Humanized immune checkpoint (ICP) and co-stimulatory molecules for preclinical biologics assessment

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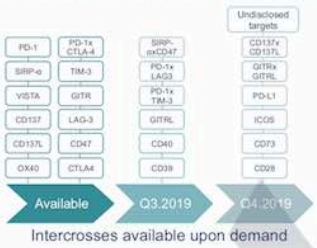
Introduction

Immuno-intervention through targeting of **activating and inhibitory immune checkpoints (ICPs)**, has shown promising results in the clinic over the last years. However, the biology of ICPs and their targeted modulation in the clinic raise several questions with regard to their potential safety and side effects: the entire activity spectrum of ICP modulators, used alone or in combination, is currently the subject of intense study.

To facilitate those researches, **we developed a pipeline of immuno-competent mouse models expressing humanized ICP instead of their mouse counterparts**; thus, compounds can be tested in the absence of endogenous cross-reacting mouse targets. The humanization strategy is target-dependent but ensures that at least the **entire extracellular domain (ECD) is from human origin**, allowing **versatility for compound testing *in vivo***. The strategy also ensures that the biology of the target, its physiological regulation and interacting partners are preserved.

Here we report a first set of data for humanized VISTA (hVISTA), hPD-1 and hOX40 models: these humanized proteins behave as their murine counterparts, display proper regulation and allow for ***in vivo* preclinical efficacy and toxicity studies of biologics targeting ICP**.

Pipeline of preclinical models

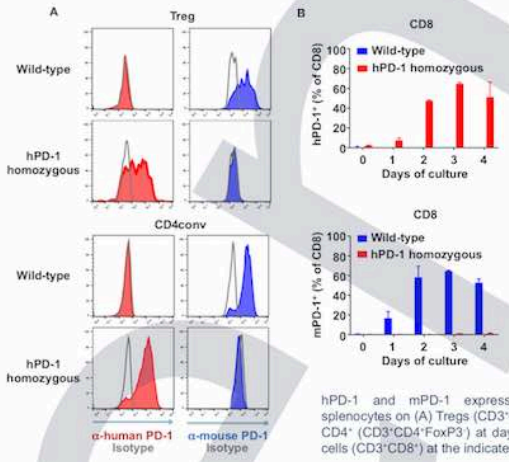


Intercrosses available upon demand

PD-1 humanized model

We developed the hPD-1 humanized model by Knockin at the mouse PD-1 locus. **The entire extracellular domain of PD-1 is from human origin allowing *in vivo* specificity and mechanism of action studies of any compound targeting extracellular human PD-1.**

hPD-1 expression pattern recapitulates mPD-1

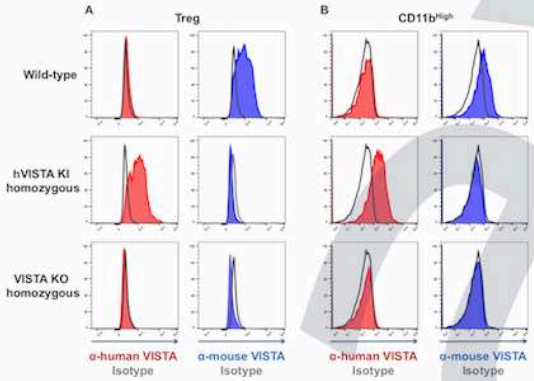


hPD-1 and mPD-1 expression on αCD3/αCD28-activated splenocytes on (A) Tregs ($CD3^+CD4^+CD25^+FoxP3^+$), conventional $CD4^+$ ($CD3^+CD4^+FoxP3^-$) at day 2 of culture and (B) on $CD8^+$ T cells ($CD3^+CD8^+$) at the indicated time points.

