Introduction
Soft tissue sarcomas (STS) represent a heterogeneous group of rare malignant tumors with a wide spectrum in terms of histologic type and prognosis (1). Prognosis of STS is dominated by local recurrence and distant metastasis. STS recur locally in less than 10% when located in the limbs and trunk wall but metastasize in 30 to 50% of cases. Quality of surgical margins is the most important factor for predicting local recurrence whereas histologic grade particularly indicates the probability of distant metastasis and overall survival (2-5). For more than 20 years, the main use of grading has been to select patients for chemotherapy. However, the utility of adjuvant chemotherapy in STS is still debated and the approach of sarcoma therapy has recently undergone shifts from non-specific cytotoxic agents towards utilization of molecularly targeted treatments. Moreover, some histotypes have shown different sensitivity to specific cytotoxic drugs. Nowadays, the value of grading is also limited by the universal use of core needle biopsies. The aim of this presentation is to report the practical value and limitations of histologic grade considering the recent modifications that occurred in the management of patients with STS and try to predict the future of grading.

Histologic grade: the most important predicting factor in STS
In 1977, Russell et al (6) proposed the first coherent and effective system for establishing the prognosis of STS. However, in their clinicopathologic classification, histologic grade was rather subjectively based on several histologic parameters. To obtain the most efficient system, histologic parameters should be selected by multivariate analysis to use only the necessary parameters summarizing all the prognostic histologic information. According to the studies that have used this methodology, the best parameters are histologic type and subtypes and/or differentiation, tumor necrosis and mitotic index whereas cellularity, nuclear atypia and pleomorphism should not be considered (7,8). Several grading systems have been reported in the early 1980s, but the most common systems used are the National Cancer Institute grading (7) and the French grading (8). The last system is precisely defined, easy to use and may be, the most widely employed. It is based on a score obtained by evaluation of 3 parameters: tumor differentiation, mitotic rate and amount of tumor necrosis. The reproducibility of this grading (agreement in 75%) was better than those of histotyping (agreement in 61%) (9). However, this reproducibility has been established in the early 1980s when immunohistochemistry was poorly efficient and with no available molecular analysis. Several studies with a multivariate analysis on metastasis have reported that the grade is the most important factor (3, 4,7). Therefore, the main value of grading has been for the most efficient use of
chemotherapy with a few studies reporting a more efficiency of chemotherapy in grade 3 tumors as compared to grade 1 or 2 tumors (3,10). Patients with a grade 1 tumor should not receive chemotherapy because it is useless and inefficient. On the other hand, chemotherapy could be useful and efficient in patients with a grade 3 tumor.

**Histologic grade: limitations and controversial points**

Histologic grade is used as a universal tool applicable to most STS but, obviously, a grading system adapted to every specific histologic type would be more efficient (11-14). However, it is totally unrealistic to develop a system for every histotype of STS. In a study on 1240 localized STS (15), it has been shown that with the exception of MPNST, histologic grade is an important predictor of metastasis in the main histologic types representing about 90% of STS.

However, it is important to keep in mind that histologic grading should not replace histologic typing. Grading is less informative than histologic type in dedifferentiated liposarcomas, round cell liposarcomas, and alveolar soft part sarcomas. Grading should not be used on tumors of intermediate malignancy such as atypical fibroxanthoma and dermatofibrosarcoma protuberans. Among poorly differentiated sarcomas, it is also important to differentiate leiomyosarcomas from undifferentiated sarcomas since the former have a worse prognosis than the latter (16, 17). Moreover, since some subtypes show a differential sensitivity to specific drugs, it is now more and more important to give the exact histotype.

Grading is recommended on representative material. However, core needle biopsies are now widely used for the primary diagnosis of STS. Although several studies (18, 19) reported a good concordance of grading between core needle biopsies and incisional biopsies, it is difficult to assess necrosis and mitotic index on core needle biopsies. Moreover, core needle biopsies are prone to sampling error with the risk of underestimation of grade, and unless obvious high-grade tumor is present, it is often inappropriate to use classical grading systems on this material.

**The future of grading**

Despite the limitations discussed above, grading identifies patients at greatest risk for distant metastasis and, therefore, pathologists will be expected to provide grades for most soft tissue sarcomas. Grading should certainly be adapted to the modern management of patients and should be complemented with clinical, radiologic, immunohistochemical and molecular parameters.

Multivariate analyses for predicting metastatic risk also retained tumor size, neurovascular or bone involvement and tumor depth in addition to histologic grade, underlining the importance not only of grade but also of clinical factors. These clinical factors are combined to histologic grade to defined staging systems. However, several staging systems have been proposed for STS and none is perfect (23-25). Nomogram is another tool combining clinical and pathological parameters and which represents a prognostic model applicable to a given patient. A nomogram for 12-year sarcoma-specific mortality has been developed and validated by Memorial Sloan Kettering (26, 27).

Extension of necrosis could be evaluated by imaging studies, which can be viewed as a type of macroscopic examination.

Mitotic index, which is difficult to evaluate on core needle biopsies, could be replaced by a mib1 score, as reported by Hasegawa et al in combination of the French grading system (28).
Grading can be considered as a morphological translation of molecular events that determine tumor aggressiveness and one can postulate that, in the future, molecular parameters will be important for prognostication of STS. In addition to efficiency and reproducibility, a molecular grading should be usable on tissue procured by microbiopsy and should certainly be histotype or, may be better, molecular type specific. Several lines of evidence suggest that sarcomas can be divided into four major genetic groups: sarcomas with specific translocation, sarcomas with specific activating or inactivating mutations, sarcomas with 12q13-15 amplification and sarcomas with a complex genomic profile. The last category represents more than 50% of STS and are mainly represented by leiomyosarcomas, myxofibrosarcomas, pleomorphic rhabdomyosarcomas, pleomorphic liposarcomas and poorly differentiated sarcomas. In order to achieve this goal, we started a genomic and expression profiling (array-CGH and Affymetrix) of 183 sarcomas with complex genetic profile and established an expression signature which is highly predictive of metastasis outcome in the whole group and the different subgroups such as limb sarcomas and leiomyosarcomas (29). This signature has been established by a bottom-up supervised strategy using genomic profile (array-CGH), histologic grade and a previously published chromosomal instability signature. The resulting signature is composed of 67 genes related to mitosis and chromosome management. This signature has been validated on an independent group of 95 sarcomas with complex genetic profile and tested in silico on GIST, breast carcinomas and lymphomas. Our goal is now to test this signature on sarcomas with a specific translocation, GIST and dedifferentiated liposarcomas, and, if possible, to adapt the signature to the different molecular categories of sarcomas.

In conclusion, histologic grade is an important prognostic factor for predicting metastasis outcome in STS but its importance is now limited due to the new therapeutic strategies and the universal use of core needle biopsies. In the future, this histologic grading will certainly be replaced by a molecular grading.

References


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18. Welker JA, Henshaw RM, Jelinek J et al. The percutaneous needle biopsy is safe and recommended in the diagnosis of musculoskeletal masses. Outcome


Morphologically Benign Lesions of Soft Tissue and Bone Which Metastasize - What Can We Do?

Introduction

Over the past several decades it has become apparent (to the dismay of surgical pathologists, clinicians and patients) that several morphologically benign tumors of soft tissue and bone may on extremely rare occasions metastasize. Recognition of and agreement on this phenomenon has not been universal among pathologists, with some for example claiming that all such cases represent “misdiagnoses” of a malignant tumor as benign (i.e., misdiagnosis of superficially located “malignant fibrous histiocytoma” as cellular fibrous histiocytoma). However, it is safe to say that there is now an evolving consensus that “benign metastasizing” soft tissue and bone tumors do occur.

“Benign metastasizing” soft tissue and bone tumors may be conceptualized as falling roughly into 3 groups. The first group includes tumors widely agreed to be benign, which may on extremely rare occasions metastasize, including benign fibrous histiocytoma (dermatofibroma) of soft tissue, and giant cell tumor and chondroblastoma of bone. The second group encompasses many of those tumors currently classified as being of borderline/intermediate malignancy by the WHO, which have a recognized ~2% risk of metastasis, despite their often very bland appearance (e.g. plexiform fibrohistiocytic tumor). The third group is the least well-defined, and includes a variety of rare mesenchymal tumors which span a histologic spectrum from apparently benign to apparently malignant, but where histologic study does not always accurately predict behavior (e.g., gastrointestinal stromal tumor, soft tissue myoepithelioma, perivascular epithelioid cell tumors).

This handout will cover the clinicopathological features of benign fibrous histiocytoma of soft tissue and giant cell tumor of bone, prototypical “benign metastasizing” tumors in those locations, as well as ossifying fibromyxoid tumor, a tumor originally considered benign but currently thought of as being of borderline malignancy.

