CROWN BIOSCIENCE

Humanized Genetically Modified Mouse Models and Humanized Target Tumor Cells

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

A JSR Life Sciences Company

FACTSHEET

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.

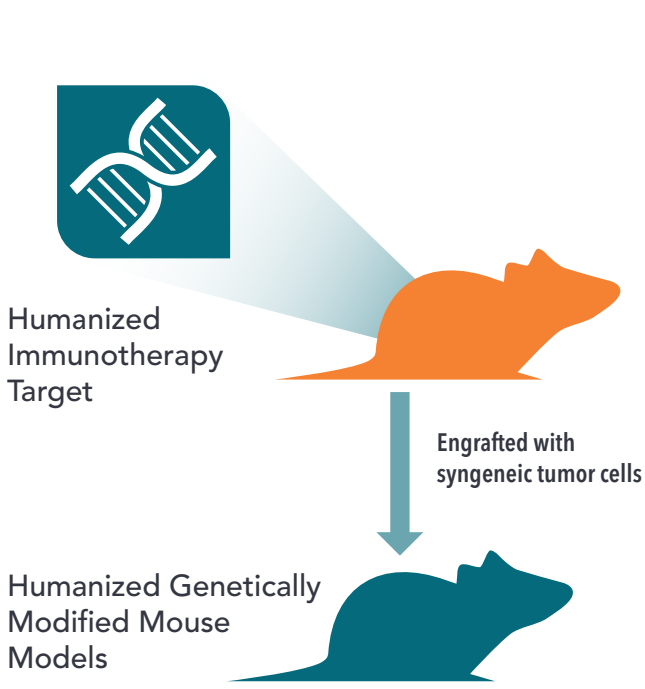
Crown Bioscience has developed humanized GEMM and target tumor cell models, allowing the evaluation of specific human biological therapies *in vivo*:

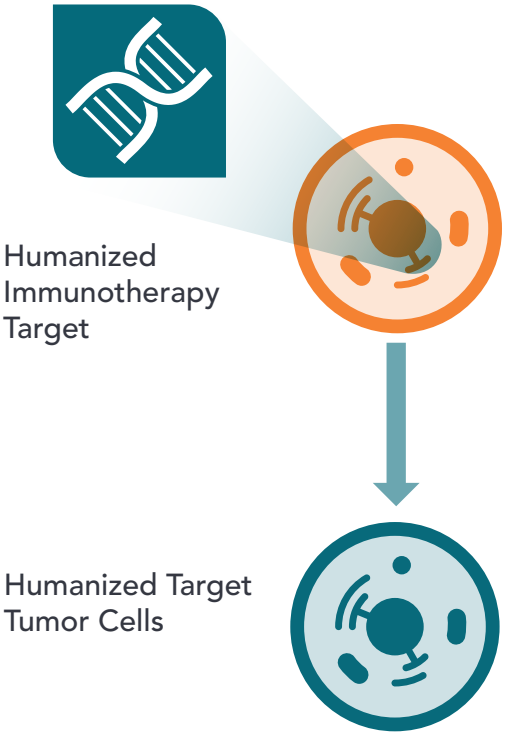
- Humanized genetically modified mouse models expressing a humanized drug target including PD-1, PD-L1, or CTLA-4
- Syngeneic tumor cells expressing humanized ligands e.g. PD-L1
- For *in vivo* studies within functioning murine immune systems
- Key checkpoint target platforms developed, including double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

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.





Humanized Drug Target Models[1]

Crown Bioscience has developed the humanized mouse models 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].

It 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 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. Both platforms can be combined as required to suit client research needs.

Case Study 1

PD-1 Humanized Mouse 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 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.

We have tested our PD-1 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**).

Within the PD-1 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.

Table 1: Humanized Mouse 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 Humanized Target Tumor Cells 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/CD137	Breeding
LAG3	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 2

Target Multiple Checkpoint Inhibitors Simultaneously Through Double Knock-In Humanized Mouse Models

With the future of immune checkpoint inhibitor use likely to be in combination regimens, we also provide double knock-in 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 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 tumor cells expressing human PD-L1 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.

