HuGEMM™ and HuCELL™ Models

CrownBio
CONNECTING SCIENCE TO PATIENTS

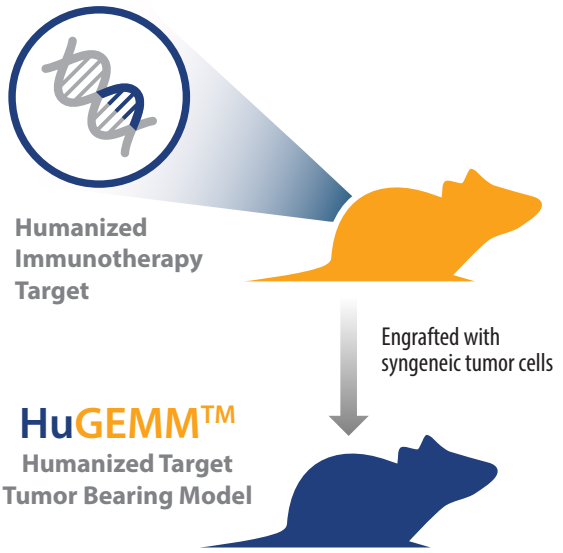
Progress your human-specific immunotherapeutics *in vivo* with our unique humanized drug target platforms

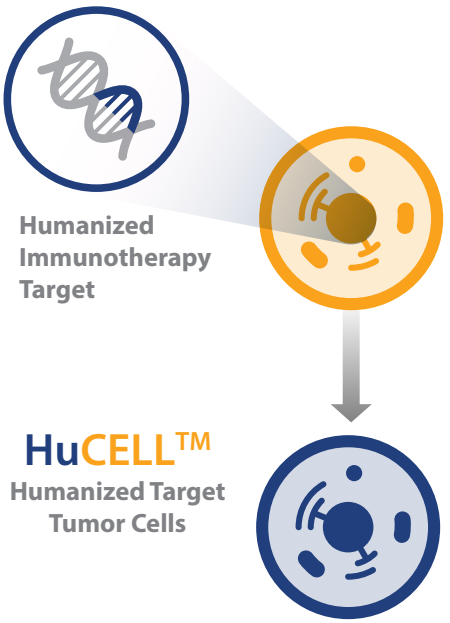
Discover the benefits of using our specific humanized target models to accelerate your immuno-oncology drug discovery programs.

The preclinical development of human-specific immunotherapeutics such as checkpoint inhibitors is currently hampered by a lack of immunotherapy models featuring human targets in the presence of a functional immune system.

CrownBio has developed HuGEMM and HuCELL models, allowing the evaluation of specific human biological therapies *in vivo*:

- HuGEMM mouse models expressing a humanized drug target including PD-1, PD-L1, or CTLA-4.
- HuCELL syngeneic tumor cells expressing humanized ligands e.g. PD-L1.
- For *in vivo* studies within functioning murine immune systems.
- Key checkpoint target platforms developed, including double knock-in models, with many more under development.





+1.855.827.6968 | busdev@crownbio.com | www.crownbio.com

HuGEMM and HuCELL Key Facts

CrownBio
CONNECTING SCIENCE TO PATIENTS

CrownBio provides a range of humanized drug target models to evaluate human-specific therapeutics within mice with a functional immune system:

- HuGEMM mouse models expressing humanized checkpoint proteins (summarized in **Table 1**).
- Complemented by HuCELL syngeneic cell lines expressing humanized target ligands e.g. MC38 expressing hPD-L1.
- Models fully validated to show human protein expression via FACS, and TGI response to human specific antibodies.
- Further models/targets under development for both the HuGEMM and HuCELL platforms.

Challenges in Developing Human-Specific Immunotherapeutics

While immunotherapy demonstrates an extremely promising treatment option for cancer patients, advancements in the field have inevitably uncovered subsequent challenges and barriers to further development.