Benign fibrous histiocytoma (BFH)

BFH, also referred to as dermatofibroma, is a common lesion of the dermis and subcutis, which are most often located in the extremities of young to middle-aged adults. There is a female predominance. The lesions may be solitary or multiple. Several variants have been described; cellular, aneurysmal, and atypical variants will be discussed in this handout.
BFH consists of a circumscribed, variably cellular monomorphic population of spindle cells arranged in fascicles and storiform patterns, which may be intermixed with multinucleated giant cells (Touton giant cells), foamy macrophages, siderophages and interstitial fibrosis. Thickened collagen bundles are classically observed at the periphery, surrounded by lesional cells. The overlying epidermis is often hyperplastic. Cellular fibrous histiocytomas are highly cellular lesions, showing a storiform or fascicular growth pattern, with little collagen deposition. They tend to be larger (up to 2.5 cm) than common dermatofibroma, often extending into subcutis (30% of cases). Mitoses are numerous, and foci of necrosis and/or vascular invasion may be observed. Intralesional aneurysmal changes are characteristic of the aneurysmal variant of fibrous histiocytoma. Vascular spaces are often bordered directly by multinucleated cells and/or siderophages. The pseudovascular lumina are frequently occupied by foamy siderophages or Touton giant cells containing lipid and hemosiderin. The atypical (pseudosarcomatous) fibrous histiocytoma variant contains numerous pleomorphic (monster) cells with enlarged hyperchromatic nuclei, resulting in a pseudosarcomatous appearance. Mitoses are sometimes numerous in this variant and atypical mitoses are occasionally seen, especially in pleomorphic areas. Recognition of features of typical BFH is the key to the diagnosis of all BFH variants. Deeply situated BFH are often more uniform in appearance, showing a storiform growth pattern and a hemangiopericytoma-like vasculature. They are often well demarcated and can be encapsulated.

Immunohistochemically, BFH are usually negative for CD34 and positive for Factor XIIIa, and KiM1p. The histiocytic cells, including multinucleated giant cells, express CD68 (clone KP1 and/or PGM1). Deep fibrous histiocytomas express CD34, at least focally, in about 30% of cases.

BFH are benign lesions. Recurrences are rare (<5% of cases), often following incomplete local excisions. Cellular, aneurysmal, and atypical variants of fibrous histiocytoma are prone to recur more frequently (up to 25% of cases). Reexcision is usually curative. Approximately 20 cases of metastasizing BFH have been reported, typically to the lungs. The primary tumors in such cases are most often of the cellular variant, although metastases have also been reported in cases of aneurysmal, atypical and conventional BFH. The lesion reported in the pulmonary pathology literature as “cystic fibrohistiocytic tumor” represents metastatic BFH in essentially all instances. There are no pathological features of the primary tumor that are predictive of metastases, although this extremely rare event seems to be somewhat more common in tumors that have recurred locally multiple times.

The differential diagnosis of cellular FH centers on fascicular spindle cell tumors, including dermatofibrosarcoma protuberans, leiomyosarcoma and the fibroma-like variant of epithelioid sarcoma. Leiomyosarcoma typically grows in longer fascicles, shows more diffuse cytoplasmic eosinophilia, lacks inflammatory cells, and is SMA/caldesmon/desmin positive in most instances. Dermatofibrosarcoma protuberans is a more monotonous proliferation of more lightly staining spindled cells arranged in storiform arrays, with diffuse infiltration of adipose tissue, and
strong CD34 expression. Fibroma-like epithelioid sarcomas typically show at least small areas of more conventional epithelioid sarcoma, with infiltrative epithelioid cells, and are cytokeratin-positive/INI-1 negative. Aneurysmal BFH should be distinguished from angiomatoid (malignant) fibrous histiocytoma, a fibrohistiocytic tumor of intermediate malignancy, typified by a fibrous capsule containing lymphoid aggregates, short fascicles and meningothelial-like whorls of histiocytoid cells often containing hemosiderin pigment, intralobular hemorrhage, and a unique desmin/EMA/CD68-positive immunophenotype. Atypical FH should be distinguished from atypical fibroxanthoma, a tumor that arises in sun-damaged skin in much older adults, and lacks the presence of areas of typical BFH at its periphery, and from superficially located pleomorphic sarcomas, in particular myxofibrosarcoma, a tumor that often occurs in the superficial soft tissues and may involve the skin. Clinical correlation with regards to the size and extent of the tumor is often helpful in this regard.

**Giant cell tumor of bone (GCT)**

GCT is relatively common, comprising 5% of all bone tumors and 20% of all benign bone tumors. GCT most often involves the epiphyseal or meta-epiphyseal region of long bones such as the femur, tibia and radius, but may also involve the sacrum, spine and short tubular bones of the hands and feet. Multicentric GCT have been reported but are extremely rare. GCT show a slight female predominance, and most often occur in skeletally mature individuals in the 3rd-4th decades of life. Clinically, GCT present as pain or swelling or with pathological fracture. Radiographically, GCT are purely lytic lesions with either well-defined, non-sclerotic margins, or poorly circumscribed margins. Cortical destruction and soft tissue extension may be present; a shell of reactive bone may be seen in soft tissue extension or in soft tissue recurrences.

Microscopically, GCT are composed of sheets of osteoclast-like giant cells with interspersed round to slightly spindled mononuclear cells. The nuclei of the mononuclear cells are uniformly bland, an important distinction from various osteoclast-rich malignancies, including osteoclast-rich osteosarcoma. Although the nuclei of the mononuclear cells and giant cells are classically described as looking similar, it is now understood that they represent different cell types. Specifically, the mononuclear cells of GCT appear to be primitive osteoblast precursors, which recruit osteoclast-like giant cells through production of RANK-L (osteoclast differentiation factor). A variety of secondary changes may be seen in GCT, including a fascicular to storiform spindle cell proliferation resembling BFH, foamy macrophages, cystic change, hemorrhage and necrosis.

Although GCT are considered benign neoplasms, they may be locally aggressive and recur locally in up to 25% of cases. The adequacy of local resection appears to be the most important predictor of local recurrence, although some authors have suggested that radiographically poorly marginated lesions (Campanacci grade 3) have a higher risk of local recurrence (presumably reflecting tumors in which adequate local resection is more difficult to achieve). Recurrent GCT, particularly those that have been treated with adjuvant radiotherapy, may
progress to high-grade sarcoma, typically resembling fibrosarcoma, undifferentiated pleomorphic sarcoma, or osteosarcoma.

Metastases, almost always to the lungs but rarely to soft tissue or lymph nodes, occur in ~2% of patients with giant cell tumors. These metastases may be solitary or multiple, tend to grow slowly, and are typically amenable to surgical resection with excellent patient outcome. There are no histologic features of the primary tumor that reliably predict metastasis, although it has been recently suggested that intratumoral hemorrhage and thrombosis is more common in GCT with lung metastases than in those without this. Larger studies are required to confirm this. The presence of soft tissue extension has also been associated with a greater likelihood of metastasis in some studies. Telomerase activation, as measured by hTERT immunohistochemistry or TRAP assays, is a common finding in GCT which appears to be associated with a greater likelihood of local recurrence, but not with a greater risk for metastasis.

The differential diagnosis of GCT includes any osteoclast-rich tumor of bone, including chondroblastoma, brown tumor of hyperparathyroidism, aneurysmal bone cyst, and osteoclast-rich osteosarcoma. Chondroblastoma typically occurs in skeletally immature individuals and contains clusters of chondroblasts with grooved nuclei as well as calcified matrix. A history of hyperparathyroidism should be sought in any patient with apparently multicentric giant cell tumors. Aneurysmal bone cyst typically occurs in younger patients in a metaphyseal location and shows a more variable microscopic appearance than does GCT. Giant cell-rich osteosarcoma occurs in the same location as does conventional osteosarcoma (metaphysis or meta-epiphysis), and is distinguished from GCT by the presence of clearly malignant-appearing stromal cells showing osteoid production. In general, bone production in GCT is found at the periphery of the tumor, as a shell of woven bone, whereas osteoclast-rich osteosarcomas show a greater abundance of centrally located bone.

**Ossifying fibromyxoid tumor of soft parts (OFMT)**

OFMT is an extremely rare mesenchymal tumor that may occur in essentially any location, usually in adults. OFMT present as relatively small, painless masses, often with a radiographically apparent shell of bone. Typical OFMT are characterized by a peripheral shell of bone in 70% of cases, lobulated growth, and small, bland cells arranged in cords and nests within a fibromyxoid stroma. The stroma of OFMT varies from highly myxoid to fibrous, and may on occasion hyalinize and calcify. A very characteristic feature of OFMT is its even and regular cell-cell spacing. Mitotic activity is usually very low. S100 protein is expressed by over 70% of typical OFMT and by a smaller percentage of atypical and/or malignant OFMT (see below). A minority of OFMT will show focal expression of cytokeratins, smooth muscle actin or desmin. Some OFMT may also rarely express other markers of putative nerve sheath differentiation, such as CD57 (Leu 7), “neuron-specific” enolase, and glial fibrillary acidic protein.
Malignant OFMT maintain the overall cytoarchitectural features of benign OFMT, but show accentuated lobularity, greatly increased cellularity with nuclear overlapping, coarse chromatin and prominent nucleoli, necrosis, vascular invasion and mitotic activity of > 2/50 HPF (Fig 17-18). Prominent spindling or extensive stromal hyalinization may be present. Bone production may either be absent or may be increased, sometimes within the center of the lesion.

