Fumarate hydratase and deuterium depletion control oncogenesis via NADPH-dependent reductive synthesis*

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Introduction

Tumor cells become oxidative pentose cycle, therefore cytoplasmic free water dependent, when their defective mitochondria disrupts low deuterium metabolic water use from the matrix and they switch to consuming pentose cycle-derived NADPH for reductive DNA and nuclear membrane fatty acid synthesis. Metabolic water by complete fat oxidation, coupled with complex-IV, contains low average ~115 ppm (parts per million) deuterium due to deuterium discrimination during photosynthesis in food. We herein report that monodeuterated, 100 ppm, 50 ppm and 25 ppm extracellular (free) water treatment[1] significantly decrease nucleotide and nuclear membrane sterol and lignoceric acid synthesis in comparison with natural 150 ppm deuterium containing water via the oxidative branch of the pentose cycle. This recapitulates metabolism after genetically restored mitochondrial fumarate hydratase in clear cell kidney tumors and mimic the metabolic impact of Glivec (imatinib mesylate, STI-571, Gleevec) and metformin treatments in other tumors.

Model & Methods

Cytotoxic effects of DDW containing D at a concentration of 25 parts per million (ppm), 50 ppm, 105 ppm and the control (150 ppm) in MIA-PaCa-2 pancreatic cancer cells were monitored by the real-time cell impedance detection xCELLigence method. Nuclear membrane turnover and nucleic acid syntheses were determined by targeted [1,2-^{13}C$_2$]-D-glucose fate associations and compared with Glivec, restored fumarate hydratase and Metformin effects, respectively, in the metabolic network as shown below.[1,2]

Cellular metabolic effects of Glivec (Gliv) and Metformin (MET)

