Factsheet: Utilizing Cell Line Derived and Patient-Derived Xenografts in Humanized NSG™ Mice

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CONNECTING SCIENCE TO PATIENTS

Evaluate human tumor response to immunotherapeutics within a functional human immune system

Executive Summary

Immunotherapy development is currently hampered by a lack of appropriate preclinical models. Many agents are evaluated in systems using murine immunity/murine tumors; however, these may not accurately reflect the response of the human immune system/human tumors to immunotherapeutic agents.

CrownBio provides a comprehensive Humanized Xenograft Platform in which human tumor models are studied in mice with fully competent human immunity (the gold standard HuNSG from The Jackson Laboratory):

- Well-characterized cell line derived xenograft models provide a platform for screening of potential drug candidates
- Our HuPrime® patient-derived xenograft (PDX) models fulfill multiple efficacy testing roles including combination therapy evaluation and population study approaches, in highly predictive models preserving the genomic integrity and heterogeneity seen in patients.

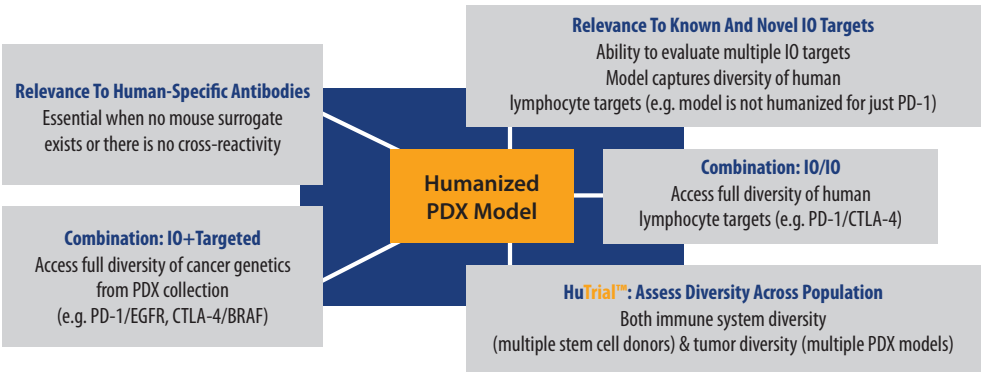
The Unmet Need for Humanized Xenograft Models

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 TECENTRIQ® (atezolizumab) for metastatic NSCLC[2].

However, development of effective new immunotherapeutics faces many challenges. We are still unsure why some patients and diseases benefit from these treatments while others do not. It is also currently unknown how to maximize the benefits from these agents e.g. through combination therapy approaches or by targeting different immune checkpoints. A lack of experimental immunotherapy models is a major obstacle toward answering these questions and developing better treatments, and there is a high unmet need for new preclinical models to help drive forward immunotherapy research and to recapitulate clinical treatments.

Humanized cell line derived xenograft and patient-derived xenograft (PDX) models are proving to have enormous value in immuno-oncology (IO) research, allowing evaluation of human tumors within a functioning human immune system. Humanized cell line derived xenograft models are well-characterized and are excellent for the screening of potential drug candidates. Humanized PDX models cover multiple functions including combination therapy studies and providing relevance to human-specific antibodies and a variety of immune targets, detailed in **Figure 1**.

Figure 1: The Value of Humanized PDX Models in Immunotherapy Drug Development



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CrownBio Immunotherapy Model Resources

CrownBio has a range of immunotherapy research platforms available for preclinical drug development, encompassing mouse immunity[3], human immunity, and a novel chimeric system[4] for development of human biologic therapeutics (fully detailed within our *In Vivo* Immunotherapy Drug Discovery Application Note).

This factsheet focuses on our human immunity resources, specifically evaluating our PDX and cell line derived xenograft models within humanized NSG (HuNSG) mice from The Jackson Laboratory. Further factsheets are available for each of our immunotherapy research resources.

Cell Line Derived and Patient-Derived Xenograft Models in Immunotherapy Drug Development

We have utilized HuNSG to evaluate our HuPrime PDX models, as well as our ValidatedXeno™ cell line derived xenograft models, and their responses to immunotherapies in a humanized setting.

Specifically we studied HuNSG cohort selection, tumor growth characteristics, donor to donor variabilty, immune cell-mediated responses, and reproducibility with the aim of enhancing anti-PD-1 antibody studies and their downstream immunophenotypic analysis.

