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Advances in the Molecular Analysis of Soft Tissue Tumors and Clinical Implications

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SYNOPSIS

The emergence of high-throughput molecular technologies has accelerated the discovery of novel diagnostic, prognostic and predictive molecular markers. Clinical implementation of these technologies is expected to transform the practice of surgical pathology. In soft tissue tumor pathology, accurate interpretation of comprehensive genomic data provides useful diagnostic and prognostic information, and informs therapeutic decisions. Most clinical molecular data will be likely generated by high-throughput genomic technologies, and reporting clinically actionable somatic mutations will be part of the routine pathology workflow, informing patient management and treatment. The purpose of this review is to introduce recently developed molecular technologies and to briefly discuss their potential for clinical implementation, focusing on various applications to the evaluation of soft tissue tumors. A special emphasis is made on practical diagnostic uses relevant to the surgical pathologist, with illustrative examples of how these technologies have helped characterize and diagnose soft tissue lesions. The concept of genomically-informed therapies will be mentioned, as an essential motivation to implement molecular tests to identify targetable molecular alterations in sarcoma.

Keywords

molecular diagnostics; targeted therapy; soft tissue tumor; sarcoma

INTRODUCTION

Recent technological advances have dramatically changed the molecular analysis of tumor samples. The development of high throughput genome-wide analytical methods, notably massively parallel sequencing technologies and associated computational algorithms, provides the ability to generate comprehensive profiles from tumor cells and tissues at several biological levels – such as genome, epigenome, or transcriptome. Such an abundance of data is transforming molecular pathology and oncology into data-intensive sciences, a transformation that is expected to have a tremendous impact in many aspects of clinical practice. From a scientific and research perspective, these technologies are perceived as disruptive, revolutionary¹ or paradigm-changing,² and have undoubtedly generated an

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impressive body of knowledge, contributing to a much improved understanding of tumor biology. In the field of diagnostic pathology of soft tissue tumors, however, the overall impact has been rather incremental, and represents the natural continuation of pioneer molecular genetic studies evolving over the last 40 years from classical cytogenetics,³ DNA hybridization techniques and FISH⁴ and PCR-based single gene assays⁵. Arguably, the biggest innovation is the accelerated pace of advances and the large volume of novel information, both truly unprecedented.

The purpose of this review is to introduce recently developed molecular technologies and to briefly discuss their potential for clinical implementation (**Table 1**), focusing on their various applications to the evaluation of soft tissue tumors. A special emphasis is made on practical diagnostic uses relevant to the surgical pathologist, with illustrative examples of how these newer technologies have helped characterize and diagnose soft tissue lesions. Finally, the concept of genomically-informed therapies in sarcoma will be mentioned, as an essential motivation to implement molecular tests to identify potentially targetable alterations. An orientative glossary of terms widely used in this field is provided in **Box 1**.

APPLICATIONS OF MASSIVELY PARALLEL SEQUENCING TECHNOLOGIES

Massively parallel sequencing (so-called *Next-generation sequencing* or NGS)

Massively parallel sequencing (MPS) technologies allow for high throughput multiplex detection of a wide range of genetic aberrations, including single nucleotide changes (SNVs), copy number alterations (CNA), insertions and deletions (*indels*) and chromosomal rearrangements.⁶ Different sequencing platforms vary in their enzymology, chemistry, signal detection, instrument, and software, variables that determine their relative strengths and limitations for each application (the specifics are beyond the scope of this manuscript, and have been extensively described in several excellent reviews).^{7,8} For all platforms, the initial step is to extract DNA or RNA molecules from the sample, break them down to fragments of a relatively homogeneous size (usually 100-300 bp long), and ligate molecular adaptors to each end to produce a library that contains the nucleic acid fragments of interest (library preparation). The fragments are then separated and immobilized on a glass surface or on synthetic beads, which permits the use of PCR amplification to create spatially distinct clusters with many identical copies of each fragment (although not all technologies use amplification steps). Multiple sequencing-by-synthesis reactions occur simultaneously, in parallel, on each of these clusters, in a cyclical process that is closely monitored by a detection system able to identify the nucleotides that are being incorporated to the template, recording their sequence as individual reads.⁹ Frequently, sequencing starts inbound from both ends of the linear target molecules, which generates *paired-end* sequencing reads that provide advantages over single end reads for mapping fragments to the reference. To maximize the mapping benefits of paired-end sequencing, input DNA may be fragmented into larger, 2-5Kb segments and then circularized, to generate rearranged DNA stretches consisting of fragments originally distant (*mate-pairs*), which provide increased ability to discover structural variation.¹⁰ Differences between platforms on each of these steps determine their performance characteristics in terms of sensitivity, accuracy, read length,

throughput, run time, coverage, error modes and cost.^{11,12} In all cases, several critical steps of computational processing, data analysis and automated variant calling¹³ are required to generate a clinically relevant report that may be useful for patient management.

