

Supplementary Materials

LT-171-861, a novel FLT3 inhibitor, shows excellent preclinical efficacy for the treatment of acute myeloid leukemia

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Supplementary Table S1. Oral and intravenous pharmacokinetics parameters of LT-171-861

Parameters	Units	IV	IG
AUC _(0-t)	ng/mL*h	3314.72	462.62
AUC _(0-∞)	ng/mL*h	3377.66	530.62
MRT _(0-t)	h	1.86	4.28
MRT _(0-∞)	h	2.1	5.95
t _{1/2z}	h	2.02	3.68
T _{max}	h	0.25	1
CL _Z	mL/h/kg	2960.63	18845.72
V _Z	mL/kg	8612.54	100186.3
C _{max}	ng/mL	2167.5	88.01

Supplementary Table S2. Clinical data for patients with AML

Patient no.	Diagnosis	Source	Blasts initial PB%	FLT3 status	Status
1	AML	PB	78%	ITD	New
2	AML	PB	54%	wt	New
3	AML	PB	63%	wt	New
4	AML	PB	71%	wt	New
5	AML	PB	42%	ITD	New
6	AML	PB	58%	wt	New
7	AML	PB	67%	ITD	Relapse
8	AML	PB	45%	ITD	Relapse
9	AML	PB	86%	ITD	Relapse
10	AML	PB	87%	ITD	Relapse

