

**High throughput sequencing in acute lymphoblastic leukemia reveals clonal architecture of central nervous system and bone marrow compartments**

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# **High throughput sequencing in acute lymphoblastic leukaemia reveals clonal architecture of central nervous system and bone marrow compartments**

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## **SUPPLEMENTARY MATERIAL**

### **Supplementary Methods**

#### *Processing of samples*

For BM samples, the total mononuclear cell fraction was isolated following centrifugation on Ficoll-Hypaque. CSF cells were pelleted by centrifugation and directly DNA directly extracted. DNA was extracted according to standardised protocols using QIAamp DNA Mini or Mirco Kit (Qiagen). DNA concentration was estimated using spectrophotometry (Nanodrop, Thermo Scientific) and then accurately quantitated by RQ-PCR using albumin as a control/reference gene. DNA and approximate cells numbers (based on 6.6 pg DNA per cell) analysed are show in supplementary table 1.

#### *High throughput sequencing*

Paired patient samples were run on separate MiSeq lanes, as well as other measures employed to prevent cross contamination.<sup>1</sup> We used a single-end 250-300 read from J to V to ensure optimal quality over the CDR3-encoding region. Indexing reads were performed to identify the 8 base pair dual indices sequences at both ends of the amplicons.

### **Supplementary references**

1. Bartram J, Mountjoy E, Brooks T, et al. Accurate Sample Assignment in a Multiplexed, Ultrasensitive, High-Throughput Sequencing Assay for Minimal Residual Disease. *J Mol Diagn.* 2016;18(4):494-506.

**Supplementary table 1. DNA input and approximate cells input**

Patient	DNA samples input		
	BM diagnosis	BM relapse	CSF relapse
CNS001	500 ng 75000 cells	500 ng 75000 cells	85 ng 12300 cells
CNS002	500 ng 75000 cells	500 ng 75000 cells	100 ng 15000 cells
CNS003	500 ng 75000 cells	500 ng 75000 cells	100 ng 15000 cells
CNS004	500 ng 75000 cells	NA	*50 ng 7500 cells
CNS005	500 ng 75000 cells	500 ng 75000 cells	100 ng 15000 cells
CNS006	500 ng 75000 cells	500 ng 75000 cells	100 ng 15000 cells
CNS007	○	500 ng 75000 cells	40 ng 6000 cells
CNS008	○	500 ng 75000 cells	50 ng 7500 cells
CNS009	○	500 ng 75000 cells	70 ng 11000 cells
CNS010	500 ng 75000 cells	6 ug 1000000 cells	100 ng 15000 cells
CNS011	○	500 ng 75000 cells	100 ng 15000 cells
CNS012	500 ng 75000 cells	1 ug 150000	100 ng 15000 cells

\*indicates CSF sample was at diagnosis. ○ No sample available; NA = not applicable