The initial description of OFMT by Enzinger et al emphasized what might be thought of as “typical OFMT” inasmuch as all of the cases were circumscribed, of low cellularity and low nuclear grade, lacked necrosis or vascular space invasion and had mitotic rates of 1-2/10HPF (with one exception). Local recurrences occurred in 7 of 41 cases with follow-up information and were generally similar to the primary lesion with the notable exception of 2 cases that showed increased cellularity and mitotic activity. One of these recurrent lesions was described as showing “a transition to a well-differentiated osteosarcoma”. That case was characterized by moderately increased cellularity and cytologic atypia and increased centrally placed hyaline matrix, but maintained the overall cytoarchitectural features of an OFMT. The other patient whose recurrent lesion showed increased cellularity suffered a contralateral soft tissue metastasis.

In the years immediately following that initial publication a number of additional series and cases were reported that for the most part described OFMT with typical histologic features and a benign clinical course. However, unquestionable examples of OFMT were also reported that either appeared histologically malignant or produced metastasis. For example, Yoshida et al first reported a tumor that lacked overtly malignant features but produced local recurrence, distant soft tissue metastasis and death. Kilpatrick and colleagues reported a series of 6 atypical OFMT, one of which appeared overtly malignant, four of which showed lesser degrees of atypicality, and one of which was a histologically typical OFMT that both recurred locally and metastasized to the lungs. Zamecnik et al described 3 histologically malignant tumors in a series of 17 OFMT, 2 of which developed recurrences and one of which metastasized to the lungs. In 2003 we published a series of 70 OFMT, noting local recurrences and metastases in 12% and 4% of “typical” OFMT (those with low nuclear grade, low cellularity and a mitotic rate < 2/50 HPF), as compared with 60% and 60% of “malignant” OFMT (those showing high nuclear grade or a combination of high cellularity and mitotic activity > 2/50 HPF). “Atypical” OFMT, defined as those tumors deviating from “typical” OFMT but not meeting criteria for “malignant” OFMT showed similar outcome to “typical” OFMT. Most recently, Miettinen and Fetsch have examined a very large series (104 cases) of purely typical OFMT (excluding all cases with any atypical features) and noted a local recurrence rate of 22%, but no metastases. Putting all of this together, it would appear that entirely banal-appearing OFMT have an approximately 15% risk of local recurrence and a 5% metastatic risk, supporting their reclassification by the WHO as mesenchymal tumors of intermediate/borderline malignancy. Malignant-appearing OFMT behave as high-grade sarcomas. Within the group of histologically typical OFMT, there are no histologic features that are predictive of metastasis.
The differential diagnosis of OFMT includes epithelioid schwannoma, epithelioid MPNST, mixed tumor/myoepithelioma, extraskeletal myxoid chondrosarcoma, and most importantly, osteosarcoma. Epithelioid schwannomas lack the bone shell and extremely uniform cell-cell spacing seen in OFMT, and often arise adjacent to a nerve. Epithelioid malignant peripheral nerve sheath tumors show much greater cytologic atypia than do OFMT, resembling melanoma. Mixed tumors/myoepitheliomas do not produce a bone shell, usually show epithelial differentiation, and express epithelial markers, such as cytokeratins much more often than do OFMT. Extraskeletal myxoid chondrosarcomas contain distinctly eosinophilic cells that grow in nests, cords and chains, often with abundant associated hemorrhage and hemosiderin deposition. Osteosarcomas lack a lobular growth pattern, show much greater cytologic atypia and pleomorphism than do even malignant OFMT, and often produce abundant lace-like osteoid, as well as malignant-appearing chondroid matrix. It should be emphasized that malignant OFMT, which may produce an osteosarcoma-like calcified matrix, maintain the overall cytoarchitectural features of typical OFMT and often arise within pre-existing typical OFMT.

Conclusions
To answer the question posed by the title of this talk and handout, it would appear that we as pathologists can do several things in response to the frustrating problem of “benign metastasizing” tumors. First and foremost, it is crucial to recognize those truly malignant tumors that may mimic these various benign neoplasms, so as to avoid underdiagnosis. It thus follows that we must also be willing to critically re-evaluate the primary tumors in patients with unexpected metastases for evidence that we may have initially been in error. Assuming that our initial diagnoses were correct, we must also be willing and able to communicate intelligently about these issues with clinicians and patients, many of whom will have great difficulty in understanding the complexities of “benign metastasizing” tumors. Third, although we as a group have not as yet identified any histologic or molecular markers predictive of eventual metastases in “benign metastasizing” tumors, it is hoped that continued study of these rare neoplasms will identify such markers. Finally, it is important that we as a discipline continue to reevaluate the proper classification of soft tissue and bone tumors, with perhaps the eventual expansion of the borderline/intermediate malignancy category to include tumors of bone, such as giant cell tumor.

Selected references
BFH


GCT


14.

OFMT


6.

11.
IMMUNOHISTOCHEMISTRY IN SARCOMA CLASSIFICATION

- IHC plays central role in sarcoma diagnosis
  - Confirm histologic impression
  - Distinguish among histologically similar tumors
  - Support diagnosis of rare tumor type
  - Support diagnosis when tumor arises in unusual location or unusual age group
IMMUNOHISTOCHEMISTRY IN SARCOMA CLASSIFICATION

• Diagnostic IHC markers for sarcomas:
  – Markers of differentiation/lineage
    » SMA, desmin, caldesmon, myogenin, S-100 protein, KIT, EMA, keratins
  – Protein correlates of molecular findings
    » ALK, MDM2/CDK4, TFE3, INI1, TLE1
Alveolar Soft Part Sarcoma

TFE3
Synovial Sarcoma

TLE1
TRADITIONAL PROGNOSTIC MARKERS FOR CANCER

- Tumor size
- Stage
- Lymph node metastases
- Grade
- Vascular invasion
**REQUIREMENTS OF NEW PROGNOSTIC MARKER FOR ACCEPTANCE IN CLINICAL PRACTICE**

- Assessment is reproducible and widely available with quality controls
- Substantial added value beyond established prognostic markers
- Results confirmed by additional independent studies

TYPES OF PROGNOSTIC MARKER STUDIES IN CANCER

• Phase I: Exploratory studies (hypothesis generating): seek association between potential marker and disease characteristics that have prognostic importance (grade, stage)

• Phase II: Exploratory studies: evaluate ability of prognostic marker to discriminate between patients at high and low risk of disease progression or death

• Phase III: Confirmatory studies (preferably large, collaborative); independent data sets

PROBLEMS WITH PROGNOSTIC MARKER STUDIES

- How to determine sample size?
- Cutpoint used to dichotomize continuous marker into “high” (positive) or “low” (negative)?
- Multivariable regression including all known prognostic markers/factors (independent significance)
- Publication bias (selective reporting)
- Lack of systematic reviews and/or meta-analyses
PROBLEMS WITH PROGNOSTIC MARKER STUDIES FOR SARCOMA

• Rare diseases – small sample sizes
• Variability in diagnosis/classification among pathologists
• Variability in treatment among oncologists
• Prospective studies nearly impossible
IMMUNOHISTOCHEMISTRY IN SARCOMA PROGNOSTICATION?

- No currently accepted clinical role, beyond classification/diagnosis (along with grade, most important prognostic factors)

- Numerous “biomarkers” evaluated, most in single retrospective studies

- “Traditional” markers (>20 yrs) such as Ki-67 and p53

- Newer markers identified through gene expression profiling
Ki-67 (MIB-1)

- Recognizes nuclear antigen
- Expressed in proliferating cells
- Preferentially late G1, S, G2, M
- Not expressed in G0
- Widely used in pathology as marker of proliferation; criterion for malignancy or grade (some organ systems)
p53

- Key role in cell cycle and apoptosis
- Tumor suppressor gene commonly mutated in diverse cancers
- Wild-type protein weak/negative by IHC
- Mutant protein extended half-life, detectable by IHC (“overexpression”)
- Strong positive staining by IHC reasonable correlation with p53 mutation
OUTLINE

1. Prognostic studies including all types of soft tissue sarcomas
2. Myogenic differentiation in pleomorphic sarcomas
3. Extent of myogenin expression in rhabdomyosarcoma
4. Tumor type-specific prognostic studies
PROGNOSTIC STUDIES
INCLUDING ALL TYPES OF
SOFT TISSUE SARCOMAS

- 174 adult soft tissue sarcomas (heterogeneous types)
- IHC for p53 and Ki-67
- >20% cut-off for each
• Decreased survival for p53+ and high Ki-67

• No significant difference among high grade sarcomas

• Neither marker prognostic significance in multivariate analysis
• 52 adult soft tissue sarcomas (heterogeneous types)

• IHC for Ki-67

• >40% cut-off = “high”
  – 84% “low” Ki-67
  – 16% “high” Ki-67
- Decreased survival for high Ki-67

- Grade not included in multivariate analysis
Jensen et al. 1998; Histopathology 32:536-46.

