#3103

Liquid Tumor Orthotopic PDX Models of Acute Leukemia

[1]Hongkui Chen, [2]*Lintao Bi, [1]Shizhu Zhao, [1]Shuai Wang, [1]Wenqiang Huang, [1]Josh Caggiula, [1]Loc Van and [1]*Danyi Wen .[1]LIDE Biotech, Shanghai, China; [2]Department of Hematology and Oncology，China-Japan Union Hospital of Jilin University, Changchun, Jilin, China;* Corresponding author.

Abstract

Liquid tumor orthotopic xenograft models were established by intra femoral injection on NCG mice using freshly prepared leukemia cells from peripheral blood (PB) or bone marrow (BM) of AML/ALL patient samples. The establishment of the orthotopic models were monitored by flow cytometry analysis of peripheral blood of engrafted mice：CD45+/CD33+ for AML and CD45+/CD19+ for B-ALL. The double positive cells in the peripheral blood of the successfully transplanted model can reach 5-15%.

Typically, it takes 120-170 days to establish the P_0 AML orthotopic model and 45-60 days to establish the P_0 ALL orthotopic model. Spleen from engrafted P_0 mice was used for P_1-P_3 reconstitution of the orthotopic model. We tested sensitivity of these orthotopic models to several medicines, such as cladribine, cytarabine, vincristine, ibrutinib and imatinib. Anti-CD47 antibody was also tested on these models. NGS profiling was used to confirm the genotype of established orthotopic PDX.

Materials and Methods

AML and ALL patient samples: Peripheral blood or bone marrow samples were obtained from AML and ALL patients at the China–Japan Union Hospital of Jilin University. The study was reviewed and approved by the Institutional Ethics Committee and written informed consent was received from all patients for study for enrollment and blood or bone marrow collection.

Establishment of the PDOX models using peripheral blood or bone marrow: 6- to 8-week-old NCG mice were injected intrafemorally with 1–2×10^6 peripheral blood or bone marrow cells of AML or ALL patients, respectively.

Flow cytometry: Engraftment of the orthotopic models was monitored by flow cytometry of peripheral blood from inoculated mice. The frequencies of CD45+CD33+ expression in AML and CD45+CD19+ expression for B-ALL were assayed. A 5–15% dual positivity indicated AML or ALL PDOX engraftment.

Additional analysis: The presence of gene mutations that commonly occur in AML and ALL patients was investigated by next generation sequencing of peripheral blood and bone marrow samples obtained from the AML and B-ALL patients and the xenografts in the PDOX mouse spleen samples. Chromosomal analysis of human AML and ALL cells in mouse spleen samples from the established PDOX models was performed by karyotyping and standard G-banding.

Standard of care in AML and ALL PDOX models: Cladribine and cytarabine as single agents were used to validate the in vivo efficacy of standard chemotherapies in the AML and ALL PDOX models.

Results

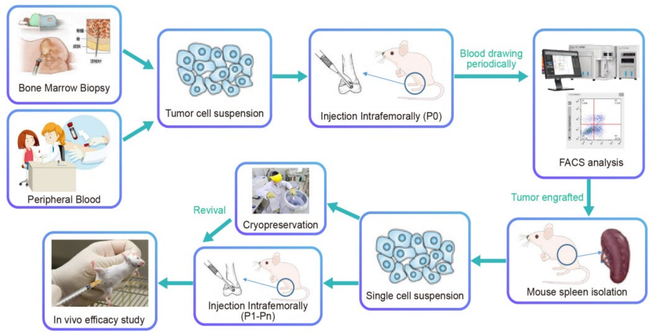


Figure 1. Flowchart of PDOX establishment using AML/ALL samples

Table 1. The list of liquid tumor models established by LIDE.

Model ID	Sex	Age	PDX pathology	PDX s.c.	PDOX i.f.
LD1-0041-362047	M	51	ALL	Available	Available
LD1-0041-362073	M	64	ALL	Available	Available
LD1-0041-362356	M	53	ALL	Available	Available
LD1-0041-362478	M	15	ALL	Available	Available
LD1-1041-362519	F	38	ALL	TBD	Available
LD1-0041-362021	M	32	ALL	TBD	Available
LD1-0040-361280	F	38	AML	Available	Available
LD1-0040-361293	F	61	AML	Available	Available
LD1-0040-362349	M	23	AML	Available	Available
LD1-0040-362384	M	59	AML	TBD	Available
LD1-0040-362030	F	56	AML	TBD	Available
LD1-0040-361780	M	49	AML	TBD	Available
LD1-0040-362224	M	75	AML	TBD	Available
LD1-0040-362369	M	57	AML	Available	Available
LD1-0040-362393	F	57	AML	Available	Available
LD1-0040-362499	M	68	AML	TBD	Available
LD1-0040-362575	F	31	AML	Available	Available
LD1-0026-362219	M	24	Burkitt's lymphoma	Available	TBD
LD2-0026-200614	M	/	CLL transit to DLBCL	Available	TBD
LD1-0026-362314	M	51	Mantle cell lymphoma	Available	TBD
LD1-0029-361915	M	62	Multiple myeloma	Available	TBD
LD1-0029-361847	M	54	Multiple myeloma	Available	TBD
LD1-0006-370728	M	68	Multiple myeloma	Available	TBD

