

Supplementary Data

Systemic Delivery of CRISPR/Cas9 with PEG-PLGA Nanoparticles for Chronic Myeloid Leukemia Targeted Therapy

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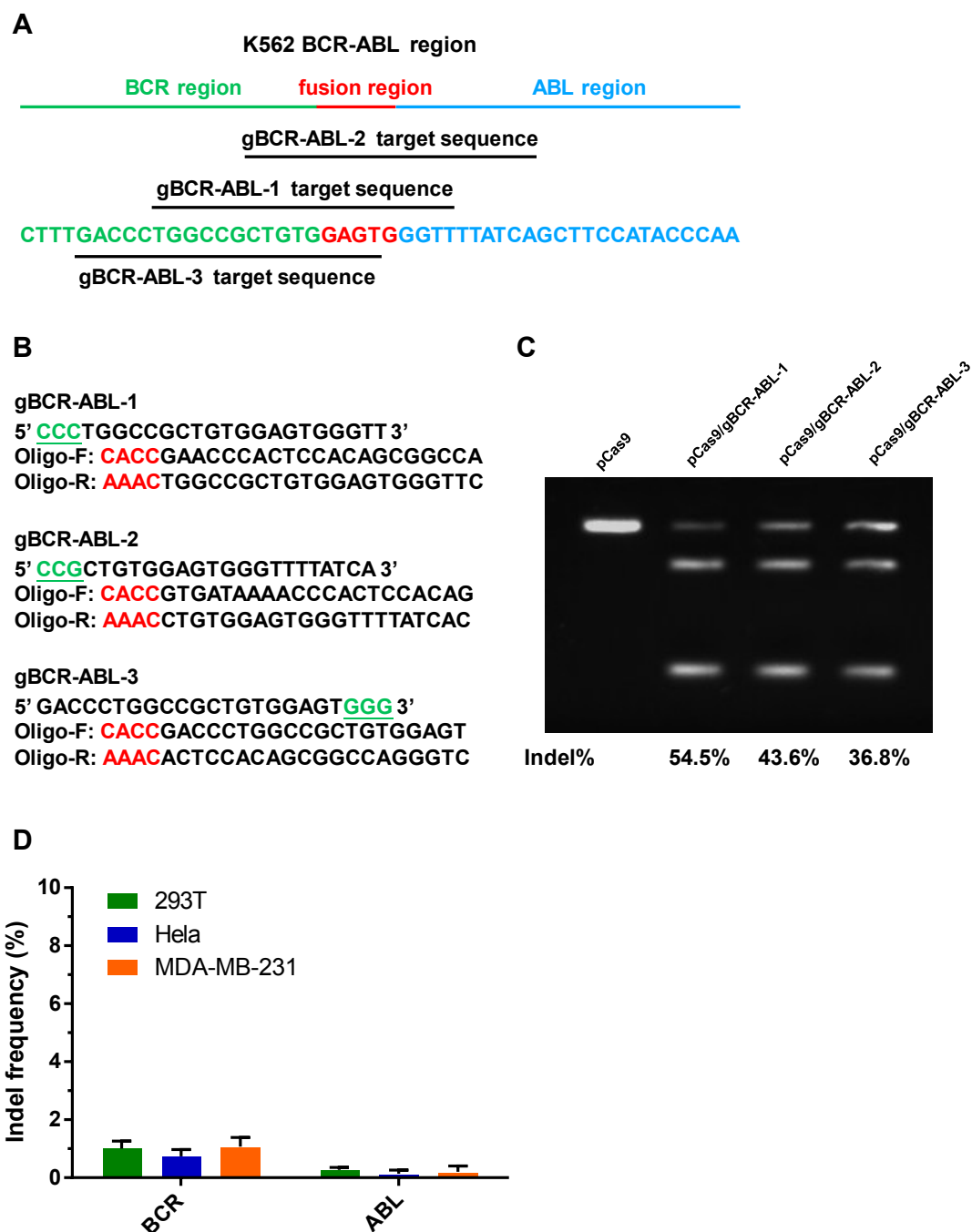


Fig. S1. Designing the sequence of gBCR-ABL for the disruption of BCR-ABL gene. (A) The scheme of gBCR-ABL-1, gBCR-ABL-2, gBCR-ABL-3 targeting the fusion region of BCR-ABL. (B) The sequences of DNA oligos for constructing the pCas9/gBCR-ABL. (C) The indel frequencies in K562 cells transfected with pCas9/gBCR-ABL-1, pCas9/gBCR-ABL-2, pCas9/gBCR-ABL-3 by Lipofectamine

LTX. (D) The indel frequencies in BCR or ABL gene of 293T, Hela and MDA-MB-231 cells transfected with CLAN_{pCas9/gBCR-ABL} at a pCas9/gBCR-ABL concentration of 2 µg/mL. The indel frequencies were detected with T7E1 at 72 h after transfection.