Within checkpoint inhibitor evaluation, the lack of models to evaluate human-specific therapeutics *in vivo* has hindered research. Surrogate anti-mouse checkpoint inhibitors were initially evaluated in syngeneic models; however, human biological therapeutics cannot be tested in syngeneic models due to species specificity issues. There is a high unmet need to develop appropriate animal models to directly evaluate anti-human PD-1, PD-L1, CTLA-4 etc. antibodies *in vivo* before moving to successful human clinical trials.

HuGEMM and HuCELL Humanized Drug Target Models[1]

CrownBio has developed the HuGEMM platform which allows the evaluation of specific human biological therapies *in vivo*, in mice with a functional murine immune system and with murine proteins (the drug target) directly replaced with their human counterpart e.g. human PD-1 knocked in to replace mouse PD-1[2].

The HuGEMM platform provides an efficient method to study a range of targeted human immunotherapies *in vivo*. Available models are shown in **Table 1**, with further models under development.

We have also developed HuCELL[3] – an associated platform for drug targets which are located on the tumor cells e.g. PD-L1. Mouse tumor cells have been engineered to express humanized ligands, with the MC38 model available expressing human PD-L1 for the evaluation of anti-human PD-L1 antibodies. HuCELL and HuGEMM models can be combined as required to suit client research needs.

Table 1: HuGEMM Model Pipeline

Single Knock-In Models		Double Knock-In Models	
Target	**Status**	**Target**	**Status**
PD-1[2]	Available	**PD-1/PD-L1**	Available
PD-L1	Available, additional HuCELL model available[3]	**PD-1/OX40**	Available
CTLA-4[4]	Available	**PD-1/CTLA-4**	Available
CD137[5]	Available	**PD-1/LAG3**	Available
OX40[6]	Available	**PD-1/CD40**	Breeding
LAG3	Available	**PD-1/CD137**	Breeding
CD40	Available	**PD-1/TIGIT**	Breeding
GITR	Validating	**PD-L1/LAG3**	Available
ICOS	Validating	**Transgenic Models**	
TIGIT	Validating	**Target**	**Status**
CD38	Validating	**CD3E**[7]	Available

Case Study 1

PD-1 HuGEMM Model Development and Characterization[1]

A chimeric human/mouse PD-1 gene (h/mPD-1) was created by recombining human PD-1 exon 2 into the mouse locus. Homozygous knock-in mice (PD-1 HuGEMM) were characterized for anti-PD-1 studies (whole chimeric protein sequences available on request).

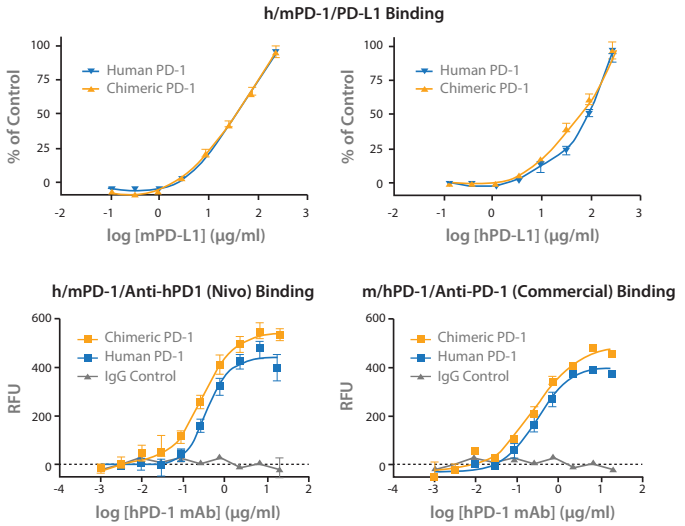
The chimeric h/mPD-1 protein was shown *in vitro* to bind to both mouse and human PD-L1 as efficiently as the human PD-1 receptor (**Figure 1, upper panel**). The chimeric protein is also recognized by anti-human PD-1 antibodies which disrupt the PD-1/PD-L1 interaction (**Figure 1, lower panel**). Human PD-1 expression was validated by FACS analysis in this model.

