A HuGEMM h/mPD1 Mouse Model for Testing Anti-Human PD-1 Therapeutics

Zhun Wang[1], Bin Cai[2], Gang Chen[2], Jinping Liu[1], Annie Xiaoyu An[1,3], Zhensheng Wang[1], Davy Xuesong Ouyang[1], Jean Pierre Wery[1], Jay Liu[2], Xin Dong[2], Henry Q.X. Li[1,3]

[1]Crown Bioscience Inc., 3375 Scott Blvd, Suite 108, Santa Clara, CA95054, [2]Nanjing Galaxy Biopharmaceutical Co. Ltd., [3]State Key Laboratory of Natural and Biomimetic Drugs, Peking University.

Introduction

The recent approval of two monoclonal antibodies targeting the immune checkpoint programmed cell death-1 (PD-1) protein has fostered the interest around the application of biological therapies targeting PD-1, including new combinatory strategies[1]. However, the lack of animal models for testing *in vivo* efficacy has hindered the research process. Syngeneic mouse models are widely used for testing surrogate anti-mouse PD-1 antibodies[2]. However they cannot serve as models for human-specific biologic therapeutics.

To address this need, we set out to develop a new model by directly replacing the mouse PD-1 protein with the human counterpart, while maintaining a functional murine immune system (HuGEMM™). These mice can be used to evaluate human biologic therapeutics *in vivo*.

Abstract

We engineered a chimeric human/mouse PD-1 gene (h/mPD-1) made up from the human exon 2&3 and mouse exon 1&4. We expressed the recombinant protein and tested its binding to both the murine and human PD-L1 ligand, as well as to the anti-human PD-1 antibody. We show that the chimeric h/mPD-1 protein can bind to both mPD-L1 and hPD-L1, as efficiently as the endogenous murine PD-1. Importantly the anti-human PD-1 antibody can also recognize h/mPD-1 and consequently block its binding to murine or human PD-L1. We knocked-in h/mPD-1 into C57BL/6 mice to generate homozygous PD-1 HuGEMM. We confirmed that the T-cells from the knock-in mice express the chimeric h/mPD-1. 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. 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 our PD-1 HuGEMM provides an urgently needed model for *testing in vivo* efficacy of human PD-1 therapeutics. We are currently working on improving our model by re-engineering the chimeric gene (version 2) to enhance the expression of the h/mPD-1 gene. We are also working on the development of HuGEMMs for other checkpoint proteins (CTLA4, OX40, 4-1BB, etc.) for targeting the corresponding biologic therapeutics.

Results

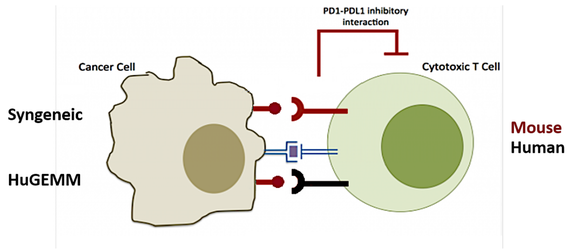

Figure 1. Concept of HuGEMM

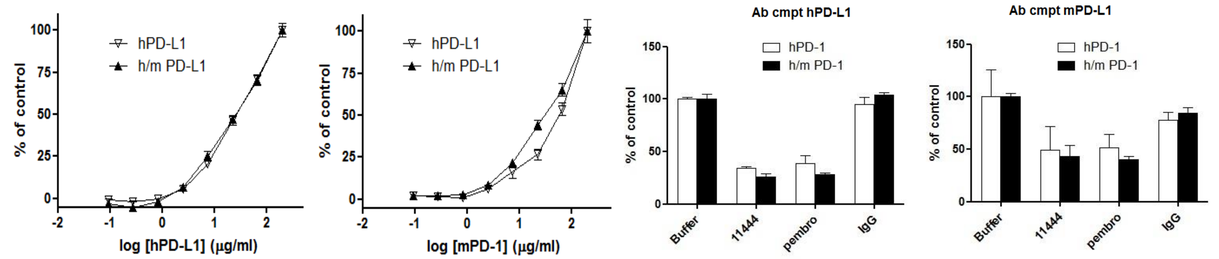

Figure 2. hPD-1 and h/mPD-1 transfected in HEK293 cells showed comparable dose-dependent responses to human or mouse PD-L1 conjugating with FITC (left) and comparable results in competition test when human or mouse PD-L1 was added with PD-1 antibody (right)

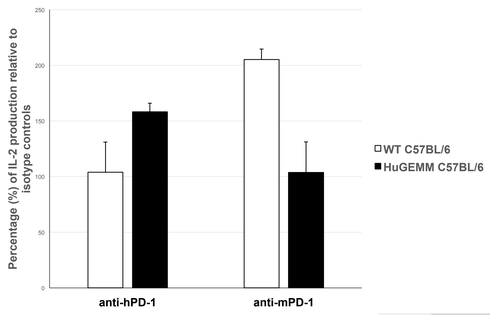

Figure 3. IL-2 production in HuGEMM mouse splenocytes relative to those from wild type C57BL/6 mice in mixed lymphocyte reaction after stimulation with anti-hPD-1 or anti-mPD1 antibody

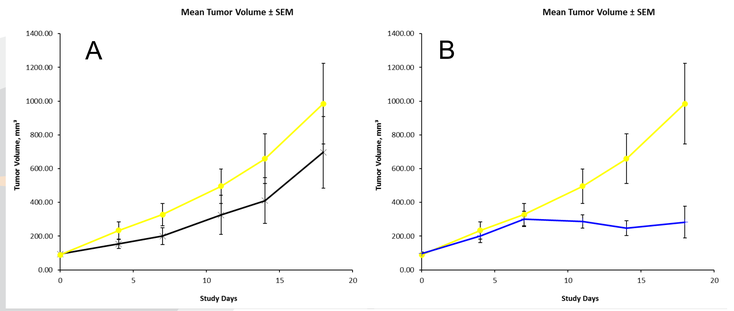

Figure 4. A. Growth curve with (yellow) & without (black) conditioning; B. Tumor response to anti-human PD-1 antibody (blue) *vs* vehicle (yellow)

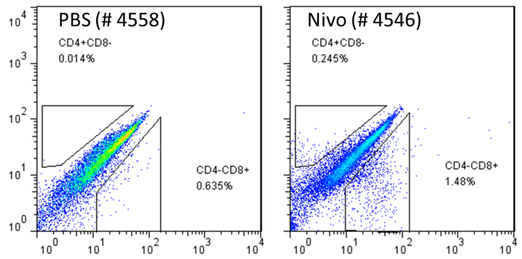

Figure 5. TILs in tumors without (left) and with anti-hPD-1 treatment (right)

Tumor	TV(mm³)	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

Conclusions

We successfully created a HuGEMM model expressing the chimeric h/mPD-1 protein (HuGEMM-huPD1) for the evaluation of anti-hPD1 antibodies and combination therapies. We find:

a) Our HuGEMM expresses h/mPD1 at low levels but can still support MC38 tumor growth under conditioning;
b) MC38 tumors respond to anti-hPD1 antibody;
c) This response correlates with increased TILs;
d) An improved version of the HuGEMM-huPD1 model has been created (V2) and is currently being validated;
e) Other HuGEMM-huChPt mice are under construction/validation

References

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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).

Contact information: HenryLi@crownbio.com