CROWN BIOSCIENCE

Diffuse Large B Cell Lymphoma PDX Models

Evaluate novel therapies and combination regimens in PDX models fully characterized for DLBCL related genes

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FACTSHEET

V1.0

Accelerate your DLBCL targeted agent and combination therapy drug discovery programs with Crown Bioscience's panel of validated, clinically-relevant patient-derived xenograft (PDX) models.

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma which is typically treated with rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP). However a significant proportion of patients relapse or are unresponsive highlighting the need for more effective treatments. Advances in understanding the molecular features of DLBCL have identified high-risk subsets with poor outcomes to R-CHOP. Novel agents to target these mutations are under development, focused on combination therapies directed at multiple mutations within a single tumor. Their preclinical efficacy testing needs models which fully capture the complex genetic interplay seen in patients. Crown Bioscience provides a diverse panel of clinically relevant DLBCL patient-derived xenograft (PDX) models representing clinical subtypes and molecular features, ideal for therapeutic evaluation of novel agents such as BTK, IRAK4, SYK and PI3Kδ inhibitors.

The Unmet Need for Novel DLBCL and ABC Treatments

DLBCL can be sorted into 2 distinct subtypes based on cell of origin and mechanism of development:

- Activated B cell-like (ABC)
- Germinal center B cell-like (GCB)

Despite differing genetic backgrounds and origins, both subtypes are commonly treated with the immunochemotherapeutic regimen R-CHOP. Patients with the ABC subtype have poorer treatment outcomes, highlighting the unmet need for ABC specific therapies[1], as well as further research to fully unravel the genetic background of the disease

ABC mutations targeting the B cell receptor (including gain of function mutations in CD79A/B in approximately 18% of ABC) and in MYD88 (around 39% of ABC) result in chronic, active or constitutive signaling, promoting tumor viability, survival, and drug resistance[2].

Treatments are under development to target these mutations - ibrutinib (Imbruvica®) inhibits BTK signaling downstream of aberrant CD79A/B, with IRAK4 inhibitors similarly blocking mutated MYD88. However, multiple mutations can occur within one tumor. Therefore, combinations of these agents (as well as other drugs in development such as SYK and PI3Kδ inhibitors) may be required to fully inhibit tumor growth.

Robust preclinical models are now needed for single agent and combination therapy evaluation, which mimic the complex signaling and mutational interplay seen in patients (i.e. the combinations of wild type and mutated genes across different subtypes). These models will provide predictive efficacy data for personalized medicine development and next step decision making.

The Crown Bioscience NHL DLBCL PDX Panel

Xenografts derived directly from primary tumor tissue (which have never been in contact with plastic) are known to be the most predictive preclinical models available for drug evaluation[3], closely reflecting patient tumors for both their histopathological and genetic profile. Crown Bioscience provides the world's largest commercial collection of PDX models, Hu**Prime**®, for translational research programs.

Establishing hematopoietic malignancy PDX (including NHL models) brings specific challenges and difficulties. However, through the evolution of our PDX development techniques, we now provide a well-characterized NHL DLBCL panel for preclinical agent evaluation. **Table 1** summarizes a selection of these models.

DLBCL PDX Panel Key Facts

Crown Bioscience provides a well-characterized panel of DLBCL PDX models, covering the main ABC and GCB subtypes, including common single or combination MYD88 and CD79B mutations, truly reflecting the patient disease background:

- To progress the preclinical evaluation of novel DLBCL agents and the combination regimens required to overcome multiple mutations within a single tumor
- PDX models from Asian and US populations, closely reflecting patient tumors for histopathological and genetic profiles and highly predictive of patient response
- Full model background, QC, and NGS genetic profiling data searchable through Crown Bioscience's online PDX database, Hu**Base**™
- Panel of models includes wild type and mutated DLBCL related genes, including:
  - common MYD88 and CD79B mutations
  - as well as EZH2, CARD11 mutations
  - BCL-6 and MYC translocations
  - combination mutation genotypes
- Ibrutinib resistance observed for a range of models/genotypes, demonstrating the need for further combination therapy evaluation
- Standard of care and radiotherapy data available for a selection of models

DLBCL PDX Panel Full Background and Genomic Profiling Available

PDX model characterization information can be found within Hu**Base**, Crown Bioscience's easily searchable PDX database, and Onco**Express™** our comprehensive oncology search engine. These resources are accessed from our website at www.crownbio.com or directly from **hubase.crownbio.com** and **oncoexpress.crownbio.com.**

Example available model data, including patient background, clinical diagnosis, and treatment history are included within **Table 1**. All Crown Bioscience models undergo in house pathology QC to confirm disease type and subtype; QC information and pathology images can be found in Hu**Base**. Standard of care and radiotherapy data are also available for a selection of models. **Figure 1** displays representative standard of care data.

