# Integrated genomic analysis to reduce chromosomal analysis for the diagnosis of pediatric hematologic malignancies: addressing the shortage of cytogenetic technologists

Chromosomal analysis (CA) has been part of the standard care for patients with hematologic malignancies since Dr. Nowell and his colleague discovered the Philadelphia chromosome in chronic myelogenous leukemia in 1961.[1] CA examines cancer-associated numerical and structural abnormalities at the single-cell level and has played a significant role in leukemia diagnosis, risk stratification, and treatment selection.[2] However, the resolution of CA in detecting chromosomal rearrangements and copy number variations is limited. Newer molecular technologies with much higher resolution and scalability, such as fluorescence *in situ* hybridization (FISH), chromosome microarray (CMA), and next-generation sequencing (NGS), have been developed and are now widely implemented to detect genomic aberrations in cancer (collectively referred to as integrated genomic analysis in this study).[3-7] CA requires both the knowledge of cancer and recognition of related aberration patterns. It usually takes 1 to 2 years of post-baccalaureate training to become a cytogenetic technologist and several more years to be an experienced cancer cytogenetic technologist. As large numbers of cytogenetic technologists retire, we are seeing a nationwide shortage of cytogenetic technologists. The situation is getting worse since the pandemic as fewer people have been entering the cytogenetic specialty.[8-10] Given this workforce challenge, we retrospectively reviewed 201 patients with pediatric hematologic malignancies who underwent CA, FISH, CMA, and NGS tests at our institution to evaluate whether integrated genomic analyses could redefine the need for conventional cytogenetics without impacting the clinical care of patients.

This study was performed in accordance with the ethical standards detailed in the Declaration of Helsinki and with the oversight of the Institutional Review Board of the Children's Hospital of Philadelphia. Genetic testing results and clinical records of 201 consecutive children, adolescents, and young adults with leukemia or lymphoma enrolled in the Children's Oncology Group (COG) clinical trials were reviewed (*Online Supplementary Table S1*). In addition to CA and targeted FISH assays required for COG participation, we performed CMA and NGS analyses in our CLIA-certified clinical laboratory. The CMA utilized the Illumina genome-wide SNP array (Illumina, San Diego, CA, USA). Our customized comprehensive hematologic malignancy NGS panel (COHEM) includes the DNA panel that interrogates 118 cancer genes known to be associated with hematologic malignancies for single nucleotide variants, small insertions and deletions (indels), and copy number variations, and an RNA panel that targets 117 cancer genes and over 700 exons for known and novel fusions by using the Anchored Multiplex PCR technology (ArcherDX, Boulder, CO, USA).[11,12] The variants identified were classified according to established guidelines.[13]

The demographics of the cohort are detailed in Figure 1A, B. The median age of the patients was 7 years (range, 1 to 24), and 113/201 (56.2%) were male. The most common

