CROWN BIOSCIENCE

CAR-T Therapy

Evaluate your CAR-T therapies *in vivo* using Crown Bioscience's novel PDX or CD19 positive cell line derived xenograft models

A JSR Life Sciences Company

FACTSHEET

V1.1

Executive Summary

As CAR-T therapies progress towards becoming approved immuno-oncology agents, validated *in vivo* preclinical models are required to enable cost-effective efficacy and safety CAR-T therapy testing.

Crown Bioscience has created a patient-derived xenograft (PDX) CAR-T therapy Translational Platform enabling the rapid validation of models for efficacy testing, in highly predictive models that truly mimic human disease.

The Translational Platform's foundation is our large, diverse collection of around 3,000 well-characterized PDX models, allowing access to a wide range of CAR-T targets. Model selection is made quick and easy via our curated online PDX database and powerful oncology model search engine, incorporating IHC model validation.

These factors enable clients to rapidly progress their preclinical CAR-T therapy evaluation against novel targets, in models which preserve the heterogeneous pathological and genetic characteristics of the original patient tumor, providing robust preclinical data before moving into the clinic.

For clients working on CD19 (the key CAR-T target antigen) we also provide a well validated Raji human Burkitt lymphoma xenograft model. Highly sensitive to CD19 targeted CAR-T therapies, the model is available now to rapidly progress your B cell malignancy research.

Background on CAR-T Therapy

Immunotherapy represents the most promising new cancer treatment approach since the first development of chemotherapies in the late 1940s[1]. The coming of age of immunotherapy as a treatment paradigm for oncology has been signified by multiple approvals for checkpoint inhibitors across a range of cancer types, including most recently Keytruda® for recurrent or metastatic head and neck squamous cell carcinoma[2].

One area of immuno-oncology which has gathered a lot of interest is adoptive cell therapy, particularly Chimeric Antigen Receptor T (CAR-T) therapies. CAR-T treatments are targeted immunotherapies, in which T cells are extracted from a patient and genetically modified *in vitro* to recognize a specific tumor-associated antigen. Following proliferation outside the patient the CAR-T cells are reinfused, effectively giving patients a "living drug". CAR-T cells subsequently multiply and selectively attack and kill only those cells displaying the surface antigen of interest, providing high tumor specificity.

Preclinical assessment of CAR-T therapies has been a key component of current clinical trial success, providing valuable information needed for the optimal performance of CAR-T cells[3]. As research moves forward, it is thought that preclinical testing will continue to play an important role in this field, complementing the data collected from clinical samples of patients treated with CAR-T cells[3]. Therefore utilizing appropriate *in vivo* preclinical models in CAR-T therapy evaluation is essential for accurate, timely, and cost-effective decision making during your CAR-T development process.

Crown Bioscience Immunotherapy and CAR-T Therapy Model Resources

Crown Bioscience has a range of immunotherapy research platforms available for preclinical drug development, encompassing mouse immunity[4],human immunity, and a novel chimeric system[5] for development of human biologic therapeutics (fully detailed within our *In Vivo* Immunotherapy Drug Discovery Application Note).

We provide two distinct platforms for *in vivo* CAR-T therapy assessment, utilizing our vast collections of:

- PDX models in an immunocompromised setting
- Cell line derived xenograft models, particularly those expressing BCMA, CD19, CD20, CD22 and other key target antigens in the CAR-T therapy field.

Utilizing PDX to Evaluate CAR-T Therapies

Xenografts derived directly from primary tumor tissue (which have never been in contact with plastic) offer the most predictive xenograft model for preclinical drug evaluation. Crown Bioscience has developed the largest commercial collection of approximately 3,000 well-characterized PDX models from US, European, and Asian populations, which represents a diversity of the oncology patient population (fully detailed within our Solid Tumor PDX FactSheet). Combining our *In Vivo* Immunotherapy Translational Technology Platform with solid tumor PDX models allows preclinical CAR-T evaluation in highly predictive models which preserve the heterogeneous pathological and genetic characteristics of the original patient tumors.

