# Isolation and characterization of *in vitro* functional anti-PD-1 antibodies from ALTHEA 4:3 VH:VL Platinum Libraries™

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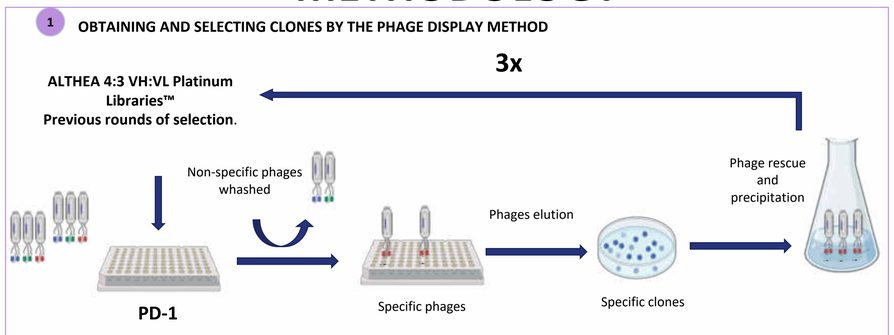
## ABSTRACT

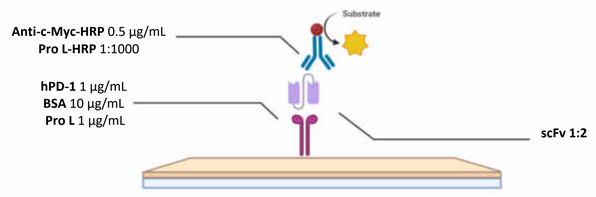
PD-1 (Programmed cell death protein 1) is expressed on the surface of T and B cells and has a role in down-regulating the immune system. It promotes self-tolerance by suppressing T cell inflammatory activity once coupled to its ligands PD-L1 and PD-L2, which are expressed on macrophages and dendritic cells. PD-L1 and PD-L2 are also highly expressed in several cancer cells, resulting in tumor immune evasion. Thus, the development of anti-PD1 antibodies blocking the PD-1:PD-L1 interaction has been shown to boost the immune system, promoting the destruction of cancer cells. In this work, ALTHEA 4:3 VH:VL Platinum Libraries™, a set of semi-synthetic phage display libraries, were used to generate a panel of anti-PD-1 antibodies with diverse binding, PD-1:PD-L1/PD-L2 blocking, and functional profiles. After three rounds of solution panning against recombinant PD-1, 88 clones were tested for PD-1 binding, yielding 13 positive single-chain variable fragments (scFvs), with five clones being unique. These unique clones were converted to human IgG4PE and assayed for blocking the PD-L1/PD-L2 interaction in ELISA and Junkat cells. The antibody, D38, was selected based on its performance in these assays. The *in vitro* functionality of D38 was assessed by expression of the interferon-gamma response in a mixed lymphocyte reaction co-culture, showing a similar functional profile to FDA-approved anti-PD-1 therapeutic antibodies (Keytruda and Opdivo). These results demonstrate that ALTHEA 4:3 VH:VL Platinum Libraries™ are a valuable source for obtaining functional anti-PD1 antibodies with a promising profile for cancer drug development.

