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Tumors of the colon can be either sporadic or hereditary, and differentiating these two scenarios is clinically important for management of the disease.

The most common hereditary cancers are those associated with familial adenomatous polyposis (FAP) and those associated with hereditary non-polyposis colorectal carcinoma syndrome (HNPCC) or Lynch Syndrome [1]. FAP patients usually are identified clinically due to extensive polyposis with associated conventional colonic adenocarcinomas. HNPCC patients are more difficult to identify based on clinicopathologic examination but may come to attention because of unusual family histories of multiple different types of carcinomas and because of the early age at which these patients can present. The complexities of identifying and managing patients with hereditary conditions, for the proband as well as the family members, necessitate a team approach to these cases. Pathologists play an important role as integral team members for working up and identifying these patients, from reviewing the histopathology to performing molecular screening tests on the tumor tissue.

HNPCC is caused by a defect in one of the DNA mismatch repair genes (MLH1, MSH2, PMS2, and MSH6) [2]. In patients with HNPCC, the deficiency in DNA mismatch repair usually is associated with earlier onset of carcinomas. These primary germine defects cause an inability to repair common somatic DNA replication errors that occur in normal cells with some regularity. Short tandem repeats, also known as microsatellites, are among the most common sites vulnerable to these replication errors. Microsatellites are short stretches of DNA, ranging from two to seven basepairs in length that are repeated a variable number of times. The number of repeats is inherited and varies from person to person, making microsatellites a very common form of genetic polymorphism.

Uncorrected replication errors in microsatellites in patients with HNPCC can be detected with a molecular assay for microsatellite instability. The microsatellite instability assay is a relatively straightforward polymerase chain reaction-based test that uses DNA extracted from tumor and normal tissues from paraffin-embedded samples. Microsatellites are examined for evidence of novel PCR amplicon in the tumor, as compared to the pattern seen in the normal tissue (Figure 1).

Current applications of microsatellite instability testing in colon cancers are in testing tumors with histologically worrisome features or for tumors in patients under the age of 50 [3]. Evidence also exists that tumors with microsatellite instability have a significantly better prognosis and may respond differently to conventional chemotherapy regimens. The prognostic and therapeutic value of microsatellite instability testing plus the fact that HNPCC also can present after age 50 are leading some institutions, including Cleveland Clinic, towards universal testing of all colon cancer cases [4].

One of the disadvantages of the microsatellite instability assay, however, is its lack of specificity. In fact, most of cases of colon cancer with microsatellite instability are not hereditary but rather by Jennifer L. Hunt, MD

BRAF and KRAS Mutation Analysis in Colon Cancer

by Jennifer L. Hunt, MD
are caused by somatic sporadic mutations in the DNA mismatch repair genes or even more commonly from methylation of one of the genes (particularly MLH1) [5]. Geneticists and clinicians treating patients identified as having microsatellite-unstable tumors subsequently must go through labor-intensive genetic counseling and time-consuming, potentially expensive germline genetic testing. In the end, many of these patients do not have a hereditary cause for their colon cancer.

Recent evidence has shown that non-hereditary tumors with high-level microsatellite instability have a high frequency of BRAF mutations, and that colon cancers not caused by the DNA mismatch repair defect pathway have a high frequency of KRAS mutations [4, 6]. Testing for BRAF and KRAS gene mutations can be performed easily on DNA extracted from the tumor tissue. The DNA is subjected to a relatively simple and rapid DNA sequencing assay. The sequence is read to detect the characteristic codon 600 mutation in the BRAF gene (Figure 2) and the codons 12 and 13 mutations in the KRAS gene. Additional evidence suggests that KRAS-positive colon cancers do not respond to drugs targeting epidermal growth factor receptor (EGFR) [7]. Therefore mutation assessment of KRAS and BRAF genes, in addition to the panel of testing for HNPCC, potentially could help identify sporadic, non-hereditary colon cancers with microsatellite instability. Patients with sporadic non-hereditary disease do not need a genetic workup and their families are not at additional risk for colon cancer over the general population.

Figure 1. This image demonstrates the pattern seen in a mononucleotide repeat unit from normal tissue (top panel) and tumor tissue (lower panel) from a case of colonic adenocarcinoma with microsatellite instability.

Figure 2. This image shows the electropherogram for sequencing of exon 15 of the BRAF gene. The arrow indicates nucleotide 1799, where a T and an A are both present. This heterozygous pattern indicates a mutation of one of the alleles.