MPS technologies can probe different input nucleic acid molecules extracting information from the genome (DNA), transcriptome (RNA, large and small, coding and noncoding), epigenome (methyl-Seq) or chromatin (ChIP-Seq), all widely used in research settings but at different levels of implementation in clinical laboratories. The amount of input material required for successful analysis is remarkably low, in the 0.1-1ug range for most platforms and applications, which can be obtained from a few FFPE tissue sections (one 20-50um thick tissue section, with >20% of tumor cells). The degradation resulting from routine FFPE tissue processing is not a significant problem for DNA-based applications; using RNA is more demanding, but continuous improvements in reagents chemistry and extraction protocols generate good quality libraries with appropriate representation in most instances.

Cancer genome sequencing

In clinical laboratories, the prevailing application of MPS technologies is sequencing different extensions of tumor genomic DNA to identify somatic aberrations. The potential advantages of a genome-wide comprehensive approach, *whole genome sequencing*, are outweighed thus far by the challenges regarding data analysis and storage, and clinical interpretation. Similarly, capturing and sequencing all protein-coding DNA segments, *exome sequencing*, is felt to generate an excess of information of questionable clinical significance, at the expense of increased analytical challenges. Hence, most clinical cancer sequencing tests involve a panel of selected cancer-related genes (*targeted cancer gene panels*), of which every exon and some introns are covered at high reading depth (usually several hundred reads), which ensures robust detection of sequence changes and manageable data analysis. It is worth noting that *any* alterations present in the sequence are detected, not just a predefined set, in contrast with so-called “genotyping” technologies (allelic-specific assays).

The output of MPS-based cancer genome sequencing tests is a multipart, usually complex report, listing at least three types of somatic alterations: 1) nucleotide variants (SNVs) and their allelic frequency, usually classified according to the predicted biological effects on the protein (missense, nonsense, silent); 2) copy number changes, an estimate of the copy number status of the locus of each gene, based on the number of reads of each position and the local and overall coverage; and 3) chromosomal rearrangements, by virtue of paired-end sequencing and the inclusion of intronic regions, which allow some fragments to be mapped to distant regions of the genome predicting a rearrangement. All these alterations are reported using a tier-based classification system that groups them according to the level of evidence supporting their clinical relevance.¹⁴ Positive or pertinent negative findings that may affect patient management are emphasized, while changes of unknown significance are merely recorded. In this setting, the concept of “actionable mutation” has been defined as an alteration that has diagnostic, prognostic or therapeutic implications in a given clinical context.¹⁵ A *PDGFRA* exon 12 mutation detected in a gastric epithelioid mesenchymal tumor biopsy, for example, would be an actionable mutation supporting a diagnosis of gastrointestinal stromal tumor, with a relatively good prognosis, predicting moderate

sensitivity to imatinib. As the biological understanding of cancer pathogenesis progresses, and novel targeted therapies are developed, the classification of each mutation will vary with more variants increasingly being considered clinically actionable.

Targeted sequencing (cancer gene panels and exome sequencing)

Different techniques, such as hybrid capture or selective circularization, allow for target enrichment during library preparation,¹⁶ so only portions of the genome are sequenced. The selection of targets can range from tens or hundreds of genes to whole exome, resulting in the exclusion of >98% of the genomic DNA. This allows for much greater depth of sequence coverage, providing excellent sensitivity for detection of even low-abundance SNVs. Also, amplifications and small *indels* can be accurately estimated with targeted approaches. The sensitivity for detection of medium to large-sized deletions and importantly, chromosomal rearrangements, is much lower despite continuous improvements in bioinformatic algorithms.¹⁷

