Rabbit PD-L1 Monoclonal Antibody Development

Rabbit reconstituent monoclonal antibody platform was developed using B cells from peripheral blood. Briefly, B cells were isolated from the whole blood of rabbits immunized with PD-L1 peptides. Immune response-positive cells were selected after they were cultured for 7 to 10 days. Rabbit IgG variable light and heavy chain were POCl3 apyridlated and cloned into vectors. Positive clones were sequenced. Both light and heavy chain were co-expressed in 200 cells for antibody expression. More than 10 positive PD-L1 clones were selected for the production of mAbs. Recombinant PD-L1 clone OR-BH7 for ICC works on both human and mouse tissues (Figure 1).

Figure 1: Positive PD-L1 OR-BH7 On Human & Mouse Tissues

Introduction

Current PD-L1 ICC immune targets T cells that are blocked by the PD-L1–PD-L1 interaction between tumor and immune cells to reactivate T cells activity (1). However, total success of PD-L1 therapy is in preventing the progression of melanoma, non-small cell lung cancer (NSCLC) and breast cancer. Many new targets and new cancer drugs are believed to be developed in the near future. Immune cells are believed to understand the role of tumor microenvironment in the T-cell regulation. Previously we have begun to look at the tumor microenvironment by evaluating the expression profile of immune cell markers CD3, CD20, CD8A, CD4, PD-L1, PD-L2 and found that LAG3 and TIM3 produced unique immune profiles between the three cancer types. This study continues screening possible markers by evaluating the same tumors for expression of DGKa. DGKa has been shown to participate in the up-regulation of inhibitory (LAG3/TIM3) antibodies and down-regulation of a few immune cells such as CD8A, CD20, CD4 (2). We also confirmed that chosen antibodies of DGKa avoided the anti-tumor activity T cells in the presence of PD-L1 positive tumor cells. Our study uses the traditional strategy (3) to investigate DGKa expression pattern in these tumors where PD-L1 expression is known.

Table 1: Antibody Information and Dilution

Design & Methods

Anti-DGKa monoclonal antibody (DGKa) functions as a biologic agent to phosphorylate the lipid-diacylglycerol kinase (DGKa) and has been characterized to negatively regulate the Rho MAPK pathway. In T cells, DGKa has been shown to regulate the phosphorylation of Rho MAPK pathway and revealed the anti-tumor activity of T cells in vivo. In this study, we blocked PD-L1 expression on the surface of NSCLC and bladder cancer cells. Each row has one lung cancer and one bladder cancer. Table 1 shows the score for L1 antibody (OR-BH7) exposure in NSCLC and bladder cancer. Each cell has been sequenced to identify unique signature profile of immune cells to elucidate the signature profile of these cancers. In this study, we evaluate NSCLC, and bladder cancer and found a few unique signatures for these cancers. In this study, we evaluate

Table 2: Lung Cancer Data Score

Table 3: Bladder Cancer Data Score

Results

Figure 4: PD-L1, CD3E, CD8A, CD20, FOXP3, LAG3 and TIM3 IHC Stain on NSCLC, Bladder Cancer, & Melanoma

Conclusion

1) DGKa clone OTI 7B6 stains mostly tumor cells and not immune cells in Bladder and Lung cancer. It has very little background and takes it as a very good ICC antibody.
2) Staining pattern showed very little overlap between PD-L1 and DGKa in both the lung cancer and bladder tumor cells.
3) In data not presented 20 more cases of lung cancer where 6 tumors showed some DGKa positive staining using only 3 of those case showed overlap with PD-L1.
4) Future goal is to review the stains.

Table 4: Lung Cancer Data Score

Table 5: PD-L1, CD3E, CD8A, CD20, FOXP3, LAG3 and TIM3 IHC Summary on NSCLC, Bladder Cancer, & Melanoma

Figure 5: PD-L1, CD3E, CD8A, CD20, FOXP3, LAG3, TIM3 IHC Summary on NSCLC, Bladder Cancer, & Melanoma

Figure 3: PD-L1 and DGKa ICC and Bladder Cancer

Figure 2: PPRE ICC PD-L1 clone: OR-BH7 and UMAB328 compared to SP 14.1 (B 8) in F-Dep approved rabbit PD-L1 clone on NSCLC (lung), not shown as bladder cancer, and melanoma. Results all PD-L1 antibodies worked similarly using manual staining.

Immunohistochemistry:

Manual IHC staining of paraffin embedded human and mouse tissues using and PD-L1 rabbit monoclonal clone SP142 (Spring Bioscience, Pleasanton, CA) with rabbit polyclonal antibodies, Cambridge, MA OR-BH7, OR-BH8, OR-BH3, OR-BH3, Rb 1:250dil (4) on Immunolab (Cambridge, MA), and UMAB101 (5) on Immunolab (Cambridge, MA). The immune markers identified from Origenes CD3, CD8A, CD20, CD4, CD45, CD20, FOXP3, LAG3, and TIM3 antibodies to Table 1 antibody assays required heat induced epitope retrieval (HER) using Origenes ACCEL, the EDTA buffer pH 9.0 for clone OR-BH7 Or-BH8 Or-BH3 or Tris pH 6.0 for clone SP142 2 Chemo Dako 3 (Dako) for 3 minutes. Biocare De-Deblocker chamber: PD-L1 staining was neutralized 210°C and incubated for 10 minutes at room temperature. Biocare, pHine 1: clone 2: rabbit anti-human HRP detection (ACCEL 1230) was used except clone 28-12 which used 2 step Nk (ProteinA/Peroxidase) and (Dako) chromogen staining according to manufacturer protocol. The seven immune marker antibodies also required unique retrieval.