WHITE PAPER

# Soluble B7-H1/PD-L1 Levels in Multiple Cancer Subtypes: High Sensitivity Measurement by Immunoassay

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## ABSTRACT

Aberrant expression of the immune checkpoint protein B7-H1/PD-L1 is evident in many forms of cancer. The evidence for increased protein levels of B7-H1/PD-L1 comes largely from immunohistochemical analysis of surgical and biopsy specimens. While several studies attempt to correlate B7-H1/PD-L1 expression with poor prognosis, there are studies that conclude no correlation based on technical difficulties associated with detecting B7-H1/PD-L1 protein expression on the cell surface. We were able to quantifiably measure soluble B7-H1/PD-L1 in conditioned medium and cell lysates from tumor cell cultures using the Human/Cynomolgus Monkey B7-H1/PD-L1 Quantikine® ELISA. We show that B7-H1/PD-L1 expression in breast, prostate, glioma, and Hodgkin's lymphoma cells correlates with PTEN expression or proliferation status. This is in agreement with previous findings showing that increases in B7-H1/PD-L1 expression levels are frequently coincident with loss of the PTEN tumor suppressor or increased activation of the phosphoinositide 3-kinase (PI 3-K) pathway. The androgen-responsive LNCaP prostate cancer cell line did not exhibit measurable B7-H1/PD-L1 in any instance, while the androgen-independent prostate cancer cell line PC-3 expressed measurable B7-H1/PD-L1 that was further inducible with PMA treatment. In all other cell lines included in our study, incubation with PMA increased the level of B7-H1/PD-L1 released into the culture media, while pretreatment with the PI 3-K inhibitor, LY294002, abrogated this increase. Our data suggest that our Quantikine® ELISA is a powerful tool for reliably and quantitatively assessing soluble B7-H1/PD-L1 levels.

## INTRODUCTION

Although the immune checkpoint protein B7-H1/PD-L1 is a commonly used cancer biomarker, controversy remains over the predictive and prognostic utility of immunohistochemical methods. There are a number of issues associated with the use of immunohistochemistry (IHC) in this context. First, B7-H1/PD-L1 expression is not homogenous across tumors, which obfuscates the generalizability of IHC data. Second, IHC results vary widely, depending on the antibody used. Third, there is no standardized method for quantifying B7-H1/PD-L1 immunoreactivity (1,2). Finally, B7-H1/PD-L1 expression is regulated by proteins such as the tumor suppressor Phosphatase and Tensin Homolog (PTEN). Decreased PTEN expression is correlated with increased B7-H1/PD-L1 expression in glioma (3,4) and breast cancer cells (5).

In light of this controversy, we wanted to determine whether the Human/Cynomolgus Monkey B7-H1/PD-L1 Quantikine® ELISA represents an easier, more reliable alternative to IHC for detecting B7-H1/PD-L1 in research settings. We tested a number of cancer cell lines that were grouped based on PTEN status as well as proliferation status. Aggressive (highly proliferative) cancer lines tested include: PC3, MDA-MB-231, U87MG, and HDLM-2. Less aggressive cancer cell lines tested include: LNCaP, MCF-7, T98G. In addition to measuring basal levels of soluble B7-H1/PD-L1, we tested the hypothesis that the PI 3-K pathway was important for Phorbol myristate acetate (PMA) induced B7-H1/PD-L1 release into conditioned media supernates and cell lysates. Our data show that basal levels of soluble B7-H1/PD-L1 can be reliably measured in conditioned media and cell lysates from most cell lines using our Quantikine® ELISA. In particular, the cell lines corresponding to more aggressive cancers in each type demonstrated higher basal levels of soluble B7-H1/PD-L1. We were also able to observe PI 3-K dependent, PMA-induced B7-H1/PD-L1 release in most of the cell lines.

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# METHODS

## Cell Culture

Human cancer cell lines (PC-3, LNCaP, MDA-MB-231, MCF-7, U87-MG, T98G, and HDLM-2) were cultured in base media supplemented with 10% FBS containing 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin, and maintained in a 5% $CO_2$ incubator at 37 °C. Cells were left untreated (Control) or treated with 20 µM LY294002 (Tocris, Catalog # 1130), 60 nM PMA (Sigma, Catalog # P1585), or pretreated with 20 µM LY294002 for 1 hour and then 60 nM PMA. Cancer cell lysates or conditioned media supernates were collected after 24 hours of treatment.

## Quantikine® ELISA Analysis

Human cancer cells cultured under various conditions were analyzed on the Human/Cynomolgus Monkey B7-H1/PD-L1 Quantikine® ELISA Kit (R&D Systems, Catalog # DB7H10). Conditioned media supernates and cell lysates were prepared and analyzed according to the procedure outlined in the product insert. Briefly, 1 µg/well of lysates were used for HDLM-2 samples and 10 µg/well of lysates were used for all other samples. Cell lysates were diluted in the calibrator diluent provided with the kit. Conditioned media was collected and centrifuged to remove particulates before evaluation to produce a conditioned media supernate. A 200 µl sample was required for duplicate data points and all samples were tested in duplicate.