The difficulty in detecting chromosomal rearrangements by sequencing targeted cancer gene panels is particularly inconvenient for the field of soft tissue tumor pathology; the inclusion of intronic sequences, as well as specific computational approaches may improve the detection of select rearrangements,¹⁸⁻²⁰ but the sensitivity is still low. This technical feature is, arguably, the main limitation of current DNA-based targeted sequencing approaches, severely limiting its diagnostic value for soft tissue tumors. Despite these limitations, anecdotal evidence indicates that identification of diagnostic gene fusions is possible using targeted cancer panels, as dramatically illustrated by the case of a 42 year old patient treated at our institution of a pulmonary lesion with clinical and morphologic features consistent with an atypical carcinoid tumor, correctly diagnosed as Ewing sarcoma by targeted MPS.²¹

Several institutions have developed customized gene panels for targeted sequencing, which are rapidly evolving as new knowledge becomes available.²² Some panels are organ or system-specific, while others are “pan-cancer” tests (**Table 2**). The largest in-house pan-cancer panels typically involve 100-300 genes, including known oncogenes and tumor suppressors. The overlap between the gene sets covered by each panel is remarkably limited (i.e. different panels interrogate different genes),²³ which reflects the lack of standardization in these early days, and speaks for the difficulty to define a clinically relevant cancer gene census.

Exome sequencing (often designated *whole exome sequencing* or *WES*, to emphasize its comprehensive nature) has the obvious advantage of probing a much larger portion of the genome, its ~300,000 exons, including all the cancer related genes present in focused panels. The reduced coverage that results may be problematic, but only in limited samples with very low tumor content. The technique is becoming increasingly facile and cost effective, and some groups have demonstrated its feasibility in a clinical environment in terms of turnaround time and other performance metrics.²⁴ In the research setting, exome sequencing has led to the identification of fusions such as *NAB2-STAT6* in solitary fibrous tumor.²⁵ The large volume of information generated by exome sequencing, however, and the computational and analytical challenges associated with the interpretation of the results have limited its clinical implementation thus far. Efforts to simplify and facilitate the clinical

implementation of exome sequencing include interesting analytical algorithms and clinical decision support tools that harness the potential of publicly available knowledge bases using user friendly interfaces and leading to interactive reports.^{24,26}

For soft tissue tumors, point mutations and *indels* detectable by targeted MPS involve an increasing number of relevant genes (**Table 3**), such as *KIT/PDGFRA* and *SDHA/B* in GIST, *CTNNB1* in desmoid tumors, *IDH1* or *IDH2* in enchondroma/chondrosarcoma, *COL2A1* in chondrosarcoma, the polycomb repressive complex components *SUZ12* or *EED* in MPNST, *PIK3CA* in myxoid liposarcoma, *KDR* in angiosarcomas, *STAG2* in Ewing sarcoma, *N/K/HRAS* and *FGFR4* in embryonal rhabdomyosarcoma, *MYOD1* in spindle cell rhabdomyosarcoma, *MED12* in leiomyomas and a very small subset of leiomyosarcomas, and *NF1* in malignant peripheral nerve sheath tumors and in an increasing number of other tumor types. *SMARCB1* is rarely genomically inactivated in epithelioid sarcoma. The various amplicons characteristic of well-differentiated / dedifferentiated liposarcoma are also readily detected as copy number gains of *MDM2* and *CDK4*, while *MYC* amplification is present in radiation-associated angiosarcoma. Mutations in *TP53* are often identified in some sarcomas (osteosarcoma, leiomyosarcoma), as well as occasional *PTEN* losses, although with little value for diagnosis or patient management at this time. *CDKN2A* inactivating events are common (malignant peripheral nerve sheath tumors, fibrosarcomatous dermatofibrosarcoma protuberans, advanced gastrointestinal stromal tumors).