Natural deuterium depletion for NADPH and reductive synthesis via mitochondria is restored by mitochindrial fumarate hydratase, Glivec and metformin
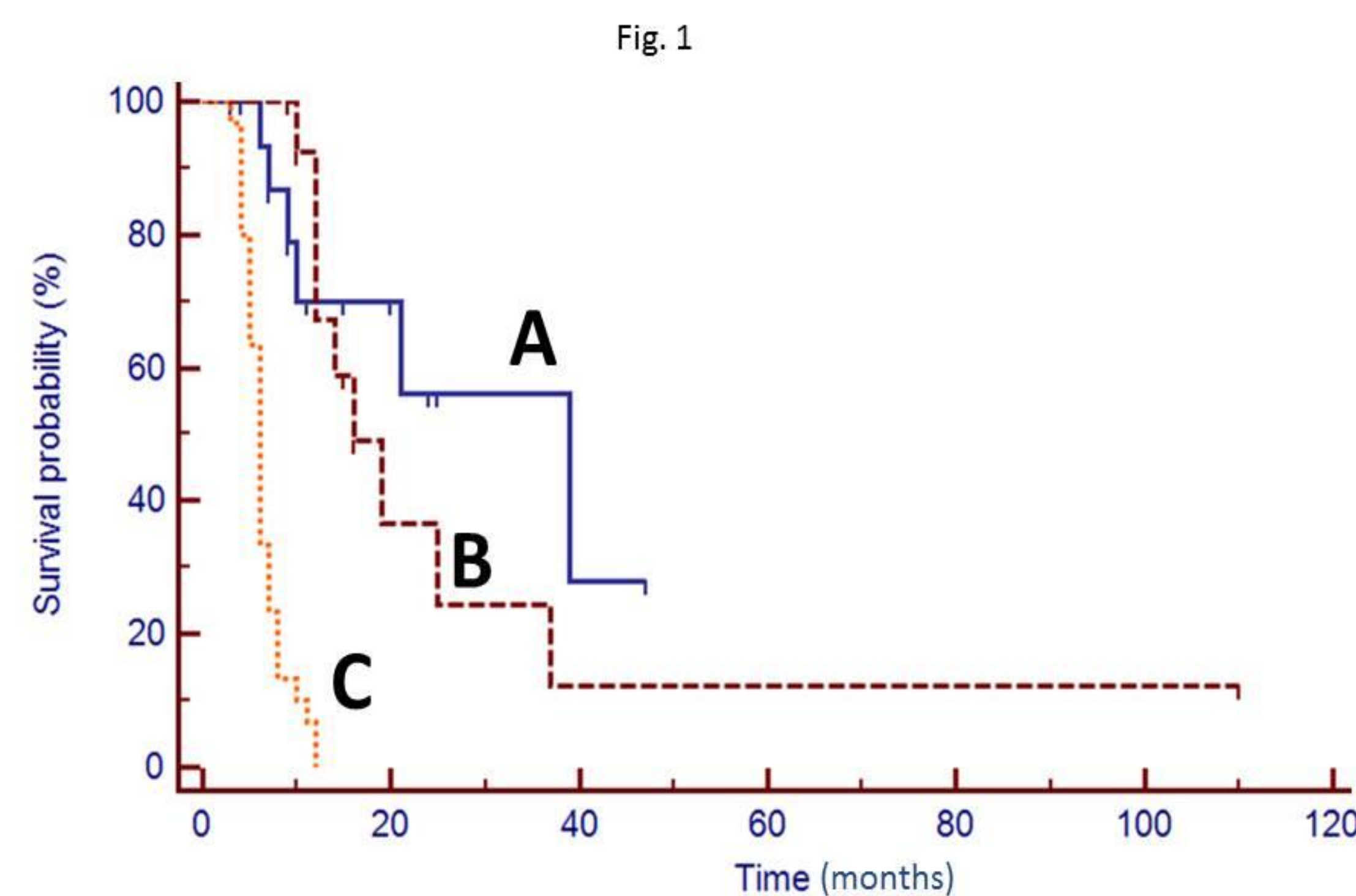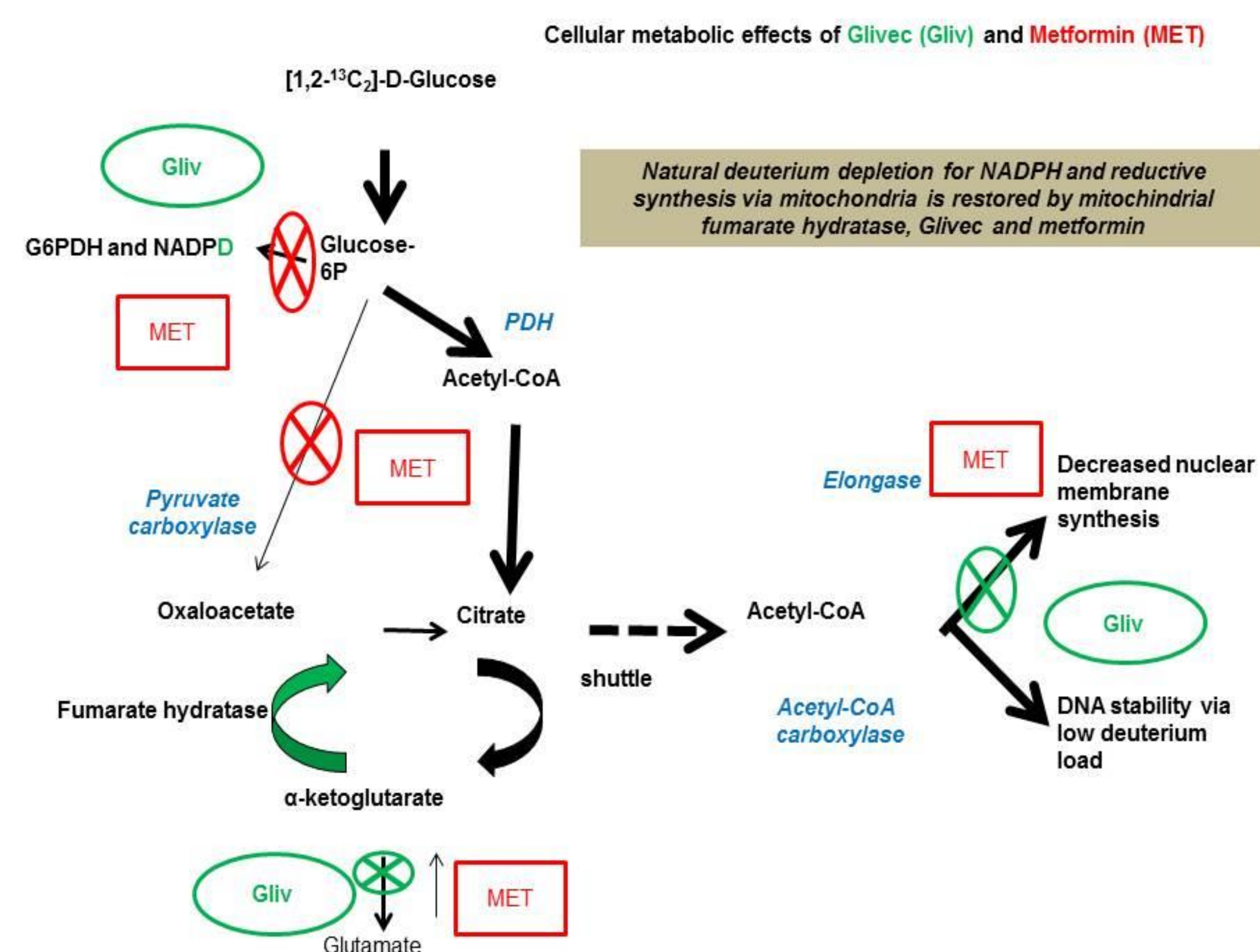
Patients with inoperable PaCa