# RESULTS

Our data indicate that the relative B7-H1/PD-L1 concentration was consistent between conditioned media supernates and cell lysates under all treatment conditions. Two prostate cancer cell lines were tested, the less aggressive, androgen sensitive LNCaP cell line and the more aggressive androgen insensitive PC-3 cell line. Although we were unable to detect B7-H1/PD-L1 in LNCaP cells, its basal expression was enhanced by PMA in PC-3 cells in a PI 3-K dependent manner (Figure 1). Similarly, PMA treatment caused a robust, PI 3-K dependent increase in soluble B7-H1/PD-L1 levels in aggressive U87-MG glioma cells but not the less aggressive T98G glioma cells (Figure 2). Although cell lysates from both breast cancer cell lines exhibited robust PI 3-K mediated PMA induction of B7-H1/PD-L1, soluble B7-H1/PD-L1 was only observed in the more aggressive MDA-MB-231 cells (Figure 3). Basal B7-H1/PD-L1 and soluble B7-H1/PD-L1 were enhanced in a PI 3-K dependent manner in the aggressive Hodgkin's lymphoma HDLM-2 cells (Figure 4).

**Figure 1**

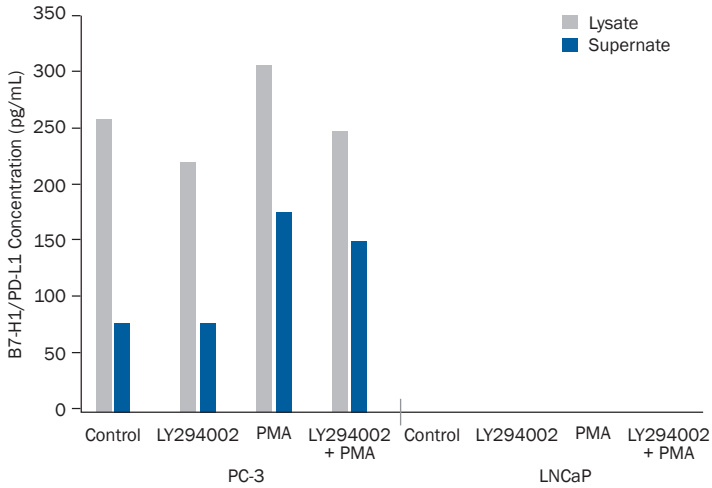


**Figure 1. Human prostate cancer cell lysates and conditioned media supernates were analyzed using the Human/Cynomolgus Monkey B7-H1/PD-L1 Quantikine® ELISA.** The effects of PI 3-K inhibitor, LY294002, on PMA-induced B7-H1/PD-L1 protein expression and release in human cancer cells was investigated. PC-3 and LNCaP, cells, were left untreated (Control), or treated with LY294002, PMA, or pretreated with LY294002 and then PMA for 24 hours. Conditioned media and cell lysates were collected and prepared simultaneously. Conditioned media was centrifuged to remove particulates before evaluation. Cell lysates were evaluated using 10 µg/well. PMA treatment increased membrane and soluble B7-H1/PD-L1 concentration in the more aggressive PC-3, but not LNCaP cells.

**Figure 2**

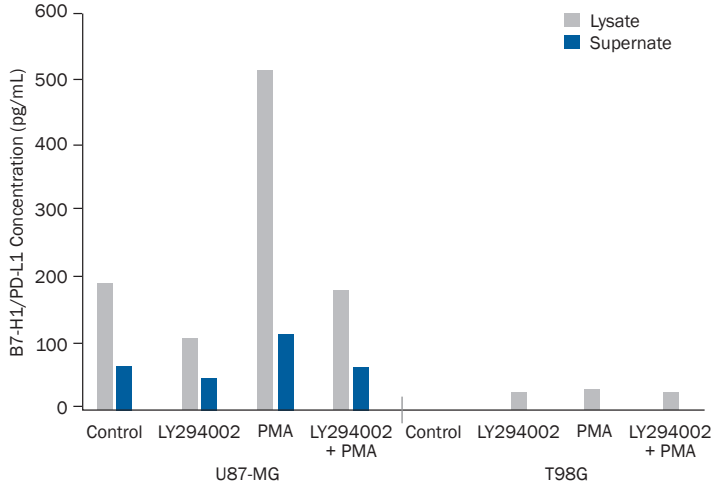


**Figure 2. Human glioma cancer cell lysates and conditioned media supernates were analyzed using the Human/Cynomolgus Monkey B7-H1/PD-L1 Quantikine® ELISA.** The effects of PI 3-K inhibitor, LY294002, on PMA-induced B7-H1/PD-L1 protein expression and release in human glioma cells was investigated. U87-MG and T98G cells were left untreated (Control), or treated with LY294002, PMA, or pretreated with LY294002 and then PMA for 24 hours. Conditioned media and cell lysates were collected and prepared simultaneously. Conditioned media was centrifuged to remove particulates before evaluation. Cell lysates were evaluated using 10 µg/well. Although basal B7-H1/PD-L1 expression was observed in lysates of both cell lines, soluble B7-H1/PD-L1 was only observed in conditioned media supernates from the more aggressive U87-MG cells. Finally, only the U87-MG cell line exhibited a 2.5-fold PI 3-K dependent increase in B7-H1/PD-L1 concentration in lysates.

