² Cori, C. F., and Cori, G. T., *J. Biol. Chem.*, 1925, lxiv, 11; 1925, lxv, 397.



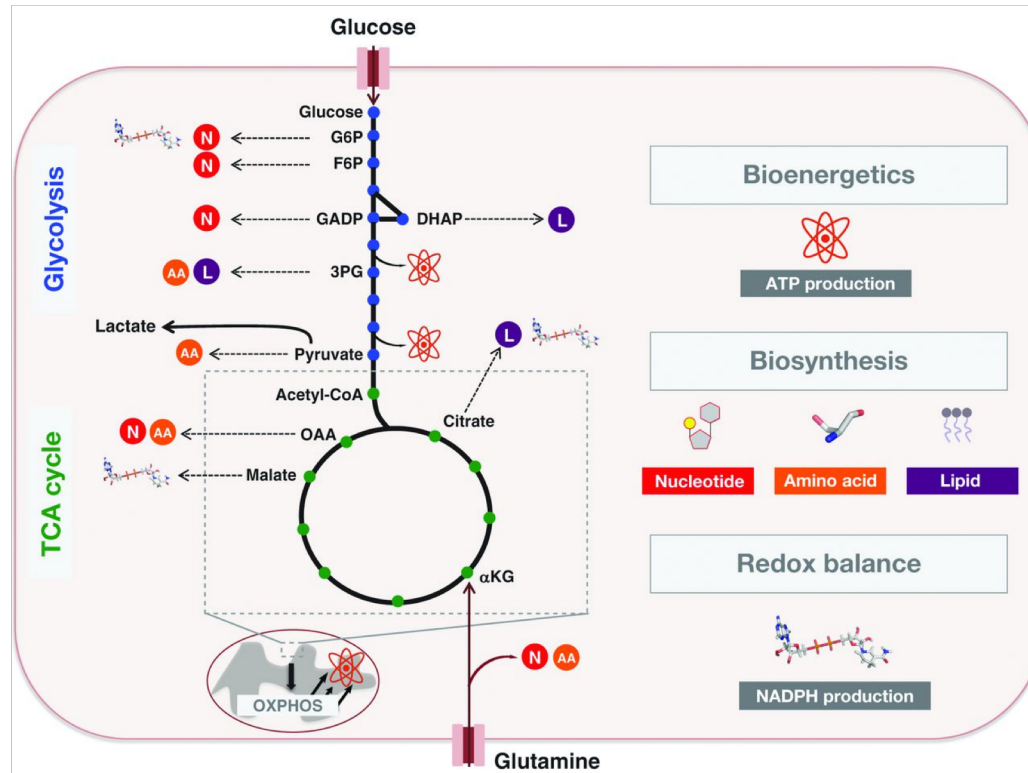
Elevated glucose uptake and conversion to lactate in tumor cells even under conditions of normal oxygen tension

The Paradox of the Warburg effect



Paradox: Tumor cells use glycolysis in spite of lower ATP yield

The Warburg effect facilitates macromolecular biosynthesis

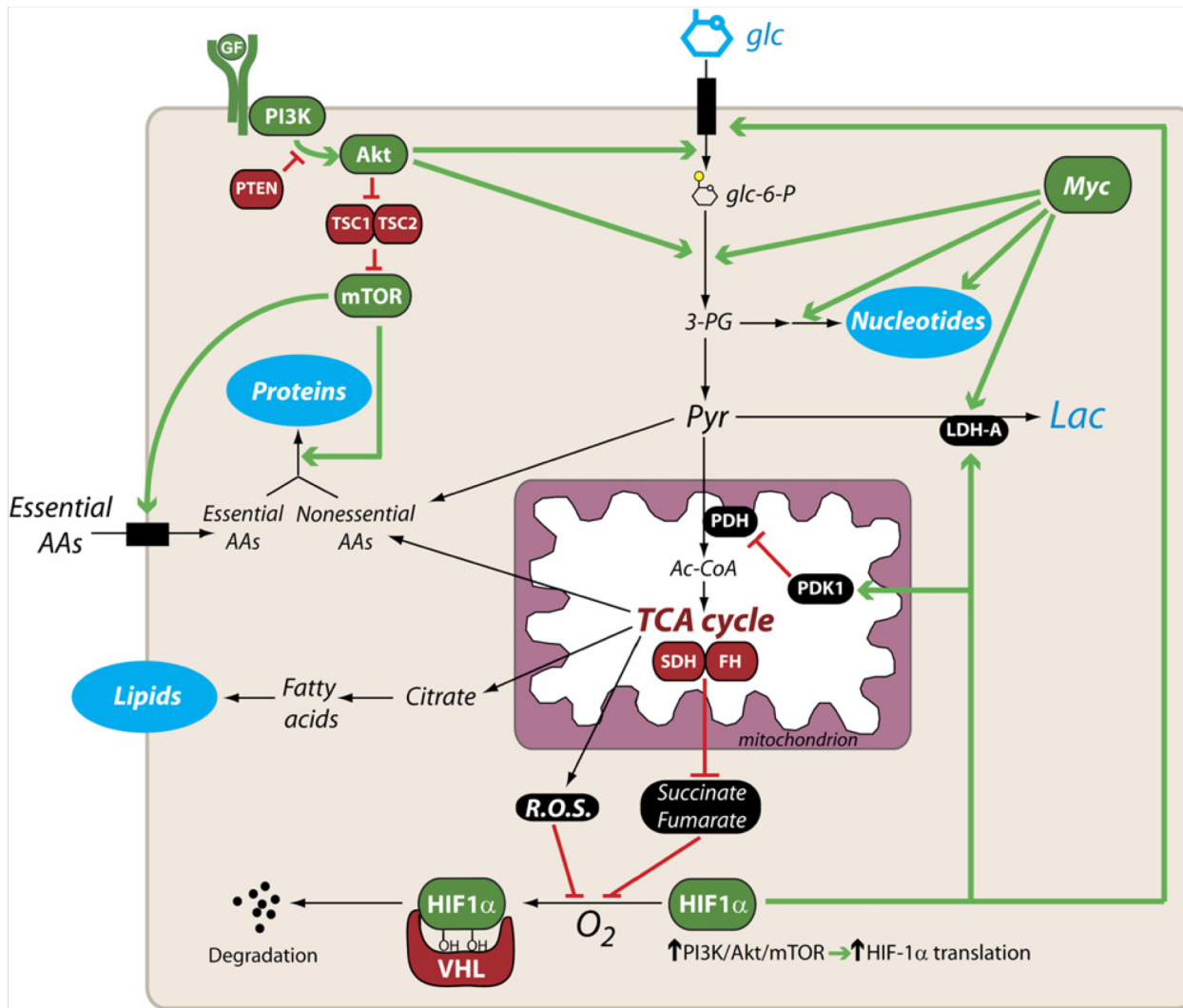


Paradox: Tumor cells use glycolysis in spite of lower ATP yield

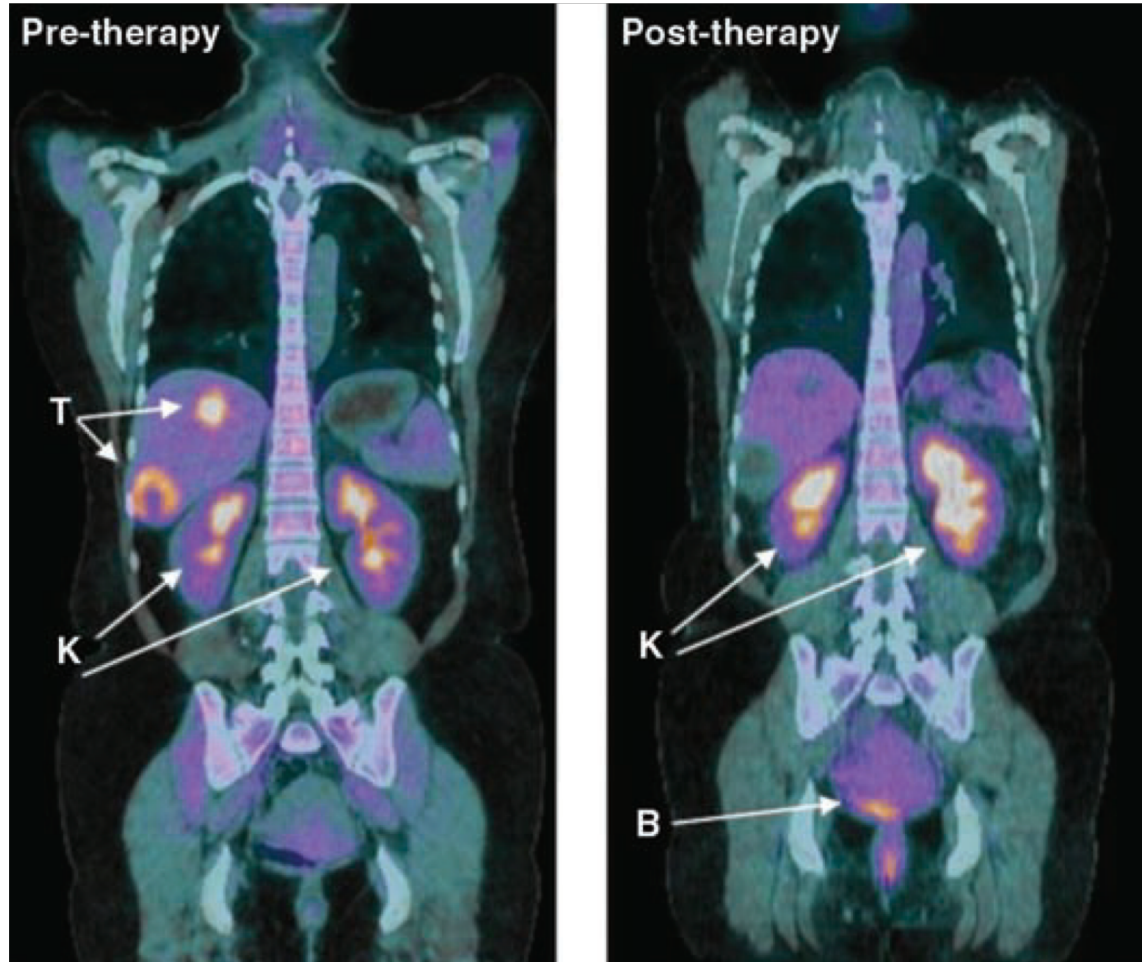
But ATP may not be limiting for cell proliferation

The major function is to provide glycolytic intermediates for biosynthesis of RNA, DNA, proteins, lipids, NADH and NADPH

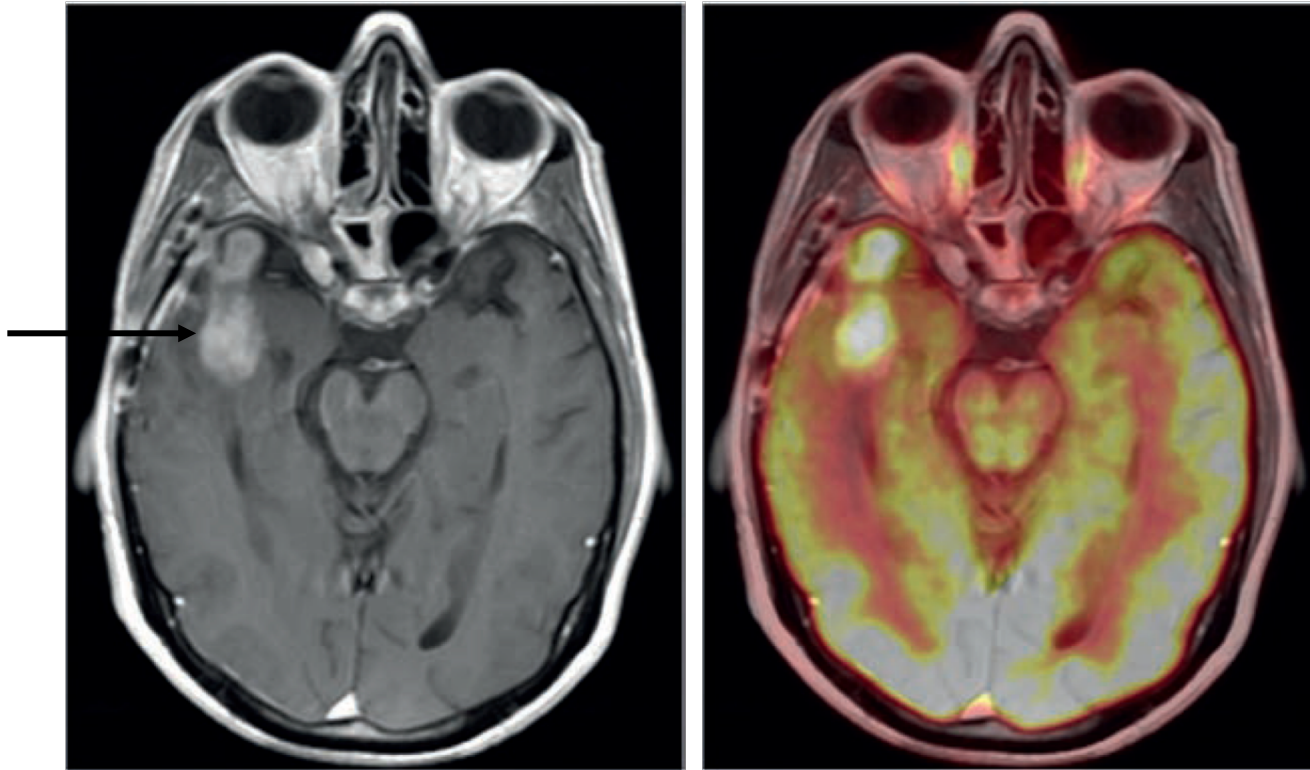
The PI3K/Akt/mTOR pathway is a major facilitator of the Warburg effect



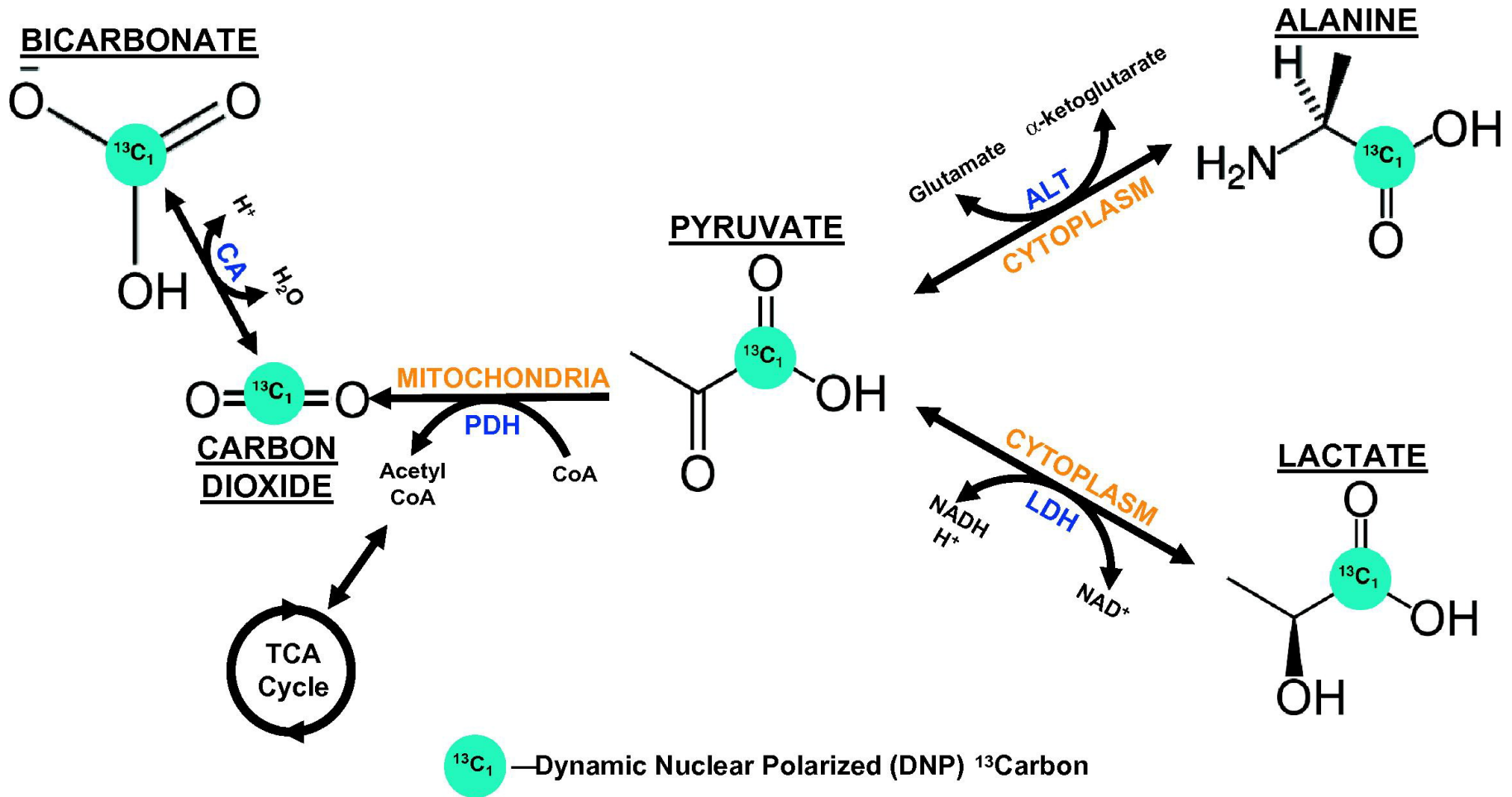
Interrogating the Warburg effect: 2- ^{18}F fluoro-2-deoxy-d-glucose (^{18}F -FDG-PET)



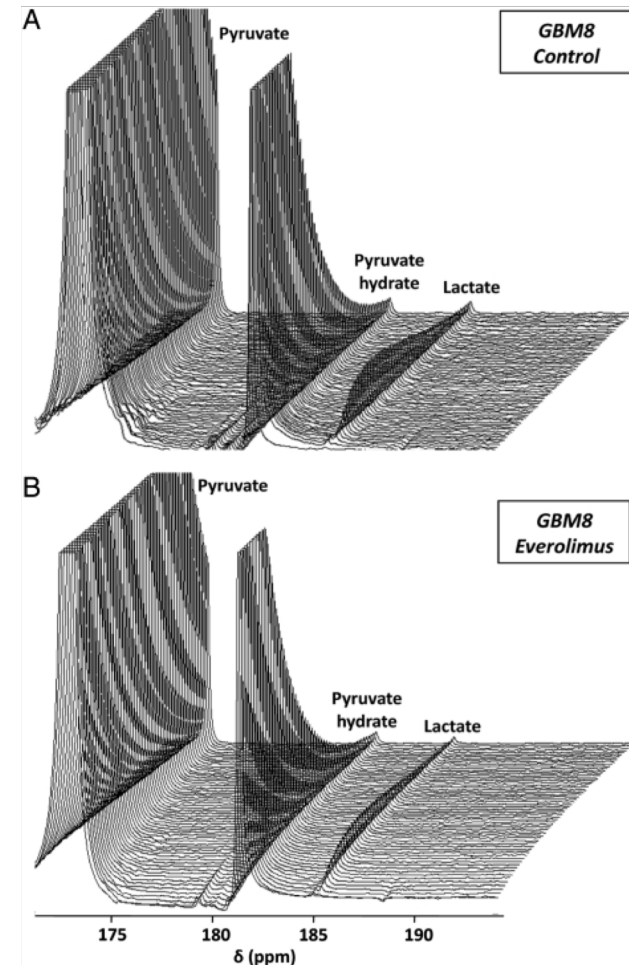
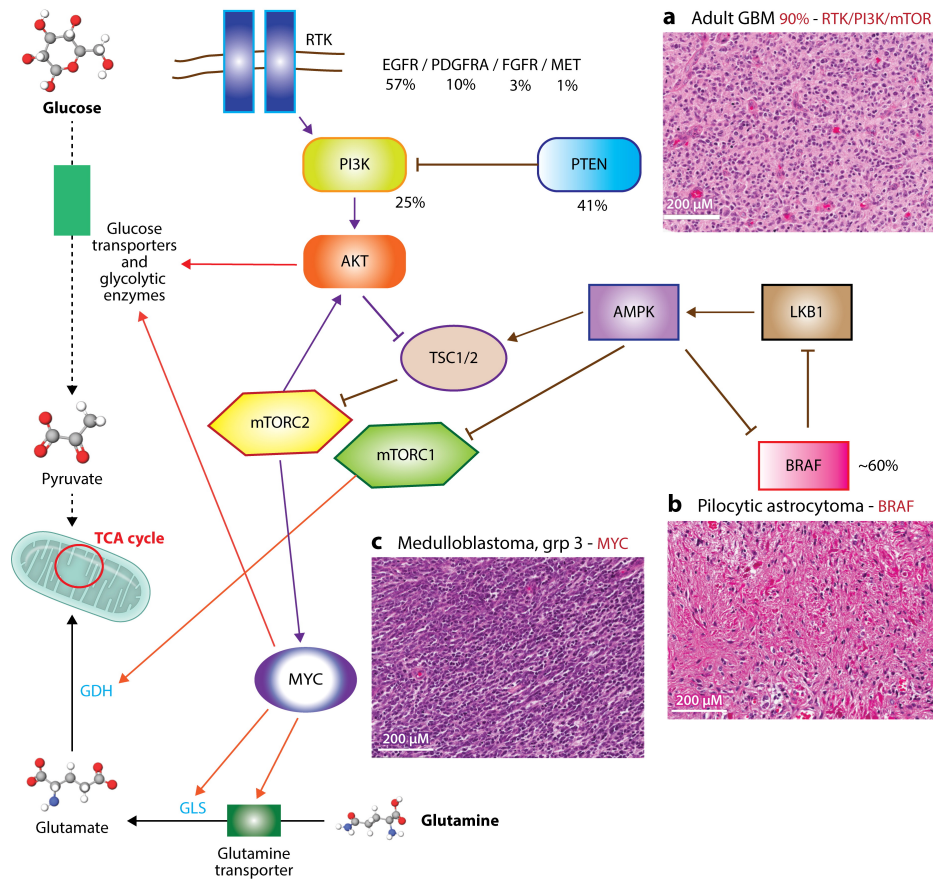
Interrogating the Warburg effect: 2- ^{18}F fluoro-2-deoxy-d-glucose (^{18}F -FDG-PET).