The "cured" animals were re-challenged with hPD-L1 MC38 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 1: Chimeric h/mPD-1 Binds to Anti-hPD-1 Antibody and to a PD-L1 Recombinant Protein

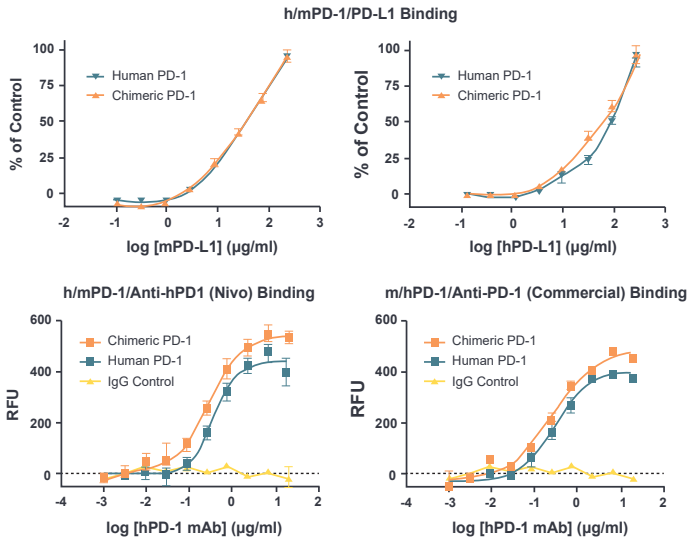


Figure 2: Model Responds to Anti-hPD-1 Treatment

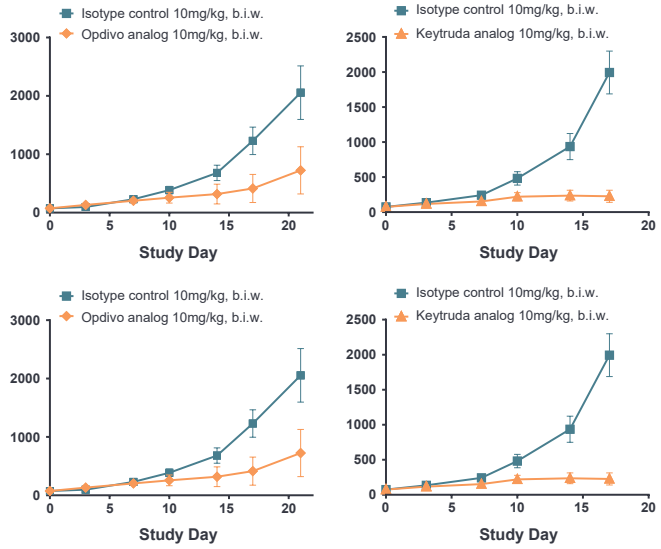


Figure 3: Model CD8⁺ Infiltration Following Anti-hPD-1 Treatment

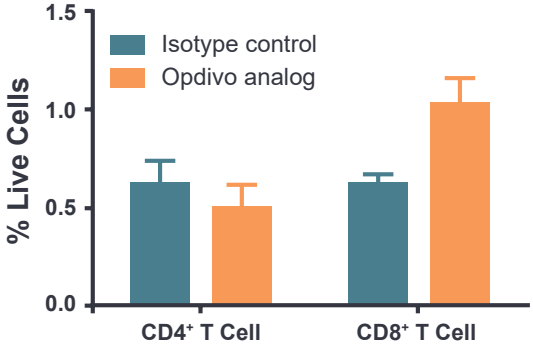
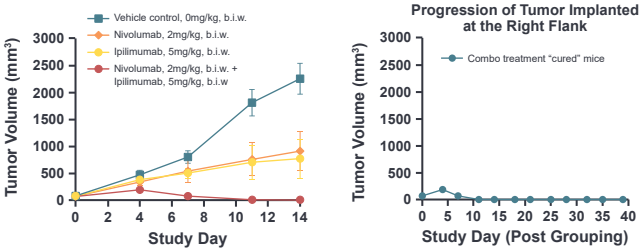


