A Brief Overview and Update on Major Molecular Genomic Alterations in Solid, Bone and Soft Tissue Tumors, and Hematopoietic As Well As Lymphoid Malignancies

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• ***Context.***—**Recent advances in comprehensive genomic profiling by next-generation sequencing have uncovered the genomic alterations at the molecular level for many types of tumors; as such, numerous small specific molecules that target these alterations have been developed and widely used in the management of these cancers.**

Objective.—**To provide a concise molecular genomic update in solid, bone and soft tissue tumors, hematopoietic as well as lymphoid malignancies; discuss its clinical applications; and familiarize practicing pathologists with the emerging cancer biomarkers and their diagnostic utilities.**

Data Sources.—**This review is based on the National Comprehensive Cancer Network guidelines and peer-reviewed English literature.**

Conclusions.—**Tumor-specific biomarkers and molecular/genomic alterations, including pan-cancer markers, have been significantly expanded in the past decade thanks to large-scale high-throughput technologies and will continue to emerge in the future. These biomarkers can be of great value in diagnosis, prognosis, and/or targeted therapy/treatment. Familiarization with these emerging and ever-changing tumor biomarkers will undoubtedly aid pathologists in making accurate and state-of-the-art diagnoses and enable them to be more actively involved in the care of cancer patients.**

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Identifying mutations that contribute to carcinogenesis is extremely important in understanding tumor biology. Mutations that give cancer cells a fundamental growth advantage, and thus promote cancer development, are considered driver mutations, and those that do not are considered passenger mutations. Our understanding of critical cancer driver mutations has been considerably deepened since the application of large-scale high-throughput comprehensive genomic profiling. Since many of these driver alterations are potentially actionable, new biomarkers in molecular diagnostics and targeted therapies using small and specific molecules have drastically increased in recent years. Thus, practicing pathologists should familiarize themselves with these emerging biomarkers. They should know not only the appropriate molecular testing to order but also how to interpret the results. In this short review, the authors summarize the current state of knowledge pertinent to the different molecular/genomic alterations and therapeutic targets of interest in solid, bone and soft tissue tumors, hematopoietic as well as lymphoid malignancies.

US FOOD AND DRUG ADMINISTRATION–APPROVED PAN-CANCER MARKERS IN SOLID TUMORS

In the past few years, pan-cancer and multihistology biomarkers have been playing more and more important roles in molecular testing and solid tumor treatment. Large panel comprehensive genomic profiling, including DNA and RNA sequencing, has made it possible to screen for common and various rare targetable alterations simultaneously. Other methods are also available to detect these biomarkers, including but not limited to immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), reverse transcription–polymerase chain reaction (RT-PCR),

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and multiplex PCR. In addition, biomarkers associated with immuno-oncology agents can also be assessed, such as mismatch repair (MMR)/microsatellite instability (MSI) signature and tumor mutation burden (TMB).[1–3]

Microsatellite Instability

Microsatellites are short tandem repeats ranging from 1 to 6 base pairs or more. The MMR proteins MLH1, PMS2, MSH2, and MSH6 can correct the replication errors of these microsatellites, induced by DNA polymerase slippage. Microsatellite instability results from deficient MMR, and MSI-high (MSI-H) tumors are characterized by hypermutations and enriched with frame-shift insertions and deletions. Traditionally, evaluation of MSI status by multiplex PCR for microsatellites and IHC for MMR protein expression has been performed mainly in colorectal cancer (CRC) and uterine endometrioid cancer, according to the recommendation of the Bethesda guidelines,[3] based on patients' age and family history. In 2017, the US Food and Drug Administration (FDA) approved MSI as the first tissue/site-agnostic (pan-cancer) biomarker to predict response to immune checkpoint inhibitor therapies.[1,4] The indications to test MSI include adult and pediatric patients with unresectable or metastatic solid tumors that have progressed after prior treatment and with otherwise no satisfactory alternative treatment options. National Comprehensive Cancer Network (NCCN) updated the list of tumors that need such test, especially universal testing of MMR or MSI in all newly diagnosed patients with colon cancer, and listed next-generation sequencing (NGS) as one of the accepted methodologies (NCCN Guidelines, Version 2.2021, Colon Cancer).[5]

Neurotrophic Receptor Kinase Fusions

Neurotrophic receptor kinase genes (*NTRK*), including *NTRK1*, *NTRK2*, and *NTRK3*, encode the TRK family proteins TrkA, TrkB, and TrkC, respectively. *NTRK* forms an in-frame fusion with partner genes and generates fusion proteins that lead to the activation of TRK kinase domain and become novel targets of tyrosine kinase inhibitors (TKIs). *NTRK* fusions are very prevalent in certain tumors: high frequency (>90%) in secretory carcinomas of the breast and salivary gland, infantile fibrosarcomas, and congenital mesoblastic nephromas; and intermediate frequency (5%–25%) in spitzoid tumors and papillary thyroid carcinoma. *NTRK* fusions are relatively rare (<5%) in non–small cell lung cancer (NSCLC), glioblastoma, gastrointestinal stromal tumor, and others.[6,7] TRK inhibitors demonstrate a high rate and sustainable response in patients positive for *NTRK* fusions.[8] A wide variety of testing methods have been developed and applied clinically in formalin-fixed, paraffin-embedded (FFPE) tissue, such as IHC, break-apart FISH, RT-PCR, DNA- and RNA-based NGS, or DNA/RNA hybrid NGS.[2,9–14] *NTRK* is the second tissue/site-agnostic (pan-cancer) biomarker approved by the FDA, indicated for unresectable or metastatic solid malignancies.