## Figure 3

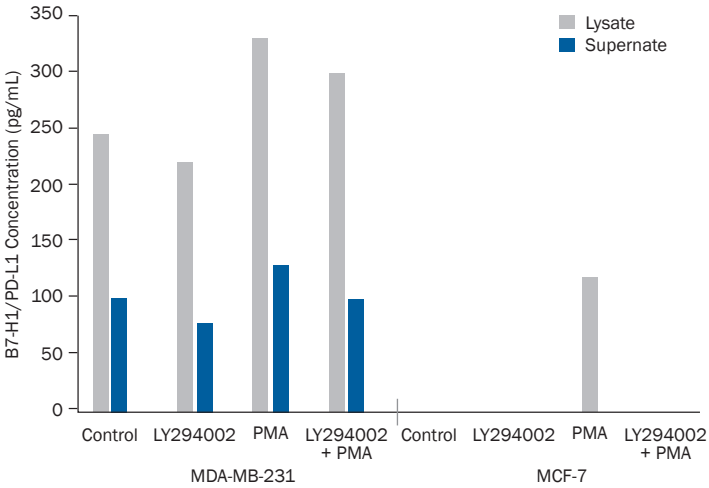


**Figure 3. Human breast cancer cell lysates and conditioned media supernates were analyzed using the Human/Cynomolgus Monkey B7-H1/PD-L1 Quantikine® ELISA.** The effects of PI 3-K inhibitor, LY294002, on PMA-induced B7-H1/PD-L1 protein expression and release in human cancer cells was investigated. MDA-MB-231 and MCF-7 cells were left untreated (Control), or treated with LY294002, PMA, or pretreated with LY294002 and then PMA for 24 hours. Conditioned media and cell lysates were collected and prepared simultaneously. Conditioned media was centrifuged to remove particulates before evaluation. Cell lysates were evaluated using 10 µg/well. Although, PMA treated, PI 3-K dependent increases in B7-H1/PD-L1 was observed in both cell lines, soluble B7-H1/PD-L1 was observed only in conditioned media supernates from the aggressive MDA-MB-231 cells.

## Figure 4

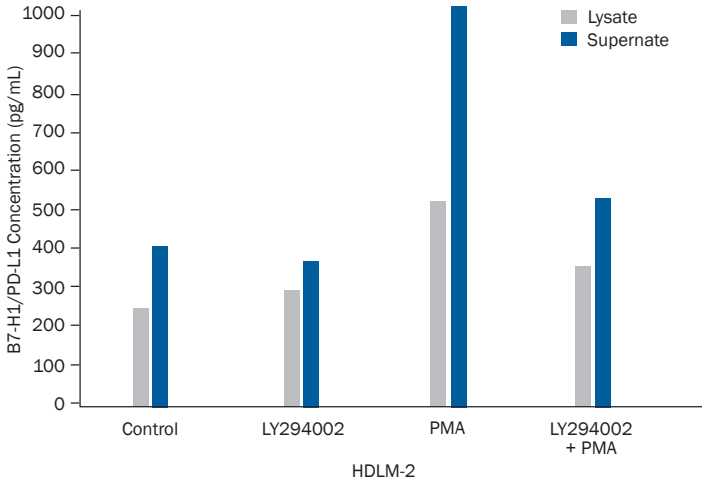


**Figure 4. Cell lysates and conditioned media supernates from Hodgkin's lymphoma cell lines were analyzed using the Human/Cynomolgus Monkey B7-H1/PD-L1 Quantikine® ELISA.** The effects of PI 3-K inhibitor, LY294002, on PMA-induced B7-H1/PD-L1 protein expression and release in human cancer cells was investigated. HDLM-2 cells were left untreated (Control), or treated with LY294002, PMA, or pretreated with LY294002 and then PMA for 24 hours. Conditioned media and cell lysates were collected and prepared simultaneously. Conditioned media was centrifuged to remove particulates before evaluation. Cell lysates were evaluated using 1 µg/well for HDLM-2 cells. Although PI 3-K dependent PMA stimulation was observed in both lysates and conditioned media supernatants, the response was particularly robust in the supernates.

# DISCUSSION

Our data indicate that we can reliably measure B7-H1/PD-L1 in conditioned media supernates and cell lysates from a number of cancer cell lines. Our Quantikine® ELISA represents an easier, more reliable alternative to immunohistochemistry for detecting B7-H1/PD-L1 in research settings. Our observation of B7-H1/PD-L1 levels in conditioned media supernates and cell lysates implies that the former can serve as a proxy for the latter in aggressive cell cancer lines that have reduced PTEN activity (3-5). Please note that the rigorous quality control measures undertaken for our Quantikine® ELISAs ensure consistent and reliable performance over time, making it the best choice for measuring PD-L1 expression levels. In conclusion, Bio-Techne is able to provide the optimal immunoassay solution for your research needs.

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