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Prognostication in soft tissue sarcomas- where do we stand ?

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Key points

- Histologic grade is the most important prognostic factor. It should be included in the pathology report along with tumor size, tumor depth, vascular invasion, peripheral tumor growth pattern, and status of surgical margins.
- Histologic typing and subtyping is a prerequisite to grading. Grade does not replace histologic typing and subtyping; both methods are complementary.
- Core biopsy specimens can be graded. Unless tumors are obviously high-grade, there is a risk for grade underestimation. Clinical data and data from imaging procedures (MRI, PET scan) should always be taken into account when evaluating core needle biopsies.
- Molecular parameters will not replace classic prognostic factors. They may have a role in predicting and/or monitoring response to therapy, and in detecting infraclinical relapses in a subset of STS.
- Nomograms and risk stratification models, including molecular approaches are major steps in sarcoma prognostication. Vascular invasion, an important prognosticator, should be included in staging systems and nomograms/risk stratification models.

Introduction

Soft tissue sarcomas (STSs) form an heterogeneous group of rare mesenchymal neoplasms with a wide range in terms of clinical presentation, situation, size, histology, and behaviour [1]. Roughly, they recur locally in about 20 to 30% of cases and metastasize, essentially to lungs and pleura, in 30 to 50% of cases; 5-year overall survival varies between 55% and 65%, regardless of stage and histology. Treatment and prognosis are interdependent factors. On one hand, accurate determination of prognosis is critical in order to guide therapeutic decisions, on the other hand, prognosis depends on therapeutic efficacy. Classical predictors of distant metastases and overall survival include histologic type and subtype, histologic grade, tumor size, tumor site and depth, tumor necrosis, mitotic activity, vascular invasion, and neurovascular or bone involvement [2-6]. Local recurrence(s) is mainly related to the quality of surgical excision (i.e. the capacity of the surgeon to resect the lesion with tumor-free margins). In the last 10 years, significant advances have been made regarding diagnostic procedures (e.g. core biopsies are increasingly used instead of

incisional biopsies) and imaging techniques, as well as molecular mechanisms involved in STS growth and maintenance. Some of these techniques (e.g. molecular biology) generated new potential prognostic factors (e.g. fusion gene products), the value of which still needs to be confirmed. The aim of this presentation is to examine the practical value and limitations of well-established and emerging prognostic factors considering the recent modifications that occurred in the management of patients with STS. GIST, which best illustrates the relationship between morphology, molecular biology, response to treatment and outcome, will not be considered here.

Histologic grade

It is currently recognized that histologic grade is the most important prognostic factor for adult STSs [2,6]. As best predictor of metastasis development and tumor mortality, histologic grade is a key parameter of the currently used 2002 TNM clinicopathologic staging system [7]. Several grading systems (two-tier, three-tier and four-tier systems) have been developed to overcome inconsistencies among pathologists in histologic typing and subtyping. All of them proved to correlate with overall survival and disease-free survival. Two three-grade systems, the FNCLCC (“French”) system and the NCI (National Cancer Institute) system are the most widely used [1,2,6]. The NCI system is based on the evaluation of tumor histologic type or subtype, location, and amount of tumor necrosis, although cellularity, nuclear pleomorphism, and mitotic count are also to be considered in certain situations. The FNCLCC system is based on a score generated by the assessment of three parameters: tumor differentiation (which is, in some ways, equivalent to histologic typing/subtyping), mitotic rate, and amount of necrosis. The goal of a grading system is to separate tumors of good prognosis (grade 1) from those of poor prognosis (grade 3). When comparing the performances of these two systems in the same group of patients with STS, the French system did better [6]. It allocated fewer patients to the noninformative grade 2 category and appeared more discriminating and efficient in the selection of patients with tumors of high malignant potential who could benefit from adjuvant chemotherapy. Discrepancies between the two grading systems were nevertheless observed in 34.6% of the cases [6].

Limitations of grading systems and controversial points

➤ Is an universal grading system applicable to most STSs preferable to a system applicable to every histologic type of STS ?

Several criticisms have repeatedly addressed to grading systems [8-11]. One is related to the fact that all grading systems have been developed and tested in the overall sarcoma group and not specifically in every histologic category. In a recent study on 1240 localized STSs, Coindre et al. [12] showed, however, that, with the exception of MPNST and rhabdomyosarcomas, histologic grade remains an important predictor of metastasis in the main histologic types of STSs (90% of STSs.). It is now clear that no current grading system performs well for every type of STS. However, given the number of histologic types of STSs, it is totally unrealistic and pointless to develop a grading system for every specific histologic type of STS. For the time being, the FNCLCC or the NCI systems, which perform adequately for the most common sarcoma types, still represent an acceptable alternative.

➤ Histologic grading, histologic typing, or both ?

It is important to keep in mind that histologic grading will not replace histologic typing. In the FNCLCC system, differentiation is almost synonymous with histotyping since determining histologic type is a prerequisite to judging differentiation. A weakness of

the FNCLCC system is the assignment of a differentiation score and the criteria used to distinguish between, for example, a well-differentiated (score 1) leiomyosarcoma and a conventional (score 2) leiomyosarcoma. In the refined version of the FNCLCC system [6], differentiation scores were partly assigned on the basis of statistical analyses. However, these analyses have never been published.

Histologic grading and histologic typing should be used in combination. It is clear that for some sarcoma types, histologic type is a synonym for grade. Well-differentiated liposarcomas/atypical lipomatous tumors are all grade 1 neoplasms, whereas Ewing sarcoma, alveolar rhabdomyosarcoma, extrarenal rhabdoid tumor and desmoplastic small round cell tumor are definitionally high-grade STSs. It is also not recommended to grade poorly-differentiated (round cell) myxoid liposarcomas, epithelioid sarcomas, clear cell sarcomas, and alveolar soft part sarcomas using the FNCLCC system as most of them belong to the noninformative grade 2 category [6]. Here, histology is more important than grade. Grade is also less informative than histologic type in a subset of STSs that includes dedifferentiated liposarcoma (many dedifferentiated liposarcomas are high-grade tumors but show a relatively low - 15-20% - metastatic rate), low-grade fibromyxoid sarcoma (a morphologically low-grade neoplasm which metastasizes in 50-70% of cases after 7-10 years), giant cell tumor of soft tissue, and myxoid chondrosarcoma [2,9].

➤ **Grading systems: three-tier or two-tier ?**

In a three-tier grading system, the grade 2 category (40% to 50% of cases) is noninformative in terms of prognosis and therapeutic decision. It is clear that, in this intermediate category which comprises a mixture of tumors of good and of bad prognosis, additional criteria are needed to discriminate between them. Ravaud et al [13] showed that a combination of grade and tumor depth was a valuable approach; superficial grade 2 tumors showed a prognosis quite similar to that of grade 1 tumors, whereas deep-seated grade 2 STSs behaved like grade 3 tumors. In the 2002 TNM system STSs are lumped in two categories only, low-grade and high grade tumors [7]. This approach removes the problem of the noninformative G2 category but does not solve the issue at all. STS of good prognosis which would have been classified as grade 2 tumors in a three-tier system are automatically reclassified as high-grade tumors in a two-tier system. As a consequence, such patients are likely to be inappropriately overtreated. One study which compared a 2 grade system to a 3 grade system demonstrated higher performance for the three tier system in predicting metastasis-free survival in extremity soft tissue sarcoma [14].

➤ **Other limitations of conventional grading systems**

Grading should be performed on untreated tumors because radiotherapy and chemotherapy can increase tumor necrosis and decrease mitotic activity.

Another weakness of any grading system is its reproducibility. The reproducibility of the FNCLCC system has been tested. It showed that evaluation of tumor grade was more reproducible than the diagnosis of histologic type (75% agreement for tumor grade versus 61% agreement for diagnosis of histologic type) [15]. It is true that some sarcomas may be difficult to classify, and, indeed, histologic grade was developed initially to overcome inconsistencies among pathologists in histologic typing and subtyping.

Pediatric STSs that are not rhabdomyosarcomas and not Ewing/PNET can be graded according to the FNCLCC system. However, they are most often graded according to the POG system [16] based on patient age, histologic type, tumor necrosis, and mitotic activity.

➤ **Are conventional grading systems adapted to core needle biopsies or fine-needle aspirations (FNA) ?**

Conventional gradings systems were developed for use on representative material, i.e. on whole untreated tumor or on open biopsies. Core needle biopsies are currently widely used for the diagnosis of primary STSs and for establishing treatment strategies [17-21]. FNA are more suitable for the evaluation and documentation of local recurrences and metastases from previously diagnosed STSs. For some authors both techniques are complementary [18]. Most studies showed that core needle biopsies have a high degree of accuracy in the diagnosis of malignancy (95%) [17-21]. False negative results are often due sampling errors or to well-differentiated liposarcomas misdiagnosed as lipomas [19]. Sarcoma typing and grading can also be performed with an acceptable degree of accuracy (70% to 80%) [19,20]. In the French Sarcoma Group, we found that histologic grade determination is accurate essentially for high-grade (G3) neoplasms. Determination of grade in the intermediate (G2) and low-grade (G1) categories is more problematic [21]. It should also be stressed that core needle biopsies should not be evaluated without a good knowledge of clinical and radiological data. Imaging procedures often provide useful information about the nature of the lump, especially regarding tumor size, borders, and amount of necrosis.

Can histologic grading systems be refined ?

Nowadays, classical grading systems should be updated to fit with the modern management of patients. Many patients receive preoperative chemotherapy or radiotherapy, rendering grading pointless at the time of surgical excision. Evaluation of STS grade (low versus high grade), of extension of tumor necrosis and response to therapy can now also be assessed using new diagnostic imaging techniques such as magnetic resonance spectroscopy, FDG-PET, and FLT-PET [22-24], and the results integrated in updated grading systems. It has also been shown that grading using a Mib-1 score instead of mitotic count has a better predictive value and is more reproducible [25] than the original version.

Prognostic impact of myogenic differentiation in STSs

In a recent re-evaluation of 100 so-called MFH, Fletcher et al. [26] observed that pleomorphic sarcomas which showed myogenic differentiation (i.e. leiomyosarcoma, rhabdomyosarcoma and myogenic sarcoma NOS) were associated with more aggressive behavior, higher metastatic rate and shorter time to metastasis than those lacking myogenic differentiation. It was the first time that myogenic differentiation, as a whole, was shown to be an adverse prognostic factor. Deyrup et al. [27] showed that this negative effect was maintained even after adjusting for tumor grade, tumor size, tumor extent, and patient age, and that increasing myoid differentiation correlated with worse survival (additive effect of myoid differentiation). Thus, patients with pleomorphic sarcomas that express myoid antigens might benefit from the development of better adjuvant therapies. Of importance, dedifferentiated liposarcomas showing heterologous myogenic differentiation do not show increased aggressiveness due to this additional feature; they behave like conventional dedifferentiated liposarcomas (i.e. without myogenic differentiation) [28].

Contribution of molecular biology and markers to STS prognostication

In the last 10 years significant achievements have been made in the molecular approach of soft tissue sarcomas (STS). For instance, it has been suggested that

fusion type could be an important prognostic factor in synovial sarcoma patients, tumors harboring the SYT-SSX1 fusion being more aggressive and having a higher propensity for metastatic dissemination than SYT-SSX2 neoplasms [29-31]. Unfortunately, this could not be confirmed in a subsequent independent study [32]. The prognostic value of fusion type in synovial sarcoma is questionable at the present time, and cannot be considered clinically relevant.

Similarly, the prognostic value of fusion type in Ewing sarcoma remains to be confirmed. Zoubeck et al. [33] showed in univariate analyses that for patients with localized disease, type I fusion gene (fusion between EWS exon 7 and FLI1 exon 6) was associated with longer relapse-free survival compared with other type of fusion genes. In a series of 112 patients, de Alava et al. [34] observed a positive relationship between type I EWS-FLI1 fusion and overall survival suggesting that EWS-FLI1 transcript structure is an independent determinant of prognosis in Ewing sarcoma. Similar results were obtained by Avigad et al. more recently [35]. In contrast, Ginsberg et al. [36] failed to find any prognostic value of fusion gene in ES in univariate analyses.

Alterations (deletions, mutations) of the p16^{INK4}, p14^{ARF}, p27^{KIP1}, and p53 tumor suppressor genes are also of great importance [37,38], often associated with aggressive behavior and poor chemoresponse [39]. Telomerase activity [40] and short telomeres [41] were also found to be strong negative prognosticators. Recently, gene expression profiling technology has led to the identification of high-risk and low-risk patient groups [42], in addition to delivering some arguments in favor of the use of anti-tyrosine kinase receptors (e.g. anti-IGF-1 receptor).

Most alveolar rhabdomyosarcomas bear either the t(2;13) (q35;q14) PAX3-FOXO1A (70-80% of cases) or the t(1;13) (p36;q14) PAX7-FOXO1A (10%) translocation. From a prognostic point of view, tumors with PAX7-FOXO1A fusion tend to behave less aggressively than PAX3-FOXO1A [43], especially in patients with metastatic disease [44]. Bone marrow involvement was also significantly more frequent in PAX3-FOXO1A-positive patients [44]. As for Ewing sarcoma, the presence of alveolar rhabdomyosarcoma tumor cells in bone marrow, as detected by RT-PCR, seems to be predictive of shortened disease-free survival and/or overall survival [45,46], suggesting that these patients should be treated more intensively.

Recently, microarray analysis of 139 primary rhabdomyosarcomas by Davicioni et al [47] revealed that alveolar RMS expressing either fusion gene share a common expression profile that is distinct from fusion-negative alveolar RMS. In addition, the authors identified a subset of genes within the PAX-FOXO1A expression signature that segregated alveolar RMS into 3 distinct prognostic categories with 5-year overall survival estimates of 7%, 48%, and 93%, independently of conventional clinical risk factors, suggesting that PAX-FOXO1A target gene signatures may provide prognostic information. Amplification and/or overexpression of N-MYC was also found to be associated with adverse outcome in alveolar rhabdomyosarcoma [48].

Prognostication using staging systems - limitations and refinements

The aim of a staging system is to provide clinicians with a tool that allows them to evaluate accurately the aggressiveness of a given STS, resulting, ideally, in tailored therapeutic modalities. It should be simple, easy to use and based on the most prognostically relevant factors. Various clinicopathologic staging systems have been proposed for STS (reviewed in [49]), but none are perfect. Most, including the 2002 TNM classification, are based on clinical and histologic parameters, including

histologic malignancy grade, tumor size, tumor depth, and the presence or absence of regional lymph node and/or distant metastases. Experience strongly suggests that some other parameters should be considered such as vascular invasion [50-52], peripheral tumor growth pattern (pushing versus infiltrative borders) [51,52], Mib-1 immunostaining [25,51,52], as well as some clinical parameters (e.g. tumor site, margin status, and prior local recurrence) [49].

Two recently developed staging systems proved to be superior to the 2002 TNM classification in identifying patients at high-risk for the development of metastases: the SIN-system based on vascular invasion, tumor size, and microscopic tumor necrosis [50], and the system of Engellau et al. based on vascular invasion, tumor size, microscopic tumor necrosis, and peripheral tumor growth pattern [52]. Interestingly, the latter system was able to detect low-risk tumors in a population of histological high-grade soft tissue sarcomas, thus avoiding overtreatment for a subset of patients [52]. Recently, it has also been suggested that tumor depth could well be deleted from staging systems since a large proportion of its prognostic weight is already included in tumor size, provided that more than two size categories (≤ 5 cm versus > 5 cm) are used [53]. In the 2002 TNM system, lumping grade 2 and grade 3 tumors in a high-grade category is another way to lose prognostic information and it increases the risk for patients to receive inappropriate neoadjuvant or adjuvant treatments (see paragraph on histologic grade). Obviously, the existence of several grading systems with their own advantages and limitations points to the need for a universal grading and staging approach in STS.

The future:

➤ Will nomograms and risk stratification models replace conventional staging and grading systems ?

A nomogram is a prognostic model that predicts disease-specific events (e.g. death) for a given patient with a given disease. Thus, it is useful for patient counselling and, also, theoretically, for selecting patients appropriate for adjuvant therapy. All important prognostic factors should be included in the model to be useful and highly predictive. A nomogram for 12-year sarcoma-specific mortality has been developed and validated by Memorial Sloan Kettering (<http://www.nomograms.org>) [54,55]. In this model, a limited number of histological types of STS have been included, and an important prognosticator, vascular invasion, is missing. A modified version of this nomogram, which is applicable to extremity STS only and which includes a three-tier grading system, has been developed and showed higher predictive value [56]. More recently, a nomogram devoted specifically to liposarcomas has been proposed [57].

Risk stratification models and nomograms are better predictors than conventional staging or grading systems for a given patient. Compared to staging systems, nomograms take several additional factors into account such as tumor site, tumor histology, patient age, gender, or tumor margins [49]. For some sarcoma types, histotype-specific risk stratification models are probably more appropriate [57]. In the near future, they will gradually include radiological, biological, and molecular parameters, and will become the gold standard for STS prognostication.

➤ Towards molecular prognostication for STSs ?

The practical value of molecular biology to sarcoma prognostication is still limited at the present time [58]. Many promising preliminary results still require validation in prospective studies before they meet with general acceptance. Despite these

limitations, it is anticipated that arrays (DNA, cDNA), histotype-specific or not, regrouping most significant molecular prognosticators will soon be available [58]. Proteomics is also a promising tool for molecular classification and prognostication of STSs [59].

Key points

- Histologic grade is the most important prognostic factor. It should be included in the pathology report along with tumor size, tumor depth, vascular invasion, peripheral tumor growth pattern, and status of surgical margins.
- Histologic typing and subtyping is a prerequisite to grading. Grade does not replace histologic typing and subtyping; both methods are complementary.
- Core biopsy specimens can be graded. Unless tumors are obviously high-grade, there is a risk for grade underestimation. Clinical data and data from imaging procedures (MRI, PET scan) should always be taken into account when evaluating core needle biopsies.
- Molecular parameters will not replace classic prognostic factors. They may have a role in predicting and/or monitoring response to therapy, and in detecting infraclinical relapses in a subset of STS.
- Nomograms and risk stratification models, including molecular approaches are major steps in sarcoma prognostication. Vascular invasion, an important prognosticator, should be included in staging systems and nomograms/risk stratification models.

References

- 1 Fletcher CDM, Unni KK, Mertens F, eds. Pathology and Genetics of Tumours of Soft Tissue and Bone. Lyon, France: IARC Press, 2002. World Health Organization Classification of Tumours, Vol 5.
- 2 Coindre JM. Grading of soft tissue sarcomas. Review and update. Arch Pathol Lab Med 2006; 130: 1448-1453.
- 3 Coindre JM, Terrier P, Bui NB, et al. Prognostic factors in adult patients with locally controlled soft tissue sarcoma : a study of 546 patients from the French Federation of Cancer Centers Sarcoma Group. J Clin Oncol 1996, 14: 869-877.
- 4 Pisters PWT, Leung DHY, Woodruff J, et al. Analysis of prognostic factors in 1041 patients with localized soft tissue sarcomas of the extremities. J Clin Oncol 1996, 14: 1679-1689.
- 5 Zagars GK, Ballo MT, Pisters PWT, et al. Prognostic factors for patients with localized soft-tissue sarcoma treated with conservation surgery and radiation therapy. Cancer 2003; 97: 2530-2543.

