HuGEMM™ and HuCELL™ Models

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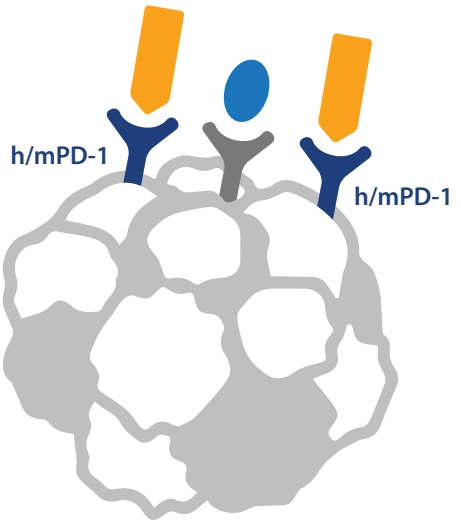
Progress your human-specific immunotherapeutics *in vivo* with our unique humanized drug target platforms

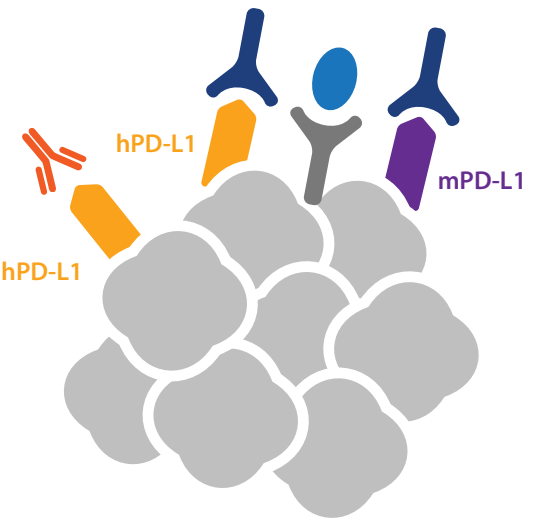
Discover the benefits of using our specific humanized target models to accelerate your immuno-oncology drug discovery programs.

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

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

- HuGEMM mouse models with murine drug target proteins e.g. PD-1 directly replaced with their human counterpart.
- HuCELL syngeneic models with engineered mouse tumor cells expressing humanized ligands e.g. PD-L1.
- For *in vivo* studies within fully functioning murine immune systems.
- Key checkpoint target platforms developed, with many more under development, including double knock-in models.





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HuGEMM and HuCELL Key Facts

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CrownBio provides a range of drug target humanized models developed in collaboration with Nanjing Galaxy Biopharmaceutical Co. Ltd., to evaluate human-specific therapeutics within mice with a fully functional immune system:

- HuGEMM mouse models created via CRISPR/Cas9 gene editing, with murine T cells expressing chimeric human/murine PD-1, CTLA-4, OX40, CD137, or TIM-3.
- Models fully validated to show human protein expression via FACS, and TGI response to human specific antibodies.
- Further models under development for a range of targets, including double knock-in models.
- Complemented by HuCELL syngeneic models with humanized ligands expressed on mouse cancer cells e.g. the MC38 syngeneic model expressing hPD-L1.
- Fully validated to confirm human PD-L1 is expressed on the cells, and for reliable TGI response when treated with a range of anti-hPD-L1 antibodies.
- Collaborations welcome on further model/target 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 these syngeneic models due to species specificity issues. There is a high unmet need to develop appropriate animal models to directly evalute 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 HuGEMM - a chimeric platform which allows the evaluation of specific human biological therapies *in vivo*, in mice with a fully 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* with models available for PD-1, CTLA-4, OX40, CD137, and TIM-3, and models under development for a range of other immuno-oncology targets including double knock-in models (**Table 1**). The concept of HuGEMM is shown in **Figure 1**, exemplified by our validated HuGEMM PD-1 (HuPD-1) model.