Table 2. FACS detection: the corresponding relationship of clinical and PDX.

The series of ALL/AML-associated antigens revealed that the immunophenotype of PDOX cells was consistent with the primary specimens for most antigens tested and that they preserved the disease characteristics of the patients that they originated from

Gene	Clinical Result	PDX Result	
		Blood	Spleen
CD45	+	+	+
CD33	+	+	+
MPO	+	+	+
CD56	+	+	+
CD117	+	+	+

Table 3. Gene detection: the corresponding relationship of clinical and PDX

Gene	Clinical Result	PDX Result				
		WES_ Mut(AF)	RNA_ Mut(AF)	RNA_TPM	RNA_TPM_ zscore_GTEx	RNA_TPM_ zscore_TCGA
c-kit/D816V	-	-	-	12.5	4.200862288	-1.086434655
CEBPA	-	-	-	117.22	1.922675063	0.460406123
NPM1exon12A	+	Trp288fs (0.312)	Trp288fs (0.355)	1558.02	4.793047232	3.38832293
FLT3/ITD	-	-	-	146.78	2.790451315	0.758331842

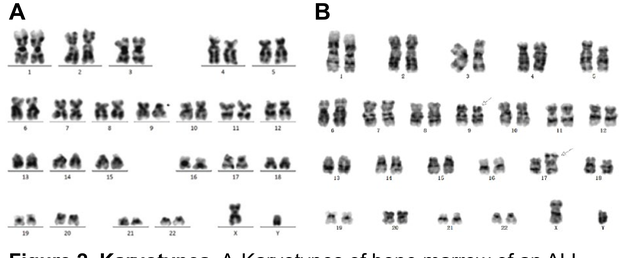
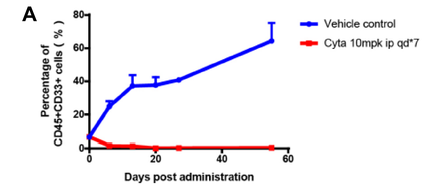


Figure 2. Karyotypes. A-Karyotypes of bone marrow of an ALL patient (model ID: LD1-0041-362021) and a-mouse bone marrow of the LD1-0041-362021 PDOX model

G-banding karyotype analysis showed normal karyotypes and revealed that the karyotype of PDX cells was similar to that of patient specimens



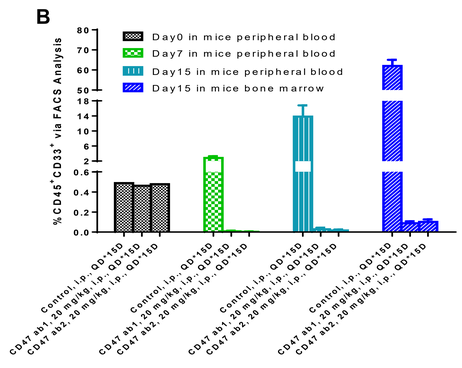


Figure 3. the correlation between clinical medication history and PDX.

A: Cytarabine or cladribine demonstrated completely tumor elimination in an AML PDOX.

B:AML PDOX model was inoculated orthotropically, and CD47 abs showed anti-cancer effect by completely inhibiting CD45+CD33+ cells in mice.

Summary and Conclusion

- The established PDOX models preserve the pathological, phenotypic, and genetic characteristics of the clinical samples, and they also have similar drug sensitivity to the corresponding patients.
- The PDOX mouse model of acute leukemia provides a clinically relevant platform for testing novel chemotherapy drugs.

Shanghai LIDE Biotech, Co. Ltd.
887 ZuChongZhi Rd, Building 77-78, 3rd F, Pudong, Shanghai 201203. P.R. China
Call 1-276-296-LIDE
Email business@lidebiotech.com
www.lidebiotech.com

Poster Download