Background

B-cell precursor acute lymphoblastic leukemia (B-ALL) accounts for 70% of all infant and childhood leukemia, and also affects adults. B-ALL is a complex disorder with germline and multiple somatic lesions in genes that regulate hematopoiesis, the cell cycle, and cellular proliferation. Patients with B-ALL harbor recurrent cytogenetic aberrations which can be detected by classical karyotyping:

- whole chromosome aneuploidy (hypodiploidy or hyperdiploidy)
- chromosomal translocations: t(9;22) [BCR-ABL1], t(4;11) [KMT2A-AFF1], t(1;19) [TCF3-PBX1]

Fluorescence in situ hybridization (FISH) studies are performed concurrently to detect B-ALL-specific submicroscopic alterations:

- balanced translocation t(12;21) [ETV6-RUNX1], KMT2A gene break apart rearrangements
- deletions involving the CDKN2A/B locus, PAX5 and IKZF1 genes.

Genomic technologies have identified a number of submicroscopic copy number alterations that have been incorporated into an integrated cytogenetic and genomic classification reported to improve risk stratification [PMID: 24957142].

In addition, distinction of clones with double hypodiploidy versus hyperdiploidy requires microarray testing using platforms that contain SNP probes to detect homozygosity (LOH). Further FISH tests are commonly indicated to elucidate chromosomal rearrangements and to identify possible fusion genes in patients with ALL. There is also great interest in the identification of BCR-ABL1-like B-ALL and B-ALL with iAMP21, both recognized in the 2016 WHO classification.

Current cytogenetic testing

In the Pittsburgh Cytogenetics Laboratory, the diagnostic evaluation for B-ALL has included classical karyotyping and the ALL FISH panel to identify BCR-ABL1 and ETV6-RUNX1 gene fusions, KMT2A gene rearrangements, and to detect deletions of CDKN2A/B, PAX5, and IKZF1 genes. However, the necessity for dividing cells, poor chromosome morphology, and a relatively low-resolution of G-banding can be limiting factors. FISH has been an invaluable technique for the identification of fusion, break apart rearrangements, and detection of deletions and duplications with a resolution of 100-250 kb on metaphase and non-dividing interphase cells. However, FISH must be targeted to selected genomic regions and does not provide genome-wide analyses or genomic resolution necessary in some cases. In addition, the continued expansion of labor-intensive FISH panels is impractical due to longer turn-around times, cost, and the inability to target all genomic regions of clinical interest.

The Pittsburgh Cytogenetics Laboratory and the Division of Hematopathology recognize the need to provide clinicians with complete and accurate results in a timely manner, which is critical for enrolling ALL patients into the appropriate treatment protocol. Therefore, we established a new approach - an INTEGRATED CYTOGENOMIC ANALYSIS into the clinical diagnosis of B-cell ALL.
## INTEGRATED CYTOGENOMIC ANALYSIS FOR B-ALL

<table>
<thead>
<tr>
<th>Sample type:</th>
<th>Bone marrow sample at the time of diagnosis or relapse. Peripheral blood if &gt;20% of blasts are present.</th>
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<tbody>
<tr>
<td>Indications:</td>
<td>Suspected or definite diagnosis of B-cell Acute Lymphoblastic Leukemia (B-ALL)</td>
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<td>Tests package performed</td>
<td>Array comparative genomic hybridization (using custom high resolution CGH+SNP microarray) in conjunction with classical karyotyping and FISH for balanced translocations t(9;22) ([BCR-ABL1]), 11q23 ([KMT2A]) break apart, t(12;21) ([ETV6-RUNX1]), CRLF2 break apart</td>
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### B-ALL specific alterations detected by custom microarray:

- Imbalances of \(CDKN2A/B\), \(PAX5\), \(IKZF1\), \(TCF3-PBX1\), \(CRLF2\), \(RB1\), \(EBF1\), \(ETV6\), \(BTG1\), \(FLT3\), \(TP53\)
- Intrachromosomal amplification of chromosome 21 [iAMP21]
- Hyperdiploidy - aneuploidy for all chromosomes including trisomy 4 and 10
- Hidden hypodiploidy and LOH regions
- Genomic imbalances resulting in tyrosine kinase fusion genes in patients with \(BCR/ABL1\)-negative ALL
- Cryptic imbalances at the balanced translocation breakpoint hotspots enabling targeted FISH confirmation
- Copy number alterations of all chromosomes enabling accurate characterization of structural rearrangements detected by classical karyotype analysis