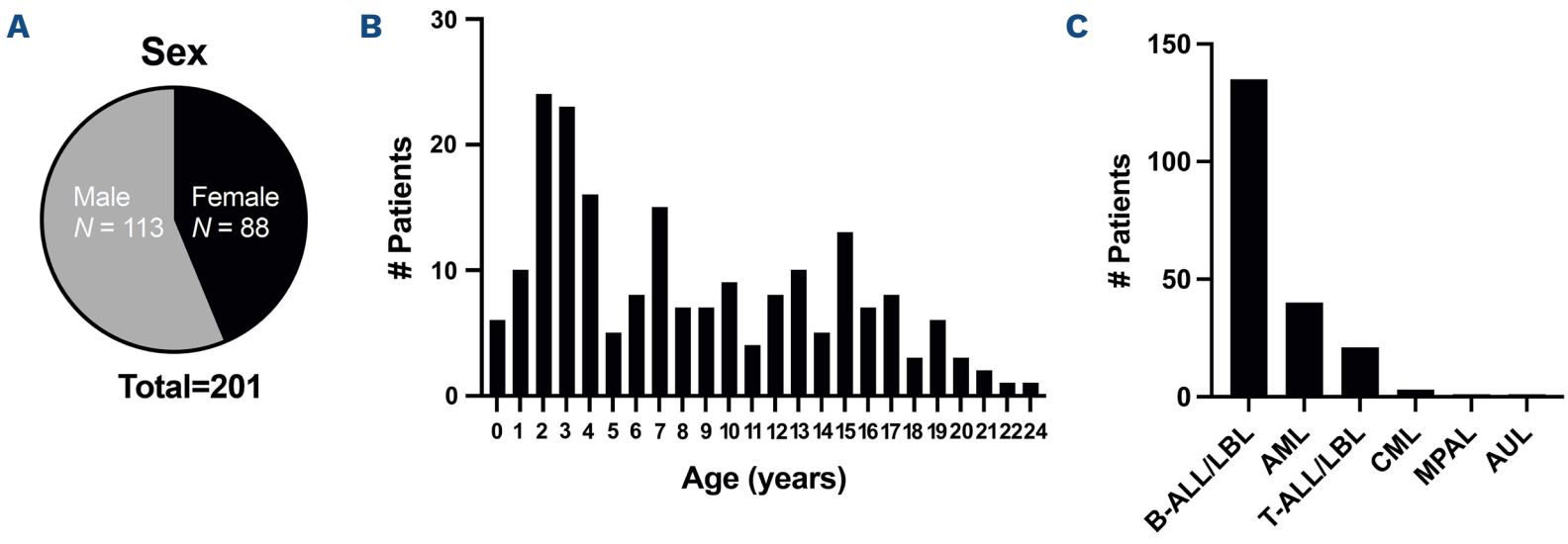


**Figure 1. Overview of the study cohort.** (A) Sex and (B) age (at sampling) of the children, adolescents, and young adults in the study cohort (N=201). (C) Number and type of leukemia and lymphoma diagnoses included in the study cohort. B-ALL/LBL: B-cell lymphoblastic leukemia/lymphoma; AML: acute myeloid leukemia/myeloid sarcoma; T-ALL/LBL: T-cell lymphoblastic leukemia/lymphoma; CML: chronic myeloid leukemia; MPAL: mixed phenotype acute leukemia; AUL: acute undifferentiated leukemia.

diagnosis was B-cell lymphoblastic leukemia/lymphoma (N=135, 67.2%), followed by acute myeloid leukemia/myeloid sarcoma (N=40, 19.9%), T-cell lymphoblastic leukemia/lymphoma (T-ALL; N=21, 10.4%), chronic myeloid leukemia (N=3, 1.5%), mixed phenotype acute leukemia (N=1, 0.05%), and acute undifferentiated leukemia (N=1, 0.05%) (Figure 1C). All patients underwent CA and FISH testing, although 22 patients (10.9%) had insufficient cell growth *in vitro*, preventing informative CA (Figure 2). The majority of cases underwent successful COHEM (200/201, 99.5%) and CMA (184/201, 91.5%) testing (*Online Supplementary Table S1*).

At least one clinically significant variant was detected in each case (*Online Supplementary Table S1*). Among 179 patients with CA results, 26 (14.5%) showed a normal karyotype (Figure 2, *Online Supplementary Table S1*). However, clinically significant genomic aberrations, including translocations, copy number variations and fusions, were identified in 17/26 (65.4%) cases via FISH, CMA, and/or COHEM testing, likely reflecting the growth advantage of normal cells over tumor cells in culture or limited CA resolution.

Among the remaining 153 cases, CA revealed an additional finding with defined clinical significance, but without impacting risk stratification or therapy (referred to as category 1), which was not detected by other methodologies in only one patient with T-ALL (0.7%) (Figure 2, *Online Supplementary Table S1*, case #12). In this case, karyotyping identified a balanced translocation between chromosomes 11 and 14 with possible breakpoints at 11p1?3 and 14q11.2 (46,XY,t(11;14)(p1?3;q11.2)) in 12 out of 20 metaphase cells. This finding was confirmed by metaphase FISH using a break-apart probe set for TCR Alpha/Delta (TRA/D) at 14q11.2 (*Online Supplementary Table S1*). Rearrangements involving *TRA* or *TRD*, which encode the T-cell receptor $\alpha$ and $\delta$ chains, respectively, have been found in 5-10% of cases of T-ALL.[14,15] We have now included this FISH probe set in our T-ALL FISH panel to ensure the detection of these important fusions.

