

Spectroscopy and Tumor Metabolism

UCSF

Learning Objectives

- Understand the important metabolic changes that occur in cancer and their cause.
- Awareness of the NMR approaches and pre-clinical model systems used to identify metabolic targets for patient imaging studies.
- Awareness of ^1H & hyperpolarized ^{13}C MRI metabolic imaging methods that can be used to assess tumor metabolism and the types of clinical questions that can be addressed.

UCSF

Metabolic studies using hyperpolarized ^{13}C

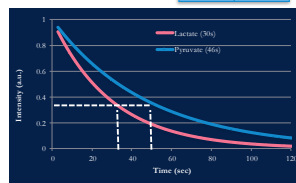
Characteristics of a successful probe

- T_1
- Rapid enzymatic conversion
- Swift transport across membranes
- Preservation of polarization during biochemical reaction
- Metabolite pool sizes

$[1-^{13}\text{C}]$ pyruvate	
T_1	Field
67	3T
48	11.7T
44	14.1T

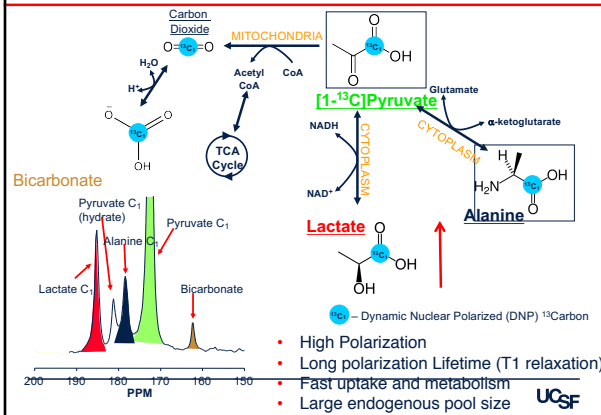
Application

- Receptor ligand binding
- Metabolism
- Perfusion and permeability

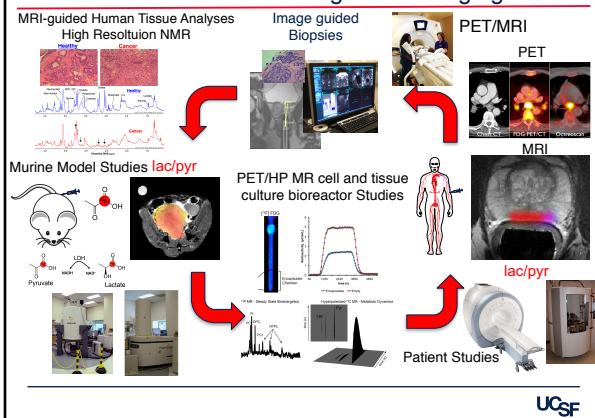


UCSF

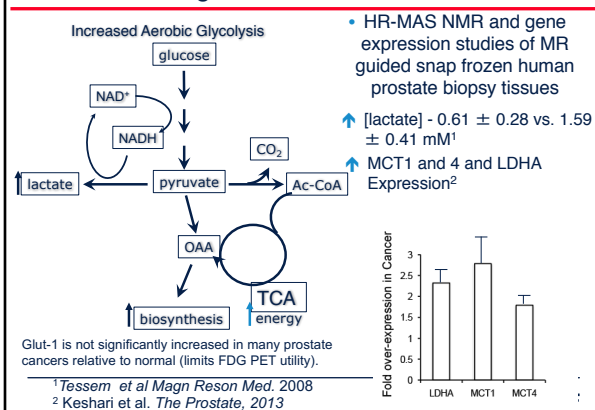
Hyperpolarized [1-¹³C]Pyruvate Metabolism



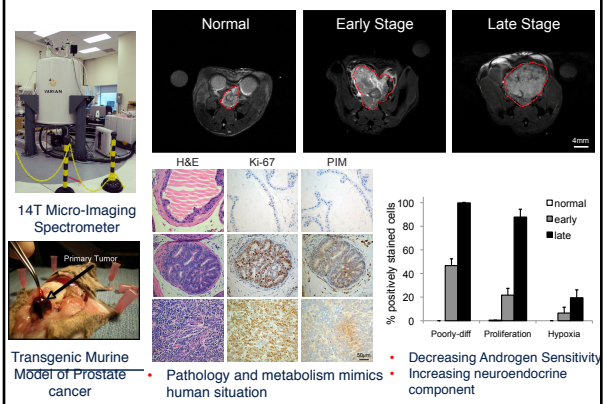
Identification of Metabolic Targets for Imaging Studies



Warburg Effect in Prostate Cancer?