Table 1: HuGEMM Model Pipeline

Single KI	Status	Double KI	Status
PD-1	Available	PD-1/PD-L1	Available (validation studies ongoing)
CTLA-4	Available	PD-1/TIM-3	Homozygous breeding
OX40	Available	PD-1/LAG3	Heterozygous breeding
CD137	Available	PD-L1/CTLA-4	Heterozygous breeding
TIM-3	Available		
PD-L1	Available		
LAG3	Undergoing validation		
TIGIT	Undergoing validation		
GITR	Homozygous breeding		
CD40	Homozygous breeding		
ICOS	Homozygous breeding		

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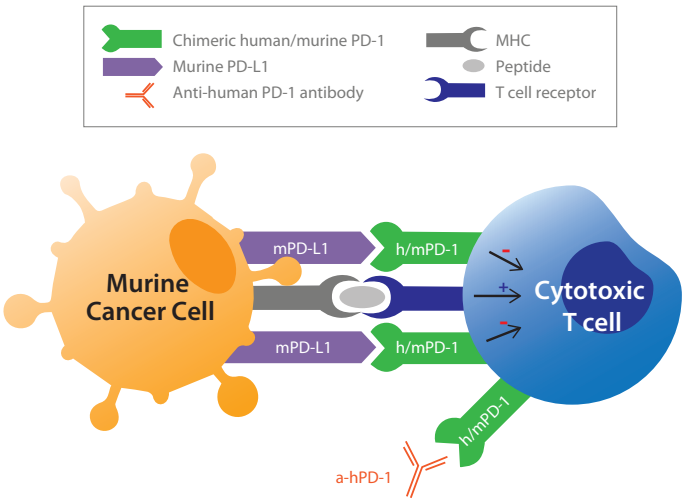
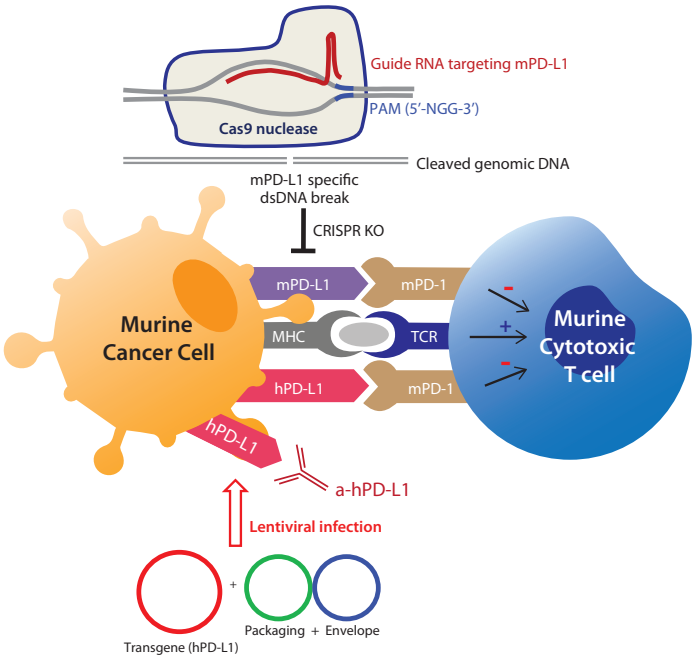
Figure 1: The Concept of HuGEMM



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 (the concept of HuCELL is shown in **Figure 2**). HuCELL and HuGEMM models can be combined as required to suit client research needs.

Figure 2: The Concept and Genetic Engineering Strategy of HuCELL



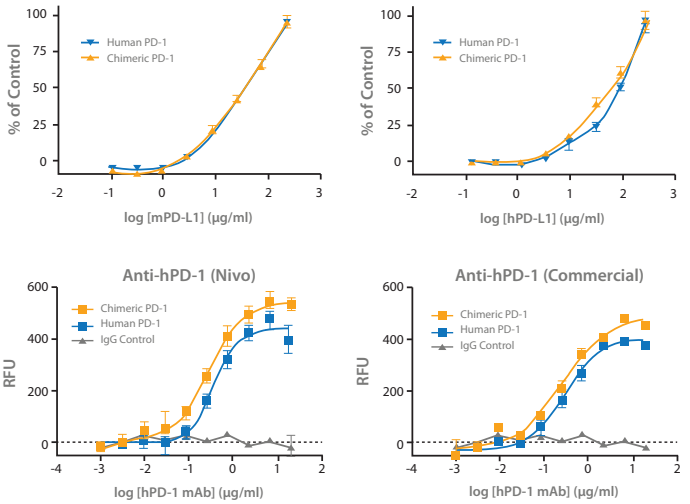
Model Development and Validation

HuGEMM HuPD-1 Model[1]

