Functional characterization of anti-PD1 antibodies

Edith González-González[1,2], Alejandra Montes-Luna[1,2], Keyla M. Gómez-Castellano[1,2], Frida Daniela Ramírez Villedas[1,2], Sandra Comparán-Alarcón[1,2], Tomas Iván Alvarez-Fosada[1,2], Sonia M. Pérez-Tapia[1,2,3], Juan C. Almagro[1,2,4]

[1]Unidad de Desarrollo e Investigación en Bioprocesos (UDIBI), Escuela Nacional de Ciencias Biológicas (ENCB), Instituto Politécnico Nacional (IPN). 11340 CDMX, México. [2]Laboratorio Nacional para Servicios Especializados de Investigación, Desarrollo e Innovación (I+D+i) para Farmacoquímicos y Biotecnológicos, LANSEIDI-FarBiotec-CONACyT, ENCB-IPN. 11340 CDMX, México. [3]Departamento de Inmunología, ENCB-IPN. 11340 CDMX, México. [4]GlobalBio, Inc. Cambridge, MA 02138

ABSTRACT

PD-1 (also known as CD279) is a type I transmembrane receptor expressed on the cell surface of T cells, B cells , monocytes , natural killer T cells and dendritic cells. PD-1 has two naturally occurring ligands: PD-L1 (B7-H1, CD274) and PD-L2 (B7-DC, CD273) and serves as an immunologic checkpoint. In patients with cancer, the antitumoral response is affected by PD-1 expression on tumoral infiltrating lymphocytes, justifying PD-1 blockade in cancer immunotherapy. This poster describes a set of assays for *in vitro* characterization and evaluation of anti-PD1 antibodies and their application to the discovery campaign in progress in our laboratory as part of the PRONACES of Immunotherapy.

STRUCTURAL FEATURES OF PD-1 AND ITS LIGANDS

PD-1 is a 288 amino acid (aa) protein, composed of one immunoglobulin (Ig) superfamily domain, a ~20 aa stalk, a transmembrane domain, and an intracellular domain of approximately 95 residues containing an immunoreceptor tyrosine-based inhibitory motif (ITIM) as well as an immunoreceptor tyrosine-based switch motif (ITSM).

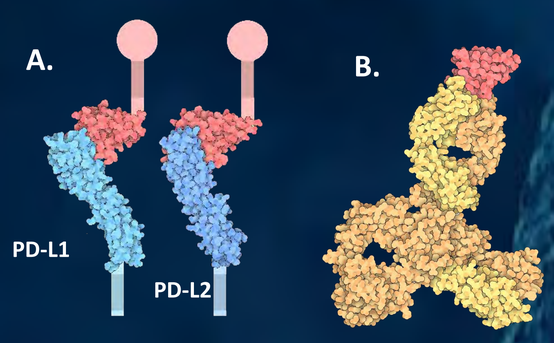

Figure 1. (A) PD-1 receptor and its ligands PD-L1 y PD-L2. (B) Pembrolizumab binding to PD-1. *(Reference 4) .*

PD-1 AND ITS LIGANDS ARE A NEW TARGET FOR CANCER THERAPY

The interaction of PD-1 with its ligands plays an essential role in the immune suppression associated with tumors. Persistent stimulation from tumor antigens induces PD-1 overexpression by tumor-infiltrating lymphocytes, whereas expression of PD-Ls is characteristic of malignant cells and various tumor-infiltrating APCs. In lymphoid malignancies, diverse mechanisms contribute to the constitutive expression of PD-Ls by tumor cells, including genetic alterations, transcriptional activation by certain oncogenic signaling pathways, viral infection, and induction by inflammatory cytokines. Binding of PD-Ls to PD-1 on T cells functionally silences the activation of tumor-associated T cells and leads to impaired cell survival and effector function, producing a tumor-permissive microenvironment.

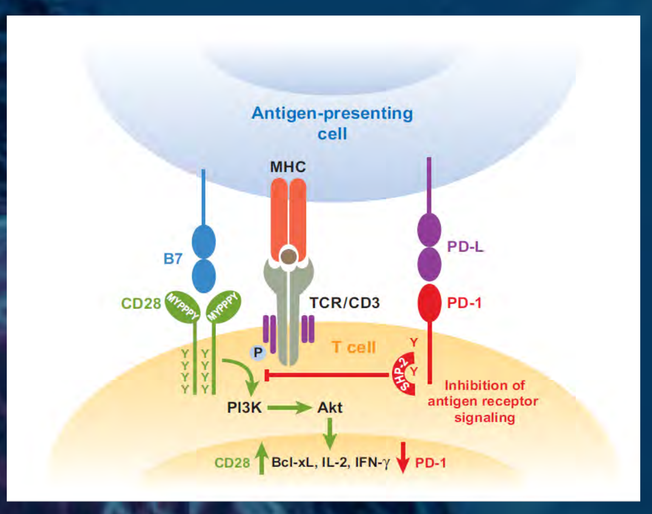

Figure 2.PD-1:PD-L1 interactions inhibit T cell Responses (see reference 1).

