Gluconeogenesis and the pentose cycle impact deuterium depleted water efficacy in anticancer therapeutics

László G. Boros1-3, Howard E. Katz4, Justine P. Roth5, Gábor Somlyai6

1Department of Pediatrics, Harbor-UCLA Medical Center, UCLA School of Medicine, Los Angeles, California, USA, 2 The Los Angeles Biomedical Research Institute (LABIOMED), Torrance, California, USA, 3 SIDMAP, LLC, Culver city, California, USA, 4Department of Materials Science & Engineering, Whiting School of Engineering, Johns Hopkins University, Baltimore, Maryland, USA., 5Departament of Chemistry, Johns Hopkins University, Baltimore, Maryland, USA., 6HYD, LLC for Cancer Research & Drug Development, Budapest, Hungary, European Union (EU)

Increased glucose decarboxylation during nucleic acid ribose synthesis occurs at the expense of glycolysis. This pathway is coupled to the recycling of drinking water, which passes through the cytoplasm after absorption. Consumption of water from the cytoplasm and cellular matrix, upon ring-opening hydrolysis in the pentose cycle, results in the production of 6-phospho-D-gluconate. Oxidation at C1 of this substrate, together with NADP+ reduction, transforms mammalian cells into megalo- and sideroblastosis with “unstable” DNA [1-3]. On the other hand, gluconeogenesis is coupled to mitochondrial substrate oxidation and the recycling of “matrix water”, which passes by substrate shuttling during respiration and hydration of metabolites in mitochondria. Results will be presented which suggest that mitochondrial matrix water (re)cycling [4, 5] during oxidation of ketogenic substrates, low in deuterium content [6], reverses the pentose cycle-transformed tumorous phenotype [4]. The anti-cancer drug Avastin® requires mitochondrial complex-I activation with normal mitochondrial morphology [7] for efficacy, which demonstrates the substitution of natural abundance water by deuterium depleted water (DDW) produced in the mitochondrial matrix. Stable isotope depletion is also involved in the efficacy of Glivec®, which is associated with increased fatty acid oxidation through the tricarboxylic acid (TCA) or Krebs cycle [8-10]. This cycle produces energy in the form of adenosine triphosphate (ATP) via the aerobic oxidation of acetate derived from carbohydrates, fats and proteins. In addition to CO₂, the reduced nicotinamide cofactor and precursors of certain amino acids are products. Clearly, the central importance of the TCA cycle in many biochemical pathways makes it a critical target for controlling cellular metabolism, growth and survival. Using DDW in place of natural abundance water and increasing deuterium by even 1 parts per million (ppm) for every 8 hours in cultures impedes the growth of MIA-PaCa and MCF-7 cancer cell lines (xCELLigence System - Roche). The effect is thought to derive from interfering with the pentose cycle and subsequently altering the structural integrity of cellular DNA in tumor cells. Further, it is proposed that “metabolic” matrix water production, upon ketogenic substrate oxidation, precipitates the DNA strand-breaking event at the accessible surface areas of deoxyribose [11] via gluconeogenesis. It follows from observations, with the anti-cancer drug Rosiglitazone®, that isotope depletion in the TCA cycle and “metabolic” water affects the deuterium content at the C5′-C3′ hydrogenic positions (Fig 1-2) of the nucleotide sugar fragment, after fatty acid chain shortening and redistribution of low-deuterium acetyl-CoA [12, 13].

The observations link DDW, containing 100, 50 and 25 ppm deuterium, which is 155 ppm in natural abundance water (non-DDW) to limited tumor cell growth and extension of median survival time in patients with inoperable advanced pancreatic cancer from 6 months with chemotherapy alone to 40 months, when DDW is added as an adjuvant within 60 days after diagnosis in an open label trial. The clinical and experimental data implicate exchange of pentose cycle-derived triose fragments (C1-C3 to C3-C5) into ribose and deoxyribose [14], and the nucleic acid sugar backbone as a sink for deuterium originating from cytoplasmic water.