Interrogating the Warburg effect: Hyperpolarized [1-¹³C]-pyruvate



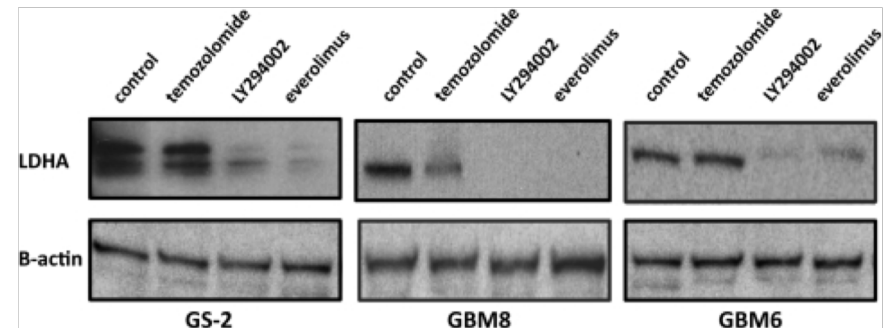
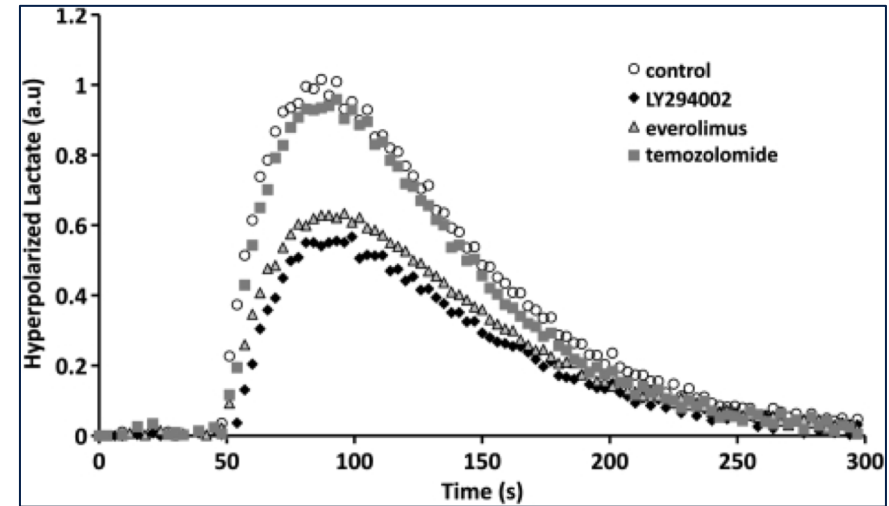
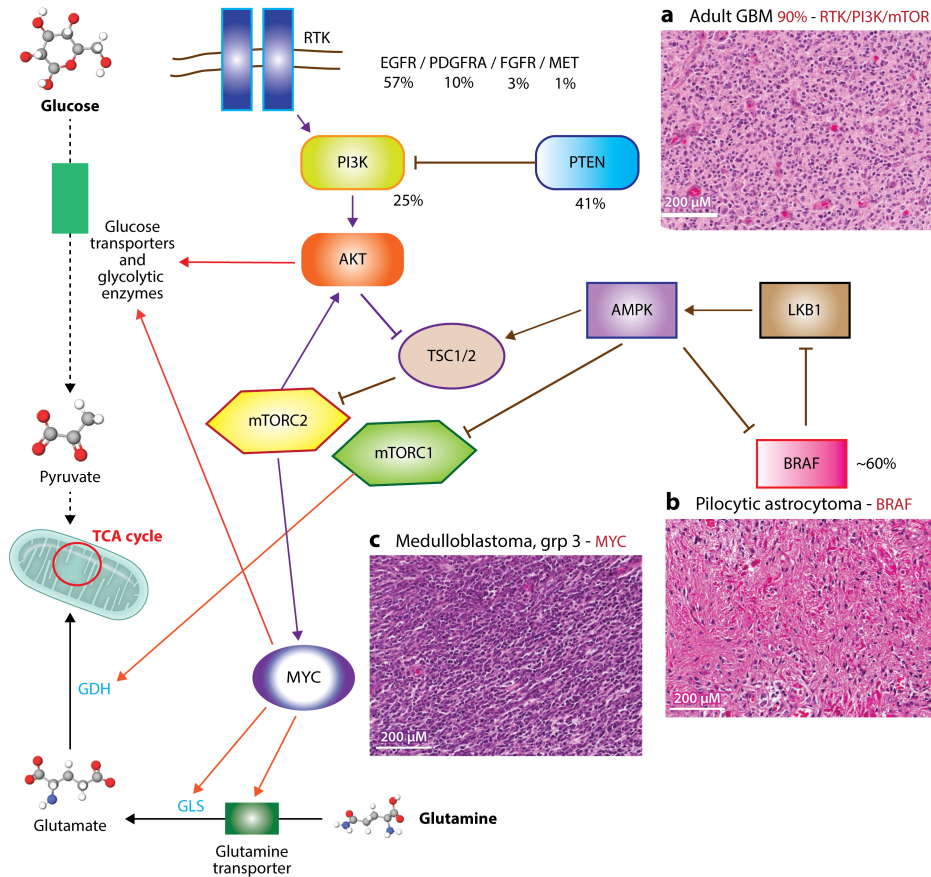
Hyperpolarized [1-¹³C]-pyruvate can monitor response to PI3K inhibition in GBMs



AR Venneti S, Thompson CB. 2017. Annu. Rev. Pathol. Mech. Dis. 12:515–45

Venkatesh et al. Neuro Oncol, 14(3): 315–325, 2012

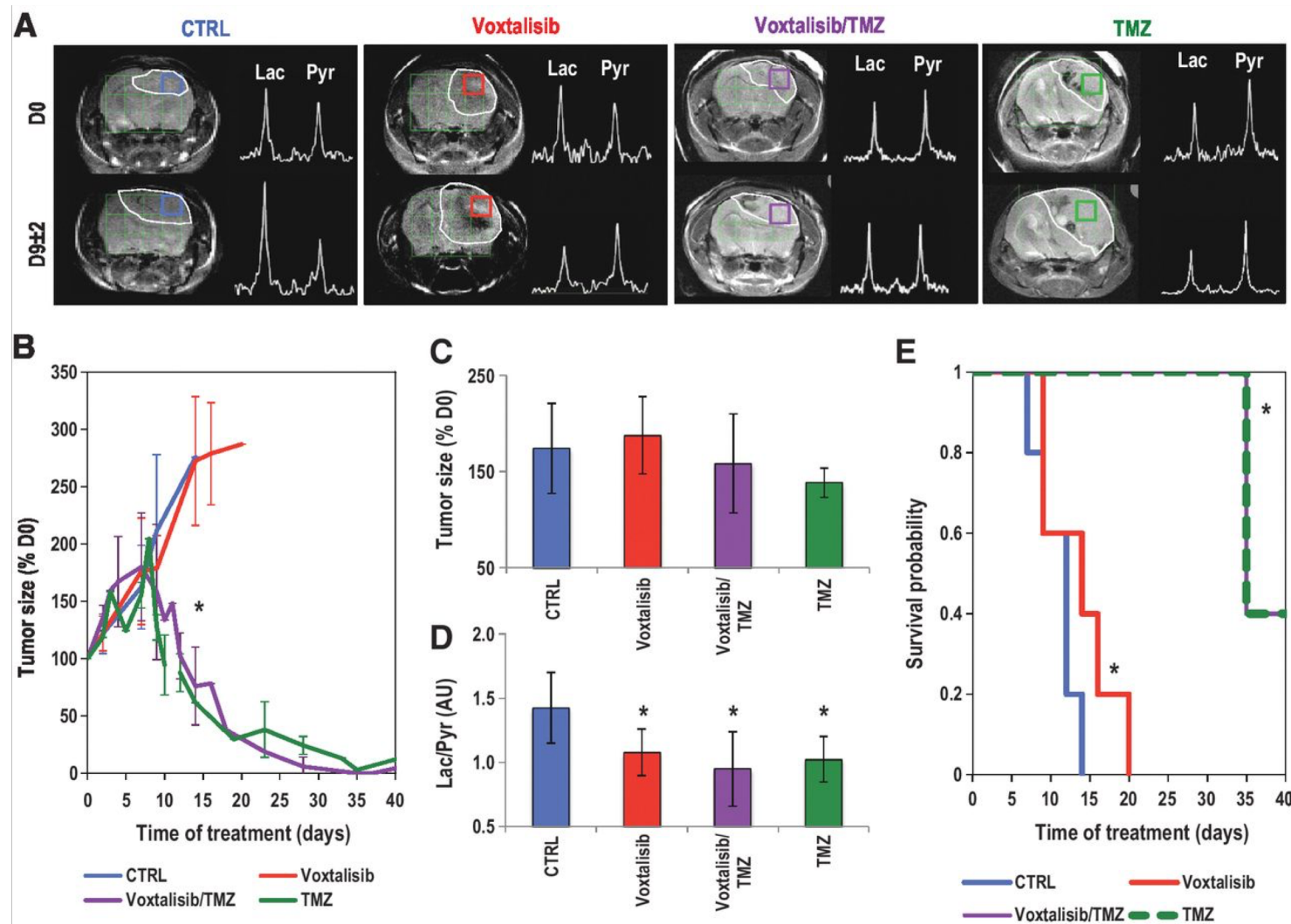
Hyperpolarized [1-¹³C]-pyruvate can monitor response to PI3K inhibition in GBMs



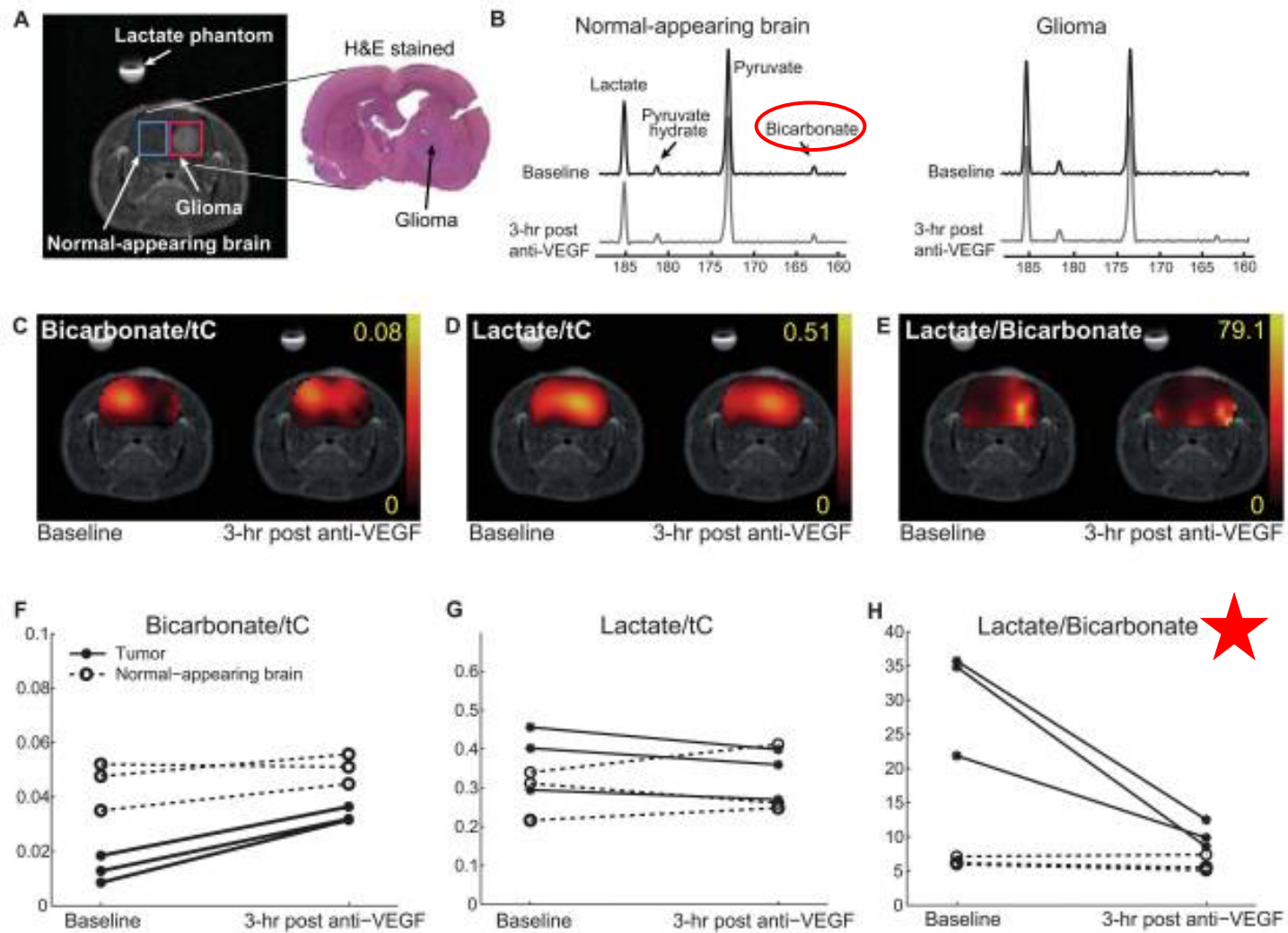
Venneti S, Thompson CB. 2017.
Annu. Rev. Pathol. Mech. Dis. 12:515–45

Reduced hyperpolarized lactate production correlates with reduced LDHA

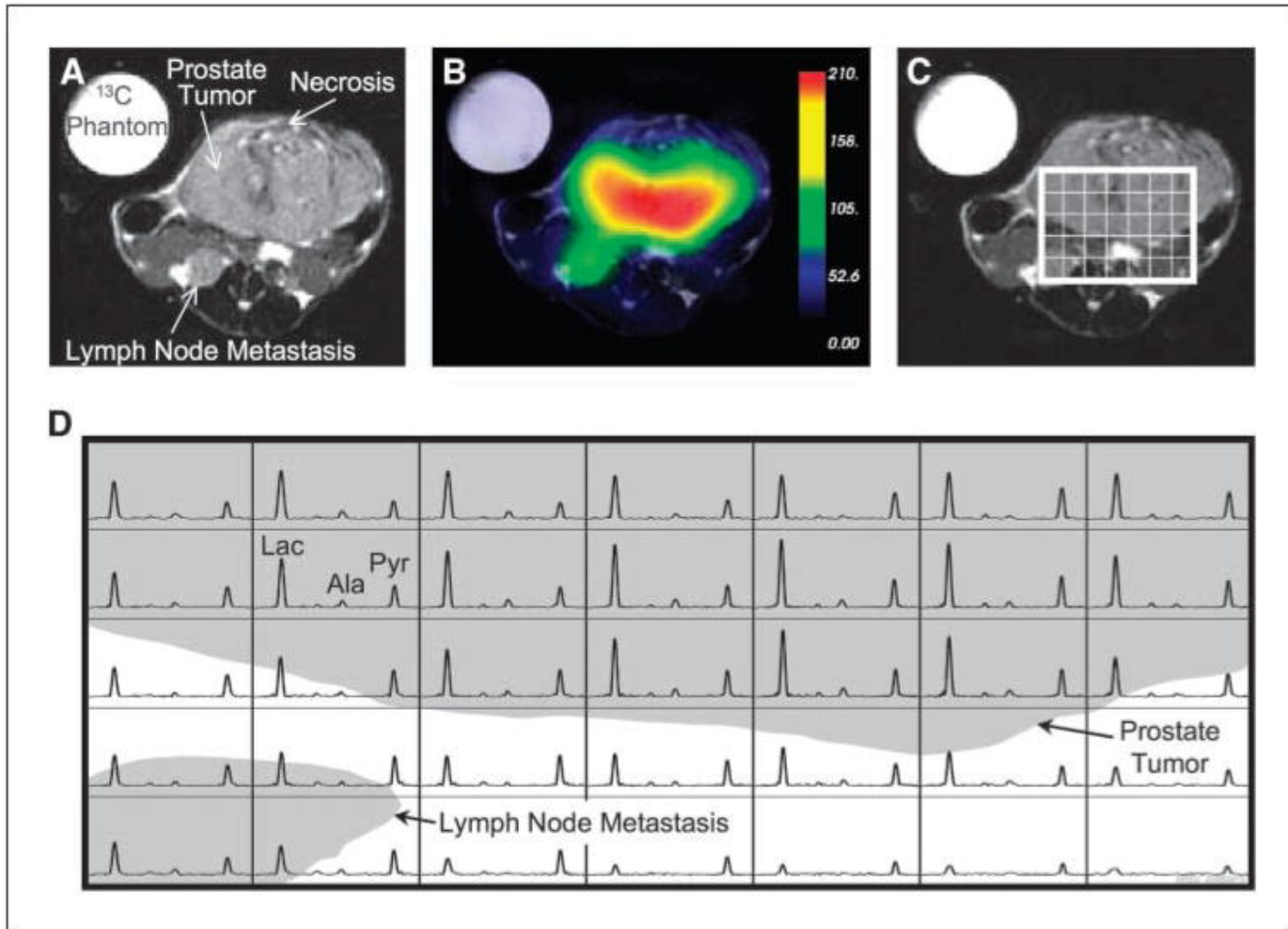
Hyperpolarized [1-¹³C]-pyruvate can monitor early response to PI3K inhibition in GBMs



Hyperpolarized [1-¹³C]-pyruvate can monitor early response to anti-VEGF treatment in GBMs



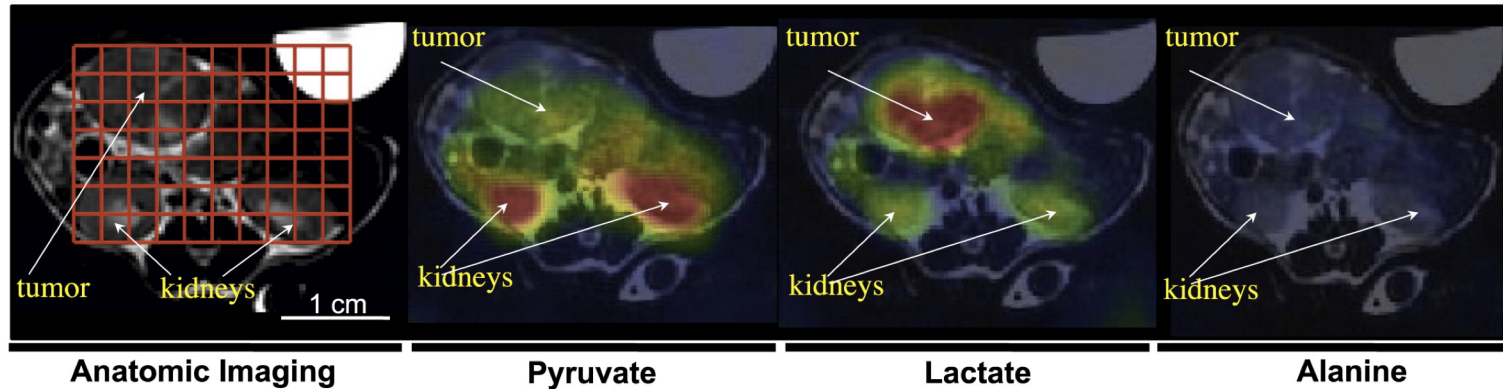
Hyperpolarized [1-¹³C]-pyruvate can tumor burden in prostate cancer