Our studies have focused on how these factors relate to cancer treatment with Keytruda®.

Evaluating MDA-MB-231 Xenograft Model Response to Keytruda

Utilizing the MDA-MB-231 model, we first established that Keytruda treatment was engaging with the expected PD-1 target via FACS analysis of $CD3^+$ lymphocytes collected from peripheral blood pre- and post treatment. In the Keytruda group post treatment, detection of PD-1 on T cells was abrogated due to engagement of Keytruda to its target therefore masking the protein (**Figure 2**).

Further experiments confirmed that HSC-derived human immune cell engraftment does not have a significant effect on tumor growth (**Figure 3**). However, as expected, further studies with this model confirmed that humanization is required for an antitumor response to Keytruda, with humanized mice displaying disease remission compared to their non-humanized counterparts (**Figure 4**).

Humanized mice which had previously responded to Keytruda treatment were re-engrafted with MDA-MB-231 tumor cells. Tumor growth was suppressed when tumor volume reached $100mm^3$ without Keytruda treatment, demonstrating an immune memory to MDA-MB-231 cells in these animals. Re-inoculation of the same cell batch into age-matched, naïve, humanized control NSG mice from three different cohorts resulted in tumor growth (**Figure 5**).

Figure 2: Anti-PD-1 Treatment Engages Target PD-1[5]

MDA-MB-231 xenograft in 2 different HSC donor cohorts were analyzed showing identical results (figure from one of the two HSC donors). $CD3^+$ lymphocytes from peripheral blood measured pre- and post-Keytruda treatment (48 hour after 2nd dose).

