CytoScan HD Suite: a comprehensive solution for analyzing karyotypes in hematological malignancies

Highlights

- Molecular karyotyping is becoming the standard for detecting and analyzing chromosomal aberrations in leukemias
- Applied Biosystems™ CytoScan™ HD Suite, including Chromosome Analysis Suite software, facilitates analysis of molecular karyotypes
- CytoScan HD Suite has successfully detected chromosomal anomalies missed by conventional karyotyping techniques

Introduction

Complex karyotypes, which can include combinations of chromosomal deletions, duplications, translocations, and inversions, are thought to be involved in the early steps leading to a number of leukemias and solid tumors. These alterations are typically associated with a poor prognostic outcome [1-5], but the mechanisms underlying mitotic errors that lead to structural chromosome alterations are still not well understood. Nevertheless, analysis of chromosomal aberrations plays an important role in the management of hematological cancers.

These chromosomal aberrations fall into a number of general categories. Deletions and duplications are loss or gain, respectively, of chromosomal segments. These can be as small as a few nucleotides up to an entire chromosome. Balanced and unbalanced translocations are exchanges of chromosomal segments. Inversions involve the reversal of the order of genes on a chromosomal arm. In each of these cases, the breakpoints of the rearrangement can produce loss-of-function alterations or novel fusion transcripts that may be oncogenic. In addition, copy-neutral loss of heterozygosity (cnLOH) can occur when one allele or linkage group in a heterozygous individual “converts” to the other allele, making the locus homozygous. If this happens in a cell with a defective tumor suppressor gene, it may contribute to a leukemia. Many of these aberrations are found in well-defined chromosomal locations. Therefore, karyotyping is instrumental in clearly identifying biomarkers that drive the evolution of the hematological malignancies associated with response or resistance to therapy, or that are known to have prognostic value and distinguish between aggressive and nonaggressive disease states.

Methods for analyzing chromosomal aberrations

In general, there are two strategies for generating a karyotype. The first strategy involves directly visualizing the chromosomes in cells, which can be accomplished by two main methods. One is by staining mitotic cells with Giemsa or other dyes that stain chromosomal segments, producing a characteristic banding pattern (G-banding). Disruption or rearrangement of the banding pattern can indicate that a large aberration (more than 10 Mb) is present in the cell. Although commonly used, G-banding requires that cells be cultured prior to staining and be stained in a specific mitotic phase. It also requires a specialized understanding of banding patterns and can only detect larger aberrations. The other direct visualization method involves fluorescence *in situ* hybridization (FISH) to specific genomic sequences. Here, whole nuclei are stained and the specific sequences probed can be quantified by counting spots lit up by the probes. Although this can give a measure of the number of copies of a locus, it is limited to a small number of known aberrations for which site-specific probes are available, and requires stained cells to have good nuclear morphology.

The second strategy for generating a karyotype uses molecular biology techniques to analyze chromosomal sequences as well as microscopic and submicroscopic changes. This has the distinct advantage that preservation of cellular morphology is not required; rather, purified genomic DNA is used for the analysis. One of the molecular karyotyping techniques makes use of next-generation sequencing (NGS). Here, representative amplicons or whole exomes are sequenced and analyzed. While these methods excel at detecting SNPs or novel sequences generated by breakpoint fusions of exons, they can miss significant regions of non-exonic DNA. In addition, the cost per sample and data analysis requirements can be relatively high, especially when looking at whole-genome copy number aberrations. While targeted NGS can identify known fusion genes and somatic mutations, whole-genome copy number and cnLOH analyses are not available in existing targeted NGS panels.

Another common molecular method is to generate karyotypes using high-density DNA microarrays. For these analyses, millions of probes tiled across the genome are arrayed onto a single chip. Hybridization to these arrays can determine which sequences are present and in how many copies. If the appropriate probes are present, they can also detect common SNP variants. The resolution of the copy number sequences is determined by the number of probes available; when probes are directly designed for predefined common deletions or duplications, these can in some cases detect events as small as 1,000 nucleotides.

The College of American Pathologists (CAP) and the American Society of Hematology (ASH) recently developed evidence-based guidelines emphasizing that molecular genetic tests should be used to complement conventional karyotyping approaches in clinical practices [6]. Molecular tests facilitate leukemia management because they help make more accurate diagnoses, aid in the evaluation of risk stratification, assist with identifying target-specific treatments, and help monitor recurrence [7-12]. Therefore, molecular testing complements traditional cytogenetic techniques. However, their ease of use, sequence precision, and relative cost suggest that molecular karyotyping analyses by high-resolution microarray may become the standard in the near future.