The median age of the 32 DDW treated patients was 61.5 years. Median duration of the treatment with DDW was 6.64 months (85 ppm, 65 ppm and 45 ppm regimen DDW). The treatment period showed a high standard deviation (SD: 12.24 months), because 9 patients (28%) consumed DDW for an extended period, more than one year. Patients started the DDW treatment at different time points at their free consent after diagnosis, which was recorded. The median elapsed time between diagnosis and entering the trial was 1.16 months (SD: 4.06 months). When data sheets were closed for standard matrix diagnostics, data processing and abstracting, in order to meet study deadline objectives (June 2011), 17 patients were still alive and offered continued DDW treatment; therefore they are included in this report with their applicable (at least) mean survival time upon study termination.

The median age of the 30 control natural deuterium-containing water consuming patients was 65.9 years (SD=9.05 years) (Female: n=16, Average age: 67 years, SD=9.24 years; Male: n=14, Average age: 64.3 years, SD=8.9). Histology in all cases confirmed pancreatic adenocarcinoma and all patients received treatment with standard gemcitabine (Gemzar 1000 mg/m² as an intravenous infusion) combined with 5 fluorouracil consistent with the EU gastrointestinal adenocarcinoma disease management protocol.

Fig. 1

FIGURE 1. *Kaplan-Meier estimator curve of survival probability* from diagnosis in A) 18 pancreatic cancer patients DDW trial within 60 days after diagnosis (MST: 39 months) with deuterium depleted water and in B) 14 pancreatic cancer patients with deuterium depleted water (DDW) joining the trial after 60 days of diagnosis (MST: 16 months). We transferred for clarity the C) 30 pancreatic cancer patients with natural deuterium containing water consumption (MST: 6.88 months in females and 5.86 months in males). All groups underwent standard cytostatic treatment due to an unresectable disease .