## METHODOLOGY

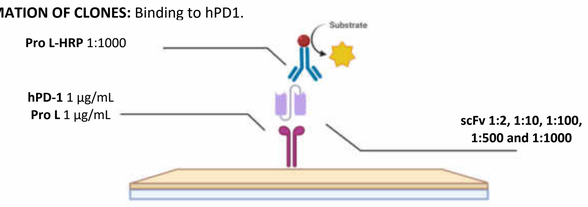
**1 OBTAINING AND SELECTING CLONES BY THE PHAGE DISPLAY METHOD**



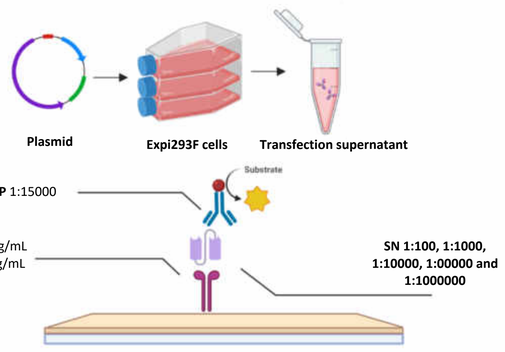

**2 SCREENING 88 CLONES:** Binding to hPD-1, BSA and Protein L



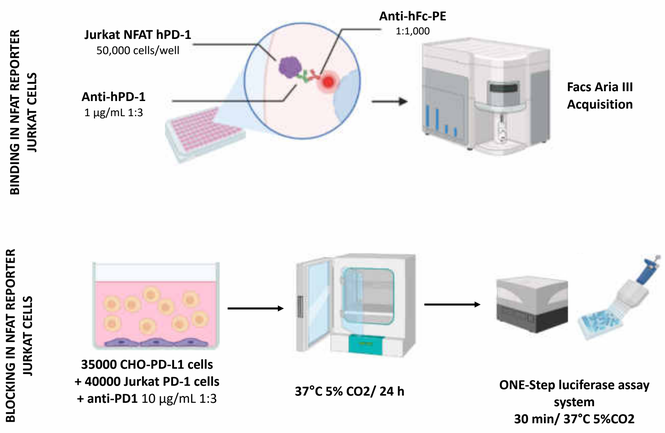

**3 CONFIRMATION OF CLONES:** Binding to hPD1.




**4 TRANFECTION AND EVALUATION of IgG:** 5 clones conversion to IgG4 on Expi293F cells.




**5 CHARACTERIZATION AND BIOLOGICAL EVALUATION OF IgGs:** Binding to hPD1-1 on ELISA/Jurkat cells, Blocking of PD-L1/PD-L2 interaction in NFAT Junkat cells and expression of IFN-gamma response in a Mixed Lymphocyte Reaction.




## RESULTS

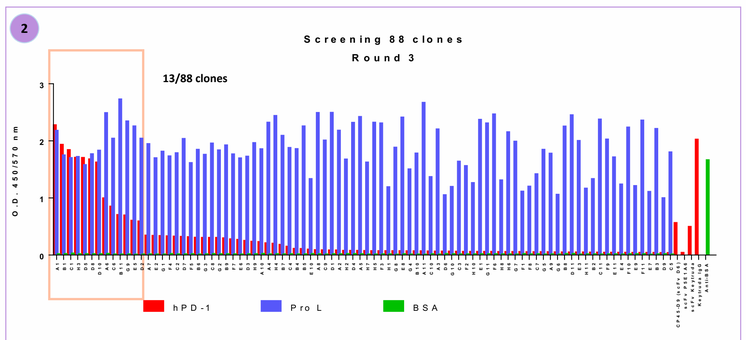



**FIGURE 1**. *Screening 88 clones: Round 3.* Evaluation of the binding to hPD-1 of 88 clones by the ELISA method.

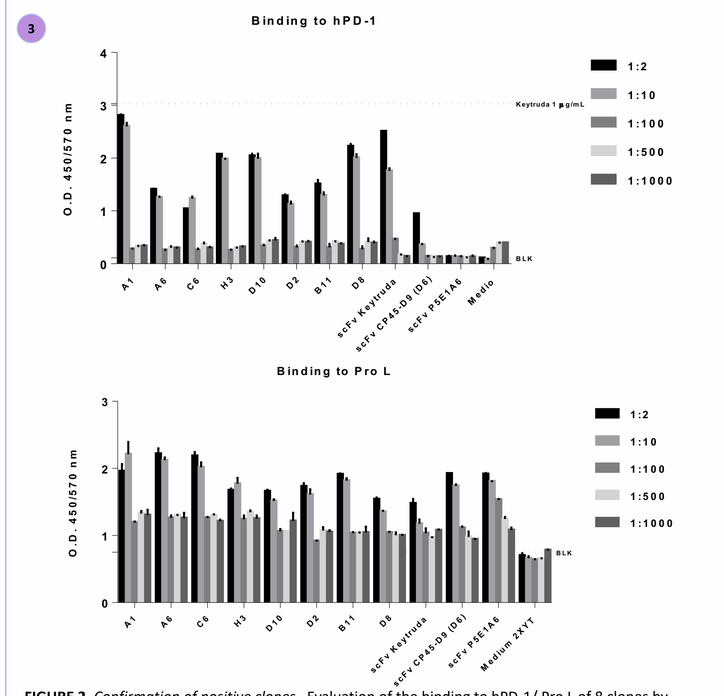



**FIGURE 2**. *Confirmation of positive clones* . Evaluation of the binding to hPD-1/ Pro L of 8 clones by the ELISA method.