EVALUATION OF ANTI-PD-1 ANTIBODIES

A robust functional characterization of antibodies at early stages of their development determines the success of the antibody-based drugs in the preclinical and clinical development processes. Two of the assays we developed and validated to screen for promising antibodies during the discovery campaign were: (1) binding to PD-1 human and mouse and (2) blockade of PD1:PD-L1 interaction.

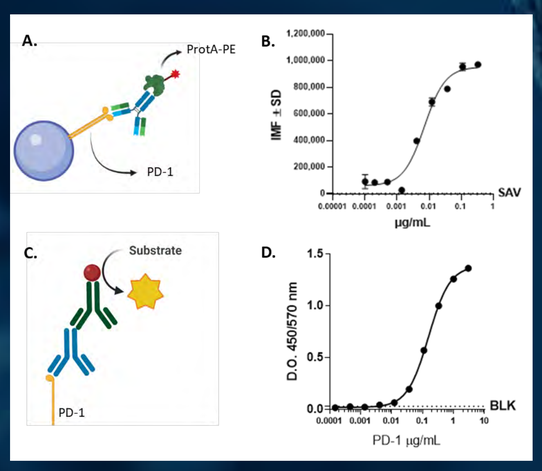

Figure 3. Anti-PD-1 antibodies. Binding to human PD-1 .

(A-B) Streptavidin beads (iQue Qbeads® DevScreen Sav) were coated with biotinylated human PD-1 protein at a concentration of 40ng/mL and incubated with the serial dilutions of antibody Pembrolizumab at 2µg/ml. Binding was evidenced by the addition of ProtA coupled to phycoerythrin (PE) and MIF was determined using a flow cytometer. (C-D) 96 wells plate were coated with PD1, incubatead over night and blocked, three fold serial dilutions of anti-PD1 antibodies were added. As detection agent anti-IgG-HRP was used . The system was revealed with TMB substrate Reagent. The color rection was stopped with Phosphoric acid 1M. The plates were read at 450 nm with correction at 570 nm using a EspectraMax M3 microplate reader

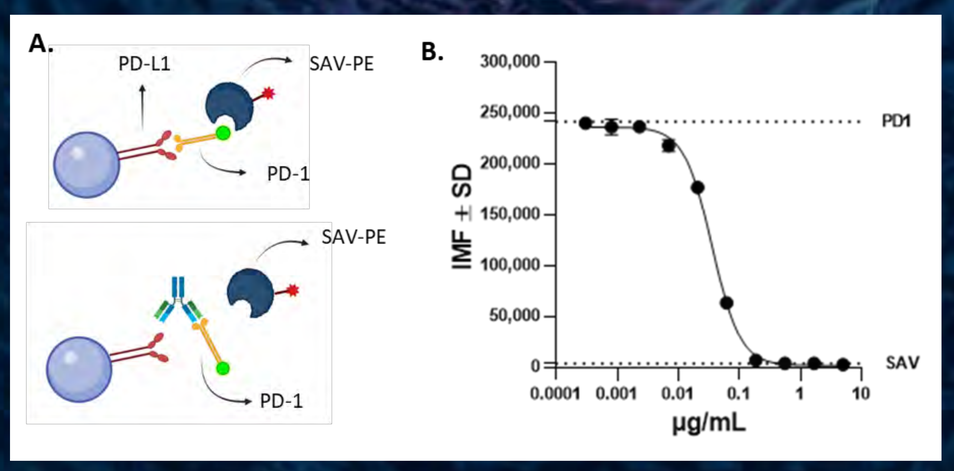

Figure 4. Blocking of PD1:PDL-1 interaction by Anti-PD-1 antibodies .

PDL-1 was coupled to SAV beads, and a mix of three-fold serial dilutions of anti-PD1 antibodies and PD1-AVI was added . the interaction was revealed with streptavidin coupled to phycoerythrin and MIF was determined using a flow cytometer.