Tumor Mutation Burden

Tumor mutation burden is a quantitative variable and defined as the total number of nonsynonymous mutations per megabase (Mb) of tumor genome. It is associated with the likelihood of tumor neoantigen generation and response to immuno-oncology therapies. Several prospective trials including multiple tumor types showed the predictive value of TMB in the response to immuno-oncology therapies and established its role as a promising biomarker.[15,16] The FDA approved pembrolizumab for adults and children with TMB-high (TMB-H) solid tumors (≥10 mutations/Mb), unresectable or metastatic solid tumors, and also approved FoundationOne CDx assay (Foundation Medicine, Inc, Cambridge, Massachusetts) as a companion diagnostic for pembrolizumab. However, the predictive value of TMB is influenced by multiple factors and varies among different panels.[2] For example, tumor-only sequencing may be compounded by some rare germline variants or somatic mutations from white blood cells (clonal hematopoiesis),[17] while tumor-normal matched sequencing can eliminate these alterations and generate more accurate TMB values. In addition, concurrent mutations from other genes may also affect the predictive value of TMB or indicate the possibility of resistance to immune checkpoint inhibitors. In NSCLC, *EGFR* and *STK11* mutations are associated with poor immuno-oncology therapy response. Loss-of-function mutations in β-2 microglobulin gene (*B2M*) and loss of human leukocyte antigen gene (*HLA*) may also be associated with poor response, regardless of the TMB-H status, since these genomic aberrations may affect specific immune signaling pathways or lead to immune dysregulation.[18–22] Therefore, it is important to consider the testing platform and other concurrent genomic alterations when interpreting the value of TMB.

EMERGING BIOMARKERS IN SOLID TUMORS

BRCA1 and *BRCA2* Mutations

Targeting DNA damage repair signaling has rapidly evolved in cancer therapy for the last decade. Poly (ADP-ribose) polymerase (PARP) is an essential and abundant DNA repair protein that plays an important role in regulation of various DNA damage repair pathways.[23] A PARP inhibitor (PARPi) is used to impair the process of single-strand break repair, consequently converting single-strand breaks into double-strand breaks (DBSs). Therefore, if DBS repair is defective owing to mutations of *BRCA1* and/or *BRCA2*, DBSs would be repaired, and as a result, the cell dies. This is the concept known as synthetic lethality.[24] Originally, PARPi was approved for treating ovarian and breast cancers. Recently, the list was expanded to include pancreatic cancer, prostatic cancer, and others (Table 1).[25] Four PARPis (olaparib, rucaparib, niraparib, and talazoparib) have been approved by the FDA for clinical use as single agent.[26] At the same time, many reports have demonstrated that the presence of a *BRCA* mutation does not always result in PARPi-associated synthetic lethality in treatment-naïve tumors. Cancer cells can also develop resistance to PARPi therapy. Hence, combination therapy may provide better therapeutic benefit than monotherapy.[27]

Fibroblast Growth Factor Receptor Alterations

Fibroblast growth factor receptors (*FGFRs*) form a family of 4 (*FGFR1-4*) highly conserved transmembrane receptor tyrosine kinases, and an additional receptor (*FGFR5*, also known as *FGFRL1*) binds fibroblast growth factor (FGF) ligands but lacks an intracellular kinase domain. The FGF signaling pathway has been implicated in oncogenesis, tumor progression, and resistance to cancer therapy across many tumor types.[28] *FGFR2* mutations were found in ~12% to 14% of endometrial cancers, ~4% of NSCLCs, and gastric cancers.[29–31] *FGFR* gene fusions are also involved in the pathogenesis of human cancer. *FGFR3* fusions are relatively

