Discovery and characterization of anti-PD-1 antibodies with therapeutic potential

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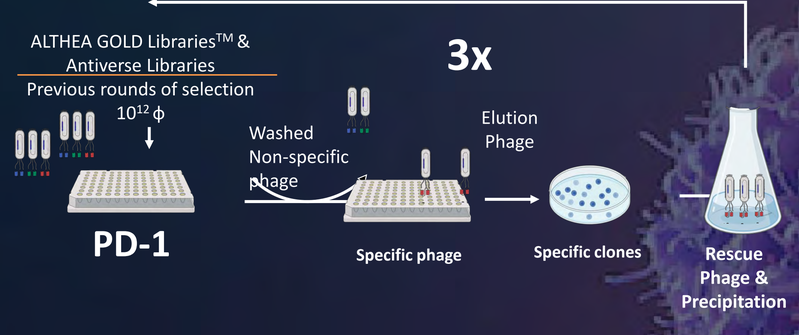
Abstract

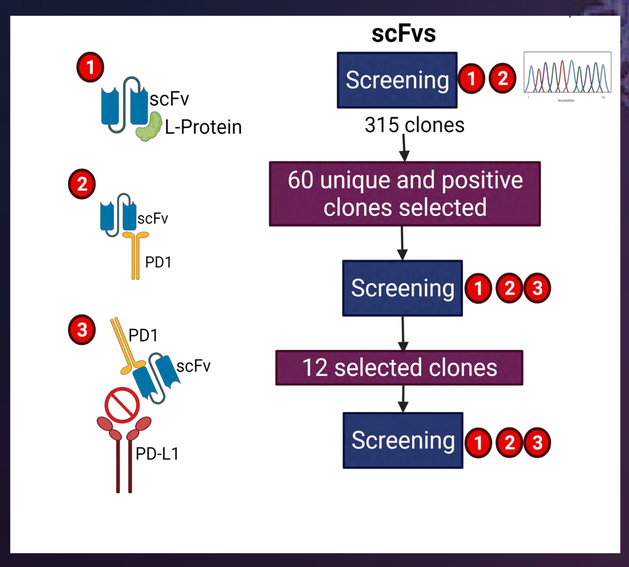
PD-1 is a member of the CD28 superfamily, which transmits inhibitory signals upon engagement with its ligands PD-L1 and PD-L2. Under normal conditions it helps to maintain immune homeostasis. However, when PD-1 is expressed on the surface of exhausted T and B cells, in a cancer or infection context, it promotes pathogenic cells expressing PD-L1 or PD-L2 evasion of the immune system by deactivating T cells cytotoxic activity. Therefore, inhibiting PD-1 by monoclonal antibodies has been a successful strategy to induce T cell mediated apoptosis of the cancer of infected cells, leading to treatment options for patients with diverse cancers including non-small cell lung cancer, melanoma, Hodgkin lymphoma, bladder, kidney and breast cancer, to mention the most relevant pathologies.

Phage display technology has demonstrated its robustness and reproducibility as a platform for human antibody discovery. To date, several dozens of approved monoclonal antibodies (mAbs) or molecules in pre-clinical development or in clinical trials have been obtained by this technology. In this poster, we present the results of employing phage display libraries for isolation of scFvs against the extracellular domain of PD-1 protein. After three rounds of pannings, novel anti-PD-1 scFv were found that exhibited specific binding to PD-1 antigen and blocking interaction with its ligand PD-L1. The expression yield in HEK293 cells, binding to PD-1, and blocking activity were evaluated in supernatants from cells transfected with nine specific sequences in a IgG4 format. The results confirmed the functional profile previously evaluated using the scFvs format. Based on these results nine IgG4s were purified and further characterized (See Poster *"Functional characterization of anti-PD!1 antibodies")*.

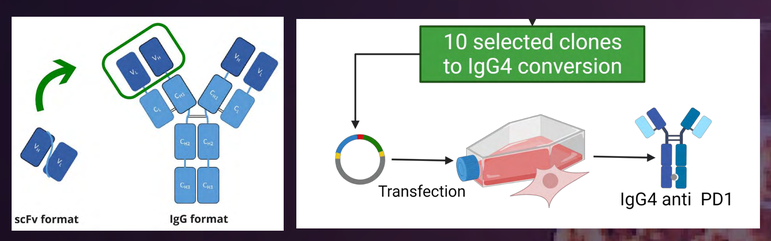
Panning. Selection of specific PD-1 scFvs



Soluble scFv screening and DNA sequencing

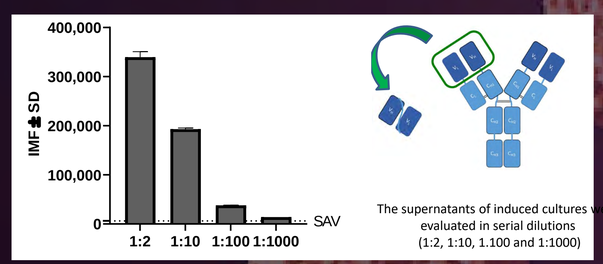


Convertion of scFv into IgG format



Generation and expresión of scFv-Keytruda (pembrolizumab)