- 216 soft tissue sarcomas (heterogeneous types)
- IHC for Ki-67 and p53
- >10% cut-off for p53
- >12% cut-off for Ki-67 (median)
• Grade strongest predictor of survival
• No effect of Ki-67 on survival for “MFH”
• Among high grade sarcomas other than “MFH”, Ki-67 strong independent predictor of survival
• 86 primary extremity soft tissue sarcomas (heterogeneous types)

• IHC for p53 and MDM2

• >10% cut-off for each
  – 57% positive for p53
  – 70% positive for MDM2
  – 46% positive for both
• 121 localized high grade soft tissue sarcomas of extremities (heterogeneous types)
• IHC for Ki-67 and p53
• >20% cut-off for each
  – Ki-67 “increased” in 69%
  – p53 “positive” in 9%
Metastasis-free survival

- Ki-67 independent prognostic significance for both metastasis and overall survival
Comments

• Ki-67 not a replacement for grading
• Ki-67 may be prognostic for (some) high grade sarcomas
• p53 aberrations nearly exclusive to high grade sarcomas
• Given marked differences in behavior for sarcoma subtypes, difficult to draw meaningful conclusions
MYOGENIC DIFFERENTIATION IN PLEOMORPHIC SARCOMAS
Fletcher et al.

- 100 “MFH” of extremities/trunk wall re-classified
- Upon re-review:
  - 29 myxofibrosarcomas
  - 20 leiomyosarcomas
  - 30 overall some form of high grade myogenic sarcoma
Myogenic tumors (stage II/III) worse metastasis-free survival
92 pleomorphic sarcomas of extremities

IHC for SMA, MSA, desmin, myoglobin

- 42 positive for at least 1 marker
Myoid differentiation independent adverse prognostic indicator
Inverse relationship between number of positive myoid markers and survival.
65 pleomorphic sarcomas of extremities re-evaluated

Upon re-review:
- 22 leiomyosarcomas
- 13 myxofibrosarcomas
- 9 other myogenic sarcomas
Myogenic differentiation only independent predictor of overall survival
Comments

- Subclassification of pleomorphic sarcomas clinically significant
- IHC plays important role identify/confirm myogenic sarcomas
- Pleomorphic sarcomas with myogenic differentiation (not only LMS, RMS) higher metastatic potential
EXTENT OF MYOGENIN EXPRESSION IN RHABDOMYOSARCOMA
Embryonal Rhabdomyosarcoma

myf4
(Solid) Alveolar Rhabdomyosarcoma

myf4
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A-RMS

focal
diffuse

E-RMS
Diffuse staining for myogenin independent predictor of overall survival
INDIVIDUAL TUMOR TYPE-SPECIFIC PROGNOSTIC STUDIES

- 35 patients with extra-uterine leiomyosarcoma
- IHC for p53 and Ki-67

- No correlation between staining for p53 or Ki-67 and overall or recurrence-free survival

- 86 patients with primary localized synovial sarcoma
- IHC for p53 and MIB-1
- >10% cut-off for MIB-1
- >25% cut-off for p53
p53 not associated with survival
MIB-1 index significantly associated with metastasis
• 49 patients with localized synovial sarcoma of extremities
• IHC for cell cycle-associated proteins, p53 and Ki-67
• >20% cut-off for Ki-67 (59% +)
• >10% cut-off for p53 (16% +)
p53 and Ki-67 associated with worse disease-specific survival
71 patients with myxoid liposarcoma

- IHC for p53
- >10% cut-off (17% positive)
Significantly higher metastatic rate for p53-positive localized tumors

- 32 patients with myxoid liposarcoma
- IHC for adipogenesis and proliferation-related proteins (RET, IGF1R, IGF2)
- >50% considered positive
Significantly lower metastasis-free survival for high expression of each
• 28 patients with leiomyosarcoma of deep somatic soft tissue
• IHC for c-myc
• >5% considered positive
Significantly lower metastasis-free and overall survival
Schirosi et al.

- 88 patients with pleuropulmonary solitary fibrous tumor
- IHC for p53, various kinases
- >5% considered positive
p53
Multivariate analysis: p53 significantly associated with disease-free and overall survival
Gene expression profiling on 51 leiomyosarcomas

Unsupervised clustering: 3 clusters (1 “muscle-enriched”)

IHC on TMA for 5 markers with high mRNA in “muscle-enriched” cluster
Multivariate model: muscle-enriched markers associated with improved survival, independent of site, grade, necrosis, and mitotic rate

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<th>Wald</th>
<th>HR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF1-response protein</td>
<td>13.0</td>
<td>4.7 (2.0-10.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>signature (Espinosa et al., 2009)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of positive</td>
<td>4.5</td>
<td>0.77 (0.6-0.98)</td>
<td>0.035</td>
</tr>
<tr>
<td>group 1/muscle-enriched</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>markers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site</td>
<td>1.8</td>
<td>0.35 (0.1-1.6)</td>
<td>0.178</td>
</tr>
<tr>
<td>Mitotic figures</td>
<td>0.8</td>
<td>1.3 (0.7-2.4)</td>
<td>0.371</td>
</tr>
<tr>
<td>Grade</td>
<td>0.32</td>
<td>1.3 (0.6-2.5)</td>
<td>0.570</td>
</tr>
<tr>
<td>Necrosis</td>
<td>0.01</td>
<td>1.0 (0.5-2.2)</td>
<td>0.910</td>
</tr>
</tbody>
</table>
Final Comments I
Prognostic Role of IHC in Sarcomas

• IHC for accurate diagnosis (critical for outcome prediction)
• Other than diagnosis, grade remains key prognostic determinant
• Myogenic differentiation in pleomorphic sarcomas prognostic significance
• Other “biomarkers” not yet ready for prime time
Final Comments II
Prognostic Role of IHC in Sarcomas

- Studies of single tumor type (uniform histology, grade, etc.)

- Gene expression profiling identifying novel prognostic markers

- Goal will be to stratify patients into lower/higher risk groups – guide selection of therapy
Final Comments III
Prognostic Role of IHC in Sarcomas

• How to establish threshold for “positive”?  
• Need for uniform reporting

• Need for pooled analyses/systematic reviews
• How best to translate to clinical practice?
Over the past 25 years, immunohistochemistry (IHC) has played a central role in the classification of mesenchymal tumors. The chief contribution of IHC to diagnosis is to distinguish among histologically similar tumors, but IHC can also be applied (often for reassurance) to support the diagnosis of rare tumor types or to support the diagnosis when a tumor arises at an unusual location or in an unusual age group. The majority of the widely available IHC markers for sarcomas suggest lines of differentiation, such as smooth muscle actin and desmin for smooth muscle or myofibroblastic tumors, myogenin (myf-4) for skeletal muscle neoplasms, and S-100 protein for nerve sheath (Schwann cell) tumors. Unfortunately, few of these traditional markers are highly specific, and therefore a panel of markers is usually needed, and the results must be interpreted carefully in the context of the histologic and clinical findings. More recently, diagnostic IHC markers have been developed that can serve as surrogates for specific molecular findings, such as ALK for inflammatory myofibroblastic tumor, MDM2 and CDK4 for well-differentiated and dedifferentiated liposarcoma, TFE3 for alveolar soft part sarcoma, INI1 for malignant rhabdoid tumor and epithelioid sarcoma, and TLE1 for synovial sarcoma. However, IHC has no currently accepted clinical role in prognostication for sarcomas, beyond its role in establishing a specific diagnosis (which, along with grade, is among the most important prognostic factors).

Traditional prognostic markers for cancer in general, which provide critical information to oncologists both for counseling patients on the likelihood of developing metastases and
selecting appropriate systemic therapies, include such factors as tumor size, stage, and grade, and
the presence of vascular invasion and lymph node metastases. In order for novel prognostic
markers to gain acceptance in clinical practice, several broad requirements should be met: (1) the
assessment must be reproducible and widely available with appropriate quality controls; (2) the
marker must have substantial added value beyond that of established prognostic markers; and (3)
the results of prognostic marker studies should be confirmed by additional independent studies.