+1.855.827.6968 | busdev@crownbio.com | www.crownbio.com

HuGEMM and HuCELL Factsheet

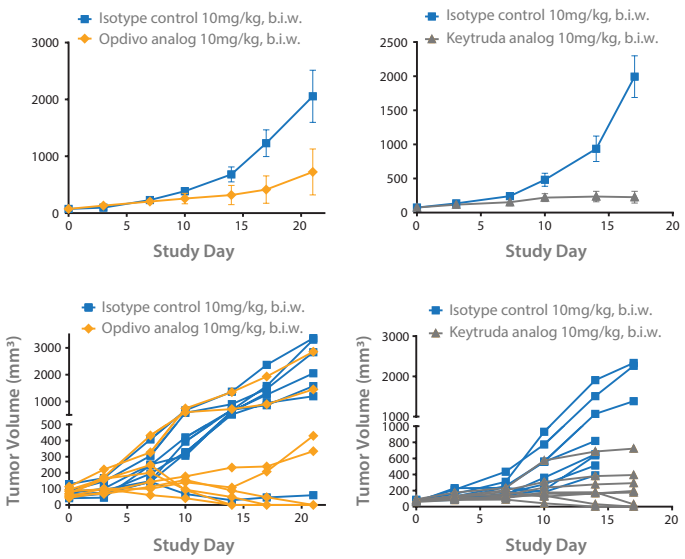
CrownBio
CONNECTING SCIENCE TO PATIENTS

Figure 1: Chimeric h/mPD-1 Binds to Anti-hPD-1 Antibody and to a PD-L1 Recombinant Protein



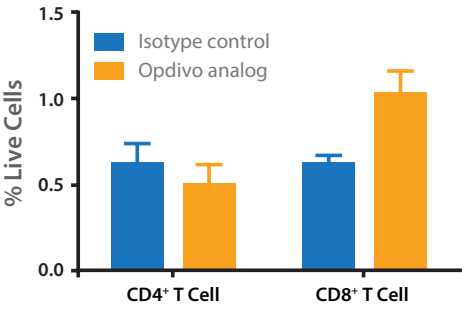
We have tested our PD-1 HuGEMM model for response to Opdivo® and Keytruda® analogs, with both treatments resulting in individual mice being "cured" (4 out of 8 mice in the Opdivo analog group, TGI 68%; 3 out of 8 in Keytruda analog group, 92% TGI) (**Figure 2**).

Figure 2: PD-1 HuGEMM Model Responds to Anti-hPD-1 Treatment



Within the PD-1 HuGEMM model system, TIL analysis has shown that CD8⁺ T cell infiltrates increase in MC38 tumors following 2 doses of anti-PD-1 therapy (**Figure 3**), which is as expected from anti-PD-1 relief of immunosuppression in this model system. Tumor volume was also shown to correlate with CD8⁺ T cell percentage post treatment.

Figure 3: PD-1 HuGEMM Model CD8⁺ Infiltration Following Anti-hPD-1 Treatment



Case Study 2

Target Multiple Checkpoint Inhibitors Simultaneously Through Double Knock-In HuGEMM Models

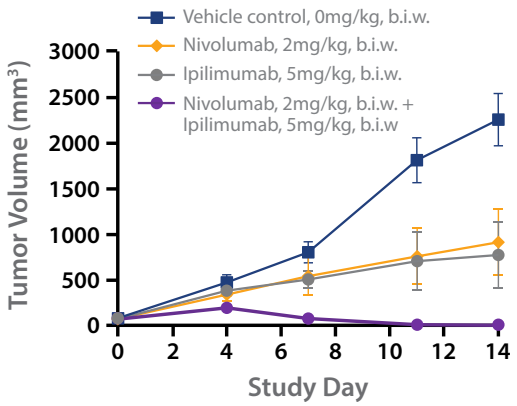
With the future of immune checkpoint inhibitor use likely to be in combination regimens, we also provide double knock-in HuGEMM models for evaluating a variety of different agent combinations (available double knock-in models shown in **Table 1**).

