Selection of an optimal anti-PD-L1 single domain antibody format for the vectorization into oncolytic vaccinia virus and the generation of bispecific immunomodulators.

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Abstract 1885
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BACKGROUND

Single domain antibodies (**sdAbs**) isolated after immunization of camelids are particularly attractive formats for their high **modularity** and **small size** allowing a better diffusion into tumors. However, the short *in vivo* half-life of sdAbs, related to the lack of a Fc domain, limits their clinical application. By replicating specifically into tumor cells, the **oncolytic vaccinia virus** (VACV) is an optimal vector to deliver and maintain **high intra-tumoral concentrations** of therapeutic sdAb. Moreover, sdAb targeting immunological targets, such as **PD-L1**, may synergize the anti-tumoral activity of VACV**. Randox & Transgene** report the selection and characterization of a **sdAb targeting the human PD-L1** and the design of optimal formats, including bispecific **anti-PD-L1-TNFSF**, for vectorization into VACV.

METHODS

Alpacas were immunized with human PD-L1 protein and sdAb coding sequences were isolated by PCR. Anti-PD-L1 sdAb binders were selected by phage display and sdAb blockers of PD-L1/PD-1 interaction were identified by ELISA. The ability of the selected sdAb to disrupt the PD-L1/PD-1 interaction was verified on transformed and primary cells. To fine-tune an optimal anti-PD-L1, several sdAb formats were designed and vectorized into VACV. The sdAb format exhibiting the best PD-L1/PD-1 blocking activity was selected by the screening of culture supernatants of several VACV-sdAb infected tumor cells. Finally, anti-PD-L1 sdAb-TNFSF fusions were designed to generate a strong TNFRSF agonists active only in a PD-L1 positive environment.

RESULTS

SdAb clone 1A1 exhibited the best **PD-L1/PD-1 blocking** activity which remained unchanged after extensive humanization (latterly becoming named **GS542**).
GS542 was vectorized in VACV as monomeric, single chain homodimer, and fused to Fc domain or antibody hinge domain to foster dimerization together with full length IgG1 avelumab as anti-PD-L1 benchmark. All constructs were expressed by infected tumor cells. The **single chain homodimer** displayed the best PD-L1/PD-1 blocking activity, superior to that of avelumab.
Moreover, **GS542-TNFSF fusions** were designed to take advantage of the natural trimerization of TNFSF to increase the avidity for PD-L1 while clustering ligands at the surface of PD-L1+ cells **to trans-activate TNFRSF** pathways. Evaluation of these GS542-TNFSF fusions showed strong TNFRSF agonist activities **depending** on the presence of **PD-L1+ cells** making these constructs safer agonists.

CONCLUSION

An anti-PD-L1 sdAb with a strong blocking activity was selected, humanized and evaluated under different VACV-vectorized formats. The single chain dimeric sdAb expressed by VACV was identified as the best PD-L1/PD-1 blocking format. Furthermore, bispecific anti-PD-L1 sdAb-TNFSF fusions that exhibited strong agonist activities were characterized within a PD-L1+ environment.

KEY FINDINGS

- ✓ **sdAb GS542** isolated from immunized alpacas with same PD-L1 blocking activity as avelumab
- ✓ **Dimeric format** of sdAb is better than avelumab once vectorized in VACV
- ✓ **SdAb-CD40L or 4-1BBL** are efficient agonists of CD40/4-1BB pathways **ONLY** in presence of PD-L1$^+$ cells

Versatile blocking anti-PD-L1 single domain antibody for arming oncolytic vaccinia virus

