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Running chromosome analysis in leukemia or not, upfront or later?

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In this letter by Li, et al [1], an alternative clinical cytogenetic testing strategy is proposed to help overcome the shortage of skillful cytogenetic technologists. The proposed workflow integrates multiple FISH panels, genomic microarray, NGS panel-based gene mutation profiling and RNA fusion testing, followed by reflex karyotyping in some patients to confirm or define the chromosome and genetic abnormalities indicated by the initial tests. The results obtained with this workflow were compared with traditional karyotyping analysis of a pediatric leukemia cohort, comprised predominantly of B cell ALL patients. The intention was to help overcome the shortage of skilled cytogenetic technologists in clinical diagnostics by skipping chromosome analysis upfront in a vast number of cases, while improving the turnaround time. This pilot strategy may be applicable for some hematological neoplasms, such as pediatric acute leukemia with simpler karyotypes or defined gains and losses, and those leukemias such as myeloma, and CLL, often with a low mitotic index in cell culture. It could achieve a short-term benefit of requiring fewer trained technologists; however, this approach may indeed compound the challenges in the long-term in training new cytogenetic technologists and advancing the analytical skills of current cytogeneticists.

At many large cancer centers, numerous leukemia clinical trials are offered, and the eligibility for these trials and stratification to appropriate study arms often depends on cytogenetic findings, and molecular gene mutation profiling. Detecting recurring chromosome abnormalities and gene mutations, listed in the current classification and guidelines, i.e., ICC, WHO 5th and NCCN, is critical in clinical decision-making and management [2, 3, 4]. Whether a patient with newly diagnosed AML has a CBF leukemia, or a complex karyotype may guide an appropriate clinical trial, as outlined in Beat AML and MyeloMATCH, which require these results by day 5 or sooner. Thus, a rapid workflow with appropriate integrated testing that includes karyotyping, selected FISH testing, and rapid small NGS panel for the most common gene mutations is in place in many cancer centers to meet clinical needs.

With prompt notification from clinicians and an efficient triaging and workflow in the laboratory, we provide a preliminary karyotyping result within three days in the majority of newly diagnosed patients with acute leukemia at MSKCC. Once an overnight culture of bone marrow or peripheral blood cells is set up, followed by harvesting, slide preparation, and chromosome banding the next morning, chromosome analysis is initiated in the afternoon to obtain a preliminary chromosome result. In our experience,

performing karyotyping analysis together with a few selected FISH tests, i.e., to detect *KMT2A/MLL* translocations, t(9;22)/*BCR*::*ABL1* fusion, and *TP53* deletions, has been excellent in most leukemia cases. Additional FISH tests may be reflexed to clarify uncertain karyotype findings or to rule out recurring chromosome translocations with breaks at the relevant loci. The concordance of karyotyping results and the findings of FISH and fusion studies was extremely high, with a few exception due to cryptic translocations, such as t(5;11)/*NUP98::NSD1*. On the other hand, certain recurring chromosome translocations or fusions, typically related to enhancer or promoter swapping or hijacking, such as inv(3)/t(3;3)/*MECOM* rearrangement, and Immunoglobulin or T cell receptor (TCR) related translocations are often under-detected by NGS based assays, including RNAseq testing. FISH testing may be run upfront for specific disease concerns, such as the *PML::RARA* fusion in APL, and is needed for some cryptic translocations, such as t(5;11)/*NUP98::NSD1*, relatively common in pediatric AML

More importantly, with improved response to chemotherapy and novel targeted treatments, many patients achieve a long survival; however, some of them are at risk of developing secondary hematological neoplasms, such as MDS or AML, following treatment for the primary cancer. These patients are often elderly and may present with a coexistent MDS. Detecting these diseases is critical, not only for a precise diagnosis, but also to guide management and treatment planning. In these situations, chromosome analysis is essential, and should be performed immediately [5, 6]. As demonstrated in Figure 1, chromosome analysis of a bone marrow aspirate specimen from a 72 year old male patient detected a hypodiploid karyotype that is characteristic in B-ALL associated with a high risk and revealed two cytogenetically distinct clones with deletion of the long arm of chromosomes 5 and 10, respectively (Figure 1A). FISH testing further confirmed the del(5q) in mature granulocytes (Figure 1B), supportive of coexistent MDS and B-ALL. Optical Genome Mapping (OGM) testing confirmed the hypodiploid clone, the relevant translocations, and deletion of 10q but cannot define the relationship of del(10q) to the hypodiploid clone, i.e., as a subclone or a separate clone as noted by chromosome analysis (Figure 1C). The del(5q) was not detected by OGM testing. This example nicely demonstrates some important technical features of karyotype analysis at the single cell level, which may define clonal relationships, including clonal evolution, complexity, and heterogeneity, and pinpoint whether chromosome translocations or copy number alterations resulted from deletions, gains, or unbalanced translocations, which may involve gene fusions. It is particularly helpful in detecting early clones, or minor clones in myeloid neoplasms, such as MDS, characterized by only a few immature blasts in bone marrow or blood samples.