Prognostic marker studies can be classified into three general groups: phase I exploratory
(hypothesis generating) studies that seek associations between potential markers and disease
characteristics that have known prognostic importance, such as stage or grade; phase II
exploratory studies that evaluate the ability of prognostic markers to discriminate between
patients at high and low risk of disease progression or death; and phase III confirmatory studies
that seek to validate the results of phase II studies using independent (preferably large and
collaborative) data sets. These latter studies would ideally be prospective and protocol-driven,
although systematic reviews or meta-analyses of phase II studies using pooled data are
reasonable alternatives. Unfortunately, nearly all prognostic studies of IHC markers are phase
II-type studies, without confirmatory follow-up studies using independent data sets. Indeed, it is
uncommon for more than one study to evaluate a specific potential prognostic IHC marker.

In addition to the lack of critical confirmatory studies and systematic reviews, there are
many other important issues that must be addressed for prognostic marker studies to yield
clinically meaningful and applicable results. For example, how should continuous markers be
dichotomized into "high" (or positive) and "low" (or negative) results? This question is
particularly relevant to IHC studies, where the evaluated markers often show a range of staining
in terms of both extent and intensity. Publication bias is also a significant issue for IHC-based
prognostic studies, since such studies that yield negative results are rarely published, and,
similarly problematic, published studies sometime omit the results of markers that failed to reach
significance. Furthermore, multivariable regression models that include all known prognostic
factors must be employed to determine whether the evaluated markers have independent
prognostic significance. There are also additional problems that pertain to prognostic marker
studies particularly relevant to sarcomas. Since sarcomas are very rare diseases, sample size is
always an issue, and prospective studies are nearly impossible to perform. In addition, there can
be variability in both diagnosis and treatment of sarcomas among different cancer centers, pathologists and oncologists.

Many potential prognostic "biomarkers" have been evaluated in sarcomas, most in single or small numbers of retrospective studies. These range from "traditional" markers such as Ki-67 and p53, which have been studied for more than 20 years, to newer markers that are being identified through gene expression profiling. Ki-67 (or MIB-1) recognizes a nuclear antigen expressed in proliferating cells, preferentially in late G1, S, G2, and M phases of the cell cycle, but not in quiescent cells (G0). Ki-67 is widely used in pathology as a marker of proliferation, and, in some organ systems, as a criterion for malignancy or grading. The p53 gene encodes a protein that plays a key role in the cell cycle and apoptosis. p53 acts as a tumor suppressor gene; a wide variety of human cancers harbor loss-of-function p53 mutations. Since the wild-type p53 protein is rapidly degraded, IHC for p53 shows negative or weak staining in normal cells, whereas mutant p53 proteins usually have an extended half-life, and therefore tumor cells harboring p53 mutations usually show strong positive staining by IHC.

The "first generation" of prognostic IHC marker studies of sarcomas included all types (and grades) of tumors. These studies often reported conflicting results with regard to the prognostic significance of positive staining for p53 and/or a high Ki-67 index, and most failed to demonstrate independent prognostic significance of these markers in multivariate analysis. In contrast, more recent individual tumor type-specific prognostic IHC marker studies of sarcomas, including synovial sarcoma and myxoid liposarcoma, have demonstrated independent prognostic significance for expression of p53 and other IHC markers. Similarly, recent studies using gene expression profiling have identified groups of markers whose expression is associated with prognosis. These results have yet to be confirmed by other groups on independent data sets, and it is not yet clear how these findings can be translated to clinical practice.

Several retrospective studies have evaluated the prognostic significance of myogenic differentiation in pleomorphic sarcomas. In the first such study by Fletcher and colleagues in 2001, 100 extremity and trunk wall tumors formerly diagnosed as "malignant fibrous histiocytoma" were re-classified applying strict diagnostic criteria, in conjunction with IHC and
electron microscopy (in select cases). Upon re-review, the most common sarcoma types were high grade leiomyosarcoma and myxofibrosarcoma. In total, 30 of the tumors were classified as some form of high grade myogenic sarcoma. When the localized myogenic sarcomas were compared to non-myogenic tumors, the myogenic tumors showed a higher rate of metastasis. In a follow-up study by Deyrup and colleagues, 92 pleomorphic sarcomas of the extremities were immunostained for the myogenic markers smooth muscle actin, muscle-specific actin, desmin, and myoglobin; 42 tumors were positive for at least one marker. Similar to the prior study, myogenic differentiation was found to be an independent adverse prognostic indicator. Furthermore, there was an inverse relationship between the number of positive myogenic markers and survival. A subsequent study by Massi and colleagues re-evaluated 65 pleomorphic sarcomas of the extremities. Similar to the study by Fletcher, the most common diagnoses on re-review were leiomyosarcoma and myxofibrosarcoma; 31 tumors in all were some form of myogenic sarcoma. Upon multivariate analysis, myogenic differentiation was the only independent predictor of overall survival. These studies confirm the prognostic value of subclassifying pleomorphic sarcomas, for which IHC plays an important role, as well as demonstrate that pleomorphic sarcomas with myogenic differentiation (not only pleomorphic leiomyosarcoma and rhabdomyosarcoma, which are known to pursue an aggressive clinical course) have a higher metastatic potential. Such prognostic information can be helpful to select patients for clinical trials of novel chemotherapeutic agents.

Previous studies have shown that alveolar rhabdomyosarcoma typically displays diffuse staining for the skeletal muscle transcription factor myf4 (myogenin), whereas embryonal rhabdomyosarcoma (which has a better clinical outcome) usually expresses myf4 in only scattered cells. A recent study by Heerema-McKenney and colleagues evaluated the prognostic significance of the extent of myf4 staining in 71 pediatric rhabdomyosarcomas (>80% nuclear staining was defined as "diffuse"). Interestingly, the authors found that diffuse staining for myf4 was an independent predictor of overall survival, after adjusting for histologic subtype, anatomic site, stage, and age.

At present, IHC plays a limited role in prognostication for sarcomas, beyond supporting accurate diagnosis (which is a critical determinant of outcome). IHC is helpful to identify
pleomorphic sarcomas with myogenic differentiation, which have a higher metastatic risk than those without such differentiation. Other potential "biomarkers" are not yet ready for routine clinical application. Before the introduction of new IHC prognostic markers, thresholds for "positive" results will need to be examined, both in terms of biological relevance and so that the results can be reliably (and reproducibly) reported. Along these lines, there is a need for more uniform reporting of the results of these sorts of studies, to allow for systematic reviews including pooled analyses of data, so that novel prognostic markers can be validated and become a routine part of evaluation by surgical pathologists.

Key words: immunohistochemistry, soft tissue sarcomas, biomarkers, p53, Ki-67
Selected references:


Jensen V, Sørensen FB, Bentzen SM, Ladekarl M, Nielsen OS, Keller J, Jensen OM. Proliferative activity (MIB-1 index) is an independent prognostic parameter in patients with high-grade soft tissue sarcomas of subtypes other than malignant fibrous histiocytomas: a


Prognostication in GIST
A New Paradigm

Brian Rubin, MD, PhD
Anatomic Pathology and Molecular Genetics
Cleveland Clinic
Lerner Research Institute
Taussig Cancer Center
GIST

- Most common mesenchymal tumor of the GI tract.
- 0.2% of all GI Tumors; 80% of GI sarcomas.
- Up to 5000 new cases/year in USA.
- Annual incidence of 7-19 cases/million.
GIST – Anatomic Location

- Stomach - 60%
- Small Bowel – 30%
- Esophagus/Colon/Rectum – 5%
- Extragastrointestinal - 1% or less
  - Omentum
  - Mesentery
KIT immunoreactivity in GISTs

Cytoplasmic Pattern

Dot-Like Pattern
KIT immunoreactivity in GISTs

Membranous Pattern
**KIT** and **PDGFRA** Mutations in 950 GISTs

Overall Mutation Frequency: **86%**

**KIT (78.5%)**
- Exon 11 (67%)
- Exon 9 (9%)
- Exon 13 (1%)
- Exon 17 (1%)

**PDGFRA (7.5% total)**
- Exon 12 (2%)
- Exon 14 (rare)
- Exon 18 (5.5%)

(35% of KIT-WT)

Heinrich M and Corless C – Personal Communication
What is KIT?

- Type III receptor tyrosine kinase
- Located on chromosome 4q
- Involved in the proliferation and maintenance of:
  - germ cells
  - hematopoietic cells (mast cells)
  - melanocytes
  - interstitial cells of Cajal
Prognosis in GIST

• GIST as a paradigm for personalized medicine.

• Areas of importance
  • To determine who should receive follow-up for patients with resectable localized disease.
  • To determine who should receive adjuvant therapy for patients with resectable localized disease.
  • To determine the type of targeted therapy for treatment of metastatic disease.
Prognostic Biomarkers in GIST
Mitotic Rate

- High mitotic rate associated with more aggressive clinical behavior in many studies.
- Mitotic rate of ≥ 5/50 HPFs had a hazard ratio of 14.6 (p <0.001) in univariate and multivariate analysis in study by DeMatteo and colleagues.