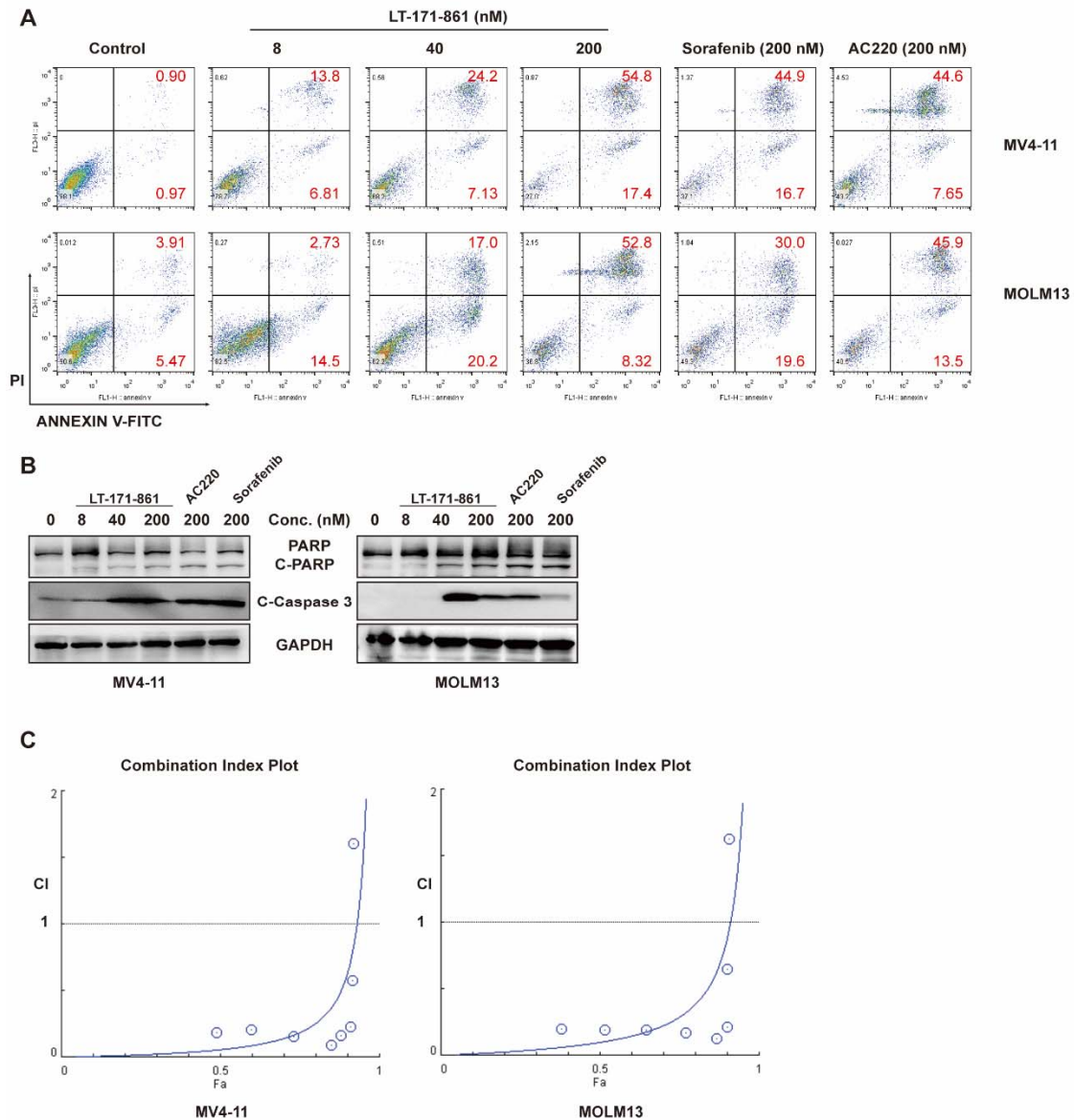


Figure S1

Supplementary Figure S1. Pro-apoptotic effects of LT-171-861 and established FLT3 inhibitors on leukemia cell lines expressing FLT3-ITD

(A) MV4-11 and MOLM-13 cells treated with indicated concentrations of LT-171-861, AC220 or sorafenib respectively for 36 h were stained by Annexin V-FITC and PI. Then flow cytometry assay was used to evaluate the ratio of dead cell. (B) MV4-11 and MOLM-13 cells were incubated with LT-171-861, AC220 or sorafenib at indicated concentrations for 36 h. PARP, and cleaved caspase-3 were evaluated in the cell lysate by western blot. (C) Combination index (CI) value calculated by Compusyn2.0 software.

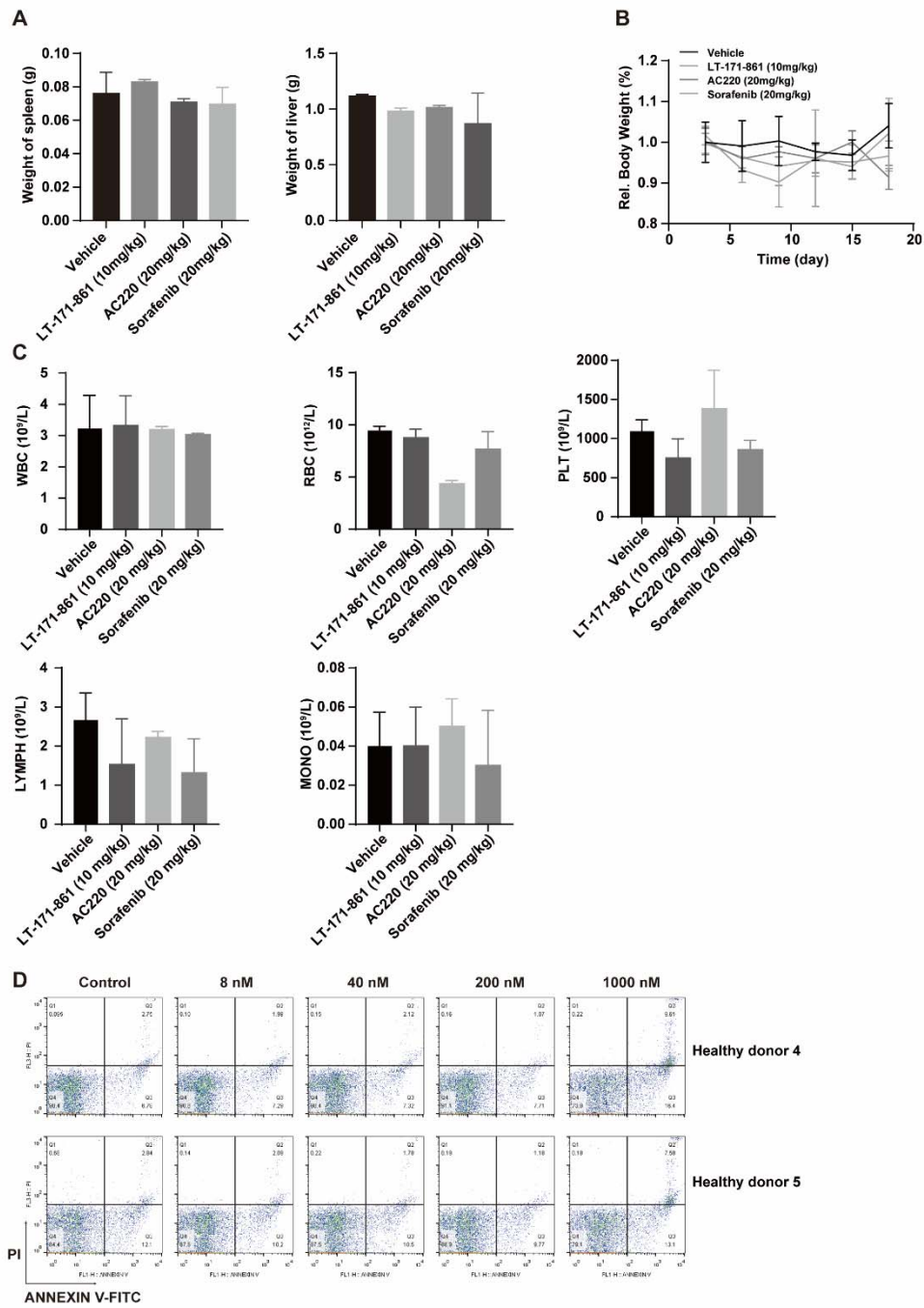


Figure S2

Supplementary Figure S2. Toxicity evaluation of LT-171-861 in C57BL/6 mice and normal PBMCs from healthy donors.