Table 1. Summary of Emerging Biomarkers in Solid Tumors

Biomarker	Alterations	Testing Methods	Testing Material	Prevalence in Different Tumor Types	FDA-Approved Targeted Therapies and Approved Tumor Type in Parentheses
BRCA1/2	Mutation	PCR, NGS (DNAseq) multiplex ligation-dependent probe amplification	FFPE tissue, liquid biopsy	*BRCA1/2* mutation Breast (∼3%), ovarian (12%–15%), pancreas (∼10%), prostate (∼12%)	Olaparib (breast, ovarian, pancreas, prostate) Rucaparib (ovarian, prostate) Niraparib (ovarian) Talazoparib (breast)
FGFR	Fusion/mutation	FISH, RT-PCR, NGS (DNAseq and RNAseq)	FFPE tissue, liquid biopsy	*FGFR2* mutation Endometrial (12%–14%), NSCLC (∼4%), stomach (∼4%) FGFR2 fusion Intrahepatic CCA (∼14%) FGFR3 fusion Bladder (3%), glioblastoma (∼3%)	Erdafinib (bladder) Pemigatinib (CCA)
HER2/ERBB2	Fusion/mutation	FISH, IHC, NGS (DNAseq)	FFPE tissue, liquid biopsy	*HER2* amplification Breast (15%–20%), GEJ and stomach (15%–20%), colorectal (2%–3%) HER2 mutation Colorectal (∼2%)	Transtuzumab (breast, stomach, and GEJ) Pertuzumab (breast) Ado-trastuzumab emtansine (breast) Fam-trastuzumab deruxtecan (breast) Laptinib (breast) Afatinib (breast)
RET	Fusion/mutation	FISH, RT-PCR, NGS (DNAseq and RNAseq)	FFPE tissue, liquid biopsy	RET fusion NSCLC (1%–2%) Thyroid (include MTC, 10%–20%) RET mutation MTC (25%–40%)	Selpercatinib (NSCLC, thyroid)
TERTp	Mutation	NGS (DNAseq and RNAseq)	FFPE tissue	*TERTp* mutation Oligodendroglioma (80%), bladder (70%), HCC (50%), thyroid (40%)	None
VEGF	Transcriptional dysregulation	N/A	N/A	ccRCC (N/A)	Sorafenib (ccRCC) Sunitinib (ccRCC)

Abbreviations: CCA, cholangiocarcinoma; ccRCC, clear cell renal cell carcinoma; DNAseq, DNA sequencing; FDA, US Food and Drug Administration; FFPE, formalin-fixed, paraffin-embedded; FGFR, fibroblast growth factor receptor; FISH, fluorescence in situ hybridization; GEJ, gastroesophageal junction; HCC, hepatocellular carcinoma; *HER2*, human epithelial growth factor receptor 2; IHC, immunohistochemistry; MTC, medullary thyroid cancer; N/A, not applicable; NGS, next-generation sequencing; NSCLC, non–small cell lung cancer; PCR, polymerase chain reaction; RNAseq, RNA sequencing; RT-PCR, reverse transcription–polymerase chain reaction; TERTp, telomerase reverse transcriptase promoter; VEGF, vascular endothelial growth factor.

common in glioblastoma (∼3%) and urothelial carcinoma (∼3%), with rare reports in lung cancer. The most common partner for *FGFR3* is *TACC3* (transforming acidic coiled-coil containing protein 3). *FGFR2* fusions have several fusion partners and are found in ∼14% of intrahepatic cholangiocarcinomas (iCCAs), and rarely in lung, thyroid, and prostate cancers (Table 1).[28–30] Two selective FGFR-TKIs have been approved to date by the FDA for the treatment of *FGFR*-driven cancers. Erdafinib was approved in 2019 for the treatment of patients with locally advanced or metastatic urothelial carcinoma with *FGFR3* or *FGFR2* genetic alterations, including R248C, S249C, G370C, and Y373C mutations and *FGFR3-TACC3* fusions.[32] Pemigatinib, a selective, potent, oral inhibitor of *FGFR1*, *FGFR2*, and *FGFR3*, was granted FDA-accelerated approval in 2020 for the treatment of patients with iCCA and *FGFR2* fusions.[33]

Human Epidermal Growth Factor Receptor 2 Amplification and Mutations

The human epidermal growth factor receptor (*HER*) family proteins are type I transmembrane growth factor receptors that function to activate intracellular signaling pathways in response to extracellular signals. The *HER* family has 4 members: *EFGR* (*HER1*, *ERBB1*), *HER2* (*ERBB2*, *HER2/neu*), *HER3* (*ERBB3*), and *HER4* (*ERBB4*). Altered *ERBB2* signaling caused by genomic amplification of *ERBB2* or mutations is oncogenic and has been observed in multiple cancer types.[34,35] *ERBB2* amplification was observed in 15% to 20% of breast cancers and a similar proportion of gastric and gastroesophageal junction (GEJ) adenocarcinomas (Table 1). Trastuzumab (Herceptin, Genetech), a therapeutic antibody targeting this receptor and approved by the FDA in 1998, was the first monoclonal antibody approved for the treatment of a solid tumor. It was further approved for the treatment for either metastatic cancer of the stomach or gastroesophageal junction GEJ adenocarcinoma in 2010. Lately, several more anti-*ERBB2* agents have been approved for breast cancer, including pertuzumab, ado-trastuzumab emtansine, fam-trastuzumab deruxtecan, as well as pan-*ERBB* small-molecule inhibitors lapatinib or afatinib.[36–38]