| IgG | Clone |
|---|---|
| D35E | PD1- IgG4 Clone A1 |
| D36E | PD1- IgG4 Clone D2 |
| D37E | PD1- IgG4 Clone H3 |
| D38E | PD1- IgG4 Clone C6 |
| D38E | PD1- IgG4 Clone B11 |

**TABLE 1**. *Conversion of unique clones of Round 3 of discovery panning to IgG4.*

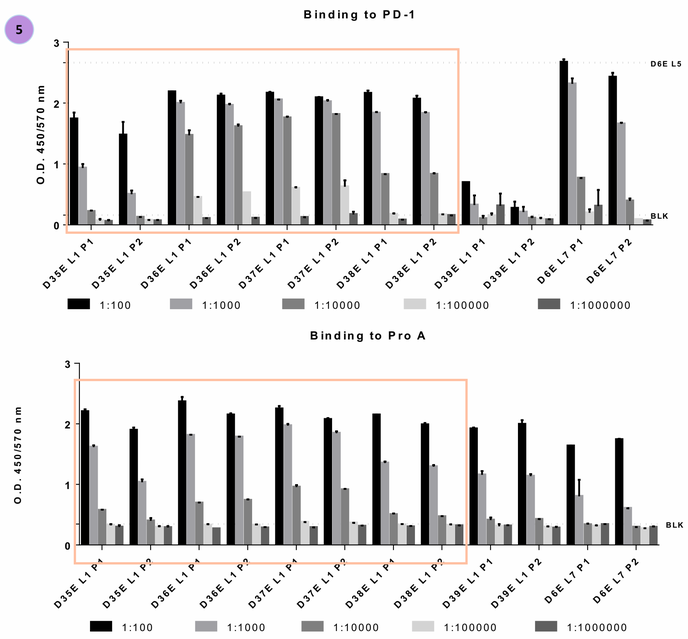



**FIGURE 3**. *Conversion of the positive unique clones to IgG4.* Evaluation of the binding of 5 clones with unique sequence to hPD-1/ Pro A by the ELISA method. For each IgG, they were transfected in duplicate in well 1 (P1) and well 2 (P2). D6E was used as a positive control (See poster No.**B127**).

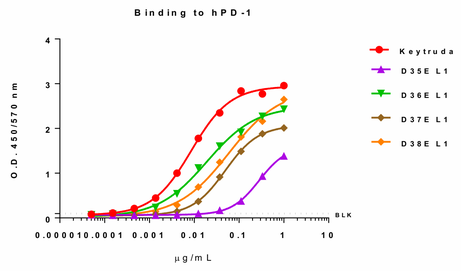



**FIGURE 4**. *Binding to hPD-1 of purified IgG4* . Evaluation of the antibodies D36E L1, D37E L1 and D38E L1 binding to hPD-1 by the ELISA method.
Keytruda was used as a positive control.

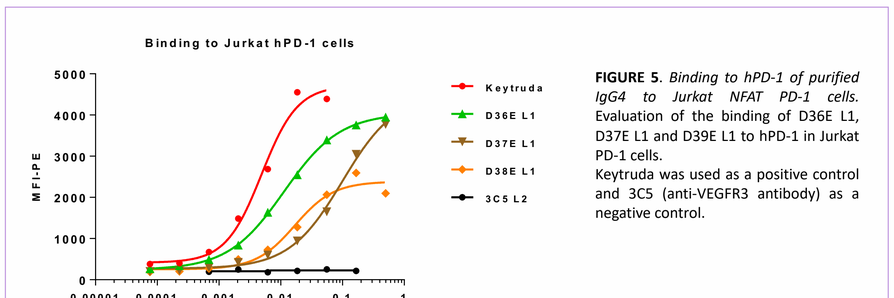



**FIGURE 5**. *Binding to hPD-1 of purified IgG4 to Jurkat NFAT PD-1 cells.* Evaluation of the binding of D36E L1, D37E L1 and D39E L1 to hPD-1 in Jurkat PD-1 cells.
Keytruda was used as a positive control and 3C5 (anti-VEGFR3 antibody) as a negative control.