Whole genome sequencing (WGS)

Sequencing the entire genome is still far from clinical application, due to logistic challenges that include relatively high costs in a CLIA environment. The mean sequencing depth is approximately 30 to 60 reads, and the high number of sequence variants requires paired tumor and normal samples to filter out the germline variants from the tumor genome calls. Even then, the functional relevance of most genetic variants detected by WGS is unknown. The clinical implementation of WGS is further hampered by the need of fresh or frozen tissue, since FFPE tissues remain problematic for WGS, and the sequencing depth required for robust mutation detection from poor-quality DNA at this coverage is not yet cost effective. As a discovery tool, WGS has led to the discovery of *STAG2* mutations in Ewing sarcoma^{27,28} and H3.3 mutations in chondroblastoma and giant cell tumor of bone.²⁹ Since these genes can be incorporated into cancer gene panels or sampled by exome sequencing, the use of WGS in clinical samples is generally considered “overkill” at the present time, but it may become more relevant in the future.³⁰

An advantage of WGS is a higher sensitivity for the detection of chromosomal rearrangements using specific algorithms to map paired-end reads;³¹ this application has been widely validated in research settings, as for the discovery of *ALK* and *RET* gene fusions in lung adenocarcinoma.^{32,33} For soft tissue tumors, most groups have favored the use of RNA-seq approaches for fusion detection. An additional feature of WGS is the ability to detect alterations in noncoding, gene regulatory regions, such as *TERT* promoter mutations first described in melanoma.³⁴ There is little information available for this type of genetic alterations in sarcoma.

Transcriptome sequencing (RNA-seq)

The use of RNA sequencing is particularly attractive for the study of soft tissue tumors, given its ability to detect structural rearrangements.³⁵ The relevance of chromosomal rearrangements in soft tissue tumor pathogenesis and their diagnostic utility is well documented,^{36,37} and recurrent rearrangements have been used as a dichotomous classifier to define a whole category of translocation-associated soft tissue tumors. Most genomic breakpoints occur at variable locations within introns, which can be quite large, so the resulting chimeric RNA transcripts are usually smaller than the corresponding rearranged genomic DNA segments. The analysis of RNA also provides information about expression levels and transcript variants (expression profiling), which contributes to a better understanding of tumor biology and has prognostic implications in certain tumor types.

The usual workflow starts with RNA extraction and enrichment for protein-coding RNA molecules, by capturing the poly-A tails. Several approaches may be used to enrich for certain types of noncoding RNAs, such as micro-RNAs, and to deplete others (such as ribosomal RNA) to increase the desired signal. The quality of RNA from old FFPE tissue blocks may be suboptimal for some applications, but some technologies are specifically designed to extract information from substantially degraded RNA molecules utilizing very short reads;^{38,39} in addition, extraction protocols continue to improve and there are good examples of successful RNA-seq application to archival material up to 10 years old.⁴⁰ Once the library is prepared and converted to cDNA, sequencing takes place by MPS as for genome-based approaches. The length of the reads (ultimately depending on the quality of the starting RNA) is a big determinant of the analytical sensitivity and the quality of the results. A number of computational algorithms are available to detect fusion transcripts in RNA-seq data, such as defuse, FusionSeq or FusionHunter (reviewed in Carrara and colleagues⁴¹); in general, the algorithms work by iteratively mapping initially discarded reads allowing for an increasing number of mismatches to the reference. The progress in this area is remarkable: recent RNA-seq studies managed to characterize the entire spectrum of kinase fusions in cancer, with direct therapeutic implications.⁴²

Applied to the study of soft tissue tumors, RNA-seq has become the most popular mode of discovery for novel fusion genes. Notable examples of fusions genes detected by RNA-seq include *YWHAE-NUTM2A/B* in high-grade endometrial stromal sarcoma,⁴³ *WWTR1-CAMTA1* in epithelioid hemangioendothelioma,⁴⁴ and *BCOR-CCNB3* in undifferentiated round cell sarcoma.⁴⁵ A slightly different RNA-based detection approach was used to identify the *HEY1-NCOA2* in mesenchymal chondrosarcoma,⁴⁶ in which the data showed variable levels of expression of different exons from both genes, leading the authors to identify the rearrangement (similar data were observed at the RNA level on the *NAB2-STAT6* study by Robinson and colleagues). At present, RNA-seq is not being used for clinical applications in soft tissue tumors, although suitable methods have been described.⁴⁷

Gene expression profiling deserves a particular mention here, since it has evolved almost completely to RNA sequencing-based methods. Despite the initial good results of microarray-based assays in research settings,^{48,49} attempts to clinically utilize gene expression profiling for sarcoma diagnosis or prognostication have generally failed, due to poor reproducibility and poor performance.⁵⁰ This may very well change with the increased

sensitivity of sequencing technologies and algorithms, which would perhaps contribute to improved subclassification of currently heterogeneous categories of sarcoma. Paradigmatic examples are leiomyosarcoma and malignant peripheral nerve sheath tumors, both heterogeneous groups of sarcoma lacking subgroup-specific molecular markers to accurately predict clinical behavior.^{51,52}