Uses of CytoScan HD Suite for karyotyping leukemia samples

CytoScan HD Suite—comprising microarrays, reagents, and analysis software—is a comprehensive, high-resolution, whole-genome solution designed to assist in the understanding and characterization of biomarkers in hematological malignancies (Figure 1). The CytoScan HD assay interrogates all relevant copy number alterations (CNAs) associated with lymphoid and myeloid disorders, using a single microarray-based assay. The assay covers all the major lymphoid disorders associated with acute lymphocytic leukemia (ALL) and chronic lymphocytic leukemia (CLL), as well as myeloid disorders associated with acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), chronic myeloid leukemia (CML), and multiple myeloma (MM). In addition to superior performance, CytoScan HD Suite does not require additional cell culture or cell arrest prior to kayotyping (Figure 2).

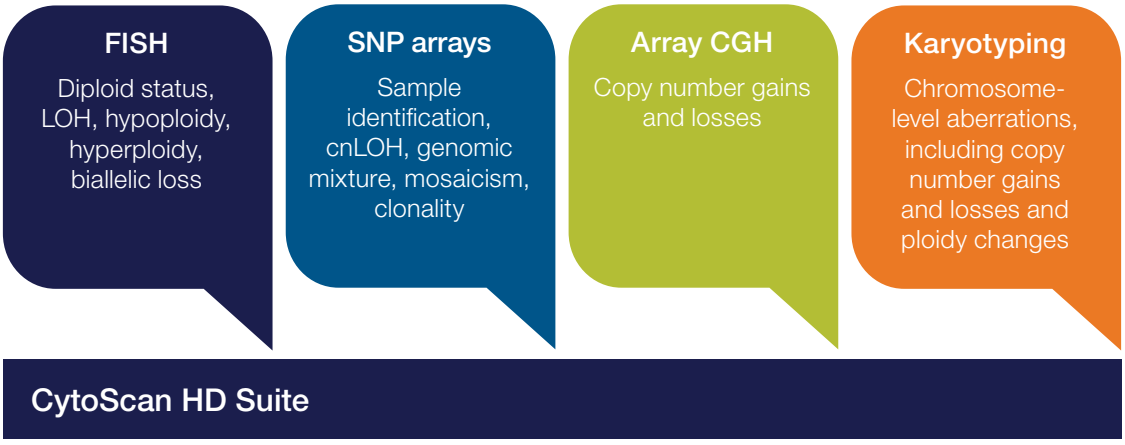

Figure 1. CytoScan HD Suite provides data otherwise only obtained from four different technologies.

- **Sample types**—blood or bone marrow
- **Sample input**—200 ng DNA
- **Sample preparation**—automated on the NIMBUS Target Preparation Instrument, or manual preparation

- **High throughput**—whole-genome copy number analysis on the Applied Biosystems™ GeneChip™ Scanner 3000 7G System

- **Data analysis**—identification of amplifications or deletions, LOH, cnLOH, ploidy, and breakpoint determination
- **Automated reporting**—Oncomine Knowledgebase Reporter for myeloid cancer

Figure 2. The CytoScan HD Suite workflow provides streamlined detection and analysis of copy number changes in lymphoid and myeloid malignancies in as little as 3 days.

Examples of CytoScan HD Suite in leukemia research

CytoScan HD Suite can detect aberrations missed by conventional karyotyping

Molecular karyotyping with microarrays has been useful for complementing and confirming results obtained by traditional karyotyping. Quite frequently, molecular karyotyping using high-resolution microarrays can detect many aberrations that are missed by conventional karyotyping methods, such as submicroscopic copy number changes and cnLOH. For example, a recent study examining anomalies in MDS found that 56% of samples had abnormal cytogenetic profiles, 38% appeared normal, and 6% were unsuccessfully analyzed. Analyzing the same samples using CytoScan HD Suite found that 73% had an abnormal karyotype [13]. These results are typical—the finer resolution of microarrays can detect smaller rearrangements than can traditional cytogenetic methods. This has led to several recommendations and guidelines among leukemia researchers for using microarrays for understanding the underlying chromosomal aberrations [12,14,15]. CytoScan HD Suite can therefore be an integral part in detecting relevant aberrations that are missed by traditional karyotyping of hematological malignancies.

The high probe density and higher resolution of microarrays can sometimes lead to new insights that would be missed by conventional karyotyping. One study detected a microdeletion, missed by conventional karyotyping but mapped using CytoScan HD Suite, in an individual with a type of anemia. This suggested to the authors that several candidate genes may be causative or contribute to the syndrome [16]. Similarly, a different study of MDS samples using CytoScan HD Suite found a microdeletion covering several tumor suppressor genes. Again, the conventional karyotype was normal and this microdeletion would have been missed without performing a molecular karyotype [17]. Using microarrays for molecular karyotyping can therefore reveal small deletions not detectable by FISH or G-banding, and generate new hypotheses on the cause of underlying pathologies.