PD-1 – scFv-Keytruda binding assay by Flow cytometry



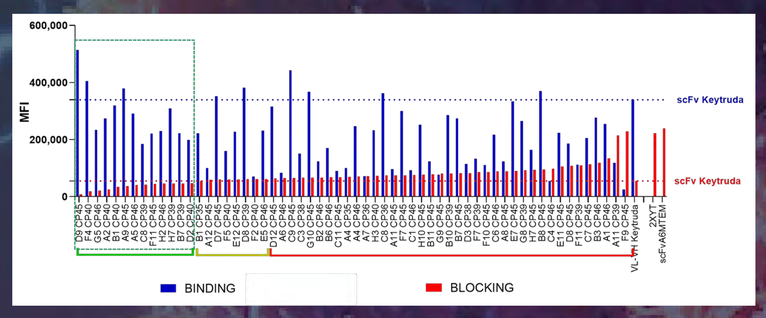
scFv-Keytruda was generated and validated as a positive control for the following anti-PD-1 (scFv format) screening.

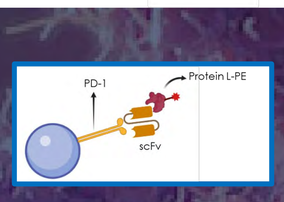
Summary of the Biopanning results & extended Screening

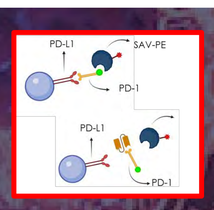
Panning strategy	3 rounds in Solid Phase Elution with trypsin and Glicine HCl
Positives Clones	47.6 % (150/315) Extended screening
Hit Rate	19 % (60/315). Obtained 60 Unique
Binding (Flow cytometry)	Differencial specific binding pattern with PD-1

Functional profile of the unique-specific-scFvs.

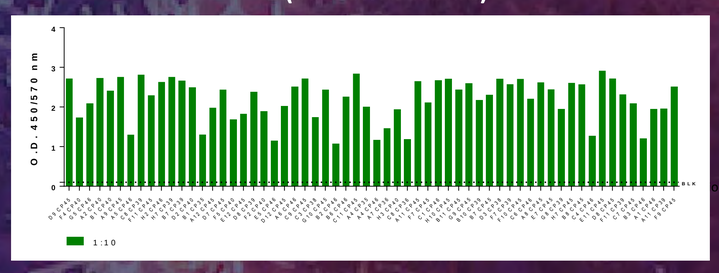
PD-1 Binding Assay & PD-1/PD-L1 Blockade Assay (60 clones tested)







Protein L Binding Assay by ELISA (for scFvs expression) (60 clones tested)

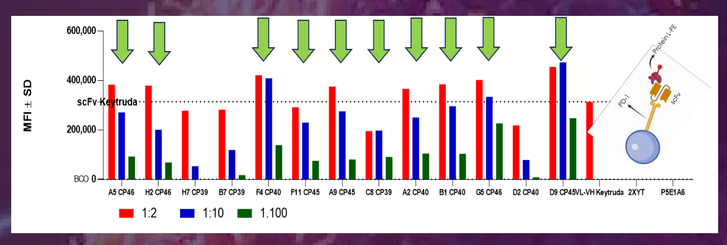


The PD-1/PD-L1 Blockade assay is a biologically relevant assay that can be used to measure the potency and stability of scFvs to block the PD-1/PD-L1 interaction.
We selected 12 promising scFvs by PD-1 binding and The PD-1/PD-L1 Blockade (green box)

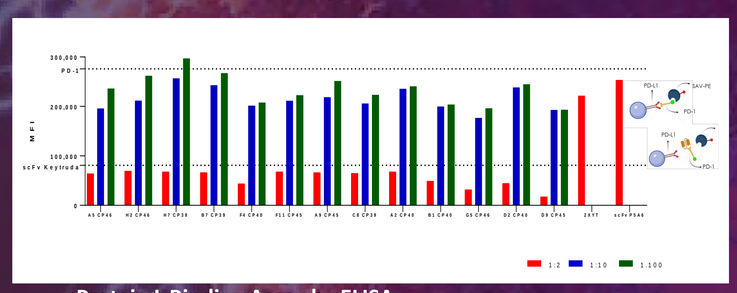
Functional confirmation of selected scFvs

In order to verify the functionality of scFv clones, We again evaluated: (1) specific bindings on PD-1 through Flow cytometry assay, (2) Blocking of PD-1/PD-L1 interaction and (3) scFv expression in supernatants of induced cultures.