Influence of mitotic activity on behavior

Hazard Ratio 14.6
(6.5-32.4)

P<0.0001 by
Univariate and
multivariate analysis

Influence of tumor size on behavior

Hazard Ratio 2.5
(1.3-4.8)

P=0.0004 by Univariate and P=.007 by multivariate analysis

Influence of tumor location on behavior

P=0.0004 by Univariate and P=.009 by multivariate analysis

Other prognostic biomarkers

- Ki67
- S-100
- CD44
- Multiple growth factors
- BCL-2
- p53
- COX-2
- p16\textsuperscript{INK4A}
- p14\textsuperscript{ARF}
- Midkine
- Several cyclins

- Rb
- MDM2
- HIF-1 alpha
- c-MYC
- DNA ploidy
- Cytogenetic complexity
- Telomerase activity
- Microvessel density
- Lack of KIT expression
- Type of KIT mutation

# NIH-Fletcher Criteria

<table>
<thead>
<tr>
<th>Risk category</th>
<th>Tumor size in greatest dimension</th>
<th>Mitotic count (per 50 HPFs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very Low</td>
<td>&lt;2 cm</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Low</td>
<td>2-5 cm</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Intermediate</td>
<td>&gt;5 cm; 5-10 cm</td>
<td>6-10; &lt;5</td>
</tr>
<tr>
<td>High</td>
<td>&gt;5 cm; &gt;10 cm; Any size</td>
<td>&gt;5; Any mitotic rate; &gt;10</td>
</tr>
</tbody>
</table>

Risk stratification by Fletcher Criteria of 259 GIST patients

# Risk Stratification of Primary GIST by Mitotic Index, Size and Site

<table>
<thead>
<tr>
<th>Mitotic Index</th>
<th>Size</th>
<th>Gastric</th>
<th>Duodenum</th>
<th>Jejunum/Ileum</th>
<th>Rectum</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 5 per 50 hpf</td>
<td>≤ 2 cm</td>
<td>None (0%)</td>
<td>None (0%)</td>
<td>None (0%)</td>
<td>None (0%)</td>
</tr>
<tr>
<td></td>
<td>&gt; 2 ≤ 5 cm</td>
<td>V-low (1.9%)</td>
<td>Low (8.3%)</td>
<td>Low (4.3%)</td>
<td>Low (8.5%)</td>
</tr>
<tr>
<td></td>
<td>&gt; 5 ≤ 10 cm</td>
<td>Low (3.6%)</td>
<td>(Insuff)</td>
<td>Moderate (24%)</td>
<td>(Insuff)</td>
</tr>
<tr>
<td></td>
<td>&gt; 10 cm</td>
<td>Mod (10%)</td>
<td>High (34%)</td>
<td>High (52%)</td>
<td>High (57%)</td>
</tr>
<tr>
<td>&gt; 5 per 50 hpf</td>
<td>≤ 2 cm</td>
<td>None+ (Insuff)</td>
<td>High (50%)</td>
<td>High (73%)</td>
<td>High (52%)</td>
</tr>
<tr>
<td></td>
<td>&gt; 2 ≤ 5 cm</td>
<td>Mod (16%)</td>
<td>(Insuff)</td>
<td>High (85%)</td>
<td>(Insuff)</td>
</tr>
<tr>
<td></td>
<td>&gt; 5 ≤ 10 cm</td>
<td>High (55%)</td>
<td>(Insuff)</td>
<td>High (90%)</td>
<td>High (71%)</td>
</tr>
<tr>
<td></td>
<td>&gt; 10 cm</td>
<td>High (86%)</td>
<td>High (86%)</td>
<td>High (90%)</td>
<td>High (71%)</td>
</tr>
</tbody>
</table>

Hpf = high power field; insuff = insufficient data; v-low = very low; mod = moderate
Data are based on long-term follow-up of 1055 gastric, 629 small intestinal, 144 duodenal, and 111 rectal GISTs.
*Defined as metastasis or tumor-related death
+Denotes small number of cases

Adapted from Miettinen and Lasota – Semin Diagn Pathol 2006; 23:70.
Significant Differences between Fletcher-NIH and Miettinen Criteria

- NIH-Fletcher appeared to overestimate the risk of GIST $\leq 2$ cm with $\leq 5$ mitotic figures per 50 HPFs.
- NIH-Fletcher appeared to overestimate the risk of large gastric GIST.
<table>
<thead>
<tr>
<th>Study</th>
<th>Site</th>
<th>Size</th>
<th>Prevalence</th>
<th>Mut. Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agaimy and Wunsch¹ (Sporadic Cajal cell hyperplasia)</td>
<td>Esophagus (Mean 8 sections)</td>
<td>0.4-1 mm (mean 0.7 mm)</td>
<td>7 of 77 (9.1%)</td>
<td>Not done</td>
</tr>
<tr>
<td>Kawanowa et al.² (microscopic GISTs)</td>
<td>Proximal – upper stomach. (mean 130 slides)</td>
<td>0.2-0.4 mm (mean 1.5 mm)</td>
<td>50 GISTs in 35 of 100 stomachs (35%)</td>
<td>KIT mutations 2/25 (8%)</td>
</tr>
<tr>
<td>Agaimy et al. ³ Gastric Sclerosing Stromal Tumors (GIST tumorlets)</td>
<td>Stomach – cardia, fundus or proximal body</td>
<td>1-10 mm (mean 4mm)</td>
<td>22.5% of autopsy stomachs</td>
<td>KIT mutations 11/24 (46%) PDGFRA mutations 1/24 (4%)</td>
</tr>
<tr>
<td>Abraham et al.⁴ “Seedling” GISTs</td>
<td>44% - gastric 50% - esophageal Mean 30 sections</td>
<td>0.2-0.3 mm (mean 1.3 mm)</td>
<td>18 GISTs in 15 of 150 esoph-gastrectomy specimens</td>
<td>Not Done</td>
</tr>
</tbody>
</table>

Incidental Gastric GIST
Incidental Gastric GIST

CD34

SMA

DES

KIT
The Future of Risk Stratification

- **Nomogram** – “a graphical interface for a statistical model using variables with additive prognostic importance to predict precisely a patient outcome”

GIST nomogram

- **Points**
- **Size (cm)**
- **Mitotic index**
  - <5/50 HPF
  - ≥5/50 HPF
- **Site**
  - Colon/rectum
  - Stomach/other
  - Small intestine
- **Total points**
- **Probability of 2-year RFS**
- **Probability of 5-year RFS**

GIST Nomogram

• Marginal improvement over Miettinen criteria.
• However – still some problems.
  • 88% 5-year recurrence-free survival for 7 cm gastric GIST with 4/50 HPFs
  • 18% 5-year recurrence-free survival for 7 cm gastric GIST with 5/50 HPFs

Targeting activating \textit{KIT} mutations with small-molecule tyrosine kinase inhibitors

Imatinib mesylate

\[ \text{Imatinib mesylate} \]

Courtesy of Paul Manley, Novartis Oncology
Imatinib Mesylate Therapy

March 3, 2000

April 5, 2000

Joensuu H et al. NEJM 2001; 344:1052
Who should be followed clinically?

- GIST $\leq 2$ cm with $\leq 5$ mits/50HPFs do not need follow-up.
- Very low to low risk categories range from 1.9%-8.5%.
- Intermediate to high-risk GIST require follow-up.
Adjuvant Imatinib prolongs recurrence free survival

Who should receive adjuvant therapy?

- No guidelines issued by FDA for who should receive therapy or for how long.
- ? High-risk GIST?
Tyrosine Kinase Inhibitors for Treatment of Metastatic GIST

• Two large phase III studies
  • progression-free survival of approximately 20 months
  • median overall survival of 50 months
Primary Imatinib Resistance

- Seen in at least 10% of GIST
- Those tumors that progress within 3-6 months of initiating therapy.
  - \textit{KIT} WT
  - \textit{KIT} exon 9 mutants
  - Most common \textit{PDGFRA} mutant (exon 18 – D842V)

GIST: KIT and PDGFRA Mutations Predict Overall Survival in Patients Treated with Imatinib

GIST: Progression Free Survival

KIT exon 9 Mutations Treated with Imatinib

Progression-free and overall survival of patients treated with imatinib depends on genotype.

Heinrich MC et al. JCO 2008 26:5352
Should we treat according to \textit{KIT/PDGFRA} Genotype?

- Imatinib and Sunitinib appear to have different efficacies in GIST of different genotype.
- \textit{KIT} exon 9 mutants may respond better to imatinib 800 mg/d or sunitinib.
- \textit{KIT} WT may respond better to sunitinib.
- Current recommendations are imatinib 400mg/d followed by imatinib 800mg/d followed by sunitinib.