KEYNOTE PRESENTATION - http://www.deuteriumdepletion.com/
These reactions involving carbon and hydrogen bonds give rise to apparent (and potentially complex) isotope effects [15-17], which might be exploited in anti-cancer chemotherapy [18, 19]. Additional applications include intramolecular deuterium disequilibrium as the result of deuterium depleting mechanisms linked with isomerase function [20] as novel NMR based biomarkers to determine risks for aneuploidy, as well as response to various deuterium depleting therapies in disease. Water distribution in tumors in comparison with that of surrounding tissues, by means of magnetic resonance imaging (MRI), is routinely observed by the deuterium oxide (D₂O)/water (H₂O) contrast, where D₂O occupying tumors cause a reduction of the signal when ¹H detection is used during flow velocity studies. Perfusion with D₂O may reduce water signals to minimum, which can subsequently be renewed with distilled low deuterium containing H₂O in various models [23]. Therefore, the replacement of low deuterium-containing metabolic matrix water due to impaired mitochondria with that of more D₂O enriched water from the circulation in the cytoplasm of cancer cells can potentially induce similar losses of the proton signal, whereby the resulting D₂O/H₂O contrast obtained with MRI contributes an effective clinically applied metabolic biomarker tool to studying tumors within various organs.
**Figure 1.** Comparison of metabolic profile changes associated with 1) natural deuterium depletion by low deuterium fatty acid oxidation. Avastin®, Glivec® and Rosiglitazone® exert similar effect and require intact mitochondria for efficacy [7, 9, 13] (Red boxes No1), and 2) low deuterium metabolic water recycling from the mitochondrial matrix during citrate, isocitrate and malate formation; the target of fumarate hydratase activation [4, 5] and hyperbaric oxygen treatment combined with a ketogenic diet [21] (Red box No2). Mitochondrial shuttles, such as the malate shuttle, pass low deuterium carrying fatty acid carbons to gluconeogenesis, where glyceraldehyde-3-phosphate becomes the source of extensive carbon exchange reactions [14] for the non-oxidative pentose cycle to maintain low deuterium saturation in C3’-C5’ pentose sugar carbon positions (Red box No3). These are the carbon sites where DNA stability, radiation- and chemotherapy derived hydroxyl radical sensitivities are regulated by hydrogen/deuterium [11] due to primary and secondary intrinsic isotope effects; as well as partially by proton tunneling [15-17]. Besides the C3’-C5’ nucleic acid sugar backbone fragment, de novo nucleic acid base syntheses, hydrogen bonding and deuterium channeling into hydrogen bonds are controlled by the serine oxidation glycine cleavage single carbon cycle pathways [22; SOGC] (Red box No4). When tumor cells revert to the Warburg phenotype and reductive carboxylation-driven mitochondria, deuterium depletion in free (drinking) water becomes an effective deuterium depleting mechanism for specific carbon sites in nucleic acid backbone sugars and the bases (Red box No5). (Blue arabic numbers are enzyme identifiers also found in ref# [12])
Figure 2. Lobry de Bruyn–Alberda–van Ekenstein aldose-ketose transformations via glucose-phosphate isomerase (GPI), triose phosphate isomerase (TPI) and other similar reactions in the non-oxidative pentose cycle deplete deuterium for various ribose and deoxyribose carbons in the presence of deuterium depleted water in cytoplasm, labeled in red squares. The 5th and 3rd carbons, with depleted deuterium, especially, in DNA deoxyribose, enhance physiological (UV light induced) and chemo- and/or radiation induced hydroxyl attacks to potentially assist anticancer therapies ref# [11].
References:


**Author Information:**

Correspondence and requests for materials should be addressed to L.G.B. (boros@labiomed.org; boros.laszlo@yahoo.com) (USA)

**Key Words:**
deuterium depletion, mitochondrial matrix metabolic water, deuterium-depleted water, NADPH, oxidative pentose cycle, median survival time, unresectable pancreatic cancer, open label study, targeted tracer fate associations study, targeted deuterium fate association study, TTFAS, TDFAS

Additional ¹³C tracer and data interpretations can be found on the World Wide Web at http://youtu.be/Pms6k9AQ3bQ regarding mammalian cell mitochondria.