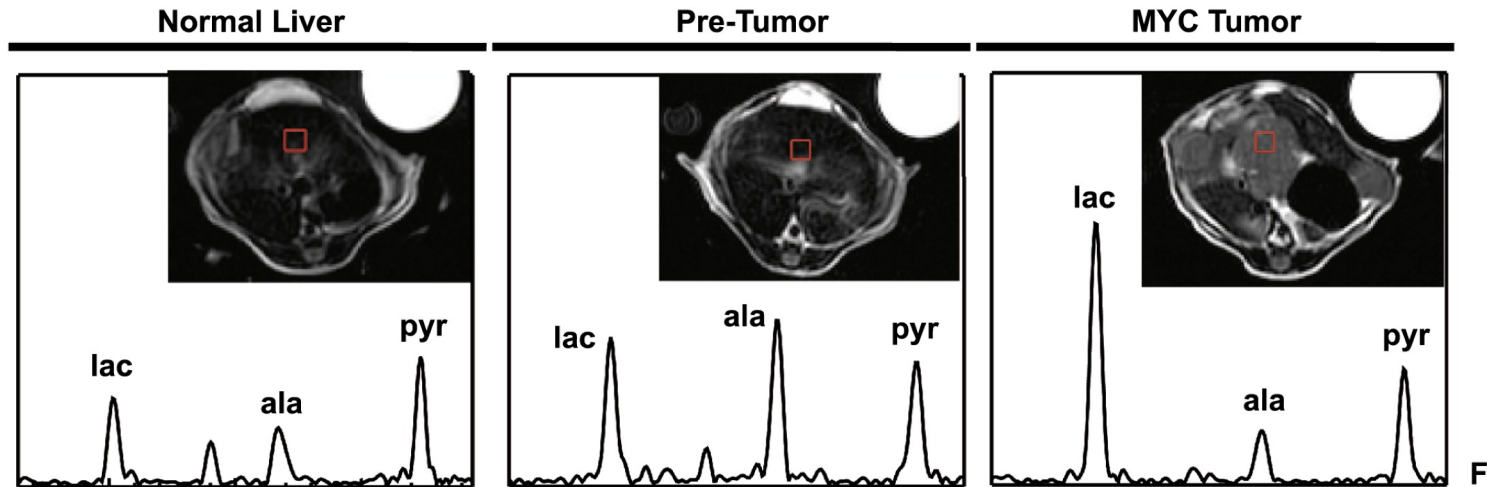
Albers, et al. Cancer Res, 68:8607-8615, 2008

Elevated hyperpolarized lactate production is observed in Myc-driven hepatocellular carcinoma

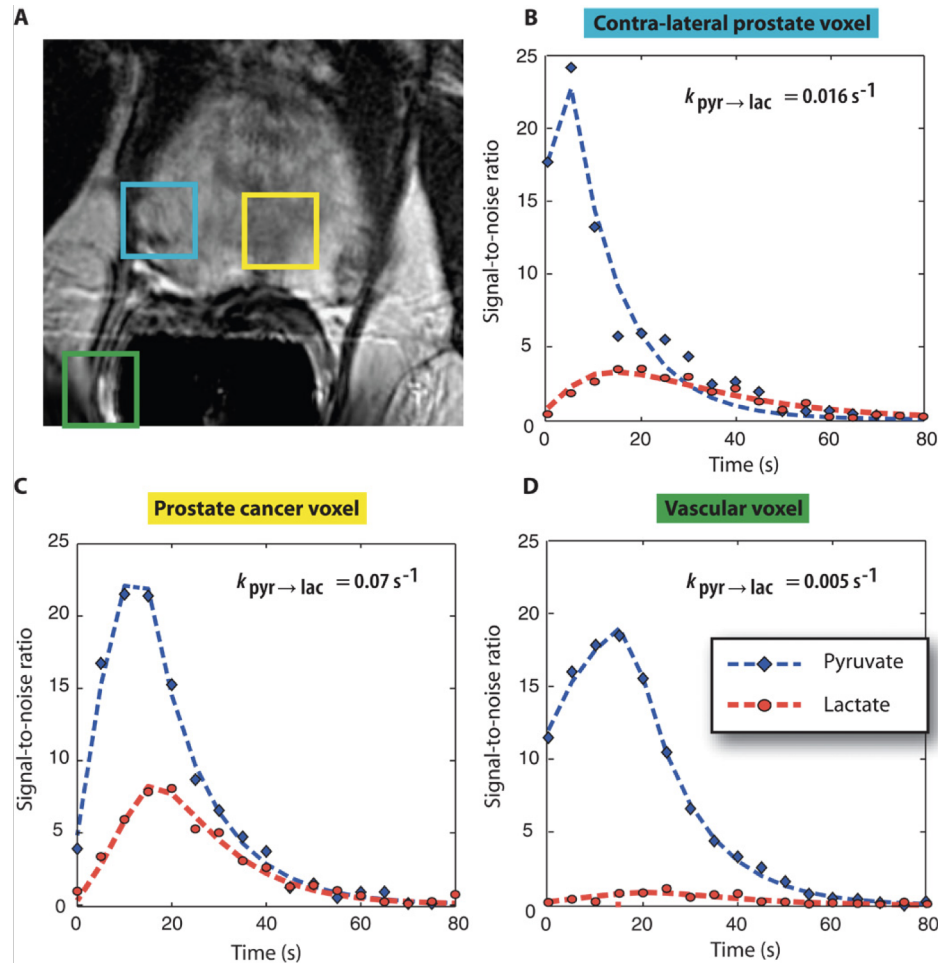
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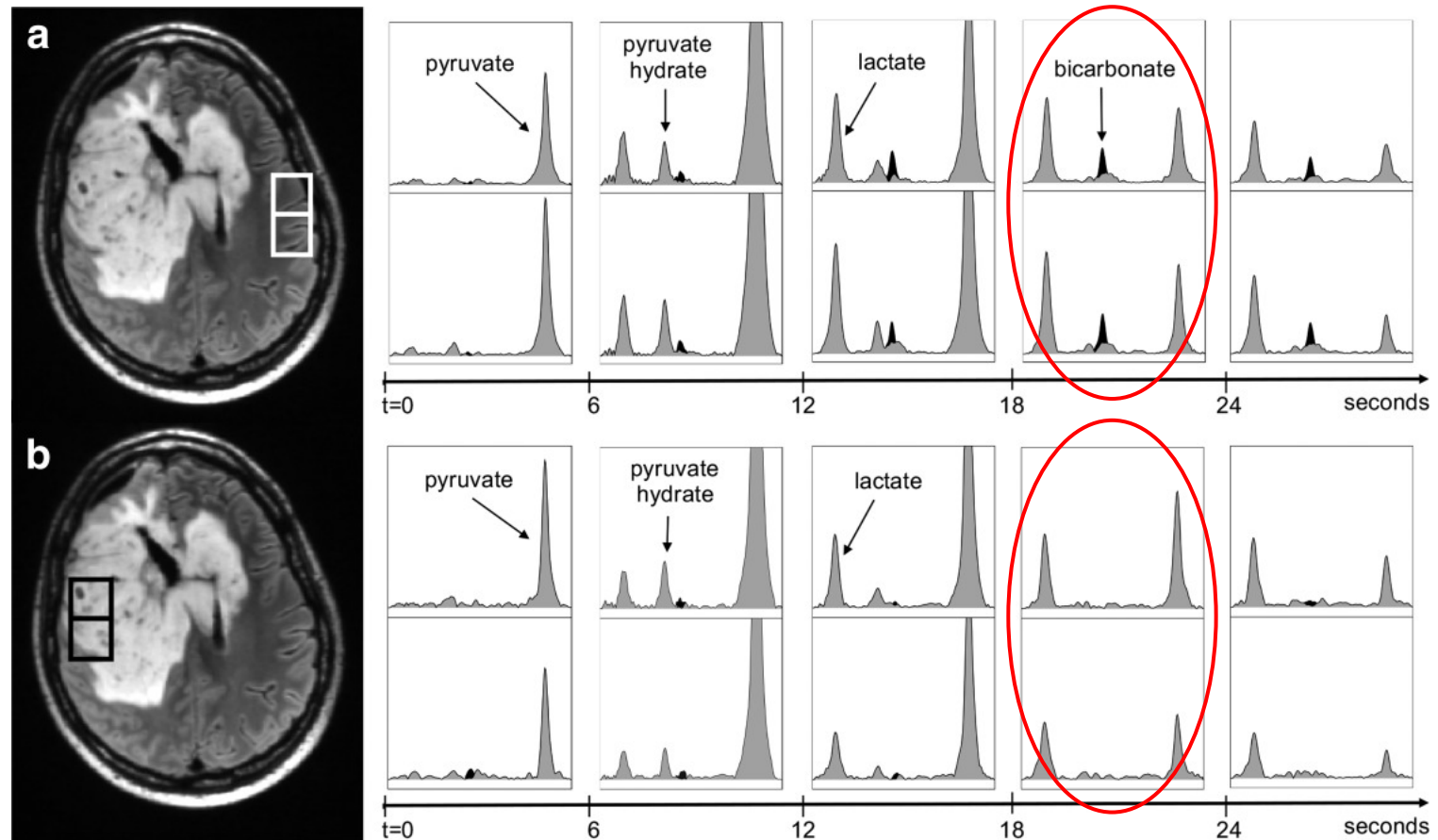


Hyperpolarized [1-¹³C]-pyruvate imaging in prostate cancer patients



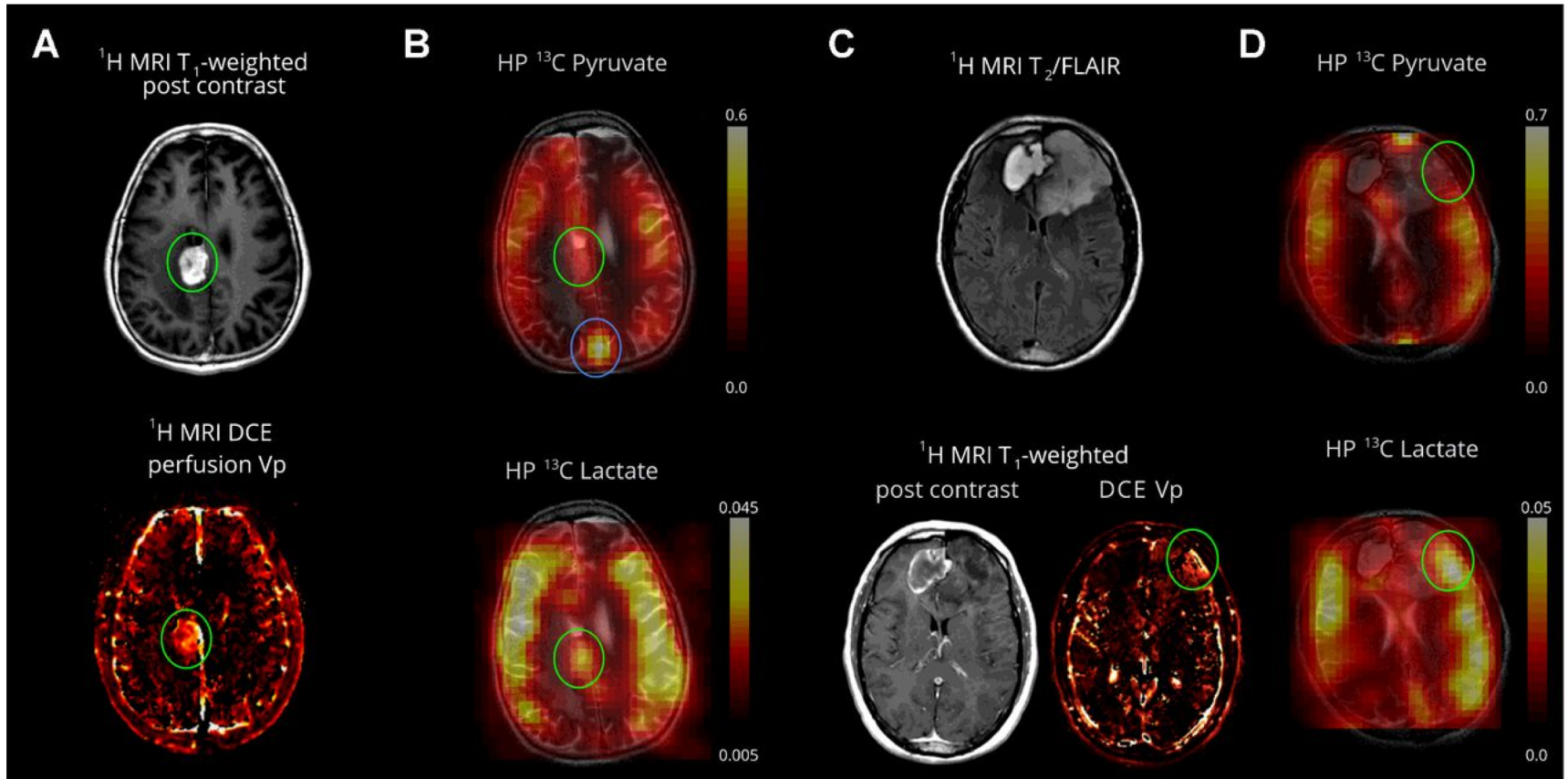
Nelson et al., Sci Transl Med , 5 (198) pp. 198ra108, 2013

Hyperpolarized $[1-^{13}\text{C}]$ -pyruvate imaging in GBM patients



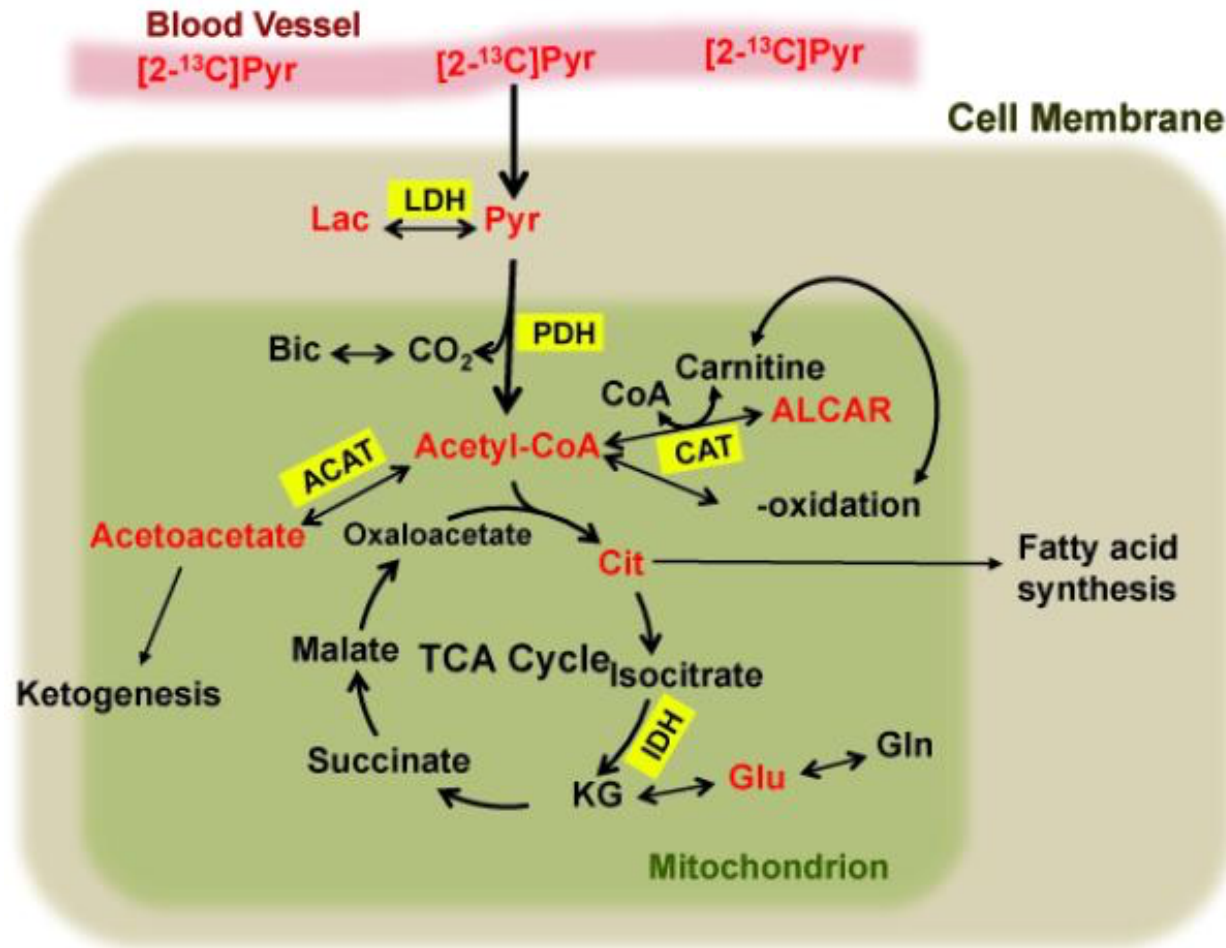
Park et al., Magn Reson Med, 80(3):864-873, 2018