Demetri GD et al. \textit{JNCCN} 2007; 5 Suppl 2:S1
Imatinib – Delayed Resistance

- Characterized by patients who show partial response or at least stable disease and then go on to develop disease progression.
- Usually happens within 2 yrs of initiation of therapy.
- Most common mechanism is intra-allelic, second site *KIT* mutations in regions that encode the ATP binding domain or activation loop of KIT.
- Two of the most common mutations, V654A and T670I sensitive to imatinib.
Resistance to Imatinib Mesylate: Recognition of Clonal Evolution

Courtesy of Dr. G.D. Demetri.
Variety of secondary mutations in a single patient

Exon 9 mutant

- Exon 9 / N822K
- Exon 9 / D820E
- Exon 9 / N822Y
- Exon 9 / D820G
- Exon 9 / V654A
- Exon 9 / N822H

Courtesy of Dr. Jonathan Fletcher
Location and biochemical properties of secondary KIT kinase mutations in TKI-resistant GIST

Summary

• Important biomarkers for risk stratification include mitotic rate, tumor size and anatomic location.
• Provides information to determine who should have clinical follow-up and adjuvant imatinib.
• \textit{KIT/PDGFR\textalpha} mutation status predicts response to imatinib/sunitinib.
• Provides theoretical basis for determining who should receive imatinib/sunitinib.
Introduction

Gastrointestinal stromal tumor (GIST) is the most common mesenchymal tumor of the intestinal tract with as many as 4,500-6000 new cases in the USA each year (1) and an annual incidence of 7-19 cases per million (2-4). GISTs may arise anywhere along the gastrointestinal tract but are most common in the stomach (60%) and small bowel (30%) (1). They also arise rarely in extragastrointestinal locations such as the omentum and mesentery (5). Approximately 85% of GIST harbor activating mutations in KIT, a receptor tyrosine kinase. Another 5-7% have activating mutations in a related receptor tyrosine kinase, platelet-derived growth factor receptor A (PDGFRA) (1, 6-7). KIT and PDGFRA mutations are mutually exclusive. Importantly, GIST is a paradigm for oncogene addiction, whereby a tumor is significantly dependent on a single oncogenic protein; KIT or PDGFRA in the case of GIST (8). Small-molecule inhibitors such as imatinib mesylate (Gleevec; Novartis Pharmaceuticals) and sunitinib malate (Sutent; Pfizer) inhibit KIT, leading to inhibition of proliferation and clinically, to partial response or stable disease (9-10). Thus, imatinib and sunitinib are targeted therapies that target an oncogenic protein (KIT).

Determining prognosis in GIST is particularly important in the context of effective targeted therapies. GIST is an important paradigm for personalized medicine. There are three major areas of potential importance in which prognosis may play an important role: 1. to determine who should receive follow-up for patients with resectable localized disease 2. to determine who should receive adjuvant therapy for patients with resectable localized disease and 3. to determine the type of targeted therapy for treatment of metastatic disease.

Risk Stratification

Numerous studies have shown that mitotic rate is by far the most important prognostic biomarker in GIST (11). Higher mitotic rate is associated with more aggressive clinical behavior. In a recent study of 127 GIST by DeMatteo and colleagues, they found that a mitotic rate ≥ 5/50 HPFs had a hazard ratio of 14.6 with a p-value of <0.001 in both univariate and multivariate analyses (12). While not nearly as strongly predictive as mitotic rate, tumor size is also an important prognostic biomarker with independent predictive value by univariate and multivariate analysis across many studies (11). Larger size predicts more aggressive biological behavior at all sites. Finally, tumor location also has independent prognostic significance (11). The most important point regarding site is that gastric GIST have a favorable prognosis as compared with GIST that occur elsewhere.
Other prognostic factors including: tumor necrosis, cellular atypia, expression of Ki67, S-100, CD44, multiple growth factors, bcl-2, p53, COX-2, p16<sub>INK4A</sub>, p14<sub>ARF</sub>, midkine, several cyclins, Rb, MDM2, HIF-1α, c-MYC, DNA ploidy, cytogenetic complexity, telomerase activity, microvessel density, and lack of KIT expression have all been assessed for prognostic significance in small series of GIST and some look promising. However, at this point, none have made their way into commonly used risk stratification schemes.

In April of 2001, the National Institutes of Health convened a consensus conference on GIST, which gave rise to the NIH-Fletcher criteria, which used size and mitotic rates to predict GIST behavior (Table 1)(13). Note that since GIST typically have low mitotic rates, the mitotic rate is based on 50 HPF. Subsequently, these criteria were validated in several large retrospective studies and performed well (see excellent review by Joensuu for summary)(11).

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</tr>
<tr>
<td></td>
<td>5-10 cm</td>
<td>&lt;5</td>
</tr>
<tr>
<td>High</td>
<td>&gt;5 cm</td>
<td>&gt;5</td>
</tr>
<tr>
<td></td>
<td>&gt;10 cm</td>
<td>Any mitotic rate &gt;10</td>
</tr>
</tbody>
</table>

More recently, Miettinen and Lasota developed criteria for risk stratification based on mitotic rate, size, and anatomic site (Table 2)(14). The criteria were developed based on long-term follow-up from 1055 gastric GIST (15), 629 GIST of the jejunum and ileum (16), 144 duodenal GIST (17), and 111 rectal GIST (18). These criteria have been adopted by the AJCC Cancer Staging Manual and College of American Pathologists Guidelines for reporting on GIST (19-20). One main difference between the NIH-Fletcher criteria and the Miettinen criteria is that the NIH-Fletcher criteria appeared to overestimate the risk of the very low risk category which essentially has no risk for aggressive behavior. In addition to the data from Miettenin’s group showing that GIST <2 cm with ≤5 mitotic figures per 50 HPFs had no metastatic potential, recent work has shown that sub-centimeter GIST are very common (21-23). In one study by Kawanowa and colleagues, they found that 35% of gastrectomy specimens contained at least one sub-centimeter GIST (23). The other main difference between the NIH-Fletcher criteria and the Miettinen criteria is that the NIH-Fletcher criteria appeared to overestimate the risk of large gastric GIST. Miettinen found that intestinal GIST >10 cm with ≤5 mitotic figures per 50 HPFs had a metastatic rate of 52% and intestinal GIST measuring 2-5 cm with mitotic figures 5 per 50 HPFs had a metastatic rate of 73%, much higher than the corresponding gastric GIST with metastatic rates of 11% and 16% (15-16).
Table 2 – Risk Stratification of Primary GIST by Mitotic Index, Size and Site

<table>
<thead>
<tr>
<th>Mitotic Index</th>
<th>Size</th>
<th>Gastric</th>
<th>Duodenum</th>
<th>Jejunum/Ileum</th>
<th>Rectum</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 5/50 HPFs</td>
<td>≤ 2 cm</td>
<td>None (0%)</td>
<td>None (0%)</td>
<td>None (0%)</td>
<td>None (0%)</td>
</tr>
<tr>
<td></td>
<td>&gt; 2 ≤ 5 cm</td>
<td>Very low (1.9%)</td>
<td>Low (8.3%)</td>
<td>Low (4.3%)</td>
<td>Low (8.5%)</td>
</tr>
<tr>
<td></td>
<td>&gt; 5 ≤ 10 cm</td>
<td>Low (3.6%)</td>
<td>Insufficient data</td>
<td>Moderate (24%)</td>
<td>Insufficient data</td>
</tr>
<tr>
<td></td>
<td>&gt; 10 cm</td>
<td>Moderate (10%)</td>
<td>High (34%)</td>
<td>High (52%)</td>
<td>High (57%)</td>
</tr>
<tr>
<td>&gt; 5/50 HPFs</td>
<td>≤ 2 cm</td>
<td>None – small number of cases</td>
<td>Insufficient data</td>
<td>High – small number of cases</td>
<td>High (54%)</td>
</tr>
<tr>
<td></td>
<td>&gt; 2 ≤ 5 cm</td>
<td>Moderate (16%)</td>
<td>High (50%)</td>
<td>High (73%)</td>
<td>High (52%)</td>
</tr>
<tr>
<td></td>
<td>&gt; 5 ≤ 10 cm</td>
<td>High (55%)</td>
<td>Insufficient data</td>
<td>High (85%)</td>
<td>Insufficient data</td>
</tr>
<tr>
<td></td>
<td>&gt; 10 cm</td>
<td>High (86%)</td>
<td>High (86%)</td>
<td>High (90%)</td>
<td>High (71%)</td>
</tr>
</tbody>
</table>

Adapted from Miettinen and Lasota (14). Data are based on long-term follow-up of 1055 gastric, 629 small intestinal, 144 duodenal and 111 rectal GISTs.

* Defined as metastasis or tumor-related death.