Selection of the appropriate PDX models for CAR-T evaluation is made easy by our curated online PDX database and our powerful new search engine (which searches models across all Crown Bioscience oncology databases). Our PDX models are fully characterized and collated with their genomic annotation, allowing rapid selection of models overexpressing antigens of interest.

We also provide a wide range of tumor tissue microarrays (TMAs) from a variety of our PDX models, allowing clients to rapidly and efficiently investigate and confirm expression of antigens of interest across hundreds of PDX samples.

Crown Bioscience and its partner have utilized our PDX CAR-T therapy Translational Platform in the assessment of glypican-3 (GPC3)-targeted and mesothelin (MSLN)-targeted CAR-T therapies for the treatment of lung and pancreatic solid tumors, respectively.

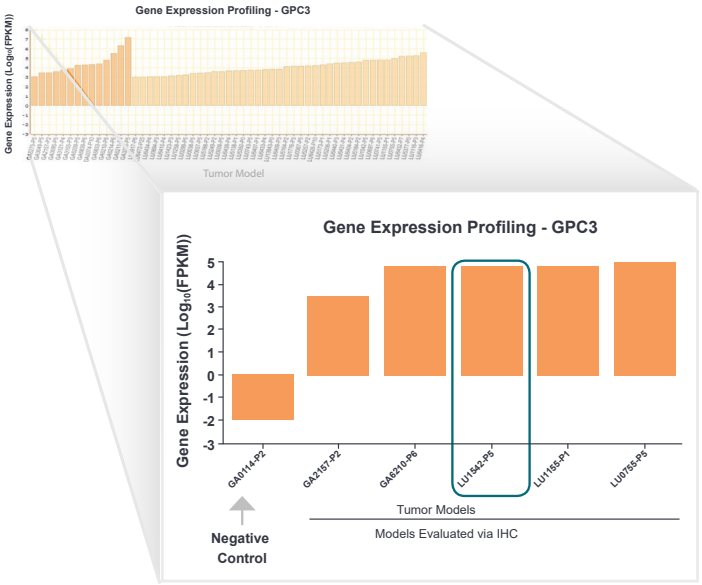
GPC3 Targeted CAR-T Therapy

GPC3 is highly expressed in hepatocellular carcinoma (HCC) and other cancers, where it attracts Wnt proteins to the cell surface and promotes tumor cell proliferation[6]. Therefore, GPC3 has emerged as a candidate oncology therapeutic target, with clinical trials ongoing for GPC3-derived peptide vaccines in HCC as well as ovarian clear cell carcinoma and pediatric cancer.

For our GPC3 case study, mRNA data stored within our PDX model database were used to identify a number of gastric and lung PDX models which express GPC3 (Figure 1). Immunohistochemistry (IHC) confirmed GPC3 expression in two of the PDXs, with the non-small cell lung cancer (NSCLC) model LU1542 (described in detail in Table 1 and GPC3 IHC shown in Figure 2) selected for CAR-T therapy evaluation.