Hyperpolarized [1-¹³C]-pyruvate imaging in GBM patients

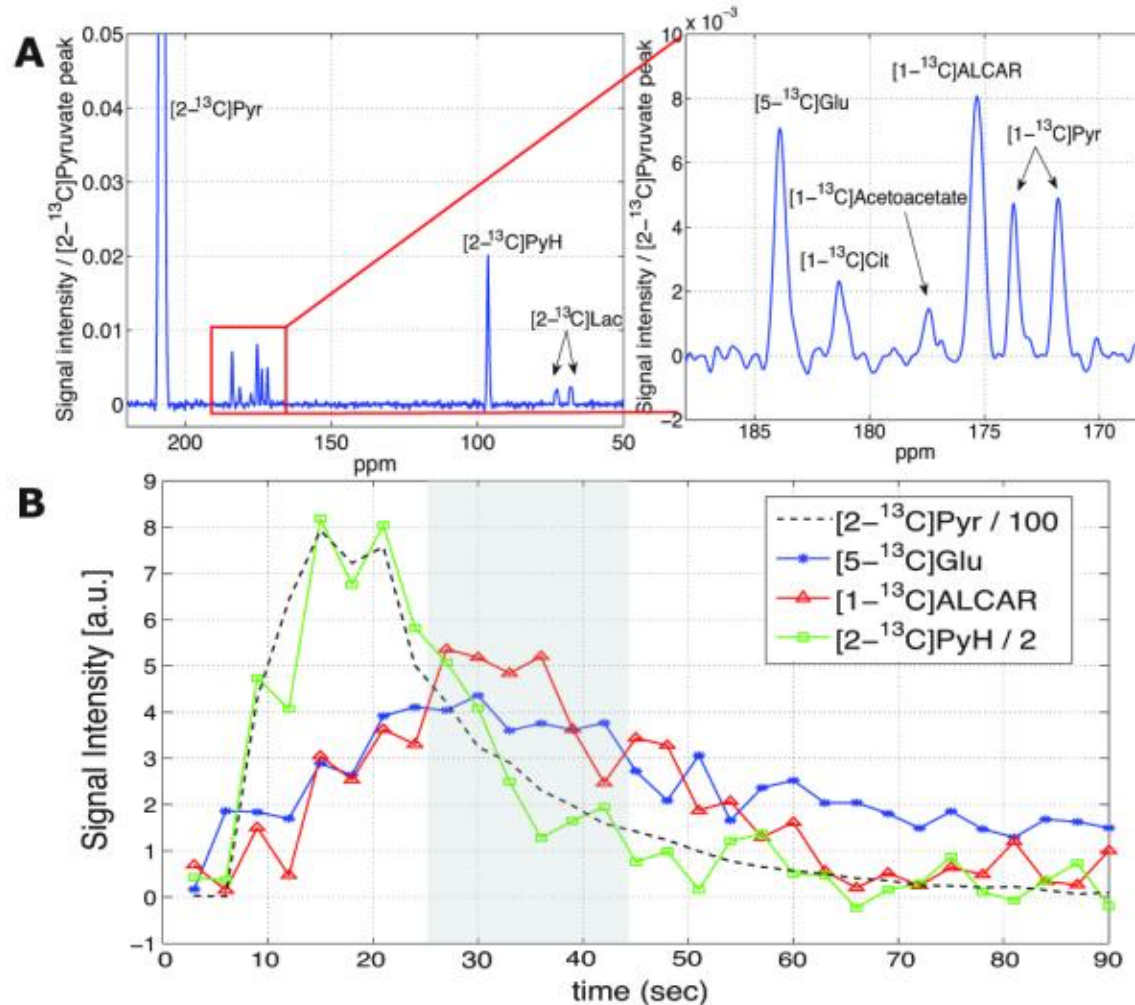


Miloushev et al., Cancer Res; 78(14); 3755-60, 2018

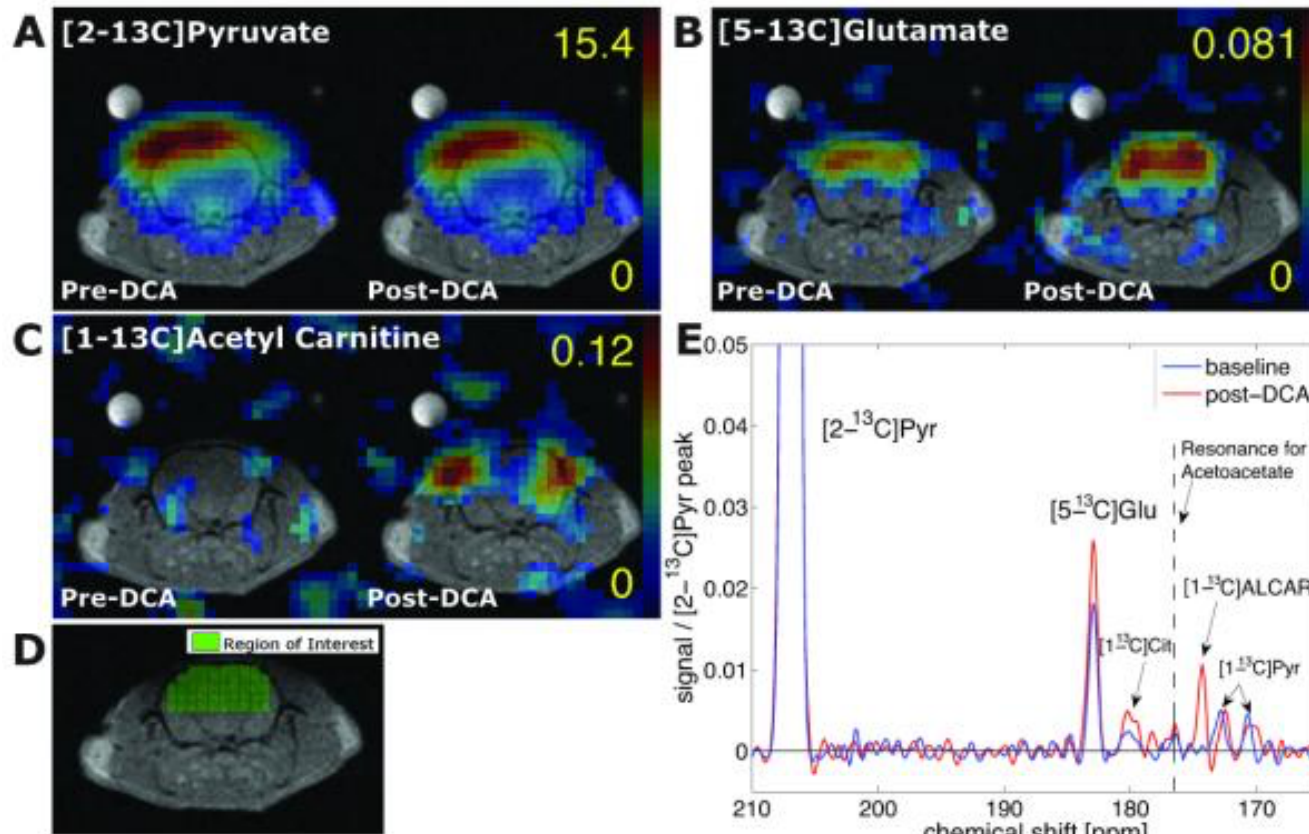
Interrogating the Warburg effect and TCA cycle: Hyperpolarized $[2-^{13}\text{C}]$ -pyruvate



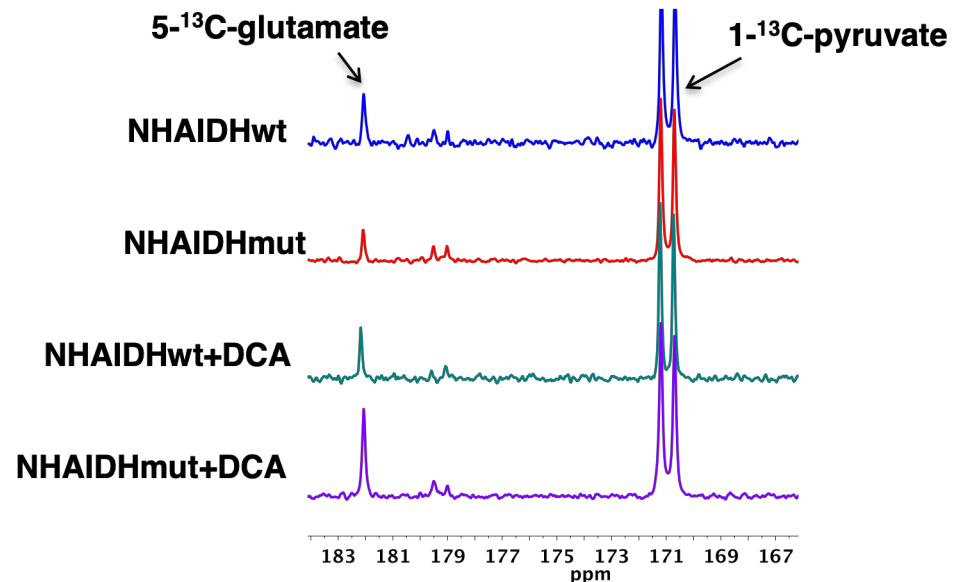
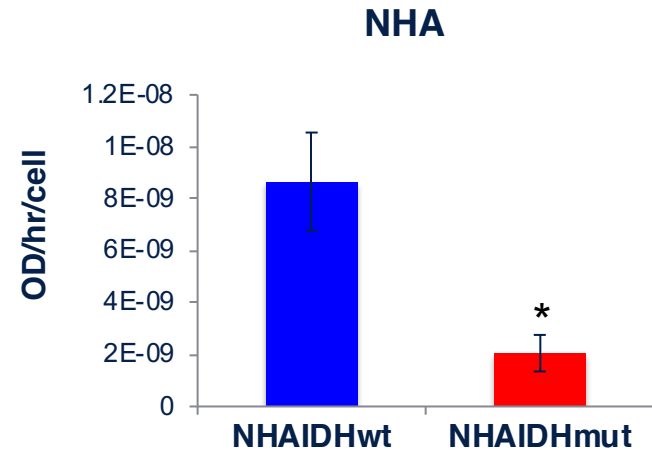
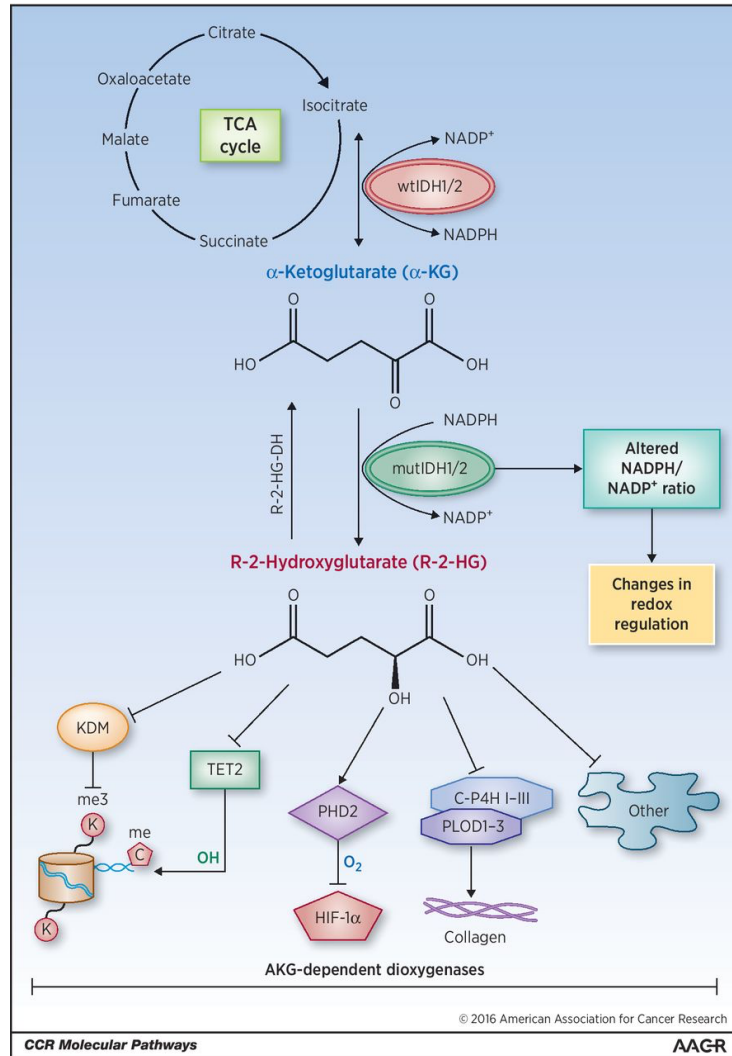
Hyperpolarized [2-¹³C]-pyruvate can monitor response to dichloroacetate therapy in the brain



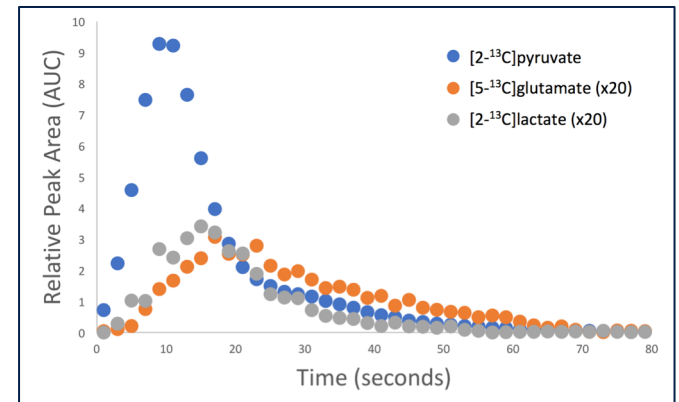
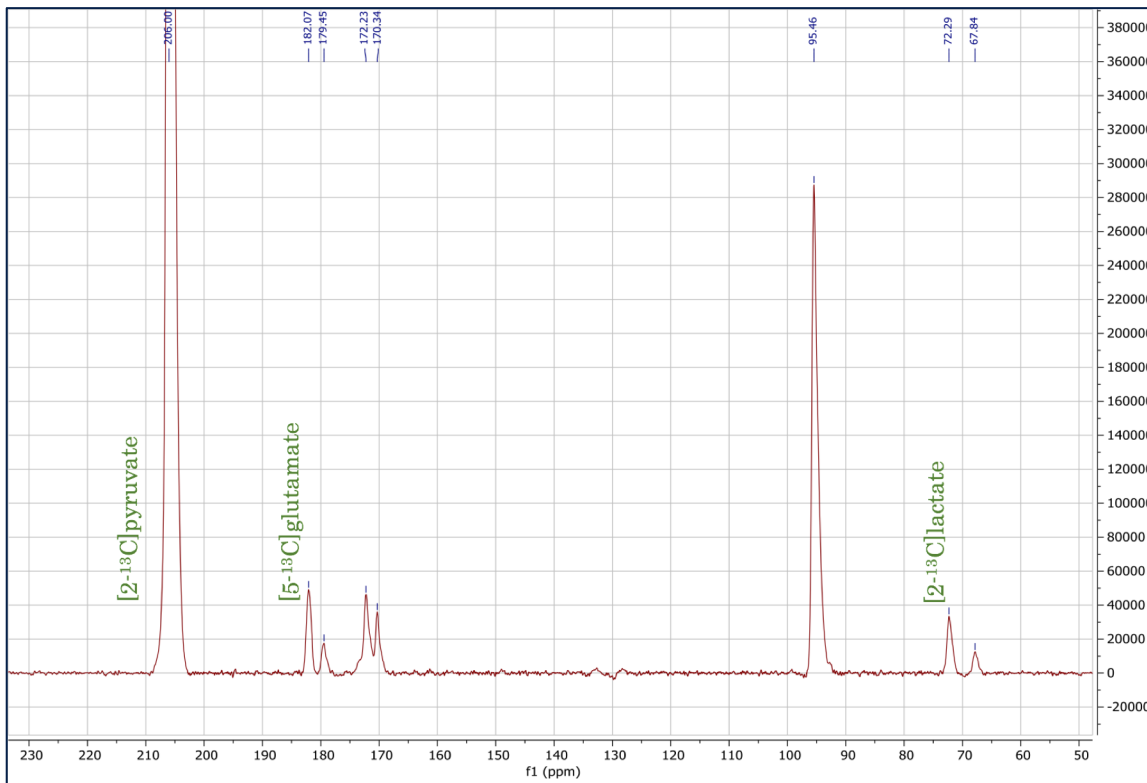
Hyperpolarized [2-¹³C]-pyruvate can monitor response to dichloroacetate therapy in the brain



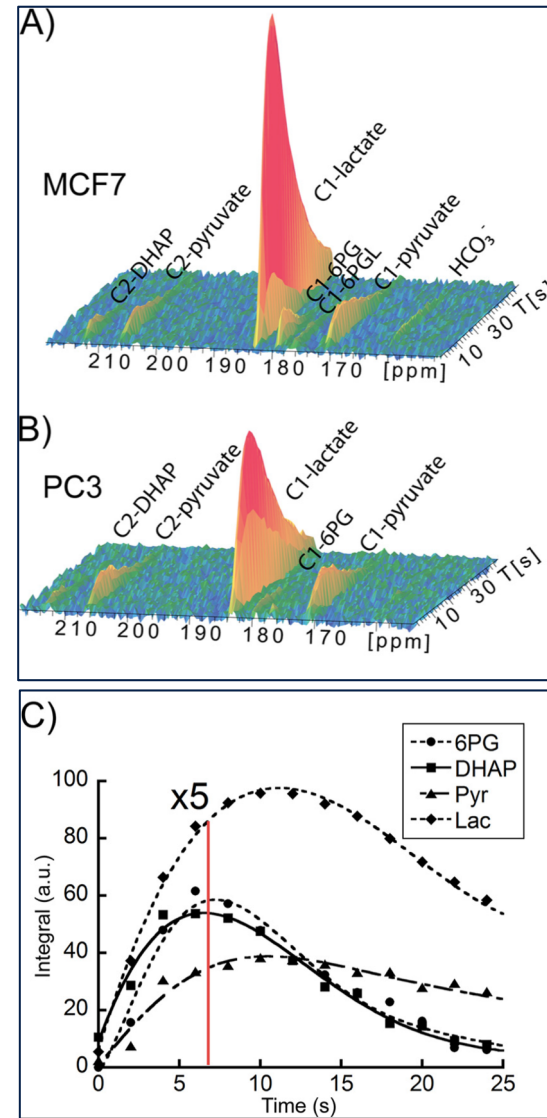
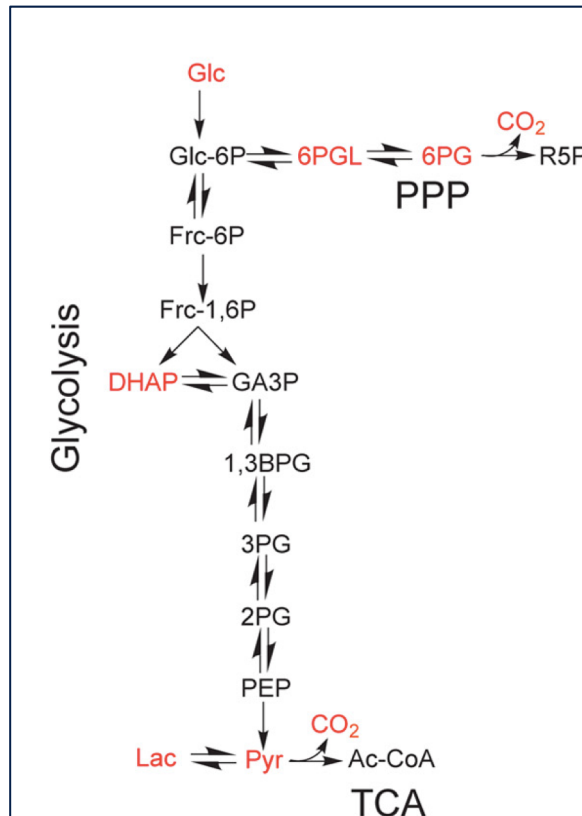
Hyperpolarized [2-¹³C]-pyruvate can monitor mutant IDH1-associated PDH down-regulation



Hyperpolarized [2-¹³C]-pyruvate is moving to the clinic

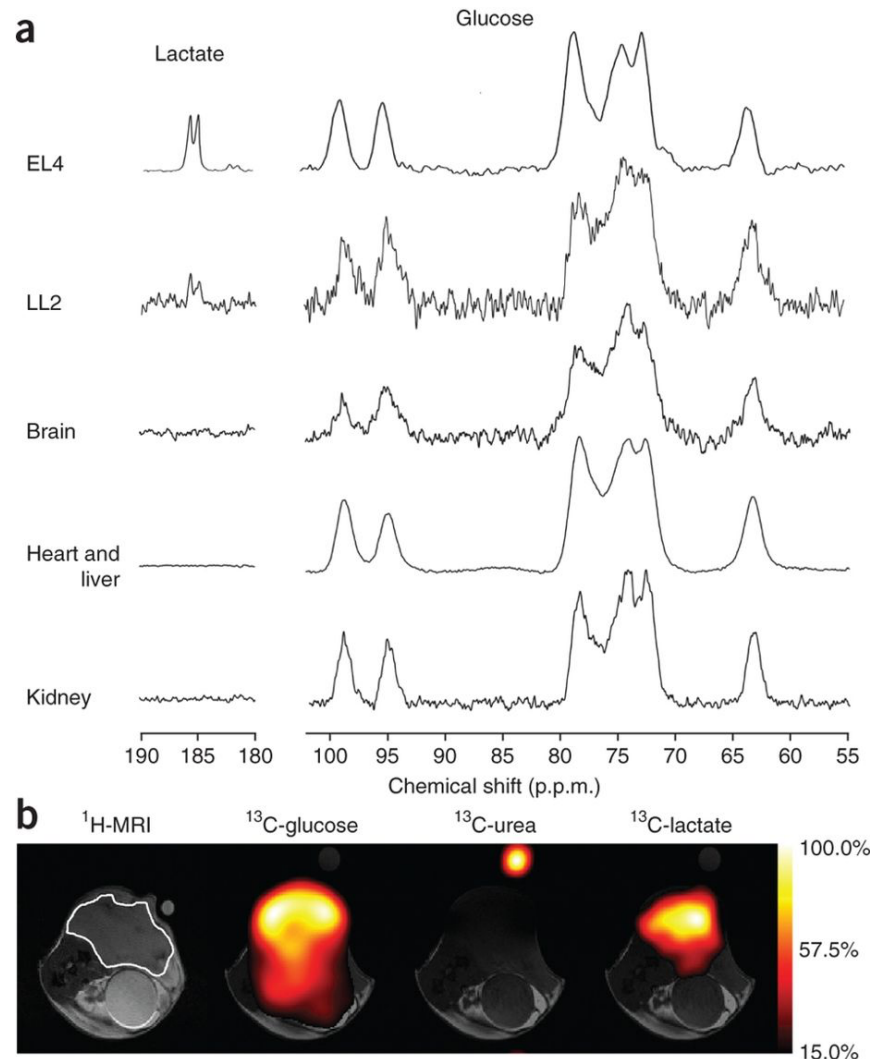


Interrogating the Warburg effect: Hyperpolarized [U- ^{13}C , U- ^2H]- glucose

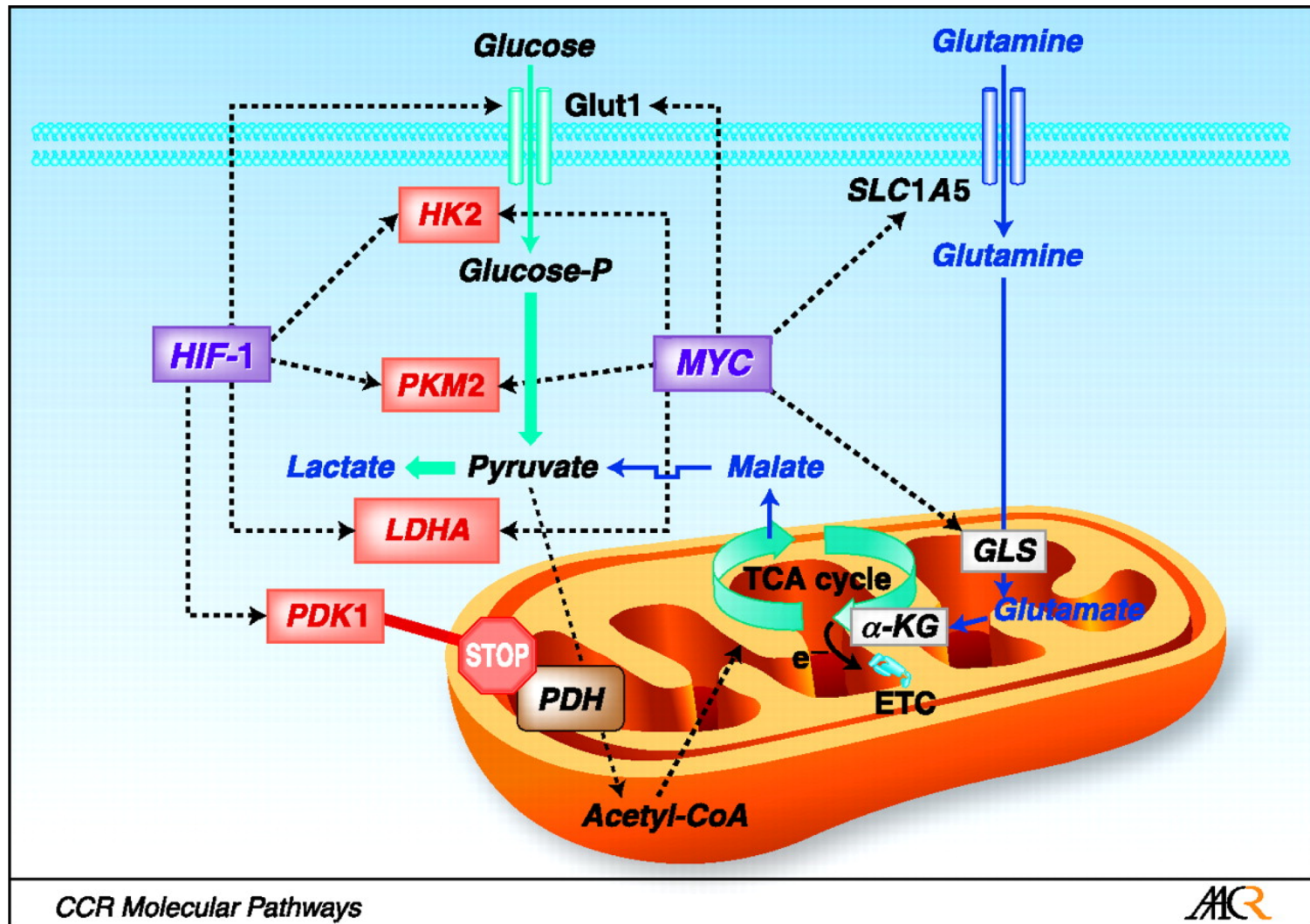


Christensen et al., J. Biol. Chem., 289:2344-2352, 2014.