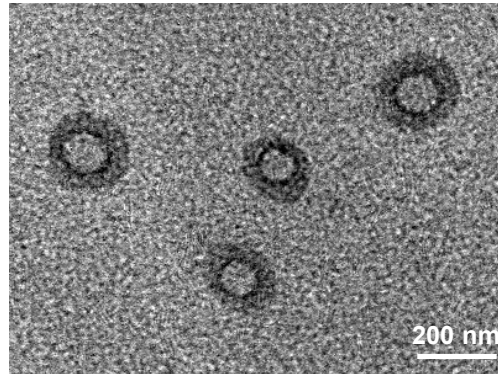


Fig. S2. The morphology of CLAN_{pCas9} was analyzed with TEM. Scal bar = 200 nm.

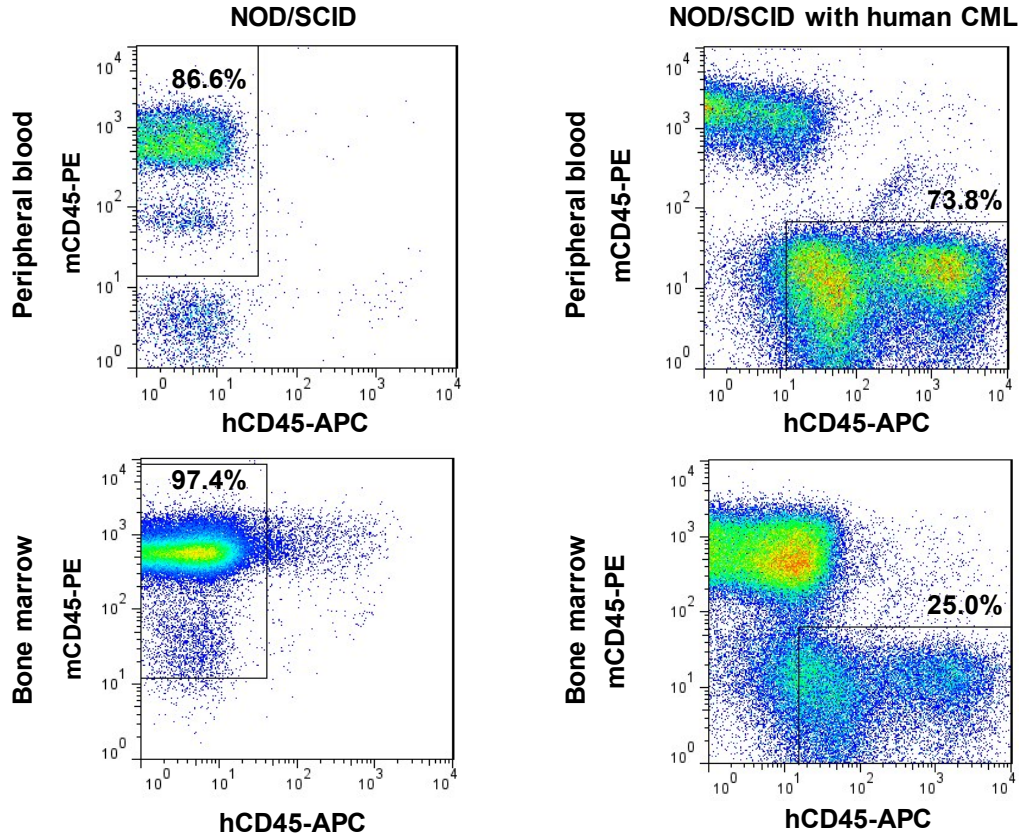


Fig. S3. The percentage of human derived-K562 cells (hCD45⁺mCD45⁺) in the blood and bone marrow of CML mouse at 28 days after transplantation. CML mouse was irradiated at the dose of 270 cGy by ¹³⁷Cs and injected with K562 cells (1×10^5 cells in 500 μ L PBS per mouse) within 24 h by tail intravenous injection.

gBCR-ABL		
PAM	Target sequence	
CTTTGACCC	TGGCCGCTGTGGAGTGGGTTTTATCAGCTTCCATACCCAA	WT
CTTTGACCC	---CGCTGTGGAGTGGGTTTTATCAGCTTCCATACCCAA	$\Delta 4$
CTTTGACC	-----GCTGTGGAGTGGGTTTTATCAGCTTCCATACCCAA	$\Delta 6$
CTTTGACCCAC	---GCTGTGGAGTGGGTTTTATCAGCTTCCATACCCAA	$\Delta 5, +2$
CTTTGACCC	---C---TGGAGTGGGTTTTATCAGCTTCCATACCCAA	$\Delta 8$
CTTTGACC	---G---GTATGTGGAGTGGGTTTTATCAGCTTCCATACCCAA	$\Delta 7, +3$

Fig. S4. The representative mutant sequences harboring indels in the BCR-ABL locus of K562 cells. hCD45⁺mCD45⁻ K562 were sorted by flow cytometry at 72 h after

CLAN_{pCas9/gBCR-ABL} injection.