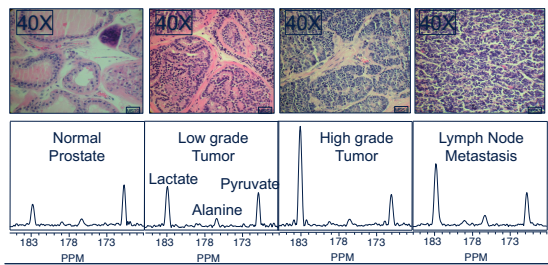


Metabolism in a Murine Model of Prostate Cancer



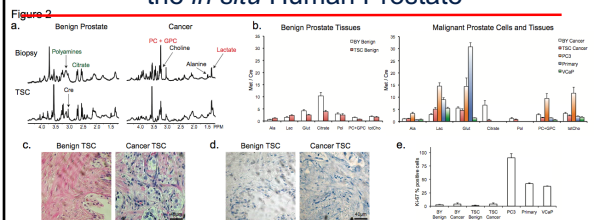
Pathologic Grade Monitored by HP ^{13}C MR

- Lactate significantly increased with progression from early to late stage/poorly differentiated prostate cancer and metastases in a transgenic mouse model of prostate cancer.



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

Prostate Tissue Slice Cultures – Better Reflect the *in situ* Human Prostate

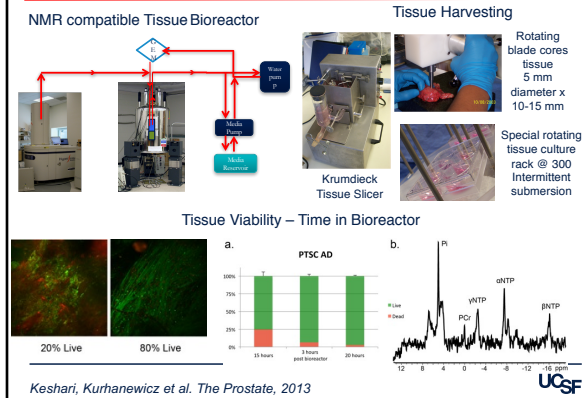


- The metabolic profile of prostate TSCs better reflect what is observed in snap frozen human biopsy samples than pre-clinical cell lines (a and b)
- The tissue slices capture the pathologic complexity of the human prostate and interactions between cancer and surrounding stroma (c).
- Critical in therapeutic investigations – Malignant TSC's proliferate at similar lower rates as *in situ* human cancer (d and e)

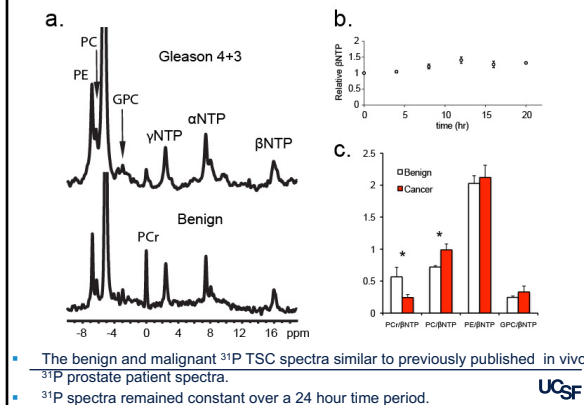
Keshari, Kurhanewicz et al. The Prostate, 2013