VISTA humanized model

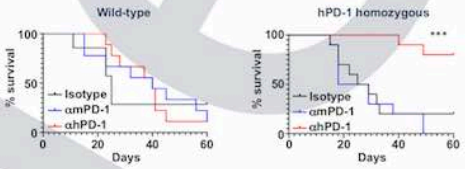
We generated a humanized VISTA model by Knockin for compound efficacy assessment: human VISTA is driven by the mouse endogenous locus. **The model design enables versatility for human VISTA compound specificity study.**

hVISTA expression pattern recapitulates mVISTA



Splenocytes isolated from WT, homozygous VISTA KO and homozygous hVISTA mice. mVISTA and hVISTA expression were analyzed on Tregs (viable, $CD3^+$, $CD4^+$, $CD25^+$, $Foxp3^+$) and $CD11b^{High}$ (viable, $CD11b^{High}$). Similar results were obtained on conventional $CD4^+$ T cells, $CD8^+$ T cells and dendritic cells.

In vivo anti-tumor activity is observed in response to anti-PD-1 treatment in hPD-1 mice



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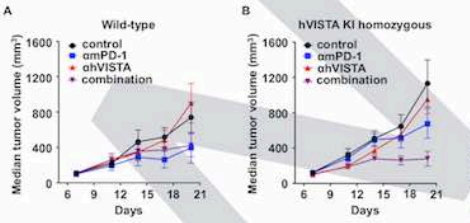
Survival of mice of indicated genotype implanted intracranially with GL261 murine glioma cells and treated bi-weekly with isotype control, anti-human PD-1 (100 µg/mouse) or anti-mouse PD-1 (2.5 mg/Kg) (Mantel-Cox test: *** p<0.001).

Anti-tumor effects in response to anti-human VISTA + anti-mouse PD-1 treatment

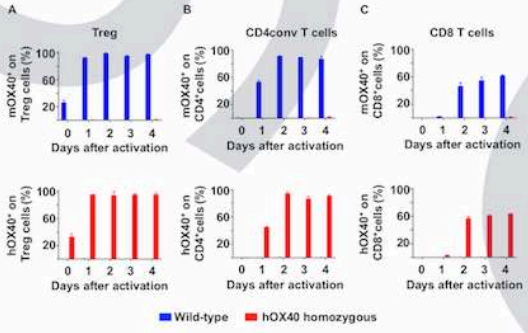


Tumor volume analysis on WT (left panel) and hVISTA mice (right panel) inoculated with MC38 tumors s.c. on day 0. Anti-mouse PD-1, anti-human VISTA or isotype treatment at day 7, 11, 15, and 19 (i.p. 20mg/kg).

OX40 humanized model

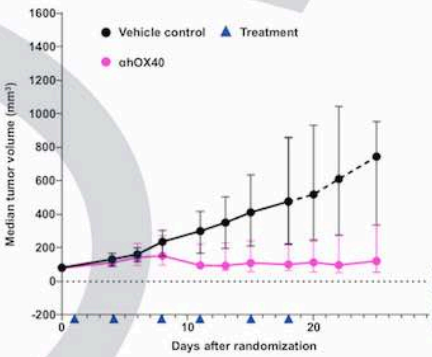
The OX40 humanized model, developed by Knockin (KI) at the mouse *OX40* locus, expresses a fully humanized OX40: human extracellular, transmembrane and intracellular domains. hOX40 expression displays physiological regulation and expression pattern. The model enables assessment of OX40-targeting compounds in immunocompetent mice.

In vivo expression of hOX40 on TILs



hOX40 and mOX40 expression on splenocytes activated with αCD3/αCD28 in presence of mIL-2. Kinetic of hOX40 and mOX40 expression on (A) Treg (viable, $CD3^+CD4^+CD25^+FoxP3^+$), and (B) conventional CD4 (viable, $CD3^+CD4^+FoxP3^-$), and (C) CD8 (viable,$CD3^+CD8^+$)Tcells. Results are expressed as mean±SD.

In vivo anti-tumor effect in response to anti-hOX40 mAb treatment



Survival of hOX40 mice inoculated with MC38 tumors s.c. on day 0 and treated with vehicle or anti-human OX40 (7.7 mg/kg) at the indicated time.

Conclusion

We report a first set of data for immuno-competent humanized hVISTA, hPD-1 and hOX40 models. These humanized targets behave as their murine counterparts, display proper regulation and provide novel tools for ***in vivo* pre-clinical efficacy and toxicity study of biologics targeting ICP**. These models have been crossed with other ICP humanized models for profiling of combo therapies in a context of antitumor response, but also for the modulation of autoimmune disease and inflammation.

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