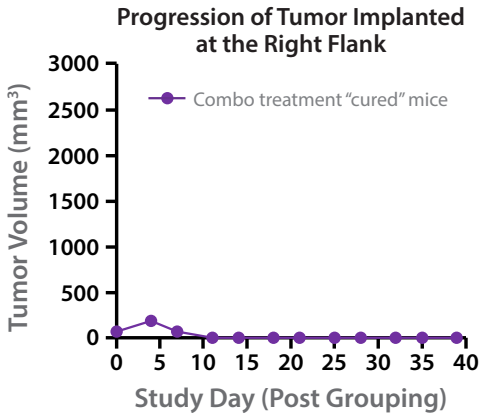
The double knock-in PD-1/CTLA-4 HuGEMM model was derived by cross breeding individual single knock-in mice, each generated by knock-in of human PD-1 or CTLA-4 cDNA. Expression of human PD-1 and CTLA-4 was verified via FACS analysis in the resulting double knock-in model, which was subsequently engrafted with MC38 HuCELL tumor cells expressing human PD-L1 (hPD-L1 HuCELL) for combination efficacy studies.

Individual treatment with anti-PD-1 (nivolumab) or anti-CTLA-4 (ipilimumab) inhibitors resulted in similar tumor growth inhibition (TGI), with the drug combination inducing complete remission in 4 out of 5 mice (**Figure 4**). The "cured" mice remained disease free up to 40 days post grouping.

+1.855.827.6968 | busdev@crownbio.com | www.crownbio.com

Figure 4: PD-1/CTLA-4 HuGEMM Model Responds to Combination Therapy

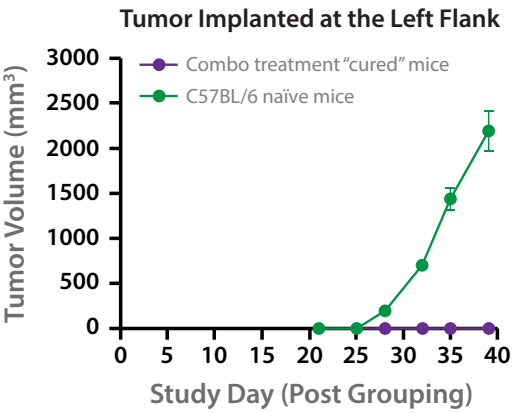




	Nivolumab (2mg/kg)	Ipilimumab (5mg/kg)	Combo
TGI (%)	61	64	103
Mice cured	1/5	0/5	4/5

The "cured" animals were re-challenged with hPD-L1 MC38 HuCELL to study a potential memory response. The mice were re-engrafted with tumor cells 21 days post original grouping, alongside treatment naïve C57BL/6 mice. Tumors were rapidly observed in the treatment naïve group; however, the previously treated animals remained tumor free, demonstrating a memory response (**Figure 5**).

Figure 5: PD-1/CTLA-4 HuGEMM Model Shows a Memory Response Following Tumor Re-Engraftment



Case Study 3

Evaluate Combination Regimens of Human-Specific Antibodies Targeting Both Tumor and T Cells by Combining HuGEMM and HuCELL Models

By combining HuGEMM mice and HuCELL tumor cells, combination regimens which target both the tumor and T cells can be assessed (e.g. combined PD-1 and PD-L1 targeting).

We have combined a HuGEMM mouse model expressing human PD-1 with hPD-L1 HuCELL tumor cells for the efficacy evaluation of a human-specific combination of PD-1 and PD-L1 inhibitors. Human gene expression was confirmed by FACS analysis in both model systems.

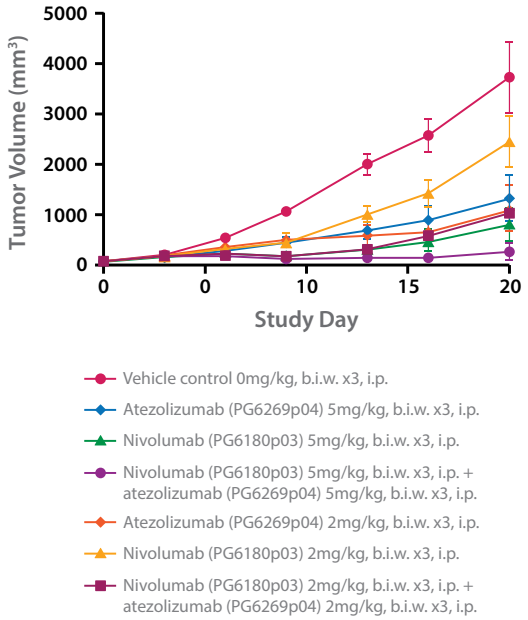
The combined model responds to both anti-PD-1 (nivolumab) and anti-PD-L1 (atezolizumab) single agent treatments, with nivolumab showing dose dependent antitumor efficacy. Combination anti-PD-1/PD-L1 results in further tumor growth inhibition compared to both single agent treatments alone (**Figure 6**). Immunoprofiling showed that, consistent with efficacy data, the combination regimen resulted in the greatest increase of infiltrating CD4+ and CD8+ T cells (**Figure 7**).