In 45 cases (29.4%, 45/153), CA revealed additional findings, mostly providing chromosome structural aberration information to confirm or augment results of FISH, CMA, and COHEM testing, although such data are not currently used for diagnosis, prognosis, or treatment selection (referred to as category 2) (Figure 2, *Online Supplementary Table S1*). These results included non-recurrent complex low-level subclonal structural variations (N=1), derivative chromosomes (N=21), isochromosomes (N=7), dicentric chromosomes (N=2), balanced translocations (N=8), three-way translocations (N=6), an inversion (N=1), an insertion (N=1), a gain of a whole chromosome (N=1), complex rear-

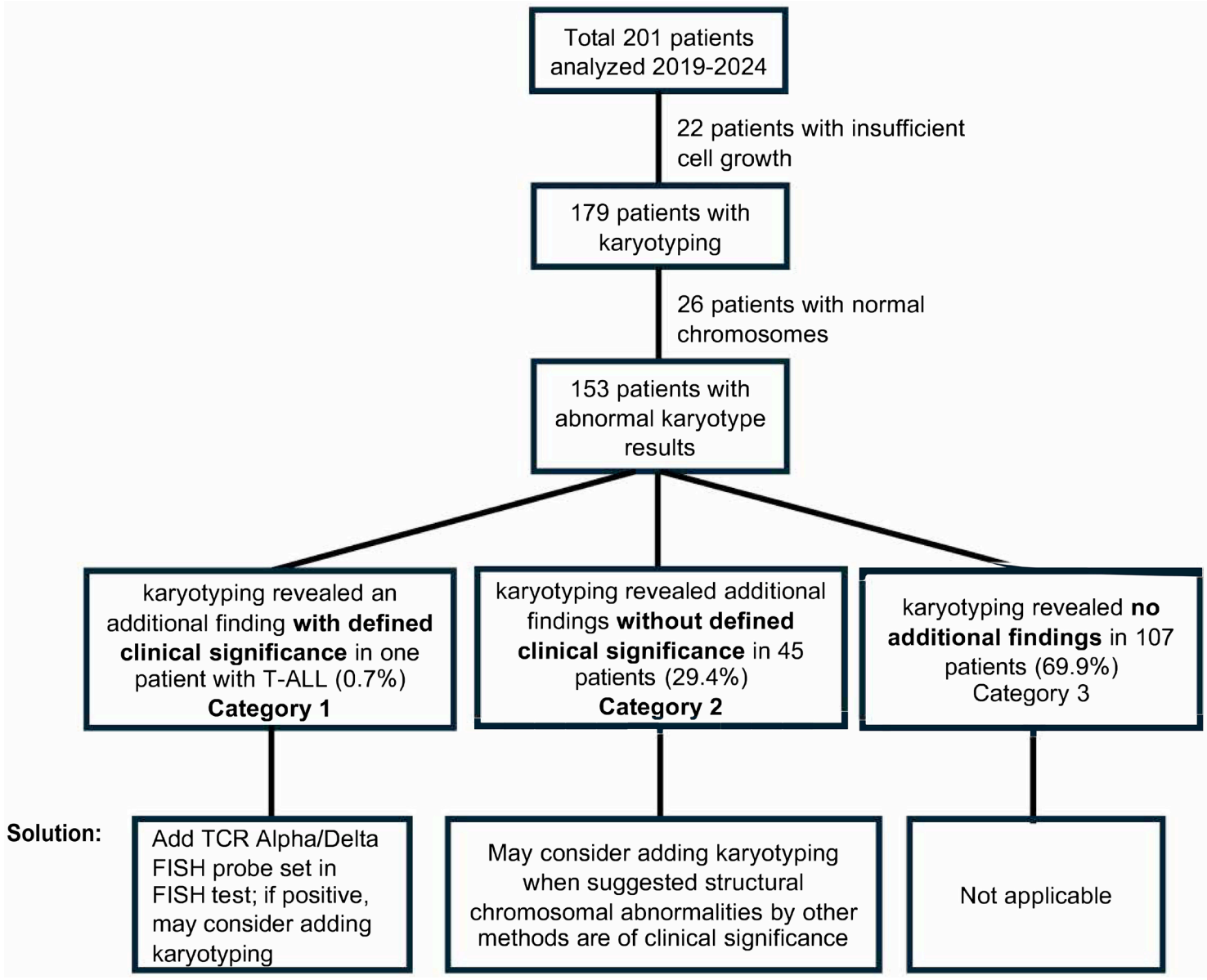


**Figure 2. Study workflow.** T-ALL: T-cell lymphoblastic leukemia; FISH: fluorescence *in situ* hybridization.

rangements involving multiple chromosomes (N=4), and (near) tetraploid genomes (N=2) (Figure 2, *Online Supplementary Table S1*). In most cases with a derivative chromosome, isochromosome, dicentric chromosome, balanced translocation, or three-way translocation, results from concurrent FISH, CMA, and/or COHEM analyses also suggested such abnormalities (*Online Supplementary Table S1*). For example, CMA identified loss of 8p23.3p11.21, 8p11.21, and 12p13.33p11.22 in patient #19, consistent with CA showing a dicentric chromosome 45,XY,dic(8;12)(p11.21;p11.22) (*Online Supplementary Table S1*). Similarly, loss of 7p together with gain of 7q identified by CMA in patient #110 was consistent with an isochromosome i(7)(q10) on karyotype (*Online Supplementary Table S1*). In patient #56, a *PICALM::MLLT10* fusion identified by COHEM testing clarified the finding of a balanced translocation between chromosomes 10 and 11 at possible breakpoints of 10p12 and 11q14.2, respectively. In patient #60, a derivative chromosome 16 [der(16)t(X;16)(p11.2;p13.1)] with a loss of 16pter->16p13.1 and a duplication of Xpter->Xp11.2 structurally resembled a ~44.7 Mb gain of chromosome Xp22.33p11.3 and a ~12.9 Mb loss of 16p13.3p13.12 identified by CMA. These results emphasize the importance of cell-based assay CA in characterizing balanced and certain unbalanced chromosomal aberrations when suspected by FISH, CMA, and NGS assay data. Although none of these structural abnormalities identified in the 45 cases had defined clinical significance, CA facilitated improved knowledge of these genetic alterations and could potentially lead to discovery of new disease-defining aberrations, especially in rare leukemia subtypes.