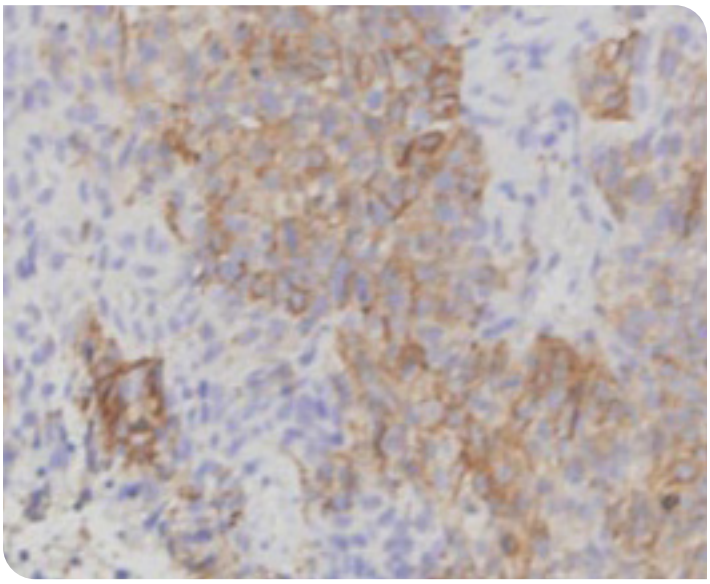
The PDX model was implanted subcutaneously in NOD/SCID mice and the animals were treated with CAR-GPC3 T cells intravenously.

Figure 1: Summary of GPC3 Expression in Gastric and Lung Cancer PDX Models



RNAseq GPC3 expression (cut off at 3), and inset showing models taken forward for IHC evaluation of GPC3 expression.

Figure 2: LU1542 PDX Model GPC3 Expression



Confirmation of LU1542 GPC3 expression by IHC. Model shown P10.

Table 1: Summary of NSCLC SCC PDX Model LU1542

HuPrime Identifier	Cancer Type and Subtype	Patient Background	Tumor Pathology Diagnosis	PDX Tumor Pathology QC	Genomic Profiling	Treatment History	Examples of Oncogene Mutation Status
LU1542	Lung cancer NSCLC, SCC	Asian male aged 68 years	Squamous cell carcinoma. No malignant cells adjacent to bronchial stump. Regional lymph nodes (LN): LN between lobules of lung (0/2), the second hilum of lung LN (0/2), inferior carina of trachea LN (0/3), superior mediastinal LN (0/6)	P0, P8: Poorly differentiated squamous cell carcinoma	Affy U219 Affy SNP 6.0 miRNA2.0 data RNAseq completed	Naive	WT: BRAF, CTNNB1, EGFR, KRAS, MAPK1, PIK3CA, PTEN AKT1 49G> A Glu17Lys homo TP53 742C> T Arg248Trp homo

The model was highly sensitive to CAR-T cell treatment, showing both tumor growth inhibition and a reduction in tumor weight (Figure 3)

MSLN Targeted CAR-T Therapy

MSLN is a cell surface antigen highly expressed in mesothelioma as well as lung, pancreas, breast, ovarian, and other cancers. It's aberrant expression plays a role in both malignant transformation of tumors and tumor aggressiveness and, combined with low expression on normal mesothelial cells, MSLN provides an attractive oncology target[7].

A range of Phase I and II clinical trials are ongoing for MSLN-targeted immunotherapeutics including CAR-T therapies, tumor vaccines, and antibody-based treatments in a variety of cancer types including pancreatic, ovarian, and mesothelioma[7].

Similar to our GPC3 trial, mRNA data stored within our PDX model database were used to identify a number of PDX models expressing MSLN, including a range of pancreatic PDX (Figure 4). Based on expression data, and a previous partial response to a MSLN ADC, PDX model PA3029 (detailed in Table 2) was chosen for CAR-T therapy efficacy evaluation.

Following subcutaneous implantion of the PDX model in mice, the animals were treated with CAR-MSLN T cells intravenously. PA3029 is highly sensitive to CAR-T cell treatment, with tumor regression observed (Figure 5).

These case study data confirm that our Translational Platform of PDX models, as well as model selection and validation through the use of our PDX model database and IHC, are valuable tools in the evaluation of novel T cell therapies.