UCSF

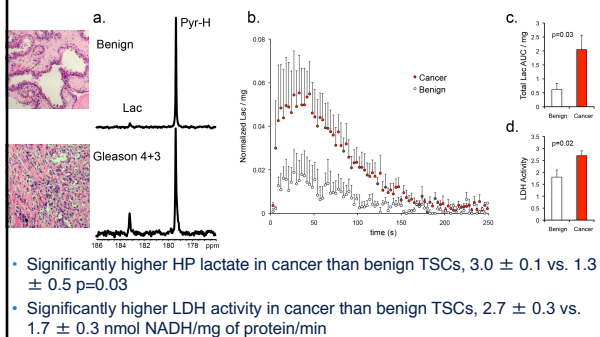
More Realistic Models-Human Tissue Slice Culture Bioreactor

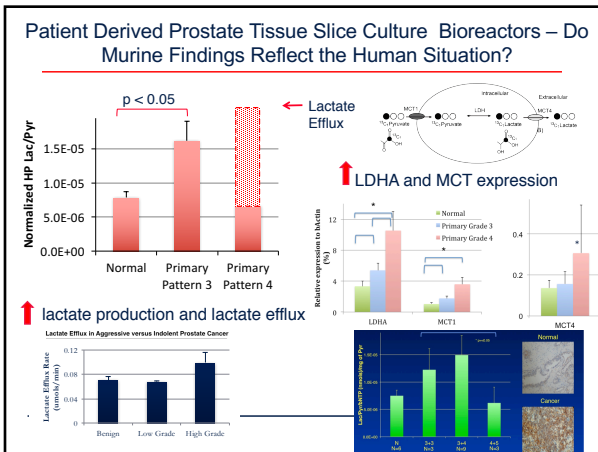


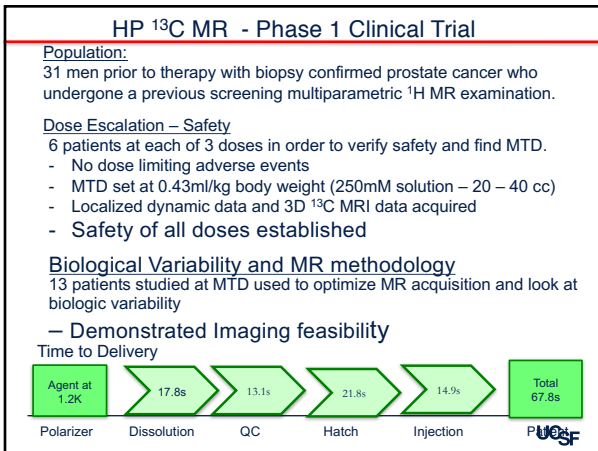
^{31}P NMR: Human Tissue Slice Culture Bioreactors