A chimeric human/mouse PD-1 gene (h/mPD-1) knocking-in human exon 2 was created by CRISPR/Cas9 gene editing. Homozygous knock-in mice (HuPD-1) 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 3, upper panel**). The chimeric protein is also recognized by anti-human PD-1 antibodies (**Figure 3, lower panel**), which disrupts the PD-1/PD-L1 interaction. FACS analysis confirms that human PD-1 is expressed on the cells of this model.

Figure 3: Chimeric h/mPD-1 Protein Binds to Anti-hPD-1 Antibody and to PD-L1

Data generated by Nanjing Galaxy Biopharmaceutical Co. Ltd.



We have validated our HuPD-1 model with 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 4**).

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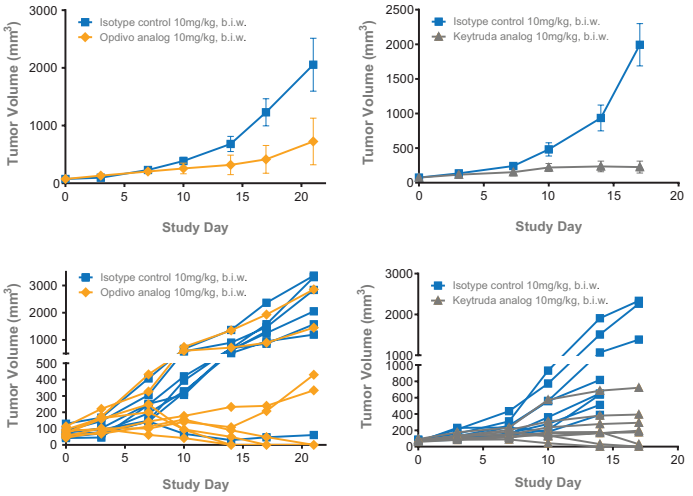
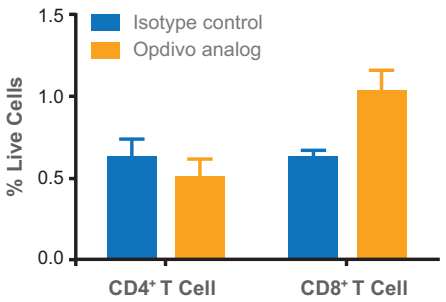
Figure 4: HuPD-1 Model Responds to Anti-hPD-1 Treatment



Within the HuPD-1 model system, TIL analysis has shown that CD8+ T cells increase in MC38 tumor infiltrates following 2 doses of anti-PD-1 therapy **(Figure 5)**, 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 5: HuPD-1 CD8+ Infiltration Following Anti-hPD-1 Treatment



HuGEMM HuCTLA-4 Model[4]

CrownBio has also validated a HuCTLA-4 HuGEMM model, with the model shown by FACS analysis to express human CTLA-4 on T cells. The chimeric CTLA-4 construct consists of human exons 2 and 3, and murine exons 1 and 4. Following engraftment with the MC38 cancer model and treatment with a Yervoy® analog, tumor growth inhibition of 94% was observed, with 2 out of 8 mice effectively "cured" (**Figure 6**). An increase in TILs is also observed following Yervoy analog treatment (**Figure 7**), confirming that this model is an appropriate platform for human anti-CTLA-4 antibody evaluation.

