Study title: Inhibition of Warburg effect with a novel combination of dichloroacetate and niclosamide for therapy in malignant pleural mesothelioma

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Background: Inhalation of asbestos fibers is the most common cause of malignant pleural mesothelioma (MPM). In 2004, the United States Food and Drug Administration approved a combination of cisplatin with pemetrexed to treat unresectable MPM. Nonetheless novel treatment is urgently needed. The objective of this study is to report the combination effect of dichloroacetate (DCA) or niclosamide (Nic) Nic in MPM.

Materials and methods: The effect of a combination of DCA and Nic was studied using a panel of MPM cell lines (H28, MSTO-211H, H226, H2052, and H2452). Cell viability was monitored by MTT assay. Glycolysis, oxidative phosphorylation, glucose, glycogen, pyruvate, lactate, citrate, succinate and ATP levels were determined by corresponding ELISA. Apoptosis, mitochondrial transmembrane potential, cell cycle analysis, hydrogen peroxide and superoxide were investigated by flow cytometry. Cell migration and colony formation were investigated by transwell migration and colony formation assays respectively. The *in vivo* effect was confirmed using 211H and H226 nude mice xenograft models.

Results and conclusion: Cell viability was reduced. Disturbance of glycolysis and/or oxidative phosphorylation resulted in downregulation of glycogen, citrate and succinate. DCA and/or Nic increased apoptosis, mitochondrial transmembrane depolarization, G2/M arrest and reactive oxygen species. Moreover, DCA and/or Nic suppressed cell migration and colony formation. Furthermore, a better initial tumor suppressive effect was induced by the DCA/Nic combination compared with either drug alone in both 211H and H226 xenograft models. In H226 xenografts, DCA/Nic increased median survival of mice compared with single treatment. Single drug and/or a combination disturbed the Warburg effect and activated apoptosis, and inhibition of migration and proliferation *in vivo*.
In conclusion, dichloroacetate and/or niclosamide showed a tumor suppressive effect in MPM *in vitro* and *in vivo*, partially mediated by disturbance of glycolysis/oxidative phosphorylation, apoptosis, ROS production, G2/M arrest, and suppression of migration and proliferation.