Additionally, *ERBB2* amplification and activating mutations were also seen in other solid tumor types such as NSCLC and CRC albeit at a lower frequency. Approximately 5% of CRCs harbor *ERBB2* alterations, including amplifications and mutations; among them, 2% to 3% are amplifications (Table 1).[39] Most *ERBB2* mutations were known activating mutations, which were considered as true driver mutations. To date, there are no agents that have been approved by the FDA for CRC; however, there are multiple multicenter clinical trials that are currently underway. The current NCCN guidelines recommend anti-*ERBB2*–targeted therapy to *RAS* wild-type, *ERBB2*-amplified metastatic CRC, based on the results of the HERACLES-A trial.[40]

Rearranged During Transfection Fusions and Mutations

While the rearranged during transfection gene (*RET)* was mutated in approximately 80% of medullary thyroid cancers, its fusions have been detected in papillary thyroid carcinoma, NSCLC, and a range of other tumor types at lower frequencies (Table 1).[41–44] The *RET* fusions in NSCLC were enriched in nonsmokers lacking other known driver mutations[45,46] and defined a new therapeutic target in this subset of lung cancers, especially with the availability of selective *RET* inhibitor selpercatinib, which demonstrated durable response and increased progression-free survival.[47,48] The FDA has approved Retevmo (selpercatinib, Eli Lilly and Company) for treating *RET* fusion–positive NSCLC, *RET* fusion–positive thyroid cancer, and *RET*-mutant medullary thyroid cancer.[49,50]

TERT Promoter Mutations

Two hotspot mutations in the *TERT* promoter (*TERTp*) region, c.124C>T (C228T) and c.146C>T (C250T), were first discovered in melanoma with ~70% prevalence.[51,52] They generated de novo binding sites for E-twenty-six (ETS) transcription factors resulting in increased *TERT* transcription. Subsequently, many studies demonstrated presence of *TERTp* mutations in 80% of oligodendrogliomas, 70% of urothelial carcinomas, 50% of hepatocellular carcinomas (HCCs), and 40% of thyroid carcinomas (Table 1).[53] The incidence of *TERTp* mutations was very low in common cancers originating from lung, colon, prostate, and breast.[53] The anti-*TERT* therapy is challenging and remains premature currently. However, the detection of urine *TERTp* promoter mutations, which can be used as simple and inexpensive noninvasive biomarkers for the early detection of bladder cancer, is very attractive and promising.[54,55]

Vascular Endothelial Growth Factor

The vascular endothelial growth factor (*VEGF*) inhibitors as targeted therapy for clear cell renal cell carcinoma could be directly traced back to the discovery of the *VHL/HIF/VEGF* axis as a central mediator of hypoxia signaling, for which the 2019 Nobel Prize was awarded.[56] There are several FDA-approved *VEGF* inhibitors, including sorafenib (Nexavar, Bayer and Onyx Pharmaceuticals) and sunitinib (Sutent, Pfizer).[57] The *VEGF* inhibitors could be used as monotherapy or combination therapy with other VEGF inhibitors, mTOR inhibitors, or immune checkpoint inhibitors.[58] Unfortunately, there are currently no predictive biomarkers/tests that could guide the best therapy for the appropriate patient.[59] The pathologic diagnosis of clear cell renal cell carcinoma is the most important criterion for *VEGF* inhibitor treatment (Table 1).[60]

The tumor-specific prevalence and FDA-approved targeted therapies of these emerging biomarkers are summarized in Table 1.

UPDATES OF MOLECULAR GENOMIC ALTERATIONS IN BONE AND SOFT TISSUE TUMORS

Bone and soft tissue tumors are not common, and they encompass a remarkably diverse spectrum of benign and malignant entities. In the last couple decades, molecular genetic abnormalities have been shown to be an important, sometimes necessary adjunct for diagnosis and therapeutics of these neoplasms.[61–63] In general, from the genomic alterations, bone and soft tissue tumors can be roughly classified into 2 categories: those neoplasms with relatively simple, consistent, and recurrent genetic aberrations (Table 2 for bone tumors, Table 3 for soft tissue tumors) and those high-grade sarcomas with complex, nonspecific cytogenetic profiles.[64–68]

