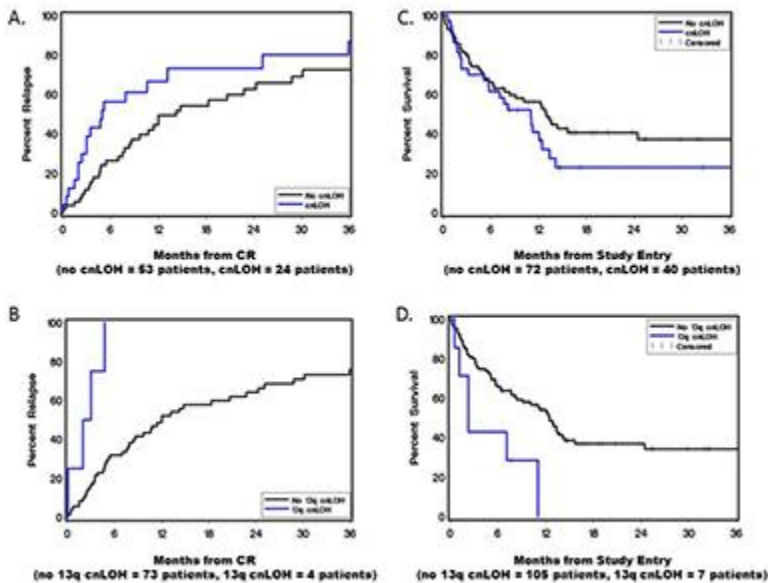


CGAT: a Vital Instrument in the Prognostic Toolbox for AML

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Kaplan-Meier curves showing the prognostic impact of cnLOH. Blue lines in A (% relapse after CR) and C (% survival) represent cnLOH; black lines represent no cnLOH. In B (% relapse after CR) and D (% survival), blue and black lines represent 13q cnLOH and no 13q cnLOH, respectively.

Image provided by Christine Gronseth

Normally, human cells contain two copies of each chromosome; one each from the mother and the father. In some cases, whole chromosomes or segments of chromosomes may appear to be from only one parent; although the copy numbers remain normal, the genetic variability is lost. This is called copy-neutral loss of heterozygosity (cnLOH), and can be identified using chromosome genomic array testing (CGAT). Detection of chromosomal DNA irregularities using genomic microarray techniques is generally important for diagnosis and prognosis of patients with acute myeloid leukemia (AML), but the World Health Organization does not currently include cnLOH among its criteria for classifying tumors of hematopoietic and lymphoid tissue. To elucidate its prognostic significance, Dr. Min Fang from Fred Hutch's Clinical Transplant Research Program (Clinical Research Division) initiated a study to correlate acquired clonal cnLOH in AML patients - which relatively often display the abnormality - with various covariates, mutations, risk classification, and outcome after treatment. The results were recently published in *Cancer*, with cytogenetics technologist Christine Gronseth at the Seattle Cancer Care Alliance (SCCA) as first author.

A total of 112 AML patients who underwent chromosome genomic array testing (CGAT) at the SCCA were included in the study. DNA was taken from blood or bone marrow samples and analyzed using a high-density microarray, including detection of single-nucleotide polymorphisms. Of the 112 patients, 40 had cnLOH; the remaining 72 comprised the control group. In the cnLOH cohort, 33 of 40 had cnLOH involving a single chromosome arm; cnLOH involving 2, 3, and 4 chromosome arms were found in 5, 1, and 1 patient(s), respectively. The most common sites of cnLOH were at 11p and 13q in this patient population.

Overall, cnLOH did not significantly impact the clinical remission rate (60 % in patients with cnLOH, and 74 % in patients without; $p = 0.14$). On the other hand, presence of the abnormality was clearly correlated with disease recurrence (1.87-fold higher in patients with cnLOH; $p = 0.02$), with a striking 6.64-fold higher relapse rate in patients with cnLOH in chromosome 13q ($p = 0.002$), also after adjustment for age, cytogenetics, and secondary versus *de novo* (without association to previous therapy) AML. The risk of death was consequently increased by a factor of 3.45 ($p = 0.05$) in this population. Further, the CGAT findings were correlated with results from molecular testing for gene mutations. Fms-related tyrosine kinase 3-internal tandem duplication (FLT3-ITD) mutation data was available for 78 of the 112 patients, and the mutation was clearly associated with cnLOH in 13q. Six patients with 13q cnLOH were tested for FLT3; all of them were positive for FLT3-ITD. Patients with FLT3-ITD mutation with 13q cnLOH had shorter duration of remission and shorter overall survival than patients with the FLT3 mutation without 13q cnLOH ($p < 0.01$).

The study is the first to demonstrate an association between a complex CGAT result (at least three CGAT abnormalities) and poor outcome, analogous to that of a complex karyotype (at least three clonal abnormalities) as determined using conventional cytogenetics. Importantly, 13 % of the patients had abnormalities detected by CGAT that would not have been found using standard techniques only. "CnLOH cannot be detected by conventional methods," Dr. Fang said. "Some of our providers have not yet fully embraced the usefulness of CGAT and are unsure about whether CGAT-identified abnormalities are equally significant as those detected by conventional cytogenetics and FISH," she continued, emphasizing her hope that this paper will highlight these concerns.

According to the authors, CGAT may be most useful for AML patients with an intermediate cytogenetic risk, from the perspective of clinical practice. The risk is enriched in patients with normal cytogenetics but abnormal CGAT - a patient population with particularly uncertain prognosis, for which the benefits of allogeneic hematopoietic cell transplantation are difficult to predict, in relation to the treatment-associated risks. Identifying prognostic factors for this patient population is one of the future goals for the investigators, Christine Gronseth explained.

Thus, the investigators found their answers: cnLOH has, indeed, prognostic significance in AML patients, and CGAT is useful for improving diagnosis and care. "Establishing the clinical utility of acquired clonal cnLOH in AML patients is an important key to personalized medicine," Gronseth said. "The detection of cnLOH is helpful not only to establish the patients' prognosis, but also to follow the disease status and progression," she added.

Patients with cnLOH were few in the current study, and the prognostic effect of aberrations in individual chromosomes was therefore not easily quantifiable. The researchers are keen on continuing the investigations, and Gronseth staked out the future direction: "We have already begun analysis of cnLOH on specific chromosome arms and hope to conduct a multi-institutional collaboration to increase the study size."

[Gronseth CM, McElhone SE, Storer BE, Kroeger KA, Sandhu V, Fero ML, Appelbaum FR, Estey EH, Fang M.](#) 2015. Prognostic significance of acquired copy-neutral loss of heterozygosity in acute myeloid leukemia. *Cancer*. DOI: 10.1002/cncr.29475. [Epub ahead of print]