Figure 6: HuCTLA-4 Responds to Anti-hCTLA-4 Treatment

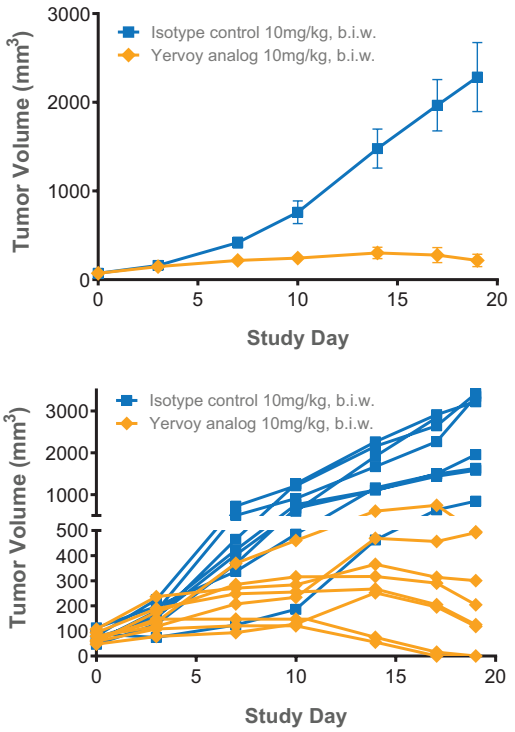
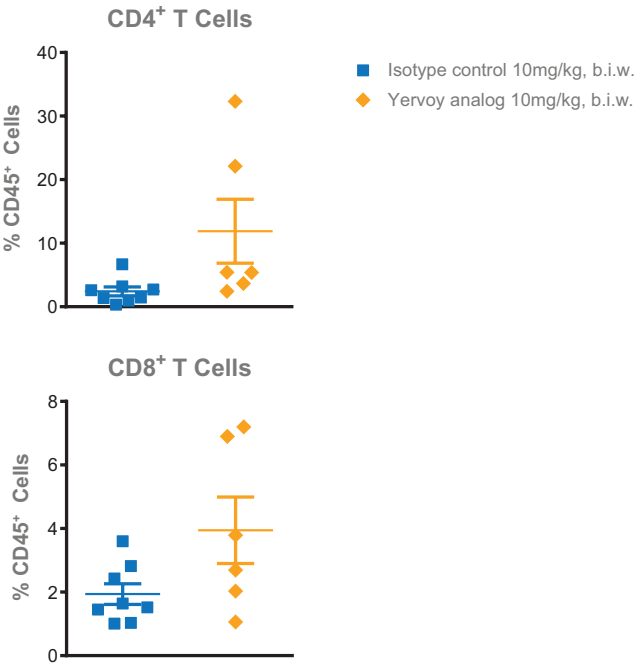


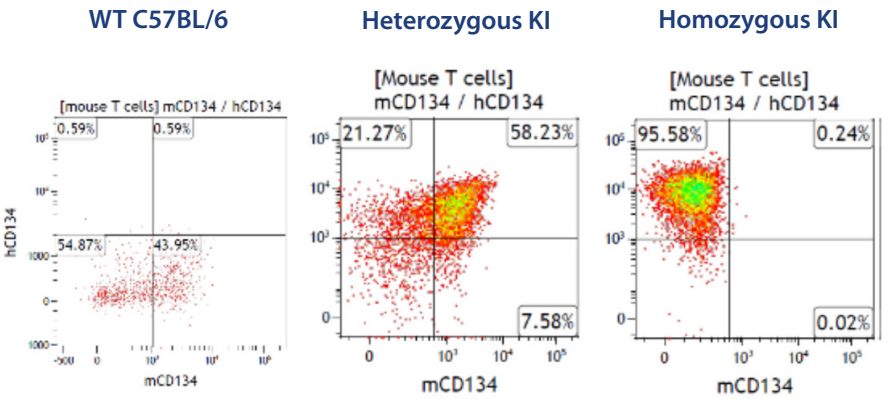
Figure 7: HuCTLA-4 TIL Following Anti-hCTLA-4 Treatment



HuGEMM HuOX40 Model[5]

The HuGEMM OX40 model was developed by replacing the entire mouse OX40 receptor gene coding region with the human counterpart, including the extracellular, transmembrane, and intracellular domains. FACS analysis confirmed expression of human OX40 on T cells of the homozygous knock-in model, with both mouse and human OX40 expressed in the heterozygous model (**Figure 8**).