Our collective data suggest that CA was not necessary for cancer genetic diagnostics in more than two-thirds of our pediatric leukemia/lymphoma cohort, which could allow cytogenetic technologists to focus critical efforts on a smaller number of CA cases and expedite FISH assays. Based on the results of this study, our laboratory implemented a new policy in which we do not routinely run CA for hematologic malignancy cases unless these data are required for enrollment on a specific clinical trial; however, efficient chromosomal preparations are performed for all cases, so that CA can be subsequently added if indicated by results of FISH, CMA, and/or NGS testing. We continue to perform CMA on all leukemia and lymphoma cases, and TCA/D FISH is added when T-ALL is suspected or confirmed. In the first 6 months after implementing the new CA policy in April 2024, we reduced our average CA volume by 43% which resulted in reduced average turnaround time for CA from 20 days to 11 days (Figure 2) for cases in which CA is required. Importantly, this reduction of CA volume has also led to a decreased average turnaround time for cancer FISH analysis from 6.8 days to 4.8 days.

In summary, our collaborative study demonstrates that while FISH, CMA, and NGS testing cover a broad spectrum of genomic alterations and are scalable, CA remains a valuable approach to identify structural configuration of chromosomes at a single-cell level and cannot be completely replaced in clinical laboratories for pediatric hematologic malignancies at this time. Nonetheless, integrated genomic analysis can effectively reduce the CA volume for a large percentage of routine cases and may, in turn, help to mitigate the current shortage of cytogenetic technologists without compromising patient care in pediatric hematologic malignancies. Importantly, we recommend proactive chromosomal preparations for all patients with triage to CA testing for a subset of cases as indicated by other test results. Ongoing developments in machine learning and artificial intelligence-based automatic karyotyping systems are expected to provide a better solution for comprehensive CA in hematologic malignancies in the future.

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**Disclosures**

SPH owns common stock in Amgen and has received honoraria from Amgen, Jazz and Servier. SRR is a consultant for AbbVie and Pfizer. MML was on the scientific advisory board of Bayer and Agilent. SKT receives research funding from Kura Oncology and Incyte Corporation, has served or is serving on scientific advisory boards for Astra Zeneca, Jazz Pharmaceuticals, Kura Oncology, Syndax Pharmaceuticals and Wugen Inc., and has received travel support from Amgen and Jazz Pharmaceuticals (all for unrelated studies). The other authors have no conflicts of interest to disclose.

**Contributions**

DL, MML and YZ conceived and designed the study, analyzed the

data, and wrote the initial version of the manuscript. All authors (DL, SKT, GW, SRR, KMB, HN, LW, DMW, BT, LFS, EM, VP, ML, MEP, STH, MML and YZ) interpreted the results and reviewed and contributed to the final version of the manuscript.

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**Data-sharing statement**

The datasets generated and analyzed for this study are available from the corresponding author upon reasonable request.

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