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Poster : #103

Establishment of a Humanized PD-1 Mouse Model for *in vivo* Pharmacological Evaluation of Anti-Human PD-1 Antibodies

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Introduction

The recent approval of two immunotherapeutic agents targeting the immune checkpoint programmed cell death-1 (PD-1) protein has ramped up the efforts of generating other investigational PD-1 antibodies and optimizing combinatory therapies[1]. However, the lack of animal models for *in vivo* efficacy evaluation has slowed down the process. Syngeneic mouse tumor models, thanks to their fully competent immune system have been widely used for testing surrogate anti-mouse PD-1 antibodies[2]. However they cannot serve as models for testing human biological therapeutics, because of species specificity issues.

To address this need, we developed the HuGEMM™ models, featuring a fully competent murine immune system. In this models we replaced the endogenous murine therapeutic target with the human counterpart, and we use them to evaluate human biological therapeutics in vivo.

Results

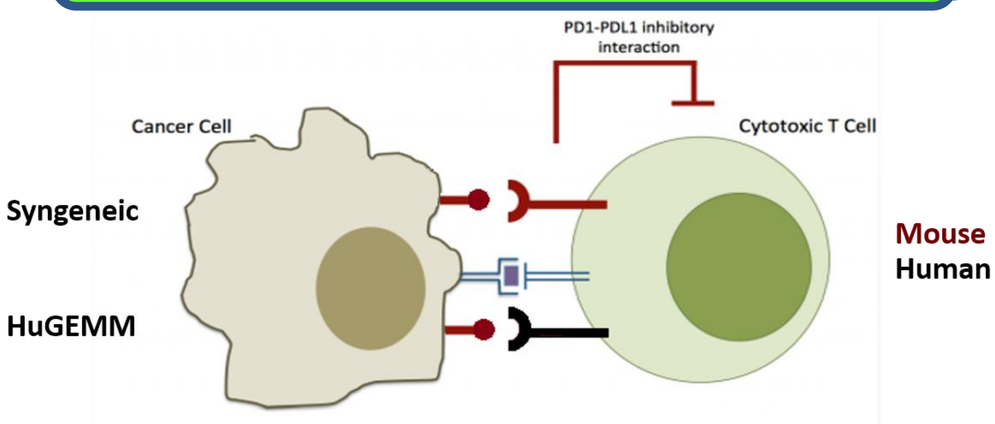

Figure 1. Concept of HuGEMM

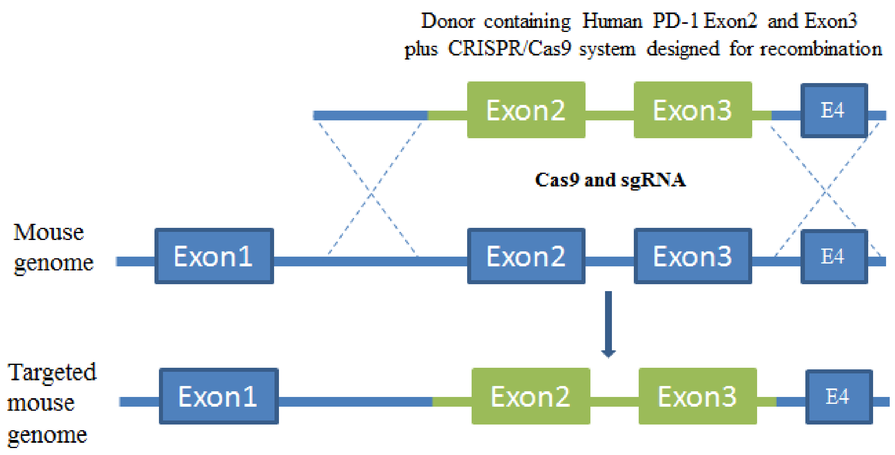

Figure 2. Strategy for generating the Pdcd1 (E2 and E3) KI in mouse

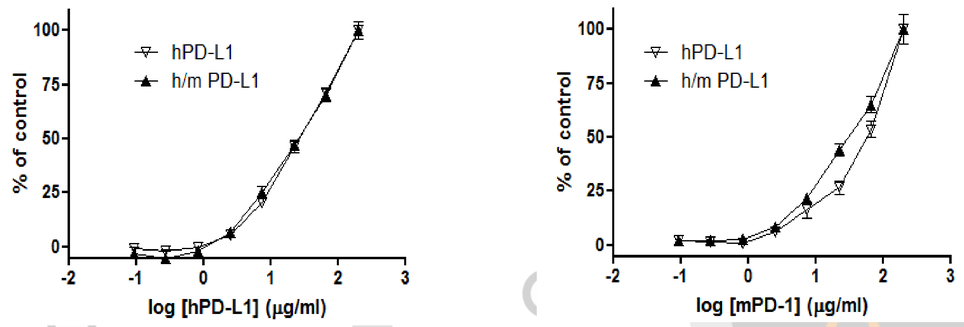

Figure 3. hPD-1 and h/mPD-1 transfected in HEK293 cells showed comparable dose-dependent response to FITC-conjugated human or mouse PD-L1

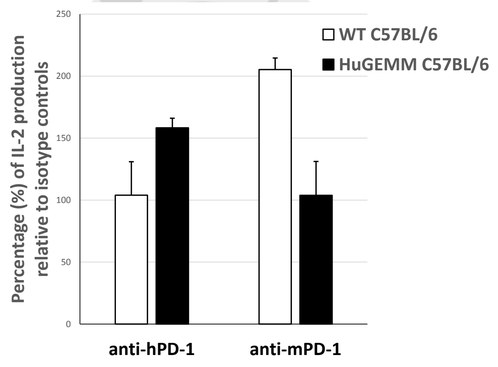

Figure 4. IL-2 production in HuGEMM mouse splenocytes relative to those from wild-type C57BL/6 mice in mixed lymphocyte reaction after stimulation with anti-hPD-1 or anti-mPD1 antibody

Tumor	TV(mm^3)	CD4(%)	CD8(%)
PBS (#4558)	1595.40	0.014	0.635
Anti-PD1 (#4546)	413.50	0.243	1.470

Table 1. Tumor volume and TILs

Abstract