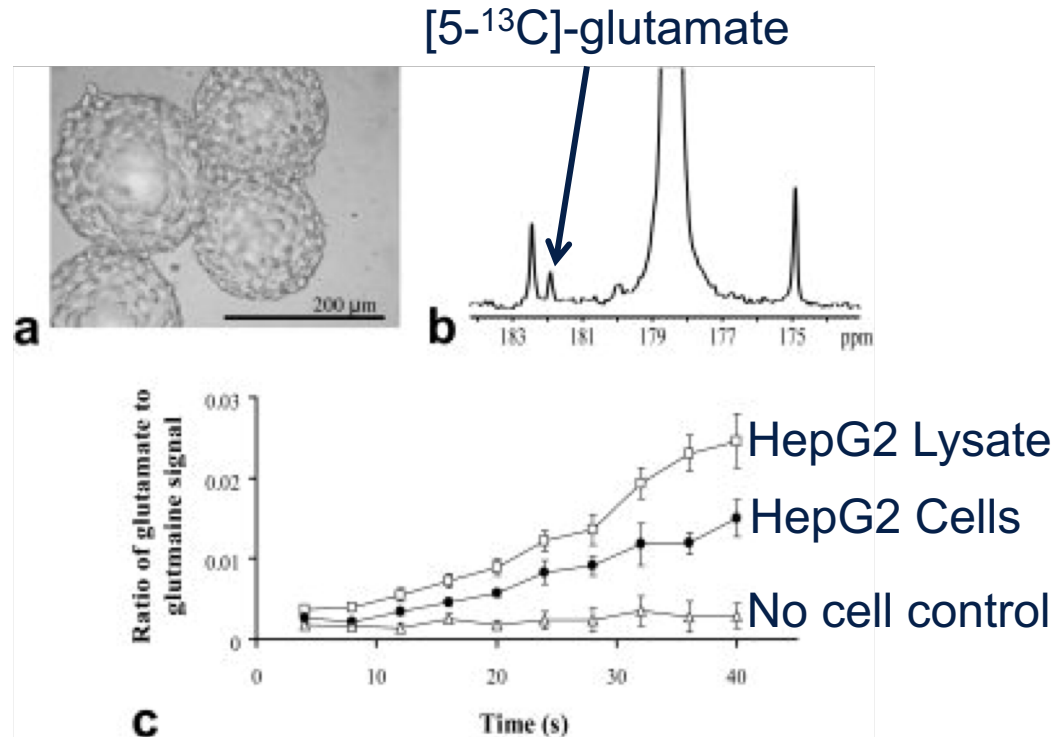
Interrogating the Warburg effect: Hyperpolarized [U- ^{13}C , U- ^2H]- glucose



Altered glutamine metabolism is a hallmark of cancer



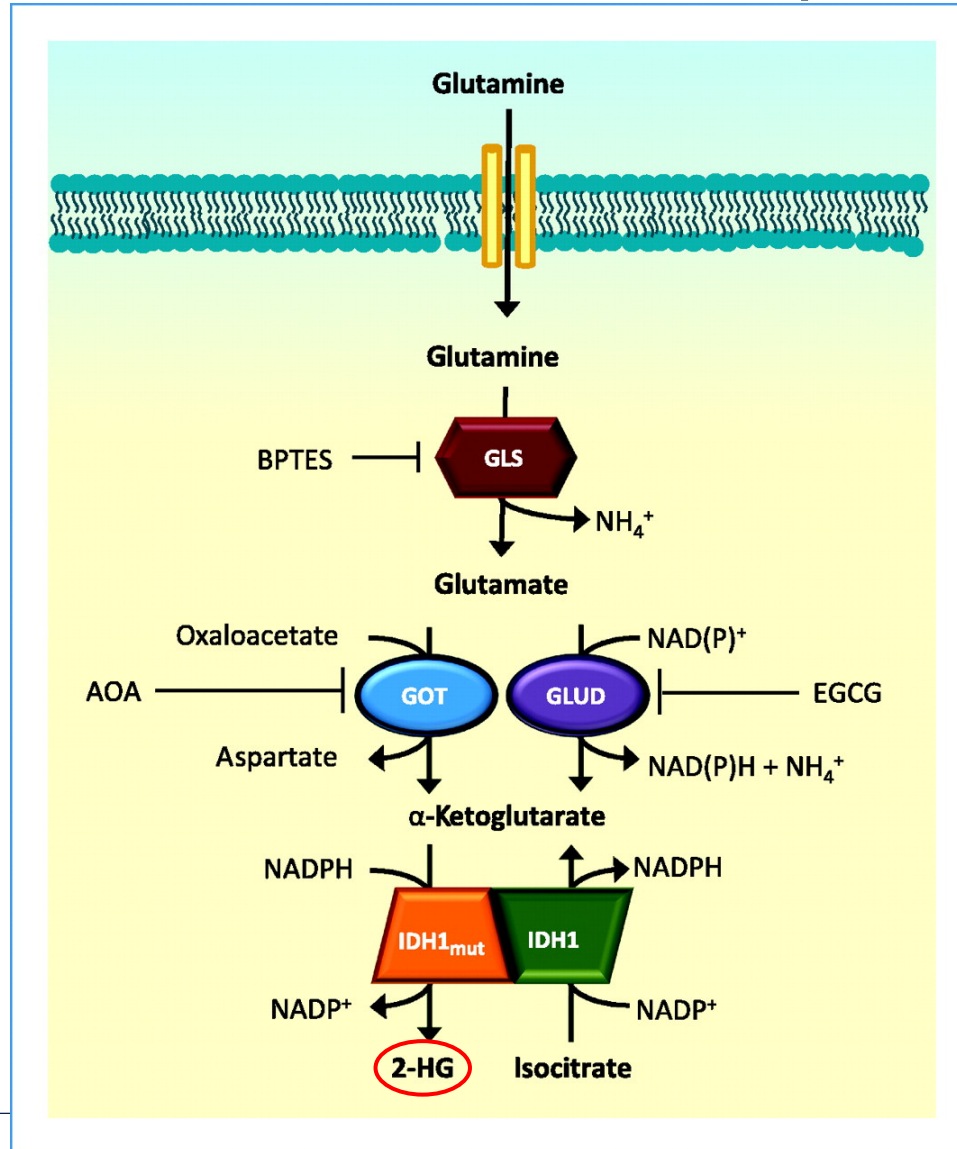
Interrogating glutaminolysis- hyperpolarized [5-¹³C]-glutamine



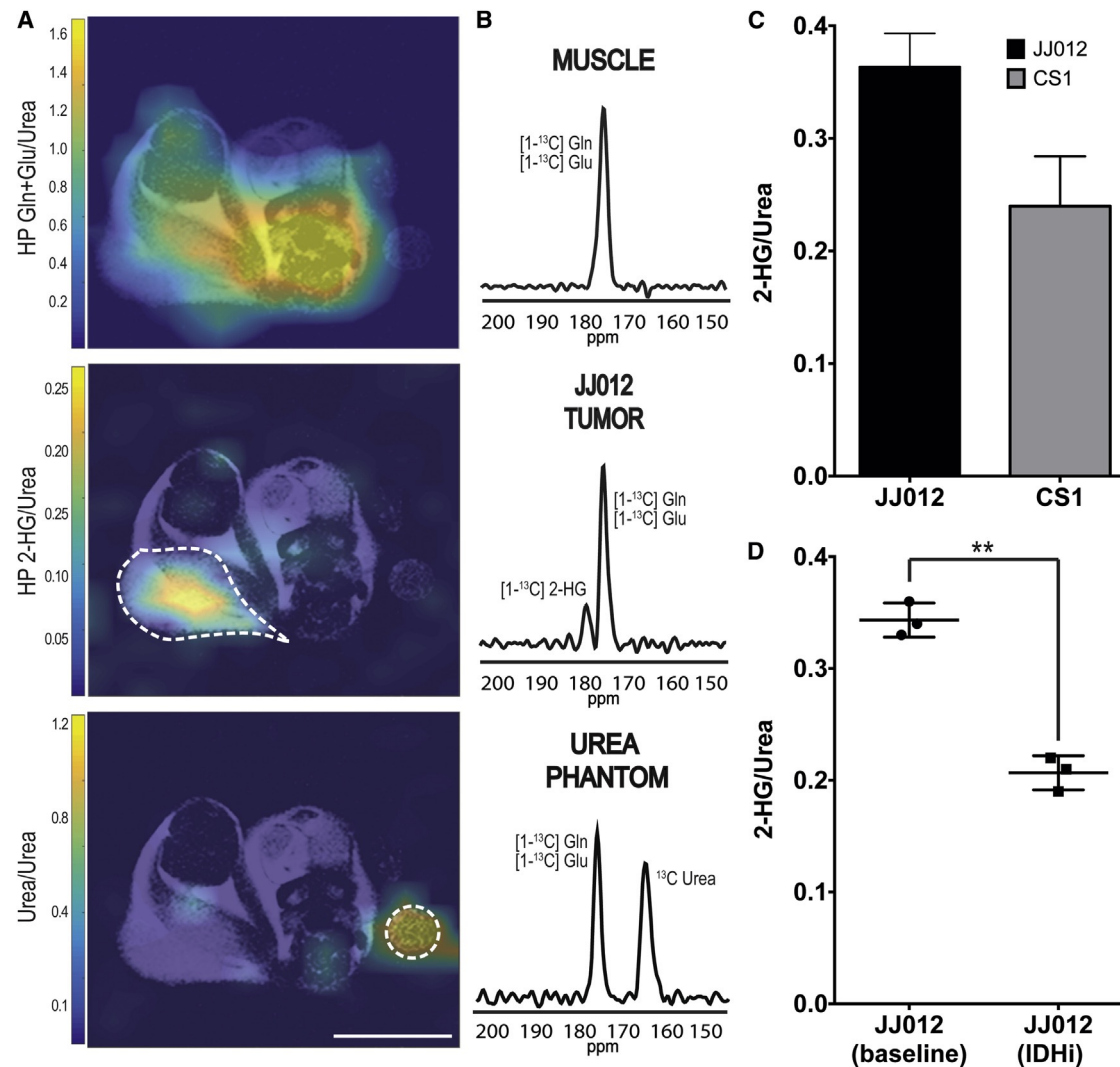
Gallagher et al., Magn Reson Med 60:253–257, 2008

Rapid, spontaneous degradation of glutamine to glutamate (product of interest) in the prep is an issue.

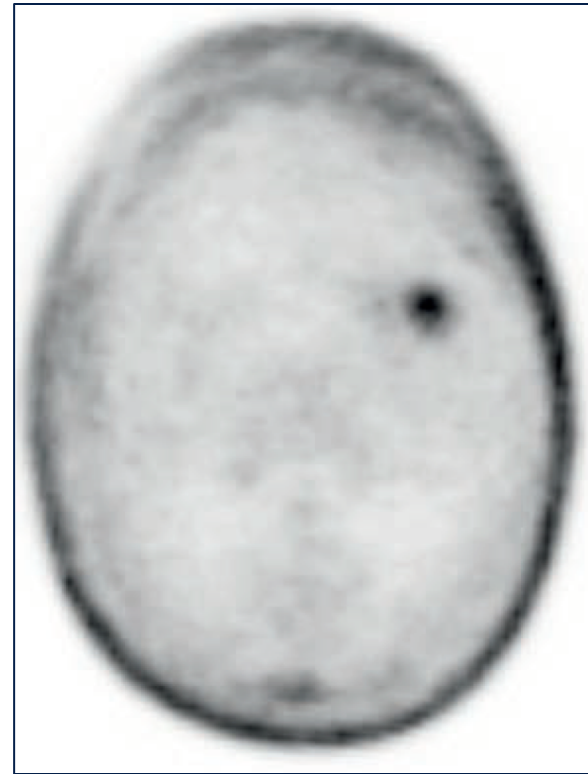
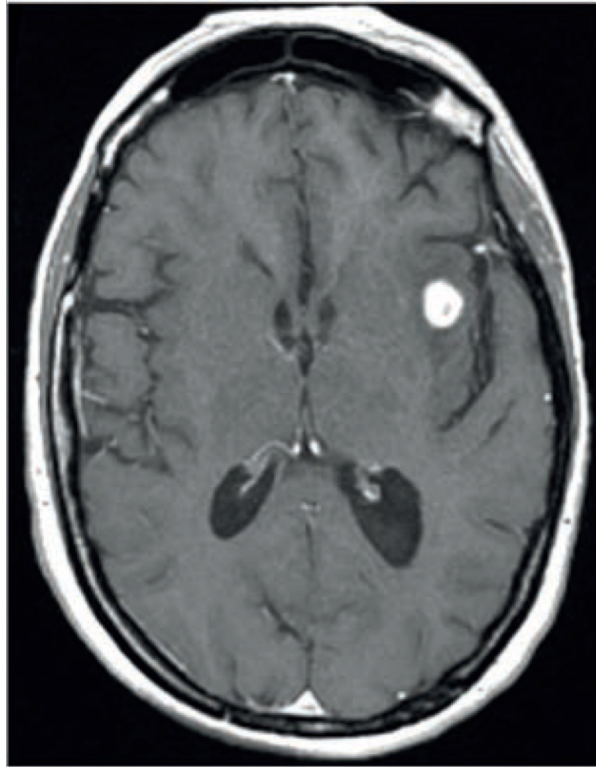
Interrogating glutamine metabolism-hyperpolarized [1-¹³C]-glutamine can detect 2-HG production



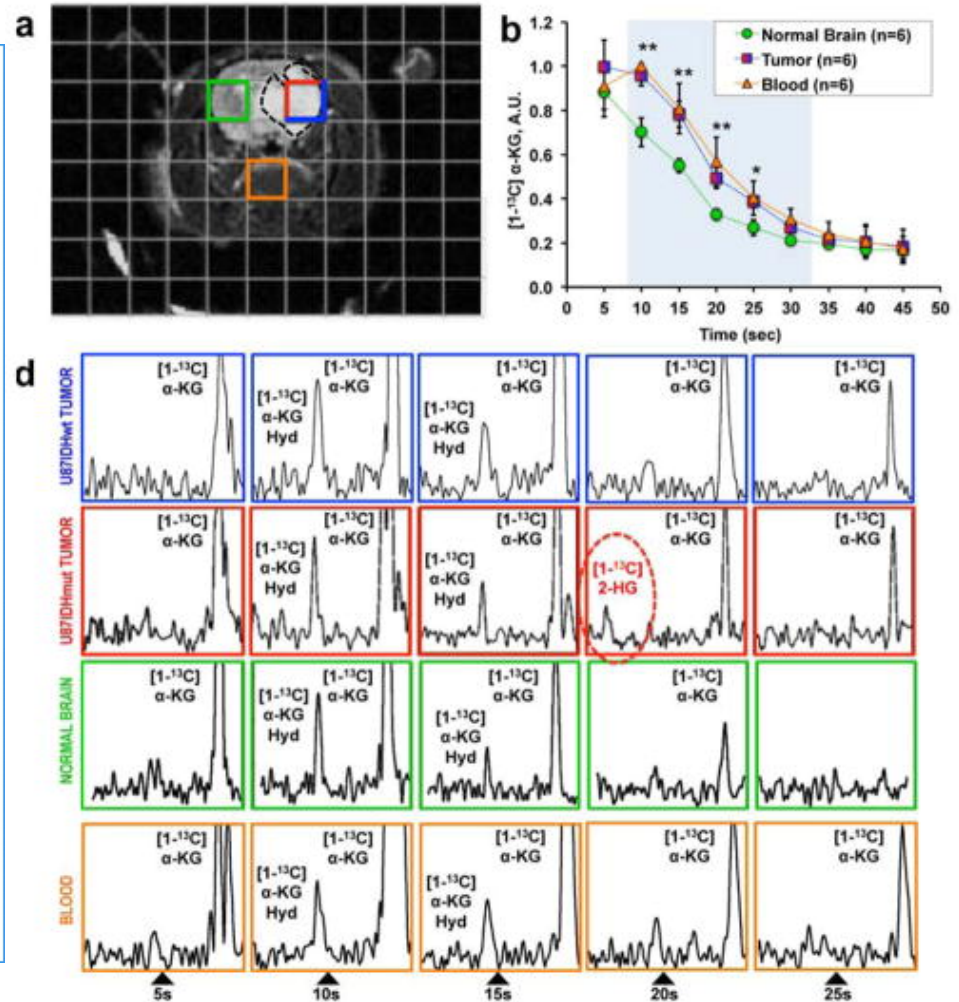
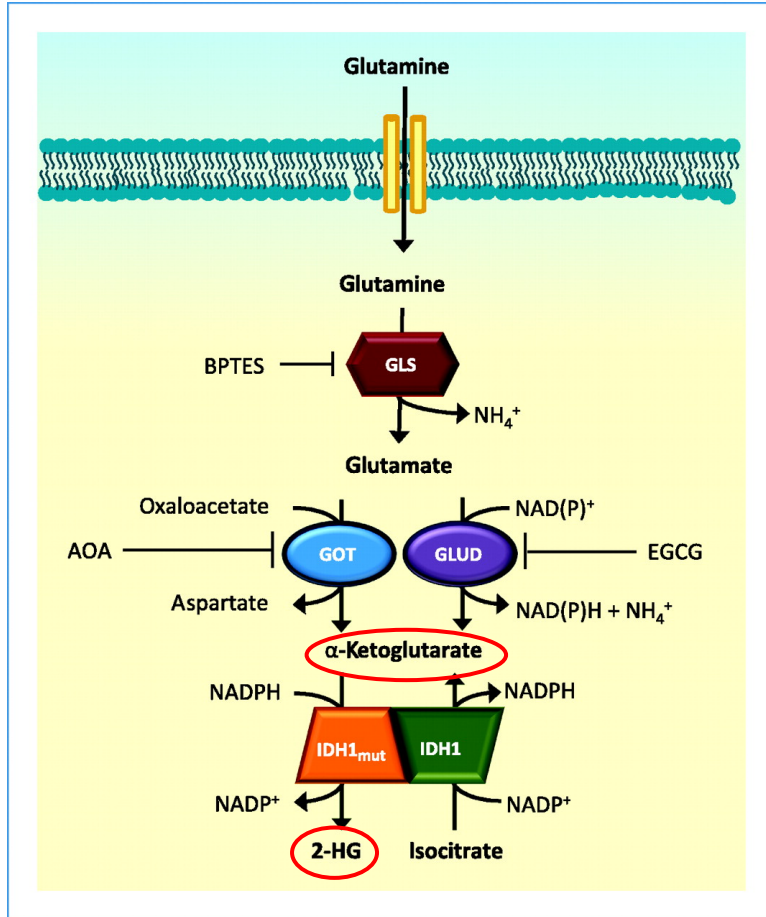
Interrogating glutamine metabolism-hyperpolarized [1-¹³C]-glutamine can detect 2-HG production