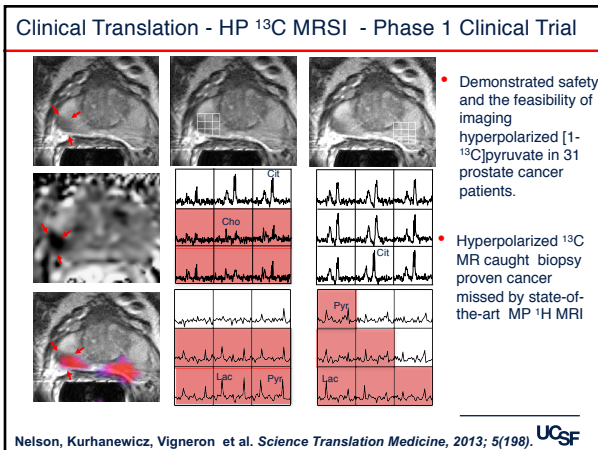


Hyperpolarized Lactate: Human Tissue Slice Culture Bioreactors



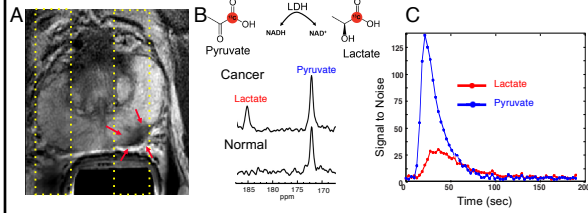






Dynamic Hyperpolarized ^{13}C MR - Phase 1 Clinical Trial

Metabolic time course of Pyruvate Uptake and Metabolism



2-D Localized Dynamic (every 3 sec.) Pyruvate and Lactate Data

- Negligible hyperpolarized lactate produced in benign prostate.
- Cancer time course - pyruvate uptake 20 seconds after injection - maximum pyruvate signal at 27s and maximum production of lactate at 45s in cancer.
- Reproducibility of uptake a metabolism (current clinical trial).

UCSF

Probe considerations (Pyruvic acid)

Choice of labeling position

• T1

• Metabolic pathways of interest –

– Organ dependent

- PC flux in liver observed with $[1-^{13}\text{C}]$ pyruvate

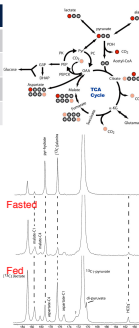
Montell ME et al. Proceedings of the National Academy of Sciences 2011;108:19084–19089.

– Pathology determined

- Glycolysis in tumors measured with $[1-^{13}\text{C}]$ pyruvate
- PDH flux detectable using $[2-^{13}\text{C}]$ or $[1,2-^{13}\text{C}_2]$ C1,C2 Pyruvate

Shiran R, Kuchanawit J, Vigneron DB. Hyperpolarized Carbon-13 MRI and MRS Studies. online.library.wiley.com/Chichester, UK: John Wiley & Sons, Ltd, 2007. doi: 10.1002/9780470520000.ch10

T1 (S) @ 3T	
$[1-^{13}\text{C}]$ Pyruvate	67
$[2-^{13}\text{C}]$ Pyruvate	39
$[1-^{13}\text{C}]$ Lactate	50
$[1,2-^{13}\text{C}_2]$ Pyruvate	44



UCSF

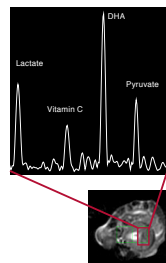
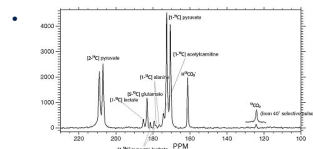
Multi-Pol

• Simultaneous polarizations of more than 2 substrates

- Spectral overlap
- Spectral bandwidth limitation

• Concurrent metabolism + perfusion information

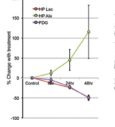
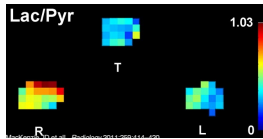
• Multiple metabolic readout



UCSF

Application of [1-¹³C]Pyruvate

- Tumor grade
- Small molecule treatment response, Radiation, chemotherapy
- PET comparison
- Inflammation
- Genetic marker – IDH mutation
- Clinical application!



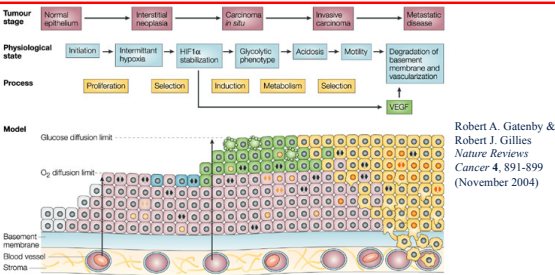
UCSF

Successful probes

Function	Substrates
Metabolism	Pyruvate, Lactate, Glucose, Fructose
Interstitial pH	Bicarbonate
Cellular necrosis	Fumarate
Fatty acid metabolism	Acetate, butyrate, propionate
Redox	Ascorbate
Perfusion	Urea, HPO1, t-butanol
Amino acid metabolism	α -Ketoisocaproate, α -ketoglutarate, glutamate
Cellular proliferation	Glutamine

