

# **Pneumoconiosis Compensation Fund Board**

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## **Final Report**

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**Project title: A novel vaccine for mesothelioma immunotherapy and prevention**

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##### **1. Background**

Mesothelioma is a type of tumor that occurs on serosal surfaces such as the pleura and peritoneum. Long term exposure and inhalation of asbestos remains one of the major causes of mesothelioma with a latency period of approximately 30 years. There are currently no effective therapy against mesothelioma except for cytoreductive surgery that can prolong patients' life up to 92 months. Chemotherapy has limited effects due to lack of penetration into the tumor cells, and attempts at immunotherapy using Flt3, IL-12, and immunotoxin only minimally helped patients survive for ~3 more years. Mesothelioma is a life-threatening malignant tumor in humans without any therapeutic cure (Robinson and Lake, 2005). Significant progresses have been made with vaccines that can prevent oncovirus-associated (e.g. HBV, HPV) liver and cervical cancers (Dorans, 2009; Hung et al., 2007; Schiller and Davies, 2004). The passive administration of tumor-specific antibodies or immune cells such as cytotoxic T lymphocytes (CTLs), lymphokine-activated killer cell (LAK) and dendritic cells (DC) has been clinically beneficial to patients (Kohrt et al., 2012; Palucka and Banchereau, 2012; Restifo et al., 2012; Vanneman and Dranoff, 2012). Despite these progresses, preventing and eradicating malignant tumor such as mesothelioma by vaccine-induced immune surveillance remains difficult even in animal model systems.

Mesothelioma highly expresses two tumor associated antigens (TAAs), Wilms tumor antigen 1 (WT1) and mesothelin (MSLN). While WT1 is readily detected in

patients with mesothelioma, MSLN could also be detected in sera from more than 71% of patients suffering from this cancer. Thus, our strategy is to develop an effective vaccine against these two sensible targets.

We have taken advantage of findings on programmed death-1 (PD-1), which serves as an inhibitory signal against activated immune cells, as a target for antigen delivery method (ADM). ADM vaccines generally achieves high level of T cell response, especially CD8<sup>+</sup> T cells which offer cytotoxic activities against tumor cells. PD-1 (or its soluble form) binds its ligands, PD-L1 and PD-L2, which are expressed on antigen presenting cells and can be used as target for antigen delivery. Apart from using soluble PD-1, we have also identified a novel PD-1 variant, ΔPD-1, that could also be used for antigen delivery (Zhou et al., 2013b). Therefore, we have constructed vaccines against mesothelioma based on fusion between soluble PD-1 or ΔPD-1, with WT1 or MSLN antigens (Specific Aim 1). To effectively test whether our vaccines offers protection and therapeutic activities against mesothelioma, we have constructed a mesothelioma cell line that can establish a solid tumor in mice, and can be monitored by *in vivo* imaging, as we present in this report (Specific Aim 2).

We hypothesized that an effective cancer vaccine should harness the immune system and reinstate immune surveillance by overcoming tumor-associated immune suppression. To test this hypothesis, we sought to determine the efficacy and immune correlates of protection of a potent soluble PD1 (sPD1)-based fusion DNA vaccine, namely sPD1-p24<sub>fc</sub>, via *in vivo* electroporation (EP) delivery against malignant mesothelioma in immune competent BALB/c mice. The model antigen p24 is derived from HIV capsid protein GAG (Zhou et al., 2013a). The uniqueness of this vaccine lies in its capacity to induce high frequency of antigen-specific CD8<sup>+</sup> T cells with broad reactivity, long-term memory, polyfunctionality and cytotoxicity by targeting the p24 to DC while triggering IL-12 production and antigen cross-presentation (Zhou et al., 2013a). Using a malignant murine mesothelioma tumor engineered to express GAG, we now demonstrate that CD8<sup>+</sup> T cells induced by sPD1-p24<sub>fc</sub>/EP are essential to achieve complete prevention and therapeutic cure of mesothelioma.

## **2. Research highlights**

- i. Intramolecular adjuvant developed at our AIDS Institute that can assist in eliciting robust CD8<sup>+</sup> T cell immunity (Zhou et al., 2013a; 2013b)

- ii. We have generated a number of fusion DNA vaccines expressing either WT1 or MSLN gene fused to intramolecular adjuvants.
- iii. *In vivo* experiments in mice have shown that WT1 vaccines can generate specific immune responses against WT1 and MSLN, but not enhanced by our sPD1 fusion strategy.
- iv. We have established a tumor model in mice using the mesothelioma cell line (AB1) that can be used for *in vivo* imaging. Based on this model, we found that our vaccine used preventively can effectively hinder the growth of tumor in mice.
- v. Using modified AB1 cell line that expresses HIV-1 GAG antigen (AB1-GAG cells), we found that our fusion DNA vaccines against the GAG subunit p24 can effectively clear mesothelioma challenge, in both protective and therapeutic settings.
- vi. The mechanism for protection and clearance of AB1-GAG cells *in vivo* is based on the functionally enhanced p24-specific CD8<sup>+</sup> T cells, which could overcome the tumor microenvironment by downregulating regulatory T cells and myeloid-derived suppressor cells (MDSCs).