To provide a rapid cytogenetics result in samples from patients with newly diagnosed leukemia, it will require a combination of an efficient workflow within a triaging setting to select the test categories and targeted FISH testing menu, based on disease types, such as AML vs ALL, and disease status, along with hematopathology and flow cytometry findings, and cytogenetic history, if available. Receiving a fresh specimen quickly and including select cell growth factors in cell cultures will increase the success of chromosome analysis. Applying automation in various cytogenetic procedures, i.e., harvesting, slide preparation, and banding in karyotyping, and in post-hybridization washing in FISH testing will help reduce staff time, and ensure consistent quality [7]. Recently, some AI based karyotyping analysis modules, which rely on numerous algorithms and calibration, have been validated and applied in clinical

cytogenetics diagnostics with an impressive improvement in chromosome analysis, with only a few seconds required to separate, identify, and compare the chromosomes. This technology will significantly help the cytogenetics laboratory to offer a rapid chromosome analysis result in many cases.

However, a precise chromosome analysis relies on skillful cytogenetic technologists who must examine, compare, and magically align chromosomes to match the banding patterns to define clones and their relationship. This can be achieved with extensive practice and work experience. Thus, there is an urgent need to advocate, introduce, and mentor new technologists to enter into this field, which for more than 50 years has been at the center in discovering recurring translocations in cancer, such as the t(9;22) in CML, and t(8;14) in Burkitt lymphoma, defining their functional consequence, and developing targeted treatment [8].

In summary, performing a high-quality karyotype analysis upfront, particularly in myeloid neoplasms, is the most efficient and economic for a clinical cytogenetics laboratory. Integrating cytogenetic analysis with selected FISH tests, and NGS based gene mutation profiling will provide optimal service for clinical needs, particularly for various clinical leukemia trials. With additional molecular testing, such as OGM, and WGS, chromosome analysis will remain as one of the key genetic diagnostic technologies in the near future.

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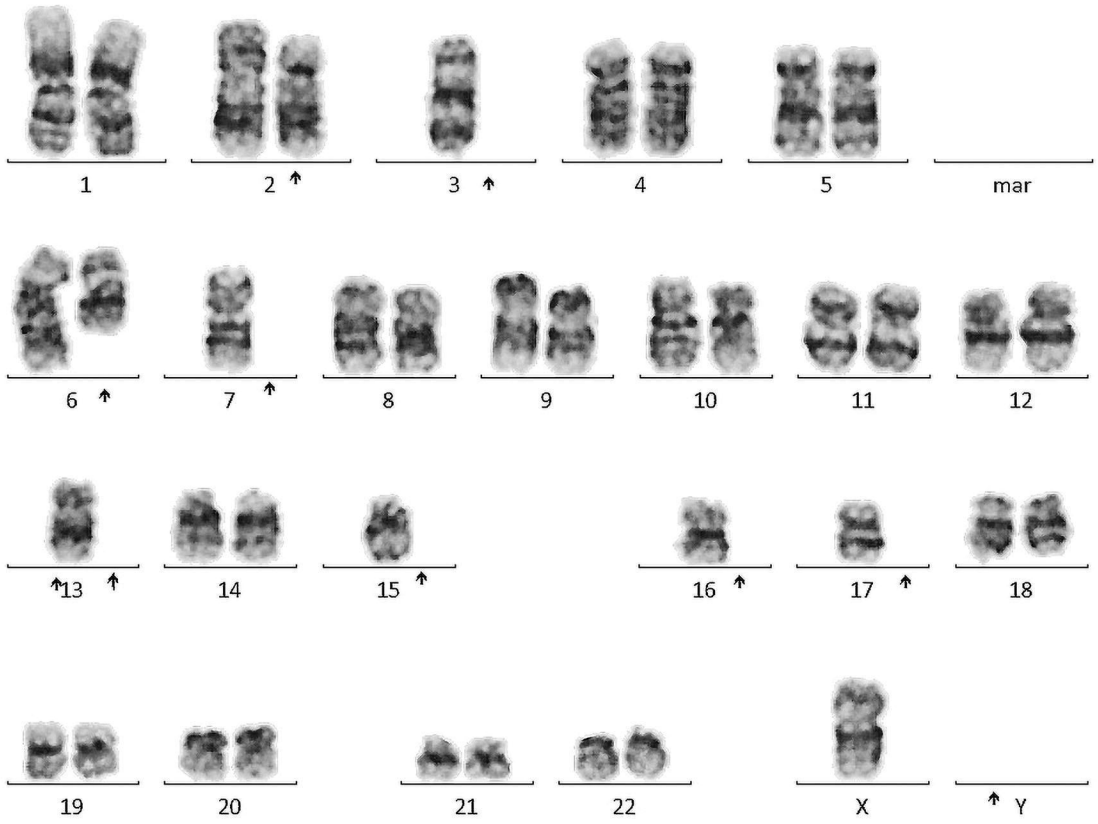
Figure 1 Legends:

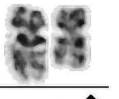
Coexistence of B-ALL and MDS in a 72-year-old male patient revealed by integrating chromosome and FISH analysis and molecular testing.

