

Clinical Genomics Laboratory
Attn: Jill M. Hagenkord, MD
723 N. 18th Street
Beirne Tower Rm 410
Omaha, NE 68178
Phone: 402.280.4382
Fax: 402.280.1855



Medical Director: Jill M. Hagenkord, MD
email: jillhagenkord@creighton.edu
Laboratory Phone: 402.280.3963
Website: <http://www.cml.md/genomics/>
Patient: XXXXXXXXX

Test: SNP oligonucleotide microarray karyotype

Patient Name: XXXXXX
Gender: Male
Date of Birth: XXXXX
Specimen Type: Frozen (OCT)
Submitters Name: XXXXXX
Submitters Institution: XXXXX

CML Accession Number: CML09-GDXXXXX
Date specimen obtained: XXXXXX
Date specimen received: XXXXXX
Report date: XXXXXX
Test Indication: Glial neoplasm

Result:

Tumor, brain, SNP oligonucleotide microarray karyotype (CML09-VSXXXX, frozen tissue):

- Gain of chromosome 7 is detected.**
- Homozygous deletion of p16 on 9p is detected.**
- Deletion of chromosome 10 (including PTEN) is detected.**
- 1p/19q co-deletion is not detected.**
- Amplification of EGFR is not detected.**
- Amplification of MDM2 is not detected.**
- 17p LOH (TP53) is not detected.**

The genomic profile of this sample suggests that it is a primary glioblastoma (WHO grade IV) originating from an astrocytic precursor.

Interpretation: This tumor shows gain of chromosome 7, which is the most frequent numerical aberration observed in both diffuse astrocytomas (WHO grade II) and anaplastic astrocytomas (WHO grade III).¹

This tumor also shows loss of 9p and chromosome 10. The p16 tumor suppressor gene on 9p is homozygously deleted. Homozygous loss of p16 occurs in 31% of glioblastoma.² Loss of chromosome 10 occurs in 60-80% of glioblastoma.³ Loss of the PTEN tumor suppressor gene on 10q23 and loss of p16 occur almost exclusively in glioblastoma and can provide means to distinguish anaplastic astrocytoma from glioblastoma.^{2,3} Loss of both 9p and 10 are detected in this sample, suggesting that this tumor is a glioblastoma.

Loss of all of chromosome 10, rather than just 10q, suggests that this is a primary glioblastoma.³

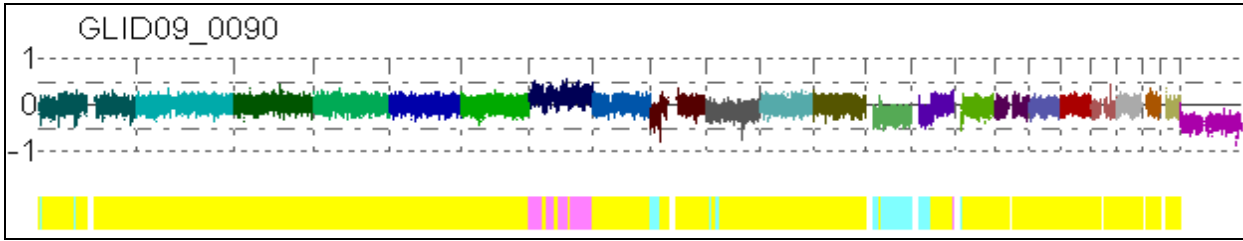
This sample does not have the 1p/19q co-deletion seen in most oligodendrogliomas. This sample does not have other key genetic abnormalities sometimes seen in glial tumors, such as LOH of 17p LOH (TP53) or amplifications of EGFR or MDM2. Point mutations in EGFR, or other genes, cannot be detected by virtual karyotyping.

Additional chromosomal abnormalities in this tumor include loss of one copy of the CDKN2C gene on 1p, loss of 13q (including RB1), and partial loss of 14q (14q11.2-q21.3). Copy number variants or other genetic variants of uncertain significance are not reported, but are archived at Creighton Medical Laboratories.