Results

FIGURE 2. Scattered plot of *daily DDW intake* and serum D concentrations with y=0.9873x+27.177 slope and intercept. (n=10; R²=0.85; after 7 days equilibrium between plasma, interstitial and intracellular water pools)
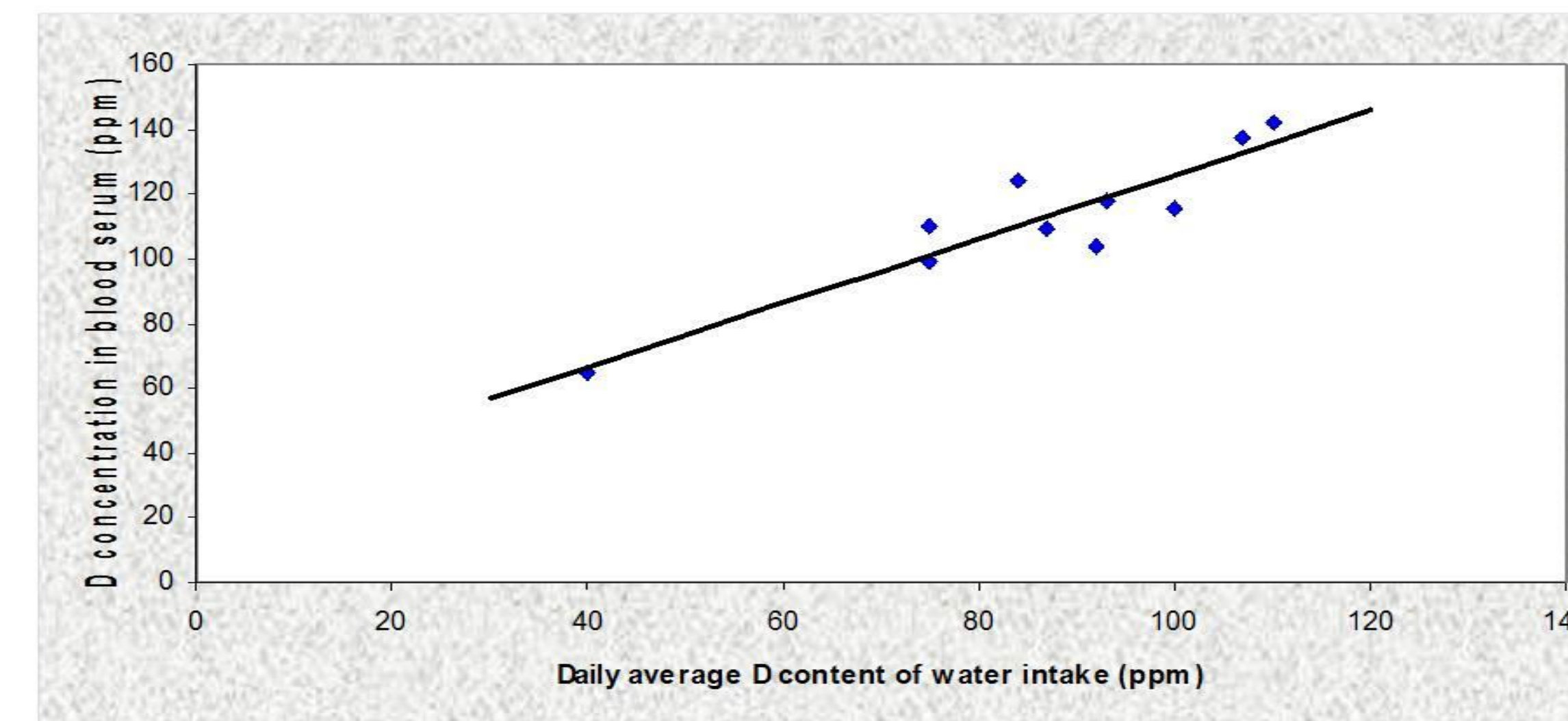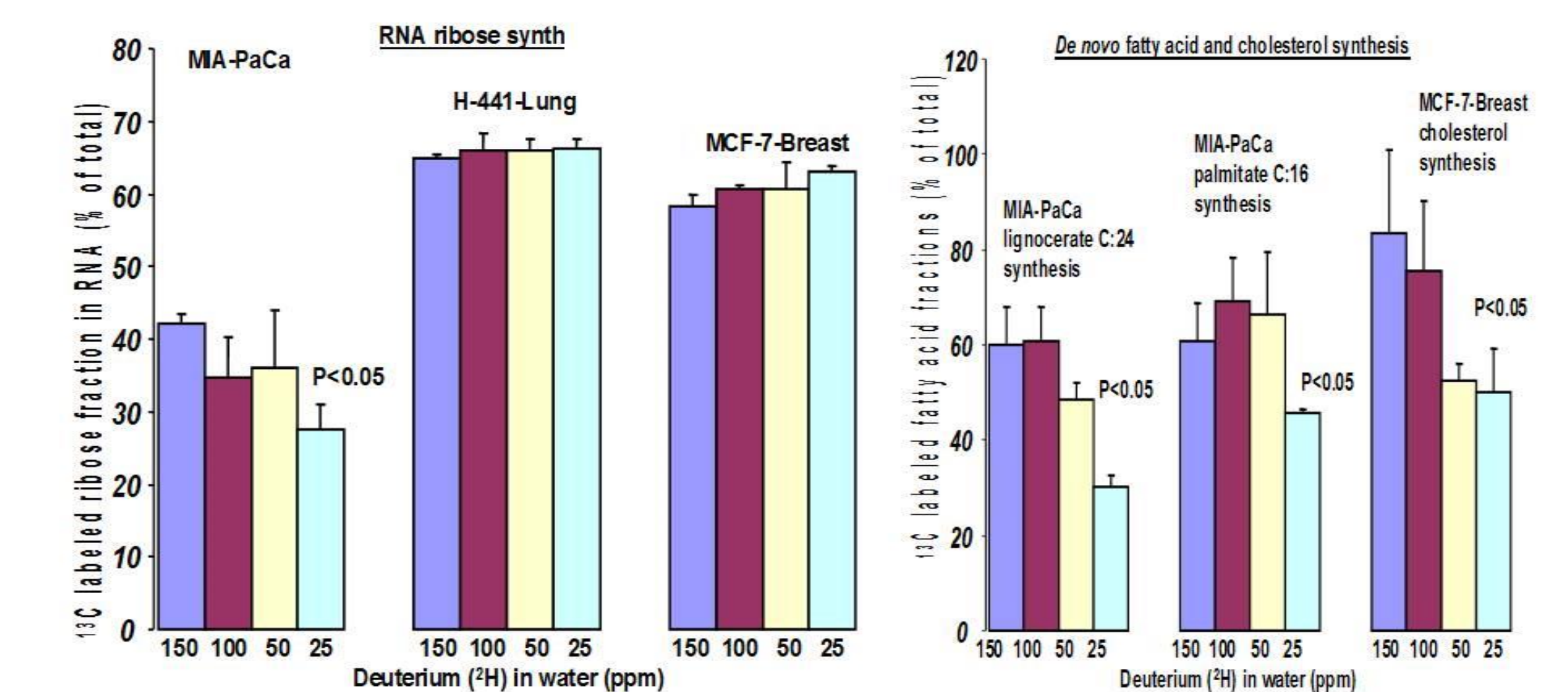