NON SEQUENCING-BASED HIGH-THROUGHPUT MOLECULAR TECHNIQUES

Array-based technologies

The technological revolution undergone by sequencing technologies over the last 5 years has essentially eclipsed any other molecular genetic techniques. Array-based technologies, however, are also able to provide high-resolution analysis of tumor genomes and transcriptomes.⁵³ Current array CGH (comparative genomic hybridization) and SNP array platforms are particularly suited to detect somatic copy number changes (deletions and amplifications) at a resolution much greater than sequencing techniques at the usual coverage. Copy number changes are extremely common in cancer, and induce significant functional changes that drive oncogenesis in proportions comparable to sequence variations.⁵⁴ In sarcoma, several oncogenic events are engaged by copy number changes, such as amplification of oncogenes (*MDM2* and *CDK4* in dedifferentiated liposarcoma and paraosteal osteosarcoma) and deletion of critical tumor suppressors (*NF1* in malignant peripheral nerve sheath tumor, *DMD* in myogenic sarcomas⁵⁵).

The use of array-based techniques is the natural evolution of karyotyping for genome-wide analysis, providing much richer information regarding gains and losses of genetic material, at higher resolution. In addition, these techniques can use interphase DNA and thus obviate the need to culture living cells to establish metaphase spreads (which, for cell culture enthusiasts, represents a painful loss!). The detection of balanced translocations, however, is not possible with high-resolution arrays and requires complementary technologies. Ideally, array-based techniques should be integrated into comprehensive, integrative genotyping approaches that include orthogonal assays based on sequencing. Such approaches have been successfully applied in soft tissue tumors for discovery purposes (leading to the identification of GRM1 fusions in chondromyxoid fibroma).⁵⁶ Pioneer experiences in the study of glioblastoma have shown very promising results in terms of feasibility and clinical implementation.⁵⁷

LOW-THROUGHPUT TECHNOLOGIES

Single-gene sequencing assays

Sanger sequencing (capillary electrophoresis, dye-terminator method) is still considered the gold standard for diagnostic detection of point mutations and *indels* in specific gene regions. For hot-spot analysis and validation purposes, Sanger sequencing provides a convenient, inexpensive and universally available method. The main limitation is its relatively low sensitivity, with a detection threshold of approximately 30% allelic frequency (problematic in cases with low tumor content, or in the detection of subclonal/heterogeneous genetic

changes). In addition, the technique remains relatively artisanal and laborious in terms of primer design and electropherogram interpretation, which leads to limited scalability. RT-PCR followed by sequencing is still widely used as validation method for gene fusion detection (although the extreme sensitivity of PCR amplification implies a high risk of 'carry-over' contamination and hence requires very stringent conditions and appropriate controls to avoid false positive results).⁵⁸

Fluorescence in situ hybridization (FISH)

FISH is a well-established approach to interrogate known chromosomal rearrangements and is still the preferred technique for the cytogenetic diagnosis of soft tissue tumors.^{59,60} Copy number gains and deletions can also be readily detected. During clinical work-up, morphologic evaluation combined with pertinent immunohistochemical stains usually narrows down the differential diagnosis to a limited number of entities that occasionally can be confirmed by FISH. In this usual workflow, FISH has mainly a role in validating or confirming a suspected diagnosis. In research, FISH has been an essential technique as part of the classical route to discover new fusion genes: after identification of recurrent rearrangements by chromosome banding techniques, FISH mapping allows to delineate breakpoints that can then be defined by RT-PCR based techniques.³⁵ Technically, FISH consists in the hybridization of fluorescently-labeled probes to specific nucleotide sequences (usually genomic DNA). The size of FISH probes is in the hundreds Kb range, and the resolution to detect rearrangements is limited by the fluorescence microscope used for evaluation (small insertions or complex rearrangements may be missed). At present, about 30 recurrent chromosomal rearrangements are known to be involved in soft tissue tumors and can be used for diagnostic purposes (reviewed by Mertens *et al*⁶⁵, and Al-Zaid *et al*⁶¹). An added value of FISH, when performed in tissue sections, is the preservation of tissue architecture and the ability to evaluate regional heterogeneity or specific cellular populations within the tumor.