Clinical Genomics Laboratory
Attn: Jill M. Hagenkord, MD
 723 N. 18th Street
 Beirne Tower Rm 410
 Omaha, NE 68178
 Phone: 402.280.4382
 Fax: 402.280.1855

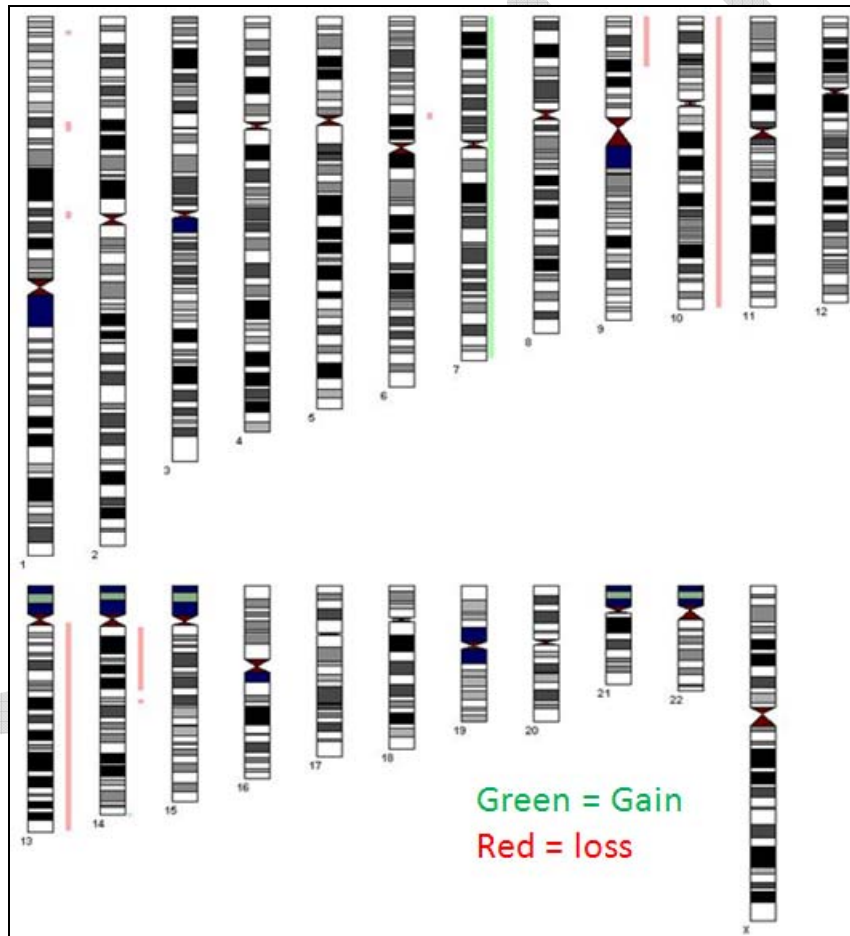


Medical Director: Jill M. Hagenkord, MD
 email: jillhagenkord@creighton.edu
 Laboratory Phone: 402.280.3963
 Website: <http://www.cml.md/genomics/>
 Patient: XXXXXXXXX

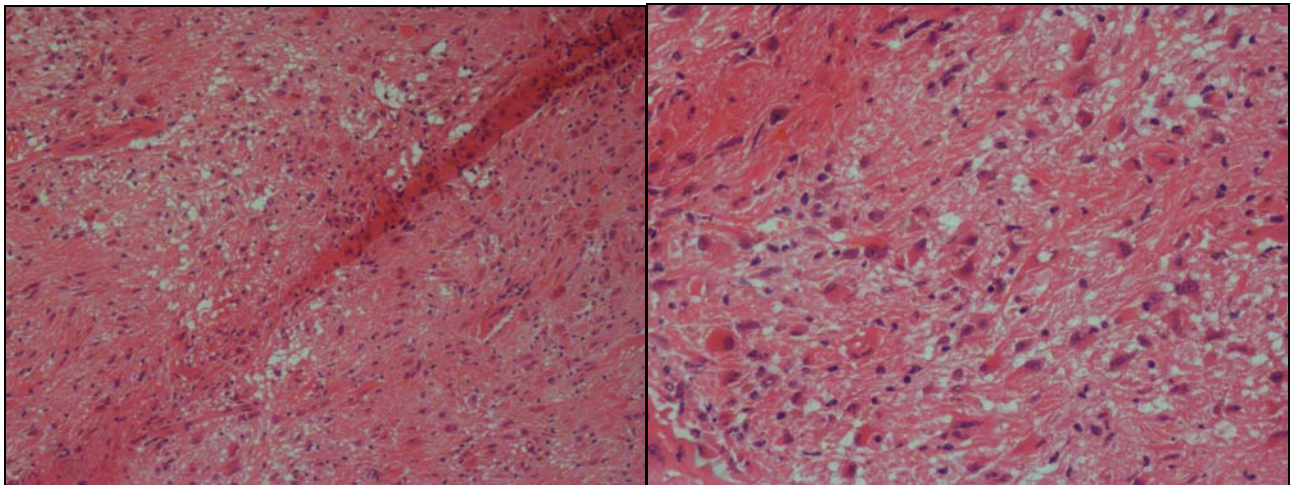


Copy Number		Color Code
Deletions		
Dark blue = 0	Yellow = 2	Pink = 3
Light blue = 1		Pink-Red = 4
		Red = amplified