CytoScan HD Suite offers advantages over conventional FISH

Another advantage of CytoScan HD Suite is that it can replace a panel of multiple FISH probes, eliminating the need to perform multiple hybridizations to query different loci. To analyze the efficacy of different techniques, Stevens-Kroef et al. [18] examined samples from 28 CLL individuals by FISH, multiplex ligation–dependent probe amplification (MLPA™) assay, and microarray. To evaluate the samples by FISH, they used four different sets of probes on over 100 interphase nuclei for each sample. In contrast, the microarray analyses were performed from a single genomic DNA preparation per sample. The microarray analyses therefore conserved material and effort. Furthermore, the results from the microarrays indicated that 57% of the samples carried additional anomalies and SNPs that were not detected by FISH or MLPA assay.

Chromothripsis is a catastrophic chromosomal rearrangement event that arises from breakage of chromosomes at multiple sites and subsequent random rejoining and repair. Chromothripsis has been associated with multiple highly aggressive cancers. Chromothripsis is typically detected by FISH, but molecular methods have started to become more widely accepted. In an effort to evaluate molecular karyotyping for detection of chromothripsis in AML, Fontana et al. [19] analyzed 395 samples using microarrays. The authors were able to identify several hallmarks of chromothripsis, including higher copy number alterations, breaks in regions that were more frequent in the samples, and mutations in the *TP53* gene. They therefore concluded that chromothripsis frequently occurs in AML, and analysis of chromothripsis in these samples can influence the prognosis and understanding of the biology of the disease.

Inclusion of SNPs on the CytoScan HD array facilitates ultrafine-scale detection

The CytoScan HD array contains 2.67 million markers for CNAs, including close to 750,000 SNPs and 1.9 million nonpolymorphic probes. The inclusion of the SNP probes provides extra resolution in specific regions, allows for confirmation of copy number changes by revealing allelic imbalance, offers the detection of cnLOH as well as breakpoint and ploidy determination, and identifies low-level mosaicism, sample heterogeneity, and clonal diversity [20]. The presence of the SNP probes also provides the opportunity to obtain variant information in addition to gross chromosomal structural information. For example, chromosomal gains, losses, missense mutations, and frameshift mutations were all detected in AML samples analyzed using CytoScan HD Suite [21]. These included novel mutations in *FOXP1*, a known contributor to human malignancies [22], that were identified and confirmed by sequencing. Therefore, the SNP probes present on the CytoScan HD array can provide other information about changes to the genome that have occurred, in addition to karyotypes.

cnLOH occurs when one of two homologous regions in a chromosome pair is lost, but through various mechanisms the other homolog becomes duplicated. In an individual with only one functional copy of a tumor suppressor gene, cnLOH can result in a loss-of-function mutation in that gene and be instrumental in leukemia genesis. To determine the significance of these changes in MDS, Yeung et al. analyzed 68 samples using FISH and CytoScan HD Suite [13]. As noted above, the microarray captured a higher frequency of chromosomal aberrations. They also noted a high frequency of cnLOH. Specifically, they found that cnLOH was most common on the 9p arm in their samples, and less frequent but detectable in other regions. They concluded that genomic array testing may provide relevant prognostic information for the management of MDS.

These examples illustrate how CytoScan HD Suite has been used to understand various hematological cancers, often detecting multiple aberrations and other anomalies that would otherwise be missed by traditional techniques.

Interpretation of CytoScan HD assay results

CytoScan HD Suite includes Chromosome Analysis Suite (ChAS) software, a simple yet powerful analysis software that enables viewing and summarizing genome-wide data from the CytoScan HD assay (Figure 3). The streamlined analysis workflow includes tools for mosaic calling and noninteger copy number reporting, a database capability for storing and querying segment data and annotations, and a histogram track display to visualize the database contents, as well as the ability to analyze up to 500 samples. Customized filters can be applied to analyze the genome at different levels of resolution. Annotation files can be created, modified, and uploaded with flagged regions that facilitate focused analysis. Reports of results can be exported as DOCX or PDF files. The software allows direct access to external databases such as NCBI, UCSC Genome Browser, Ensembl, and OMIM. Moreover, results can be exported to Ion Torrent™ Oncomine™ Reporter, which summarizes the relevant cancer-driver variants in a clear and simple report that links sample-specific variants to labels, guidelines, and global clinical trials. These features of ChAS and Oncomine Reporter enable rapid analysis and interpretation of molecular karyotyping results.