Figure 8: HuOX40 Model T Cells Express Human OX40

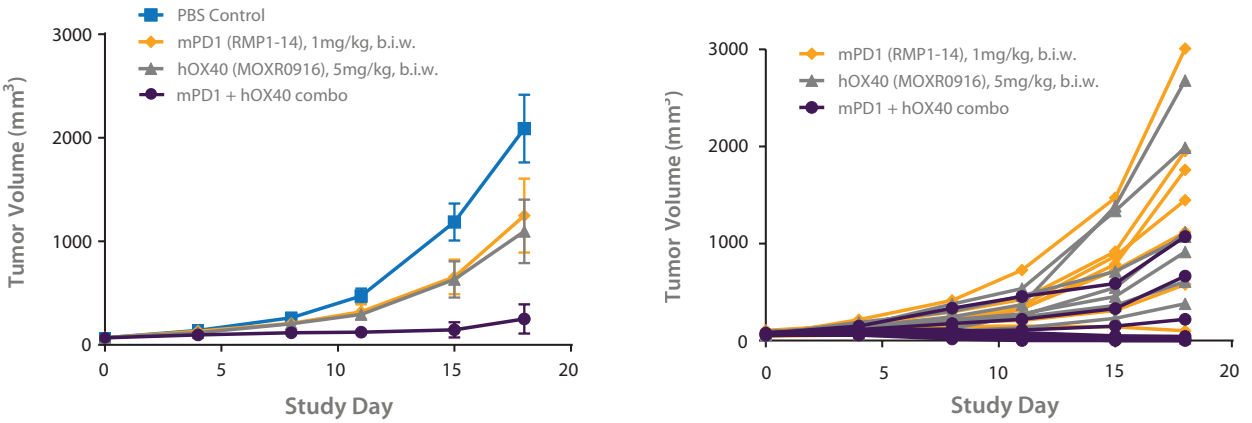


Both heterozygous and homozygous knock-in models have been validated with anti-OX40 antibodies, and in combination studies with anti-mPD-1 antibodies. The heterozygous knock-in, expressing both human and murine OX40, can be used to assess both human-specific OX40 antibodies and their murine analogs.

A human OX40 antibody (MOXR0916 analog) and murine PD-1 antibody where shown to induce TGI of the MC38 model implanted in heterozygous HuOX40 mice, of 49% and 41%, respectively.