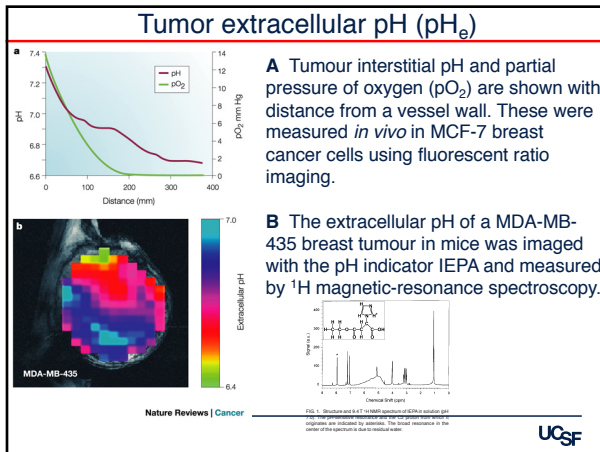
UCSF

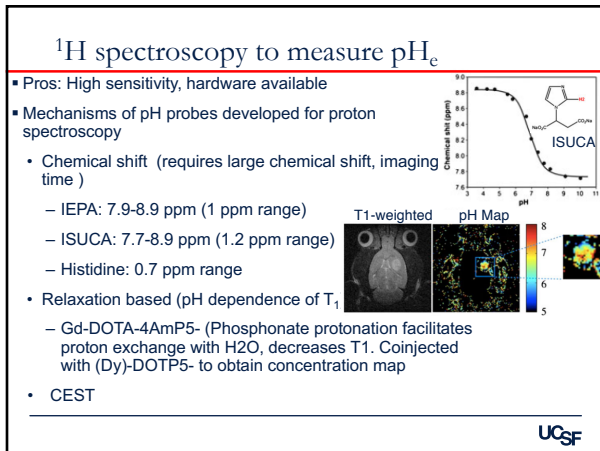
The Stages of Epithelial Tumour Growth and Metabolism

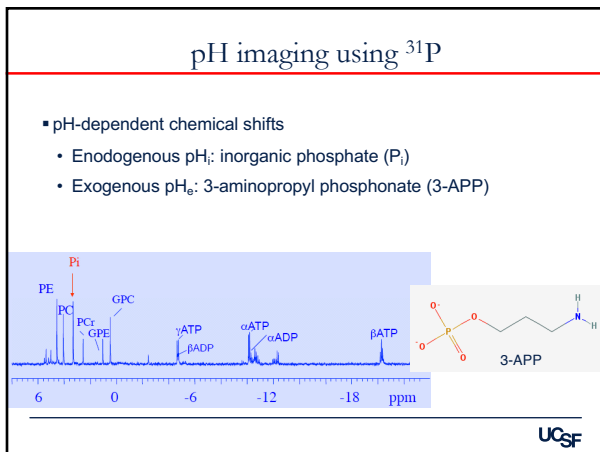


Normal epithelial cells (grey) become hyperproliferative (pink) following induction. As they reach the oxygen diffusion limit, they become hypoxic (blue), which can either lead to cell death (apoptotic cells shown with blebbing) or adaptation of a glycolytic phenotype (green), which allows cells to survive. As a consequence of glycolysis, lesions become acidotic, which selects for motile cells (yellow) that eventually breach the basement membrane.

UCSF

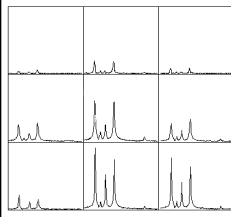






HP Pyruvate and Bicarbonate - Acidic Interstitial pH

Increased tumor lactate → Increased MCT4 expression → Acidic interstitial pH



pH from peak areas

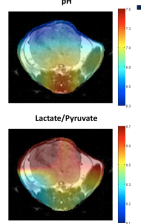
$$pH = pK_a + \log_{10} \left(\frac{[HCO_3^-]}{[CO_2]} \right)$$
 (pKa = 6.17)

6.92	7.15	6.98
7.22	7.11	7.12
7.28	7.37	7.30

Gallagher FA,
 et al., Nature
 2008;453:940-944

Estrella, V et al, Cancer Res 73,
 1524-1535; Gatenby, R. A., and
 Gillies, R. J. (2004) Nat Rev Cancer
 4, 897-899.