HuGEMM and HuCELL Factsheet

CrownBio
CONNECTING SCIENCE TO PATIENTS

Figure 6: PD-1 HuGEMM and PD-L1 HuCELL Responds to Combination Therapy

To avoid interference from endogenous murine PD-L1 and for optimal efficacy results a double knock-in PD-1/PD-L1 HuGEMM mouse was utilized in this study in combination with hPD-L1 HuCELL

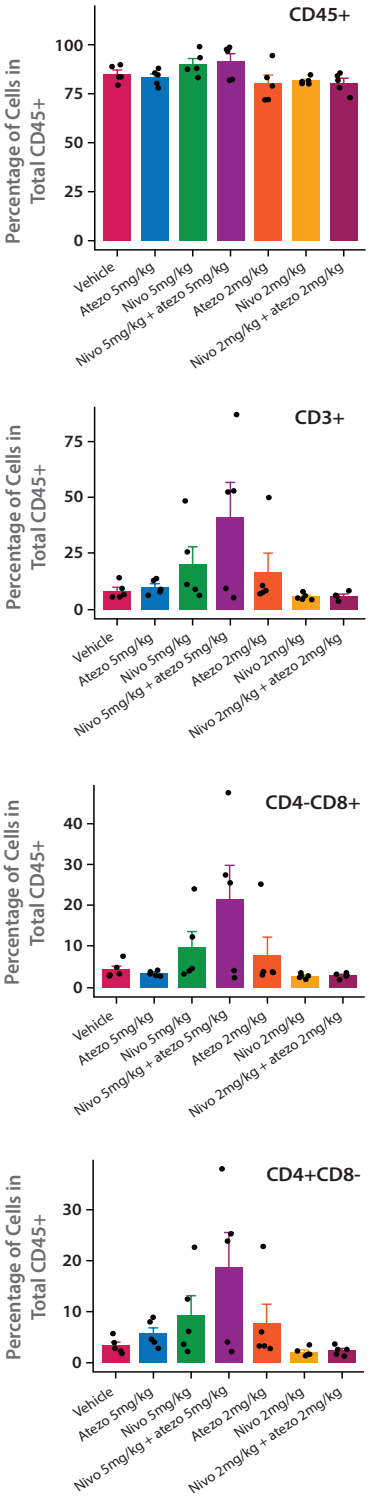


	Atezolizumab		Nivolumab		Combo	
	2mg/kg	5mg/kg	2mg/kg	5mg/kg	2mg/kg	5mg/kg
TGI (%)	72	66	35	80	74	95

Easily Search our HuGEMM Data in MuBase®

The data from our HuGEMM platform are all stored within our easy to use, proprietary online database, MuBase. A wide variety of data including model background, mouse strain, histopathology, genomic profiling (RNAseq), and standard of care data are also captured in the database. This allows researchers to quickly and easily search for models of interest to meet their research needs. MuBase can be accessed directly from **mubase.crownbio.com** or from our website at **www.crownbio.com**, and a factsheet detailing full MuBase utilities is available from **www.crownbio.com/resources**.