Figure 4: PD-1/CTLA-4 Model Responds to Combination Therapy



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

Case Study 3

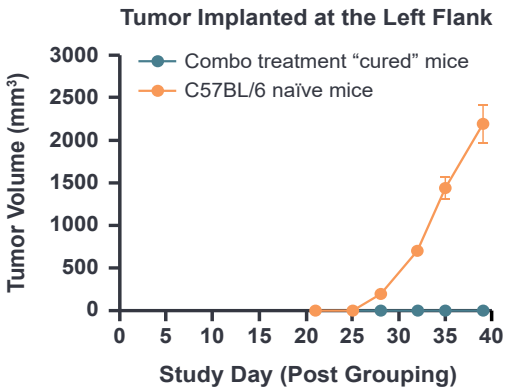
Evaluate Combination Regimens of Human-Specific Antibodies Targeting Both Tumor and T Cells by Combining humanized Mouse and humanized target tumor cells

By combining both platforms 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 mouse model expressing human PD-1 with hPD-L1 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**).

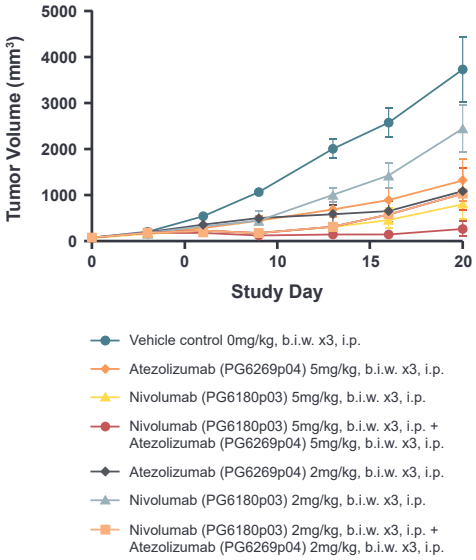
Figure 5: PD-1/CTLA-4 Model Shows a Memory Response Following Tumor Re-Engraftment



Easily Search Data in Our IO Murine Models Database

The data from our humanized mouse platform are all stored within our easy to use, proprietary online database. 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. The database can be accessed directly from our website at **https://www.crownbio.com/databases**

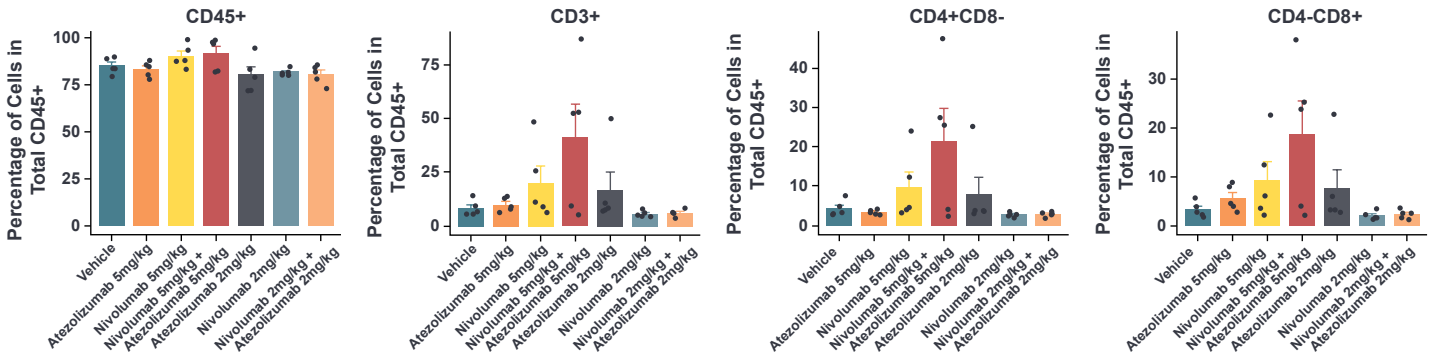
Figure 6: PD-1/PD-L1 Responds to Combination Therapy



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

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

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



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.

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

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

References

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Get in touch

Sales
US: +1 858 622 2900
UK: +44 (0)1530 234871
busdev@crownbio.com
www.crownbio.com