These specific recurrent genomic alterations often lead to changes in transcription and altered signaling pathways. Many of these simple genetic alterations and tumor-specific biomarkers can be detected by commonly used methods in real practice, including IHC, FISH, multiplex PCR, and NGS. Knowledge of these genetic abnormalities and methods is useful for rendering an accurate diagnosis in daily practice. For example, the differential diagnoses for a lytic bone lesion with giant cell–rich histology might include giant cell tumor of bone, chondroblastoma, or aneurysmal bone cyst to list a few. Immunohistochemistry study using mutant-specific antibodies of histone H3F3 pG34W and pK36M would help distinguish giant cell tumor of bone from chondroblastoma (Table 1), while a positive FISH test result for *USP6* gene rearrangement would help render a diagnosis of aneurysmal bone cyst.[69] Recently, a number of molecularly defined rare soft tissue neoplasms have emerged and continue to expand, including *EWSR1-SMAD3*–positive fibroblastic tumors and neurotrophic tyrosine receptor kinase (*NTRK*)-rearranged spindle cell neoplasms.[63,70] Kao et al[71] recently described a subset of soft tissue tumors with *NTRK1* rearrangements as "lipofibromatosis-like neural tumors." They are characterized by mildly atypical spindle cells with infiltrative growth pattern in the subcutis and expression of S100 and CD34. Other mesenchymal tumors with fusions involving *NTRK2* and *NTRK3* rearrangement also have a wide spectrum of morphologic, immunophenotypic features and often carry a variable risk of malignancy.[72] Recognizing these new entities might be quite challenging, and detection of these kinase fusions genetically is often required for definitive diagnosis. Some patients may develop lung metastases, and they can be treated with tyrosine receptor kinase inhibitors.

A major group of high-grade bone and soft tissue sarcomas, including undifferentiated pleomorphic sarcomas, leiomyosarcomas, angiosarcomas, high-grade osteosarcomas, and chondrosarcomas, are genetically heterogeneous. They often harbor complex karyotypes, without recurrent genetic alterations. Clinical genetic tests are not generally helpful for differential diagnosis. However, massive parallel sequencing of primary bone and soft tissue tumors has revealed the full spectrum of driver gene alterations, and these findings can be used for the differential diagnosis in many given challenging cases. For example, most high-grade chondroblastic osteosarcomas can be distinguished from other histologic mimics such as chondrosarcoma,

Table 2. Bone Tumors With Simple Genetic Alterations and Available Diagnostic Tests		
Tumor	**Genetic Alteration**	**IHC/FISH With Targeted Genes in Parentheses**
Osteochondroma	*EXT1/2*	
Osteoblastoma/osteoid osteoma	*FOS/FOSB* rearrangement	IHC (FOSB)/FISH
Enchondroma	*IDH1/2*	
Chondroblastoma	*H3F3B pK36M* mutation	IHC (H3F3 K36M)
Synovial chondromatosis	*FN1-ACVR2A*	FISH
Chondromyxoid fibroma	*GRM1* rearrangement	FISH
Extraskeletal myxoid chondrosarcoma	*EWSR1-NR4A3*	FISH (ESWR)
Mesenchymal chondrosarcoma	*HEY1-NCOA2*	FISH
	IRF2BP2-CDX1	
Fibrous dysplasia	*GNAS1* mutation	
Well-differentiated osteosarcoma	*MDM2*, *CDK4* amplification	IHC (MDM2), FISH
Giant cell tumor of bone	*H3F3A pG34W* mutation	IHC (H3F3 G34W)
Aneurysmal bone cyst	*USP6* rearrangement	FISH (*USP6*)
Bizarre parosteal osteochondromatous proliferation	t(1;17)(q32;q21); inv(7); inv(6)	
Subungual exostosis	*COL12A1* and *COL4A5*	
Parosteal lipoma	*HMGA2*	
Small round cell tumors of bone and soft tissue	*EWSR1-FLI1*	FISH
	EWSR1–non-*ETS* fusions	
	CIC-rearranged	
	BCOR	

Abbreviations: ACVR2A, activin A receptor type 2A; BCOR, BCL6 corepressor; CIC, capicua transcriptional repressor; COL12A1, collagen type XII α-1 chain; COL4A5, collagen type IV α-5 chain; ETS, transcription factor Ets; EWSR1, Ewing sarcoma RNA-binding protein 1; EXT1/2, exostosin-1/2; FISH, fluorescence in situ hybridization; FLI1, Friend leukemia integration 1; FN1, fibronectin 1; FOS/FOSB, FBJ murine osteosarcoma viral oncogene homolog/B; GNAS1, guanine nucleotide–binding protein, α stimulating; GRM1, glutamate metabotropic receptor 1; HEY1, hes-related family bHLH transcription factor with YRPW motif 1; HMGA2, high-mobility group AT-hook 2; H3F3A/B, H3.3 histone A/B; IDH1/2, isocitrate dehydrogenase 1/2; IHC, immunohistochemistry; inv, inversion; MDM2, mouse double minute 2; NCOA2, nuclear receptor coactivator 2; NGS, next-generation sequencing; NR4A3, nuclear receptor subfamily 4 group A member 3; RT-PCR, reverse transcription–polymerase chain reaction; t, translocation; USP6, ubiquitin-specific peptidase 6.

which possesses *IDH1/2* mutations,[73] and/or *COL2A1* mutations.[74] Giant cell–rich osteosarcoma can be distinguished from a subset of malignant giant cell tumors, which harbor mutant H3.3 G34W that can be assessed by IHC.[75]