- 6 Guillou L, Coindre JM, Bonichon F, et al. Comparative study of the National Cancer Institute and French Federation of Cancer Centers Sarcoma Group grading systems in a population of 410 adult patients with soft tissue sarcoma. *J Clin Oncol* 1997; 15: 350-362.
- 7 International Union Against Cancer: TNM Classification of malignant tumours. LH Sobin and Ch Wittekind (eds). 6th edition. New York, USA, Wiley-Liss, 2002.
- 8 Kilpatrick SE. Histologic prognostication in soft tissue sarcomas: grading versus subtyping or both ? A comprehensive review of the literature with proposed practical guidelines. *Ann Diagn Pathol* 1999;3: 48-61.
- 9 Brown FM, Fletcher CDM. Problems in grading soft tissue sarcomas. *Am J Clin Pathol* 2000; 114: S82-S89.
- 10 Oliveira AM, Nascimento AG. Grading in soft tissue tumors: principles and problems. *Skeletal Radiol* 2001; 30: 543-559.
- 11 Deyrup AT, Weiss SW. Grading of soft tissue sarcomas: the challenge of providing precise information in an imprecise world. *Histopathology* 2006; 48: 42-50.
- 12 Coindre JM, Terrier P, Guillou L, et al. Predictive value of grade for metastasis development in the main histologic types of adult soft tissue sarcomas : a study of 1240 patients from the French Federation of Cancer Centers Sarcoma Group. *Cancer* 2001; 91: 1914-1926.
- 13 Ravaud A, Bui NB, Coindre JM, et al. Prognostic variables for the selection of patients with operable soft tissue sarcomas to be considered in adjuvant chemotherapy trials. *Br J Cancer* 1992; 66: 961-969.
- 14 Kandel RA, Bell RS, Wunder JS, et al. Comparison between a 2- and 3-grade system in predicting metastatic-free survival in extremity soft-tissue sarcoma. *J Surg Oncol* 1999; 72, 77-82.
- 15 Coindre JM, Trojani M, Contesso G, et al. Reproducibility of a histopathologic grading system for adult soft tissue sarcoma. *Cancer* 1986; 58: 306-309.
- 16 Parham DM, Webber BL, Jenkins JJ, et al. Nonrhabdomyosarcomas soft tissue sarcomas of childhood: formulation of a simplified system for grading: *Mod Pathol* 1995; 8:705-710.
- 17 Welker JA, Henshaw RM, Jelinek J et al. The percutaneous needle biopsy is safe and recommended in the diagnosis of musculoskeletal masses. Outcome analysis of 155 patients in a sarcoma referral center. *Cancer* 2000; 89: 2677-2686.
- 18 Domanski HA, Akerman M, Carlen B, et al. Core-needle biopsy performed by the cytopathologist. A technique to complement fine-needle aspiration of soft tissue and bone lesions. *Cancer Cytopathol* 2005; 105: 229-239.
- 19 Hoerber I, Spillane AJ, Fisher C, et al. Accuracy of biopsy techniques for limb and limb girdle soft tissue tumors. *Ann Surg Oncol* 2001; 8: 80-87.

- 20 Mitsuyoshi G, Naito N, Kawai A, et al. Accurate diagnosis of musculoskeletal lesions by core needle biopsy. *J Surg Oncol* 2006; 94: 21-27.
- 21 Ray-Coquard I, Ranchère-Vince D, Thiesse P, et al. Evaluation of core needle biopsy as a substitute to open biopsy in the diagnosis of soft-tissue masses. *Eur J Cancer* 2003, 2021-2025.
- 22 Vaidya SJ, Payne GS, Leach MO, et al. Potential role of magnetic resonance spectroscopy in assessment of tumour response in childhood cancer. *Eur J Cancer* 2003; 39: 728-735.
- 23 Bastiaannet E, Groen H, Jager PL, et al. The value of FDG-PET in the detection, grading and response to therapy of soft tissue and bone sarcomas. A systematic review and meta-analysis. *Cancer Treat Rev* 2004; 30: 83-101.
- 24 Cobben DCP, Elsinga PH, Suurmeijer AJH, et al. Detection and grading of soft tissue sarcomas of the extremities with ¹⁸F-3'-fluoro-3'-deoxy-L-thymidine. *Clin Cancer Res* 2004; 10: 1685-1690.
- 25 Hasegawa T, Yamamoto S, Yokoyama R, et al. Prognostic significance of grading and staging systems using MIB-1 score in adult patients with soft tissue sarcoma of the extremities and trunk. *Cancer* 2002; 95: 843-851.
- 26 Fletcher CD, Gustafson P, Rydholm A, et al. Clinicopathologic re-evaluation of 100 malignant fibrous histiocytomas: prognostic relevance of subclassification. *J Clin Oncol* 2001; 19: 3045-3050.
- 27 Deyrup AT, Haydon RC, Huo D, et al. Myoid differentiation and prognosis in adult pleomorphic sarcomas of the extremity: an analysis of 92 cases. *Cancer* 2003; 98: 805-813.
- 28 Bui Nguyen Binh M, Guillou L, Hostein I, et al. Dedifferentiated liposarcomas with divergent myosarcomatous differentiation developed in the internal trunk. A study of 27 cases and comparison to conventional dedifferentiated liposarcomas and leiomyosarcomas. *Am J Surg Pathol* 2007 ; 31 : 1557-1566.
- 29 Kawai A, Woodruff J, Healey JH, et al. SYT-SSX gene fusion as a determinant of morphology and prognosis in synovial sarcoma. *N Engl J Med* 1998 ; 338: 153-160.
- 30 Nilsson G, Skytting B, Xie Y, et al. The SYT-SSX1 variant of synovial sarcoma is associated with a high rate of tumor cell proliferation and poor clinical outcome. *Cancer Res* 1999 ; 59 : 3180-3184.
- 31 Ladanyi M, Antonescu CR, Leung DH, et al. Impact of SYT-SSX fusion type on the clinical behavior of synovial sarcoma: a multi-institutional retrospective study of 243 patients. *Cancer Res* 2002; 62: 135-140.
- 32 Guillou L, Benhattar J, Bonichon F, et al. Histologic grade, but not SYT-SSX fusion type, is an important prognostic factor in patients with synovial sarcoma: a multicenter, retrospective analysis. *J Clin Oncol* 2004; 22: 4040-4050.

- 33 Zoubek A, Dockhorn-Dworniczak B, Delattre O, et al. Does expression of different EWS chimeric transcripts define clinically distinct risk groups of Ewing tumor patients? *J Clin Oncol*. 1996 ;14 : 1245-1251.
- 34 De Alava E, Kawai A, Healey JH, et al. EWS-FLI1 fusion transcript structure is an independent determinant of prognosis in Ewing's sarcoma. *J Clin Oncol*. 1998 ;16:1248-1255.
- 35 Avigad S, Cohen IJ, Zilberstein J, et al. The predictive potential of molecular detection in the nonmetastatic Ewing family of tumors. *Cancer* 2004; 100: 1053-1058.
- 36 Ginsberg JP, de Alava E, Ladanyi M, et al. EWS-FLI1 and EWS-ERG gene fusions are associated with similar clinical phenotypes in Ewing's sarcoma. *J Clin Oncol* 1999; 17: 1809-1814.
- 37 de Alava E, Antonescu CR, Panizo A, et al. Prognostic impact of P53 status in Ewing sarcoma. *Cancer* 2000 ;89 :783-792.
- 38 Honoki K, Stojanovski E, McEvoy M, et al. Prognostic significance of p16^{INK4a} alteration for Ewing sarcoma: a meta-analysis. *Cancer* 2007; 110: 1351-1360.
- 39 Huang HY, Illei PB, Zhao Z, et al. Ewing sarcomas with p53 mutation or p16/p14ARF homozygous deletion: a highly lethal subset associated with poor chemoresponse. *J Clin Oncol* 2005; 23:548-558.
- 40 Ohali A, Avigad S, Cohen IJ, et al. Association between telomerase activity and outcome in patients with nonmetastatic Ewing family of tumors. *J Clin Oncol* 2003; 21: 3836-3843.
- 41 Avigad S, Naumov I, Ohali A, et al. Short telomeres: a novel potential predictor of relapse in Ewing sarcoma. *Clin Cancer Res* 2007; 13: 5777-5783.
- 42 Ohali A, Avigad S, Zaizov R, et al. Prediction of high risk Ewing's sarcoma by gene expression profiling. *Oncogene* 2004; 23: 8997-9006.
- 43 Kelly KM, Womer RB, Sorensen PH, et al. Common and variant gene fusions predict distinct clinical phenotypes in rhabdomyosarcoma. *J Clin Oncol* 1997; 15: 1831-1836.
- 44 Sorensen PH, Lynch JC, Qualman SJ, et al. PAX3-FKHR and PAX7-FKHR gene fusions are prognostic indicators in alveolar rhabdomyosarcoma: a report from the Children's Oncology Group. *J Clin Oncol* 2002; 20: 2672-2679.
- 45 Kelly KM, Womer RB, Barr FG, et al. Minimal disease detection in patients with alveolar rhabdomyosarcoma using a reverse transcriptase-polymerase chain reaction method. *Cancer* 1996; 78: 1320-1327.
- 46 Athale UH, Shurtleff SA, Jenkins JJ, et al. Use of reverse transcriptase polymerase chain reaction for diagnosis and staging of alveolar rhabdomyosarcoma,

Ewing sarcoma family of tumors, and desmoplastic small round cell tumor. *J Pediatr Hematol Oncol* 2001; 23: 99-104.

47 Davicioni E, Finckenstein FG, Shahbazian V, et al. Identification of a PAX-FKHR gene expression signature that defines molecular classes and determines the prognosis of alveolar rhabdomyosarcomas. *Cancer Res* 2006; 66: 6936-6946.

48 Williamson D, Lu YJ, Gordon T, et al. Relationship between MYCN copy number and expression in rhabdomyosarcomas and correlation with adverse prognosis in the alveolar subtype. *J Clin Oncol* 2005; 23: 880-888.

49 Kotilingam D, Chelouche Lev D, Lazar JF, et al. Staging soft tissue sarcoma : evolution and change. *CA Cancer J Clin* 2006; 56: 282-291.

50 Gustafson P, Akerman M, Alvegard TA et al. Prognostic information in soft tissue sarcoma using tumour size, vascular invasion and microscopic tumour necrosis – the SIN-system. *Eur J Cancer* 2003; 39: 1568-1576.

51 Engellau J, Bendahl PO, Persson A, et al. Improved prognostication in soft tissue sarcoma: independent information from vascular invasion, necrosis, growth pattern, and immunostaining using whole-tumor sections and tissue microarrays. *Hum Pathol* 2005; 36: 994-1002.

52 Engellau J, Samuelsson V, Anderson H, et al. Identification of low-risk tumours in histological high-grade soft tissue sarcomas. *Eur J Cancer* 2007; 43: 1927-1934.

53 Rydholm A, Gustafson P. Should tumor depth be included in prognostication of soft tissue sarcoma ? *BMC Cancer* 2003; 3: 1-5.

54 Kattan MW, Leung DHY, Brennan MF. Postoperative nomogram for 12-year sarcoma-specific death. *J Clin Oncol* 2002; 20: 791-796.

55 Eilber FC, Brennan MF, Eilber FR, et al. Validation of the postoperative nomogram for 12-year sarcoma-specific mortality. *Cancer* 2004; 101: 2270-2275.

56 Mariani L, Miceli R, Kattan MW, et al. Validation and adaptation of a nomogram for predicting the survival of patients with extremity soft tissue sarcoma using a three-grade system. *Cancer* 2005; 103: 402-408.

57 Moore Dalal K, Kattan MW, Antonescu CR, et al. Subtype specific prognostic nomogram for patients with primary liposarcoma of the retroperitoneum, extremity, or trunk. *Ann Surg* 2006; 244: 381-391.

58 Oliveira AM, Fletcher CDM. Molecular prognostication for soft tissue sarcomas: are we ready yet. *J Clin Oncol* 2004; 22: 4031-4034.

59 Suehara Y, Kondo T, Fujii K, et al. Proteomic signatures corresponding to histological classification and grading of soft-tissue sarcomas. *Proteomics* 2006; 6: 4402-4409.

Epithelioid Vascular Tumors of Bone and Soft Tissue

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Introduction

Endothelial cells distinguished by their epithelioid morphology have been recognized for many years. Rosai first characterized them as “histiocytoid” in appearance in 1979 and soon thereafter they became known by the more popularized term "epithelioid". By the early and mid 1980's this nomenclature was incorporated into classification schemes of epithelioid vascular tumors that included reactive, benign and malignant proliferations such as variants of bacillary angiomatosis, epithelioid hemangioma, spindle cell hemangioma, epithelioid hemangioendothelioma and epithelioid angiosarcoma. Subsequently, several new uncommon entities including composite hemangioendothelioma and epithelioid sarcoma-like hemangioendothelioma were described. As a group, these tumors can originate in a variety of different organ systems however, most develop in the soft tissues and skeleton. The epithelioid endothelial cell is common to them all, however, it is very important to distinguish amongst them as they have significant differences in their clinical behavior and biological potential and consequently their treatment and prognosis.

The Epithelioid Endothelial Cell

The hallmark of epithelioid endothelial cells is their polyhedral shape and abundant densely eosinophilic cytoplasm which frequently contains one or more large vacuoles and their large nuclei. The vacuoles represent the earliest stage of vessel lumen formation and may be empty or contain intact or fragmented red blood cells. As vacuoles from neighboring cells fuse they create vascular spaces of varying degrees of differentiation. This process is best developed in the benign lesions where well-formed vessels are numerous and the lining epithelioid endothelial cells bulge into the lumens in a “tombstone “ or “hobnail-like” fashion. In malignant neoplasms the tumor cells are often arranged in cords and sheets with only subtle evidence of vessel lumen formation, which may only be in the guise of intracytoplasmic vacuoles. It is important to note, however, that a solid cord or sheet-like growth pattern can be seen in all types of epithelioid vascular neoplasms, irrespective of their biologic potential and should be recognized as a key diagnostic pitfall because, in our experience, it can cause a benign lesion to

be misconstrued as a malignancy. As with many types of tumors, the histologic significance of architecture in epithelioid vascular tumors must be evaluated in the context of other compelling morphological features.

The nuclear features of the epithelioid endothelial cells vary according to the biologic nature of the tumor. In reactive conditions and benign neoplasms they are hyperlobated, have irregular or grooved membranes, vesicular chromatin and small or sometimes prominent nucleoli. In the more aggressive tumors, namely, epithelioid hemangioendothelioma and epithelioid angiosarcoma, the nuclei differ in that they exhibit a significantly greater degree of pleomorphism and hyperchromasia.

Epithelioid endothelial cells express all of the characteristic vascular endothelial markers including Ulex europeus lectin, Factor VIII related antigen, CD34, CD31, and Fli-1. Epithelioid vascular tumors have not been systematically studied for the expression of GLUT-1 and D2-40, however, the limited information available suggests that they are negative for GLUT-1 and epithelioid hemangioendothelioma and angiosarcoma express D2-40. Additionally, epithelioid endothelial cells frequently demonstrate strong and diffuse staining for a variety of molecular weight cytokeratins and epithelial membrane antigen. This is another diagnostic hazard, as other types of tumors composed of epithelioid cells that are in the histological differential diagnosis, such as carcinoma and epithelioid sarcoma also express keratin and EMA.

Ultrastructurally epithelioid endothelial cells contain abundant cytoplasmic filaments, mitochondria, lysosomes, pinocytotic vesicles and Weibel-Palade bodies and are surrounded by basal lamina.

No genetic abnormalities have been found to be specific for epithelioid endothelial cells.

Bacillary Angiomatosis

Bacillary angiomatosis (BA), previously known as epithelioid angiomatosis is a distinctive reactive vascular proliferation elicited by infection by *Bartonella* (formerly *Rochalimaea*) *henselae* and *quintana* organisms, which are small gram negative rods. These pathogens are introduced into humans, who serve as reservoirs, by blood-sucking arthropods (body louse- *Pediculus humanus* and cat flea) or in the case of the former by a direct cat scratch or bite. Once infection occurs an intraerythrocyte bacteremia develops and the organisms target endothelial cells where they grow within and around the cells. These species of bacteria are unique in their ability to produce vasoproliferative lesions by secreting mitogens which result in the development of new capillaries from preexisting ones. Histologically, the proliferating endothelial cells can cause confusion with epithelioid hemangioma, epithelioid hemangioendothelioma, Kaposi's sarcoma and angiosarcoma.

BA usually occurs in immunocompromised hosts especially those infected with HIV, however, it has also been reported to rarely involve immunocompetent persons. The lesions present as erythematous cutaneous nodules that are frequently multiple and may be wide spread. Extracutaneous lesions may also develop and have been reported in

the mucus membranes, lymph nodes, bone marrow, liver, spleen and soft tissue. Clinically, the cutaneous manifestations can mimic pyogenic granuloma, Kaposi's sarcoma and angiosarcoma.

Microscopically, the vascular proliferation in BA has a spectrum of findings. The vessels are frequently arranged in vague lobules and range from small, well formed capillaries lined by flattened to plump endothelial cells to interanastomosing cords and nests of epithelioid endothelial cells. The epithelioid endothelial cells may have prominent nucleoli, cytoplasmic vacuoles, numerous mitoses and regions of necrosis. Present in all cases are interstitial edema, and perivascular collections of neutrophils with karyorrhectic and fine granular basophilic debris. The Warthin-Starry silver stain shows clumps of pleomorphic bacilli 1 to 3 microns in length located in the areas of basophilic debris.

Cutaneous BA is polypoid, frequently ulcerated and its base is surrounded by a hyperplastic squamous collarette. These architectural features as well as the lobular distribution of the vessels can mimic pyogenic granuloma. However, pyogenic granuloma lacks the diffuse infiltrate of neutrophils and granular debris and importantly, epithelioid cells are either not prominent or are absent. The presence of neutrophils and granular debris are also helpful in separating BA from epithelioid hemangioma. Epithelioid hemangioendothelioma lacks the inflammation and micro-organisms but also has a prominent myxoid or hyaline stroma that is not present in BA. Epithelioid angiosarcomas show a greater degree of cytologic atypia, atypical mitoses and do not have a significant inflammatory infiltrate and certainly no micro-organisms.

Treatment is the administration of antibiotics, namely erythromycin which result in resolution of the lesions. Untreated, patients can develop fatal systemic disease.

Epithelioid Hemangioma

Epithelioid hemangioma, previously designated angiolymphoid hyperplasia with eosinophilia and histiocytoid hemangioma, is a well recognized clinicopathologic entity. It has been most extensively documented in the skin and subcutis, and has also been described in bone, lymph nodes, lung, penis, eye, tongue, breast, arteries, colon, heart, spleen, and testis. The skeleton, it turns out, is probably the second most common location for this benign neoplasm.

In the superficial tissues epithelioid hemangioma usually presents as a solitary or cluster of small, pink to red-brown, dome-shaped nodules in the head and neck region of adults. Typically, the tumor has been noted months to several years prior to diagnosis and a few patients may also have associated regional adenopathy. In our experience with over 50 cases arising in bone, the patients range in age from 10-75, average 35 years and present with pain localized to the involved site. In the skeleton the tumors tend to involve the long tubular bones (38%), distal lower extremities (18%), flat bones (18%), vertebrae (16%), and small bones of the hands (8%). Radiographically, epithelioid hemangioma is lytic with well-defined margins. In a minority of instances the tumor is expansive, and especially in the small tubular bones, there may be cortical destruction and a periosteal reaction in conjunction with a soft tissue mass.

