# PD-L1 is Overexpressed on Daughter Cells Differentiated from Glioblastoma Cancer Stem Cells in an Inflammatory Environment and Enables Tumor Suppression of CD8 Cells

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## Introduction

One of the reasons for limited success with immunotherapy in glioblastoma has been the negative regulation of activated T cells. PD-L1, otherwise known as B7-H1, the third member of a transmembrane glycoprotein of the B7 family of costimulatory molecules, has been reported to be responsible for glioma immunosuppression.

## Methods

We assessed the expression of PD-L1 on human glioma cancer stem cells in immunodeficient mice and in culture in the presence or absence of interferon gamma. 8 glioma cancer stem cell lines and their daughter cells were tested for PD-L1 expression in the presence and absence of interferon gamma. Co-cultures with human CD4 and CD8 cells were performed with each of the CSC lines and their differentiated daughter cells to determine the effect of PD-L1 expression on T cell survival.

## Conclusions

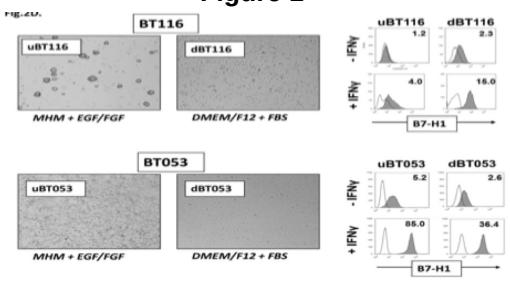
PD-L1 is highly expressed in differentiated glioma cells in comparison to undifferentiated cell brain cancer stem-like cells. In the setting of inflammation, PD-L1 is overexpressed and induces CD8 > CD4 T cell killing. This mechanism of T cell suppression is invoked by daughter cells rather than cancer stem cells and may make CSCs more susceptible to T cell killing if CSC antigens can be differentially targeted.

## Results

PD-L1 is clearly expressed by glioma cells during their infiltration into the brain tissues of non-obese diabetic (NOD) mice that were injected with undifferentiated glioma cells. We observed higher expression of PD-L1 in differentiated glioma cells than in undifferentiated cells after addition of proinflammatory cytokine, IFN-gamma (Fig. 1). Only differentiated glioma CSCs induce T cell killing preferentially of CD8 cytotoxic T cells more than CD4 helper T cells ($p < 0.05$). The T cell survival was restored when an anti-PD-L1 antibody was incubated with the glioma cells prior to coculture with T cells (Fig. 2).

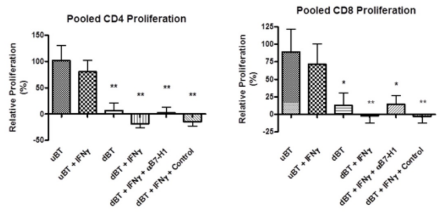
Among malignant glioma patients, PD-L1 (B7-H1) has been reported to be an important negative regulator of T cells activation and is known to be highly expressed in glioma cells especially under pro-inflammatory conditions.

**Figure 1**




Previously, we have found that differentiated daughter tumor cells (d) are more immunosuppressive compared to undifferentiated cancer stem cells (u) with a unique immunological pattern in both cells. Specifically, the major histocompatibility complex (MHC) class I and II are all expressed in these cells, but not CD80 and CD86.

**Figure 2**




Furthermore, only IFN- induced differentiated daughter cells expressed PD-L1. Differentiated cells show more inhibition of T cells after co-culturing compared to undifferentiated cells. Only the differentiated cells were able to inhibit T cell proliferation. . Blocking with B7-H1 antibodies reversed the inhibition of T cell proliferation of both CD8 and CD4 cells by differentiated glioblastoma tumor cells.

## Learning Objectives

By the conclusion of this session, participants should be able to: 1) Describe the importance of immunosuppression in glioma immunotherapy 2) Discuss the role of PD-L1 expression in glioma immunosuppression 3) Identify an effective treatment strategy to target glioma cancer stem cells.

## References

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