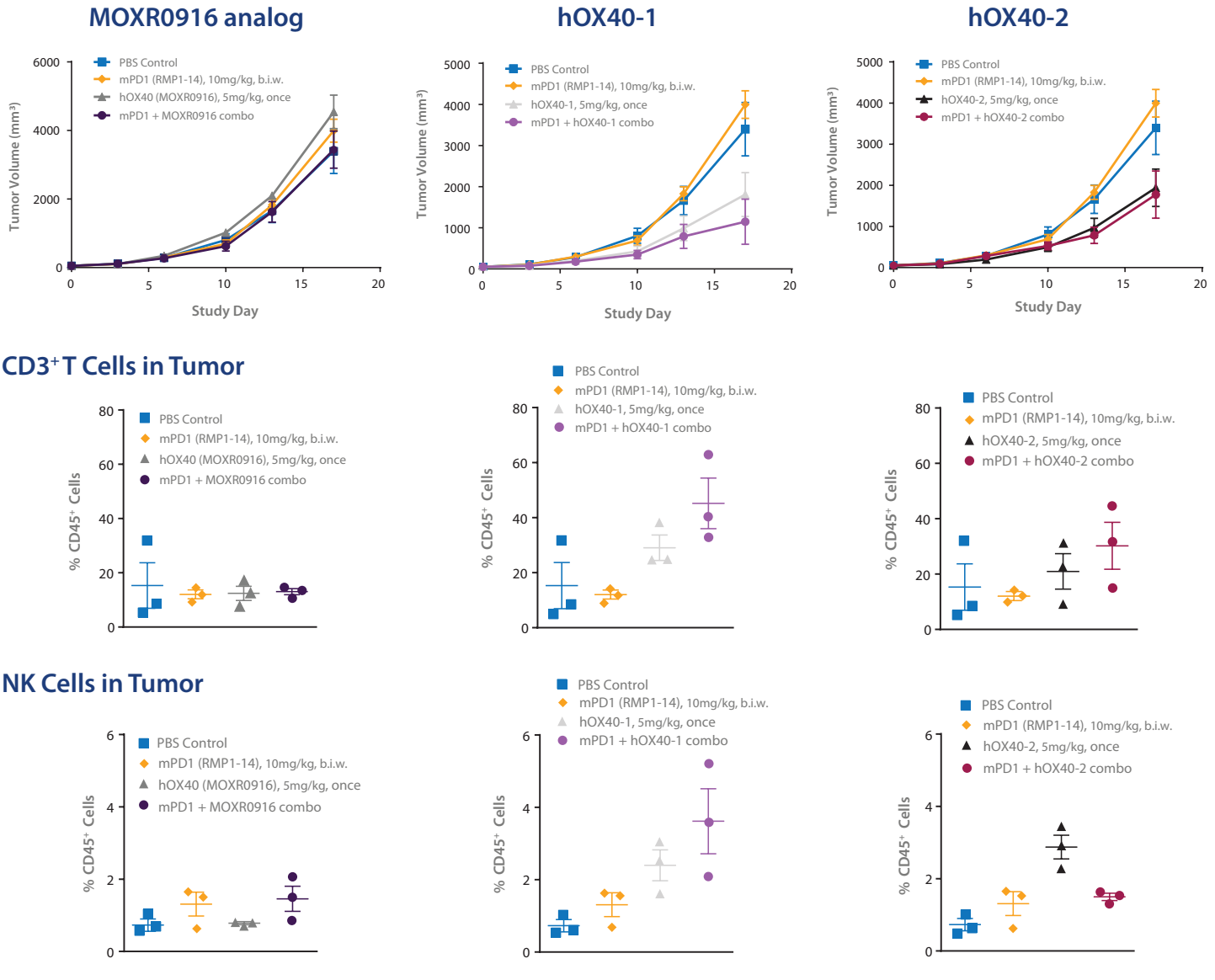
Combination therapy increased this to 91% TGI, with four out of eight mice showing full tumor regression, being effectively "cured" (**Figure 9**). A murine OX40 antibody (OX86) induced a lower response of 19% TGI; however, combination with an mPD-1 antibody still resulted in 81% TGI and two mice with complete tumor regression.

Figure 9: Heterozygous HuOX40 Model Responds to Anti-hOX40 and Combination Anti-mPD-1 Therapy



The homozygous model has been used to assess a range of human-specific OX40 antibodies, to compare efficacy and TILs following treatment of the B16-F10 model. Anitbodies showing the greatest TGI (e.g. test antibody 1) corresponded with an increase in CD3 T cells and NK cells (**Figure 10**).

Figure 10: hOX40 Antibody Efficacy Corresponds with Increased Tumor Infiltrating Lymphoctyes



HuGEMM HuCD137 Model[6]

A HuCD137 model has been created, with the construct comprised of human exons 4 through 7 inclusive, and murine exons 1 through 3, plus 8 and 9. FACS analysis was used to confirm that human CD137 is expressed within this homozygous knock-in model.

The homozygous model has been validated with anti-hCD137 antibodies alone or in combination with anti-hPD-1 agents, a clinically relevant combination therapy. Urelumab and utomilumab were shown to have varying TGI effects on the MC38 model implanted in HuCD137 mice, with an improved response following urelumab combination with an anti-PD-1 therapeutic (**Figure 11**). FACS analysis showed that the combination treatment also increased CD8⁺ T cells in both the tumor and liver (**Figure 12**). The heterozygous model has also been validated with human and mouse CD137 antibodies.

