Novel Humanized CTLA-4 Mouse Model for Assessment of Efficacy of Biologics

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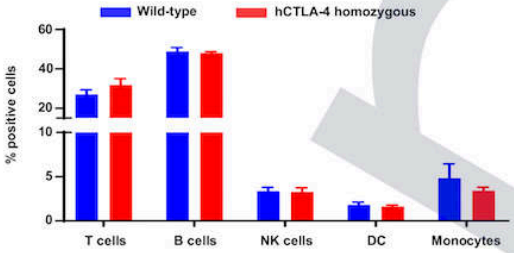
Abstract

Immuno-intervention through targeting of activating and inhibitory immune checkpoints (ICPs) has shown promising results in the clinic over the last years. The entire activity spectrum of ICP modulators, used alone or in combination, is currently the subject of intense study. To facilitate this research, we developed a pipeline of immunocompetent 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 at least that the entire extracellular domain 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 the validation of the humanized CTLA4 model.

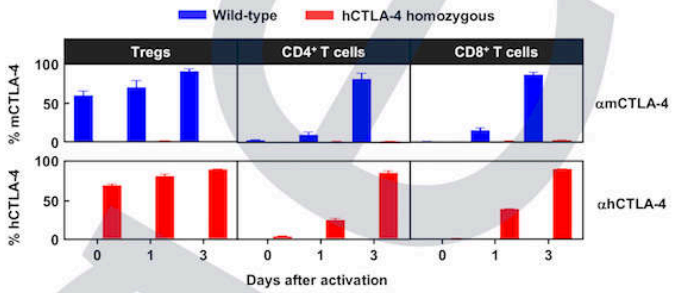
Ex vivo experiments

Normal immune cell distribution in hCTLA-4 mice suggests the presence of a functional CTLA-4



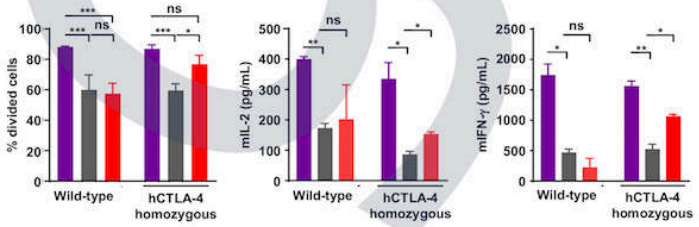
Splenocytes were isolated from wild-type and homozygous hCTLA-4 mice. Analysis of splenic cell population gated on live cells: T cells ($CD3^+$ $CD19^-$), B cells ($CD3^-$ $CD19^+$), NK cells ($CD3^-$ $CD19^-$ $NKp46^+$), DC ($CD3^-$ $CD19^-$ $CD11b^+$ $CD11c^+$) and monocytes ($CD3^-$ $CD19^-$ $CD11b^+$ $CD11c^-$). n=3.

Human CTLA-4 expression recapitulates mouse CTLA-4



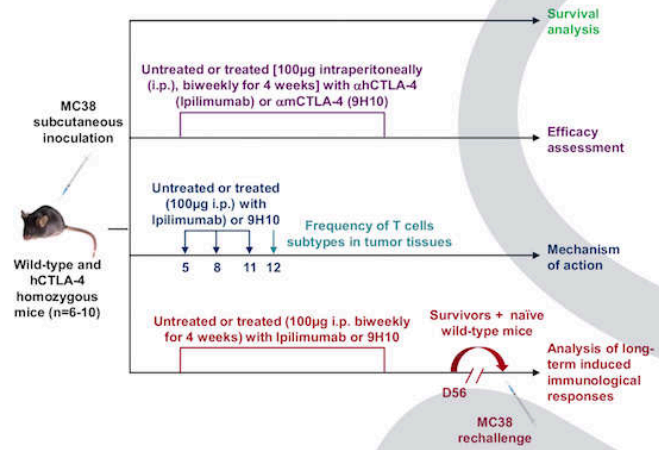
Splenocytes isolated from wild-type and homozygous hCTLA-4 mice activated with αCD3. Mouse and human CTLA-4 expression evaluated at D+3 on Tregs (viable, $CD3^+CD4^+CD25^+Foxp3^+$), conventional $CD4^+$ (viable, $CD3^+CD4^+Foxp3^-$) at day 3, and $CD8^+$ (viable, $CD3^+CD8^+$) T cells at indicated time points. Representative data of two independent experiments (n=2).