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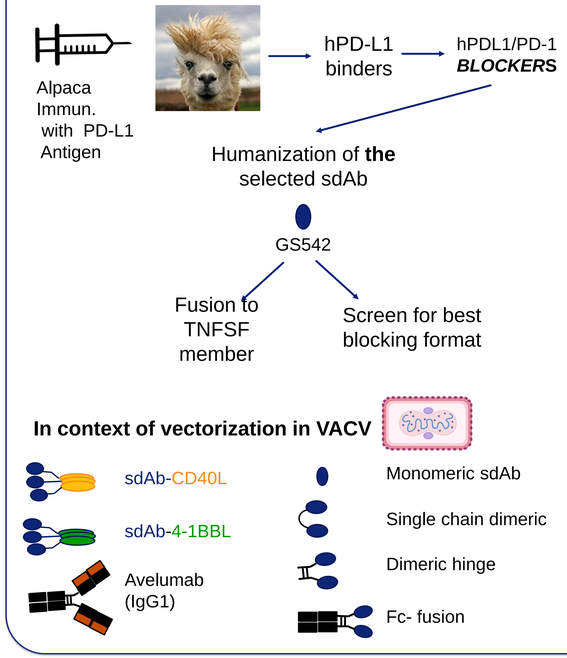
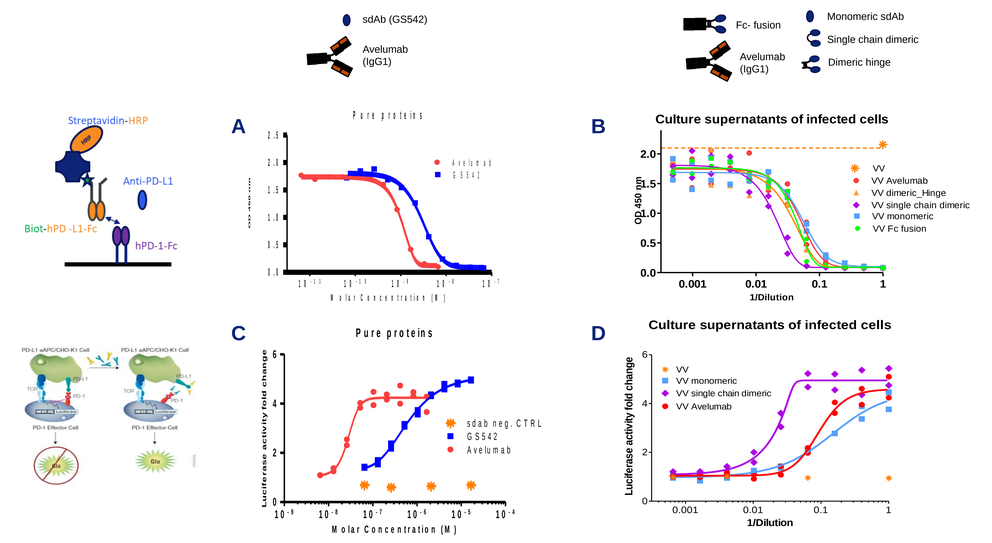
sdAb ISOLATION & DESIGNS FOR VECTORIZATION

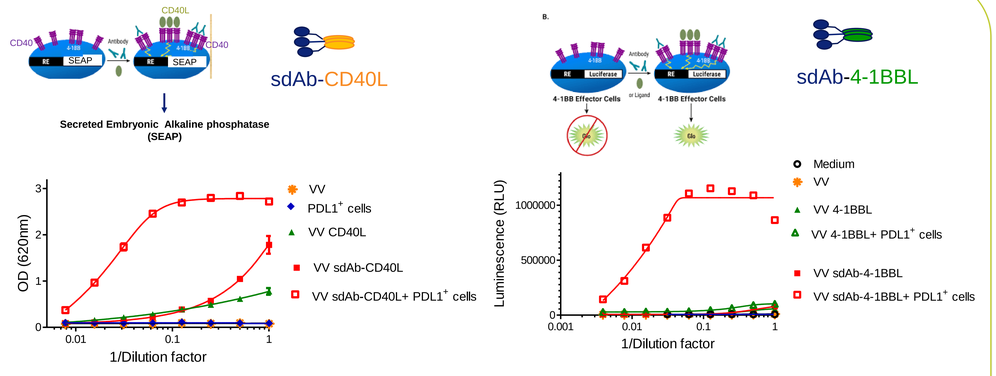


Dimeric fusion is the best PD-L1 blocking format



On two independent assays: competition ELISA (top) and cellular assay (bottom), the single chain dimeric format vectorized in VACV generated the best PD-L1/PD-1 blocking activity in supernatant of infected tumor cells. Graphs A and C are results obtained with avelumab and monomeric sdAb as purified proteins. Graphs B and D are results obtained with supernatant of HT29 cells infected with different VV

Fusions sdAb-TNFSF are strong agonists in presence of PDL1$^+$ cells



Vectorized fusions of anti-PD-L1 sdAb to either CD40 Ligand (left) or 4-1BB ligand (right) produced by VACV-infected cells are activating CD40 or 4-1BB pathways in cellular assays ONLY in presence of human PD-L1 expressing cells (here gastric cancer cell line Hs746T)