Immunohistochemistry as a surrogate for molecular alterations

Although immunohistochemistry is formally not considered a molecular technique, the remarkable impact of recent molecular genetic discoveries in the development of useful immunohistochemical markers deserves consideration. Considering that immunohistochemistry is fully integrated in the surgical pathology workflow, it seems clear that the greatest clinical impact of molecular discoveries is achieved when a corresponding immunohistochemical assay is developed. For soft tissue tumors, this rapid translation has successfully occurred on multiple instances in recent years.⁶² The usual course of events is the discovery of a molecular alteration in a given soft tissue tumor type by gene expression profiling or MPS technologies, followed by the development and validation of antibodies with appropriate specificity and sensitivity to be used for immunohistochemistry. Following this paradigm, gene expression profiling has led to the discovery of DOG1 as a diagnostic marker for GIST,⁶³ TLE1 for synovial sarcoma,⁶⁴ and MUC4 for low-grade fibromyxoid sarcoma.⁶⁵ Overexpression of STAT6, TFE3 or ALK as a result of chromosomal rearrangements in solitary fibrous tumor, alveolar soft part sarcoma and inflammatory myofibroblastic tumors, respectively, can also be detected by robust immunohistochemical techniques. Gene amplifications (*MDM2*, *CDK4*, *MYC*) and deletions (*RB1*, *SMARCB1*)

also correlate with protein expression and can be used for diagnostic purposes. Anecdotally, some experts have referred to the development of these markers as ‘next-gen immunohistochemistry’, which reflects how significant a change is perceived in the field (of note, the same designation has been also proposed for mass spectrometry-based methods for protein identification in tissues⁶⁶). It is pertinent to highlight that immunohistochemistry is very susceptible to preanalytical variables, requires intensive quality controls to be performed routinely, and requires expert interpretation.

DRUG TARGET IDENTIFICATION

One of the main forces driving the clinical implementation of high-throughput molecular techniques is the perception that high-density genetic information will enable better discrimination of patients with similar phenotypic and clinical presentations, ultimately allowing for a refined selection of the most appropriate treatment. Different variations of this theme are currently encompassed under the concept of *precision medicine*, the most recent designation for what has been also called personalized medicine or individualized medicine.⁶⁷ The emergence of the designation *precision medicine* is much more than a terminological issue, and its rapid adoption reflects the widespread acceptance of its main principle, that the disruptive nature of high-throughput molecular technologies will fundamentally transform patient care. The tremendous excitement surrounding precision medicine is exemplified by ambitious academic endeavors (such as a report from the Committee on a Framework for Developing a New Taxonomy of Disease),⁶⁸ the deployment of large-scale research initiatives (such as the Presidential Precision Medicine Initiative),⁶⁹ and the considerable revenue of specialized biotechnology companies,⁷⁰ all of which are taking place amid great enthusiasm in the academic medical community.⁷¹ In oncology, the prevailing opinion is that genomic information, layered on top of “traditional” clinicopathological variables, will inform therapeutic selection and improve patient outcomes.⁷² This genomics-driven paradigm in oncology will require validation in clinical trials with innovative designs (involving molecularly stratified groups matched to their drugs in umbrella protocols, and likely requiring serial biopsies, for example),⁷³ but anecdotal evidence and extraordinary responses observed in multiple instances contribute to a general sense of optimism.⁷⁴

Successful clinical implementation of genomics-driven oncology will depend on the development of effective targeted agents. It is estimated that 550 genes encode drug targets utilized by currently established pharmaceuticals, only a small fraction of which corresponds to cancer targets, while the druggable genome is predicted to comprise ~1,000 genes (2-5% of the genome).⁷⁵ At present, many potentially druggable mutations are identified for which there are no effective inhibitors. Conversely, most tumors harbor at least one targetable genomic alteration.⁷⁶ In soft tissue tumors, several clinically relevant drug targets have been identified through improved understanding of tumor genomics,⁷⁷ but thus far only 5 targeted agents are FDA approved for sarcoma patient treatment: the RANK ligand inhibitor *denosumab* for the treatment of giant cell tumor; the multikinase inhibitor *pazopanib* for several soft tissue sarcomas; and the receptor tyrosine kinase inhibitors *imatinib*, *sunitinib* and *regorafenib* for the treatment of gastrointestinal stromal tumor. A series of up to 20 additional compounds are at different stages of clinical development, including the ALK

inhibitor *crizotinib*, the mTOR inhibitor *sirolimus*, and the CDK4 inhibitor *palbociclib*, as reviewed elsewhere.^{61,78} The molecular abnormalities underlying activation of these oncogenes can be readily detected by MPS technologies.