### Advantages:

- Precise copy number profiling of the whole genome at a resolution significantly higher than conventional karyotyping or FISH, even in samples with normal karyotype or culture failure (See Page 3)
- Detection of mono- and bi-allelic intragenic alterations that are below the resolution of FISH analysis (Page 4)
- Detection of additional genomic alterations of prognostic and diagnostic significance that are not included in the ALL FISH panel (see Page 5)
- Cost-effective replacement of multiple FISH assays by a single microarray test
- Rapid turnaround time
- Integrated analysis incorporates results of classical karyotyping, FISH and microarray facilitating accurate molecular diagnosis, risk stratification and optimal treatment options
- Integrated cytogenetic and genomic analysis defines a cost-effective disease monitoring approach using patient-specific markers for follow up, eliminating unnecessary testing

### Limitations:

- Low-level (less than 10%) mosaicism for clonal aberrations will not be detected by microarray

### Reflex FISH studies:

FISH studies will be performed to confirm balanced rearrangements detected by karyotype & to evaluate possible rearrangements suggested by microarray

### Additional FISH studies:

Available upon request for the \(IGH\), \(ABL1\), \(ABL2\), \(JAK2\), \(CSF1R\), \(PDGFRB\), \(NUP98\), \(NUP214\) gene rearrangements (see list of FISH probes at [http://pittgenetics.com/PDFfiles/ONC%20PROBES.pdf](http://pittgenetics.com/PDFfiles/ONC%20PROBES.pdf))
The collective findings of microarray, FISH and karyotype studies provide comprehensive and accurate analysis of the cancer genome, including evaluation for B-ALL specific alterations (microarray, red arrows); structural chromosome abnormalities observed by karyotype (microarray, blue arrows); and clinically significant submicroscopic balanced rearrangements (FISH, white arrows).
PRECISE COPY NUMBER PROFILING BY MICROARRAY ANALYSIS

Left panel: The IKZF1 exon 1 deletion identified by a custom microarray.
Right panel: FISH using the IKZF1 gene-specific probe showing two red signals, failing to detect IKZF1 gene alteration that is outside of FISH targeted region.

Left panel: Bi-allelic deletion (107 kb and a 45 kb in size) of the PAX5 gene detected by microarray in a patient with B-ALL.
Right panel: Interphase cell showing normal (false negative) FISH results for the PAX5 locus, as both intragenic deletions are below the level of detection by FISH assay.

Left panel: Bi-allelic deletion of the CDK2A/2B locus is identified by a custom CGH microarray.
Right panel: Lack of a red signal (white arrow) is detected by interphase FISH, corresponding to the 273 kb deletion. However the resolution of the FISH probe is insufficient to detect a 33 kb deletion residing on the other allele.
B-ALL SPECIFIC ALTERATIONS IDENTIFIED BY HIGH RESOLUTION CUSTOM MICROARRAY ANALYSIS

Our custom genome-wide microarray platform contains CGH and SNP oligonucleotide probes enabling assessment of copy number alterations and LOH detection in a single assay. The microarray design has an enhanced coverage for 898 targeted genes implicated in oncogenesis as well as breakpoint “hot spot” intervals associated with recurrent balanced rearrangements [PMID: 26299921; 28214896].

- **RB1**: Bi-allelic deletion (4.2 Mb and 165 kb in size) of the RB1 gene identified by microarray. Copy number alterations involving RB1 have been associated with a poor-risk genetic profile and increased risk of relapse.

- **BTG1**: A 316 kb deletion involving the BTG1 gene detected in a patient with B-ALL. BTG1 is a recurrent target in pediatric ALL, deletions of which occur predominantly in patients with ETV6-RUNX1 (~20%) or BCR-ABL1 (26%) fusions.

- **ABL1**: About 60% of cytogenetically balanced rearrangements have cryptic copy number alterations at the breakpoints, enabling targeted FISH testing. A 5 kb deletion was observed in intron 1 of the ABL1 gene, a common breakpoint hotspot, indicative of a BCR-ABL1 gene rearrangement, confirmed by FISH.
How to order Integrated Cytogenomic Analysis for B-ALL

1. Download the Oncology Cytogenetics Requisition form from our website (http://www.pittgenetics.com/PDFfiles/Oncology%20Cytogenetics%20Requisition%20form.pdf).

2. Provide diagnosis, such as B-ALL. Specify indication of a new diagnosis or a relapse sample.
   - Note: Integrated Cytogenomic Analysis is not recommended on remission samples nor as a test for minimal residual disease.

   - Karyotype, FISH and microarray studies will be completed in parallel.
   - Reflex FISH for IGH will be performed in cases positive for the CRLF2 gene rearrangement.

4. Individual FISH testing can be ordered from a list of probes for follow up studies.

CONTACT US:
PITTSBURGH CYTOGENETICS LABORATORY
Tel: 412-641-5558
Fax: 412-641-2255
Maureen Sherer, MSc
Section Supervisor, Oncology
Svetlana Yatsenko, MD
Lab Director