Epithelioid hemangioma of the skin and subcutis is multifocal in as many as 50%, whereas, only 18% of those developing in the skeleton affect more than one bone. Involvement of widely separate sites, including simultaneous involvement of skin and bone and skin and lymph node is uncommon.

Epithelioid hemangioma of the skin and subcutis ranges in size from 0.2 to 8 cm, whereas in bone they are often larger varying in size from 2.5-15 cms (mean 5 cm). The tumors are solid, well-circumscribed, tan-red and hemorrhagic and have a lobular architecture. In the skin and subcutis they are often associated with a large caliber, thick-walled vessel that exhibits changes suggestive of previous injury.

The hallmark of epithelioid hemangioma is large polyhedral endothelial cells that either line numerous well-defined vascular spaces or grow in solid cords and sheets which can produce a densely cellular mass. The tumor cells have oval, kidney bean shaped, lobated, grooved, vesicular nuclei with variably sized nucleoli. The cytoplasm is eosinophilic, abundant and some cells contain conspicuous, round, clear, cytoplasmic vacuoles which may harbor fragments of red blood cells. In some cases, there is abundant intralesional hemorrhage which is usually associated with proliferating, cytologically bland, spindle cells. Nuclear atypia, mitotic activity and necrosis are generally limited. The stroma frequently contains a prominent inflammatory cell infiltrate rich in lymphocytes, including follicles with germinal centers, and variable numbers of eosinophils and other mononuclear cells. In some cases, particularly those in bone, the inflammatory infiltrate is sparse or absent all together.

Epithelioid hemangioma of the skin and subcutis has limited growth potential, and is usually not very aggressive. Accordingly, marginal surgical excision is generally the treatment of choice, which is associated with non-aggressive recurrences in approximately one-third of cases. Rare examples of tumors spontaneously resolving have been reported.

Treatment of bone tumors usually consists of intralesional curettage or local resection. Untreated tumors have usually remained stable, and recurrences have been relatively infrequent.

No metastases have been reported, however, tumors involving bone and other distant sites and types of tissue which likely reflects multicentric disease, have rarely been described. Evans et al. argue that these latter cases are manifestations of metastases and use this to bolster their opinion that epithelioid hemangioma (as defined in the skin and subcutis) of bone is non-existent and simply represents a misdiagnosed hemangioendothelioma - a tumor with metastatic potential. Evidence they cite to support this assertion, I feel, is not very convincing, and includes the observations that epithelioid hemangioma of bone usually does not involve medium or large-sized vessels, especially arteries, and shows a greater degree of histologic variability. In contention, medium to large-sized vessels are normally not present in the medullary cavity of bones, and small vessels, if indeed the site origin may be obliterated by the expanding tumors (which are usually larger than those originating in the skin and subcutis). Additionally, most, if not all, of the morphologic variants observed in osseous tumors have been described in classic lesions originating in the skin. Also relevant is the fact that hemangioendothelioma of bone, not otherwise specified or qualified, is not a distinct diagnostic entity in current classification systems.

Epithelioid hemangioma can be confused with various benign diseases and neoplasms. It simulates eosinophilic granuloma because of the tissue eosinophilia and the morphologic characteristics of the endothelial cells. However, the Langerhans cells in Langerhans cell histiocytosis do not have cytoplasmic vacuoles, do not form vascular lumens, are S-100 protein positive and Factor VIII negative and have different ultrastructural features. Kimura's disease differs from epithelioid hemangioma in the clinical findings and the absence of epithelioid endothelial cells.

Epithelioid hemangioma can be difficult to distinguish from epithelioid hemangioendothelioma and angiosarcoma. This is most evident in cases in which the endothelial cells tend to grow in solid sheets and arise in blood vessels with infiltration through the vessel walls into the surrounding soft tissue. The lack of myxoid-hyaline stroma, hyperchromasia, atypical mitoses, necrosis and anastomotic network as well as the presence of the inflammatory infiltrate are important differential points.

Spindle Cell Hemangioma

Spindle cell hemangioma (SCH) is a morphologically unusual vascular tumor that was first described by Weiss and Enzinger in 1986 as a variant of a hemangioendothelioma. Subsequently it has been recognized as a benign lesion whose hallmark is the admixture of cavernous hemangioma-like and Kaposi's sarcoma-like spindle cell regions.

SCH develops in all age groups but approximately 50% of patients are young adults in the 3rd-5th decade of life; the sexes are equally affected. Clinically, the tumor presents as a painless firm superficial mass that has usually been present for years or even a decade prior to diagnosis. In approximately 40% of patients the lesions are multifocal, but, tend to involve a single localized area of the body.

SCH commonly arises in the dermis and subcutis of the distal extremities; tumors originating in bone are extraordinarily rare. It is not uncommon for them to involve or be completely intravascular in growth (50%) and this may be the mechanism by which multiple lesions arise in a relatively restricted region. Grossly, the mass is red-blue, nodular and ranges from several millimeters to several centimeters in greatest dimension. Histologically, SCH is composed of two elements, which share some histologic features. The first is a cavernous hemangioma-like component consisting of large, dilated, thin-walled vascular spaces that are lined by flattened or somewhat plump cytologically banal endothelial cells. Occasionally the lumen is expanded by calcified thrombi or phleboliths. Surrounding and admixed with the cavernous spaces is the second element which consists

of fascicles of spindle cells that likely represent a combination of collapsed vessels, fibroblasts, and cells with features of pericytes. Some of the spindle cells delineate slit-like vascular spaces that are filled with red blood cells. The spindle cells have elongate nuclei containing finely granular chromatin and small nucleoli. They are not pleomorphic and there is minimal mitotic activity. Scattered amongst the spindle cells are cords and small groups of larger epithelioid cells that have vacuolated cytoplasm. Some of the vacuoles contain red blood cells and others are large and empty mimicking adipocytes.

Immunohistochemically, the cells lining the cavernous spaces express endothelial markers, however, the spindle cells are frequently negative. A variety of etiologies have been proposed for the development of SCH including a reactive process caused by bouts of repeated thrombosis and recanalization, hamartomatous growth, and neoplastic proliferation. Interestingly, SCH has been described in patients with a variety of disease including Maffucci's syndrome, congenital lymphedema, and Klippel-Trenaunay syndrome.

In addition to the other epithelioid vascular tumors, Kaposi's sarcoma is an important lesion in the differential diagnosis of SCH. Kaposi's sarcoma occurs in chronic, lymphadenopathic, transplantation associated and AIDS related forms. Clinically, SCH and AIDS related Kaposi's sarcoma have some overlapping features in that they affect young adults, are frequently superficial in location, and have a tendency to be multifocal. However, an important difference is that patients with SCH have not been reported to be infected with HIV. Histologically, SCH and Kaposi's sarcoma can be distinguished because Kaposi's sarcoma does not have cavernous areas or epithelioid cells, and SCH does not demonstrate the hyaline globules seen in some of the cells of Kaposi's sarcoma. Although the clinical course of Kaposi's sarcoma is variable it is more aggressive than SCH and in the AIDS related variant involvement of lymph nodes and viscera is common. Also, Kaposi's sarcoma has been shown to be closely related to human herpes virus 8 which has not been described in SCH.

The recommended initial treatment of SCH is conservative excision. In more than 50% of cases, however, new lesions may develop in the same region and they may either be followed or excised.

Epithelioid Hemangioendothelioma

Epithelioid hemangioendothelioma (EHE) is an uncommon endothelial tumor that most frequently arises in the soft tissues, liver, lung, and skeleton. It usually behaves as a low-grade sarcoma, however, a minority are aggressive and life threatening. Cytogenetic studies have revealed inconsistent findings including a t(1;3)(p36;25), t(10;14)(p13;q24) and gains and deletions involving chromosomes 11 and 12.

Epithelioid hemangioendothelioma can be seen in most age groups and has its peak frequency in the 2nd and 3rd decades. The tumor affects males and females equally and develops in whites disproportionately more often than members of other races. In the soft tissues the tumor presents as slowly growing somewhat painful solitary mass that arises in the subcutis or the deeper muscle. Frequently, it is angiocentric and is associated with a medium sized vessel, especially a vein.

In contrast, tumors that develop in the skeleton demonstrate multifocal involvement in approximately one-third to one-half of cases and involvement may be in the form of multiple sites in a single bone or separate lesions in multiple bones simultaneously. The tumor tends to arise in the extremities, pelvis and spine. In the extremities, the long bones, as well as the small bones of the hands and feet are commonly involved and typically cause localized pain that may be associated with swelling. Some patients also have disease in the soft tissues, liver or lung at the time of diagnosis of the skeletal disease. The radiographic features are variable; most lesions range in size from 1-5 cm and are round or elongate and predominately lytic. The margins may be well delineated or poorly defined, and the adjacent bone is usually sclerotic.

Epithelioid hemangioendothelioma is pale tan in color, and lacks the red, hemorrhagic appearance of conventional hemangiomas. Microscopically, it is composed of large epithelioid and spindle endothelial cells with round or elongate nuclei, prominent nucleoli, and abundant eosinophilic cytoplasm. Intracytoplasmic lumens appear as vacuoles that may contain intact or fragmented red blood cells. The vacuoles may coalesce to form primitive vascular channels, recapitulating embryonic angiogenesis, however well-formed blood vessels are not prominent in most cases. Instead, the tumor cells are arranged in cords and nests which are embedded within a myxoid to hyalinized ground substance that may resemble cartilaginous matrix. Only in a minority of cases is a prominent inflammatory infiltrate present. In some cases the spindle cells are prominent and are arranged in intersecting fascicles. The neoplastic

cells generally show limited cytologic atypia and mitotic activity, but in some cases, nuclear hyperchromasia and pleomorphism are significant and mitoses numerous, making it difficult to distinguish the neoplasm from high-grade angiosarcoma.

The tumor cells express the full spectrum of immunohistochemical endothelial markers including Factor VIII, CD34, and CD31 and, like epithelioid endothelial cells in general, may also exhibit intense and extensive positive staining for keratin and epithelial membrane antigen. The tumor cells usually do not stain with antibodies to S-100 protein, and desmin. Ultrastructurally, the neoplastic cells contain abundant intermediate filaments, pinocytotic vesicles, intracytoplasmic lumens and basal lamina material.

Epithelioid hemangioendothelioma is treated by complete surgical excision if feasible. However, multifocal lesions may be difficult to excise and may require radiation or thermal ablation. Soft tissue primaries metastasize in almost 50% of cases, but many of these involve regional lymph nodes. Only 10-15% of patients with cytologically banal tumors die of disease reflecting their somewhat indolent growth and ability to be cured by excision of metastatic sites, when present. Predicting the outcome for patients with osseous epithelioid hemangioendothelioma is problematic as the clinical behavior is not always predicted by the morphologic features of the tumor. In one large series, 20% of patients succumbed to disease; of those who died most had concurrent visceral tumors. In the absence of parenchymal organ involvement, epithelioid hemangioendothelioma of bone usually behaves in an indolent fashion and infrequently metastasizes. They may locally recur following curettage or slowly enlarge and destroy bone if left untreated.

The differential diagnosis of epithelioid hemangioendothelioma includes other epithelioid vascular neoplasms particularly epithelioid hemangioma and angiosarcoma. Epithelioid hemangioendothelioma is distinguished from epithelioid hemangioma by its characteristic hyalinized stroma and the paucity of well-formed vessels. Angiosarcoma also lacks the hyalinized stroma and usually shows a greater degree of cytologic atypia and mitotic activity. The epithelioid features of the tumor cells, their cohesive nature, and intracytoplasmic vacuoles can also mimic metastatic adenocarcinoma. The staining of tumor cells of epithelioid hemangioendothelioma for epithelial markers can further complicate this distinction. Accordingly, immunohistochemistry employing antibodies directed against endothelial markers should be performed on unusual epithelioid tumors of bone. Fortunately, metastatic carcinomas do not stain for endothelial markers, and the myxoid or hyalinized stroma is distinct from the

desmoplasia seen in metastatic carcinoma. The cells in cartilaginous tumors do not form cohesive nests, stain immunohistochemically for S-100 protein, and are negative for endothelial markers.

Composite Hemangioendothelioma

Composite hemangioendothelioma is a rare neoplasm composed of elements that recapitulate vascular tumors of different types that range in biology from benign to malignant. Less than 20 cases have been reported and most have affected young and middle aged adults; several patients have been young children. The tumors tend to arise in the dermis or subcutis and three cases have originated in the oral cavity. One patient had Maffucci's syndrome. The tumors present as a slowly enlarging mass of frequent long duration, in fact, in some instances, the tumors were first noted at the time of birth. Composite hemangioendotheliomas are poorly defined and infiltrative and the dominant histologic component is a central area of retiform hemangioendothelioma admixed and surrounded by areas that have the appearance of epithelioid hemangioendothelioma. Other components that have been noted include foci that display the features of spindle cell hemangioma, lymphangioma, angiomatosis, arteriovenous malformation, and angiosarcoma. The angiosarcoma element is usually present in only small amounts.

Biologically, composite hemangioendothelioma is locally aggressive and has recurred in approximately 50% of cases, but has only rarely metastasized (lymph nodes and soft tissue). Excision with negative margins is the goal of therapy, however, this may be difficult to achieve because of the large size of some of the lesions.

Epithelioid sarcoma-like Hemangioendothelioma

Epithelioid sarcoma-like hemangioendothelioma is another very rare type of epithelioid vascular neoplasm of low grade malignancy. Only seven cases have been reported and they tend to arise in young adults (17-54, mean 23 years) within the superficial or deep soft tissues, most frequently the extremities. The tumors are usually several centimeters in size and grow as nodule or sheets of large cells with prominent deeply eosinophilic glassy cytoplasm. Many of the cells are polyhedral and they sometimes blend with those that are spindle shaped. The nuclei are gun metal gray and demonstrate minimal to moderate atypia; mitoses are infrequent. Vascular channel formation and hemorrhage are absent, however, intracytoplasmic vacuolization suggestive of cytoplasmic lumen formation is often noted. Immunohistochemically, the tumors express keratin, EMA, CD 31, and Fli-1, but are negative for CD 34. Biologically these tumors have demonstrated recurrence following excision and local soft tissue metastasis.

The differential diagnosis includes epithelioid sarcoma and epithelioid hemangioendothelioma. Epithelioid sarcoma differs in that it is usually composed of nodules of tumor with central necrosis, which is lacking in epithelioid sarcoma-like hemangioendothelioma, and is negative for CD 31, but positive for CD 34. Epithelioid hemangioendothelioma differs morphologically in that the tumor cells grow in cords, have a myxohyaline stroma, and frequently arise from a vessel.

Epithelioid Angiosarcoma

Epithelioid angiosarcoma refers to a variant of angiosarcoma that is composed of neoplastic cells that have an epithelioid morphologic appearance. These tumors are usually poorly differentiated and biologically aggressive. Epithelioid angiosarcoma is the most aggressive of epithelioid vascular tumors and has been described to arise in a variety of different organs including skin, soft tissue, bone, adrenal, breast, bladder, lung, thyroid, gastrointestinal tract, heart, and great vessels.

In our experience most angiosarcomas of soft tissue and bone are of the epithelioid type. All age groups are affected but the majority of patients are middle aged adults. Some tumors may arise in the background of previous radiation, in an arterio-venous fistula, be associated with genetic diseases such as neurofibromatosis, Klippel-Trenaunay, and Maffucci's syndromes, or arise in association with a different type of neoplasm. In the soft tissues these tumors usually present as a rapidly growing aggressive mass in the superficial or deep soft tissues of the extremities, followed by the trunk and head and neck. In the skeleton the long bones, especially the femur are frequently affected followed by the spine and small bones of the distal extremities; approximately 60% of bone lesions are multifocal. Radiographically, the tumors present as lytic, poorly defined masses that frequently destroy the cortex and extend into the soft tissues.

The tumors are friable hemorrhagic masses that range in size from several centimeters to greater than 14 cms. Histologically, they grow with an infiltrative pattern and are composed of large polyhedral cells with prominent nucleoli and abundant eosinophilic cytoplasm that may contain clear vacuoles similar to those in the other types of epithelioid endothelial cell tumors. The cells may grow in solid sheets, form well-developed vascular channels, assume papillary structures mimicking papillary endothelial hyperplasia, and contain cystically dilated spaces filled with blood. In many cases the epithelioid cells transition into those that are spindle shaped which can be arranged in fascicles. Nuclear pleomorphism is severe, mitoses including atypical forms are often numerous, hemorrhage is abundant, and necrosis is commonplace. In some tumors a prominent neutrophilic may be present.

Immunohistochemically, the tumors express one or more of the endothelial markers including Factor 8 related antigen, CD 31, CD 34 and Fli-1. A significant percentage of cases also strongly express keratin and EMA.

Ultrastructurally the epithelioid cells are frequently surrounded by basal lamina, and have intercellular and intracellular lumina. Intermediate filaments arranged in whorls are common, pinocytotic vesicles are usually seen and some tumor cells have tonofilament-like structures. Weibel-Palade bodies are rare or absent.

Biologically these tumors are aggressive and need to be treated with side complete excision, if possible. At least 50% of patients with soft tissue epithelioid angiosarcoma and almost all patients with bone primaries die of metastatic disease.

The differential diagnosis includes a variety of lesions, but one of the most important is metastatic carcinoma. Metastatic carcinoma can be difficult to distinguish from epithelioid angiosarcoma, especially in the skeleton. Both tumors can involve multiple bones, affect older individuals, and are composed of sheets of epithelioid tumor cells that may express epithelial markers. Helpful histologic features in correctly identifying epithelioid angiosarcoma include the

presence of well-formed vascular channels, cytoplasmic vacuoles that are mucin negative and contain intact or fragments of red blood cells, and an intratumoral neutrophilic infiltrate. Lastly, most carcinoma do not express endothelial markers.

References

Bacillary Angiomatosis

1. LeBiot PE, Egbert BM, Stoler MH, Strauchen JA, Berger TG, Yen TSB, Bonfiglio TA, English CK and Wear DJ. Epithelioid hemangioma-like vascular proliferation in AIDS: manifestation of cat scratch disease bacillus infection? *Lancet* 1988;1:960-3.
2. LeBoit PE, Berger TG, Egbert BM, Beckstead JH, Benedict Yen TS and Stoler MH. Bacillary Angiomatosis. The histopathology and differential diagnosis of a pseudoneoplastic infection in patients with human immunodeficiency virus disease. *Am J Surg Pathol* 1989;13(11):909-20.
3. Walford N, Van Der Wouw PA, Das PK, Ten Velden JJAM and Hulsebosch HJ. Epithelioid angiomatosis in the acquired immunodeficiency syndrome: morphology and differential diagnosis. *Histopathology* 1990;16:83-8.
4. [Dehio C](#). Molecular and cellular basis of bartonella pathogenesis. [Annu Rev Microbiol](#). 2004;58:365-90.