Interrogating glutamine metabolism by PET-(2S,4R)-4-[^{18}F]fluoro-L-glutamine

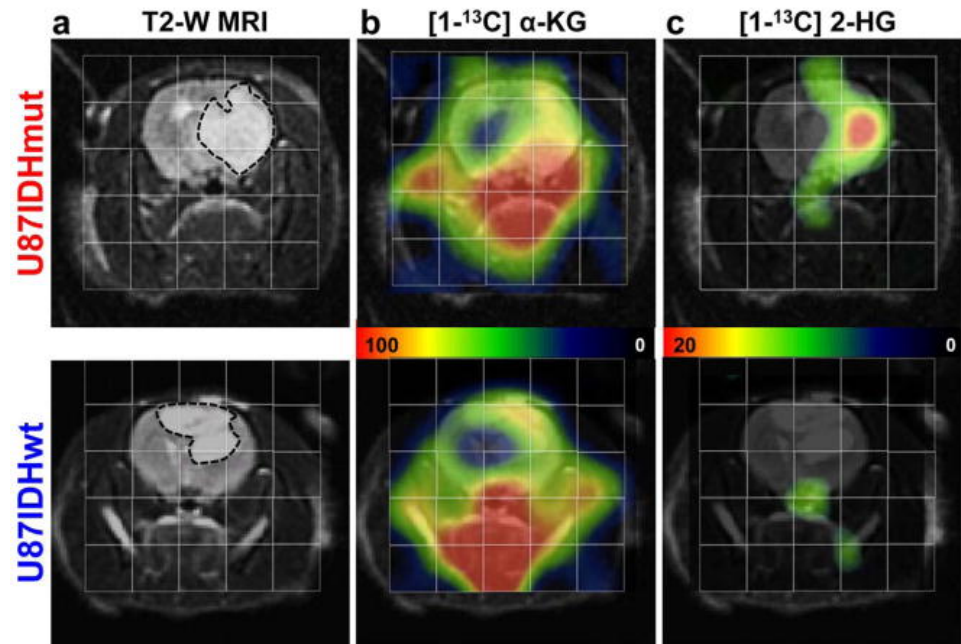
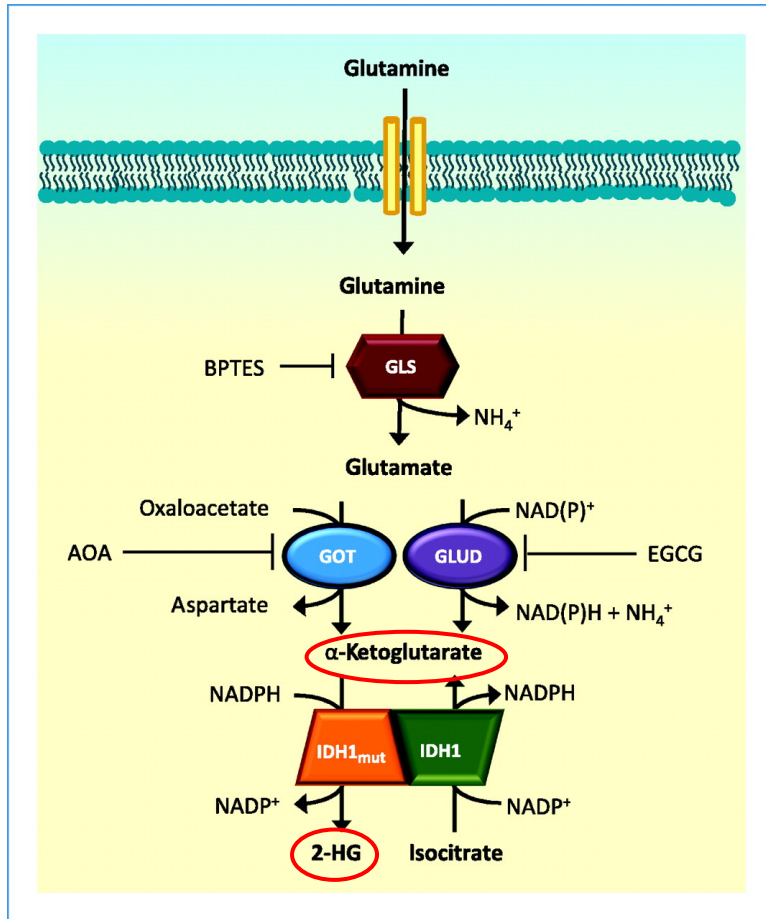


Interrogating oncometabolite production- hyperpolarized $[1-^{13}\text{C}]\text{-}\alpha\text{-ketoglutarate}$



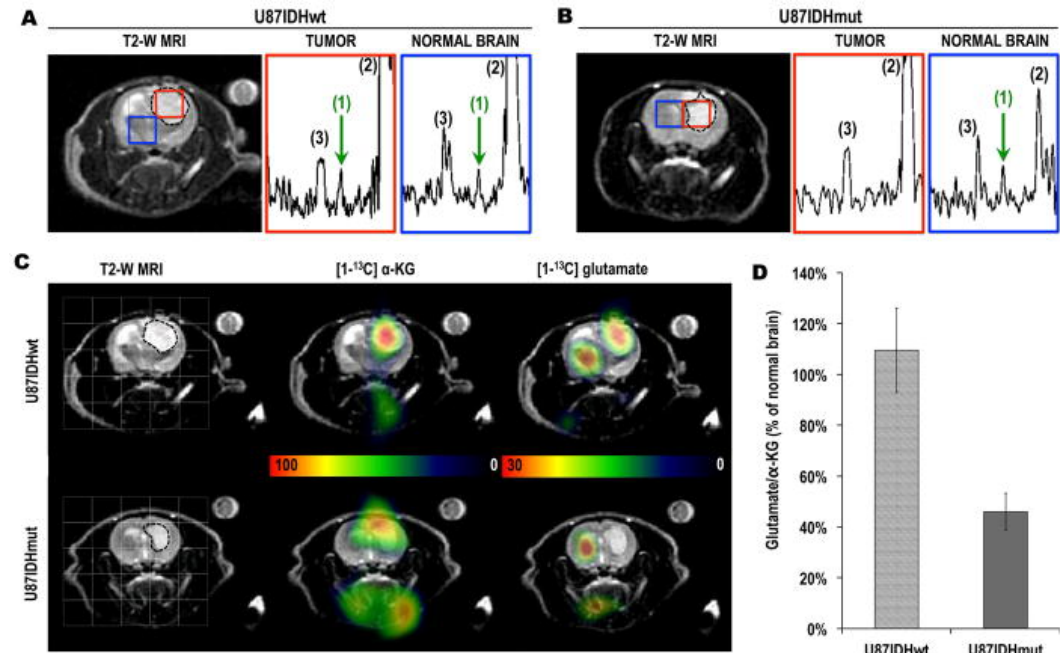
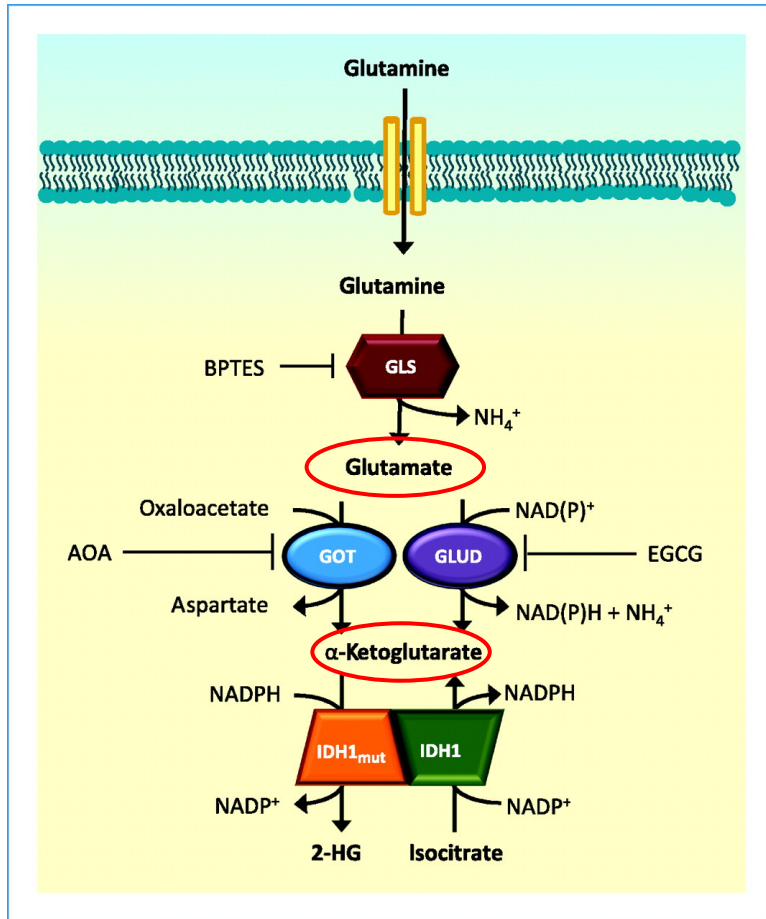
Chaumeil et al. Nat Commun, 4: 2429, 2013.

Interrogating oncometabolite production- hyperpolarized [1-¹³C]-α-ketoglutarate



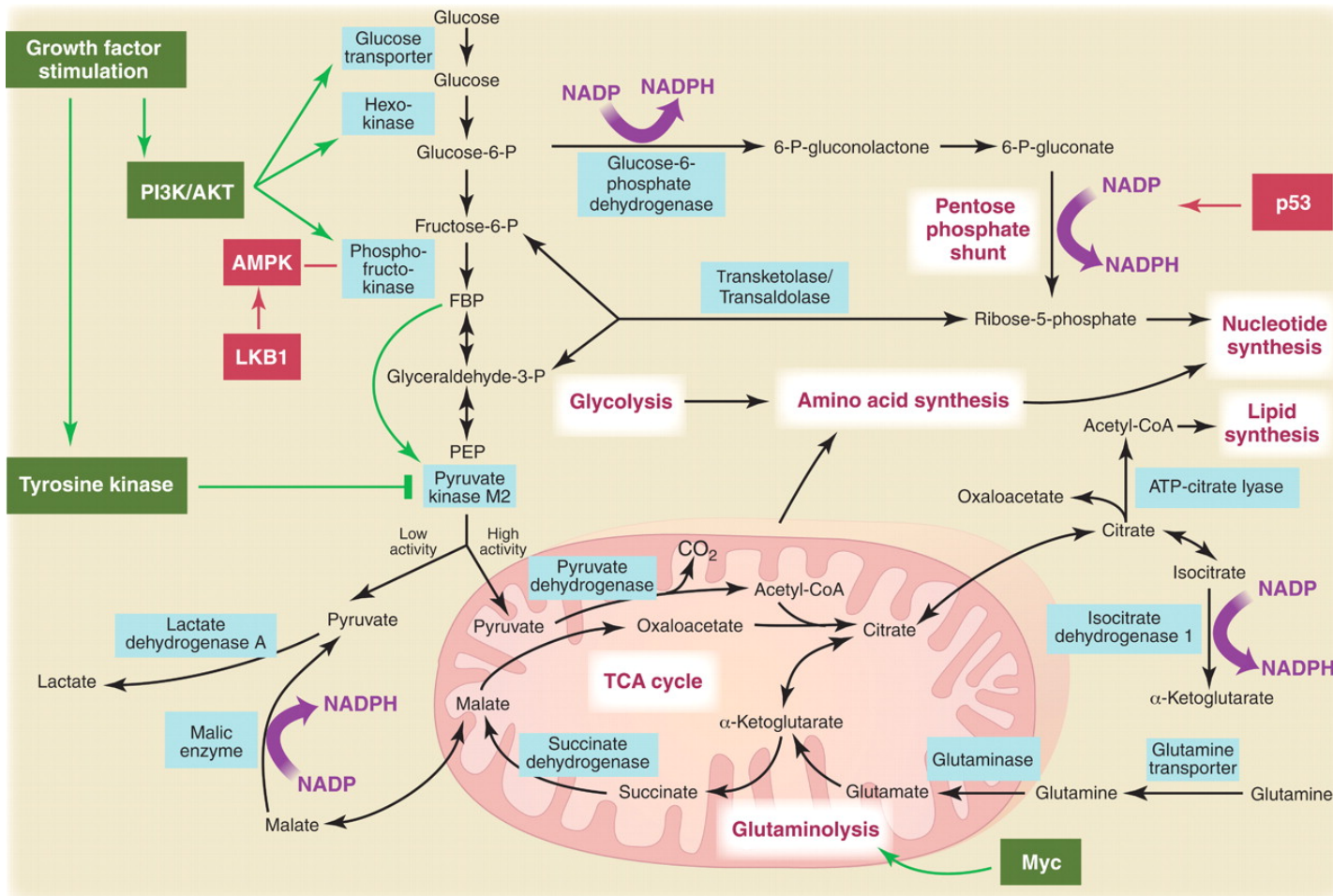
Chaumeil et al. Nat Commun, 4: 2429, 2013.

Interrogating transamination- hyperpolarized [1-¹³C]-α-ketoglutarate

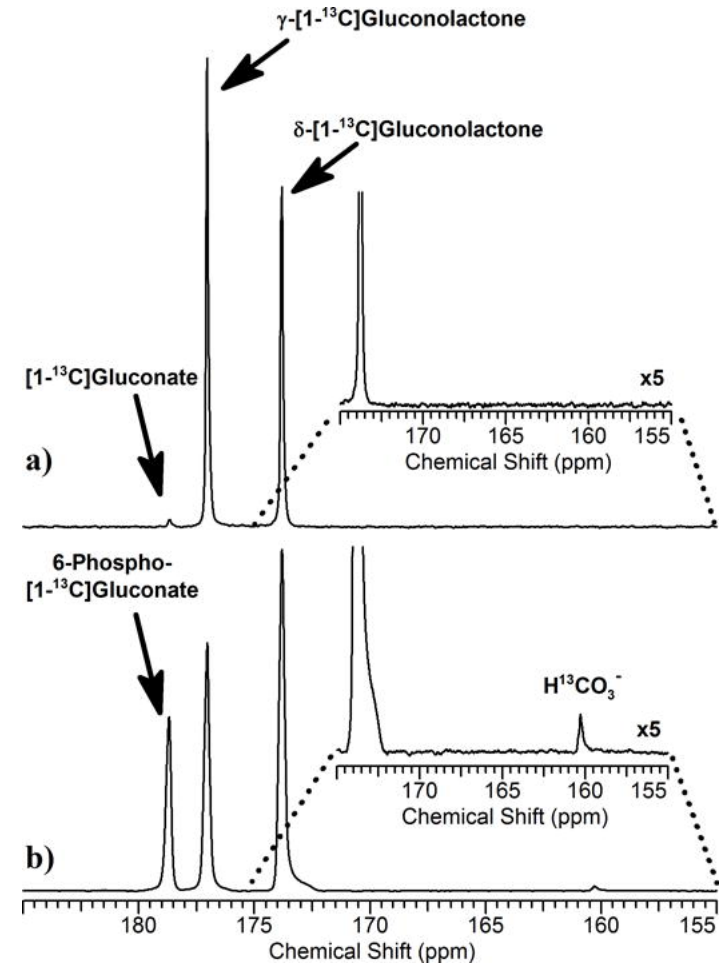
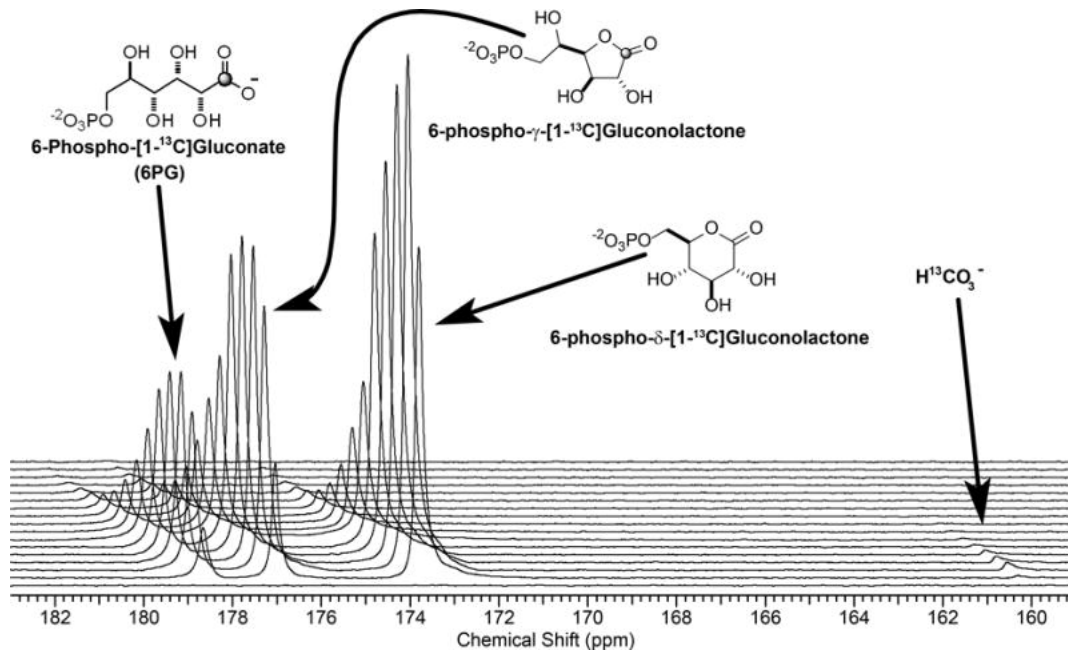


Chaumeil et al. Cancer Res, 74(16): 4247–4257, 2014.