MOLECULAR GENOMIC PATHOLOGY UPDATES OF HEMATOPOIETIC AND LYMPHOID MALIGNANCIES

In addition to the recurrent cytogenetic abnormalities and common gene mutations in acute myeloid leukemia (AML), outlined in the revised 4th edition of the World Health Organization (WHO) *Tumours of Haematopoietic and Lymphoid Tissues*,[76] comprehensive genomic profiling in AML has shown a number of genes commonly altered in AML, which can be subdivided into the following 8 functional categories: (1) signaling genes including *KIT*, *FLT3*, *PTPN11*, *KRAS*, and *NRAS*; (2) DNA methylation–associated genes including *DNMT3A*, *TET2*, *IDH1*, and *IDH2*; (3) myeloid transcription factor gene fusions including t(8;21) and inv(16)/t(16;16) or transcription factor mutations (*RUNX1*, *CEBPA*); (4) nucleophosmin gene 1 (*NPM1*); (5) tumor-suppressor genes including *TP53*, *WT1*, and *PHF6*; (6) chromatin-modifying gene mutations in *ASXL1* and *EZH2* or *KMT2A* fusions; (7) spliceosome-complex genes *SRSF2*, *SF3B1*, *U2AF1*, and *ZRSR2*; and (8) cohesin-complex genes *STAG2* and *RAD21*68. Among these genes, the mutational analysis of *FLT3*, *NPM1*, and *CEBPA* has shown improvement in risk stratification for patients who do not have karyotypic abnormalities. Therefore, the current WHO classification recognizes AML with mutated *NPM1* and AML with biallelic mutation of *CEBPA* as specific AML classification categories, and AML with mutated *RUNX1* as a provisional entity.[76]

While European LeukemiaNet recommends PCR assessment of minimal/measurable residual disease (MRD) in patients with *PML-RARA*, *RUNX1-RUNX1T1*, *CBFB-MYH11*, or *NPM1*-mutated AML,[77] the presence of persistent *DTA* mutations (mutations in *DNMT3A*, *TET2*, *ASXL1*), which are frequently found in age-related clonal hematopoiesis of indeterminate potential, does not correlate with relapse. Furthermore, mutations in *FLT3-ITD*, *FLT3-TKD*, *KRAS*, *NRAS*, *IDH1*, *IDH2*, and *MLL-PTD*, should not be used as single MRD markers. These markers may be useful when combined with a second MRD marker.

In contrast to the nonrandom balanced translocations in AML, complex karyotypes are frequent in myelodysplastic syndrome (MDS). *TP53* mutations are often found in cases with complex karyotype and predict poor clinical outcomes. Myelodysplastic syndrome often shows gene mutations overlapping with AML, since both are myeloid neoplasm; however, more frequently observed in MDS are those affecting epigenetic regulation and RNA splicing, including DNA methylation (*TET2*, *DNMT3A*, and *IDH1/IDH2*),[78,79] chromatin/histone modification (*KMT2D*, *EZH2*, *PRC2*, *ARID2*, and *ASXL1*),[80] and RNA splicing (*SF3B1*, *SRSF2*, *U2AF1*, *U2AF2*, *ZXRSR2*, *SF1*, and *SF3A1*).[81] Mutations in *DDX41*, *RUNX1*, *GATA2*, and *TP53* can be present in the germline and responsible for AML and MDS predisposition.

Great progress has been made in the field of B-acute lymphoblastic leukemia (B-ALL) in the last 2 decades. For example, gene expression profile has helped identify a distinct subtype of B-ALL with no Philadelphia (*Ph*) chromosome [t(9;22)(q34.1;q11.2)] but that resembles *Ph*-positive cases with poor prognosis, thus this category of B-ALL is called *Ph*-like ALL and is associated with a high risk