Virtual Karyotype of CML09-GDXXXXXX, whole genome view.
 Chromosomes are color-coded and plotted in order with chr 1 on the left and X on the right. The log2ratio is shown as a smoothed average over 10 SNPs. Hidden Markov Model (HMM) for copy number is coded as indicated at left.



Virtual karyogram of CML09-GDXXXXXX



H&E photomicrographs of CML09-GDXXXX at 10x (right) and 20x (left).

Chromosome	Left boundary	Right boundary	Width (Mb)	Aberration	Copy Number	ISCN record
1	6.832780838	7.455851078	0.6231	Loss	1	arr cgh 1(p36.31p36.23)x1
1	48.52213287	53.27660751	4.7545	Loss	1	arr cgh 1(p33p32.3)x1
1	89.68990326	93.50794983	3.8180	Loss	1	arr cgh 1(p22.2p22.1)x1
6	44.60948563	47.96319199	3.3537	Loss	1	arr cgh 6(p21.1p12.3)x1
7	0.141322002	158.6050568	158.4637	Gain	3	arr cgh 7(p22.3q36.3)x3
9	21.523955	22.158464	0.6345	Loss	0	arr cgh 9(p21.3)x0
9	0.102326572	23.76858902	23.6663	Loss	1	arr cgh 9(p24.3p21.3)x1
10*	0.098685056	135.5008087	135.4021	Loss	1	arr cgh 10(p15.3q26.3)x1
13	17.63258743	114.3269272	96.6943	Loss	1	arr cgh 13(q11q34)x1
14	19.49575233	49.51488113	30.0191	Loss	1	arr cgh 14(q11.2q22.1)x1
14	52.99915314	53.7518959	0.7527	Loss	1	arr cgh 14(q22.2)x1

Genomic position break points and ISCN nomenclature for CML09-GDXXXX. Asterisk indicates that only a subset of cells (or alleles, if tumor is polyploidy) has the abnormality.

Summary of Genetic Lesions in Glial Tumors

In astrocytoma grade I, a normal karyotype is observed most frequently.³ Among the cases with abnormal karyotypes, chromosomal gains are seen most frequently in chromosomes 5 and 7.³ Gain of chromosomes 7q and 8q are the most frequent numerical aberration observed in diffuse astrocytomas (WHO grade II).³ Deletions of LOH of 22q (17%) and 6 (14%) are also seen in diffuse astrocytomas.³ The TP53 tumor suppressor gene is located at 17p13.1. TP53 mutations and/or 17p LOH are common in grade II and III astrocytomas.^{2,3}

In addition to loss of TP53 function, loss of 10q occurs in 35-60% of anaplastic astrocytomas (WHO grade III). PTEN is a tumor suppressor gene located at 10q23.31. Loss of PTEN is prognostically unfavorable.² LOH 22q in anaplastic astrocytomas occurs at a frequency similar to that of low-grade astrocytomas (25%), while LOH 19q is significantly more frequent (46%) in anaplastic astrocytomas.³ LOH 6q occurs in approximately 33% of anaplastic astrocytomas. EGFR amplification is very uncommon in anaplastic astrocytoma (<10%), and may be associated with significantly shorter survival.³

Molecular abnormalities in glioblastoma (WHO grade IV) are numerous and include deletions/mutations of p16 (9p) and PTEN (10q), LOH of 17p (p53), mutations in p53, and amplification of EGFR and MDM2.²

Clinical Genomics Laboratory
Attn: Jill M. Hagenkord, MD
723 N. 18th Street
Beirne Tower Rm 410
Omaha, NE 68178
Phone: 402.280.4382
Fax: 402.280.1855



Medical Director: Jill M. Hagenkord, MD
email: jillhagenkord@creighton.edu
Laboratory Phone: 402.280.3963
Website: <http://www.cml.md/genomics/>
Patient: XXXXXXXXXX