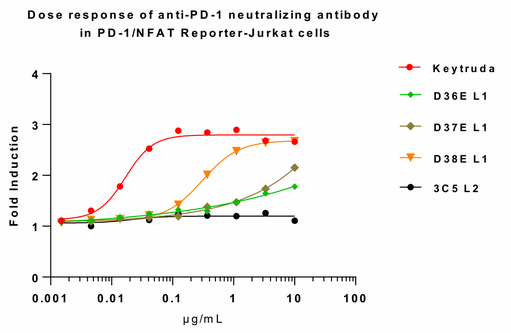
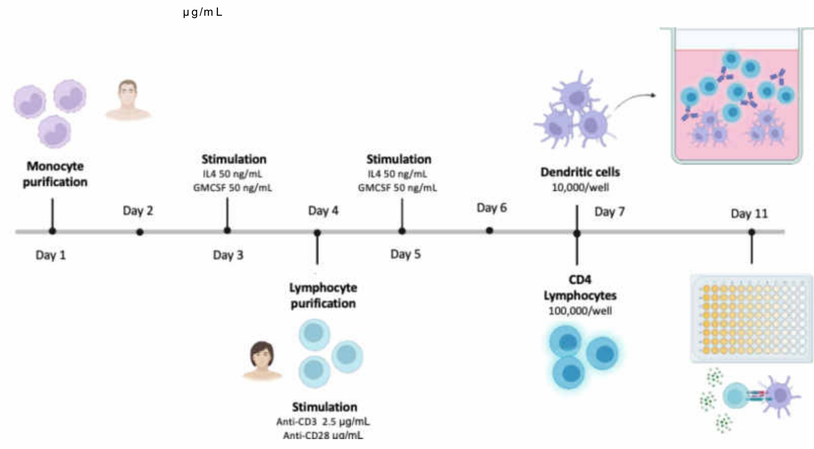



**FIGURE 6**. *Blocking in NFAT reporter Jurkat cells.*. Evaluation of the blocking of D36E L1, D37E L1 and D38E L1 in NFAT reporter Jurkat cells
Keytruda was used as a positive control and 3C5 (anti-VEGFR3 antibody) as a negative control.



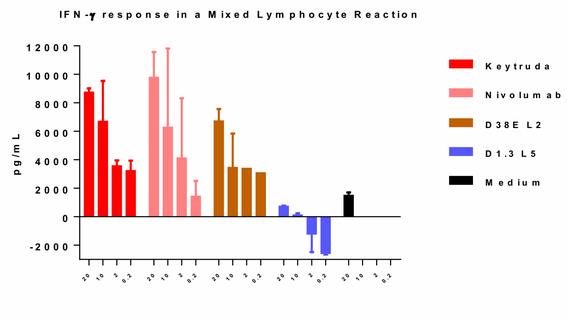




**FIGURE 7**. Expression of IFN-gamma response in a Mixed Lymphocyte Reaction. Evaluation of the production of IFN-gamma in a MLR. Keytruda and Nivolumab were used as positive controls and 3C5 (anti-VEGFR3 antibody) as a negative control.

## CONCLUSION

The D38 antibody obtained from ALTHEA 4:3 VH:VL Platinum Libraries™ proved to be a candidate to block the interaction *in vitro* between PD-1 and its ligand PD-L1. This activity could promote cancer cells destruction by stimulating the immune system. More importantly, it has shown to induce increased expression of cytokines such as IFN-gamma, which contributes to the antitumor immune response through its immunostimulatory and immunomodulatory effects. Thus, D38 seems to be a promising antibody to develop an anti-PD-1 therapeutic antibody to treat cancer.

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