**Supplementary table 2.** Detailed clone information across compartments

	Diagnosis BM		BM relapse		CSF relapse	
<b>CNS001</b>	IGHV4-34*01/IGHD2-2*01/IGHJ5*02	85%	IGHV4-34*01/IGHD2-2*01/IGHJ5*02	0.002%	IGHV4-34*01/IGHD2-2*01/IGHJ5*02	0
	IGHV6-1*01/IGHJ5*02	4%	IGHV6-1*01/IGHJ5*02	88%	IGHV6-1*01/IGHJ5*02	78%
<b>CNS002</b>	IGHV1-69*06/IGHD5-12*01/IGHJ4*02	36%	IGHV1-69*06/IGHD5-12*01/IGHJ4*02	45%	IGHV1-69*06/IGHD5-12*01/IGHJ4*02	5%
	IGHV3-72*01/IGHD2-8*02/IGHJ4*02	11%	IGHV3-72*01/IGHD2-8*02/IGHJ4*02	0	IGHV3-72*01/IGHD2-8*02/IGHJ4*02	0
	IGHV1-18*01/IGHD2-2*02/IGHJ6*02	10%	IGHV1-18*01/IGHD2-2*02/IGHJ6*02	0	IGHV1-18*01/IGHD2-2*02/IGHJ6*02	0
	IGHV3-74*01/IGHD4-23*01/IGHJ6*02	3%	IGHV3-74*01/IGHD4-23*01/IGHJ6*02	0	IGHV3-74*01/IGHD4-23*01/IGHJ6*02	0
	IGHV3-74*01/IGHD5-12*01/IGHJ4*02	0.1%	IGHV3-74*01/IGHD5-12*01/IGHJ4*02	34%	IGHV3-74*01/IGHD5-12*01/IGHJ4*02	0.1%
	IGHV3-74*01/IGHD7-27*01/IGHJ6*02	0	IGHV3-74*01/IGHD7-27*01/IGHJ6*02	0.6%	IGHV3-74*01/IGHD7-27*01/IGHJ6*02	43%
	IGHV3-74*01/IGHJ6*02	0	IGHV3-74*01/IGHJ6*02	0.06%	IGHV3-74*01/IGHJ6*02	14%
<b>CNS003</b>	IGHV1-46*01/IGHD3-3*01/IGHJ4*02	91%	IGHV1-46*01/IGHD3-3*01/IGHJ4*02	50%	IGHV1-46*01/IGHD3-3*01/IGHJ4*02	94%
<b>CNS004</b>	IGHV4-34*01/IGHD3-16*01/IGHJ2*01	40%	NA		IGHV4-34*01/IGHD3-16*01/IGHJ2*01	22%
	IGHV6-1*01/IGHD3-9*01/IGHJ4*01	16%			IGHV6-1*01/IGHD3-9*01/IGHJ4*01	41%
<b>CNS005</b>	IGHV1-8*01/IGHD2-2*02/IGHJ6*03	55%	IGHV1-8*01/IGHD2-2*02/IGHJ6*03 <sup>†</sup>	v-v replaced	IGHV1-8*01/IGHD2-2*02/IGHJ6*03 <sup>†</sup>	v-v replaced
	IGHV3-23/IGHD2-2*02/IGHJ6*03	0.005%	IGHV3-23/IGHD2-2*02/IGHJ6*03	40%	IGHV3-23/IGHD2-2*02/IGHJ6*03	39%
	IGHV1-2*04/IGHD3-22*01/IGHJ6*03	0.01%	IGHV1-2*04/IGHD3-22*01/IGHJ6*03	48%	IGHV1-2*04/IGHD3-22*01/IGHJ6*03	45%
<b>CNS006</b>	IGHV6-1*01/IGHD3-10*01/IGHJ6*02	26%	IGHV6-1*01/IGHD3-10*01/IGHJ6*02	0.9%	IGHV6-1*01/IGHD3-10*01/IGHJ6*02	0
	IGHV3-30-3*01/IGHJ4*02	2%	IGHV3-30-3*01/IGHJ4*02	10%	IGHV3-30-3*01/IGHJ4*02	1%
	IGHV3-11*06/IGHJ4*02	0	IGHV3-11*06/IGHJ4*02	41%	IGHV3-11*06/IGHJ4*02	0
	IGHV1-3*01/IGHD2-8*01/IGHJ4*02	2%	IGHV1-3*01/IGHD2-8*01/IGHJ4*02	11%	IGHV1-3*01/IGHD2-8*01/IGHJ4*02	1%
	IGHV3-30-3*01/IGHD2-8*01/IGHJ4*02	2%	IGHV3-30-3*01/IGHD2-8*01/IGHJ4*02	10%	IGHV3-30-3*01/IGHD2-8*01/IGHJ4*02	1%
	IGHV3-23*01/IGHD2-8*01/IGHJ4*02	0	IGHV3-23*01/IGHD2-8*01/IGHJ4*02	7%	IGHV3-23*01/IGHD2-8*01/IGHJ4*02	0
	IGHV6-1*01/IGHD2-8*01/IGHJ4*02	0.4%	IGHV6-1*01/IGHD2-8*01/IGHJ4*02	0.5%	IGHV6-1*01/IGHD2-8*01/IGHJ4*02	26%
	IGHV3-30*18/IGHJ4*02	0.01%	IGHV3-30*18/IGHJ4*02	0.7%	IGHV3-30*18/IGHJ4*02	21%
	IGHV7-4-1*01/IGHJ6*02	0.02%	IGHV7-4-1*01/IGHJ6*02	0.1%	IGHV7-4-1*01/IGHJ6*02	16%