Are there ways of improving risk criteria for GIST? There have already been several suggestions that look interesting. For instance, DeMatteo and colleagues have proposed the use of a nomogram (“a graphical interface for a statistical model using variables with additive prognostic importance to predict precisely a patient outcome”), which is based on anatomic location, tumor size, and mitotic rate (24). Risk scores associated with each prognostic biomarker are added together and the 2-year and 5-year recurrence-free survival can be read from the nomogram. In comparison with the Miettinen criteria, the nomogram performed marginally better. However, as pointed out by Joensuu, there are problems with the nomogram, which is based on the same three prognostic biomarkers as the Miettinen criteria (11). For instance, the nomogram predicts about 88% 5-year recurrence-free survival for a patient with a 7 cm gastric GIST with four mitotic figures per 50 HPFs, but only 18% recurrence-free survival for a similar case with five mitotic figures per 50 HPFs (11).

Risk stratification to determine need for follow-up

Assessing risk of aggressive behavior of primary GIST is useful for determining who should be followed-up clinically. Based on the Miettinen criteria, it is easy to argue that
patients with GIST at all locations that are completely resected and measure ≤2 cm with ≤ 5 mitotic figures per 50 HPFs, do not have to be followed. However, it is not clear cut for the very low or low risk categories with probabilities of metastasis ranging from 1.9% - 8.5%. Currently these decisions are being made by oncologists and their patients, who determine the mode and length for follow-up. Given the cost of follow-up and potential for radiation damage from repeated abdominal CT scans, this is not a trivial issue.

**Risk-stratification in the adjuvant setting**

Based on its success in treating patients in the metastatic setting, clinical trials were conducted to evaluate imatinib in the adjuvant setting. In a pivotal, large, randomized, double-blind, placebo-controlled trial, with 400mg/d of imatinib for 12 months, there was a marked improvement in 1 year recurrence-free survival compared with placebo (97% versus 83%; \( p <0.00001 \)) (25). These results led to FDA approval for usage of imatinib in the adjuvant setting for GIST. Unfortunately, no guidelines were given for either which risk categories should receive adjuvant imatinib or for the duration of treatment. Most experts agree that high-risk GIST should receive adjuvant imatinib but the duration is problematic and other risk categories are less clear (26). Again, deciding who should and should not receive adjuvant imatinib is important, especially considering a cost of approximately twenty thousand dollars per year, only for imatinib, not to mention all of the ancillary costs from blood tests, doctor visits and so forth.

**Prediction of therapeutic response in the metastatic setting**

Currently, both imatinib (first line) and sunitinib (second line) are approved for use in treating metastatic GIST. Extensive analysis in phase I, II, and III trials revealed that imatinib is very useful in the treatment of metastatic GIST. Patients in two large phase III studies achieved progression-free survival of approximately 20 months and a median overall survival of 50 months in either the 400 mg/day or 800 mg/day arms of the studies (27-28). Molecular analysis revealed that response to imatinib corresponds to genotype (29-30). **Primary imatinib resistance**, which is seen in at least 10% of GIST patients, is defined as those tumors that progress within 3 to 6 months of initiating therapy. While in general, GIST with KIT exon 11 mutations (the most common mutations) respond well to imatinib, GIST that are likely to have primary resistance are those that are KIT and PDGFRA wild-type, those that have a KIT exon 9 mutation, and those that have the most common PDGFRA mutation, D842V (29). KIT exon 9 mutant GIST appear to respond to the higher dose of imatinib (800mg/day) (30). Sunitinib has been shown to be effective in stabilizing a subset of patients who were intolerant of or exhibited primary resistance to imatinib (10). A phase III, placebo-controlled trial in patients who were either intolerant or resistant to imatinib showed a median progression free survival of 24.1 weeks in the treatment arm versus 6 weeks in the placebo arm (10). Interestingly, KIT and PDGFRA sequence analysis revealed that KIT and PDGFRA wild-type and KIT exon 9 mutants were more likely to respond to sunitinib than KIT exon 11 mutants (31). In other words, imatinib and sunitinib appear to be most effective in treating GIST with different genotypes and genotype appears to predict response. This suggests that patients should be stratified by genotype in terms of whether or not they receive imatinib or sunitinib and
the dose but this remains to be examined prospectively in a carefully controlled clinical trial. Furthermore, an argument can be made to give imatinib at 800mg/day to patients with KIT exon 9 mutations (30). At the current time, imatinib is FDA approved as the first line therapy for recurrent/metastatic GIST while sunitinib is used in those patients who are intolerant of or progress on imatinib at the higher dose of 800 mg/day (26).

**Delayed imatinib resistance**, is characterized by patients who show partial response or at least stable disease and then go on to develop disease progression. This usually happens within 2 years of treatment. KIT gene sequence analysis has revealed that delayed (also known as acquired or secondary) imatinib resistance is due to secondary intra-allelic, second site KIT mutations in regions that encode the ATP binding domain (encoded by exons 13 or 14) or the activation loop of KIT (encoded by exons 17 or 18) in about 50% to 66% of cases (Fig. 1) (32-33). One of the more interesting aspects of delayed resistance is that it is characterized by actively proliferating nodules that appear to grow out as individual clones from tumors (33). Gene sequence analysis reveals that each clone appears to be unrelated as they frequently contain different secondary mutations. Luckily, two of the more common delayed resistance mutations, V654A and T670I are sensitive to sunitinib (Fig. 1 from (34)) (31). Currently, KIT and PDGFRA gene sequence of tumors from patients with delayed imatinib resistance does not play a role in determining therapy.

![Fig. 1 – Location of secondary KIT mutations in imatinib or sunitinib resistant GIST](image)

**Bibliography**

USCAP 2010 - ISBSTP COMPANION MEETING

Prognostic and predictive molecular testing in soft tissue sarcomas - where do we stand?

Marc Ladanyi, Memorial Sloan-Kettering Cancer Center, New York, NY

A. Prognostic Markers: Fusion type

<table>
<thead>
<tr>
<th>Sarcoma</th>
<th>Favorable fusion type</th>
<th>Unfavorable fusion type</th>
<th>Reproducibility</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ewing Sarcoma</td>
<td>EWS-FLI1 type 1</td>
<td>EWS-FLI1 other types</td>
<td>+</td>
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<tr>
<td>Synovial Sarcoma</td>
<td>SYT-SSX2</td>
<td>SYT-SSX1</td>
<td>+/-</td>
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<tr>
<td>ARMS</td>
<td>PAX7-FKHR</td>
<td>PAX3-FKHR</td>
<td>++</td>
<td>(9;10)</td>
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</table>

B. Prognostic Markers: other mutations (excluding GIST)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sarcoma</th>
<th>Impact</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>P53 mutation</td>
<td>Ewing Sarcoma Myxoid Liposarcoma</td>
<td>Unfavorable</td>
<td>(11-13)</td>
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<tr>
<td>P16/CDKN2A loss</td>
<td>Ewing Sarcoma Myxoid Liposarcoma</td>
<td>Unfavorable</td>
<td>(11;13;14)</td>
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<tr>
<td>MYCN amplification</td>
<td>Neuroblastoma</td>
<td>Unfavorable</td>
<td>(15)</td>
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<tr>
<td>PIK3CA mutation</td>
<td>Myxoid Liposarcoma</td>
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</table>
C. Predictive Markers: Fusion type

<table>
<thead>
<tr>
<th>Sarcoma</th>
<th>Fusion</th>
<th>Predictive value</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFSP</td>
<td>COL1A1-PDGFB</td>
<td>Imatinib response</td>
<td>(17) (18)</td>
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<td>Giant cell tumor of tendon sheath/PVNS</td>
<td>COL6A3-CSF1</td>
<td>Imatinib response</td>
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<tr>
<td>Myxoid Liposarcoma</td>
<td>FUS-CHOP, EWS-CHOP</td>
<td>Trabectedin response</td>
<td>(21;22)</td>
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<tr>
<td>ASPS</td>
<td>ASPL-TFE3</td>
<td>MET TKI response</td>
<td>(23) (24)</td>
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<td>Clear Cell Sarcoma</td>
<td>EWS-ATF1</td>
<td>MET TKI response</td>
<td>(24-26)</td>
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<tr>
<td>Ewing Sarcoma</td>
<td>EWS-FLI1, EWS-ERG</td>
<td>IGF1R Ab therapy response</td>
<td>(27-30)</td>
</tr>
</tbody>
</table>

TKI: tyrosine kinase inhibitor

D. Predictive Markers: other mutations (excluding GIST)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sarcoma</th>
<th>Predictive value</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDM2 amplification</td>
<td>WD/DD Liposarcoma</td>
<td>MDM2 inhibitor (nutlin) response</td>
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<td>PEComa</td>
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<td>Neuroblastoma</td>
<td>ALK TKI response</td>
<td>(34-37)</td>
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<td>ALK fusion</td>
<td>Inflammatory myofibroblastic tumor</td>
<td>ALK TKI response</td>
<td>(37)</td>
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<tr>
<td>KDR mutation</td>
<td>Angiosarcoma</td>
<td>KDR TKI response</td>
<td>(38)</td>
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Reference List