A. Chromosome analysis of bone marrow specimen detected a hypodiploid karyotype with clonal evolution, including doubling cells, and two cytogenetically distinct clones with deletion of the long arm of chromosomes 5 and 10, respectively. TP53 and TET2 mutations were detected earlier in a peripheral blood sample. Arrows point to loss of the relevant chromosomes and del(5q), and del(10q).
B. FISH testing confirmed del(5q) in granulocytes, supporting a diagnosis of MDS, despite minimal dysplastic morphological evidence. Two cells show del(5q) with one orange signal (EGR1, 5q31) and two internal control green signals (5p15.2).
C. Optimal Genomic Mapping (OGM) study (circos plot) confirmed the hypodiploid clone with loss of chromosomes Y, 3, 7, 13, 15, 17 (red bar), der(2q;16q) and del(10q), but did not reveal del(5q).

A



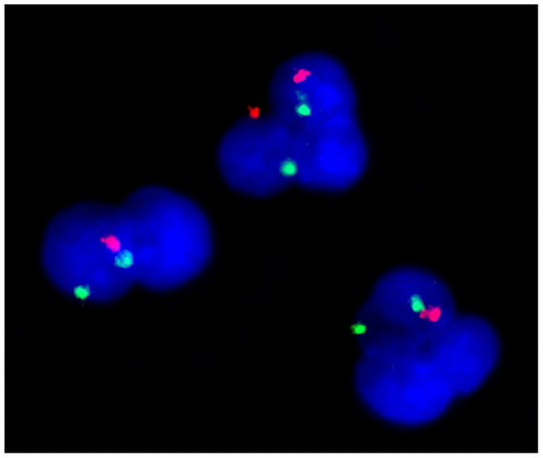
del(5)(q22q35)[2]



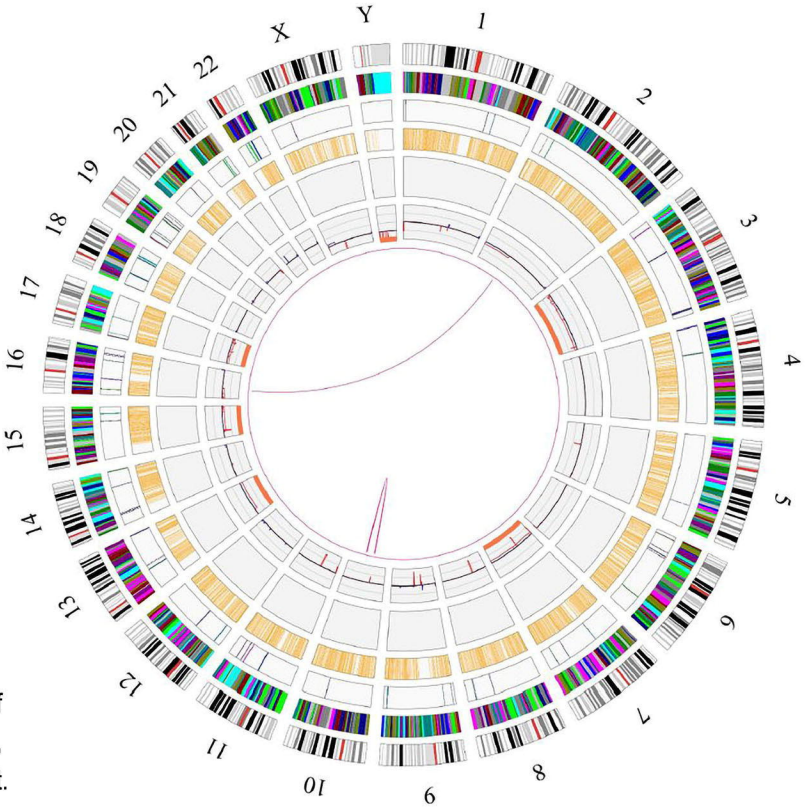
del(10)(q22q24)[6]

39,X,-Y,der(2;16)(q10;p10),-3,del(6)(q13q21),-7,-13,add(13)(p11.1),-15,-17[5]/78,idemx2[1]

B FISH confirms del(5q) (3.7%), in granulocytes



C



OGM test confirms hypodiploidy with loss of chromosomes Y, 3, 7, 13, 15, 17 (red bar), der(2;16), and del(10q), but not del(5q). Circos plot.