Table 1 also confirms the genomic profiling information available for our models within Hu**Base**, allowing detailed searching, comparison, and selection of appropriate models based on their genetic background.

Table 1: HuPrime NHL DLBCL PDX Panel of Well-Characterized Models

HuPrime ID	Disease Subtype	Patient Background	Patient Pathology Diagnosis	PDX Genomic Profiling Available
LY0257	NHL DLBCL ABC	Asian female, treatment naive	NHL (large B-cell) IHC: CD20(+), CD45RO(-), CD3(-), CD15(-), CD79α(+), CD30(-), CK(-), CD56(-)	P5: Affy U219, SNP 6.0, RNAseq P9: WES
LY2214	NHL DLBCL GCB	Asian female, aged 54. Treatment naive	NHL (large B cell) IHC: CD3(-), CD45RO(-), CD20(+), CD79α(+), Ki-67(80%), CD10(-), CD30(-), ALK(-), BCL-6(-), MUM1(-)	P0: Affy SNP 6.0 P4: WES P5: RNAseq
LY2264	NHL DLBCL ABC	Asian male, aged 61. Treatment naive	NHL (DLBCL) IHC: CD3(-), CK(-), CD20(+), Vim(+/-), CD79α(+/-), CD45RO(-), Ki-67(80%), CD30(-), HMB45(-), Mart-1(-), Pax-5(+), Bc1-6(+), EMA(-), CK7(-), P63(-), ALK(-), CD10(-)	P3: WES P4: Affy SNP 6.0, RNAseq
LY2266	NHL B cell ABC/GCB	Asian male, aged 67. Treatment naive	NHL (B cell lymphoma with plasma cell differentiation) IHC: CK(-), CD20(+), CD3(-), CD79α(+), Ki-67(40%), CD5(-), CyclinD1(-), CD138(+/-), CD56(-), CD10(-), Bc1-6(-), Pax-5(+), MUM1(+/-), CD45RO(-)	P1: Affy U219, SNP 6.0 P3: WES P4: RNAseq
LY2298	B cell lymphoma ABC	Asian female, aged 60. Treatment naive	B cell lymphoma of right forehead IHC: CD20(++), CD79α(+), Ki-67(+80%), CD56(-), CD3(-), CD45RO(-), NSE(-), GFAP(-), Syn(-), S-100(-)	P2: Affy U219, SNP 6.0, WES P4: RNAseq
LY2318	NHL DLBCL GCB	Asian male, treatment naive	NHL, DLBCL	P4: RNAseq
LY2345	NHL DLBCL ABC/GCB	Asian female, aged 56. Treatment naive	NHL (consider large B cell lymphoma) IHC: CD3(-), CD45RO(-), CD20(+), CD79α(+), Pax-5(+), CD5(+/-), CD10(+), BCL-6(-), CyclinD1(-), MUM1(+)	P1: Affy SNP 6.0 P3: RNAseq, WES
LY3604	NHL DLBCL ABC	Asian female, aged 82. Treatment naive	DLBCL	P5: RNAseq
LY14019	NHL DLBCL	Caucasian male, aged 74. Pretreated. Biopsy site: right side brain tumor	NHL, DLBCL	Ongoing
LY3786	NHL DLBCL	Asian female, aged 57. Treatment naïve	Diffuse large B cell lymphoma with necrosis	P4: WES P6: RNAseq
LY6701	DLBCL GCB	Asian male, aged 32. Treatment naïve. Biopsy site: Right love of liver	NHL (diffuse large B cell lymphoma)	P4: WES P6: RNAseq
LY6933	NHL DLBCL ABC/GCB	Asian female, aged 46. Treatment naïve	Diffuse large B cell lymphoma, derived from activated b lymphocyte	P4: WES P4: RNAseq
LY6934	NHL DLBCL ABC/GCB	Asian female, treatment naïve	Diffuse large B cell lymphoma	P6: WES P6: RNAseq
LY9602	DLBCL Double-hit NHL	caucasian male, aged 77, pretreated	B-Cell lymphoma, unclassified, with features intermediate between DLBCL and BL IHC: CD 20(1~2+), CD79a(3+), CD19(3+), Cyclin D1 (-) (P1).	Ongoing
LY2301	NHL DLBCL ABC	Asian male, aged 25, treatment naïve	Diffuse large B cell lymphoma. Reactive hyperplasia of lymph node.	P4: WES P2: RNAseq

Full Mutational and Fusion Analysis Available Including DLBCL Related Genes

Part of Crown Bioscience's PDX characterization package involves mutation analysis. As standard this covers hotspot mutations in 12 common oncogenes and tumor suppressor genes e.g. TP53, PTEN, MET, MAPK1, etc.