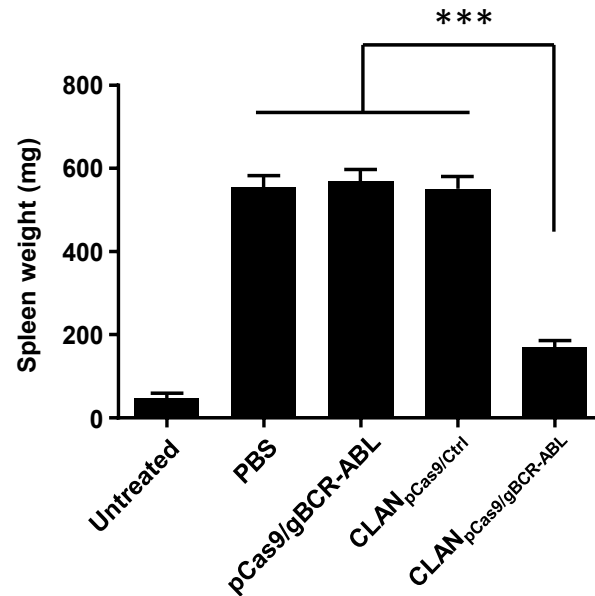


Fig. S5. The spleen weight of CML mice treated with CLAN_{pCas9/gBCR-ABL} and other formulations. Data are shown as means $\pm$ SD (n = 10), *** $p < 0.001$.

Table S1. The primer sequences for qRT-PCR and PCR.

Primers	F primer (5'-----3')	R primer (5'-----3')
BCR-ABL qPCR	GGAGCTGCAGATGCTGACCACC	TCAGACCCTGAGGCTCAAAGTC
GADPH qPCR	ATCAAGAAGGTGGTGAAGCAGGCA	TGGAAGAGTGGGAGTTGCTGTTGA
BCR-ABL	GCCACTGGATTTAAGCAGAGTTCAA	CTTTCTTTCATCAATAAGAATTCTT
Off-target 1	AAAAACTGCAACTGCTGCTGGGCCA	TGTGCTCGTTGTTCTGCAGCATGAT
Off-target 2	GACAAGCGGCGAGTGCCACTGCAAG	ACAGACTCTGGACAAGGAGCCTGTG
Off-target 3	GCCAAAGCGTTTGAGTTAAGTACC	AGACAAGCTGTGCTGTTTCAGCGTT
Off-target 4	GCAGTGCGTGTGAGGTAGGAGTAAA	CCTTCTCCACGAACCCACAAACGC
Off-target 5	TCATCCGAGGGTCCTCGCTCCTGAA	GTCCCGTCATCAGGAAGCCCAACA
Off-target 6	TTGAATCAGCCACAACGTGACCA	GTGCAAGCTCCTTTGCCTCTGAGTC
Off-target 7	GAACCAGCACAGTGATAGGGCCGCC	CCTCCTTGAGCTGCTCCTCGTGGGC
Off-target 8	ATGGAGCCTCACGTCTACTCCCTTC	TTCAATTCTATCCTTGGGAGGCAGC
BCR	CCACTGGATTTAAGCAGAGTTCAA	CCAGCCTTCACTGTTCTGACAG
ABL	CCTGTTAATTTGTTTGCTTTCCTT	CTGTTGATCCATGAAAATGTATAA

Table S2. The on-target and potential off-target sites of gBCR-ABL. The mismatched bases were in red.

Targets	sequence	mismatches	UCSC gene	locus
On-target	AACCCACTCCACAGCGGCCA			
Off-target 1	AGCCATCCCACAGCGGCCA	4MMs [2:5:6:8]	NM_001335	chr11:-65650085
Off-target 2	GACCCTCTCCACAGGGCCA	3MMs [1:6:15]	NM_001408	chr1:+109808262
Off-target 3	GGCCCACTCCTCAGCGTCCA	4MMs [1:2:11:17]	NM_025135	chr18:-34324158
Off-target 4	GACCCACCCGCTGCGGCCA	4MMs [1:8:11:13]	NM_024007	chr5:+158524126
Off-target 5	TACCGGCTCCACAGCGGCGA	4MMs [1:5:6:19]	NM_213605	chr8:+146033626
Off-target 6	GGCCCACTCCGCAGAGGCCA	4MMs [1:2:11:15]	NM_001105538	chr17:-4451298
Off-target 7	AACCCAAGCCACAGCGACCC	4MMs [7:8:17:20]	NM_201383	chr8:+145002221
Off-target 8	AATCCCATCCACAGAGGCCA	4MMs [3:6:7:15]	NM_001190456	chr15:+69019881