Figure 11: HuCD137 Model Responds to Anti-hCD137 and Combination Therapy

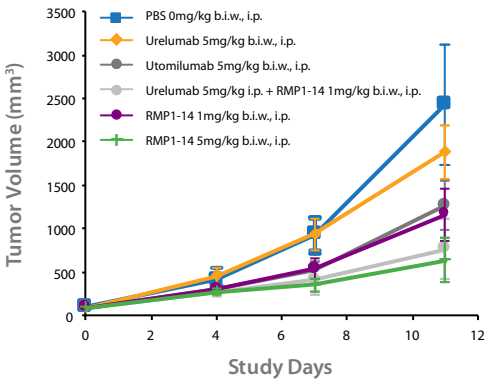
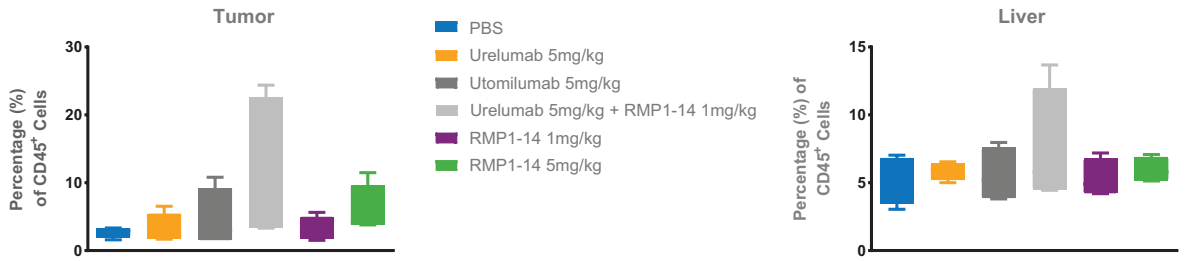


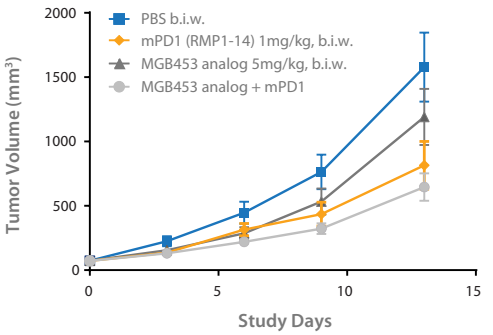
Figure 12: Combination Anti-CD137 and Anti-PD-1 Increases CD8⁺ T Cells in Tumor and Liver



HuGEMM HuTIM-3[7]

Our HuTIM-3 model was generated via a construct containing human exons 2 through 5 inclusive, and murine exons 1, 6, and 7. Validation treating the MC38 model implanted in HuTIM-3 mice with a human specific anti-TIM-3 antibody showed only 26% TGI; however, this increased to 62% when combined with an anti-mPD-1 antibody (**Figure 13**; 51% TGI for anti-mPD-1 treatment alone).

Figure 13: HuTIM-3 Model Responds to Combination Treatment



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HuCELL MC38 Model[8]

As detailed above, we have validated a MC38 HuCELL model expressing hPD-L1, with knock-in at exon 3 for human PD-L1. FACS analysis confirms that in culture only human PD-L1 is expressed on these cells; however, in an *in vivo* system cells expressing murine PD-L1 corresponding to tumor infiltrating lymphocytes are also detected.