Epithelioid Hemangioma

1. Welss GC and Whimster IW. Subcutaneous angiolymphoid hyperplasia with eosinophils. *Br J Derm* 1969;81:1-15.
2. Rosai J, Gold J, and Landy R. The histiocytoid hemangioma: A unifying concept embracing several previously described entities of skin, soft tissue, large vessels, bone and heart. *Hum Pathol* 1979;10:707-30.
3. Olsen TG and Helwig EB. Angiolymphoid hyperplasia with eosinophilia. *J Am Acad Dermatol* 1985;12:781-96.
4. Googe PB, Harris NL and Mihm MC. Kimura's disease and angiolymphoid hyperplasia with eosinophilia: Two distinct histopathologic entities. *J Cutan Pathol* 1987;14:263-71.
5. Urabe A, Tsuneyoshi M and Enjoji M. Epithelioid hemangioma versus Kimura's disease. A comparative clinicopathologic study. *Am J Surg Pathol* 1987;11:758-66.
6. Fetsch JF and Weiss SW. Observations concerning the pathogenesis of epithelioid hemangioma. (Angiolymphoid hyperplasia) *Modern Pathol* 1991;4:449-55.
7. Allen PW, Ramakrishna B and MacCormac LB. The histiocytoid hemangiomas and other controversies. *Pathol Ann* 1992;27:51-87.
8. O'Connell JX, Kattapuram SV, Mankin HJ, Bhan AK and Rosenberg AE. Epithelioid hemangioma of bone. A tumor often mistaken for low-grade angiosarcoma or malignant hemangioendothelioma. *Am J Surg Pathol* 1993;17:610-7.
9. Tsang WY and Chan JK. The family of epithelioid vascular tumors. *Histol Histopathol* 1993;8:187-212.
10. Keel, SB. and Rosenberg, AE. Hemorrhagic epithelioid and spindle cell hemangioma: a newly recognized, unique vascular tumor of bone. *Cancer* 1999;85:1966-72.
11. O'Connell JX, Nielsen GP, and Rosenberg AE. Epithelioid vascular tumors of bone: a review and proposal of a classification system. *Adv Anat Pathol* 2001;8:74-82.
12. Evans, HL., Raymond, AK. and Ayala, AG. Vascular tumors of bone: A study of 17 cases other than ordinary hemangioma, with an evaluation of the relationship of hemangioendothelioma of bone to epithelioid hemangioma, epithelioid hemangioendothelioma, and high-grade angiosarcoma. *Hum Pathol* 2003;34:680-9.
13. Fetsch, JF, Sesterhenn IA, Miettinen, M and Davis CJ, Jr. Epithelioid hemangioma of the penis: a clinicopathologic and immunohistochemical analysis of 19 cases, with special

reference to exuberant examples often confused with epithelioid hemangioendothelioma and epithelioid angiosarcoma. *Am J Surg Pathol* 2004;28:523-33.

14. Floris, G, Deraedt, K., Samson, I., Brys, P. and Sciot, R. Epithelioid hemangioma of bone: a potentially metastasizing tumor? *Int J Surg Pathol* 2006;14:9-15.
15. Evans, HL. Expert Commentary. *Int J Surg Pathol* 2006;14:16..
16. Rosenberg, AE. Expert Commentary. *Int J Surg Pathol* 2006;14:17-20.

Spindle cell Hemangioma

1. Weiss SW and Enzinger FM. Spindle cell hemangioendothelioma. A low-grade angiosarcoma resembling a cavernous hemangioma and Kaposi's sarcoma. *Am J Surg Pathol* 1986;10:521-30.
2. Scott GA and Rosai J. Spindle cell hamangioendothelioma. Report of seven additional cases of a recently described vascular neoplasm. *Am J Dermatopathol.* 1988;10:281-8.
3. Fletcher CDM, Beham A and Schmid C. Spindle cell hemangioendothelioma: a clinicopathological and immunohistochemical study indicative of a non-neoplastic lesion. *Histopathology* 1991;18:291-301.
4. Fletcher CD. The non-neoplastic nature of spindle cell hemangioendothelioma. *Am J Clin Pathol* 1992;98:545-6.
5. Fanburg JC, Meis-Kindblom JM and Rosenberg AE. Multiple enchondromas associated with spindle-cell hemangioendotheliomas. An overlooked variant of Maffucci's syndrome. *Am J Surg Pathol* 1995;19:1029-1038.
6. Perkins P and Weiss SW. Spindle cell hemangioendothelioma. An analysis of 78 cases with reassessment of its pathogenesis and biologic behavior. *Am J Surg Pathol* 20;1196-1204,1996.

Epithelioid hemangioendothelioma

1. Weiss, S. W. and Enzinger, F. M. Epithelioid hemangioendothelioma: a vascular tumor often mistaken for a carcinoma. *Cancer* 1982;50:970-81.
2. Tsuneyoshi M, Dorfman HD, Bauer TW. Epithelioid hemangioendothelioma of bone. A clinicopathologic, ultrastructural and immunohistochemical study. *Am J Surg Pathol* 1986; 10:754-764.
3. Weiss SW, Ishak KG, Dail DH, Sweet DE, Enzinger FM. Epithelioid hemangioendothelioma and related lesions. *Sem Diag Pathol* 1986; 3:259-287.
4. [Bollinger BK](#), [Laskin WB](#), [Knight CB](#). Epithelioid hemangioendothelioma with multiple site involvement. Literature review and observations. [Cancer](#).1994;73:610-5.
5. Kleer, C. G., Unni, K. K. and McLeod, R. A. Epithelioid hemangioendothelioma of bone. *Am J Surg Pathol* 1996;20:1301-11.
6. [Mendlick MR](#), [Nelson M](#), [Pickering D](#), [Johansson SL](#), [Seemayer TA](#), [Neff JR](#), [Vergara G](#), [Rosenthal H](#), [Bridge JA](#) Translocation t(1;3)(p36.3;q25) is a nonrandom aberration in epithelioid hemangioendothelioma. [Am J Surg Pathol](#). 2001;25:684-7.

Composite hemangioendothelioma

1. [Fukunaga M](#), [Suzuki K](#), [Saegusa N](#), [Folpe AL](#). Composite hemangioendothelioma: report of 5 cases including one with associated Maffucci syndrome. [Am J Surg Pathol](#). 2007 Oct;31(10):1567-72.

2. Naylor SJ, Rubin BP, Calonje E, et al. Composite hemangioendothelioma: a complex low-grade vascular lesion mimicking angiosarcoma. *Am J Surg Pathol.* 2000;24:352–356.

Epithelioid sarcoma-like hemangioendothelioma

1. [Billings SD](#), [Folpe AL](#), [Weiss SW](#) Epithelioid sarcoma-like hemangioendothelioma. *Am J Surg Pathol.* 2003 Jan;27(1):48-57.

Epithelioid Angiosarcoma

1. Fletcher CDM, Beham A, Bekir S, Clarke AM, Marley NJ. Epithelioid angiosarcoma of deep soft tissue: a distinctive tumor readily mistaken for an epithelial neoplasm. *Am J Surg Pathol* 1991;15:915-24.
2. [Meis-Kindblom JM](#), [Kindblom LG](#). Angiosarcoma of soft tissue: a study of 80 cases. *Am J Surg Pathol.* 1998;22:683-97.
3. [Deshpande V](#), [Rosenberg AE](#), [O'Connell JX](#), [Nielsen GP](#). Epithelioid angiosarcoma of the bone: a series of 10 cases. *Am J Surg Pathol.* 2003;27:709-16.
4. [Fayette J](#), [Martin E](#), [Piperno-Neumann S](#), [Le Cesne A](#), [Robert C](#), [Bonvalot S](#), [Ranchère D](#), [Pouillart P](#), [Coindre JM](#), [Blay JY](#). Angiosarcomas, a heterogeneous group of sarcomas with specific behavior depending on primary site: a retrospective study of 161 cases. *Ann Oncol.* 2007;18:2030-6.
5. [Abraham JA](#), [Hornicek FJ](#), [Kaufman AM](#), [Harmon DC](#), [Springfield DS](#), [Raskin KA](#), [Mankin HJ](#), [Kirsch DG](#), [Rosenberg AE](#), [Nielsen GP](#), [Deshpande V](#), [Suit HD](#), [DeLaney TF](#), [Yoon SS](#). Treatment and outcome of 82 patients with angiosarcoma. *Ann Surg Oncol.* 2007;14:1953-67.

Cartilaginous tumours: lessons from normal chondrogenesis

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Introduction

The normal growth plate demonstrates chondrocytes in different stages of proliferation and differentiation (1). This process is tightly regulated amongst others by the Indian hedgehog (IHH) / ParaThyroid Hormone Like Hormone (PTHrH) pathway (2). During the past years parallels between chondrocyte growth and differentiation in the growth plate and in tumours have become obvious. This was highlighted by the identification of the *EXT* genes mutated in MO and their involvement in signalling pathways in the normal growth plate. Moreover, cartilaginous tumours are nearly exclusively found in bones arising from endochondral ossification. Cartilage tumours constitute a heterogeneous group of neoplasms that have in common the production of cartilage matrix by tumour cells. In analogy with haematopoietic malignancies, it is becoming clear that the different different cartilaginous tumours represent different stages of chondrogenesis.

Osteochondroma and secondary peripheral chondrosarcoma

Osteochondroma is a mostly asymptomatic cartilage capped bony outgrowth representing ~35% of all primary benign bone tumours (3). They develop and increase in size in the first decade, ceasing to grow when the growth plates close at puberty. Osteochondromas morphologically resemble the normal growth plate. Malignant transformation towards secondary peripheral chondrosarcoma is low (<1% of cases). About 17% of osteochondromas arise in the context of multiple osteochondromas (MO) syndrome (4), an autosomal dominant disorder (5;6). Osteochondroma was initially considered a disturbance in the orientation of normal bone growth. It is now regarded a true neoplasm, based on cytogenetic abnormalities, aneuploidy and loss of heterozygosity (LOH) found in the cartilaginous cap (7-9). In MO malignant transformation towards secondary peripheral chondrosarcoma occurs in 1-5% (5;10).

Two genes, *EXT1* and *EXT2*, have been identified for MO (11-13). *EXT* expression is confined to proliferating and prehypertrophic chondrocytes of the growth plate (14). The gene products catalyze heparan sulphate (HS) polymerization (15-18). HS proteoglycans (HSPG) are large macromolecules composed of HS chains linked to a protein core. In *Drosophila* *EXT* proteins are required for gradient formation of wingless (Wg; human homologue: Wnt), Hedgehog (Hh; human homologues Indian Hh, Sonic Hh and Desert Hh) and decapentaplegic (Dpp; human homologues TGF-beta and BMP signalling) (19-24).

Loss of the remaining *EXT1* wild type allele was demonstrated in hereditary osteochondromas (9). In sporadic solitary osteochondromas *EXT1* homozygous deletions are found (25) confined to the cartilaginous cap, confirming that two hits are required for

both sporadic and hereditary osteochondroma development. At the expression level *EXT* mRNA levels are low. In both solitary and hereditary osteochondromas decreased expression and intracellular accumulation of shorter or less complex HS chains as well as HSPGs was found (26). Of the pathways predicted to be affected by defective HSPGs IHH signalling was shown to be active in osteochondroma (27), while being downregulated in peripheral chondrosarcoma (27). PTHLH signalling, downstream of IHH, is however absent in osteochondroma (28;29) and active in peripheral chondrosarcoma (27;28). Moreover, Bcl-2, which is a downstream effector of PTHR1, can be used as a diagnostic marker for secondary peripheral chondrosarcoma (28). TGF-beta is a good candidate to stimulate PTHLH signalling in the absence of IHH (27). FGF signalling is absent in osteochondroma (29).

Osteochondroma look-alikes

Dysplasia Epiphysealis Hemimelica (DEH) and metachondromatosis (MC) are cartilage lesions to be considered in the differential diagnosis of solitary and hereditary osteochondromas. Both are rare disorders with DEH demonstrating cartilaginous overgrowth of an epiphysis (30;31) and MC exhibiting synchronous enchondromas and osteochondromas (32-34). While DEH is non-hereditary, MC has an autosomal dominant mode of inheritance (32-34) but the disorder has not been mapped in the human genome so far. Histologically, clumping of chondrocytes within a fibrillary chondroid matrix is characteristic for DEH, while osteochondromas and MC display the characteristic growth plate architecture (35). The *EXT* genes are normally expressed in DEH and MC. IHH/PTHLH signalling molecules were expressed in DEH and MC suggesting this pathway to be active (35). This is in contrast to osteochondroma in which PTHLH signalling is downregulated. Thus, lesions of DEH and MC are separate entities from osteochondroma as confirmed by their different cDNA and protein expression profiles. *EXT* downstream targets, downregulated in osteochondroma, are expressed in DEH and MC, suggesting *EXT* signalling is not disturbed.

Enchondroma and central chondrosarcoma

Enchondroma usually presents in the long bones in the third or fourth decade (36). Malignant transformation towards conventional central chondrosarcoma occurs in <1% of cases (4). Conventional central chondrosarcoma is designated as such based on its location centrally within the medullar cavity and is the most common chondrosarcoma subtype. Conventional chondrosarcoma is classified into three grades predicting local recurrence and metastasis (37). Our previous studies have shown that central and peripheral chondrosarcoma, despite similar morphology, have a different genetic background (38). The *EXT* genes, that are involved in the genesis of osteochondroma and secondary peripheral chondrosarcoma, are not affected in central chondrosarcoma, since *EXT* expression levels are normal (Schrage et al, manuscript submitted) and mutations are absent. IHH signalling in enchondromas and chondrosarcomas was shown to be less active as compared to growth plate specimens (39). However, the hedgehog target genes *PTCH1* and *GLI1* are expressed (39;40). Moreover, chondrosarcoma cell proliferation could be decreased using inhibitors of hedgehog signalling, suggesting that hedgehog signalling is important for central chondrosarcoma cell proliferation (40). PTHLH signalling is active in enchondroma and central chondrosarcoma and increases with

histological grade stimulating chondrocyte proliferation (29;39;41-43). In vitro the treatment of chondrosarcoma cells with an anti-PTHrP antibody induces apoptosis and accelerates differentiation of the chondrocytes (44).

While most enchondromas are solitary, some patients demonstrate multiple enchondromas with a marked unilateral predominance particularly affecting the limbs (45), known as Ollier disease. The risk of malignant transformation is increased (10-35%) (45). In 2002 a mutation was reported in the Parathyroid Hormone Receptor type I (PTHr1) gene in 2 of 6 patients with Ollier disease (46). However, in a large multi-institutional series of 31 patients no mutations were found, suggesting PTHr1 may be the responsible gene in only a small minority of patients (47).

Chondroblastoma

Chondroblastoma accounts for approximately 1% of benign bone tumours and is characterized by high cellularity with relatively immature, rounded or polygonal, chondroblast-like cells, admixed with multinucleated osteoclast like giant-cells. Small areas of chondroid matrix with focal calcification can be found (48). Electron microscopy, histochemical analysis and tissue culture have shown that the predominant cell is most similar to an epiphyseal chondrocyte, suggesting a chondrogenic origin of chondroblastoma, with the giant cells being reactive (49). Studies of the extracellular matrix composition and gene expression patterns demonstrate that osteoid and fibrous matrix is being formed, suggesting that chondroblastoma is a bone-forming rather than a cartilage-forming neoplasm (50). However, EXT downstream targets were studied and in chondroblastoma growth plate signalling pathways were shown to be active since components of both Ihh/PTHrP as well as FGF signalling are expressed (51). This suggests chondroblastoma to originate from a mesenchymal cell committed towards chondrogenesis (51).

Chondromyxoid fibroma

Chondromyxoid fibroma is a rare benign tumour comprising less than 1% of all skeletal neoplasms. It is characterized by lobularly arranged areas of spindle or stellate cells with abundant mucomyxoid or chondroid matrix, separated by more cellular areas with spindle-shaped or rounded cells admixed with a varying number of multinucleated giant cells (48). As for chondroblastoma, a cartilaginous origin was originally proposed, based on ultrastructural studies and positive S-100 immunohistochemistry (52). Histologically, the morphological features resemble different steps of chondrogenesis in terms of cellular morphology (ranging from spindled to rounded cells) and extracellular matrix (ranging from fibrous to cartilaginous). There is a striking resemblance to normal chondrocytes in 3D culture (53). However, despite morphological similarities, PTHrP signaling was higher in articular chondrocyte pellets as compared to chondromyxoid fibroma, while Bcl-2 was absent (53), suggesting Bcl-2 to be crucial for neoplastic chondrogenesis.

Rare chondrosarcoma subtypes

Based on cell type/differentiation, matrix formation and architecture, combined with radio-diagnostic and clinical presentation several rare subtypes of chondrosarcoma are discerned, together constituting approximately 15% of all chondrosarcomas. These

include dedifferentiated chondrosarcoma, mesenchymal chondrosarcoma and clear-cell chondrosarcoma.

Dedifferentiated chondrosarcoma represents approximately 10% of all chondrosarcomas and is a highly anaplastic sarcoma next to a (usually low-grade) malignant cartilage-forming tumour, with a remarkably sharp interface between both components (54). This bears histological similarities to the process of dedifferentiation that occurs in chondrocytes *in vitro* in which chondrocytes become spindle shaped and lose the expression of cartilage specific molecules (55). The tumour bears a dismal prognosis (56). The rare genetic reports on dedifferentiated chondrosarcoma demonstrate that both components share identical genetic aberrations (57;58) with additional genetic changes in the anaplastic component (57-60) indicating a common precursor cell with early diversion of the two components. Growth plate signalling molecules have not yet been studied in dedifferentiated chondrosarcoma.

Mesenchymal chondrosarcoma constitutes <2% of all chondrosarcomas and is a highly malignant lesion characterized by scattered areas of differentiated cartilage admixed with undifferentiated small round-cells occurring in bone and soft tissue of young patients (61). The undifferentiated cells bear some resemblance to the resting cells of the growth plate. Indeed, studying cell differentiation and matrix gene expression, all steps of chondrogenesis are found in mesenchymal chondrosarcoma, suggesting it to be a neoplasm of differentiating pre-mesenchymal chondroprogenitor cells (reviewed in (55)). Moreover, SOX-9, the master regulator of chondrogenic differentiation, is expressed by the small cells of mesenchymal chondrosarcoma confirming phenotypic similarities to early chondrogenic condensation (62). Immunohistochemical analysis of 3 cases demonstrated activity of protein kinase C (c-PKC)-alpha and PDGFR α pathways and expression of the anti-apoptotic protein BCL2, confirming the similarities to mesenchymal cells involved in chondrogenesis (63). IHH expression was shown to be restricted to the matrix rich areas, but nothing is known about the activity of the pathway (64).

Clear cell chondrosarcoma accounts for <2% of all chondrosarcomas and is a low-grade malignant lesion characterized by tumour cells with clear, empty cytoplasm, strongly resembling the hypertrophic cells of the growth plate. This was confirmed by the expression of collagen type X and osteonectin (55). Expression of PTHLH has been demonstrated suggesting PTHLH signaling to be involved in proliferation (65). Expression of IHH was shown by immunohistochemistry (64) but nothing is known about the activity of the pathway.

Conclusion

The elucidation of the EXT genes for multiple osteochondromas and their function initiated the investigation of growth plate signaling molecules in the different benign and malignant cartilage tumours. Similar to the classification of haematopoietic malignancies, based on morphology, on the phenotypic expression of differentiation markers such as collagen types and on the expression of growth plate signaling molecules, the classification of cartilaginous tumours is better delineated, with each subtype representing a different phase in chondrocyte differentiation.