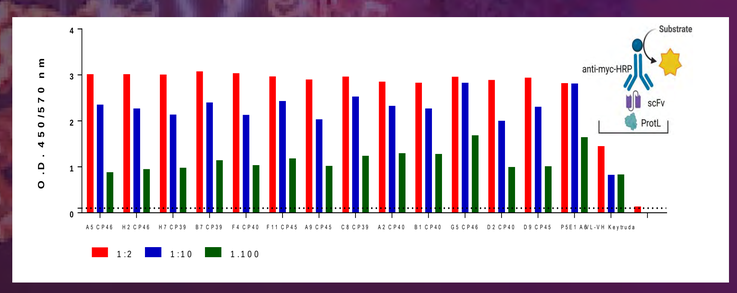
PD-1 Binding Assay (13 clones tested)



PD-1/PD-L1 Blockade Assay

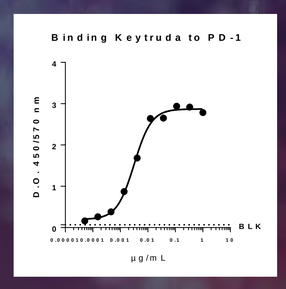


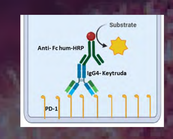
Protein L Binding Assay by ELISA



After normalization of the results, 9 of 13 clones resulted in good candidates to be converted into IgG4 format. Those were selected due to its binding and blockade activity

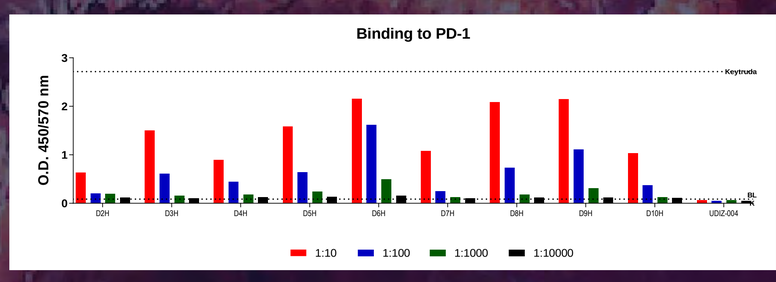
PD-1 - Keytruda binding assay has been standarized and validated by ELISA.

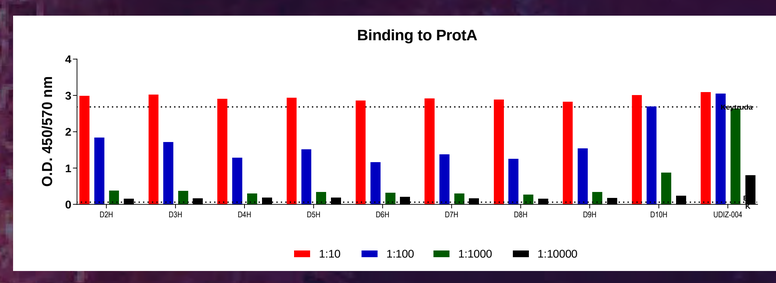


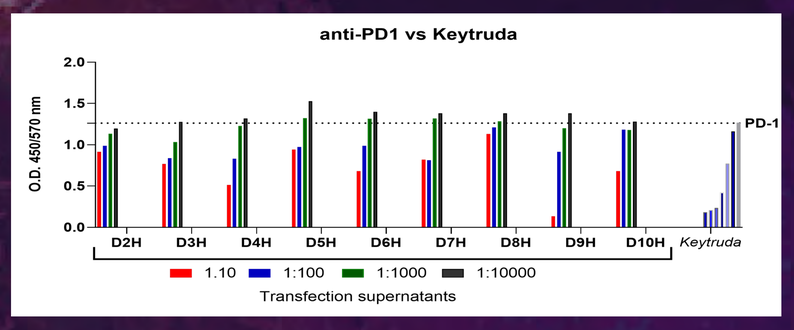


human IgG4 Conversion

To assess the performance of the scFvs in therapeutic format, nine scFvs were converted to hIgG4.
VH and VL regions of the scFvs were amplified by PCR and cloned in TGEX expression vector







Name scfv	Name IgG4
scfv_C8_CP39	D2H
scfv_B1_CP40	D3H
scfv_F4_CP40	D4H
scfv_A9_CP45	D5H
scfv_D9_CP45	D6H
scfv_F11_CP45	D7H
scfv_A5_CP46	D8H
scfv_H2_CP46	D9H
scfv_G5_CP46	D10H

9 selected clones have similar behavior using scFV or IgG4 format. The best binders and competitors with Keytruda, D4H, D5H, D6H, D8H y D9H, were expressed and purified to perform the biological and physicochemical characterization .

Conclusions

Keytruda was generated in our laboratory through molecular biology techniques in order to be used as positive control.

Our phage displayed platforms have successfully been used to develop several biologically active monoclonal antibodies.

Panning using rhPD-1 lead to isolation of 60 positive clones out of 315.

Nine of the clones were selected and converted into IgG4, with four having the best activity.

These four clones are in the process of further characterization to determine the best clones for therapeutic development.

References:

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