CONCLUDING REMARKS

The extraordinary development of high-throughput molecular technologies has the potential to transform the practice of surgical pathology. At present, appreciable changes are restricted to large academic centers and oncology institutes in which research and patient care-related activities are closely intertwined; but with continuously declining costs and increasing accessibility, it is fair to assume that some form of variably comprehensive genetic data will be available for most cancer samples. Accurate interpretation of these data may support the diagnostic process and inform therapeutic decisions. In soft tissue tumor pathology, integration of genetic information, specifically chromosomal rearrangements, has been part of the diagnostic process over the last two decades. Novel diagnostic, prognostic and predictive markers will be described and incorporated into clinical practice. In upcoming years, most molecular data will be generated by MPS technologies and, possibly, high-density arrays, both of which will eventually replace FISH for most clinical applications. Recording clinically actionable somatic mutations and pertinent negatives will be part of the routine pathology report, and will inform therapeutic decisions from the medical oncology team. In the immediate future, the biggest contributions of high-throughput genetic technologies will impact the research arena, generating knowledge and increasing understanding of the molecular pathogenetic basis of soft tissue tumors. Once knowledge bases are saturated, a huge impact in patient care is expected, with the ultimate result of improved outcomes for sarcoma patients.

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BOX 1**Glossary of terms commonly used in cancer genomics.**

Shotgun sequencing	Sequencing strategy in which the DNA/RNA is sheared generating numerous random short fragments to create a sequencing library.
Alignment or mapping	Matching sequence reads to a reference.
Paired-end sequencing	Sequencing linear DNA fragments from both ends.
Mate-pairs	Sequence reads corresponding to both ends of a unique DNA fragment.
Hybrid capture	Enrichment method to select target sequences from a pool, by tagging them with oligos or capture probes.
Read	String of text that corresponds to an single DNA fragment (data output from the sequencer).
Coverage / depth	Often used interchangeably to refer to the number of individual reads for each specific position on a target sequence.
Reference genome	Fully assembled version of a genome that can be used as a map to locate new sequences (current human version is GRCh38.p3, released by the Genome Reference Consortium in April 2015)
Variants	Differences at specific positions between two aligned sequences.
Variant calling	Process of detecting sequence variants, which is variably automated through computational algorithms.
SNV	Single Nucleotide Variant: A single base difference between a fragment and the reference.
SNP	Single Nucleotide Polymorphism: An SNV that is frequent in the population (present in at least 1% of individuals).
VUS	Variant of Unknown Significance: Variants for which the functional effect is unknown.
<i>Indel</i>	Structural aberration of the DNA in which a segment is either deleted or inserted (usually applied to small deletions or insertions).
CAN	Copy Number Alteration: Numeric alteration of the DNA by which the number of copies of a segment is increased (copy number gain) or reduced (copy number loss).
Allelic fraction	The proportion of a specific variant (allele) among all variants being observed for that locus.
Actionable mutation:	A sequence variant that provides clinically useful diagnostic, prognostic or therapeutic information.
Targetable mutation:	A sequence variant that confers sensitivity to a specific therapy.
Driver mutation:	A mutation that provides selective advantage to a clone and hence contributes significantly to cancer initiation and/or progression (in contrast with passenger mutations, which provide no significant fitness improvement).
Precision medicine:	Emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person using high-density datasets. In oncology, often used as synonymous of genomics-driven patient care.

KEY POINTS

- The recent development of high throughput genome-wide analytical methods enables comprehensive profiling of the genome, epigenome, or transcriptome of tumor samples in a clinical environment.
- Massively parallel sequencing technologies detect somatic mutations, *indels* and copy number changes in tumor samples generating clinically relevant information for cancer patient management.
- RNA-seq is best suited for the detection of chromosomal rearrangements, although most clinical laboratories still resort to FISH for diagnostic purposes.
- Genomic alterations detected in soft tissue tumors provide biological information complementary to clinicopathological variables, and substantiate therapeutic decisions or enrolment in clinical trials.