FIGURE 3. Decreased *cell impedance and count* (Cell Index) after treatment with low concentrations of D (25 ppm, 50 ppm or 105 ppm) in MIA PaCa-2 pancreatic cancer cells (n=3 +SD; *p<0.05 in DDW treated cultures in comparison with 150 ppm control)
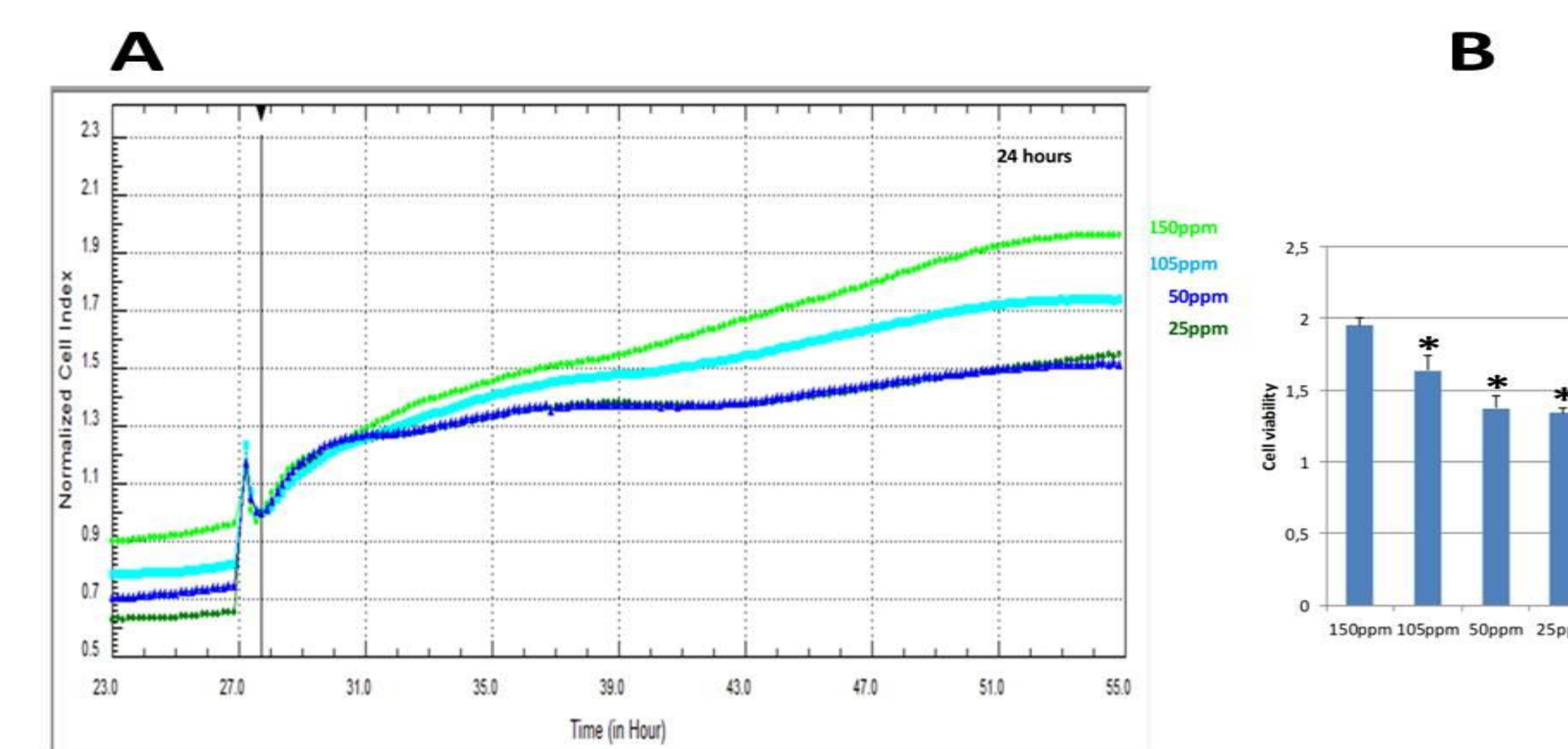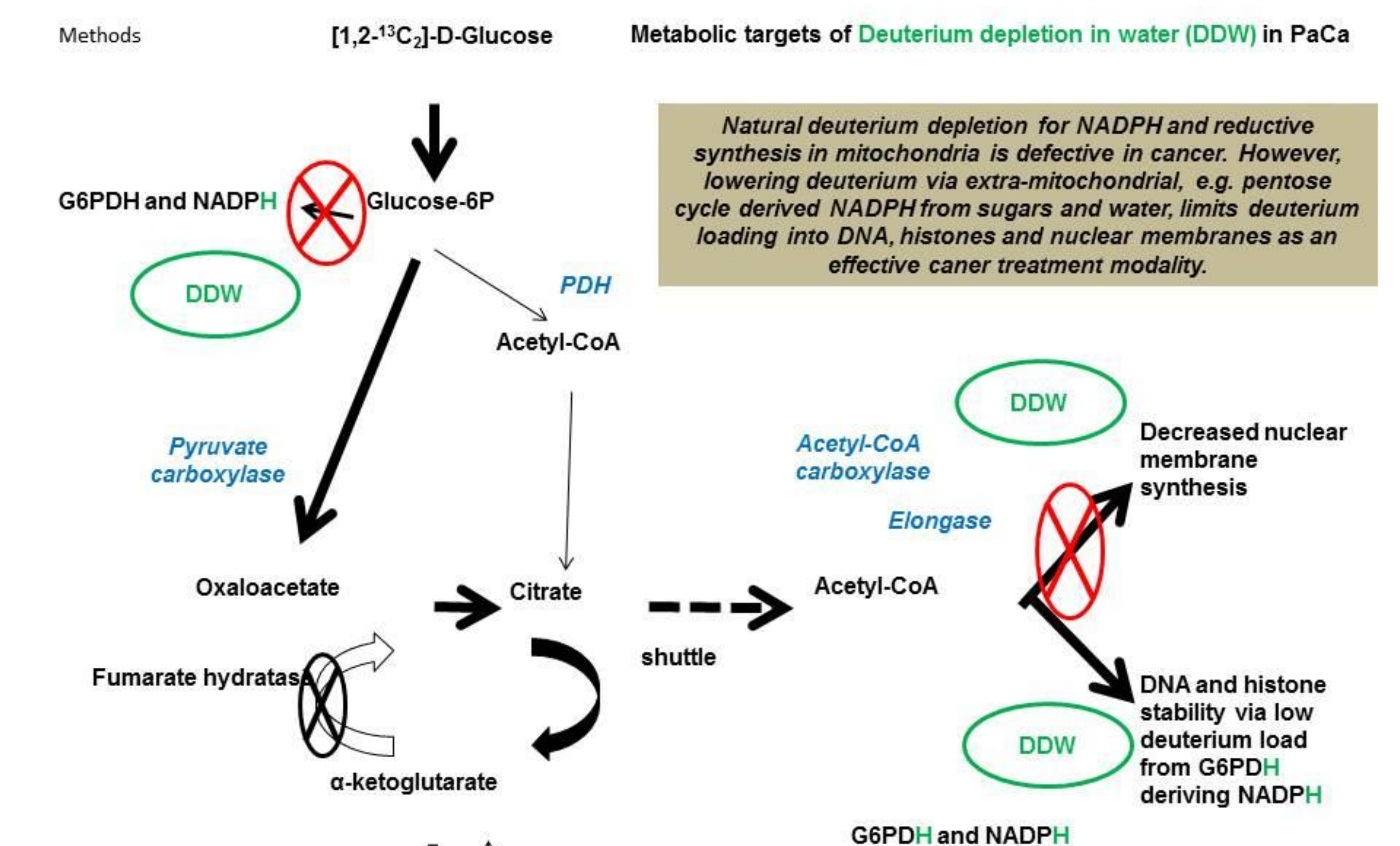