Figure 7: Combination Therapy Results in the Greatest Tumor Infiltrating Lymphocyte Increase



+1.855.827.6968 | busdev@crownbio.com | www.crownbio.com

HuGEMM and HuCELL Factsheet

CrownBio
CONNECTING SCIENCE TO PATIENTS

Summary

Immunotherapy research and agents such as anti-PD-1 and PD-L1 antibodies are showing considerable success in oncology; providing both patient benefits and commercial success for the pharmaceutical industry. However, progress in the field is hindered through a lack of experimental immunotherapy models featuring a competent immune system for evaluation of human-specific therapeutics.

CrownBio provides novel HuGEMM and HuCELL platforms with murine proteins (the drug target) on host T cells or tumor cells directly replaced with their human counterparts, allowing the evaluation of specific human biological therapies *in vivo*, in mice with a functional murine immune system.

Our validated HuGEMM models express the human target and respond to human antibodies, confirming they are appropriate platforms for human-specific immunotherapy evaluation. Our validated MC38 HuCELL model expresses human PD-L1. When engrafted in HuGEMM mice, MC38 HuCELL provide the ideal model to evaluate combinations of human-specific anti-PD-1/PD-L1 antibodies *in vivo*.

Double knock-in HuGEMM models provide the ideal platform for evaluating combination regimens of immune checkpoint inhibitors, with combined HuGEMM and HuCELL allowing evaluation of human-specific combination treatments targeting both tumor and T cells.

References

[1]Li Q-X, Feuer G, Ouyang X *et al.* Experimental animal modeling for immuno-oncology. *Pharmacol Ther* 2017;173: 34-46.
[2]Wang Z, Cai B, Chen G *et al.* HuGEMM™: Human/Mouse PD-1 Chimeric Knock-In Mice for Anti-Human PD-1 Evaluation. [abstract] UCSD Moores Cancer Center Annual Symposium; 2016 February 26; San Diego.
[3]Qiao M, Zhang J, Ding J *et al.* Development of a humanized mouse model for direct evaluation of anti-human PD-L1 antibodies [abstract]. In: Proceedings of the 107th Annual Meeting of the American Association for Cancer Research; 2016 Apr 16-20; New Orleans, LA. Philadelphia (PA):AACR; *Cancer Research* 2016;76(14 Suppl): Abstract nr5179.
[4]Ouyang DX, Wang Z, Qu GJ *et al.* Distinct Response to CTLA-4 Antibodies By Different Tumors Implicates Different Mechanisms of Action. [abstract] In: Proceedings of the 28th EORTC – NCI – AACR Symposium on Molecular Targets and Cancer Therapeutics; 2016 Nov 29-Dec 2; Munich, Germany. *Eur J Cancer* 2016;68(Suppl 1): Abstract nr 327.
[5]Ouyang DX, Chen G Wang Z *et al*. Establishment of a CD137 Humanized Mouse Model for Efficacy Assessment of Agonistic Anti-CD137 Therapeutic Antibodies. [abstract]. In: Proceedings of the American Association for Cancer Research Annual Meeting 2017; 2017 Apr 1-5; Washington, DC. Philadelphia (PA): AACR; *Cancer Res* 2017;77(13 Suppl): Abstract nr 1658.
[6]Huang X, Zheng L, Ouyang W *et al*. Utilizing Human OX40 Knock-In Mice (HuGEMM™) to Assess the Antitumor Efficacy of OX40 Agonistic Antibodies [abstract]. In: Proceedings of the 2017 AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics; 2017 Oct 26-30; Philadelphia, Pennsylvania. Philadelphia (PA): AACR, Abstract nr A207.
[7]Yang M, Zhang M, Verploegen S et al. Establishment of a human CD3ϵ transgenic mouse model to assess anti-tumor efficacy of human T-cell-redirecting bispecific antibodies [abstract]. In: Proceedings of the American Association for Cancer Research Annual Meeting 2018; 2018 Apr 14-18; Chicago, IL. Philadelphia (PA): AACR; *Cancer Res* 2018;78(13 Suppl):Abstract nr 5669.

Contact Sales
US: +1.855.827.6968
UK: +44 (0)870 166 6234
busdev@crownbio.com

Schedule Scientific Consultation
Request a consultation to discuss your project.
consultation@crownbio.com

Explore Scientific Data
Log into MuBase to review HuGEMM model data.
hubase.crownbio.com