Pneumoconiosis Compensation Fund Board research grant: final report 2017

Study title: In vitro and *in vivo* study of arginase in treatment of malignant pleural mesothelioma

Investigators and affiliations:

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Abstract

Objective: Malignant pleural mesothelioma (MPM) is notoriously hard to treat. Pegylated arginase (PEG-BCT-100 or BCT-100) has recently shown anti-cancer activities in acute myeloid leukemia, hepatocellular carcinoma and melanoma. This investigation aims to study the effects of PEG-BCT-100 on MPM.

Methods: A panel of 5 mesothelioma cell lines (H28, 211H, H226, H2052 and H2452) was employ to investigate the *in vitro* activities of BCT-100 using crystal violet staining. The *in vivo* activities of BCT-100 were examined using 211H and H226 nude mice xenografts. Protein expression and arginine concentration were studied by Western blot and ELISA respectively. Cellular localization of BCT-100 was identified by immunohistochemistry and immunofluorescence staining. TUNEL assay was used to study cellular apoptotic events.

Results: Argininosuccinate synthetase expression was found in H28, H226, and H2452 cells as well as 211H and H266 xenografts. Ornithine transcarbamylase was untraceable in all cell lines and xenograft models. BCT-100 decreased *in vitro* cell viability with IC_{50} values at 13-24 mU/ml (72 hours) across various cell lines. BCT-100 (60 mg/kg) significantly suppressed tumor growth ($p<0.01$) and prolonged median survival ($p<0.01$) in both xenograft models. Combining BCT-100 with pemetrexed or cisplatin gave no advantages over single agents. Serum and intratumoral arginine levels were effectively reduced by BCT-100. BCT-100 was accumulated in the cytosol of tumor cells. Apoptosis (PARP cleavage in 211H xenografts; Bcl-2 downregulation, and cleavage of caspase 3 and PARP in H226 xenografts; positive TUNEL staining in both) and G1 arrest (suppression of cyclin A2, D3, E1 and CDK4 in 211H xenografts; downregulation of cyclin A2, E1, H and CDK4 in H226 xenografts) were observed with BCT-100 treatment. Moreover, expression of proliferative factor Ki67 was decreased in BCT-100 treatment arms.

Conclusions: BCT-100 inhibited tumor growth and prolonged median survival partially mediated by depletion of intratumoral arginine resulting in apoptosis and G1 arrest in mesothelioma xenograft models. The findings provide scientific background to support further clinical development of BCT-100 in treatment of MPM.