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ORIGINAL ARTICLE

# A multicenter, cross-platform clinical validation study of cancer cytogenomic arrays

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Cytogenomic microarray analysis (CMA) offers high resolution, genome-wide copy number information and is widely used in clinical laboratories for diagnosis of constitutional abnormalities. The Cancer Genomics Consortium (CGC) conducted a multiplatform, multicenter clinical validation project to compare the reliability and inter- and intralaboratory reproducibility of this technology for clinical oncology applications. Four specimen types were processed on three different microarray platforms—from Affymetrix, Agilent, and Illumina. Each microarray platform was employed at two independent test sites. The results were compared in a blinded manner with current standard methods, including karyotype, FISH, or morphology. Twenty-nine chronic lymphocytic leukemia blood, 34 myelodysplastic syndrome bone marrow, and 30 fresh frozen renal epithelial tumor samples were assessed by all six laboratories. Thirty formalin fixed paraffin embedded renal tumor samples were analyzed at the Affymetrix and Agilent test sites only. All study samples were initial diagnostic samples. Array data were analyzed at each participating site and were submitted to caArray for central analysis. Laboratory interpretive results were submitted to the central analysis team for comparison with the standard-of-care assays and for calculation of intraplatform reproducibility and cross-platform concordance. The results demonstrated that the three microarray platforms 1) detect clinically actionable genomic changes in cancer compatible to standard-of-care methods; 2) further define cytogenetic aberrations; 3) identify submicroscopic alterations and loss of heterozygosity (LOH); and 4) yield consistent results within and between laboratories. Based on this study, the CGC concludes that CMA is a sensitive and reliable technique for copy number and

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LOH assessment that may be used for clinical oncology genomic analysis.

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Genomic copy number alterations are important biological markers for cancer diagnosis, prognosis, and therapeutic decision-making (1,2). Chromosomal copy number changes have been incorporated into oncology management guidelines for hematologic malignancies such as chronic lymphocytic leukemia (CLL), myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) (3–5), and can be used to distinguish solid tumor subtypes as in renal cell carcinoma (6). Historically, metaphase chromosome analysis and fluorescence in situ hybridization (FISH) have been used in clinical laboratories to detect chromosome copy number changes. However, the requirement for dividing cells, the potential for preferential growth of normal cells compared with that of tumor cells, and the low resolution of metaphase chromosome analysis limit the clinical utility of this approach (7). Likewise, the targeted nature of FISH assays can result in underestimation of the degree of genomic aberrations, since genetic alterations that are not targeted by specific FISH assays will not be detected. In the past few years, cytogenomic microarray analysis (CMA) has emerged as a reliable tool to evaluate chromosome copy number alterations. The clinical utility for detection of constitutional chromosomal aberrations has made the technology a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies (8). CMA offers high resolution, genome-wide chromosome copy number information independent of cell division, and in the case of arrays with single nucleotide polymorphism (SNP) detection capabilities, can also provide genotype and loss of heterozygosity (LOH) status. For these reasons, there is increasing interest in using CMA for clinical oncology applications.

The Cancer Genomics Consortium (CGC; http://www.cancergenomics.org/) is a group of clinical cytogeneticists, molecular geneticists, and molecular pathologists who are interested in applying microarray technologies to cancer diagnosis and cancer research. One of the missions of the consortium is to perform multicenter cancer genome translational research, with the goal of enabling the use of genomic tools to better serve cancer patients and further cancer research. The Cancer Cytogenomic Microarray Quality Control (CCM-QC) study was a multiplatform, multicenter clinical validation project designed to assess the efficacy and reproducibility of this microarray technology for clinical oncology applications. This study evaluated DNA extracted from four specimen types commonly analyzed for specific genetic aberrations in the clinical laboratory: peripheral blood from CLL patients, bone marrow aspirates from MDS patients, and frozen and formalin-fixed paraffin-embedded (FFPE) tumor tissues from renal epithelial tumor (RET) patients. Chromosome copy number analyses were performed with cytogenomic microarrays from three different manufacturers to test the hypothesis that CMA can reliably detect clinically important genomic aberrations in cancer. The specific aims were (1) to assess the concordance of cytogenomic microarray results with established standards; (2) to evaluate the reproducibility of the analysis within and across laboratories; and (3) to verify that the same genomic DNA processed on different microarray platforms would yield the same clinical results. Our findings demonstrated that cytogenomic microarray analysis is a sensitive and reliable technique for copy number assessment that can be used for genomic analysis in the clinical oncology setting.

## Materials and methods

### Participating laboratories

Six laboratories participated in the study through an open invitation to CGC member laboratories. The participating laboratories were selected by the CCM-QC committee based on their reported experience using cytogenomic microarrays, experience with clinical cytogenetics/cytogenomics, publication record, and vendor recommendations. Two laboratories were selected for each of the three platforms used in the study: The Children's Hospital of Philadelphia (CHOP) and Medical University of South Carolina (MUSC) as Illumina sites, Baylor College of Medicine (BCM) and the University of Medicine and Dentistry of New Jersey (UMDNJ) as Agilent sites, and Creighton University Medical Center (CUMC-a) and Columbia University Medical Center (CUMC-b) as Affymetrix sites (Figure 1A). The study was approved by all participating institutions' internal review boards. The reagents used for the study were provided by the vendors.

### Specimens

For 29 CLL and 34 MDS specimens, a minimum of 10 μg DNA was obtained from discarded specimens donated by GenPath, BioReference Laboratories, and the University of Pittsburgh Cytogenetics Laboratory. DNA samples were prepared from the original sample by the contributing laboratories and sent to the University of Massachusetts Memorial Cytogenetics Laboratory (central lab 1), where they were reassessed with a NanoDrop for optical density (OD) 260 nm/280 nm and 260 nm/230 nm ratios before they were coded and distributed to test sites. The clinical cytogenetic data accompanying the specimens included pathology reports confirming disease diagnosis (for all but six cases), the reported karyotype and FISH findings, as well as a karyogram and FISH images. The laboratory cut-offs for all FISH assays were also submitted. Submitting laboratories used the same commercial probes in the FISH assays. FISH assay cutoffs, FISH images and karyograms were reviewed by two experienced American Board of Medical Genetics and Genomics–certified cytogeneticists (J.L. and P.M.M.). All chromosome abnormalities were represented by at least one karyogram; all FISH abnormalities were represented by at least two nuclei images. Detailed specimen information for the CLL and MDS cases is summarized in Supplemental Tables S1 and 2.

For frozen and FFPE renal tumor samples, 30 cases representing the most frequent renal epithelial tumor subtypes