Figure 3: LU1542 PDX Model Response to GPC3-Targeted CAR-T Therapy: Tumor Growth Inhibition and Reduction in Tumor Weight

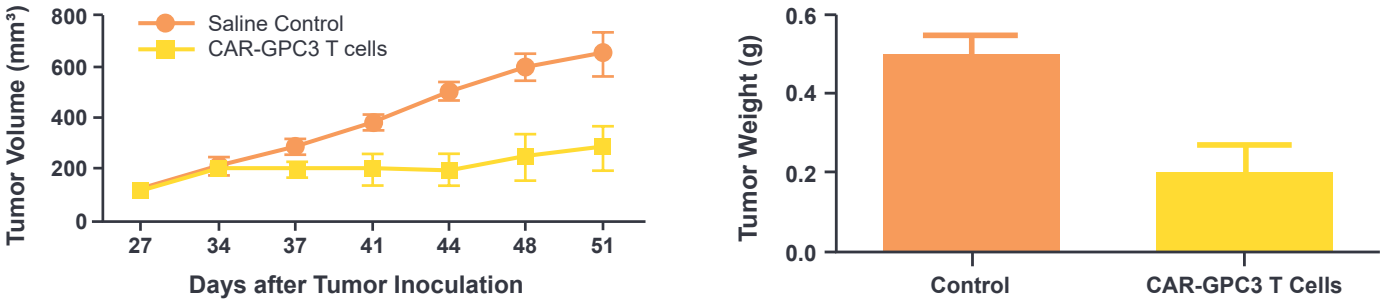


Figure 4: Summary of MSLN Expression in Pancreatic Cancer PDX Models

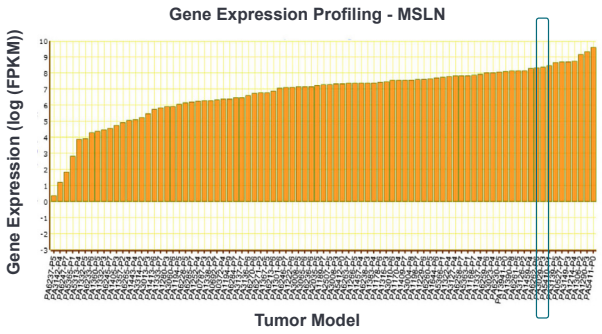


Figure 5: PA3029 PDX Model Response to MSLN-Targeted CAR-T Therapy: Tumor Growth Inhibition

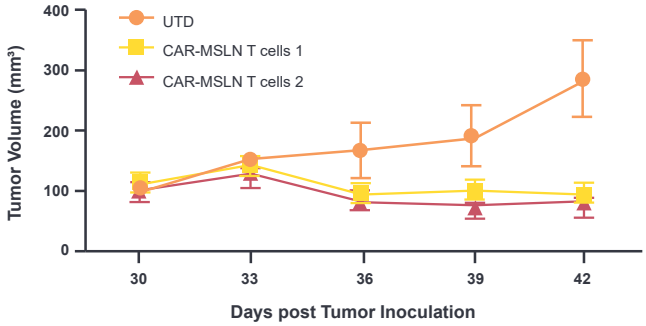


Table 2: Summary of Pancreatic Ductal Adenocarcinoma PDX Model PA3029

HuPrime Identifier	Cancer Type and Subtype	Patient Background	Tumor Pathology Diagnosis	PDX Tumor Pathology QC	Genomic Profiling	Treatment History	Examples of Oncogene Mutation Status
PA3029	Pancreatic, ductal adenocarcinoma	Asian female aged 70 years	Ductal adenocarcinoma of head of pancreas, moderately to poorly differentiated	Pa, P4: Moderately differentiated adenocarcinoma	RNAseq completed	Naive	WT: AKT1, BRAF, EGFR, MAPK1, PIK3CA KRAS 35G>A Gly12Asp

Evaluation of CAR-T Therapies Targeting CD19

The majority of CAR-T therapies in development target CD19, a B cell surface protein which is expressed throughout B cell development, and is therefore present on nearly all B cell malignancies. This makes CD19 the main target for CAR-T therapies in hematological malignancies such as chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), and B cell lymphoma.