<b>CNS007</b>	NA	IGHD6-13*01/IGHJ4*02 IGHD2-2*02/IGHJ6*02 IGHD2-2*02/IGHJ4*02	37% 15% 0	IGHD6-13*01/IGHJ4*02 IGHD2-2*02/IGHJ4*02 IGHD2-2*02/IGHJ4*02	45% 0 11%		
<b>CNS008</b>	NA	IGHV3-30-3*01/IGHD3-9*01/IGHJ6*03	95%	IGHV3-30-3*01/IGHD3-9*01/IGHJ6*03	64%		
<b>CNS009</b>	NA	IGHV1-46*01/IGHD5-12*01/IGHJ4*02 IGHV3-71*02/ IGHD6-6*01/IGHJ4*02	34% 31%	IGHV1-46*01/IGHD5-12*01/IGHJ4*02 IGHV3-71*02/ IGHD6-6*01/IGHJ4*02	48% 40%		
<b>CNS010</b>		IGHV3-15*01/IGHD6-19*01/IGHJ6*02 IGHV4-59*01/ IGHD3-10*01/IGHJH3*02 IGHV3-33*01/ IGHD6-6*01/IGHJ2*01 IGHV1-46*01/ IGHJ4*02 IGHV4-34*01/ IGHJ2*01	31% 24% 12% 6% 0.0001%	IGHV3-15*01/IGHD6-19*01/IGHJ6*02 IGHV4-59*01/ IGHD3-10*01/IGHJH3*02 IGHV3-33*01/ IGHD6-6*01/IGHJ2*01 IGHV1-46*01/ IGHJ4*02 IGHV4-34*01/ IGHJ2*01	0.05% 0 0 0 0.12%	IGHV3-15*01/IGHD6-19*01/IGHJ6*02 IGHV4-59*01/ IGHD3-10*01/IGHJH3*02 IGHV3-33*01/ IGHD6-6*01/IGHJ2*01 IGHV1-46*01/ IGHJ4*02 IGHV4-34*01/ IGHJ2*01	41% 0 0 0 46%
<b>CNS011</b>	NA	IGHV4-34/ IGHD3-9*01 /IGHJ6*02 IGHV3-33*01/IGHD3-10*02/IGHJ3*02	0.54% 0.33%	IGHV4-34/ IGHD3-9*01 /IGHJ6*02 IGHV3-33*01/IGHD3-10*02/IGHJ3*02	54% 34%		
<b>CNS012</b>		IGHV1-2*04/ IGHD7-27*01/ IGHJ2*01 IGHV7-4-1*02/ IGHD5-12*01/ IGHJ6*02	32.4% 31.7%	IGHV1-2*04/ IGHD7-27*01/ IGHJ2*01 IGHV7-4-1*02/ IGHD5-12*01/ IGHJ6*02	0.58% 0.01%	IGHV1-2*04/ IGHD7-27*01/ IGHJ2*01 IGHV7-4-1*02/ IGHD5-12*01/ IGHJ6*02	54% 35.2%

NA = sample not available; <sup>†</sup>Patient CNS005: The dominant clone in the diagnostic BM IGHV1-8\*01/ IGHD2-2\*02/IGHJ6 \*03 (55%) is not detectable at relapse, but on further analysis this has been V-V replaced (Supplementary figure 2), where by IGHV1-8\*01 is replaced to creating the IGHV3-23/ IGHD2-2\*02/IGHJ6 \*03 clone at relapse (40% and 39% in BM and CNS respectively). The v-v replaced clone is detected at low level (0.005%) in the diagnostic BM, indicating the presence of clonal evolution at diagnosis.

IGHD7-27\*01

Clone 1 43% AAATGAACAGTCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCAAGAGCCCTGGGGACTACTACGGTATGGACGTCTGGGGCCAAG  
|||||  
Clone 2 14% AAATGAACAGTCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCAAGAGACTTCTACTACTACTACGGTATGGACGTCTGGGGCCAAG

IGHJ6\*02 ATTA4CTACTACTACTACGGTATGGACGTCTGGGGCCAAG

IGHV3-74\*01 CACGGCTGTGTATTACTGTGCAAGAGA

**Supplementary Figure 1. Analysis of clones in CSF sample CNS002.** The dominant clones in the CSF (clone 1 74\*01/IGHD7-27\*01/IGHJ6\*02 and clone 2 IGHV3-74\*01/IGHJ6\*02) share the same IGHV and IGHJ gene usage but the CDR3 regions are different and unrelated.

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AGATCTGAGGACACGGCCGTGTATTACTGTGCGAGAGG IGHV1-8*01
IGHV1-8*01/IGHD2-2*02/IGHJ6*03 AGATCTGAGGACACGGCCGTGTATTACTGTGCGAGAGCCACAGGAATATTGTTAGTAGTACCAGCTGCTATAGAGGGCTACTACTACTACTACATGGACGCTCTGGGGCAAAGGGACCACGGTCACC
IGHV3-23*01/IGHD2-2*02/IGHJ6*03 GAGGACACGGCCGTATATTACTGTGCTTAAAGACCCCTGATGGTGTTGTTAGTAGTACCAGCTGCTATAGAGGGCTACTACTACTACTACATGGACGCTCTGGGGCAAAGGGACCACGGTCACC
AGGATATTGTTAGTAGTACCAGCTGCTATACC IGHD2-2*02
ATTACTACTACTACTACTACATGGACGCTCTGGGGCAAAGGG IGHJ6*03
GAGGACACGGCCGTATATTACTGTGCGAAAAGA IGHV3-23*01

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**Supplementary Figure 2. V-V replacement in CNS005.** The dominant clone in the diagnostic BM IGHV1-8\*01/IGHD2-2\*02/IGHJ6\*03 55% is not detectable at relapse, but on further analysis this has been V-V replaced whereby IGHV1-8\*01 is replaced to create the IGHV3-23/IGHD2-2\*02/IGHJ6\*03 clone at relapse