IDH-Mutant Leukemia Cells by CRISPR Base-Editing

Cell lines and cell culture:

Parental **K562**, **K562-IDH1^{R132H} (clone #C3, Xu lab #111)** and **K562-IDH2^{R140Q} (clone #B4, Xu lab #119)**

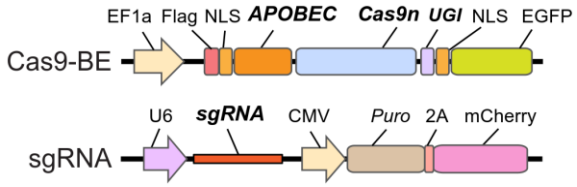
Parental **MOLM-13**, **MOLM-13-IDH1^{R132H} (clone #F8, Xu lab #108)** and **MOLM-13-IDH2^{R140Q} (clone #F6, Xu lab #115)**

All the cell lines were cultured in the RPMI-1640 medium with 10% FBS. The details of these cells lines are all described in the paper (1).

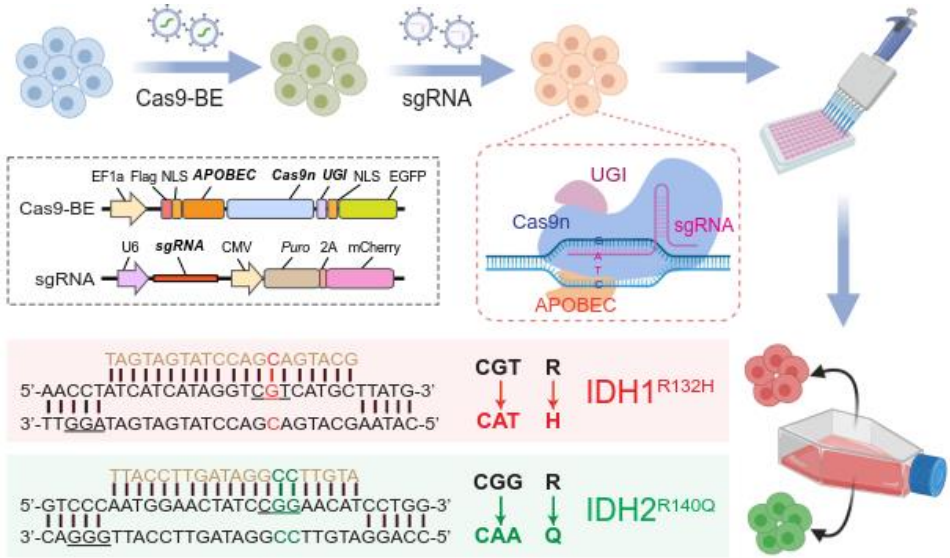
Plasmids used for generating the cell lines:

1. CRISPR/Cas9 base editor (Cas9-BE; Addgene, #110869)
2. pSLQ1651 (Addgene, #100549)
3. psPAX2 (Xu lab #660)
4. VSVG (Xu lab #409)

Schematic of the vectors:



Schematic of CRISPR base editing of IDH1^{R132H} or IDH2R^{140Q} mutations in leukemia cells:



Method for Generation of IDH-Mutant Leukemia Cells by CRISPR Base Editing:

We employed the optimized CRISPR base editing method (2) to generate hotspot mutations including IDH1^{R132H} (CGT to CAT), and IDH2^{R140Q} (CGG to CAA) in K562 and MOLM-13 human

leukemia cells, respectively. The CRISPR/Cas9 base editor (Cas9-BE; Addgene, 110869) contains $BE3^{RA}$ (Cas9n with codon optimization and deletion of premature poly(A) sites), cytidine deaminase APOBEC, uracil glycosylase inhibitor (UGI) domain, and two nuclear-localization signal (NLS) sequences at the N- or C-terminus. sgRNAs were designed to target individual hotspot mutations in IDH1 or IDH2 and cloned into pSLQ1651 vector (Addgene, 100549) with mCherry. Leukemia cells were transduced with lentiviruses expressing Cas9-BE and sgRNA for each mutation, which were packaged in HEK293T cells and sorted for EGFP and mCherry positive cells. Genomic DNA (gDNA) was extracted from single cell-derived clones and used for targeted sequencing. Raw fastq files were mapped to IDH1 or IDH2 reference sequences using Bowtie2 with default parameters (3). Mapped reads were extracted from mapping files and aligned together. For each position of piled reads, the counts of bases (A, T, C and G) and corresponding mutation frequencies for each position were calculated. The mutation frequencies at the base-edited sites in single cell-derived clones (two independent clones for each mutation) are shown below.

Representative allelic frequencies of $IDH1^{R132H}$ (CGT to CAT, red) and $IDH2^{R140Q}$ (CGG to CAA, green) mutations in base-edited K562 and MOLM-13 leukemia cells by targeted sequencing:

Gene	Amino Acid Mutation	Nucleotide Mutation	K562		MOLM-13	
			Clone no.	Mut. Freq. (%)	Clone no.	Mut. Freq. (%)
IDH1	p.Arg132His	c.395 G>A	C3	58.69	F8	67.42
			E6	65.04	G8	80.80
IDH2	p.Arg140Gln	c.419 G>A c.420 G>A	B4	49.64 49.63	C8	49.17 48.44
			C1	64.41 31.96	F6	66.12 66.24

References:

1. Lyu J, Liu Y, Gong L, Chen M, Madanat YF, Zhang Y, Cai F, Gu Z, Cao H, Kaphle P, Kim YJ, Kalkan FN, Stephens H, Dickerson KE, Ni M, Chen W, Patel P, Mims AS, Borate U, Burd A, Cai SF, Yin CC, You MJ, Chung SS, Collins RH, DeBerardinis RJ, Liu X, Xu J. Disabling Uncompetitive Inhibition of Oncogenic IDH Mutations Drives Acquired Resistance. Cancer Discovery 2022 Oct 12:CD-21-1661. doi: 10.1158/2159-8290.

2. Zafra MP, Schatoff EM, Katti A, Foronda M, Breinig M, Schweitzer AY, et al. Optimized base editors enable efficient editing in cells, organoids and mice. Nature Biotechnology 2018;36(9):888-93 doi 10.1038/nbt.4194.

3. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nature Methods 2012;9(4):357-9 doi 10.1038/nmeth.1923.