The increased sensitivity for detecting small aberrations introduces some constraints on interpreting results in the absence of a matched normal control [15]. For example, it has been recommended that only copy number variants larger than 5 Mb should be interpreted as abnormal so that benign variants that are present in the population don't lead to false-positive calls. However, copy number variants smaller than 5 Mb can be considered aberrant when they affect known tumor-related genes. Aberrations in T cell receptor or immunoglobulin genes should be excluded, since these loci undergo extensive rearrangement during normal immune system development. Large regions of cnLOH (>10 Mb) extending to the telomeres can be considered abnormal. In addition, if there is a matched

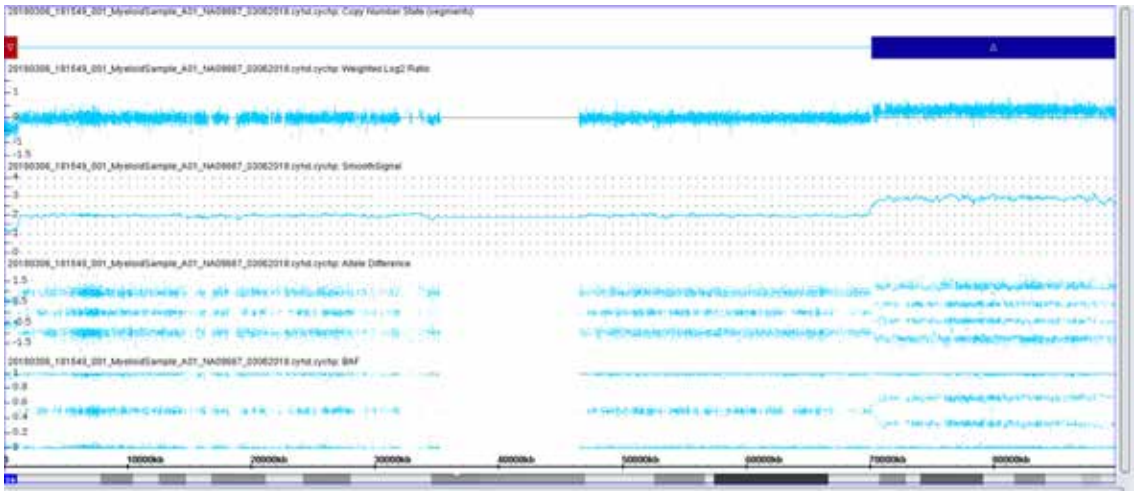

Figure 3. CytoScan HD Suite data in ChAS software. The dark red segment (upper left) signifies a loss in chromosome 16p13.3. The probe-level data—the shift down in the weighted log_2 ratio and smooth signal tracks, as well as the lack of a heterozygous track in both the allele difference and BAF (B-allele frequency) tracks—shown below the dark red segment support the call of loss in this region. The dark blue segment (upper right) in 16qter shows a duplication of this region on chromosome 16. The probe-level data show a shift in the weighted log_2 ratio track, as well as the splitting of the heterozygous track in both allele difference and BAF tracks, which are all evidence of a duplication in this region.

normal control, interpretation of copy number variants in a leukemia sample is considerably less complicated or constrained.

There are some limitations to molecular karyotyping with microarrays. For example, detecting aberrations smaller than the probe spacing may not be possible. In addition, some balanced translocations cannot be detected using CytoScan HD Suite or any other microarray. Individual cell-to-cell differences, revealing clonal history, cannot be recovered since whole genomic DNA from a blood collection is used instead of individual cells to generate the karyotype, but they may appear as mosaics. Nevertheless, the CytoScan HD Suite provides a powerful solution for analyzing molecular karyotypes.

Conclusions

CytoScan HD Suite provides a comprehensive, high-resolution, whole-genome solution to assist with the understanding and characterization of leukemia research samples. The microarray provides the broadest coverage and highest performance for detecting chromosomal aberrations, SNPs, and cnLOH with greater than 99% sensitivity and can reliably detect copy number changes across the genome at high resolution (Table 1); for most genes the resolution is 25–50 kb. CytoScan HD Suite covers all the major leukemia disorders, including ALL, CLL, AML, MDS, CML, and MM. Surpassing the limitations of current database curation, the whole-genome array allows novel findings to be cataloged for future discoveries and annotations, which are often missed by targeted designs. In addition, ChAS software vastly simplifies the interpretation of raw data, allowing the visualization of amplifications and deletions, ploidy and breakpoint determinations, and LOH and cnLOH. Together with simple reporting from Oncomine Reporter, these features make CytoScan HD Suite an ideal solution for analyzing hematological cancers.

Table 1. Comparison of technologies for analyzing karyotypes.

	Whole-chromosome aneuploidy	Small copy number alterations	Polyploidy	Balanced rearrangement	cnLOH	SNP variants
G-banding	+++	–	++	+++	–	–
FISH (queried loci only)	+++	++	++	++	–	–
MLPA analysis	+	+++	+	–	–	–
Targeted NGS	–	+	–	+	–	+++
CytoScan HD Suite	+++	+++	+++	–	+++	++

+++: excellent, ++: good, +: fair, –: not recommended.

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