

Clinical Genomics Laboratory
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Website: <http://www.cml.md/genomics/>
 Patient: XXXXXX

Patient Name: XXXXXXX
 DOB: XXXXXXX
 Gender: F
 Specimen Type: peripheral blood
 Submitters Name: XXXXXXX
 Submitters Institution: XXXXXXX

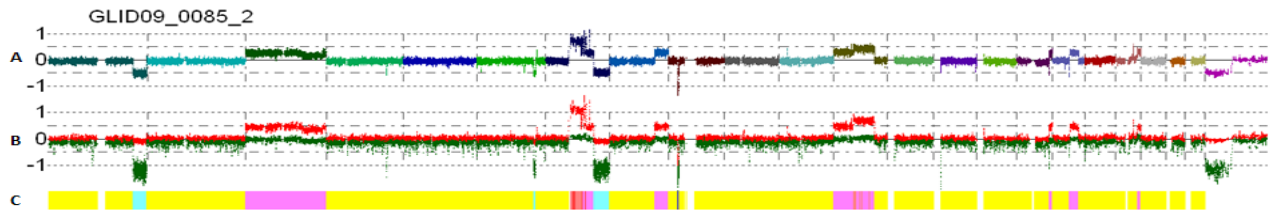
CML Accession Number: XXXXXXX
 Date specimen obtained: XXXXXXX
 Date specimen received: XXXXXXX
 Report date: XXXXXXX
 Test Indication: CLL

Result:

Peripheral blood, SNP oligonucleotide microarray karyotype:

- Partial trisomy 12** **arr snp 12(p13.33-q23.2)**
- Deletion of 6q** **arr snp 6(q24.1-q24.3)**
- No copy number changes or loss of heterozygosity is detected at 11q22, 13q14, or 17p.**
- Multiple other chromosomal abnormalities are detected (see interpretation)**

Interpretation: Partial trisomy 12 (intermediate risk) and an interstitial deletion of 6q (associated with higher stage) were detected in this sample. Deletions at the other chromosomal loci commonly included in CLL FISH panels (11q22, 13q14, and 17p) were not detected. The prognostic significance of the 6q deletion is unclear; however the deletion seen here does fall in the 6q minimally deleted region for CLL (6q21-q27).¹ The genome shows complex karyotypic abnormalities (see table and image below), including a homozygous deletion of the p16 tumor suppressor gene on chromosome 9p. In addition, some lesions were present only in a subset of cells/alleles (asterisked in table). This can be due to either the presence of subclones or a tetraploid genome. However, trisomy 12 in CLL is associated with aneuploidy,³ and the maintenance of heterozygous call bars in this sample (not shown) in regions of apparent deletion support the possibility of a tetraploid cancer genome. A cell-based assay would be necessary to determine this definitively.



Copy Number Color Code		
Deletions	Normal/diploid	Gains
Dark blue = 0	Yellow = 2	Pink = 3
Light blue = 1		Pink-Red = 4
		Red = amplified

Whole genome view SNP array karyogram for this sample. Chromosomes are plotted in numeric order from left (chromosome 1) to right (X chromosome). A) Log2ratio, zero = copy number of 2. B) Allele-specific analysis of copy number. C) The copy number Hidden Markov Model (HMM) is color-coded as indicated to the left.

The following is a summary of clinically relevant genetic lesions in chronic lymphocytic leukemia:

Trisomy 12 is a common clonal abnormality in B cell CLL occurring in 16% of cases, and it is associated with an intermediate prognosis.¹ Loss of the ATM tumor suppressor gene on the long arm of chromosome 11 has been reported in 18% of cases with B cell CLL and is associated with an adverse prognosis⁴. Loss of ATM is usually associated with extensive adenopathy and advanced disease⁴. The 13q14 deletion represents the most frequent chromosomal rearrangement in B cell CLL (55%), and when it is the sole abnormality it

confers a more favorable prognosis.⁴ Typically, it occurs in patients with highly stable and indolent disease often requiring no treatment.⁵ Homozygous loss of 13q14.3 (D13S319) may be associated with a more aggressive disease.⁶ Loss of the TP53 at 17p occurs in about 7% of CLL cases and it is the strongest predictor of poor survival⁷ and is associated with failure to respond to either alkylating agents⁸ or fludarabine.⁹ Deletions at 6q occur in 6% of patients.⁴ These patients tend to have higher white blood cell counts and more extensive lymphadenopathy at presentation¹⁰, but the prognostic significance of the 6q lesion itself is unclear.¹

Copy number polymorphisms, if present, are not reported here, but are archived in the CML Clinical Genomics Laboratory.