Table 3. Soft Tissue Tumors With Simple Genetic Alterations and Available Diagnostic Tests		
Tumor	**Genetic Alteration**	**IHC/FISH With Targeted Genes in Parentheses**
Lipoma	*HMGA2*	
Lipoblastoma	*PLAG1* fusion	FISH (PLAG1)
Chondroid lipoma	t(11;16) (*C11orf95-MRTFB*)	
Spindle cell/pleomorphic lipoma	Loss of *RB1*	IHC RB1 loss
Atypical lipomatous tumor Well-differentiated/dedifferentiated liposarcoma	*MDM2*, *CDK4* amplification	IHC and FISH (MDM2)
Myxoid liposarcoma	*FUS-DDIT3* *EWSR1-DDIT3*	FISH
Nodular fasciitis	*MYH9-USP6*	FISH (USP6)
Myositis ossificans	*USP6* rearranged	FISH (USP6)
Desmoid fibromatosis	*APC* or *CTNNB1* mutation	
Angiofibroma of soft tissue	*AHRR-NCOA2*	
EWSR1-SMAD3–positive fibroblastic tumor (emerging)	*EWSR1-SMAD3*	FISH
NTRK-rearranged spindle cell neoplasm	*NTRK* rearranged	
Solitary fibrous (emerging)	*NAB2-STAT6*	IHC (STAT6)
Dermatofibrosarcoma protuberans	*COL1A1-PDGF-B*	FISH
Infantile fibrosarcoma	*NTRK* rearranged	FISH/IHC
Inflammatory myofibroblastic tumor	*TPM3-ALK*	FISH/IHC (ALK)
Low-grade fibromyxoid sarcoma	*FUS-CREB3L2/ESWR1*	FISH (FUS/ESWR1)
Sclerosing epithelioid fibrosarcoma	*EWSR1/FUS* rearranged	FISH (EWSR1/FUS)
Alveolar rhabdomyosarcoma	*PAX3-FOXO1A* *PAX7-FOXO1A*	FISH
Angiomatoid fibrous histiocytoma	*FUS-ATF1* *EWSR1-ATF1*	FISH
Alveolar soft part sarcoma	*TFR3-ASPL*	FISH
Clear cell sarcoma	*EWSR1-ATF1*	FISH
Infantile fibrosarcoma	*ETV6-NTRK3*	FISH
Synovial sarcoma	*SS18-SSX1*	FISH
Gastrointestinal stromal tumor	*KIT, PDGFRA, BRAF* mutation	
Endometrial stromal sarcoma	*JAZF1-JJAZ1*	FISH
Epithelioid hemangioma	*FOS/FOSB* rearranged	FISH/IHC (FOS/FOSB)
Epithelioid hemangioendothelioma	*WWTR1-CAMTA1* *YAP1-TFE3*	FISH/ IHC (TFE3, CAMTA1)
Pseudomyogenic hemangioendothelioma	*FOSB* rearranged	FISH/IHC (FOSB)
Myopericytoma	*PDGFRB*	
Desmoplastic small round cell tumor	*EWSR1-WT1*	FISH

Abbreviations: ALK, anaplastic lymphoma kinase; ASPL, alveolar soft part sarcoma locus; ATF1, cyclic AMP–dependent transcription factor 1; COL1A1, collagen type I α-1; CREB3L2, cyclic AMP–responsive element-binding protein 3–like protein 2; DDIT3, DNA damage–inducible transcript 3; ETV6, ETS variant transcription factor 6; EWSR1, Ewing sarcoma RNA-binding protein 1; FISH, fluorescence in situ hybridization; FOS/FOSB, FBJ murine osteosarcoma viral oncogene homolog/B; FOXO1A, forkhead box o1; FUS, fused in sarcoma; HMGA2, high-mobility group AT-hook 2; IHC, immunohistochemistry; MDM2, mouse double minute 2; MYH9, myosin heavy chain 9; NGS, next-generation sequencing; NTRK, neurotrophic tyrosine receptor kinase; PAX3/7, paired box gene 3/7; PDGFB, platelet-derived growth factor subunit B; PLAG1, pleiomorphic adenoma gene 1; RT-PCR, reverse transcription–polymerase chain reaction; SMAD3, mothers against decapentaplegic homolog 3; SS18, synovial sarcoma 18 subunit; SSX1, synovial sarcoma X family member 1; TFE3, transcription factor E3; TPM3, tropomyosin α-3 chain; USP6, ubiquitin-specific peptidase 6; WT1, Wilms tumor 1 transcription factor.

of relapse and frequently harbors *IKZF1* alterations. *Ph*-like B-ALL is a heterogeneous disease and is further subdivided into 5 subgroups by the type of cytokine receptor or kinase fusion present: (1) rearrangements of cytokine receptor–like factor 2 (*CRLF2*) in ~50% of the cases; (2) *ABL*-class fusions; (3) *EPOR* or *JAK2* rearrangements; (4) other *JAK-STAT*–activating mutation; (5) *Ras* only; and (6) no kinase alteration.[82] Recently, 2 new subtypes of *Ph*-like B-ALL, namely PAX5alt and PAX5 P80R, were discovered by integrated genomic analysis.[83] Despite these genetic complexities, most *Ph*-like ALL cases show targetable mutations involving ABL class or *JAK-STAT* signaling pathways. The success of Gleevac (Novartis) for chronic myeloid leukemia (CML) and combining TKIs with chemotherapy for *Ph*-positive ALL have inspired clinical trials incorporating relevant TKIs including *ABL* inhibitors (imatinib or dasatinib) and *JAK* inhibitors (ruxolitinib) in *Ph*-like ALL.[84] Based on the experience with TKI therapy in CML and *Ph*-positive ALL, future challenges in *Ph*-like ALL will include possible drug-resistant kinase domain mutations after long-term TKI exposure.