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References

- (1) Hogendoorn PCW, Bovée JVMG, Karperien M, Cleton-Jansen AM. Skeletogenesis: Genetics. In: Cooper DN, editor. *Nature Encyclopedia of the Human Genome*. London: Nature Publishing Group; 2003. p. 306-13.
- (2) Kronenberg HM. Developmental regulation of the growth plate. *Nature* 2003 May 15;423(6937):332-6.
- (3) Khurana J, Abdul-Karim F, Bovée JVMG. Osteochondroma. In: Fletcher CDM, Unni KK, Mertens F, editors. *World Health Organization classification of tumours. Pathology and genetics of tumours of soft tissue and bone*. Lyon: IARC Press; 2002. p. 234-6.
- (4) Mulder JD, Schütte HE, Kroon HM, Taconis WK. *Radiologic atlas of bone tumors*. 2 ed. Amsterdam: Elsevier; 1993.
- (5) Bovée JVMG, Hogendoorn PCW. Multiple osteochondromas. In: Fletcher CDM, Unni KK, Mertens F, editors. *World Health Organization classification of tumours. Pathology and genetics of tumours of soft tissue and bone*. Lyon: IARC Press; 2002. p. 360-2.
- (6) Hameetman L, Bovée JVMG, Taminiau AHM, Kroon HM, Hogendoorn PCW. Multiple Osteochondromas: Clinicopathological and Genetic Spectrum and Suggestions for Clinical Management. *Hereditary Cancer in Clinical Practice* 2004 Dec 15;2(4):161-73.
- (7) Bridge JA, Nelson M, Orndal C, Bhatia P, Neff JR. Clonal karyotypic abnormalities of the hereditary multiple exostoses chromosomal loci 8q24.1 (EXT1) and 11p11-12 (EXT2) in patients with sporadic and hereditary osteochondromas. *Cancer* 1998;82:1657-63.
- (8) Mertens F, Rydholm A, Kreicbergs A, Willen H, Jonsson K, Heim S, et al. Loss of chromosome band 8q24 in sporadic osteocartilaginous exostoses. *Genes Chromosomes Cancer* 1994;9:8-12.
- (9) Bovée JVMG, Cleton-Jansen AM, Wuyts W, Caethoven G, Taminiau AHM, Bakker E, et al. EXT-mutation analysis and loss of heterozygosity in sporadic and hereditary osteochondromas and secondary chondrosarcomas. *Am J Hum Genet* 1999;65(3):689-98.
- (10) Bertoni F, Bacchini P, Hogendoorn PCW. Chondrosarcoma. In: Fletcher CDM, Unni KK, Mertens F, editors. *World Health Organisation classification of tumours. Pathology and genetics of tumours of soft tissue and bone*. Lyon: IARC Press; 2002. p. 247-51.
- (11) Ahn J, Ludecke H-J, Lindow S, Horton WA, Lee B, Wagner MJ, et al. Cloning of the putative tumour suppressor gene for hereditary multiple exostoses (EXT1). *Nature Genet* 1995;11:137-43.
- (12) Wuyts W, Van Hul W, Wauters J, Nemtsova M, Reyniers E, Van Hul E, et al. Positional cloning of a gene involved in hereditary multiple exostoses. *Hum Mol Genet* 1996;5(10):1547-57.
- (13) Stickens D, Clines G, Burbee D, Ramos P, Thomas S, Hogue D, et al. The EXT2 multiple exostoses gene defines a family of putative tumour suppressor genes. *Nature Genet* 1996;14:25-32.
- (14) Stickens D, Brown D, Evans GA. EXT genes are differentially expressed in bone and cartilage during mouse embryogenesis. *Dev Dyn* 2000 Jul;218(3):452-64.
- (15) Lind T, Tufaro F, McCormick C, Lindahl U, Lidholt K. The putative tumor suppressors EXT1 and EXT2 are glycosyltransferases required for the biosynthesis of heparan sulfate. *J Biol Chem* 1998;273(41):26265-8.

- (16) McCormick C, Leduc Y, Martindale D, Mattison K, Esford LE, Dyer AP, et al. The putative tumour suppressor EXT1 alters the expression of cell-surface heparan sulfate. *Nature Genet* 1998;19:158-61.
- (17) Simmons AD, Musy MM, Lopes CS, Hwang L-Y, Yang Y-P, Lovett M. A direct interaction between EXT proteins and glycosyltransferases is defective in hereditary multiple exostoses. *Hum Mol Genet* 1999;8(12):2155-64.
- (18) McCormick C, Duncan G, Goutsos KT, Tufaro F. The putative tumor suppressors EXT1 and EXT2 form a stable complex that accumulates in the golgi apparatus and catalyzes the synthesis of heparan sulfate. *Proc Natl Acad Sci USA* 2000;97(2):668-73.
- (19) Han C, Belenkaya TY, Khodoun M, Tauchi M, Lin X, Lin X. Distinct and collaborative roles of Drosophila EXT family proteins in morphogen signalling and gradient formation. *Development* 2004 Apr;131(7):1563-75.
- (20) Bellaiche Y, The I, Perrimon N. Tout-velu is a drosophila homologue of the putative tumour suppressor EXT1 and is needed for Hh diffusion. *Nature* 1998;394:85-8.
- (21) The I, Bellaiche Y, Perrimon N. Hedgehog movement is regulated through tout velu -dependant synthesis of a heparan sulfate proteoglycan. *Mol Cell* 1999;4(4):633-9.
- (22) Toyoda H, Kinoshita-Toyoda A, Selleck SB. Structural analysis of glycosaminoglycans in drosophila and caenorhabditis elegans and demonstration that tout-velu, a drosophila gene related to EXT tumor suppressors, affects heparan sulfate in vivo. *J Biol Chem* 2000;275(4):2269-75.
- (23) Bornemann DJ, Duncan JE, Staatz W, Selleck S, Warrior R. Abrogation of heparan sulfate synthesis in Drosophila disrupts the Wingless, Hedgehog and Decapentaplegic signaling pathways. *Development* 2004 Mar 31;131(9):1927-38.
- (24) Takei Y, Ozawa Y, Sato M, Watanabe A, Tabata T. Three Drosophila EXT genes shape morphogen gradients through synthesis of heparan sulfate proteoglycans. *Development* 2004 Jan;131(1):73-82.
- (25) Hameetman L, Szuhai K, Yavas A, Knijnenburg J, van Duin M, Van Dekken H, et al. The role of EXT1 in nonhereditary osteochondroma: identification of homozygous deletions. *J Natl Cancer Inst* 2007 Mar 7;99(5):396-406.
- (26) Hameetman L, David G, Yavas A, White SJ, Taminiau AHM, Cleton-Jansen AM, et al. Decreased EXT expression and intracellular accumulation of HSPG in osteochondromas and peripheral chondrosarcomas. *J Pathol* 2007;211:399-409.
- (27) Hameetman L, Rozeman LB, Lombaerts M, Oosting J, Taminiau AHM, Cleton-Jansen AM, et al. Peripheral chondrosarcoma progression is accompanied by decreased Indian Hedgehog (IHH) signalling. *J Pathol* 2006;209(4):501-11.
- (28) Hameetman L, Kok P, Eilers PHC, Cleton-Jansen AM, Hogendoorn PCW, Bovée JVMG. The use of Bcl-2 and PTHLH immunohistochemistry in the diagnosis of peripheral chondrosarcoma in a clinicopathological setting. *Virchows Arch* 2005 Mar 3;446:430-7.
- (29) Bovée JVMG, Van den Broek LJCM, Cleton-Jansen AM, Hogendoorn PCW. Up-regulation of PTHrP and Bcl-2 expression characterizes the progression of osteochondroma towards peripheral chondrosarcoma and is a late event in central chondrosarcoma. *Lab Invest* 2000;80:1925-33.
- (30) Murphey MD, Choi JJ, Kransdorf MJ, Flemming DJ, Gannon FH. Imaging of osteochondroma: variants and complications with radiologic-pathologic correlation. *RadioGraphics* 2000 Sep;20(5):1407-34.
- (31) Silverman FN. Dysplasia epiphysealis hemimelica. *Semin Roentgenol* 1989 Oct;24(4):246-58.
- (32) Bassett GS, Cowell HR. Metachondromatosis. Report of four cases. *J Bone Joint Surg Am* 1985 Jun;67(5):811-4.

- (33) Herman TE, Chines A, McAlister WH, Gottesman GS, Eddy MC, Whyte MP. Metachondromatosis: report of a family with facial features mildly resembling trichorhinophalangeal syndrome. *Pediatr Radiol* 1997 May;27(5):436-41.
- (34) Kennedy LA. Metachondromatosis. *Radiology* 1983 Jul;148(1):117-8.
- (35) Bovée JVMG, Hameetman L, Kroon HM, Aigner T, Hogendoorn PCW. EXT-related pathways are not involved in pathogenesis of Dysplasia Epiphysealis Hemimelica and Metachondromatosis. *J Pathol* 2006;209(3):411-9.
- (36) Lucas DR, Bridge JA. Chondromas: enchondroma, periosteal chondroma, and enchondromatosis. In: Fletcher CDM, Unni KK, Mertens F, editors. *World Health Organization classification of tumours. Pathology and genetics of tumours of soft tissue and bone*. Lyon: IARC Press; 2002. p. 237-40.
- (37) Evans HL, Ayala AG, Romsdahl MM. Prognostic factors in chondrosarcoma of bone. A clinicopathologic analysis with emphasis on histologic grading. *Cancer* 1977;40:818-31.
- (38) Bovée JVMG, Cleton-Jansen AM, Kuipers-Dijkshoorn N, Van den Broek LJCM, Taminiau AHM, Cornelisse CJ, et al. Loss of heterozygosity and DNA ploidy point to a diverging genetic mechanism in the origin of peripheral and central chondrosarcoma. *Genes Chrom Cancer* 1999;26:237-46.
- (39) Rozeman LB, Hameetman L, Cleton-Jansen AM, Taminiau AHM, Hogendoorn PCW, Bovée JVMG. Absence of IHH and retention of PTHrP signalling in enchondromas and central chondrosarcomas. *J Pathol* 2005;205(4):476-82.
- (40) Tiet TD, Hopyan S, Nadesan P, Gokgoz N, Poon R, Lin AC, et al. Constitutive hedgehog signaling in chondrosarcoma up-regulates tumor cell proliferation. *Am J Pathol* 2006 Jan;168(1):321-30.
- (41) Amling M, Posl M, Hentz MW, Priemel M, Delling G. PTHrP and Bcl-2: essential regulatory molecules in chondrocyte differentiation and chondrogenic tumors. *Verh Dtsch Ges Path* 1998;82:160-9.
- (42) Kunisada T, Moseley JM, Slavin JL, Martin TJ, Choong PF. Co-expression of parathyroid hormone-related protein (PTHrP) and PTH/PTHrP receptor in cartilaginous tumours: a marker for malignancy? *Pathology* 2002 Apr;34(2):133-7.
- (43) Pateder DB, Gish MW, O'Keefe RJ, Hicks DG, Teot LA, Rosier RN. Parathyroid hormone-related Peptide expression in cartilaginous tumors. *Clin Orthop* 2002 Oct;(403):198-204.
- (44) Miyaji T, Nakase T, Onuma E, Sato K, Myoui A, Tomita T, et al. Monoclonal antibody to parathyroid hormone-related protein induces differentiation and apoptosis of chondrosarcoma cells. *Cancer Lett* 2003 Sep 25;199(2):147-55.
- (45) Mertens F, Unni KK. Enchondromatosis: Ollier disease and Maffucci syndrome. In: Fletcher CDM, Unni KK, Mertens F, editors. *World Health Organization Classification of Tumours. Pathology and genetics of tumours of soft tissue and bone*. Lyon: IARC Press; 2002. p. 356-7.
- (46) Hopyan S, Gokgoz N, Poon R, Gensure RC, Yu C, Cole WG, et al. A mutant PTH/PTHrP type I receptor in enchondromatosis. *Nat Genet* 2002 Mar;30(3):306-10.
- (47) Rozeman LB, Sangiorgi L, Bruijn IH, Mainil-Varlet P, Bertoni F, Cleton-Jansen AM, et al. Enchondromatosis (Ollier disease, Maffucci syndrome) is not caused by the PTHR1 mutation p.R150C. *Hum Mutat* 2004;24(6):466-73.
- (48) Schajowicz F, Sobin LH. *World health organization. International histological classification of tumours. Histological typing of bone tumours*. 2 ed. Berlin: Springer-Verlag; 1993.
- (49) Springfield DS, Capanna R, Gherlinzoni F, Picci P, Campanacci M. Chondroblastoma. A review of seventy cases. *J Bone Joint Surg [Am]* 1985;67:748-55.

- (50) Aigner T, Loos S, Inwards C, Perris R, Perissinotto D, Unni KK, et al. Chondroblastoma is an osteoid-forming, but not cartilage-forming neoplasm. *J Pathol* 1999 Dec;189(4):463-9.
- (51) Romeo S, Bovée JVMG, Jadnanansing NAA, Taminiau AHM, Hogendoorn PCW. Expression of cartilage growth plate signalling molecules in chondroblastoma. *J Pathol* 2004 Jan;202(1):113-20.
- (52) Zillmer DA, Dorfman HD. Chondromyxoid fibroma of bone: thirty-six cases with clinicopathologic correlation. *Hum Pathol* 1989;20:952-64.
- (53) Romeo S, Bovée JVMG, Grogan S, Taminiau AHM, Eilers PHC, Cleton-Jansen AM, et al. Chondromyxoid fibroma resembles *in vitro* chondrogenesis, though differs in expression of signalling molecules. *J Pathol* 2005;206(2):135-42.
- (54) Milchgrub S, Hogendoorn PCW. Dedifferentiated chondrosarcoma. In: Fletcher C.D.M., Unni KK, Mertens F, editors. World health organization classification of tumours. Pathology and genetics. Tumours of soft tissue and bone. 2002. p. 252-4.
- (55) Aigner T. Towards a new understanding and classification of chondrogenic neoplasias of the skeleton--biochemistry and cell biology of chondrosarcoma and its variants. *Virchows Arch* 2002 Sep;441(3):219-30.
- (56) Grimer RJ, Gosheger G, Taminiau A, Biau D, Matejovsky Z, Kollender Y, et al. Dedifferentiated chondrosarcoma: Prognostic factors and outcome from a European group. *Eur J Cancer* 2007 Sep;43(14):2060-5.
- (57) Bovée JVMG, Cleton-Jansen AM, Rosenberg C, Taminiau AHM, Cornelisse CJ, Hogendoorn PCW. Molecular genetic characterization of both components of a dedifferentiated chondrosarcoma, with implications for its histogenesis. *J Pathol* 1999;189:454-62.
- (58) Ropke M, Boltze C, Neumann HW, Roessner A, Schneider-Stock R. Genetic and epigenetic alterations in tumor progression in a dedifferentiated chondrosarcoma. *Path Res Pract* 2003;199(6):437-44.
- (59) Grote HJ, Schneider-Stock R, Neumann W, Roessner A. Mutation of p53 with loss of heterozygosity in the osteosarcomatous component of a dedifferentiated chondrosarcoma. *Virchows Arch* 2000 May;436(5):494-7.
- (60) Coughlan B, Feliz A, Ishida T, Czerniak B, Dorfman HD. p53 Expression and DNA ploidy of cartilage lesions. *Hum Pathol* 1995;26:620-4.
- (61) Nakashima Y, Park YK, Sugano O. Mesenchymal chondrosarcoma. In: Fletcher C.D.M., Unni KK, Mertens F, editors. World health organization classification of tumours. Pathology and genetics. Tumours of soft tissue and bone. 2002. p. 255-6.
- (62) Wehrli BM, Huang W, De CB, Ayala AG, Czerniak B. Sox9, a master regulator of chondrogenesis, distinguishes mesenchymal chondrosarcoma from other small blue round cell tumors. *Hum Pathol* 2003 Mar;34(3):263-9.
- (63) Brown RE, Boyle JL. Mesenchymal chondrosarcoma: molecular characterization by a proteomic approach, with morphogenic and therapeutic implications. *Ann Clin Lab Sci* 2003;33(2):131-41.
- (64) Park HR, Park YK. Differential expression of runx2 and Indian hedgehog in cartilaginous tumors. *Pathol Oncol Res* 2007;13(1):32-7.
- (65) Masui F, Ushigome S, Fujii K. Clear cell chondrosarcoma: a pathological and immunohistochemical study. *Histopathology* 1999;34(5):447-52.

The Role and Relevance of Mutational Analysis in GI Stromal Sarcomas (GIST)

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Key Points

- CD117 staining intensity does not predict imatinib response
- Kinase mutations are important in GIST biology
 - *KIT* mutations are most common in exons 9 (10%) and 11 (65%)
 - *PDGFRA* mutations occur in ~7% of GISTs
 - About 15% of GISTs have no detectable mutation ('wild-type' GISTs)
- The best predictor of imatinib response is kinase genotype

	<i>KIT</i> exon 11	<i>KIT</i> exon 9	Wild-type
Objective response	64-67%	34-37%	23-37%
Progressive disease	3%	17%	19%
Progression-free survival	25-27 mo	9-16 mo	13-14 mo

- CD117-negative GISTs often have *KIT* mutations and respond to imatinib
- Up to one third of *PDGFRA* mutations are potentially sensitive to imatinib and other kinase inhibitors
- Reasons against genotyping GISTs:
 - Cost (\$600 to \$1100)
 - Time (2-4 weeks)
 - Not necessary for a routine diagnosis of GIST
- Reasons for genotyping GISTs:
 - Molecular confirmation in unusual cases
 - Predicting benefit of imatinib in the treatment of advanced cases
 - Decisions regarding adjuvant use of imatinib
 - Dose selection: some oncologists favor 800 mg for *KIT* exon 9 tumors

Introduction

During the past decade, gastrointestinal stromal sarcomas (GISTs) have emerged from historic anonymity to center stage in the emerging field of molecularly targeted therapies for solid tumors. The nearly simultaneous discovery of oncogenic kinase mutations in GISTs and the introduction of kinase inhibitor therapies have led to a rapid evolution in our understanding of these tumors and the biology that defines them. The pace of these developments has been greatly accelerated by interfacing translational research studies with observations made in patients, that is, moving from the bench to the bedside and back again. While parallel stories are emerging in adenocarcinoma of the lung, renal cell carcinoma, colorectal carcinoma and breast carcinoma, lessons learned from the molecularly targeted treatment of GISTs provide a remarkably informative picture of the advantages and limitations of targeted therapeutics, or so-called 'biologics.'

Historical Emergence of GIST

GI stromal sarcomas are mesenchymal neoplasms that arise in the muscle wall of the gastrointestinal tract, usually the stomach or small bowel, and may spread over serosal surfaces in the abdomen and/or form metastases in the liver. The tumors range from less than 1 cm to more than 40, with an average of around 5 cm. They are generally well-circumscribed, have a fleshy pink/tan cut surface, and may show areas of hemorrhagic necrosis and cystic degeneration.