We constructed a chimeric human/mouse PD-1 gene (h/mPD-1) containing the human exon 2&3 and the murine exon 1&4. We expressed the recombinant protein and tested its binding to the PD-L1 ligand from both murine and human origin, as well as to the anti-human PD-1 antibody. Our data demonstrated that the chimeric h/mPD-1 protein can bind robustly to both mPD-L1 and hPD-L1, as efficiently as the endogenous murine PD-1. Importantly, h/mPD-1 also recognizes the anti-human PD-1 antibody, which prevents h/mPD-1 from binding to mouse or human PD-L1. We knocked-in the h/mPD-1 into C57BL/6 mice to create homozygous PD-1 HuGEMM. We confirmed that T-cells from HuGEMM mice express the chimeric h/mPD-1, both *in vivo* and *ex vivo*. However, h/mPD-1 expression level in the HuGEMM is lower than the endogenous mPD-1 in the wild-type C57BL/6 mice under induction (only ~10%). Subcutaneous syngeneic engraftments of MC38 cells in h/mPD-1 HuGEMM, grow less robustly when compared to the same grafts in the wild-type mice. Furthermore, the response to anti-mouse PD-1 by MC38 tumors in wild-type mice is stronger than the observed response to anti-human PD-1 by MC38 tumors grown in HuGEMM. This diversity of responses may result from the lower expression level of m/hPD-1 in the HuGEMM, leading to a stronger autoimmune response that inhibits tumor growth. We could speculate that if the growth of MC38 tumors does not rely on the inhibition of the PD-1/PDL-1 signaling, it also may not be responsive to anti-PD-1 therapeutics. Interestingly, we devised a method to artificially enhance MC38 tumor growth in HuGEMM. Under this condition, MC38 tumors start to become also responsive to anti-human PD-1 antibody, as we were able to show in a preliminary study.

Taken together our data suggests that our PD-1 HuGEMM provides an urgently needed model for the evaluation of the *in vivo* efficacy of PD-1 therapeutics. We are currently, re-engineering the chimeric gene (version 2) in order to raise the expression of h/m PD-1 to the intact mouse PD-1 level, to improve this model. In the meantime, we are also engineering HuGEMMs for other checkpoint targets (e.g. CTLA4, PD-L1, OX40, 4-1BB, etc.) for evaluating other checkpoint therapeutics.