FIGURE 4. *Cisplatin* at 20 μM, 40 μM concentration, for 24hs, 48hs or 65hs inhibited cell viability of MIA PaCa-2 pancreatic cells dose dependently. (n=3 +SD; *p<0.05 in DDW treated cultures in comparison with 50 ppm control; #p<0.05 in DDW treated cultures in comparison with 20 μM cisplatin).
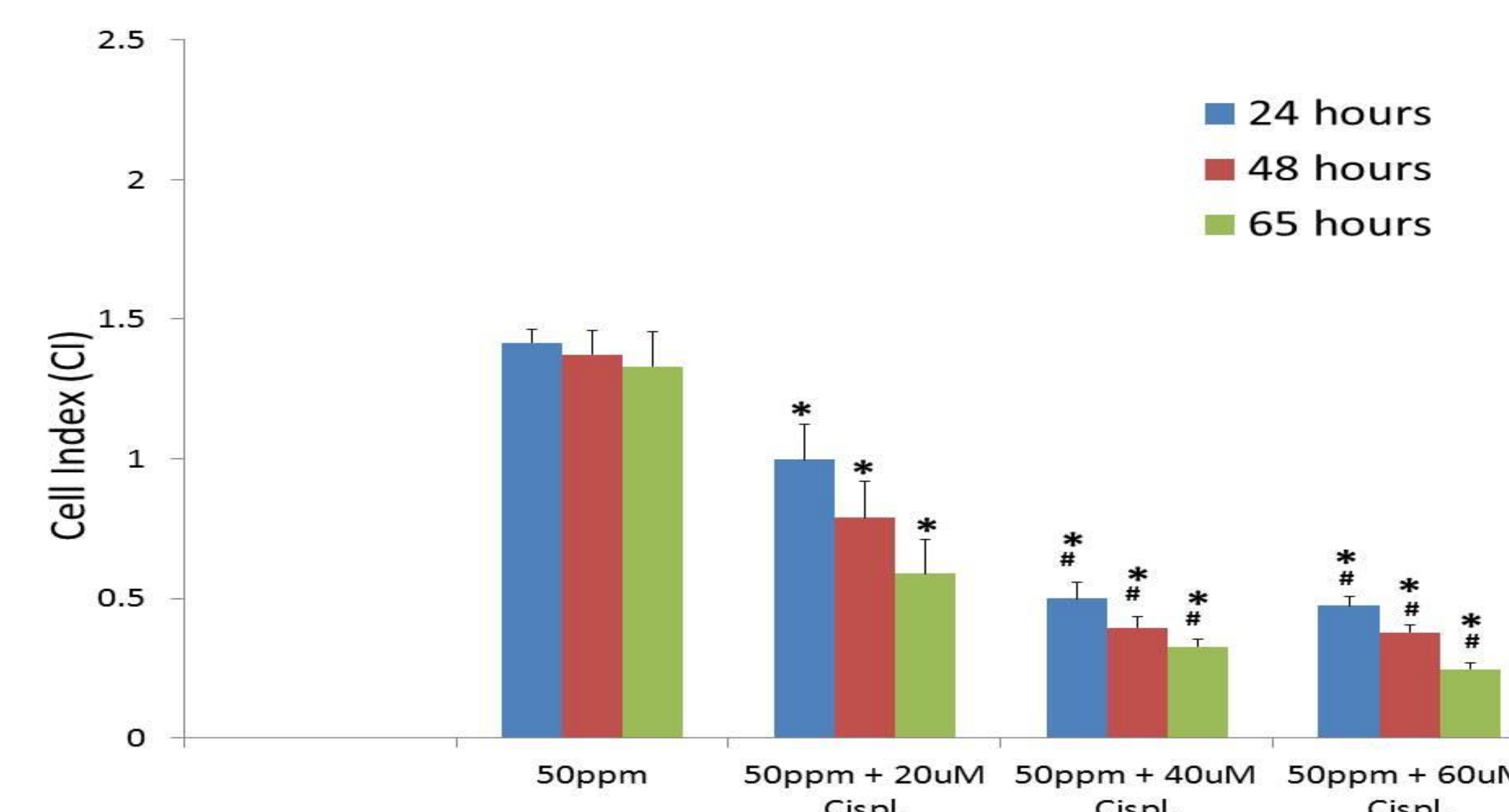
FIGURE 5. Limited *RNA ^{13}C labeling* is closely associated with the limited synthesis of nuclear membrane lignocerate (C24:0) in MIA-PaCa and cholesterol in breast cancer cells.

Conclusion

Natural deuterium depletion for NADPH and reductive synthesis in mitochondria is defective in cancer. However, lowering deuterium via extra-mitochondrial, e.g. pentose cycle derived NADPH from sugars and water, limits deuterium loading into DNA, histones and nuclear membranes as an effective cancer treatment modality.

All extra-mitochondrial NADPH synthesis pathways are targeted by DDW; particularly that of the direct glucose oxidizing arm of the pentose phosphate pathways, cytoplasmic fumarate hydratase and the serine oxidation glycine cleavage reactions to repair oncogenic NADPH producing glycolysis. Deuterium depleted sugars and water may be included as non-toxic anti-cancer treatment modalities for prevention and treatment.

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2. Cantoria MJ, Boros, L. G., Meuillet, E. J. Contextual inhibition of fatty acid synthesis by Metformin involves glucose-derived acetyl-CoA and cholesterol in pancreatic tumor cells. *Metabolomics.* 2013. doi: 10.1007/s11306-013-0555-4
3. Boros LG, Lee WN, Cascante M. Imatinib and chronic-phase leukemias. *N Engl J Med.* 2002: 347(1): 67-68. doi: 10.1056/NEJM200207043470106