In the 1950s, GISTs were classified as members of the smooth muscle family of tumors, which is understandable given the prominent spindle cell morphology in these tumors and their association with the muscularis propria. With the introduction of electron microscopy, and subsequently of immunohistochemistry, the notion that GISTs are a distinct entity began to take hold, leading to the proposal in 1983 by Mazur and Clark that they be called "stromal tumors".¹ Ten years later, the observation that most stromal tumors arising in the GI tract are immunopositive for CD34 led to general acceptance of this new classification. During the 1990's a number of investigators noted similarities between GISTs and the interstitial cells of Cajal, which are the pacemaker cells for gut peristalsis. Studies during this period showed that Cajal cells express KIT tyrosine kinase (CD117) and are developmentally dependent on this kinase.² This led to publications by two groups in 1998 showing that GISTs commonly express CD117.^{3,4} It is now well established that 95% of GISTs are unequivocally positive for CD117.

GIST Diagnosis

The introduction of CD117 as a marker for GIST helped to define the range of morphologies observed in these neoplasms, from rather bland spindle cell proliferations to highly cellular epithelioid tumors with significant nuclear pleomorphism. The spectrum of CD117 staining in GISTs is considerable, both in intensity and in cellular distribution. KIT is a glycoprotein receptor tyrosine kinase that normally resides at the cell surface, but there is evidence that mutant KIT can accumulate intracellularly, which may explain the prominent cytoplasmic staining in many GIST cases. Expression of S-100, smooth muscle actin and muscle specific actin is evident in 10-30% of cases, but strong staining for desmin is rare and should raise doubts about the diagnosis of GIST. It should be noted that approximately 5% of GISTs are negative for CD117 expression –

the so-called KIT-negative GISTs. As discussed below, kinase genotyping can provide a molecular confirmation of the diagnosis in such tumors.

Other immunomarkers may prove useful in sorting through the long morphologic differential for GISTs. For example, PKC θ is a member of the protein kinase C family that is expressed in virtually all GISTs and very few other cell types. Immunohistochemical staining for PKC θ has been documented in GISTs, but the commercially available antibodies to this protein have high backgrounds.⁵⁻⁷ Staining for PDGF receptor alpha (PDGFRA), a receptor tyrosine kinase that is closely related to KIT, has been explored as a means of identifying KIT-negative GISTs, but PDGFRA is expressed in other mesenchymal tumors and a high quality commercial antibody has yet to emerge. More promising is DOG-1 (Discovered On GIST-1), a surface membrane protein of unknown function.⁸(van de Rijn et al., in press) A polyclonal DOG-1 antibody is now available and a monoclonal may be marketed in 2008.

Clinical Features

GISTs most commonly present in the stomach (60%) and small intestine (25%), but they also occur in the colon, rectum, esophagus, mesentery and omentum (15% together). Case reports and small series have established that GISTs occasionally arise in the appendix, the gallbladder, the retroperitoneum, and in para-vaginal and para-prostatic tissue. Primary GISTs do not develop outside of the abdomen.

Based on a number of epidemiological studies, the annual incidence of new GIST cases in the U.S. is estimated between 3,300 and 6,000. The mean age at diagnosis is around 60 years.⁹ Only 2.7% of gastric GISTs and 0.6% of small bowel GISTs are detected in patients under 21 years.

Clinical symptoms associated with GIST include fatigue, abdominal pain, dysphagia, satiety, and obstruction. Rarely, patients will suffer tumor-related hypoglycemia. The workup often reveals anemia related to mucosa bleeding or intratumoral hemorrhage. Following resection, GISTs may recur locally, spread throughout the serosal surfaces of the abdomen and/or metastasize to the liver. Advanced disease is associated with metastases to distant sites, including lung, bone and rarely the brain.

KIT Mutations in GIST

In early 1998, Hirota and colleagues published their groundbreaking discovery of KIT kinase mutations in GISTs.³ Studies from dozens of laboratories worldwide have since confirmed that 60-80% of GISTs harbor a *KIT* gene mutation, that these mutations lead to constitutive activation of the kinase, and that mutant KIT is an excellent therapeutic target in GISTs.¹⁰

KIT is a type III receptor tyrosine kinase that is closely related to platelet-derived growth factor receptors alpha and beta (PDGFRA and PDGFRB), as well as to macrophage colony-stimulating-factor receptor (CSF1R) and Fl cytokine receptor (FLT3). Binding of stem cell factor to KIT results in receptor homodimerization and kinase activation. The resulting phosphorylation of specific tyrosines on KIT and a number of secondary signaling molecules promotes signaling through several downstream pathways.

The most common mutations in KIT affect the juxtamembrane domain encoded by exon 11. Two-thirds of GISTs harbor an in-frame deletion, insertion, substitution, or

combination thereof, in this exon. Approximately 10% of GISTs have a mutation in an extracellular domain encoded by exon 9. More rarely, mutations occur in the kinase I (exon 13) or kinase II (exon 17) domain.

The importance of KIT mutations in GIST development is supported by several lines of evidence. First, when expressed in transfected cell lines, mutant forms of KIT show constitutive kinase activity in the absence of stem cell factor.^{3,11,12} Second, mutant KIT is oncogenic, supporting the growth of stably transfected BA/F3 cells in nude mice.³ Third, phosphorylated KIT is detectable in GIST tumor extracts. Fourth, patients with a heritable KIT mutation are at high risk for the development of multiple GISTs. And finally, mice engineered to express mutant KIT show ICC cell hyperplasia and develop stromal tumors that resemble human GISTs.^{13,14}

PDGFRA Mutations In GISTS

Studies of GIST extracts from tumors lacking *KIT* gene mutations revealed phosphorylation of the alpha receptor for platelet derived growth factor (PDGFRA).¹⁵ A close homologue of KIT, PDGFRA has similar extracellular and cytoplasmic domains, including a split kinase domain. Sequencing of these tumors turned up mutations in the juxtamembrane domain (exon 12) and activation loop (exon 18) of *PDGFRA*. It is now established that approximately 30% of GISTs that are wild-type for *KIT*, and 5-8% of GISTs overall, have *PDGFRA* gene mutations. *KIT* and *PDGFRA* mutations are mutually exclusive.¹⁶⁻¹⁸

When expressed in transfected cell lines, mutant forms of PDGFRA have constitutive kinase activity in the absence of PDGF-A ligand.^{15,16} As indicated above, phosphorylated PDGFRA is detectable in GIST tumor extracts and the activated downstream pathways are identical to those in *KIT*-mutant GISTs.^{15,19} Patients with a heritable *PDGFRA* mutation are at risk for the development of multiple GISTs.^{20,21}

The literature on *PDGFRA*-mutant GISTs emphasizes several distinctive pathologic features, including a striking predilection for the stomach, epithelioid morphology, myxoid stroma, nuclear pleomorphism, and variable (occasionally absent) expression of CD117.^{18,22-26} The unusual epithelioid morphology of these tumors raises the question as to whether they are true 'GISTs' or whether they should be classified as some other form of stromal tumor. There are, however, compelling similarities between *PDGFRA*-mutant and *KIT*-mutant GISTs. Both types of tumors are immunopositive for DOG-1, and both express PKC θ .^{6,8,22} In addition, both genotypes are associated with cytogenetic changes that are distinctive for GIST.^{15,27} Further, KIT protein is detectable in many *PDGFRA*-mutant tumors and vice-versa (which underscores the danger of assigning a genotype by immunohistochemistry).^{8,15,17,19} **It should be noted that not all *PDGFRA*-mutant GISTs are epithelioid; some have spindle cell features that are indistinguishable from *KIT*-mutant GISTs.**

Molecular Classification of GISTS

Despite their similarities, it would be improper to regard *KIT*-mutant and *PDGFRA*-mutant GISTs as simply the same tumor with alternate oncogenic kinases. Rather, they are representatives of a family of closely related tumors, which also includes the 'wild-type' GISTs. This puzzling subset, representing ~15% of all GISTs, has no detectable *KIT* or *PDGFRA* mutations despite all best efforts to find them. What drives

the growth of wild-type GISTs remains unknown, but it is interesting that phosphorylated KIT is detectable in these tumors, suggesting that KIT is still activated.⁶

Classification of GISTs by kinase genotype, as outlined in Table 1, provides a framework for understanding their biology. The type and distribution of *KIT* and *PDGFRA* mutations in GISTs varies with anatomic location (Table 1). Whereas *KIT* exon 9 mutations are found almost exclusively in GISTs arising within the small intestine and colon, the most common *PDGFRA* mutation, D842V, is detectable only in GISTs arising in the stomach, mesentery and omentum. Other *KIT* and *PDGFRA* mutations, by contrast, can be found in GISTs throughout the GI tract, from the esophagus to the rectum.

To date, approximately two dozen kindreds with heritable mutations in *KIT* or *PDGFRA* have been identified (Table 1).^{6,14,20,28-34} The penetrance in these kindreds appears to be high, as most affected members will develop one or more GISTs by middle age; however, in many patients the tumors do not follow a malignant course. Only two kindreds with a *PDGFRA* mutation have been identified.^{20,21}

Approximately 1-2% of GISTs arise in pediatric patients. The majority of these present as multinodular, epithelioid growths in the stomach, which are slow to progress but can metastasize and threaten the life of the patient. Unlike GISTs in adults, these tumors commonly involve local lymph nodes. Most pediatric GISTs occur in girls and are negative for *KIT* and *PDGFRA* mutations. Pediatric GISTs are sometimes associated with pulmonary chondromas and/or paragangliomas – referred to as Carney triad.³⁵ The gene for this rare constellation, which is not heritable, has yet to be identified.

There is a clear association between GISTs and type I neurofibromatosis.^{21,31,36-39} Indeed, one group estimated that 7% of NF1 patients develop one or more GISTs, which tend to arise in the small intestine but do not readily metastasize.³⁶ While NF1-related GISTs stain strongly positive for CD117, they are almost universally negative for *KIT* and *PDGFRA* mutations.

KIT-Negative GISTs

In approximately 5% of GISTs staining for CD117 is completely negative, or at most equivocally positive, which leaves the morphologic diagnosis somewhat in question. A common misconception is that all of these tumors harbor *PDGFRA* mutations, but the actual figure is more in the range of 30%.¹⁶⁻¹⁸ **Over half of CD117-negative tumors have *KIT* gene mutations, usually in exon 11**, which has significant therapeutic implications. Whether a GIST can be negative for CD117 staining *and* wild-type for *KIT* and *PDGFRA* is not entirely clear, as the diagnosis must then be based strictly on exclusion.

GIST Development and Prognosis

In patients with a germline *KIT* gene mutation, multifocal proliferations of morphologically benign, CD117-positive Cajal cells are commonly observed, possibly representing the earliest stage of GIST development. Interestingly, minute growths (1 to 10 mm) of Cajal/GIST-like cells are present in 22% to 35% of thoroughly examined stomachs from the general population.⁴⁰⁻⁴² The frequency of *KIT* mutations in such ‘micro-GISTs’ ranges from 46% to 85%.

Many groups have noted that *KIT* exon 11 mutations are a negative prognostic factor in clinically detected GISTs.⁴³⁻⁵⁰ In particular, deletions involving codons 557 &

558 have been associated with malignant behavior.^{25,51,52} As a group, *PDGFRA*-mutant GISTs appear to be less aggressive than *KIT*-mutant GISTs,^{53,54} yet *PDGFRA*-mutant tumors can still progress and kill patients. Once GISTs become metastatic, kinase genotype does not factor into overall survival.⁵⁵ Thus, while a particular kinase mutation may set the initial course of a GIST, the prognosis at the time of clinical presentation is clearly influenced by other genetic events.

Unfortunately, our knowledge of these additional mutations remains limited, and current recommendations for assessing the risk of progression of a newly diagnosed primary GIST are based on three simple parameters: tumor size, tumor location, and mitotic index (mitoses per 50 high power fields). The risk assessment scheme presented in Table 2 is based on the work of Miettinen and colleagues at the AFIP, whose considerable efforts in studying the outcome of patients prior to the advent of modern therapies have provided the most complete data available.^{9,56,57}

Treatment of GIST

The primary treatment for a resectable GIST is surgery, which cures most patients with a low- or intermediate-risk tumor. The lesion should be removed intact and with clear margins, but wide margins are not required. Lymphadenectomy is unnecessary, save for pediatric GISTs.

Treatment options for patients with unresectable or metastatic GIST were poor until the advent of the kinase inhibitor imatinib (GleevecTM). An orally bioavailable 2-phenylpyrimidine derivative that was developed in the late 1990s as a treatment for chronic myelogenous leukemia, imatinib reliably achieves disease control in 70-85% of patients with advanced GIST. The median progression-free survival is 20–24 months; for some patients the overall survival now exceeds 7 years.

Kinase Mutations Predict Response to Imatinib therapy

A consistent observation in clinical studies of imatinib for the treatment of GIST is that genotypically-defined subsets of GIST have different outcomes. Based on phase III trial data for 768 genotyped GISTs, the objective response rates are as follows: *KIT* exon 11 – 64-67%, *KIT* exon 9 – 34-37%, and wild-type – 23-37%. Kinase genotype also correlates with progression-free survival and overall survival. **The median time to tumor progression in patients whose GIST has a *KIT* exon 11 mutation is more than 1 year longer than in patients whose tumor has a *KIT* exon 9 or wild-type genotype.**

The above results reflect pooled data from clinical studies in which imatinib doses ranged from 400-800 mg per day. In a recent subset analysis of the EORTC/AustralAsia phase III trial, the progression-free survival of GIST patients with *KIT* exon 9 mutations was significantly better when they were treated with 800 mg per day as compared with 400 mg.⁵⁸ This has been confirmed in a recent meta-analysis of both the North American and Europe/AustralAsian phase III trials (manuscript in preparation). In contrast, patients whose GIST had *KIT* exon 11 mutations did equally well on either dose. **Many GIST experts now recommend routine tumor genotyping and dose selection based on the presence or absence of a *KIT* exon 9 mutation.**

Treatment of *PDGFRA*-mutant GIST with imatinib

Only small numbers of patients with *PDGFRA*-mutant GIST were included in the original phase I-III trials. Among six patients whose GIST harbored the most common *PDGFRA* mutation (D842V) there were no objective responses. Correspondingly, this form of *PDGFRA* is fully resistant to the effects of imatinib in vitro.^{16,59-61} It is important to note, however, that **up to one third of *PDGFRA* mutations are sensitive to imatinib in vitro, and individual patients with these types of GIST have shown excellent responses to imatinib.** Thus, genotyping has an important role in predicting the imatinib responsiveness of *PDGFRA*-mutant GISTs.

Adjuvant Use of Imatinib

A trial of 12 months of adjuvant imatinib vs placebo was conducted for patients with a resected GIST ≥ 3 cm. More than 600 patients were enrolled in anticipation that it would take several years to determine the effectiveness of adjuvant therapy. In April 2007, however, the trial was ended early when an interim analysis revealed a significant difference in the rate of disease recurrence: 3% for patients receiving imatinib versus 17% for those who took placebo. Subset analyses, including the impact of tumor genotype, are ongoing, but many oncologists routinely prescribe 12 months or more of imatinib for patients with resected high risk GIST.

Imatinib Resistance

A minority of patients experience continued tumor growth on imatinib within the first 6 months of treatment, which is referred to as *primary* resistance. Compared with patients who have *KIT* exon 11-mutant tumors, those with exon 9-mutant or wild-type tumors are over-represented in this group. Aside from the *PDGFRA* D842V mutation, however, the causes for primary resistance remain largely unknown. Patients who benefit from treatment beyond 6 months will often show growth in one or more lesions between 12 and 36 months of treatment, which is referred to as *secondary* resistance. **At least 80% of secondary resistance is related to the acquisition of new mutations that interfere with drug binding.** Recent experiments using high-sensitivity real-time PCR indicate that imatinib-resistant GISTs are often heterogeneous, with up to 3 other acquired mutations detectable at low levels in the background of a dominant resistance mutation (Liegler et al. manuscript submitted).

New Kinase Inhibitors and Other Treatment Targets

Success with imatinib has spurred the development of many new kinase inhibitors with activity against *KIT* and *PDGFRA*. Among these is sunitinib (SutentTM), an orally bioavailable “multi-targeted” inhibitor that also blocks VEGFR2 and may impede tumor angiogenesis. Sunitinib is FDA-approved for the treatment of GIST patients who are intolerant of, or resistant to, imatinib. Based on an extended phase II trial, **it appears that the best responses to sunitinib are in patients with *KIT* exon 9-mutant or wild-type tumor.**⁶²

There are many other kinase inhibitors in clinical development, but one can safely predict that most will eventually fail if used as a monotherapy. Multi-agent treatment modalities are needed, perhaps in the form of a kinase inhibitor ‘cocktail’, or a

combination of a kinase inhibitor with another targeted therapeutic. One target that holds some promise is HSP90,⁶³ an inhibitor of which (IPI-504) is in a phase II trial.

Conclusions

Progress in our understanding of GIST biology laid the groundwork for the first breakthroughs in GIST treatment. In turn, the outcomes of GIST patients being treated with kinase inhibitors are informing ongoing studies of drug resistance, downstream signaling, apoptosis and synergistic strategies for tumor suppression. This interplay between basic tumor biology and the effects of targeted therapeutics in patients provides a model for accelerating the development of novel, rationally-based treatment strategies.

Kinase genotype has emerged as a principal factor in the evaluation of GISTs, particularly those tumors that are overtly malignant or have a high risk of recurrence. In addition to helping to establish the diagnosis of GIST in unusual cases, genotyping can be very useful to physicians and patients in deciding on imatinib dose, in estimating the likelihood and duration of benefit, and potentially in selecting second-line therapies. For all these reasons, the 2007 National Comprehensive Cancer Network (NCCN) guidelines for GIST support routine kinase genotyping for all newly diagnosed high-risk and malignant tumors.

Table 1. Molecular Classification of GISTs

Genetic type	Relative Frequency	Anatomic Distribution	Germline Examples
<i>KIT</i> Mutation	75-80%		
Exon 8	Rare	Small bowel	1 Kindred
Exon 9	10%	Small bowel, colon	None
Exon 11	65%	All sites	Several kindreds
Exon 13	1%	All sites	2 Kindreds
Exon 17	1%	All sites	2 Kindreds
<i>PDGFRA</i> Mutation	5-7%		
Exon 12	1%	All sites	1 Kindred
Exon 14	<1%	Stomach	None
Exon 18 D842V	5%	Stomach, mesentery, omentum	None
Exon 18 other	1%	All sites	1 Kindred
Wild-type	~15%	All sites	None
Carney triad-related	Rare	Stomach	Not heritable
NF1-related	Rare	Small bowel	Numerous

Table 2. Risk Stratification of Primary GIST by Mitotic Index, Size and Site

Modified from Miettinen & Lasota, 2006.⁶⁴

Data based on long-term follow-up of 1055 gastric, 629 small intestinal, 144 duodenal and 111 rectal GISTs.^{9,56,57}

#Defined as metastasis or tumor-related death.

*Denotes small numbers of cases.