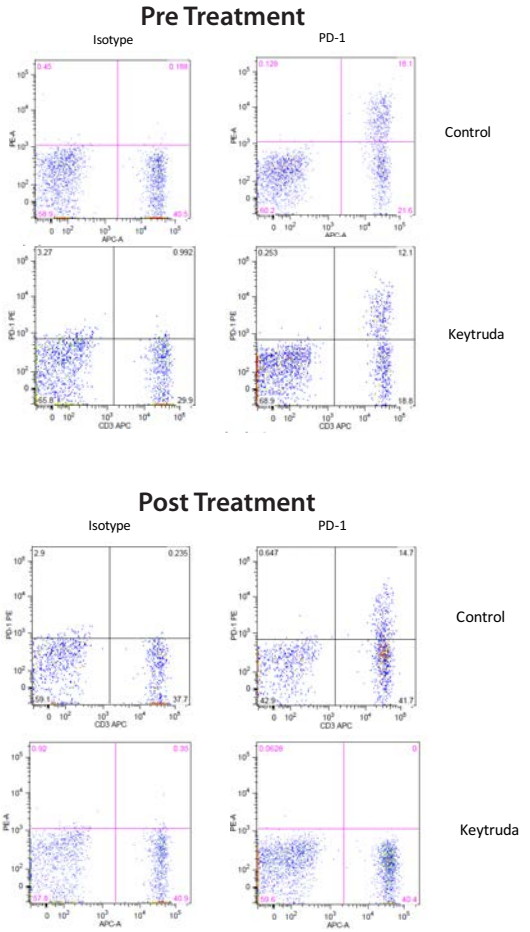

Figure 3: Humanization of NSG Mouse Does Not Affect MDA-MB-231 Growth

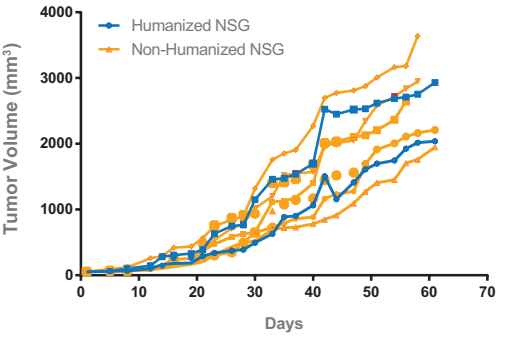

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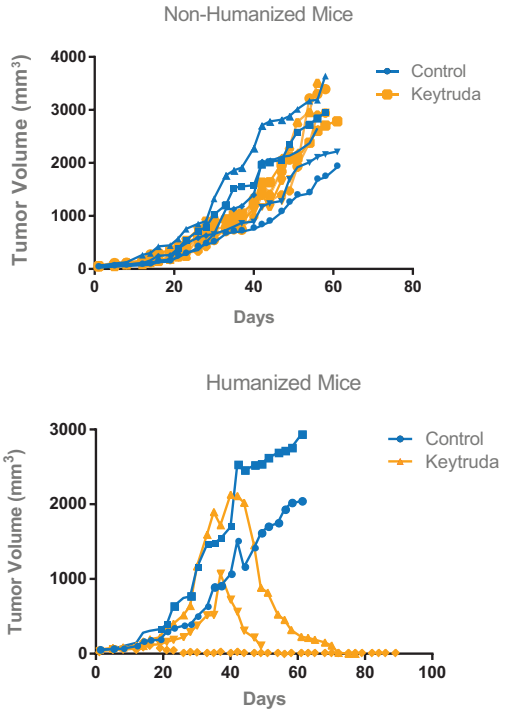
Figure 4: Humanization of NSG Mouse is Necessary for Keytruda Effect: MDA-MB-231 Model[5]
Keytruda dosing: 10mg/kg first dose, 5mg/kg every five days continuously.



Evaluating SCLC and TNBC PDX Model Response to Keytruda

Our PDX studies have focused on understanding Keytruda response across a population of HSC donors, to enable development of a tumor profile of responders and non-responders.

We have utilized SCLC and TNBC PDX models (fully detailed in our *In Vivo* Immunotherapy Drug Discovery and TNBC Application Notes, respectively). We evaluated the effects of Keytruda in a PDX model matrix, combining seven SCLC PDX models and one TNBC model with five HSC donors (selected based on elevated CD3 counts >15%) in an N of 1 study design.

Keytruda efficacy varied with differing effects observed for:

- different donors with the same tumor
- the same donor with different tumors

which clearly underscores the inherent donor to donor variability in this humanized model system. **Figure 6** shows the variation in TGI for the SCLC models via waterfall plot, with **Figure 7** showing variation in individual response across the donor population.

Figure 5: MDA-MB-231 Rechallenge of Keytruda Responder Animals Demonstrates an Immune Memory Response
Re-engraftment indicated with (↓). New, age-matched controls overlaid on the left figure.

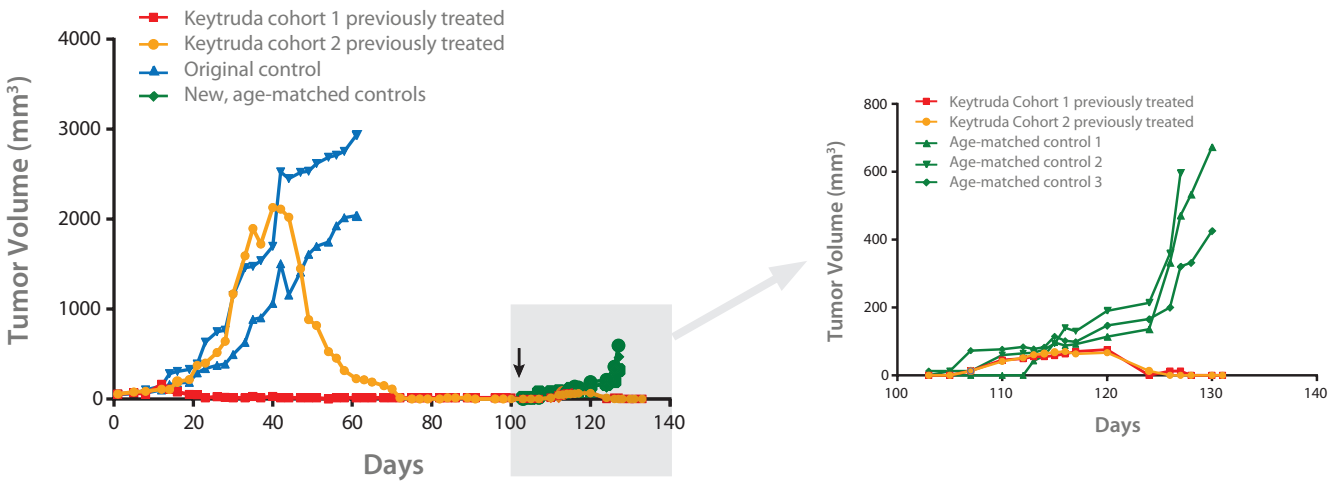


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Figure 6: Variation in Tumor Growth Inhibition Following Anti-PD-1 Treatment: SCLC PDX

