

**Pneumoconiosis Compensation Fund Board research grant: final report 2013**

***Study title: In vitro study of arsenic trioxide in treatment of malignant pleural mesothelioma***

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## **Abstract**

**Objective:** Malignant pleural mesothelioma (MPM) has been a global health problem for the past decades. It has been shown that arsenic trioxide (ATO) can suppress thymidylate synthase (TYMS) in colorectal cancer and induce apoptosis in acute promyelocytic leukemia (APL). Therefore, we conducted this study to examine the effect of ATO in mesothelioma.

**Materials and methods:** A panel of 5 mesothelioma cell lines was used to study the effects of ATO, pemetrexed and cisplatin on cell viability. The TYMS expression (protein and mRNA), pRB1 and E2F1 protein expression and TYMS activity after ATO treatment were investigated. TYMS knockdown and overexpression were carried out. Phosphatidylserine externalization, mitochondrial membrane depolarization and expression of apoptotic/anti-apoptotic proteins induced by ATO were explored. With the help of nude mice xenograft model, the *in vivo* effect of ATO was studied.

**Results:** ATO showed anti-cancer effects with clinically achievable concentrations (1.7-7  $\mu$ M) in 5 mesothelioma cell lines. Combination of ATO with pemetrexed and/or cisplatin did not show any synergism. After incubation with ATO, downregulation of TYMS protein (H28, H2052 and H2452 cells) and mRNA expression (H28 cells), suppression of pRB1 (H28 cells) and E2F1 (H28 and H2052 cells) expression, reduction of specific (H2052 and H2452 cells) and total TYMS activity (H28, H2052 and H2452 cells) were demonstrated. Cell viability was decreased by 25% in H28 cells with TYMS knockdown. Overexpression of TYMS led to increased resistance to ATO in H28 cells. Effects of ATO on apoptosis were also studied in mesothelioma cell lines. Phosphatidylserine externalization was detected in H2452 cells. Mitochondrial membrane depolarization was detected in all cell lines. Downregulation of Bcl-2 (211H, H266 and H2052 cells) and Bcl-xL (H2052 cells), and upregulation of Bak (211H, H226 and H2052 cells) were observed. Expression of cleaved caspase-3 (all cell lines) was increased. In H226 xenograft model, the relative tumor volumes were reduced as well as cleaved caspase-3 was elevated and localized to nucleus in ATO treatment arm.

**Conclusion:** ATO has potent antiproliferative and cytotoxic activity in mesothelioma *in vitro* and *in vivo* through TYMS and E2F1 downregulation and apoptosis. This provides scientific ground for future clinical application of ATO in treatment of

mesothelioma. However, there is no observed synergism in combining ATO with current standard chemotherapy (pemetrexed or cisplatin) in mesothelioma.