Tumor Parameters		Risk of Progressive Disease [#] (%)			
	Size	Gastric	Jejunum/Ileum	Duodenum	Rectum
Mitotic Index ≤ 5 per 50 hpf	≤ 2 cm	None (0%)	None (0%)	None (0%)	None (0%)
	> 2 ≤ 5 cm	Very low (1.9%)	Low (4.3%)	Low (8.3%)	Low (8.5%)
	> 5 ≤ 10 cm	Low (3.6%)	Moderate (24%)	(Insuff. data)	(Insuff. data)
	> 10 cm	Moderate (10%)	High (52%)	High (34%)	High (57%)
Mitotic Index > 5 per 50 hpf	≤ 2 cm	None*	High*	(Insuff. data)	High (54%)
	> 2 ≤ 5 cm	Moderate (16%)	High (73%)	High (50%)	High (52%)
	> 5 ≤ 10 cm	High (55%)	High (85%)	(Insuff. data)	(Insuff. data)
	> 10 cm	High (86%)	High (90%)	High (86%)	High (71%)

References

1. Mazur, M. T. & Clark, H. B. Gastric stromal tumors. Reappraisal of histogenesis. *Am. J. Surg. Pathol.* **7**, 507-519 (1983).
2. Isozaki, K. *et al.* Disturbed intestinal movement, bile reflux to the stomach, and deficiency of c-kit-expressing cells in Ws/Ws mutant rats. *Gastroenterology* **109**, 456-464 (1995).
3. Hirota, S. *et al.* Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science* **279**, 577-580 (1998).
4. Kindblom, L. G., Remotti, H. E., Aldenborg, F. & Meis-Kindblom, J. M. Gastrointestinal pacemaker cell tumor (GIPACT): gastrointestinal stromal tumors show phenotypic characteristics of the interstitial cells of Cajal. *Am. J Pathol* **152**, 1259-1269 (1998).
5. Blay, P. *et al.* Protein kinase C theta is highly expressed in gastrointestinal stromal tumors but not in other mesenchymal neoplasias. *Clin Cancer Res.* **10**, 4089-4095 (2004).
6. Duensing, A. *et al.* Protein Kinase C theta (PKCtheta) expression and constitutive activation in gastrointestinal stromal tumors (GISTs). *Cancer Res.* **64**, 5127-5131 (2004).
7. Motegi, A. *et al.* PKC theta, a novel immunohistochemical marker for gastrointestinal stromal tumors (GIST), especially useful for identifying KIT-negative tumors. *Pathol. Int.* **55**, 106-112 (2005).
8. West, R. B. *et al.* The novel marker, DOG1, is expressed ubiquitously in gastrointestinal stromal tumors irrespective of KIT or PDGFRA mutation status. *Am J Pathol* **165**, 107-113 (2004).
9. Miettinen, M., Sobin, L. H. & Lasota, J. Gastrointestinal Stromal Tumors of the Stomach: A Clinicopathologic, Immunohistochemical, and Molecular Genetic Study of 1765 Cases With Long-term Follow-up. *Am J Surg. Pathol* **29**, 52-68 (2005).
10. Corless, C. L., Fletcher, J. A. & Heinrich, M. C. Biology of gastrointestinal stromal tumors. *J Clin Oncol* **22**, 3813-3825 (2004).
11. Heinrich, M. C. *et al.* Inhibition of c-kit receptor tyrosine kinase activity by STI 571, a selective tyrosine kinase inhibitor. *Blood* **96**, 925-932 (2000).
12. Tuveson, D. A. *et al.* STI571 inactivation of the gastrointestinal stromal tumor c-KIT oncoprotein: biological and clinical implications. *Oncogene* **20**, 5054-5058 (2001).
13. Rubin, B. P. *et al.* A knock-in mouse model of gastrointestinal stromal tumor harboring kit K641E. *Cancer Res.* **65**, 6631-6639 (2005).
14. Sommer, G. *et al.* Gastrointestinal stromal tumors in a mouse model by targeted mutation of the Kit receptor tyrosine kinase. *Proc Natl. Acad. Sci. U. S. A* **100**, 6706-6711 (2003).
15. Heinrich, M. C. *et al.* PDGFRA Activating Mutations in Gastrointestinal Stromal Tumors. *Science* **299**, 708-710 (2003).
16. Hirota, S. *et al.* Gain-of-function mutations of platelet-derived growth factor receptor alpha gene in gastrointestinal stromal tumors. *Gastroenterology* **125**, 660-667 (2003).

17. Pauls, K., Merkelbach-Bruse, S., Thal, D., Buttner, R. & Wardelmann, E. PDGFRalpha- and c-kit-mutated gastrointestinal stromal tumours (GISTs) are characterized by distinctive histological and immunohistochemical features. *Histopathology* **46**, 166-175 (2005).
18. Wasag, B. *et al.* Differential expression of KIT/PDGFR mutant isoforms in epithelioid and mixed variants of gastrointestinal stromal tumors depends predominantly on the tumor site. *Mod. Pathol.* **17**, 889-894 (2004).
19. Kang, H. J. *et al.* Correlation of KIT and platelet-derived growth factor receptor alpha mutations with gene activation and expression profiles in gastrointestinal stromal tumors. *Oncogene* **24**, 1066-1074 (2005).
20. Chompret, A. *et al.* PDGFRA germline mutation in a family with multiple cases of gastrointestinal stromal tumor. *Gastroenterology* **126**, 318-321 (2004).
21. de Raedt, T. *et al.* Intestinal neurofibromatosis is a subtype of familial GIST and results from a dominant activating mutation in PDGFRA. *Gastroenterology* **131**, 1907-1912 (2006).
22. Debiec-Rychter, M. *et al.* Gastrointestinal stromal tumours (GISTs) negative for KIT (CD117 antigen) immunoreactivity. *J Pathol.* **202**, 430-438 (2004).
23. Medeiros, F. *et al.* KIT-negative gastrointestinal stromal tumors: proof of concept and therapeutic implications. *Am J Surg Pathol* **28**, 889-894 (2004).
24. Sakurai, S. *et al.* Myxoid epithelioid gastrointestinal stromal tumor (GIST) with mast cell infiltrations: a subtype of GIST with mutations of platelet-derived growth factor receptor alpha gene. *Hum. Pathol.* **35**, 1223-1230 (2004).
25. Tzen, C. Y. & Mau, B. L. Analysis of CD117-negative gastrointestinal stromal tumors. *World J. Gastroenterol.* **11**, 1052-1055 (2005).
26. Wardelmann, E. *et al.* Association of platelet-derived growth factor receptor alpha mutations with gastric primary site and epithelioid or mixed cell morphology in gastrointestinal stromal tumors. *J. Mol. Diagn.* **6**, 197-204 (2004).
27. Wozniak, A. *et al.* Array CGH analysis in primary gastrointestinal stromal tumors: cytogenetic profile correlates with anatomic site and tumor aggressiveness, irrespective of mutational status. *Genes Chromosomes. Cancer* **46**, 261-276 (2007).
28. Beghini, A. *et al.* Germline mutation in the juxtamembrane domain of the kit gene in a family with gastrointestinal stromal tumors and urticaria pigmentosa. *Cancer* **92**, 657-662 (2001).
29. Hirota, S. *et al.* Familial gastrointestinal stromal tumors associated with dysphagia and novel type germline mutation of KIT gene. *Gastroenterology* **122**, 1493-1499 (2002).
30. Isozaki, K. *et al.* Germline-activating mutation in the kinase domain of KIT gene in familial gastrointestinal stromal tumors. *Am. J Pathol.* **157**, 1581-1585 (2000).
31. Kang, D. Y. *et al.* Multiple Gastrointestinal Stromal Tumors: Clinicopathologic and Genetic Analysis of 12 Patients. *Am. J. Surg. Pathol.* **31**, 224-232 (2007).
32. Maeyama, H. *et al.* Familial Gastrointestinal Stromal Tumor With Hyperpigmentation: Association With a Germline Mutation of the c-kit Gene. *Gastroenterology* **120**, 210-215 (2001).

33. Nishida, T. *et al.* Familial gastrointestinal stromal tumours with germline mutation of the KIT gene. *Nature Genetics* **19**, 323-324 (1998).
34. O'riain, C. *et al.* Gastrointestinal Stromal Tumors: Insights From a New Familial GIST Kindred With Unusual Genetic and Pathologic Features. *Am. J. Surg. Pathol.* **29**, 1680-1683 (2005).
35. Carney, J. A. Gastric stromal sarcoma, pulmonary chondroma, and extra-adrenal paraganglioma (Carney Triad): natural history, adrenocortical component, and possible familial occurrence. *Mayo Clin. Proc.* **74**, 543-552 (1999).
36. Andersson, J. *et al.* NF1-associated gastrointestinal stromal tumors have unique clinical, phenotypic, and genotypic characteristics. *Am. J. Surg. Pathol.* **29**, 1170-1176 (2005).
37. Maertens, O. *et al.* Molecular pathogenesis of multiple gastrointestinal stromal tumors in NF1 patients. *Hum. Mol. Genet.* **15**, 1015-1023 (2006).
38. Miettinen, M., Fetsch, J. F., Sobin, L. H. & Lasota, J. Gastrointestinal Stromal Tumors in Patients With Neurofibromatosis 1: A Clinicopathologic and Molecular Genetic Study of 45 Cases. *Am. J. Surg. Pathol.* **30**, 90-96 (2006).
39. Stewart, D. R. *et al.* Mitotic recombination as evidence of alternative pathogenesis of gastrointestinal stromal tumours in neurofibromatosis type 1. *J. Med. Genet.* **44**, e61 (2007).
40. Agaimy, A. *et al.* Minute gastric sclerosing stromal tumors (GIST tumorlets) are common in adults and frequently show c-KIT mutations. *Am. J. Surg. Pathol.* **31**, 113-120 (2007).
41. Corless, C. L., McGreevey, L., Haley, A., Town, A. & Heinrich, M. C. KIT mutations are common in incidental gastrointestinal stromal tumors one centimeter or less in size. *Am. J. Pathol.* **160**, 1567-1572 (2002).
42. Kawanowa, K. *et al.* High incidence of microscopic gastrointestinal stromal tumors in the stomach. *Hum. Pathol.* **37**, 1527-1535 (2006).
43. Ernst, S. I. *et al.* KIT mutation portends poor prognosis in gastrointestinal stromal/smooth muscle tumors. *Laboratory Investigation* **78**, 1633-1636 (1998).
44. Lasota, J., Jasinski, M., Sarlomo-Rikala, M. & Miettinen, M. Mutations in exon 11 of c-Kit occur preferentially in malignant versus benign gastrointestinal stromal tumors and do not occur in leiomyomas or leiomyosarcomas. *Am. J. Pathol.* **154**, 53-60 (1999).
45. Li, S. Q. *et al.* Analysis of KIT mutation and protein expression in fine needle aspirates of gastrointestinal stromal/smooth muscle tumors. *Acta Cytologica* **44**, 981-986 (2000).
46. Singer, S. *et al.* Prognostic Value of KIT Mutation Type, Mitotic Activity, and Histologic Subtype in Gastrointestinal Stromal Tumors. *J Clin. Oncol.* **20**, 3898-3905 (2002).
47. Taniguchi, M. *et al.* Effect of c-kit mutation on prognosis of gastrointestinal stromal tumors. *Cancer Res* **59**, 4297-4300 (1999).
48. Andersson, J. *et al.* Gastrointestinal stromal tumors with KIT exon 11 deletions are associated with poor prognosis. *Gastroenterology* **130**, 1573-1581 (2006).
49. Cho, S. *et al.* Deletion of the KIT gene is associated with liver metastasis and poor prognosis in patients with gastrointestinal stromal tumor in the stomach. *Int. J. Oncol.* **28**, 1361-1367 (2006).

50. Liu, X. H. *et al.* Prognostic value of KIT mutation in gastrointestinal stromal tumors. *World J Gastroenterol.* **11**, 3948-3952 (2005).
51. Martin, J. *et al.* Deletions affecting codons 557-558 of the c-KIT gene indicate a poor prognosis in patients with completely resected gastrointestinal stromal tumors: a study by the Spanish Group for Sarcoma Research (GEIS). *J. Clin. Oncol.* **23**, 6190-6198 (2005).
52. Wardelmann, E. *et al.* Deletion of Trp-557 and Lys-558 in the juxtamembrane domain of the c-kit protooncogene is associated with metastatic behavior of gastrointestinal stromal tumors. *Int. J Cancer* **106**, 887-895 (2003).
53. Lasota, J., Dansonka-Mieszkowska, A., Sobin, L. H. & Miettinen, M. A great majority of GISTs with PDGFRA mutations represent gastric tumors of low or no malignant potential. *Lab Invest* **84**, 874-883 (2004).
54. Lasota, J., Stachura, J. & Miettinen, M. GISTs with PDGFRA exon 14 mutations represent subset of clinically favorable gastric tumors with epithelioid morphology. *Lab Invest* **86**, 94-100 (2006).
55. Gold, J. S. *et al.* Outcome of metastatic GIST in the era before tyrosine kinase inhibitors. *Ann. Surg. Oncol.* **14**, 134-142 (2007).
56. Miettinen, M. *et al.* Gastrointestinal stromal tumors, intramural leiomyomas, and leiomyosarcomas in the duodenum: a clinicopathologic, immunohistochemical, and molecular genetic study of 167 cases. *Am. J Surg. Pathol.* **27**, 625-641 (2003).
57. Miettinen, M., Makhlof, H., Sobin, L. H. & Lasota, J. Gastrointestinal Stromal Tumors of the Jejunum and Ileum: A Clinicopathologic, Immunohistochemical, and Molecular Genetic Study of 906 Cases Before Imatinib With Long-term Follow-up. *Am. J. Surg. Pathol.* **30**, 477-489 (2006).
58. Debiec-Rychter, M. *et al.* KIT mutations and dose selection for imatinib in patients with advanced gastrointestinal stromal tumours. *Eur. J. Cancer* **42**, 1093-1103 (2006).
59. Corless, C. L. *et al.* PDGFRA Mutations In Gastrointestinal Stromal Tumors: Frequency, Spectrum and In Vitro Sensitivity To Imatinib. *J. Clin. Oncol.* **23**, 5357-5364 (2005).
60. Heinrich, M. C. *et al.* Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. *J Clin Oncol* **21**, 4342-4349 (2003).
61. Weisberg, E. *et al.* Effects of PKC412, nilotinib, and imatinib against GIST-associated PDGFRA mutants with differential imatinib sensitivity. *Gastroenterology* **131**, 1734-1742 (2006).
62. Heinrich, M. *et al.* Sunitinib (SU) response in imatinib-resistant (IM-R) GIST correlates with KIT and PDGFRA mutation status. 2006 ASCO Annual Meeting Proceedings 24(18S), 520S. 2006. Ref Type: Abstract
63. Bauer, S., Yu, L. K., Demetri, G. D. & Fletcher, J. A. Heat shock protein 90 inhibition in imatinib-resistant gastrointestinal stromal tumor. *Cancer Res.* **66**, 9153-9161 (2006).
64. Miettinen, M. & Lasota, J. Gastrointestinal stromal tumors: pathology and prognosis at different sites. *Semin. Diagn. Pathol.* **23**, 70-83 (2006).

The Role of Pathology in Treatment of Sarcomas: From Grading to Histotyping

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Key points

- Soft tissue sarcoma is a rare variant of cancer in which statistically powered clinical trials are difficult to be performed.
- The utility of adjuvant chemotherapy in soft tissue sarcomas is still debated.
- Specific histotypes have shown differential sensitivity to specific chemotherapeutic agents.
- The advent of molecular target therapy has further increased the importance of accurate morphologic subtyping of sarcomas.

Soft tissue sarcomas represents a distinctively heterogeneous group of rare malignancies, with an overall incidence, of around 5/100,000/year. From the clinical standpoint approximately 20 to 30% of cases recur locally and 30 to 50% of cases metastasize, most often to lungs. 5-year overall survival varies between 55% and 65%, regardless of stage and histology. Both rarity and heterogeneity represents main factors affecting on one hand diagnostic accuracy, and on the other the feasibility of statistically powered clinical trials.

In the past standard treatment was mainly represented by surgery, variably associated to radiotherapy in the attempt to improve local control (1,2) The use of chemotherapy in advanced disease as well as in the adjuvant setting has proved most fruitful in pediatric sarcomas (rhabdomyosarcoma, Ewing's family of tumors, infantile fibrosarcoma), and osteosarcoma. On the other hand, the real efficacy of systemic treatment in adults' soft tissue tumors is still source of debate (3). Two major issues which have been addressed by a number of clinical trials are the efficacy of adjuvant chemotherapy in the localized disease and whether a polychemotherapy with doxorubicin and ifosfamide is superior to doxorubicin alone in the advanced disease. By and large, the conclusion from these trials was that adjuvant chemotherapy is ineffective, or at best marginally effective, and that polychemotherapy is not superior to single agent chemotherapy. A meta-analysis was published in 1997, and it was unable to detect any statistically significant advantage in survival (4). However, trials included in this meta-analysis had not used ifosfamide in

addition to doxorubicin. Actually, a “second-generation” study from the Italian Sarcoma Group (ISG) employing the combination of doxorubicin and ifosfamide showed an improvement in survival (5). Interestingly, chemotherapy did not affect the metastatic rate, indicating that chemotherapy most likely delays rather than prevent systemic spread. Recently another meta-analysis was preliminarily reported (6), showing the same degree of absolute benefit in relapse-free survival and overall survival, but at least the difference in survival appeared now to be significant. However, this study did not incorporate preliminary report from an EORTC Soft Tissue and Bone Sarcoma Group clinical trial, which appears to be totally negative (7). Disappointingly, after many years of such trials, the uncertainty regarding the utility of adjuvant chemotherapy does not seem to be narrowed, and as a consequence the community of sarcoma researchers remains divided on its efficacy. Patients all over the world, especially if they are high-risk, are often offered adjuvant chemotherapy as an option in conditions of uncertainty.

Whatever the treatment options, for years clinicians have not been showing much interest into histotyping of soft tissue sarcomas. As matter of fact, most of decision making was eventually made on grading (8-10). Certainly grading has proved to be one of the most important tool in order to stratify patients into prognostically meaningful categories, however certainly it was not meant for predicting response to adjuvant therapy. As mentioned before adjuvant chemotherapy still does not represent a standard treatment. Of course, in consideration of the rarity of soft tissue sarcomas, most clinical studies have been performed on histologically heterogeneous series, assuming that all histotypes would respond at the adjuvant treatment as a single disease. As a consequence, it seems likely that two main problems have somewhat hampered the clinical studies investigating the potential utility of adjuvant chemotherapy in soft tissue sarcoma: the use of a rather restricted range of effective drugs (anthracyclins and ifosfamide), and their indiscriminate use in a very heterogeneous collection of distinct tumor types. However, it is easy to understand that clinical trials on very rare tumors are unfeasible by definition. As an example, the incidence of angiosarcoma is in the order of 0.05/100,000/year. In a large country (or area) of 100,000,000 people, one would find a few dozens new patients each year. Even increasing the usual proportion of patients enrolled into clinical trials (hardly exceeding 5%), it would be difficult to set up a clinical trial on hundreds such patients in a reasonable number of years. Nonetheless, despite the impossibility to set up a randomized clinical trial on angiosarcoma, angiosarcoma itself has contributed to prove that selected groups of sarcomas may show specific sensitivity to different agents. In fact it has now

become clear that angiosarcomas may respond to cytotoxic agents like taxanes, which are inactive in almost all other soft tissue sarcomas (11). It has been also very recently shown that antiangiogenic drugs seem also to work in angiosarcomas more than in most other sarcoma subtypes, further underlying the relationships between morphology and therapy (12). In general, it has become evident in recent years that some sarcoma subtypes may indeed show specific response patterns to medical therapy. Amongst adult soft tissue sarcomas, this has been the case for trabectedin (mainly active in leiomyosarcoma and myxoid liposarcoma) (13,14), gemcitabine (most active in leiomyosarcomas and undifferentiated high grade sarcomas) (15,16), not to mention imatinib that in addition to its stunning success in gastrointestinal stromal tumors (17-21), has also proved effective in dermatofibrosarcoma protuberans (DFSP) (22), chordoma (23), and desmoid fibromatosis (24). Therefore, even if one overlooks the peculiarities of the natural history of these subgroups, recent developments with chemotherapy, and even more with molecular targeted therapies, makes this split unavoidable.