Table 1

Molecular techniques discussed in this review

Technique	Alterations detected	Limitations	Research contributions	Clinical implementation
Massively parallel sequencing technologies (next-generation sequencing or NGS)				
Cancer genome sequencing				
Targeted sequencing (cancer gene panels and exome sequencing)	SNV, CNA	Not comprehensive, transl detection	Mutational Landscape	+
Whole genome sequencing (WGS)	SNV, CNA, Transl	Cost / depth		+/-
Transcriptome sequencing (RNA-seq)	Transl	RNA workflow	Gene fusions	-
Non sequencing-based high-throughput molecular techniques				
Array CGH	CNA	No detection of transl or SNVs	Somatic CNA landscape	+
SNP arrays	CNA			+
Low-throughput technologies				
Single-gene sequencing assays	Hotspot SNV	Low throughput, not scalable	Historical landmark discoveries	++
Fluorescence in situ hybridization (FISH)	Transl, CNA	Fluorescence, cost, Low throughput, not scalable	Translocation mapping	++
Immunohistochemistry as a surrogate for molecular alterations	Protein Expression	Low throughput	(Many)	+++

Abbreviations: CGH: comparative genomic hybridization; SNP: single nucleotide polymorphism; SNV: single nucleotide variant; CNA: copy number alteration; Transl: translocation and rearrangements

Table 2
Representative cancer gene panel tests for targeted sequencing at several US academic laboratories

Institution	Test name	Number of genes sequenced (introns)	References
BWH/DFCI	Oncopanel	275 (91)	(21)
MSKCC	MSK-IMPACT	341 (33)	(79)
Washington University (St. Louis)	Comprehensive Cancer Gene Set v2	48 (6)	(80)
The Jackson Laboratory for Genomic Medicine	JAX Cancer Treatment Profile	190 (0)	(81)
University of Washington, Seattle, Washington	UW-OncoPlex	194 (15)	(82)
University of Pittsburgh (PA)	ThyroSeq v2 (thyroid)	13 (42)	(83)
Knight Diagnostic Laboratories (Portland, Oregon)	GeneTrails genotyping panels (organ specific; "solid tumors panel" considered here)	37 (0)	(84)

Table 3

Clinically actionable genetic alterations commonly encountered in soft tissue tumors

Type of alteration (main clinical use)	Genes	Entities
Point mutation / small <i>indel</i> s (potential therapeutic targets)	<i>KIT/PDGFRα, SDHA/B</i>	Gastrointestinal stromal tumor
	<i>CTNIB1</i>	Desmoid tumor
	<i>IDH1, IDH2</i>	Enchondroma/chondrosarcoma
	<i>COL2A1</i>	Chondrosarcoma
	<i>SUZ12, EED</i>	Malignant peripheral nerve sheath tumor
	<i>PIK3CA</i>	Myxoid liposarcoma
	<i>KDR</i>	Angiosarcoma
	<i>STAG2</i>	Ewing sarcoma
	<i>N/K/HRAS, FGFR4</i>	Embryonal rhabdomyosarcoma
	<i>MYOD1</i>	Spindle cell rhabdomyosarcoma
	<i>MED12</i>	Leiomyoma (and small subset of leiomyosarcoma)
	<i>NF1</i>	Malignant peripheral nerve sheath tumor and others
	<i>MDM2, CDK4</i>	Dedifferentiated liposarcoma
	<i>MYC</i>	Postradiation sarcoma
	<i>MYOCD</i>	Leiomyosarcoma
Amplification (diagnostic markers)	<i>NF1</i>	Malignant peripheral nerve sheath tumor and others
	<i>TP53</i>	Osteosarcoma, leiomyosarcoma, and others
	<i>CDKN2A</i>	Malignant peripheral nerve sheath tumor, fibrosarcomatous DFSP, advanced gastrointestinal stromal tumor
Deletion (diagnostic markers)	<i>RB1</i>	Spindle cell lipoma, mammary-type myofibroblastoma, and others
	Fusion oncogenes (constitutively active chimeric kinases, transcription factors)	Tumor-type specific (reviewed by Mertens ³⁵)
Rearrangement (diagnostic markers)		