The MC38 model has been extensively validated with anti-PD-L1 antibodies at varying doses and with mice from multiple vendors, with results showing model reliability across the studies:

- Study 1, Day 14 of treatment, C57BL/6 mice from Vendor 1: 78%TGI with Roche anti-PD-L1 antibody (h/m cross-reactive) and 52-64% with BMS analog/CrownBio PD-L1 antibodies (human only) (**Figure 14**). TIL analysis revealed an increase in $CD8^+$ T cells corresponding with atezolizumab efficacy (**Figure 15**)
- Study 2, Day 13 of treatment, C57BL/6 mice from Vendor 1: 78% TGI for BMS anti-PD-L1 analog
- Study 3, Day 13 of treatment, C57BL/6 mice from Vendor 2: 43-57% TGI for 1-10mg/kg BMS anti-PD-L1 analog, with no clear dose response observed.

Figure 14: HuCELL MC38 hPD-L1 Model Responds to Anti-hPD-L1 Treatment

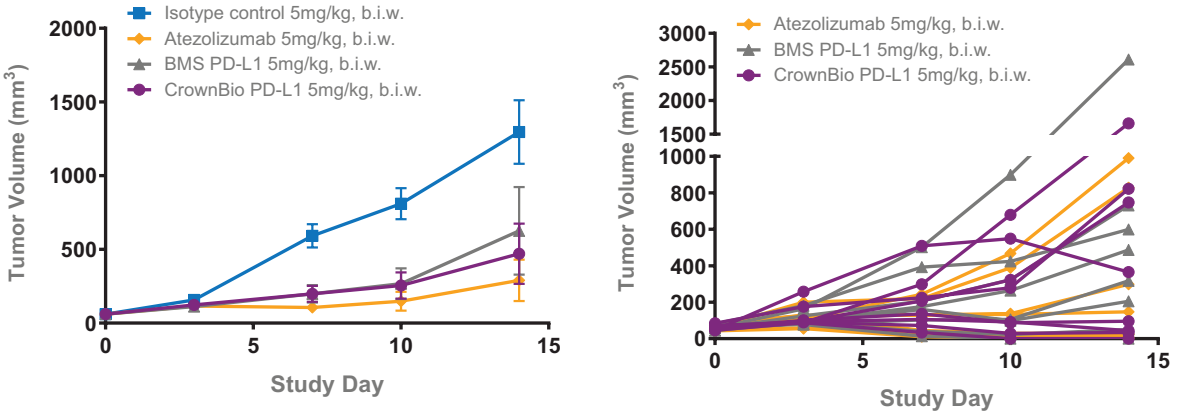
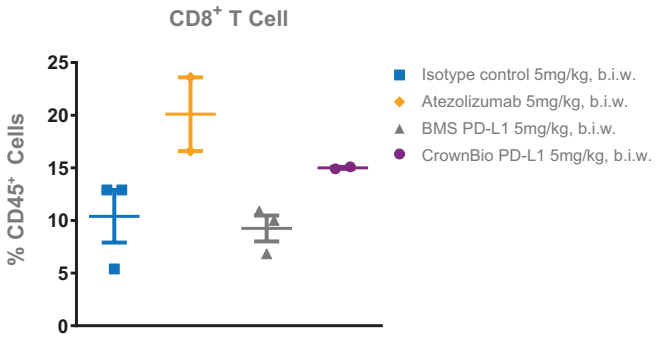


Figure 15: HuCELL MC38 hPD-L1 Model TIL Following Anti-PD-L1 Treatment



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 availble at **www.crownbio.com/publications/factsheets/**.

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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 fully competent immune system for evaluation of human-specific therapeutics.

CrownBio provides novel chimeric 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 fully functional murine immune system.

Our validated HuPD-1, HuCTLA-4, HuOX40, HuCD137, and HuTIM-3 HuGEMM models respond to their respective human antibodies, confirming that the models are appropriate platforms for human antibody evaluation. Currently, the next generation of HuGEMM mice are under development for other checkpoint/co-stimulation targets (PD-L1, CD40, etc. as well as double knock-ins) to enable evaluation of immuno-oncology monotherapies and combinations.

Our validated MC38 HuCELL model expresses human PD-L1, and responds to human antibodies when engrafted in immuno-competent mice. Combination with PD-L1 HuGEMM models currently under development will provide a model system with a full complement of hPD-L1 across both tumors and host dendritic cells.

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