Amplification of EGFR, loss of PTEN, and loss of p16 occur almost exclusively in glioblastoma and can provide means to distinguish anaplastic astrocytoma from glioblastoma.^{2,3}

Amplification of EGFR indicates that the tumor may be responsive to EGFR inhibitors.^{2,3} EGFR is the most commonly amplified gene in glioblastoma.³ Studies correlating EGFR amplification with outcome in glioblastoma have been contradictory. In anaplastic astrocytoma (WHO grade III), EGFR amplification has been associated with shorter survival.³

TP53 mutations deregulate control of cell growth and are they are the genetic hallmark of secondary glioblastoma. An alternate mechanism of TP53 deregulation is amplification of the MDM2 gene. MDM2 amplification is observed in 10% of glioblastoma without p53 mutations.³ Point mutations in TP53, or other genes, cannot be assessed by virtual karyotyping.

Mutations TP53 or LOH of 17p are associated with a astrocytoma phenotype while co-deletions of 1p/19q are associated with an oligodendroglioma phenotype.⁴ EGFR amplification is rare in oligodendrogliomas.³

Up to 90% of WHO grade II oligodendrogliomas have LOH 1p and 19q.⁵ Tumors with the 1p/19q co-deletion tend to have classic morphology. Most oligodendrogliomas show losses of one entire copy of 1p and 19q while partial deletions are rare.³ Oligodendrogliomas without losses of 1p and 19q frequently have more astrocytic features.⁴ The rate of combined 1p/19q co-deletion in grade III oligodendrogliomas is 50-70%.⁴ The 1p /19q co-deletion is predictive of radio-chemosensitivity in grade III oligodendroglioma tumors and mixed oligoastrocytomas.¹

Several genetic aberrations in oligodendroglioma are associated with decreased progression-free survival and inversely associated with 1p/19q co-deletions, including TP53 mutations or 17p LOH, loss of chromosome 10, EGFR amplifications, and homozygous deletion of the p16 tumor suppressor gene on 9p21.⁴ Several chromosomal aberrations other than 1p/19q deletion are found at more than random frequency in oligodendroglioma, most frequently gains on chromosome 7 and losses on chromosomes 4, 6, 11p, 14, and 22q.³

Methods: DNA was extracted from frozen, OCT-embedded tumor following pathologist review of the frozen section slide. 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 in when performed on fresh DNA. The assay was performed according to the manufacturer's protocol. Analysis was performed using Affymetrix™ GTYPE 2.0.1 and CNAGv3.0⁶ software programs. OneClickCGH was used to generate the karyogram and table of genomic position breakpoints (InfoQuant, LTD, London UK). The normal reference DNA used for analysis was chosen by the CNAG software from a library of data files obtained from normal specimens.

References:

1. Magnani I. Nervous System: Glioma: an overview 2008. *Atlas Genet Cytogenet Oncol Haematol*. <http://AtlasGeneticsOncology.org/Genes/GliomaOverviewID5763.html>.
2. *Tumors of the Central Nervous System*. Vol 7. Washington DC: American Registry of Pathology; 2007.
3. *WHO Classification of Tumours of the Central Nervous System*. 4th ed. Lyon: IARC; 2007.

Clinical Genomics Laboratory

Attn: Jill M. Hagenkord, MD

723 N. 18th Street
Beirne Tower Rm 410
Omaha, NE 68178
Phone: 402.280.4382
Fax: 402.280.1855



Medical Director: Jill M. Hagenkord, MD

email: jillhagenkord@creighton.edu

Laboratory Phone: 402.280.3963

Website: <http://www.cml.md/genomics/>

Patient: XXXXXXXXX

4. Hartmann C, von Deimling A. Molecular pathology of oligodendroglial tumors. *Recent Results Cancer Res.* 2009;171:25-49.
5. Hartmann C, Mueller W, von Deimling A. Pathology and molecular genetics of oligodendroglial tumors. *Journal of Molecular Medicine.* 2004;82(10):638-655.
6. Yamamoto G, Nannya Y, Kato M, et al. Highly sensitive method for genomewide detection of allelic composition in nonpaired, primary tumor specimens by use of affymetrix single-nucleotide-polymorphism genotyping microarrays. *Am J Hum Genet.* Jul 2007;81(1):114-126.

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.

SAMPLE