We have validated a Raji human Burkitt lymphoma xenograft model for CAR-CD19 T cell therapy. Burkitt lymphoma is a cancer of the lymphatic system, particularly B lymphocytes, which provides a model highly sensitive to CD19 targeting. Without treatment, our Raji model rapidly reaches termination; however, treatment with CAR-CD19 T cell therapy results in 100% model survival (Figure 6).

Summary

Immunotherapy has come of age as an oncology treatment paradigm, with multiple approvals across a range of cancer types for agents such as checkpoint inhibitors. Another area of immuno-oncology garnering interest is CAR-T therapy, potentially providing a highly specific standard of care therapy in difficult-to-treat hematological malignancies. Validated preclinical models are required for evaluation of novel CAR-T therapies for both efficacy and safety testing.

Crown Bioscience provides two distinct platforms for *in vivo* CAR-T evaluation. Our vast collection of PDXs allows the evaluation of CAR-T therapies in highly predictive models which preserve both the heterogeneous pathological and genetic characteristics of the original patient tumors.

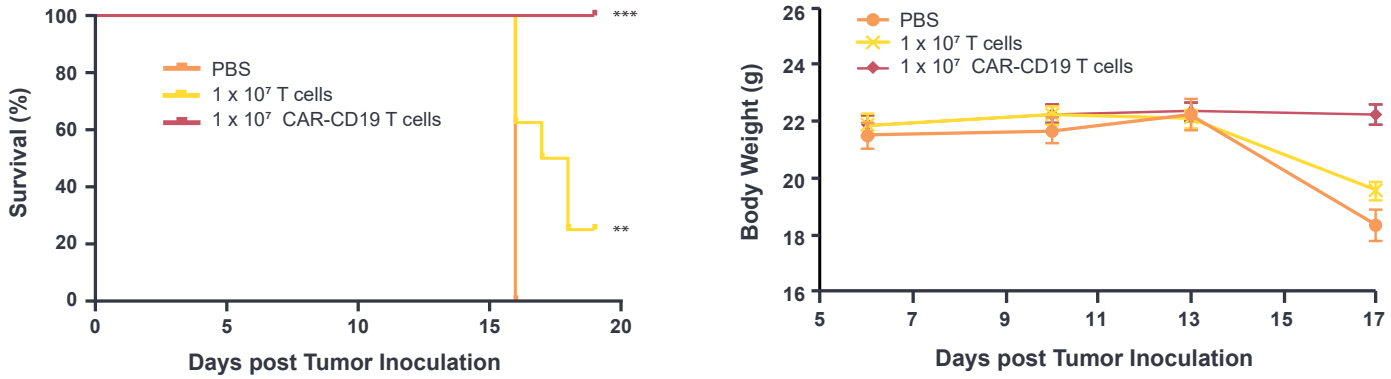
We have collated our models in an easy to use online database (capturing genomic annotation and full characterization data) as well as in TMAs, allowing the rapid selection of models to fit a variety of research needs.

Our completed PDX case studies utilize GPC3 and MSLN-targeted CAR-T therapies, which were shown to induce tumor growth inhibition in antigen expressing NSCLC and pancreatic PDX models, respectively. This confirms our PDX Translational Platform, as well as our selection and validation techniques, as valid tools in the evaluation of novel T cell therapies.

Our validated CD19 positive Raji model allows CD19-targeted CAR-T therapy evaluation, the key antigen in the CAR-T field. Treatment of our Raji human Burkitt lymphoma xenograft with CAR-CD19 T cell therapy results in 100% model survival.

Crown Bioscience can be contacted at **busdev@crownbio.com** for any further questions or information required on our CAR-T platforms, or for more information on our *in vivo* immunotherapy models, or other products and services.

Figure 6: Raji Burkitt Lymphoma Model Response to CD19-Targeted CAR-T Therapy: Body Weight and Model Survival **p<0.01, ***p<0.001.



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