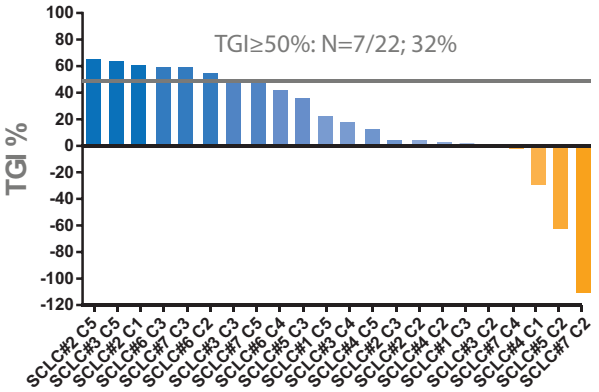
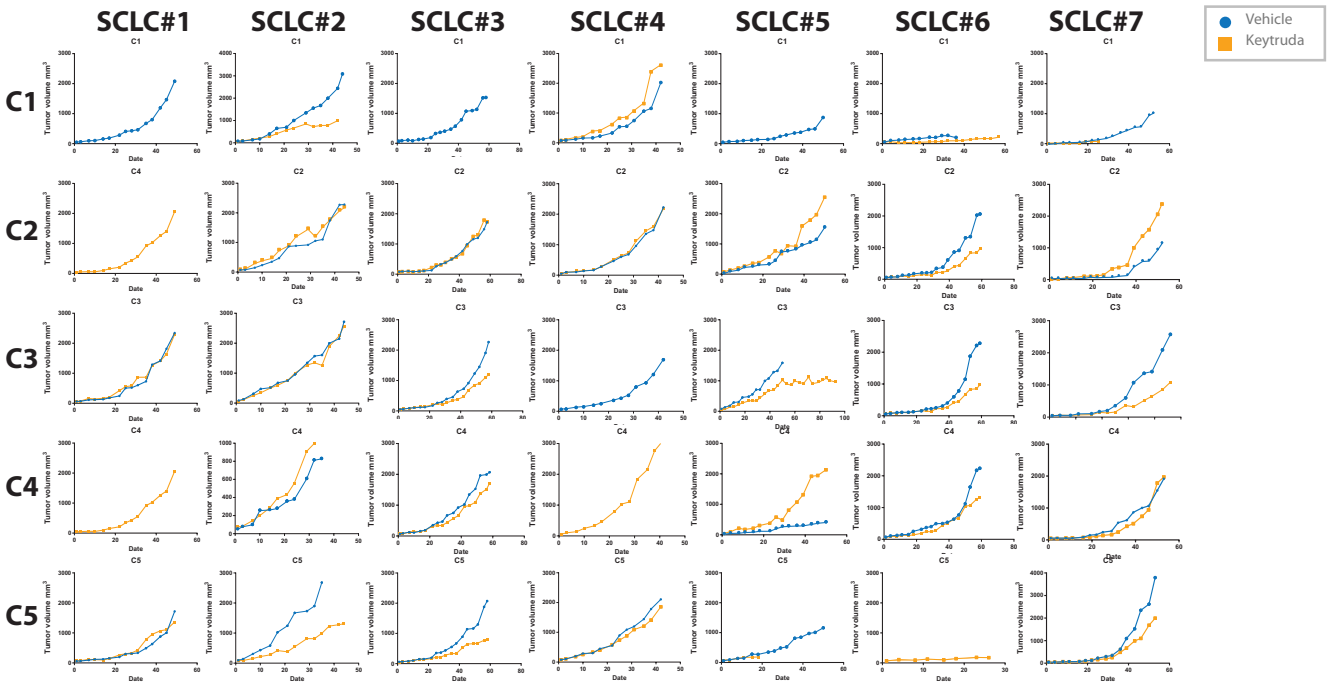


Figure 7: Variation of Anti-PD-1 Responses Across a Donor Population: SCLC PDX[5]

	SCLC #1	SCLC #2	SCLC #3	SCLC #4	SCLC #5	SCLC #6	SCLC #7
C1	NA	+	NA	-	NA	-	NA
C2	NA	-	-	-	-	+	-
C3	-	-	+	NA	+	+	-
C4	NA	NA	+/-	NA	-	+	-
C5	-	+	+	+/-	NA	NA	+/-



Selected tumor/donor pairings underwent comprehensive immunophenotypic analysis to study TIL characterization and tumor histology, comparing Keytruda responders vs non-responders. Response to Keytruda was typically associated with elevated TIL levels and increased tumor cell death and necrosis. These analyses were used to suggest a predictive signature for Keytruda response (**Figure 8**).