Wilson, Keshari, et. al. Magn Reson. 2010; 205(1),141-157

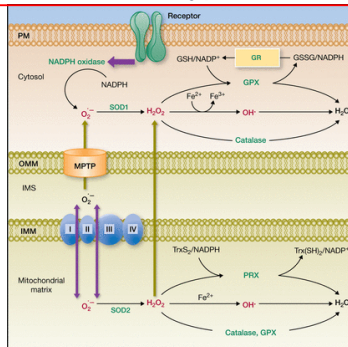


- toxic to normal cells
- promotes a degradation of the extracellular matrix by proteinases
- increases angiogenesis through the release of VEGF
- inhibits the immune response to tumor antigens.

UCSF

The Cancer Metabolic Phenotype - ROS

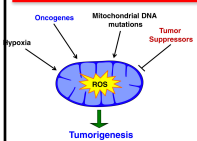
- ROS= reactive oxygen species
- Source— mitochondrial electron transport chain
- Major ROS species - Superoxide and hydrogen peroxide
- Antioxidant scavenges ROS (vit C, GSH/NADPH, Vit E)



Nogueira, V., & Hay, N. (2013). Molecular Pathways: Reactive Oxygen Species Homeostasis in Cancer Cells and Implications for Cancer Therapy. Clinical Cancer Research: an Official Journal of the American Association for Cancer Research, 19(16), 4309-4314.
<https://doi.org/10.1158/1078-0432.CCR13-1434>

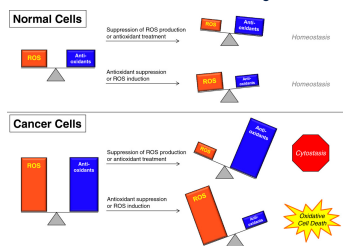
UCSF

ROS & Cancer



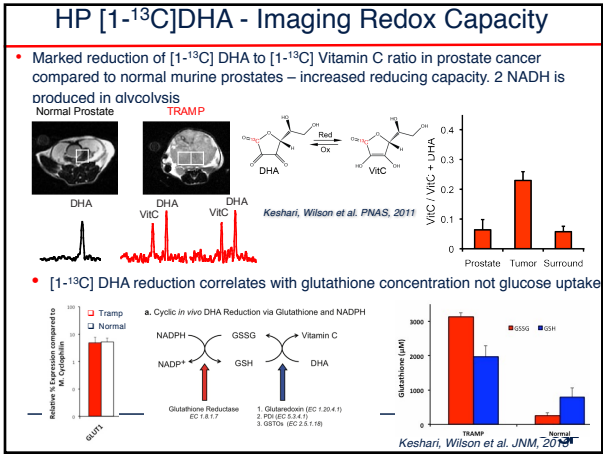
- Fine balance of cellular redox capacity
- In cancer → persistent prooxidative state due to highly metabolic state
 - ROS overproduction
 - Low levels or inactivation of antioxidant mechanisms

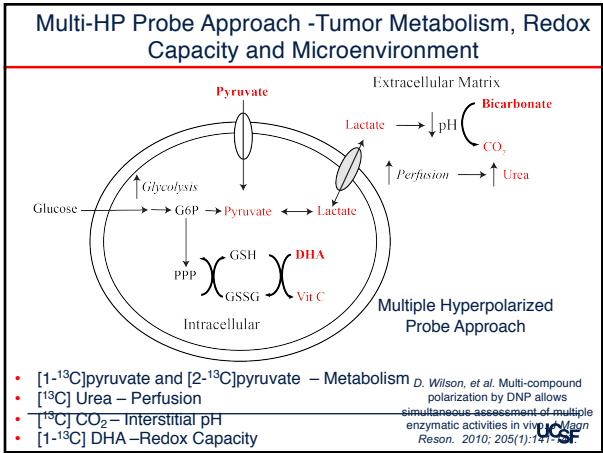
Mitochondrial ROS increases → tumorigenesis.