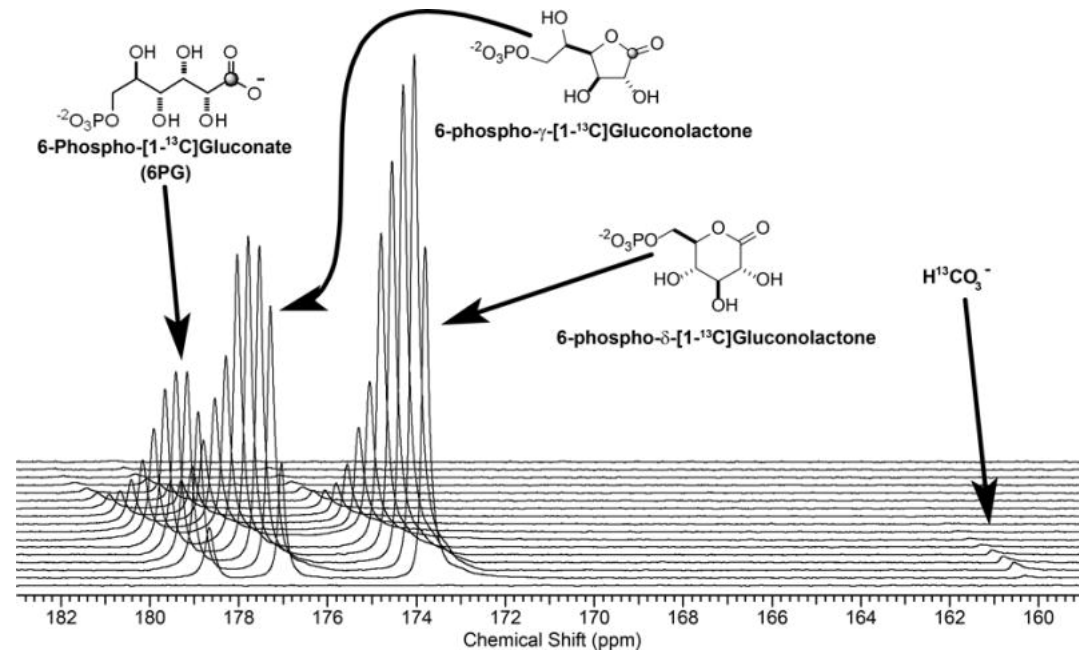
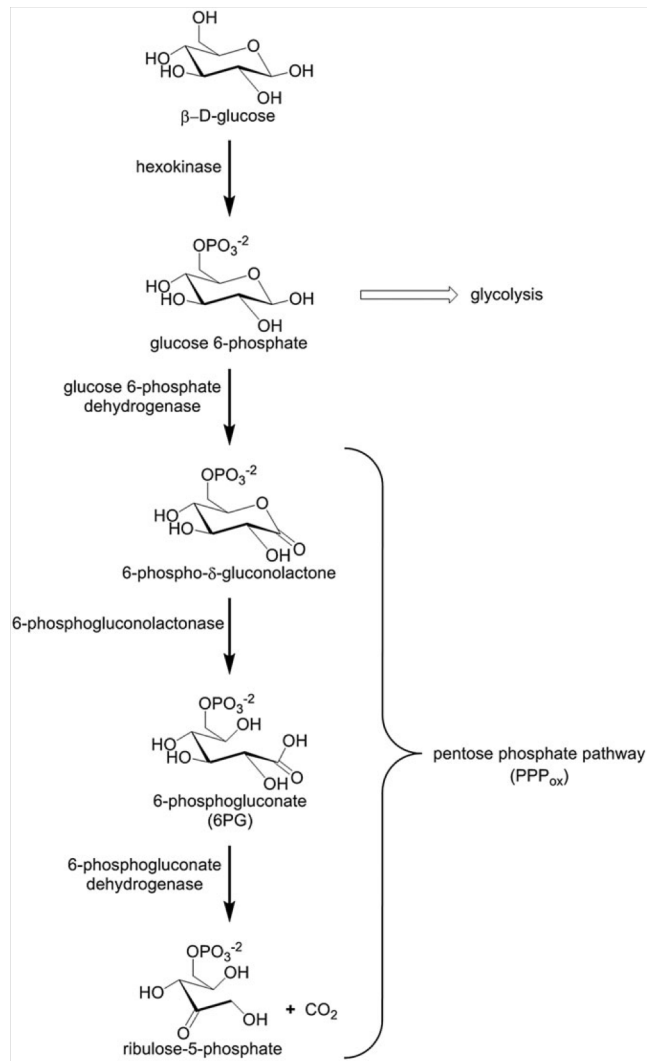
Interrogating the pentose phosphate pathway- hyperpolarized δ -[1- ^{13}C]-gluconolactone



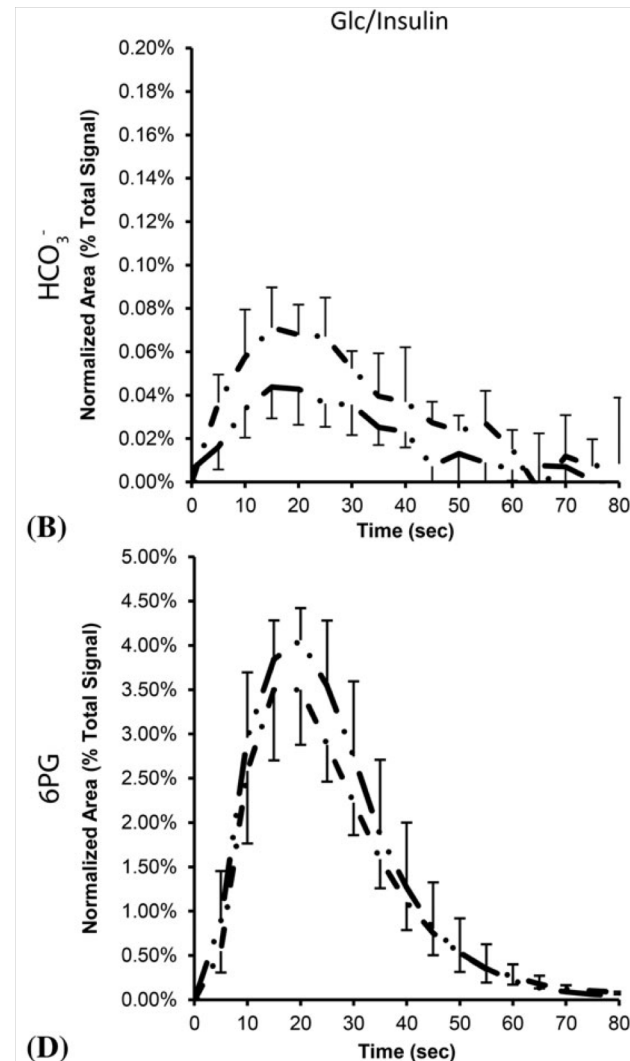
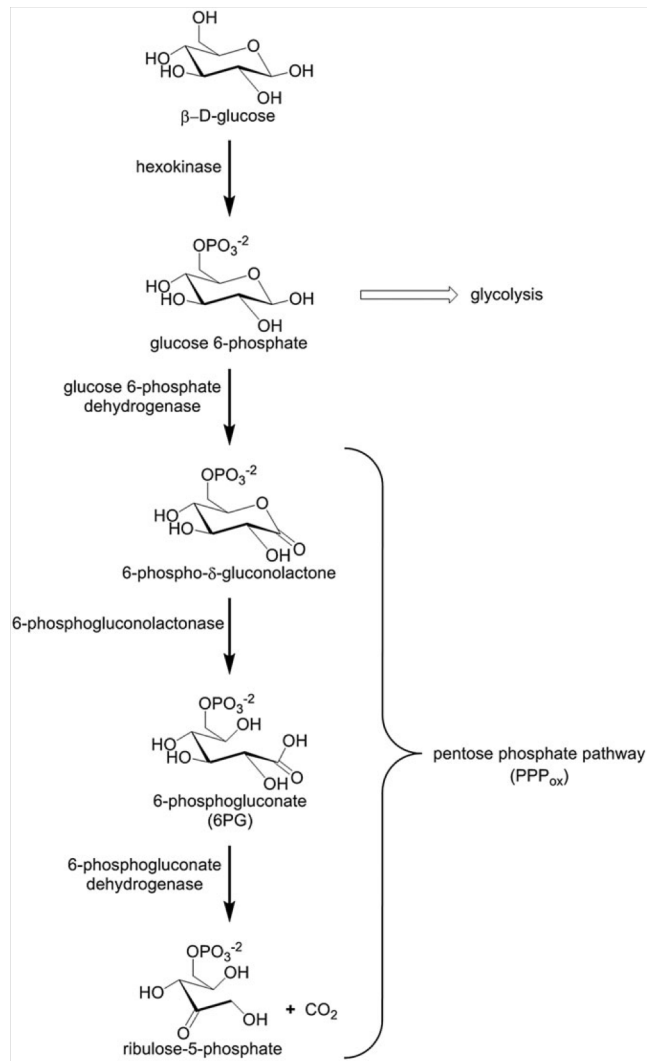
Interrogating the pentose phosphate pathway- hyperpolarized δ -[1- ^{13}C]-gluconolactone



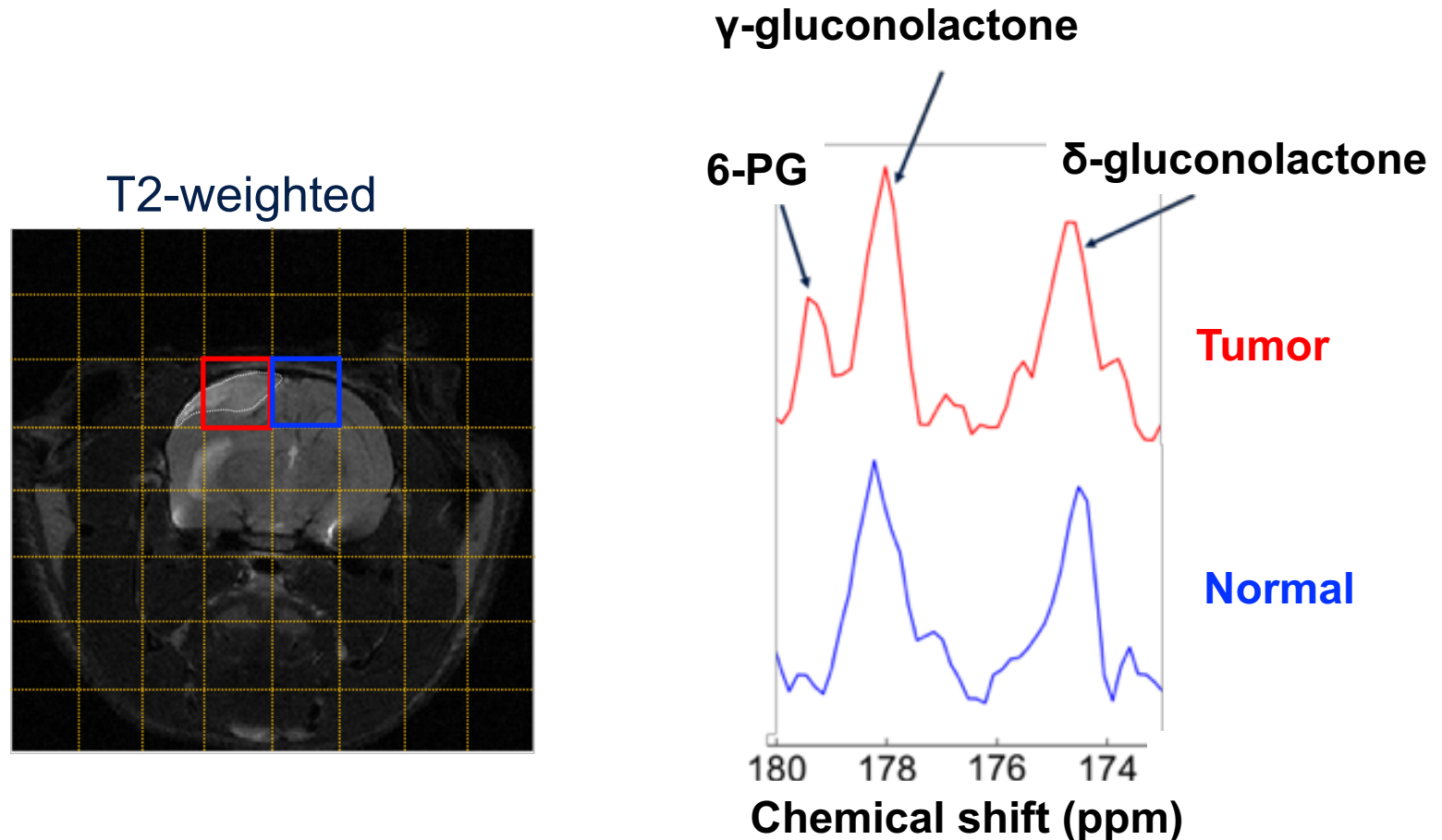
Interrogating the pentose phosphate pathway- hyperpolarized δ -[1- ^{13}C]-gluconolactone



Interrogating the pentose phosphate pathway- hyperpolarized δ -[1- ^{13}C]-gluconolactone



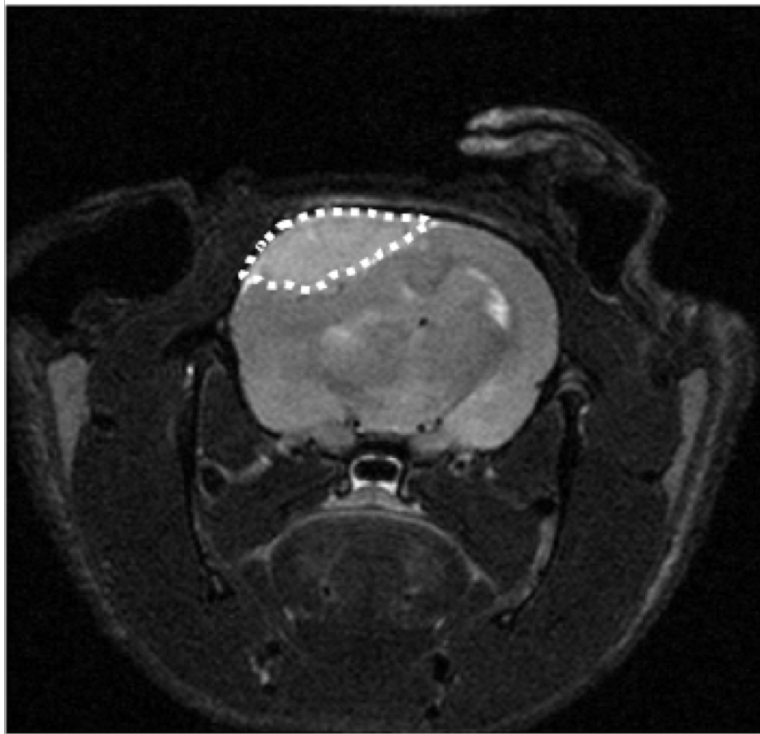
Interrogating the pentose phosphate pathway- hyperpolarized δ -[1- ^{13}C]-gluconolactone



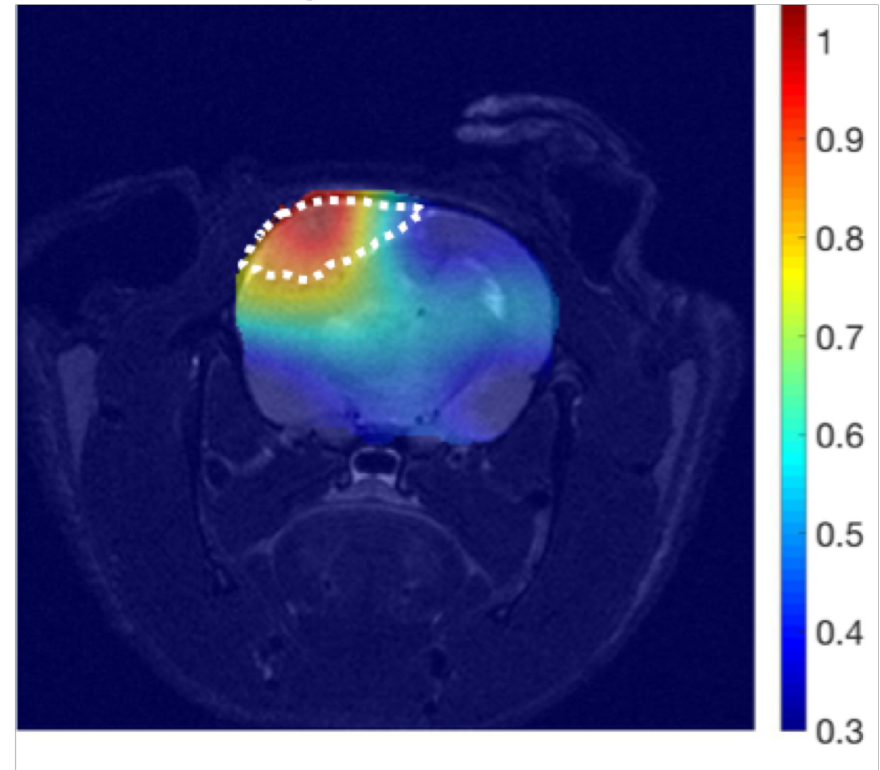
Batsios et al., unpublished data

Interrogating the pentose phosphate pathway- hyperpolarized δ -[1- ^{13}C]-gluconolactone