Using genomic profiling data we can further investigate specific genes of interest on a cancer type by type basis. For NHL DLBCL models this covers mutations in MYD88 and CD79B, as well as other relevant genes in ABC and GCB development (**Table 2**). All supporting information is found within **HuBase**, where any other genes can be searched for further mutational status information.

Ibrutinib Resistance Observed for Many Models with Both WT and Mutated CD79B and MYD88

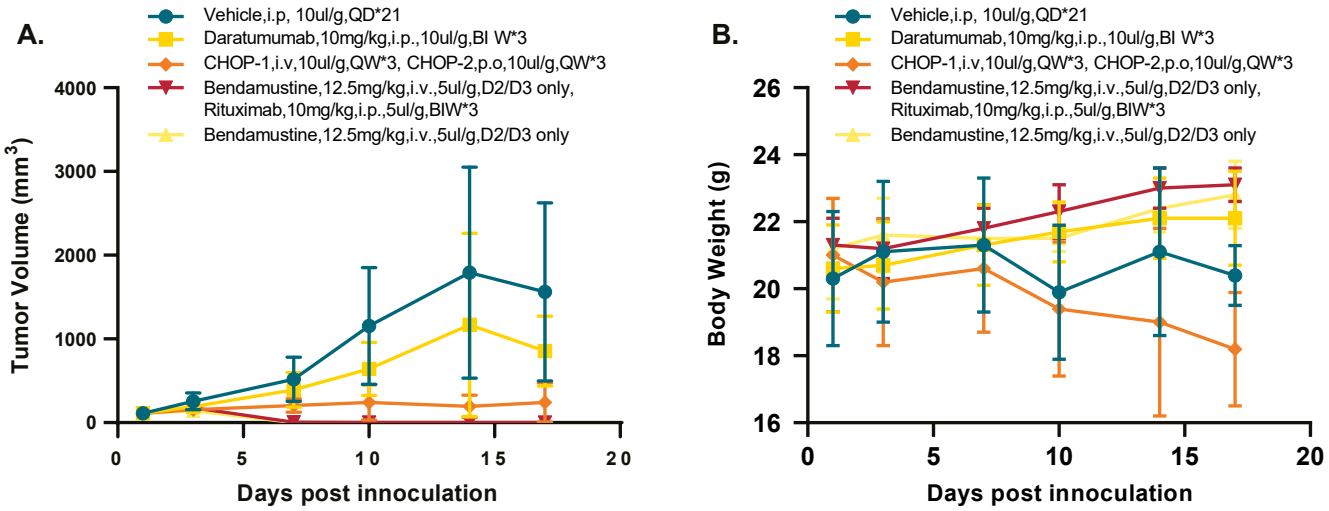
Ibrutinib is in late stage clinical trials for DLBCL treatment, having previously been approved to treat CLL, mantle cell lymphoma, and Waldenström macroglobulinemia (a NHL subtype)[4]. We have benchmarked our models with ibrutinib, with the majority of models proving to be resistant to treatment. This demonstrates the need for combination therapy evaluation in DLBCL, to simultaneously target the multiple mutated signaling pathways active within a single tumor.

Table 2 allows for comparison of responses to ibrutinib with subtype and MYD88 and CD79B mutations. Resistance occurs for ABC and GCB subtype models carrying both wild type and mutated genes. **Figure 2** displays representative response and resistance *in vivo* data.

Table 2: A Range of Disease Relevant Mutations and Translocations Found in the NHL DLCBL PDX Panel