Whole exome sequencing (WES) of peripheral T-cell lymphomas (PTCLs) identified recurrent epigenetic factor mutations in *TET2*, *DNMT3A*, and *IDH2* as well as highly prevalent Gly17Val mutation in *RHOA*, a small GTPase that regulates diverse biological processes, in angioimmunoblastic T-cell lymphoma, adding to the molecular heterogeneity of PTCLs.[85] More recent studies revealed recurrent genetic abnormalities of *KMT2C*, *SETD1B*, *YTHDF2*, and *PDCD1* in PTCL, not otherwise specified (NOS), with or

without T-follicular helper (TFH) phenotype.[86] Mutually exclusive from TFH-related subtype is *TP53/CDKN2A*-altered subtype, represented by loss-of-function alterations in transcription factor *IKZF2* and immune surveillance pathways including components of the class I major histocompatibility complex (MHC) (HLA-A and HLA-B), MHC class II transactivator (CIITA), immune checkpoints (CD274), cell adhesion molecules (CD58), and death signaling (FAS).[85] Less frequently, mutations in *FYN*, *ATM*, *B2M*, and CD58 implicating SRC signaling, which impairs DNA damage response with resultant escape from immune surveillance mechanisms, also contribute to the pathogenesis of PTCL-NOS.[85] Gene expression profile helped to define 2 prognostically relevant molecular subgroups within PTCL-NOS: *PTCL-GATA3* and *PTCL-TBX21. PTCL-GATA3* is characterized by frequent loss (by copy number abnormalities) or mutation of tumor suppressor genes targeting the *CDKN2A/B-TP53* axis and *PTEN-PI3K* pathways. Gain/amplification of *STAT3* and *MYC* can also occur in PTCL-GATA3. PTCL-TBX21 shows mutations of genes regulating DNA methylation and fewer copy number abnormalities, primarily targeting cytotoxic effector genes. The presence of these distinct genetic pathways in subgroups of PTCL-NOS provides biological rationale for targets in future clinical trials.[87]

Whole exome sequencing has also helped to decipher the pathogenesis of organ/system-specific rare T-cell lymphomas, notably hepatosplenic T-cell lymphomas (HSTCLs) and primary T-cell lymphomas of the gastrointestinal tract. Whole exome sequencing has revealed that 62% of HSTCLs harbor chromatin-modifying genes including *SETD2*. In addition, HSTCL also possesses *STAT5B* mutations.[88] By the same token, WES has unveiled that *SETD2* is the most commonly silenced gene in monomorphic epitheliotropic intestinal T-cell lymphoma (formerly known as enteropathy-associated T-cell lymphoma II), and *JAK-STAT* pathway is the most frequently mutated pathway.[89]

Molecular genomic advances using WES, whole genome sequencing (WGS), and epigenomic/methylation analysis have further refined mature B-cell lymphomas/leukemias. In chronic lymphocytic leukemia (CLL), WES and WGS have revealed recurrent commonly mutated genes including kelch-like 6 (*KLHL6*), *NOTCH1*, myeloid differentiation primary response gene 88 (*MYD88*), *POT1*,[90] exportin 1 (*XPO1*),[91] and *SF3B1*.[92] These new molecular/genomic findings have advanced our understanding of CLL beyond the 2 molecularly and clinically distinct subtypes of CLL, based on the presence or absence of somatic hypermutation of the immunoglobulin heavy chain variable (IGHV) regions. *TP53* mutations associate with unmutated *IGHV* gene with worse prognosis in CLL.[93] While *MYD88* L265P was present in other B-cell lymphomas, lymphoplasmacytic lymphoma (LPL) and immunoglobulin M (IgM) monoclonal gammopathy of undetermined significance (MGUS) account for more than 90% and more than 50% of *MYD88* L265P mutations, thus IgM MGUSs have been separated from other non-IgM MGUSs in the current WHO classification.[76] Of note, primary central nervous system large B-cell lymphoma had higher incidence (68.8%) of *MYD88* mutations including L265P.[94] *BRAF* V600E was present in virtually 100% of hairy cell leukemia (HCL), but virtually absent in HCL variant. With regard to diffuse large B-cell lymphoma (DLBCL), genomic profiling using model-based clustering of genetic mutations[95] has defined 5 subtypes, namely *BCL2* (good prognosis), *NOTCH2* (intermediate prognosis), *MYD88* (poor prognosis), *SOCS1/SGK1*, and *TET2/SGK1. TP53* mutations in the *MYD88* subtype confer even poorer prognosis but no effect in the *NOTCH2* subtype.

In summary, our knowledge and understanding of new molecular genomic alterations are rapidly evolving and ever-changing. These new understandings help us not only refine or define known entities, but also unearth new ones. Powered by the rapid and high-throughput sequencing technology and epigenetic/methylation analysis, our understanding of the pathogenesis of tumors is advancing rapidly and becoming more informative. The ultimate combination and integration of genetic and epigenetic landscapes will guide more precise and personalized management of cancers of all types and from all organs.

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