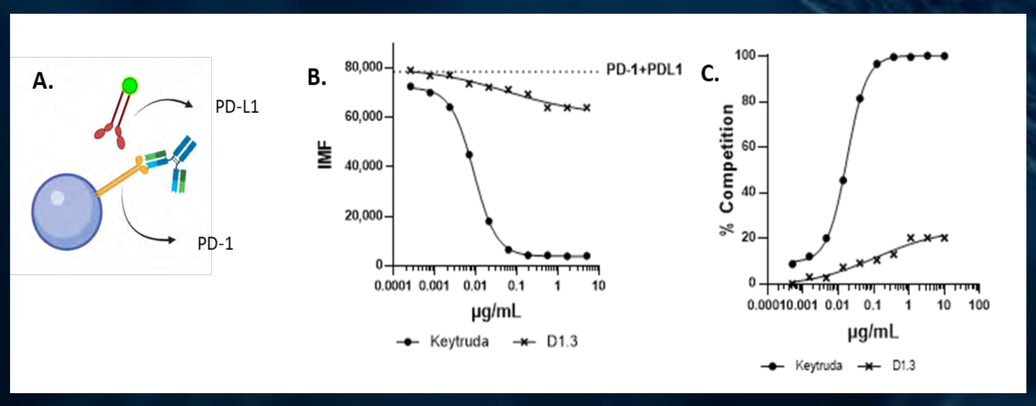

Figure 5.Competition assay for the PD-1 receptor by flow cytometry

Competition of antibody and PD-L1 ligand for the PD-1 receptor was performed by coupling PD1 to SAV beads, then a 1:1 mixture of serial dilutions of anti-PD1 antibody and PD-L1 ligand was added at constant concentration. The assay was revealed with streptavidin-PE. D1.3 corresponds to an unrelated antibody and was used as a negative control

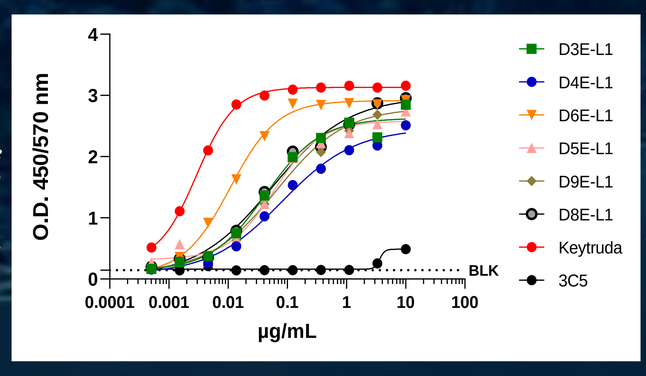

Figure 6. Comparison of the antibodies to be evaluated with the reference (pembrolizumab)

Evaluation of the binding to PD-1 of the first candidates by the ELISA method D1.3 corresponds to an unrelated anti- lysozyme antibody and was used as a negative control.

CONCLUSION

The assays developed up to this point for the characterization of totally human anti-PD1,IgG4 antibodies, have demonstrated their usefulness to evidence the recognition of their target molecule and to evaluate their blocking activity.

At this moment in the UDIBI we are developing complementary methodologies that allow us to advance in the biological and functional characterization of our anti-PD1 antibodies.

REFERENCES

1. *Keir, M. E., Butte, M. J., Freeman, G. J., & Sharpe, A. H. (2008). PD-1 and its ligands in tolerance and immunity. Annu. Rev. Immunol., 26, 677-704.*
2. *Chen, L., & Han, X. (2015). Anti–PD-1/PD-L1 therapy of human cancer: past, present, and future. The Journal of clinical investigation, 125(9), 3384-3391.*
3. *Zak, K. M., Grudnik, P., Magiera, K., Dömling, A., Dubin, G., & Holak, T. A. (2017). Structural biology of the immune checkpoint receptor PD-1 and its ligands PD-L1/PD-L2. Structure, 25(8), 1163-1174.*
4. *Han, Y., Liu, D., & Li, L. (2020). PD-1/PD-L1 pathway: current researches in cancer. American journal of cancer research, 10(3), 727.*
5. *David Goodsell, December 2016, doi:10.2210/rcsb_pdb/mom_2016_12*

GlobalBio Engineering better antibodies

CONACYT

UDIBI UNIDAD DE DESARROLLO E INVESTIGACIÓN EN BIOTERAPÉUTICOS

IPN

www.udibi.com.mx