Healthy C57BL/6 mice (n=6) were intravenously injected with vehicle or LT-171-861 (10 mg/kg) every other day and were orally administered with AC220 (20 mg/kg) or sorafenib (20 mg/kg) once per day for 18 days. 24 h after the last administration, weights of spleens and livers (A), relative body weights (B), and total peripheral blood evaluations (C) populations were analysed. WBC=white blood cells; RBC=red blood cells; PLT=platelets; LYMPH=lymphocytes; MONO=monocytes. Means \pm SD are shown. (D) PBMCs isolated by Ficoll from healthy donors were incubated with indicated concentrations of LT-171-861 for 36 h. Cells then stained with Annexin V-FITC/PI and evaluated by flow cytometry. Annexin V⁺/PI⁻ and Annexin V⁺/PI⁺ cells were considered dead cells.

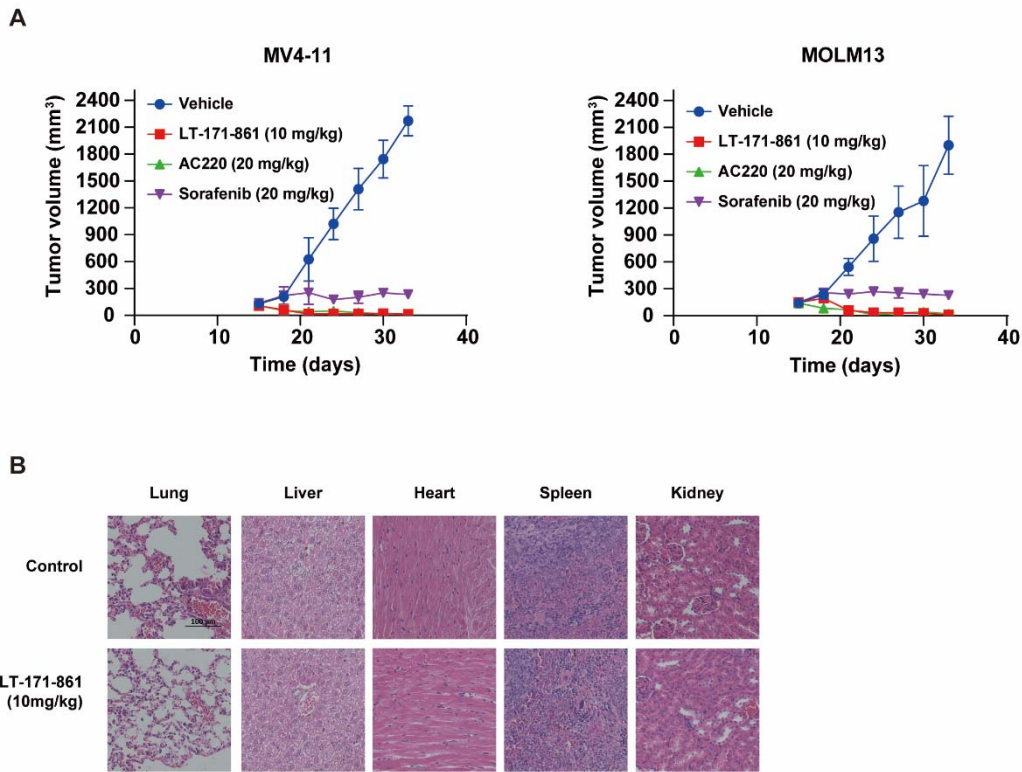


Figure S3

Supplementary Figure S3. Anti-leukemia activity of LT-171-861 and established FLT3 inhibitors AC220 and sorafenib in xenograft mouse model.

(A) Mice (n=6) administered with LT-171-861 intravenously every two days and with AC220 (20 mg/kg) or sorafenib (20 mg/kg) orally once a day for 21 days. Tumor volumes were measured by caliper. Mean \pm SD were shown. (B) Mice were sacrificed when they exhibited hind-limb paralysis after LT-171-861 treatment, and their tissues were resected and fixed in 4% formalin for HE analysis. Images were acquired at $\times 20$ magnification.