References
B-cell chronic lymphocytic leukemia (CLL), the most common form of adult leukemia in the United States, is a malignant neoplasm of small B-cells that typically involves the peripheral blood and bone marrow and may also involve the lymph nodes (1). A minority of patients with lymph node involvement but minimal to no involvement of the bone marrow or peripheral blood historically have been classified as small lymphocytic lymphoma (SLL). Currently, however, CLL and SLL are thought to represent different clinical manifestations of the same disorder.

FISHing for Prognosis in B-cell Chronic Lymphocytic Leukemia

*by James R. Cook, MD, PhD*

B-cell chronic lymphocytic leukemia (CLL), the most common form of adult leukemia in the United States, is a malignant neoplasm of small B-cells that typically involves the peripheral blood and bone marrow and may also involve the lymph nodes (1). A minority of patients with lymph node involvement but minimal to no involvement of the bone marrow or peripheral blood historically have been classified as small lymphocytic lymphoma (SLL). Currently, however, CLL and SLL are thought to represent different clinical manifestations of the same disorder.

CLL generally presents in older adults, with a median age at diagnosis of 72 years. Most patients are initially asymptomatic, with the first diagnosis often made incidentally during peripheral blood examination for other reasons. Some patients, however, may have fatigue, anemia, or recurrent infections. The diagnosis of CLL usually is straightforward and can be accomplished by morphologic evaluation of the peripheral blood together with flow cytometry studies to confirm the clonal nature and expected phenotype of the B lymphocytes.

The clinical course in CLL is generally indolent, and many patients may not require therapy of any sort for many years. Given that many cases of CLL are first diagnosed over the age of 70, many patients may go on to die of other causes without ever needing to be treated for their CLL. However, the prognosis in CLL is heterogeneous, and there is a minority of patients who follow a more aggressive clinical course that may require chemotherapy within months of initial diagnosis. There is, therefore, great clinical interest in assays that can provide prognostic information to guide therapy choices in CLL.

Several prognostic markers have been described in CLL over the last 10 years (2). Analysis of the sequence of the immunoglobulin heavy chain genes in CLL has shown that patients with many acquired mutations in the immunoglobulin heavy chain variable regions (\(IgV_H\)) (so-called “mutated CLL”) have a better prognosis...
than cases in which the $\text{IgV}_H$ gene remains in the germline configuration ("unmutated CLL"). In routine practice, however, sequencing of $\text{IgV}_H$ can be technically difficult and time consuming, and other researchers have attempted to identify other markers that may be easier to perform. Using flow cytometry, it is known that expression of the ZAP-70 protein in CLL is associated with unmutated $\text{IgV}_H$ and shorter survival. However, flow cytometric studies of this cytoplasmic protein have proven technically demanding and difficult to standardize between laboratories.

In recent years, it has become clear that subgroups of CLL can be defined on the basis of cytogenetic abnormalities. Frequent abnormalities found in CLL include deletions of the p53 oncoprotein locus ($\text{TP53}$) at chromosome 17p13, deletion of the $\text{MLL}$ gene at chromosome 11q23, trisomy of chromosome 12, and deletions on the long arm of chromosome 13. These cytogenetic abnormalities were first described using metaphase cytogenetic analysis. In CLL, however, metaphase cytogenetic studies can be very difficult because CLL cells tend to grow very poorly in culture, and it therefore can be difficult to identify metaphases for karyotyping. For this reason, fluorescence in situ hybridization (FISH) studies, which do not require dividing cells, have emerged as the technique of choice for cytogenetic evaluation of CLL.

In FISH analysis, smears of peripheral blood or bone marrow are prepared, and fluorescently labeled DNA probes complementary to the genomic regions of interest are hybridized to the target cell nuclei. Under a fluorescent microscope, the number of signals for each genomic locus is recorded (Figure 1). The resulting FISH patterns separate patients into distinct prognostic categories (Table 1), as shown in numerous clinical studies. In one large report, patients with deletions of 17p13 ($\text{TP53}$) or 11q23 ($\text{ATM}$) displayed a median treatment-free interval of only nine and 13 months, respectively, while patients with deletions of chromosome 13q as an isolated abnormality had a median of 92 months before treatment was needed (3). Patients with trisomy 12 or none of these abnormalities had an intermediate prognosis, with median treatment-free intervals of 33 and 49 months, respectively.

FISH profiling in CLL is a rapid, robust assay for identifying prognostically significant, genetically defined subsets in CLL. This information provides additional guidance to patients with CLL and their caregivers in making treatment decisions.