This includes TIL trends of:

- Increased $CD45^+$ for responders vs non-responders
- Increased $CD3^+$ for responders vs non-responders
- Increased $CD4^+$ for responders vs non-responders
- Increased $CD3^+/CD8^+$ for responders vs non-responders
- No difference between PD-1 in responders vs non-responders
- Elevated levels of macrophages in non-responders compared to responders

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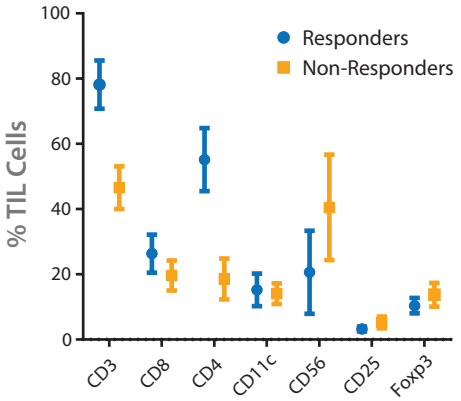
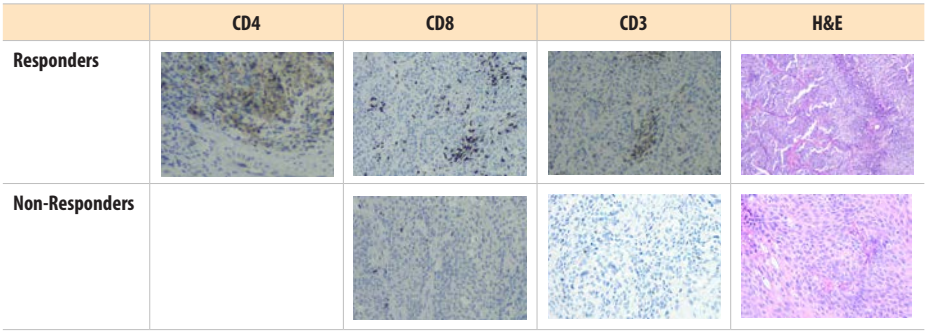
Figure 8: Potential Keytruda Responder Immunophenotypic Signature and Histology[5]





Conclusions

Immunotherapy research and agents such as anti-PD-1 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 fully competent immune systems, including humanized models.

CrownBio provides a range of platforms for preclinical drug development over a variety of different cancer types, with models incorporating either mouse, human, or chimeric immunity. Reconstitution with a human hematopoietic system offers a unique opportunity to study immunotherapeutics within a human tumor microenvironment. Utilizing HuPrime, the largest panel of commercially available PDX models, in humanized mice not only evaluates the function of the target, but also the immune response in models that preserve the genomic integrity and heterogeneity seen in patients.

HuNSG have been utilized in immunotherapeutic studies with our PDX and cell line derived xenograft models. The HSC donor variability creates a challenge for these preclinical model systems as well as in clinical trials; however, we are in the process of understanding the immuno-phenotypic profiles of responder and non-responder populations to help predict a favorable outcome for immunotherapy in this system, and across our immunotherapy platforms.

CrownBio can be contacted at **busdev@crownbio.com** for any further questions or information required on our *in vivo* immunotherapy models, or for information on other CrownBio products and services.

References

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[3]Wang Z, An X, Liu J *et al.* Response to Checkpoint Inhibition by GEMM Breast Cancer Allograft. [abstract]. In: Proceedings of the AACR-NCI-EORTC International Conference: Molecular Targets and Cancer Therapeutics; 2015 Nov 5-9; Boston, MA. Philadelphia (PA): AACR; *Molecular Cancer Therapeutics* 2015;14(12 Suppl 2):Abstract nr B97.
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[5]Izadi H, Yan D, Thatte J *et al.* Development of Patient-Derived Xenograft Models in Humanized Mice to Evaluate Efficacy of Anti-Cancer Immunotherapeutic Agents. [abstract] UCSD Moores Cancer Center Annual Symposium; 2016 February 26; San Diego.

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