T2-weighted

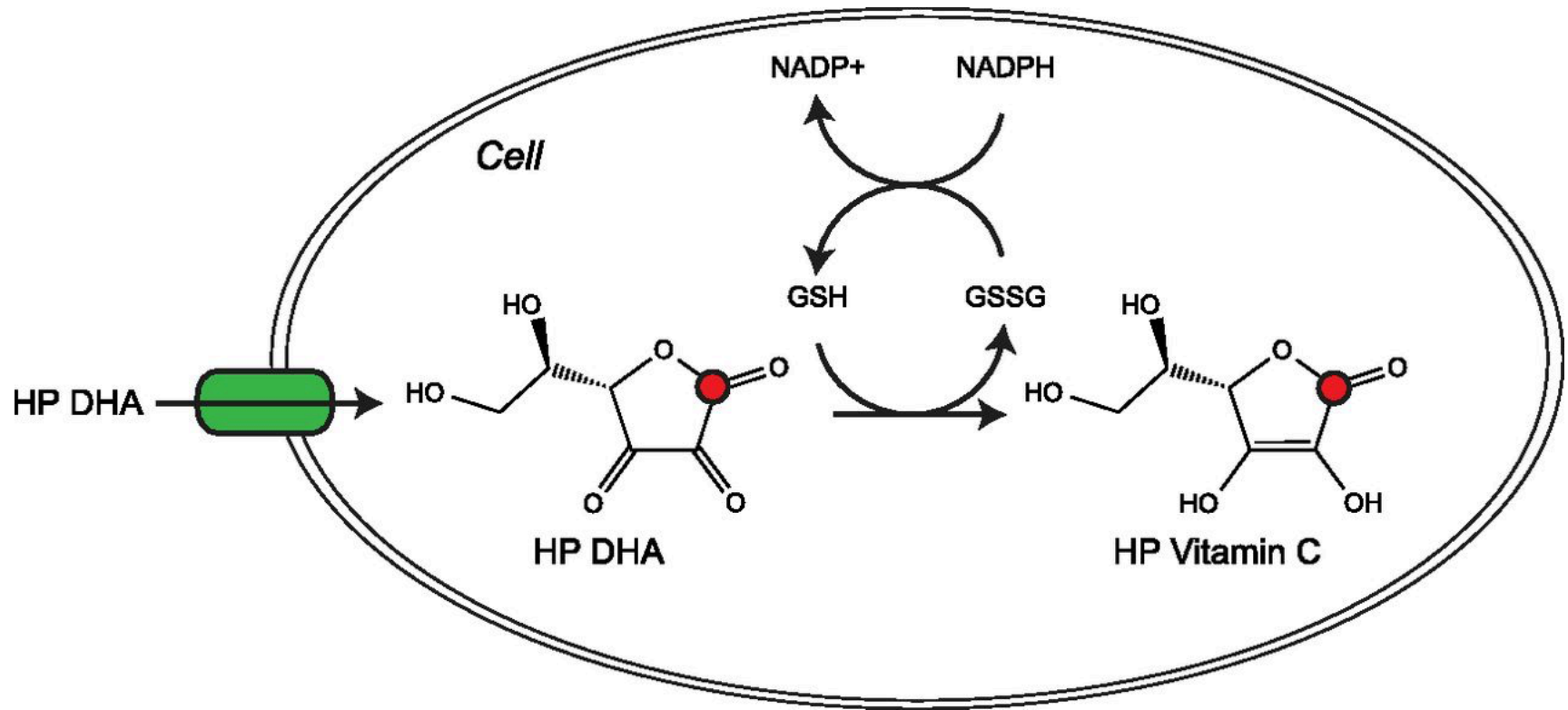


6-PG/gluconolactone



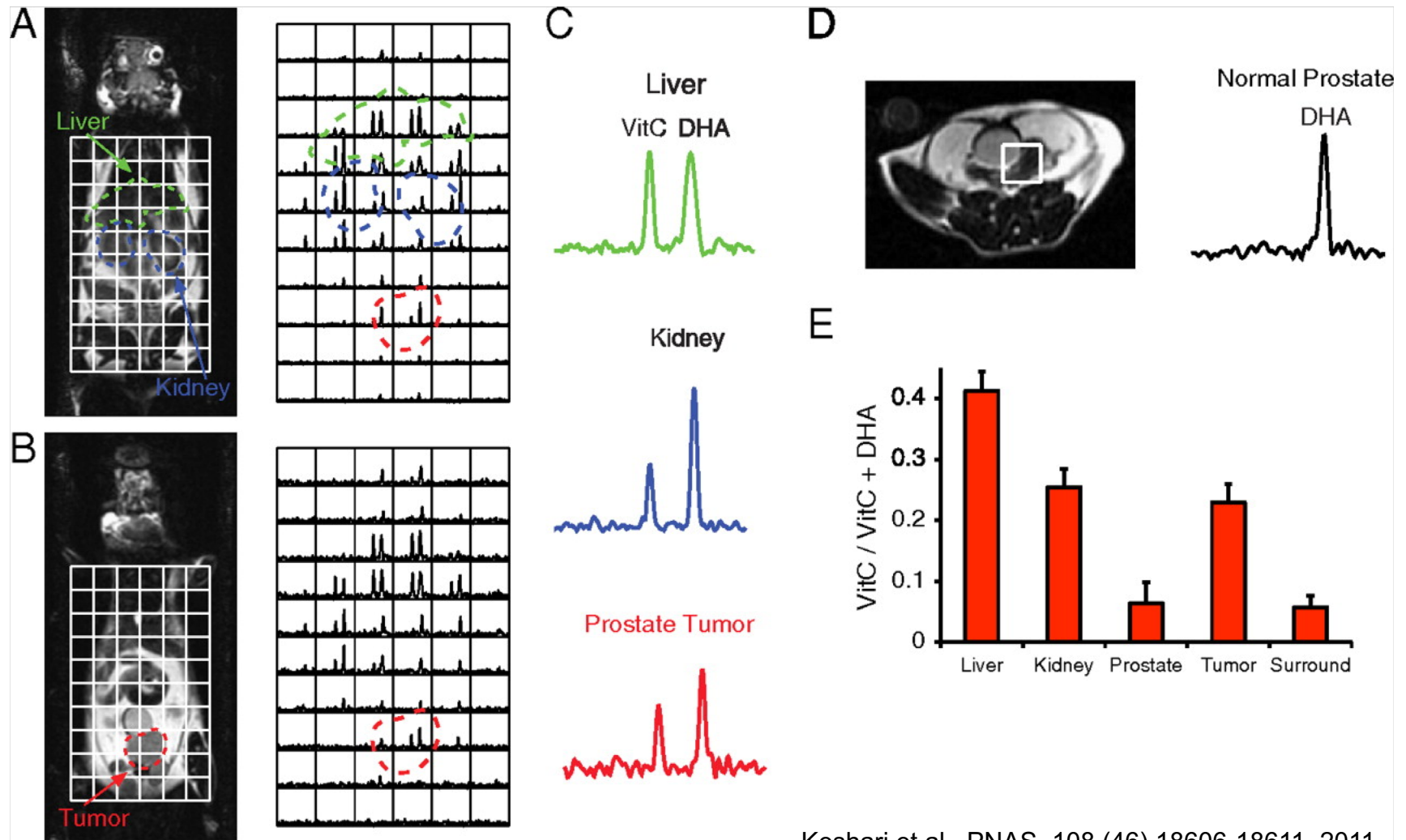
Batsios et al., unpublished data

Interrogating redox status: Hyperpolarized [1-¹³C]-dehydroascorbic acid



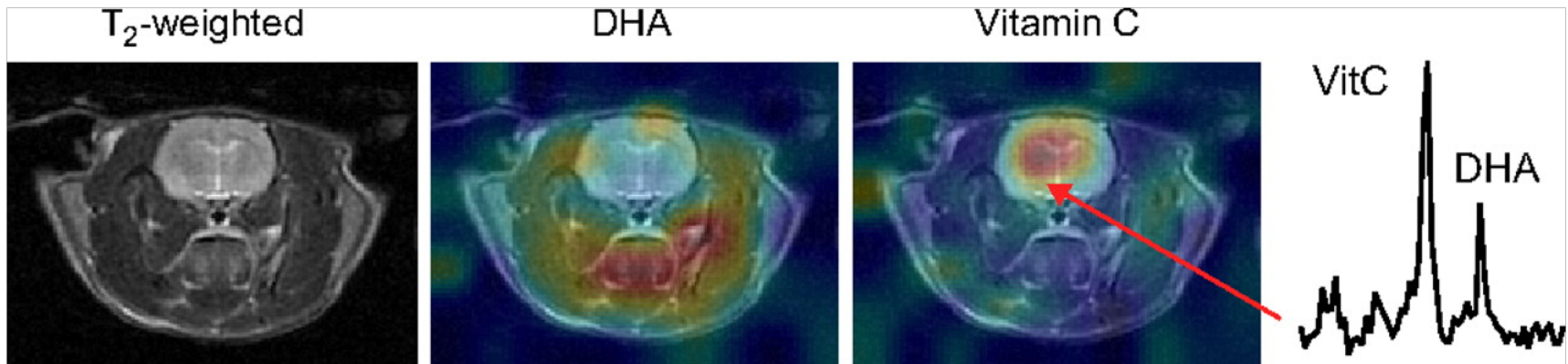
Keshari et al., Diabetes, 64(2): 344-352, 2015

Interrogating redox status: Hyperpolarized [1-¹³C]-dehydroascorbic acid



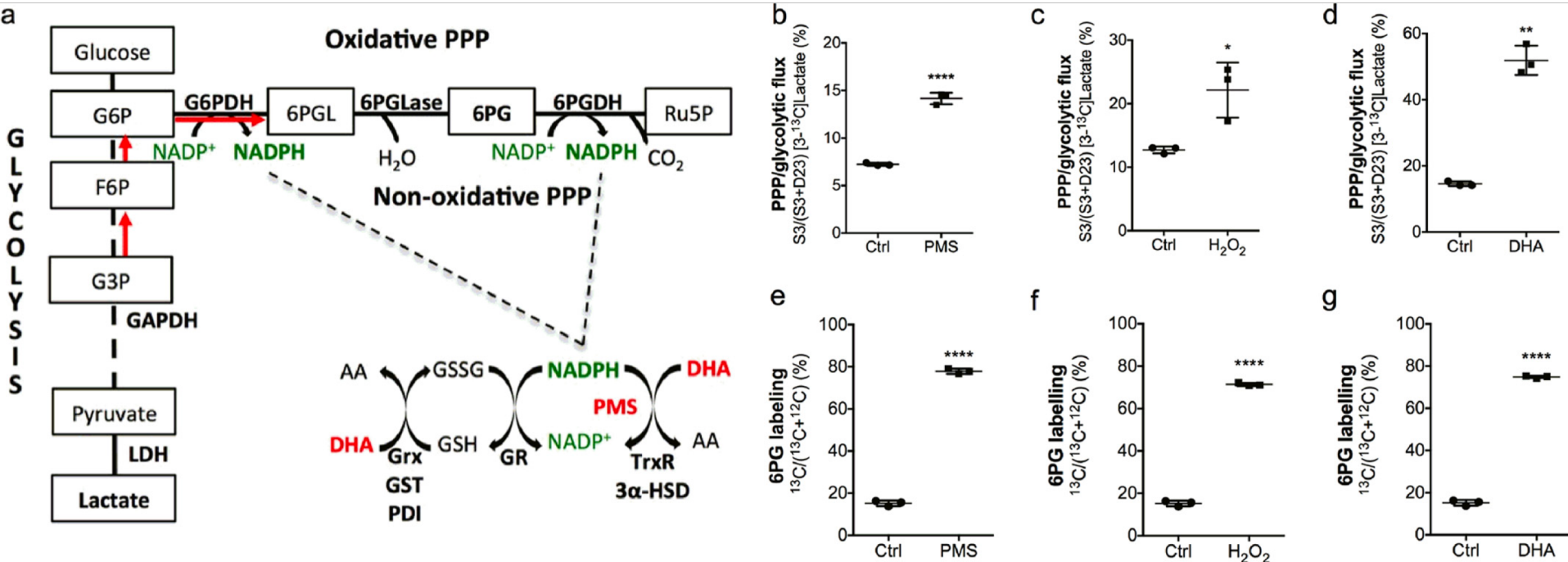
Keshari et al., PNAS, 108 (46) 18606-18611, 2011

Interrogating redox status: Hyperpolarized [1-¹³C]-dehydroascorbic acid

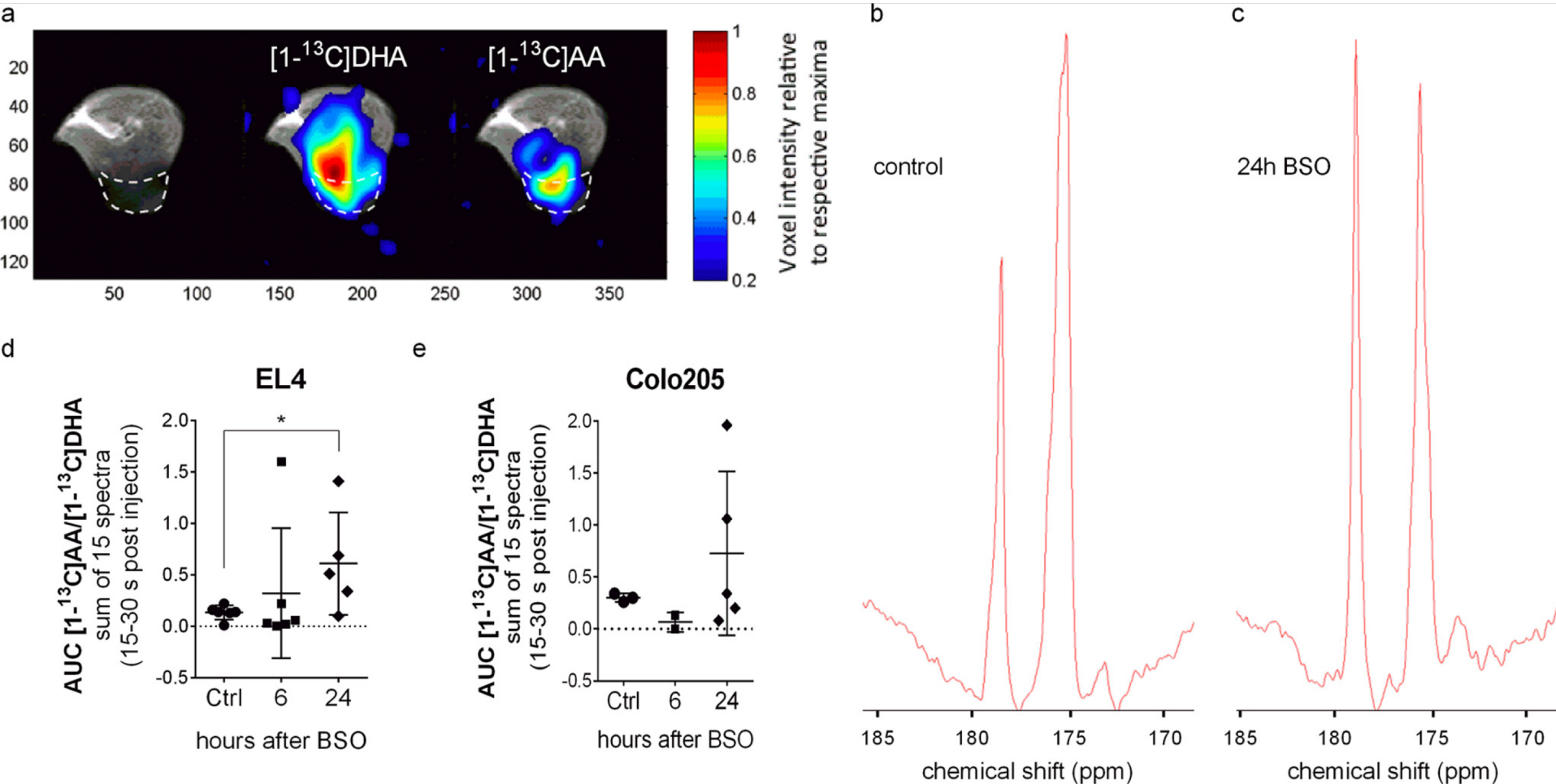


Keshari et al., PNAS, 108 (46) 18606-18611, 2011

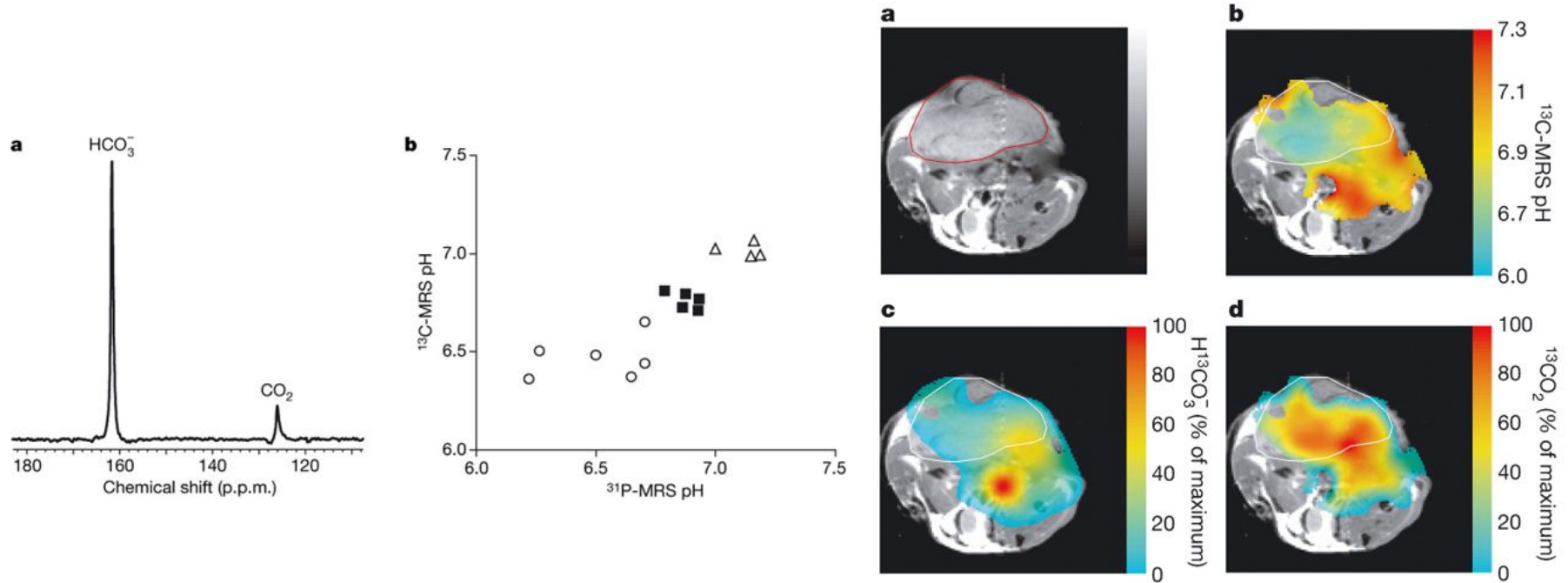
Hyperpolarized $[1-^{13}\text{C}]\text{DHA}$ reduction depends not only on GSH levels, but also on PPP flux



Hyperpolarized $[1-^{13}\text{C}]$ DHA reduction depends not only on GSH levels, but also on PPP flux

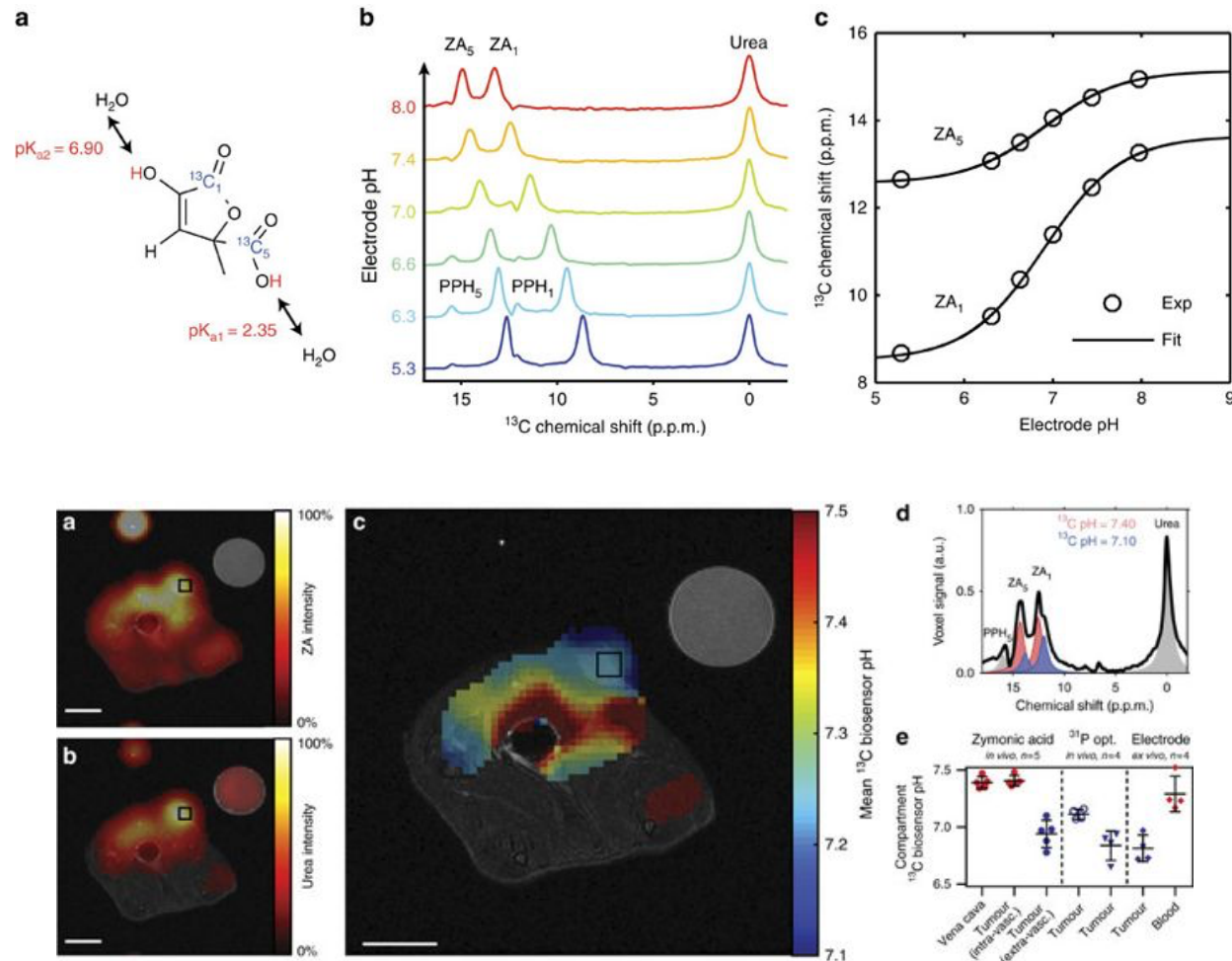


Interrogating pH: Hyperpolarized bicarbonate $\text{H}^{13}\text{CO}_3^-$



Gallgher et al., Nature, 453, 940–943, 2008

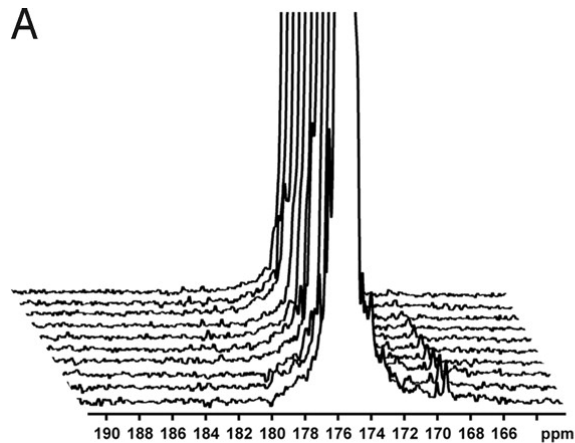
Interrogating pH: Hyperpolarized zymonic acid



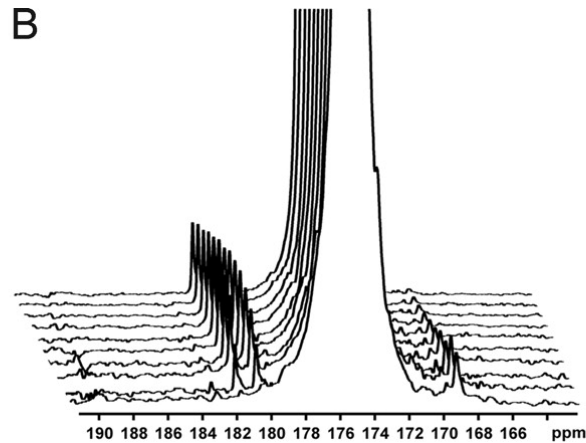
Duwel et al., Nature Communications, 15126, 2017

Interrogating cell death: Hyperpolarized [1,4- ^{13}C]-fumarate

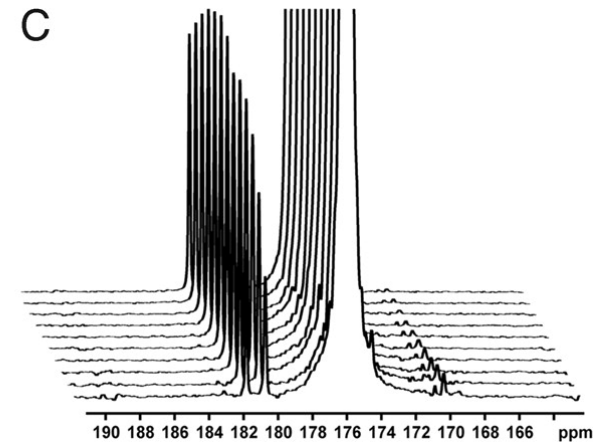
Untreated
EL4 cells



Etoposide treated
EL4 cells



EL4 lysate



Gallagher et al. PNAS, 106 (47): 19801-19806, 2009.

Summary

- Several hyperpolarized ^{13}C -MRS probes are currently available- hopefully more will come!
- In designing your experiments, consider the biology and the mechanism you are trying to image
- For more information, read
 - *Chaumeil et al., Studies of Metabolism Using ^{13}C MRS of Hyperpolarized Probes, Methods in Enzymology, Volume 561, Pages 1-71, 2015*
 - *Lumata et al., Hyperpolarized ^{13}C Magnetic Resonance and Its Use in Metabolic Assessment of Cultured Cells and Perfused Organs, Methods in Enzymology, Volume 561, 73-106, 2015*