### References


### Table 1. Influence of FISH Abnormalities on Time to Treatment

<table>
<thead>
<tr>
<th>Category</th>
<th>Number of patients</th>
<th>Median treatment-free interval (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Del(17p)</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Del(11q)</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td>Trisomy 12</td>
<td>14</td>
<td>33</td>
</tr>
<tr>
<td>No FISH abnormality</td>
<td>18</td>
<td>49</td>
</tr>
<tr>
<td>Del(13q) only</td>
<td>36</td>
<td>92</td>
</tr>
</tbody>
</table>

Adapted from Dohner et al. 3

![Figure 1. FISH analysis of B-cell chronic lymphocytic leukemia using probes specific for the chromosome 12 centromere (green signal) and a locus on chromosome 13q (red signal). This case displays 3 green signals, indicating the presence of trisomy 12, a finding associated with intermediate prognosis in B-cell chronic lymphocytic leukemia.](image)
Neoplasms composed of well-differentiated adipose tissue (fat) are among the most common soft tissue tumors. They can be classified as lipoma or atypical lipomatous tumor (ALT)/well-differentiated liposarcoma (WDLPS). Accurate classification can be problematic as many ALT/WDLPS lack cytologic atypia or lipoblasts, essentially mimicking benign lipomas at the histological level.

Distinction between lipoma and ALT/WDLPS is critical as lipomas do not exhibit locally aggressive behavior and do not require clinical follow-up. On the other hand, ALT/WDLPS exhibit locally aggressive behavior and require long-term follow-up. Furthermore, lipoma and ALT/WDLPS are treated surgically; however, the type of surgery that is performed differs radically, depending on the diagnosis. Excision of ALT/WDLPS requires a complete cuff of normal/non-neoplastic tissue surrounding the neoplasm. Otherwise, the patient is at high-risk for local recurrence with the need for additional surgeries. ALT/WDLPS is very difficult to eradicate.
once it has recurred, and patients often are subject to repeated local recurrence and large, disfiguring surgeries. Furthermore, occasional cases of ALT/WDLPS transform into high-grade sarcoma, known as dedifferentiated liposarcoma, with the capacity for distant metastasis. Lipomas can be excised without a margin of soft tissue with little to no potential for local recurrence.

Fortunately, while the histological appearance of lipoma and ALT/WDLPS can be very similar, they are vastly different at the genetic level. Lipomas tend to harbor simple rearrangements involving the long arm of chromosome 12, specifically the q13-q15 region, which contains several oncogenes. Rearrangements in this region frequently involve the \textit{HMGA2} gene. Some cases of lipoma contain structural abnormalities of chromosome 6p21. In contrast to lipomas, ALT/WDLPS harbor giant marker and ring chromosomes containing many copies of the 12q13-15 region including \textit{MDM2}, which encodes a protein that is important in the degradation of TP53, an extremely important tumor suppressor that is involved in many different kinds of cancer. Thus, lipomas contain one copy of \textit{MDM2} while ALT/WDLPS contain many copies of \textit{MDM2}, i.e., \textit{MDM2} is amplified in ALT/WDLPS.

\textbf{Assay Development}

The difference in \textit{MDM2} copy number between lipoma and ALT/WDLPS has been exploited to develop a DNA-based fluorescence \textit{in situ} hybridization (FISH) assay composed of fluorescently labeled DNA probes that bind specifically to two regions of chromosome 12, the centromeric region and \textit{MDM2}. The \textit{MDM2} probe was developed in-house while the CEP12 probe to the centromere of chromosome 12 was obtained from Abbott Molecular, Abbott Park, Ill.. The probes are labeled with different fluorophores such that the centromere fluoresces red, and \textit{MDM2} fluoresces green. Quantitation of the relative intensity of the fluorescent signals allows determination of whether \textit{MDM2} is amplified. An \textit{MDM2}/centromere signal ratio of greater than two is considered amplified and is diagnostic of ALT/WDLPS (Figure 1).

We have shown that this test holds up well, even in core needle biopsy specimens (unpublished data). Thus, core needle biopsy is sufficient to guide the surgeon to perform the best operation for each well-differentiated lipomatous tumor. At the patient level, this means that each patient receives the appropriate operation for his or her tumor, resulting in better outcomes and greater patient satisfaction.

\textbf{References}

About the Authors

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New Technical Briefs

The following technical briefs have recently been released by the Pathology and Laboratory Medicine Institute:

- Estimated Glomerular Filtration Rate
- FISH for Plasma Cell Myeloma
- BCR/ABL RT-PCR Assay for Residual Disease in CML
- PSA Reference Range Update

These technical briefs are available as downloadable pdfs at www.clevelandclinic.org/pathology/