References

1. Pardoll, D.M. The blockade of immune checkpoints in cancer immunotherapy. Nature reviews Cancer 12, 252-264 (2012).

2. Allard, B., Pommey, S., Smyth, M.J. & Stagg, J. Targeting CD73 enhances the antitumor activity of anti-PD-1 and anti-CTLA-4 mAbs. Clinical cancer research : an official journal of the American Association for Cancer Research 19, 5626-5635 (2013).

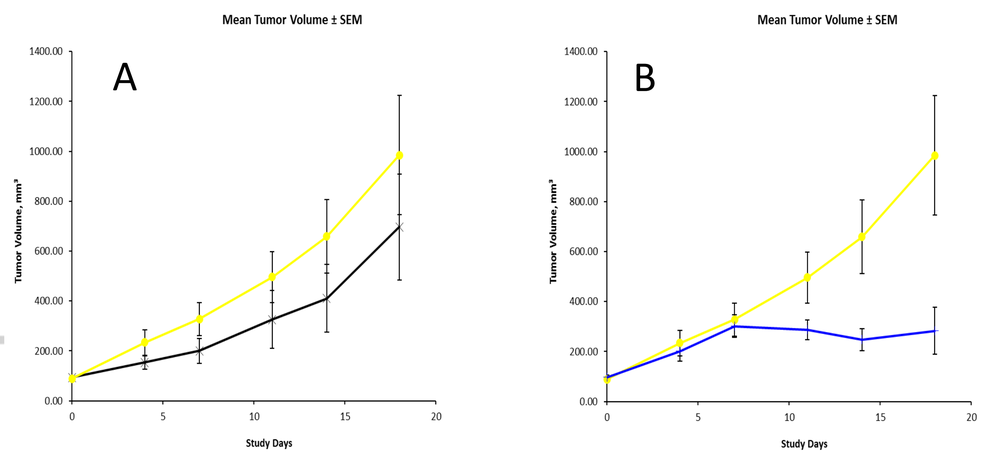

Figure 5. A. Growth curve with (yellow) & without (black) conditioning; B. Tumor response to anti-human PD1 antibody (blue) *vs* vehicle (yellow)

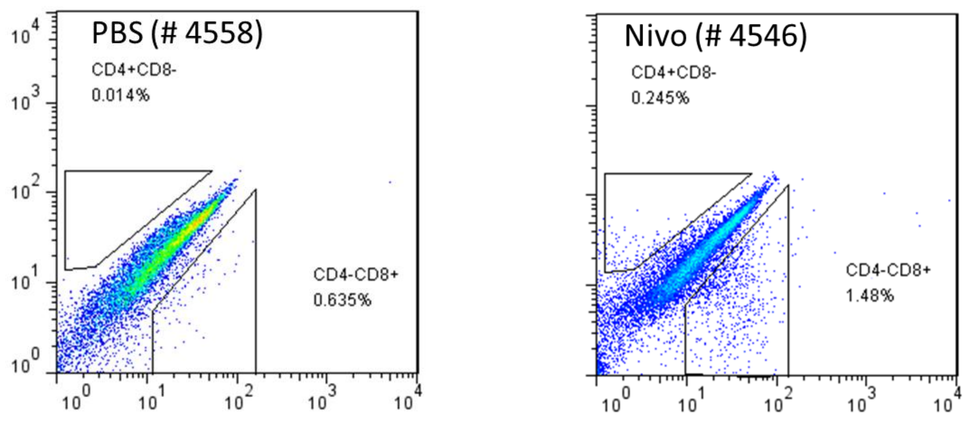

Figure 6. TILs in tumors without (left) and with (right) anti-hPD1 treatment

Conclusions

We have successfully created HuGEMM-HuPD1 (ver1) model for evaluating anti-human PD1 antibody and combination therapies.

a) HuGEMM-HuPD1 with low huPD1 expression can still support MC38 tumor growth under conditioning;
b) MC38 tumors respond to anti-human PD1 antibody
c) The response is correlated to the increased TILs
d) A new version of HuGEMM-huPD1 (Ver2) has been created and currently been validated for evaluating anti-human PD1 antibody
e) Other HuGEMM-huChPt mice are being created and validated

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