Suppressive function of Treg is specifically decreased in presence of αhCTLA-4 mAb



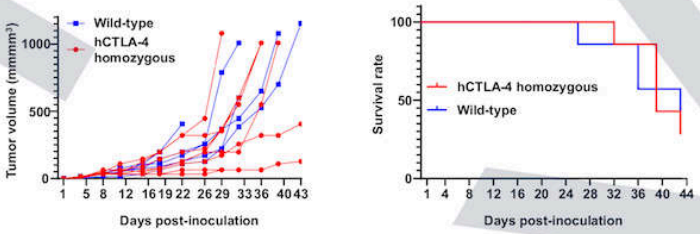
Splenocytes isolated from wild-type and homozygous hCTLA-4 mice were activated with αCD3. Mouse and human CTLA-4 expressions were evaluated at the indicated time point on viable Tregs ($CD3^+CD4^+CD25^{high}Foxp3^+$), $CD4^+$ T cells ($CD3^+CD4^+Foxp3^-$) and $CD8^+$ T cells ($CD3^+CD8^+$). Unpaired T-test: *p<0.5, **p<0.001, ***p<0.0001, ns: not significant.

Experimental design for *in vivo* experiments

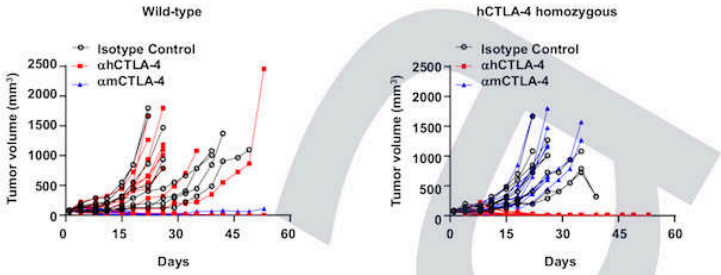


In vivo experiments

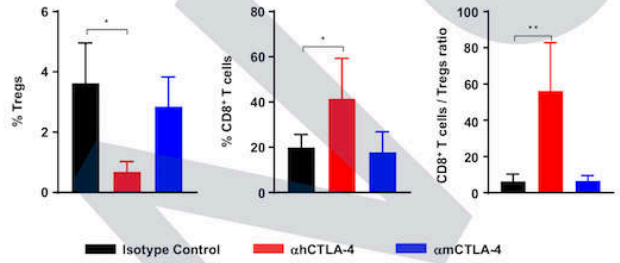
Wild-type and hCTLA-4 mice have similar tumor uptake, and survival rate in MC38 tumor model



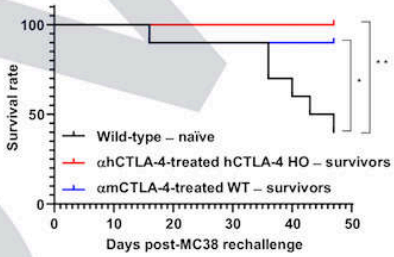
Anti-tumor growth effect demonstrated in response to ipilimumab in hCTLA-4 mice



Ipilimumab treatment increases $CD8^+$ T cells / Tregs ratio in tumor



Treatment with ipilimumab induces long-term tumor-specific immunological memory



Conclusions

These data show that hCTLA4 model enables:

- Assessment of efficacy
- Mechanism of action of biologics
- Study of long-term induced immunological responses

The hCTLA-4 is a novel preclinical model to evaluate therapies directed against human CTLA-4 in mice with fully functional immune systems. This model is being crossed with other ICP humanized models for assessment of combo therapies and bispecifics agents.