- ROS contributes to cancer initiation/proliferation
- protumorigenic signaling pathways

UCSF







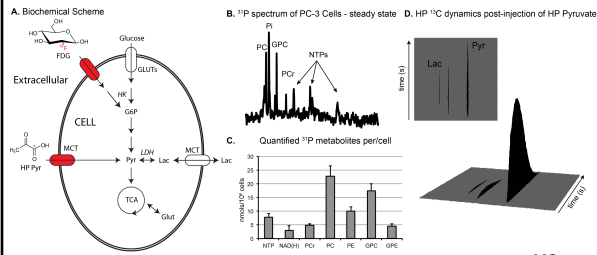
Metabolism Summary

- There is a significant interplay between the tumor micro-environment and metabolism with the hypoxic micro-environment up-regulating HIF-1a and LDH-A expression and LDH activity resulting in increased production and efflux of lactate potentially promoting progression and metastases.
- Therapeutic resistance of advanced prostate cancer is associated with an emergence of cancer phenotypes (CPRC, NEPC) that rely more heavily on glycolysis and glutaminolysis.
- PET and MRI molecular imaging techniques can be used to exploit these metabolic changes to detect and assess the aggressiveness and therapeutic responsiveness of prostate cancer in individual patients – personalized medicine.
- There may be opportunities to target cancer-specific metabolism as a means of therapy.
- Realistic pre-clinical models and sufficient correlative pathologic, metabolomic, genomic and proteomic studies are necessary to understand and clinically translate the findings from pre-clinical metabolism studies.

UCSF

Metabolism Targeted Therapy - Inhibition of NAD⁺

- (A) Biochemical Scheme of cellular metabolism when utilizing hyperpolarized [¹³C] pyruvate (HP Pyr) and [¹⁸F]-deoxyglucose (FDG)
 (B) Representative ³¹P NMR spectra of 15 x 10⁶ living PC-3 cells within the 5mm MR/PET bioreactor.
 (C) Quantitation of the ³¹P NMR spectra.
 (D) ¹³C NMR spectra acquired on PC-3 cells post injection of 7mM HP Pyr (3 sec temporal resolution) in a MR/PET compatible bioreactor.



Keshari KR, Wilson, DM, Van Criekinge, M. "Metabolic Response of Prostate Cancer to Nicotinamide Phosphoribosyltransferase Inhibition in a Hyperpolarized MR/PET Compatible Bioreactor" submitted JNM 2014.

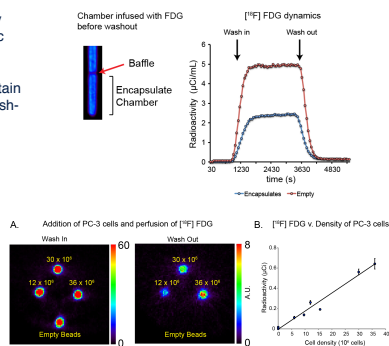
Metabolism Targeted Therapy - Inhibition of NAD⁺

Injection of FDG into an empty chamber and the characteristic wash-in/out profile.

Empty encapsulates do not retain the FDG tracer signal after wash-out.

- A. Imaging of 4 bioreactors containing increasing # of cells. (left) a wash-in of FDG of 40 min and analogous wash-out of 40 min.

- B. (B) Plot of accumulated radioactivity after wash-out of the tracer, with a linear correlation ($R^2=0.98$), as expected for increasing cell number. s



UCSF

Metabolic Monitoring of Inhibition of NAD⁺

Demonstration of translatable biomarker response in PC-3 cells by NAMPT inhibition using GNE-617, which prevents the regeneration of NAD⁺.

- (A) HP ¹³C NMR spectra - decrease in HP Lactate with treatment and increase in HP Alanine.
- (B) decrease in FDG accumulation - changes in glycolysis due to NAMPT inhibition.
- (C) mRNA expression of cells with treatment - reaffirming that the biomarker changes observed are due to a decrease in the cofactor NAD