Copy Number HMM	Chromosome	StartSNP	StartPos	EndSNP	EndPos	Start Cytoband	End Cytoband	Size Mb
1	1	254531	210,884,098	258093	244,111,184	1q41	1q44	33.2271
3	3	280392	48,603	298771	199,282,215	3p26.3	3q29	199.2336
2*	3	293498	139,831,293	298771	199,282,215	3q22.3	3q29	59.4509
1	6	349032	140,627,416	349498	145,809,505	6q24.1	6q24.3	5.1821
5	7	358401	61,635,018	361300	100,754,972	7q11.21	7q22.1	39.1200
3	7	361289	100,572,745	362783	118,537,922	7q22.1	7q31.31	17.9652
>5	7	358401	61,635,018	358412	62,350,279	q11.21	q11.21	0.7153
>5	7	358659	67,400,602	35683	67,659,413	q11.22	q11.22	0.2588
>5	7	360970	94,961,325	361060	95,812,868	q21.3	q21.3	0.8515
>5	7	36201	108,646,310	362062	109,341,009	q31.1	q31.1	0.6947
1	7	362772	118,337,984	366013	157,385,079	7q31.31	7q36.3	39.0471
3	8	377485	112,327,156	380941	146,225,933	8q23.3	8q24.3	33.8988
1	9	384318	21,523,955	384865	26,129,486	9p21.3	9p21.2	4.6055
0	9	384335	21,657,825	384597	23,928,481	9p21.3	9p21.3	2.2707
3	12	420470	145,326	430683	100,590,405	12p13.33	12q23.2	100.4451
2*	12	420470	50,446	425382	49,084,602	12p13.33	12q13.13	49.0342
1	16	465824	79,717,993	465895	80,241,738	16q23.2	16q23.2	0.5237
3	16	465824	79,717,993	466866	88,666,241	16q23.2	16q24.3	8.9482
3	17	469268	41,719,833	470731	63,976,278	17q21.31	17q24.2	22.2564
4	19	481610	44,541,942	481655	45,715,441	19q13.2	19q13.2	1.1735
3	19	482035	55,514,220	482562	63,700,378	19q13.33	19q13.43	8.1862
1	X	494859	159,978	497307	65,735,314	Xp22.33	Xq12	65.5753
3	X	497307	65,735,314	500566	154,353,200	Xq12	Xq28	88.6179

Table 1 Genomic position and cytoband breakpoints for GLID09_0085. Asterisks indicate abnormalities that were present in only a subset of alleles/cells.

Methods: DNA was extracted from EDTA anti-coagulated peripheral blood sample following a density-based enrichment for B lymphocytes. Whole genome comparative genomic hybridization was done using Affymetrix 250K Nsp SNP array which can detect uniparental disomy and copy number changes as small as 500kb. The assay was performed according to the manufacturer's protocol. All controls performed as expected. Analysis was performed using Affymetrix GTYPE 2.1 and CNAGv3.0 software programs. The normal reference DNA used for analysis was chosen by the CNAG software from a library of data files obtained from normal specimens.

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References:

1. Cotter FE, Auer RL. Genetic alteration associated with chronic lymphocytic leukemia. *Cytogenet Genome Res.* 2007;118(2-4):310-319.
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This SNP based oligonucleotide microarray was developed by Affymetrix, Inc (Santa Clara, CA, USA) and its performance determined by the Genomics Laboratory of Creighton Medical Laboratories for the sole purpose of identifying the gain or loss of DNA copy numbers and regions of loss of heterozygosity. This microarray will not detect balanced chromosomal aberrations, such as Robertsonian translocation, reciprocal translocations, inversion or balanced insertions, nor imbalances in regions that are not represented on the microarray, nor low-level mosaicism or tumor burden. This method cannot detect epigenetic events, such as aberrant methylation, or point mutations. The method is based on relative copy number estimates; therefore polyploidy cannot be reliably detected without a cell-based assay. Clinical implications of chromosomal aberrations may be unknown at the time of analysis. This test is used for clinical purposes. It has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. Pursuant to the requirement of CLIA'88, this laboratory has established and verified the test's accuracy and precision.