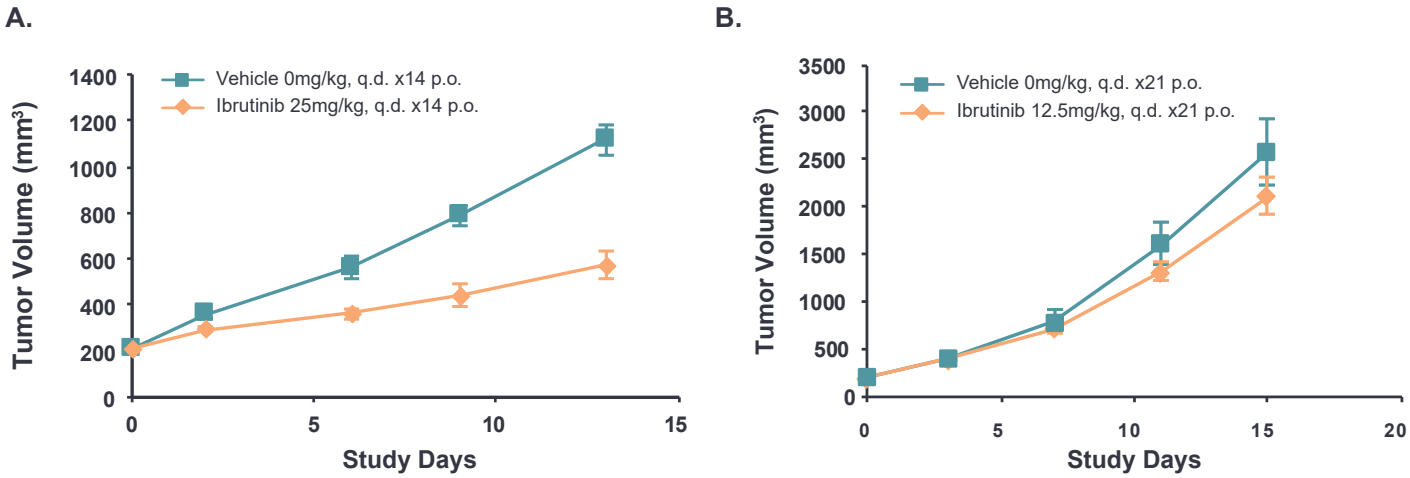
HuPrime ID	Subtype	MYD88	CD79A	CD79B	EZH2	CARD11	TNFAIP3 (A20)	PTEN	Translocations	Ibrutinib Response
LY0257	ABC	L273P	WT	WT	WT	WT	WT	WT	BCL-6	Resistant
LY2214	GCB	WT	WT	WT	WT	WT	WT		MYC	Resistant
LY2264	ABC	L273P	WT	E192A Y197S	D185H	WT	WT	WT	NA	Partial response
LY2266	ABC/GCB	WT	WT	WT	WT	F596V (low quality)	WT	WT	NA	Resistant
LY2298	ABC	L273P	WT	Y197N	WT	F596V (low quality)	WT	WT	NA	Resistant
LY2318	GCB	WT	WT	WT	WT	WT	WT	WT	NA	Ongoing
LY2345	ABC/GCB	WT	WT	WT	D185H	A687V	F127C I194T	WT	NA	Resistant
LY3604	ABC	WT	WT	WT	WT	WT	WT	WT	NA	Resistant
LY3786	"NHL DLBCL"	WT	WT	WT	WT	WT	WT	WT	WT	Ongoing
LY6701	"GCB"	WT	WT	WT	WT	WT	WT	D19E K183IfsTer15	WT	Ongoing
LY6933	ABC/GCB	WT	WT	L180CfsTer32	D185H	WT	A437EfsTer37	WT	BCL-6	Resistant
LY6934	ABC/GCB	WT	WT	WT	WT	WT	WT	WT	BCL-6	Partial response
LY9602	DLBCL	p.K108N	WT	WT	WT	WT	WT	WT	WT	Resistant
LY2301	ABC	WT	WT	WT	WT	WT	WT	WT	WT	Resistant

Figure 1: SOC data for LY9602



A: LY9602 Tumor Volume B: LY9602 Body Weight

Figure 2: NHL DLBCL PDX Models Partially Responsive and Resistant to Ibrutinib



A: LY2264 MYD88 L273P and CD79B E192A, Y197S. B: LY0257 MYD88 L273P and CD79B WT.

Summary

Crown Bioscience has developed a panel of DLBCL PDX models, which offer well-characterized, highly predictive xenografts for preclinical single agent and combination therapy evaluation.

As next generation NHL treatments may provide a personalized medicine approach based on disease subtype and background, our models have been fully profiled for the main gene mutations and translocations associated with DLBCL development including MYD88 and CD79B. The Crown Bioscience DLBCL PDX panel covers both ABC and GCB subtypes and a variety of wild type and mutated phenotypes, providing a comprehensive collection to evaluate novel combination strategies.

The DLBCL PDX panel has been benchmarked with ibrutinib treatment, allowing comparison and selection of models based upon response versus genetic profile. Standard of care treatment data is also available for a variety of our models, as required to fit research needs.

References

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