The unveiling of the molecular mechanisms involved in the carcinogenesis of several subgroups of lesions and the possibility to utilize specific molecules targeting those mechanisms certainly represents the most important advance. In parallel with the well known application of tyrosine kinase inhibitors in GIST and DFSP, it has become evident that different non mutational alterations of targets such as PDGFRA and PGFRB may explain the clinical responses observed in patients affected by chordomas and desmoid fibromatosis (25,26). Of great interest is also the fact the myxoid liposarcoma, shown to be exquisitely sensitive to the marine derived alkaloid trabectedin (13,14), tend to exhibit differential sensitivity on the basis of the type of chimeric transcript derived from the fusion of the genes FUS and DDIT3 (27). This last observation not only further demonstrates the existence of histotype-related sensitivity, but also that within the same histotype specific molecular aberration may predict the chance of response.

The current evolution of therapeutic approach to soft tissue sarcoma certainly represent the offspring of the marriage between pathology and genetics. Both classic cytogenetics and molecular genetics has not only refined and validated classification schemes, but has also provided information that are being gradually incorporated in the clinics. A good example is certainly represented by the possibility to target well differentiated/dedifferentiated liposarcoma (known to be characterized by *MDM2* gene amplification and consequent MDM2 protein overexpression) with the MDM2 antagonist Nutlin-3a, capable of inducing apoptosis and growth arrest in neoplastic cells (28).

In conclusion, any attempt to identify effective systemic treatments for sarcomas must take into account not only the great morphologically heterogeneity that characterized this fascinating group of malignancies, but also the underlining molecular mechanisms. This seems to be crucial not only for molecular targeted therapies but, as appears to happen with trabectedin, also for agents exerting apparently less specific mechanisms of action. The main consequence is that accurate morphologic diagnosis will gain even greater importance, either as a direct tool to select the best therapeutic option, or as a crucial step to allow identification of the relevant molecular abnormalities to be targeted.

References

1. Pisters PWT et al. Analysis of prognostic factors in 1041 patients with localized soft tissue sarcomas of the extremities. *J Clin Oncol* 1996; 14:1679-1689.
2. Yang JC et al. A randomized prospective study of the benefit of adjuvant radiation therapy in the treatment of soft tissue sarcomas of the extremity. *J Clin Oncol* 1998; 16:197-203.
3. Pisters PWT et al. Evidence base recommendations for local therapy for soft tissue sarcomas. *J Clin Oncol* 2007; 25:1003-1008.
4. Sarcoma Meta-analysis Collaboration. Adjuvant chemotherapy for localised resectable soft-tissue sarcoma of adults: meta-analysis of individual data. *Lancet* 1997; 350: 1647–1654
5. Frustaci S et al. Adjuvant chemotherapy for adult soft tissue sarcomas of the extremities and girdles: results of the Italian randomized cooperative trial. *J Clin Oncol* 2001; 19:1238-1247.
6. Pervaiz N; Colterjohn N; Farrokhyar F; et al. A systematic meta-analysis of randomized controlled trials for adjuvant chemotherapy for localized resectable soft tissue sarcoma. *Connective Tissue Oncology Society Ann Meet*, 2007; # 835.
7. Woll PJ, van Glabbeke M, Hohenberger P, et al. Adjuvant chemotherapy (CT) with doxorubicin and ifosfamide in resected soft tissue sarcoma (STS): Interim analysis of a randomised phase III trial. *J Clin Oncol*, 2007 ASCO Annual Meeting Proceedings Part I. Vol 25, No. 18S (June 20 Supplement), 2007: 10008.
8. Coindre JM, Terrier P, Bui NB, et al. Prognostic factors in adult patients with locally controlled soft tissue sarcoma : a study of 546 patients from the French Federation of Cancer Centers Sarcoma Group. *J Clin Oncol* 1996, 14: 869-877.

9. Guillou L, Coindre JM, Bonichon F, et al. Comparative study of the National Cancer Institute and French Federation of Cancer Centers Sarcoma Group grading systems in a population of 410 adult patients with soft tissue sarcoma. *J Clin Oncol* 1997; 15: 350-362.
10. Coindre JM. Grading of soft tissue sarcomas. Review and update. *Arch Pathol Lab Med* 2006; 130: 1448-1453.
11. Fata F, O'Reilly E, Ilson D, et al. Paclitaxel in the treatment of patients with angiosarcoma of the scalp or face. *Cancer* 1999; 86: 2034-7.
12. D'Adamo DR, Keohan M, Schuetze S, et al. Clinical results of a phase II study of sorafenib in patients (pts) with non-GIST sarcomas (CTEP study #7060). *J Clin Oncol* 2007 ASCO Annual Meeting Proceedings Part I. Vol 25, No. 18S (June 20 Supplement), 2007: 10001.
13. Le Cesne A, Blay JY, Judson I, et al. Phase II study of ET-743 in advanced soft tissue sarcomas: a European Organisation for the Research and Treatment of Cancer (EORTC) soft tissue and bone sarcoma group trial. *J Clin Oncol* 2005; 23: 576-84.
14. Grosso F, Jones RL, Demetri GD, et al. Efficacy of trabectedin (ecteinascidin-743) in advanced pretreated myxoid liposarcomas: a retrospective study. *Lancet Oncol* 2007; 8: 595-602.
15. Hensley ML et al. Gemcitabine and docetaxel in patients with unresectable leiomyosarcoma: results of a phase II trial. *J Clin Oncol* 2002; 20: 2824-2831.
16. Maki RG, Wathen JK, Patel SR, Priebat DA, et al. Randomized phase II study of gemcitabine and docetaxel compared with gemcitabine alone in patients with metastatic soft tissue sarcomas: results of sarcoma alliance for research through collaboration study 002. *J Clin Oncol*. 2007;25:2755-63
17. Rubin BP, et al. Gastrointestinal stromal tumor. *Lancet* 2007; 369:1731-1741.
18. Joensuu H, Roberts PJ, Sarlomo-Rikala M, et al. Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumour. *N Engl J Med* 2001; 344:1052-1056.
19. van Oosterom AT, Judson I, Verweij J, et al. Safety and efficacy of imatinib (STI571) in metastatic gastrointestinal stromal tumours: a phase I study. *Lancet* 2001; 358:1421-1423

20. Demetri GD, von Mehren M, Blanke CD, et al. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N Engl J Med* 2002; 47: 472-480
21. Demetri GD, van Oosterom AT, Garrett CR et al. Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. *Lancet* 2006; 368:1329-38.
22. McArthur GA, Demetri GD, van Oosterom A, et al. Molecular and clinical analysis of locally advanced dermatofibrosarcoma protuberans treated with imatinib: Imatinib Target Exploration Consortium Study B2225. *J Clin Oncol* 2005; 23: 866-73.
23. Casali PG, Messina A, Stacchiotti S, et al. Imatinib mesylate in chordoma. *Cancer* 2004; 101: 2086-97.
24. Heinrich MC, McArthur GA, Demetri GD, et al. Clinical and molecular studies of the effect of imatinib on advanced aggressive fibromatosis (desmoid tumor). *J Clin Oncol* 2006; 24: 1195-203.
25. Tamborini E, et al. Molecular and biochemical analyses of platelet-derived growth factor receptor (PDGFR) B, PDGFRA, and KIT receptors in chordomas. *Clin Cancer Res.* 2006; 12: :6920-6928.
26. Signoroni S, Frattini M, Negri T et al. Cyclooxygenase and platelet derived growth factor receptors as potential targets in treating aggressive fibromatosis. *Clin cancer Res* 2007; 13: 5034-5040.
27. Grosso F, Forni C, Frapolli A et al. Sensitivity of myxoid-round cell liposarcoma (MRCL) to trabectedin (T) may be related to a direct effect on the fusion transcript. *J Clin Oncol*, 2007 ASCO Annual Meeting Proceedings Part I. Vol 25, No. 18S (June 20 Supplement), 2007: 10008
28. Singer S, Socci ND, Ambrosini G, et al. Gene expression profiling of liposarcoma identifies distinct biological types/subtypes and potential therapeutic targets in well-differentiated and dedifferentiated liposarcoma. *Cancer Res.* 2007; 67:6626-6636

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THE EVOLUTION OF SOFT TISSUE TUMOUR TAXONOMY:
WHAT STILL NEEDS TO BE DONE?

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Bullet points:

- There has been progressive improvement in soft tissue tumor classification schemes over the past 20-30 years, but problems remain.
- Some outdated diagnostic concepts need to be abolished (e.g. MFH) or substantially revised or redefined (e.g. hemangiopericytoma and adult-type fibrosarcoma).
- Some tumor nomenclature is illogical or misleading and may need to be reappraised (e.g. synovial sarcoma and extraskeletal myxoid chondrosarcoma).
- Classification among the broad group of vascular tumors is hampered by our lack of understanding regarding pathogenesis, line(s) of differentiation and biologic status/potential.

- The impact of genetic analysis has been positive, but, as more data emerge, the results pose challenges for both morphologic and molecular classification schemes.

INTRODUCTION

For many years, classification schemes have provided very important underpinnings in the diagnosis, clinical management and research of human tumors. Furthermore, the trend towards the development of widely accepted consensus classification schemes, both within individual subspecialty groups (e.g. hematopathology) or more broadly by the World Health Organization (WHO), have helped greatly to ensure more reproducible diagnosis across larger national and international patient groups, thereby enabling more effective and meaningful analysis of both clinical and more basic research studies, while also allowing more accurate interpretation of clinical trials data.

In this regard, the classification of soft tissue tumors has made very substantial steps forward in the past 20-30 years, based upon the gradual introduction of more reproducible and objective diagnostic criteria (often facilitated by immunohistochemistry), the arrival of cytogenetic and molecular genetic data which have in large part further facilitated objectivity, as well as a willingness to question poorly substantiated dogmas based on limited data combined with the recognition that ‘histogenesis’ has very little meaning in the context of mesenchymal neoplasia. In purely pragmatic terms, the modern classification of these lesions is now largely based on evidence of a specific line of differentiation or else on genotypic data. In some tumor types (e.g. rhabdomyosarcoma), classification is based on a combination of these approaches but it is also worth noting that a remarkable proportion of the tumors in which we have no real understanding of the precise line of differentiation (for example, alveolar soft part sarcoma, synovial sarcoma and desmoplastic small round cell tumor), can be sharply and strictly defined by their karyotype and related fusion gene(s).

The 2002 WHO Classification took major steps in better defining biologic potential (benign, intermediate locally aggressive, intermediate rarely metastasizing and malignant), even acknowledging that occasional benign tumors may give rise to distant metastasis, and “grasped the nettle” in terms of allocating more tumors to the category of uncertain differentiation and acknowledging that so-called ‘malignant fibrous histiocytoma’ is not a definable entity. However, there do remain nosologic problems, uncertainties and contradictions in the current classification of soft tissue tumors which will need to be addressed in the years to come. The aim of this brief overview is to highlight some of these areas.

OUTDATED DIAGNOSTIC CONCEPTS

Changes in nomenclature, particularly if the terms in question have been used for many years, are painful and often resisted for no clear reason other than ‘discomfort’ or the false belief that retaining outdated terminology will continue to be of value to non-specialist colleagues, especially clinicians. However, such resistance is not intellectually rigorous, particularly if there are extensive data to support more logical alternatives. Several such areas will need to be addressed in any future classification scheme, primary examples among which are as follows:

1. Since it is now generally accepted that the majority of pleomorphic sarcomas can be meaningfully subclassified and that such subclassification has clinical and prognostic relevance, then there really is no justification for retaining the term ‘malignant fibrous histiocytoma’ (MFH) in any future classification scheme since the pleomorphic, giant cell and inflammatory variants are not definable or reproducible entities. The so-called myxoid and angiomatoid subsets undoubtedly represent discrete tumor types, better allocated to the fibroblastic and uncertain differentiation categories, respectively. The 2002 WHO Classification paved the way for this change, in explaining the largely meaningless nature of the ‘MFH’ terminology;
2. For very similar reasons, since it is now recognized that the large majority of lesions formerly classified as so-called hemangiopericytoma in fact have nothing whatever to do with pericytes (most of them representing solitary fibrous tumours), then, since we now have a more sharply defined group of pericytic neoplasms, presently known as myopericytomas (until such time as the loosely used term ‘hemangiopericytoma’ can be more meaningfully applied), then a case could be made for dropping use of the term ‘hemangiopericytoma’, at least for the foreseeable future;
3. While it is clear that there is a group of sarcomas which show fibroblastic differentiation (foremost among which are myxofibrosarcoma and low-grade fibromyxoid sarcoma), the concept of ‘adult fibrosarcoma’ as used in the past, defined by high cellularity and a herringbone growth pattern, for all practical purposes seems not to exist, other than as a pattern of dedifferentiation in dermatofibrosarcoma protuberans (DFSP). The overwhelming majority of tumors which would have been classified as adult-type fibrosarcoma in years gone by would nowadays be classified more meaningfully as either monophasic synovial sarcoma or malignant peripheral nerve sheath tumor. The process of dropping this terminology, in large part, has already happened through a process of

attrition, but a more stringent and meaningful re-definition of fibroblastic sarcomas in adult patients would be very useful going forward.

NOMENCLATURAL ANOMALIES

Whether or not to address the issue of tumors with inappropriate names is more of a nebulous problem, insofar as there are good arguments, for example, that the concept of ‘synovial sarcoma’ is meaningfully defined and understood both in the research and clinical arenas, even if we fully understand that these tumors have nothing whatever to do with synovium. Nevertheless, such classificatory feebleness would likely not be tolerated in other areas of science and arguments, now 25 years old, that these lesions would better be classified as ‘carcinosarcomas’ of soft tissue may ultimately prevail and would certainly be easier to defend. Other examples which might be easier to address, in that they would likely have less direct clinical or practical impact, include extraskeletal myxoid chondrosarcoma, which is now well understood to have nothing whatever to do with cartilage and so-called angiomatoid ‘MFH’, which bears no relationship to the formerly popular category of ‘fibrohistiocytic’ tumors but which is undoubtedly a discrete and now genetically well-defined tumor type. One could also make a case that the term ‘hemangioendothelioma’ needs to be more sharply refined in the future, since tumors within this category, which have often been lumped together as being of ‘intermediate’ or ‘borderline’ biologic potential, in fact span a substantial spectrum of clinical behavior – thus refinement of these terms would likely facilitate greater clinical understanding.

LACK OF BIOLOGIC UNDERSTANDING

Vascular lesions represent an area in which there is substantial uncertainty and ambiguity as to the manner in which these lesions develop, whether or not there is any significance to designating them as hemangiomas or malformations and whether or not there is any importance in determining whether these lesions show blood vascular or lymphovascular differentiation. At the present time, some of these questions are likely unanswerable. While intuitively we tend to think of vascular tumors which are composed of mixed vessel types, including larger specialized vessels such as veins and arteries, as being malformations or ‘hamartomas’, there really is no rational basis for this belief, particularly since these tumors may develop at any age and they often show a propensity for persistent/recurrent growth. Furthermore, the development of larger more specialized vascular structures is only the morphologic counterpart of very well-

differentiated lesions composed of mixed cell types in other organ systems, such as pulmonary ‘hamartoma’ and Peutz-Jegher polyps, both of which are now regarded as clonal neoplastic processes. There is some sense that classification of vascular lesions is increasingly based on clinical and behavioral parameters, rather than morphology, but the former are very poorly reproducible and this is an area which is beginning to give an impression of ‘witchcraft’. Part of the problem in this regard likely relates to the fact that there are few, if any, genetic data regarding most types of benign vascular lesion, combined with the fact that clonality *per se* is no longer regarded as being definitional for a neoplastic process. Similarly, the long-standing importance which has been attached to distinguishing between blood vascular and lymphovascular lesions seems quite illogical not only because daily experience demonstrates that many vascular lesions contain vessels of both types but also, given that lymphatics develop from the venous system during embryogenesis, then it is hardly surprising if there may be substantial overlap in the phenotype of these vessels. At the present time, however, progress in this area is likely to be slow while available data are so limited and controversial.

THE IMPACT OF GENETICS

There is no question that the development of cytogenetic and molecular genetic technologies, as applied to soft tissue tumors, has had enormous impact in advancing both classification and understanding of these diseases – outstanding examples, among others, include the proof that biphasic and monophasic synovial sarcoma represent variants of a single entity, that the embryonal and alveolar subtypes of rhabdomyosarcoma are biologically and prognostically quite distinct, that Ewing’s sarcoma, peripheral primitive neuroectodermal tumor and peripheral neuroepithelioma are all essentially a single entity and, similarly, that the myxoid and round cell variants of liposarcoma are phenotypic variants of a single tumor type. Increasingly, arguments are made that molecular genetic classification should supercede morphologic or phenotypic classification and such an approach is becoming the standard of care in large areas of leukemia diagnosis and therapy. However, as more data are gathered, areas of confusion are also beginning to appear, which may complicate the clinical utility of this technology, at least in the short term. Examples both in favor and against the greater use of genetic data in determining classification that may help to provoke discussion in this area are as follows:

1. It is recognized that there are variably subtle but undoubted morphologic similarities between spindle cell lipoma, mammary-type myofibroblastoma and cellular angiofibroma

– yet these lesions each retain clinical or phenotypic differences. However, data are now emerging that all of these lesions share the same underlying genetic signature, usually loss of the 13q14 chromosomal region. Arguments could therefore be made for co-classifying these lesions, yet it seems to me more logical, given their clinicopathologic differences, to regard them as closely related but not identical. One can well imagine that a topic such as this may become controversial, especially since the same argument could be applied for DFSP and giant cell fibroblastoma.

2. Because fluorescence *in situ* hybridization (FISH) is easier to employ in paraffin-embedded tissue and is much less prone to false-positive results than RT-PCR, then FISH is becoming increasingly popular for the molecular diagnosis of soft tissue tumors. In this regard, one of the most widely applied tests is based on the use of split-apart probes around the *EWSR1* gene. It has been recognized for some time that *EWSR1* gene rearrangements are present in a variety of quite different tumor types – not only Ewing's/PNET but also extraskeletal myxoid chondrosarcoma, clear cell sarcoma and desmoplastic small round cell tumor. However, more recent data have also shown that this gene is rearranged in so-called angiomatoid 'MFH' and personal experience indicates that it is also rearranged in a subset of myoepithelial tumors of soft tissue. While these data suggest that this gene is of enormous importance in mesenchymal tumorigenesis, it seems likely that more tumors showing this rearrangement will continue to be uncovered and this is likely to have practical daily impact both in terms of potential diagnostic confusion and also in selection of suitable technology for testing. Purely practical considerations suggest that it will be much more difficult for RT-PCR to become the test of choice on a broad scale in hospitals of all sizes.
3. Examples of fusion genes shared by quite different tumor types are increasingly recognized, making clear that morphologic correlation will almost always be an absolute necessity, rather than pathologists being able to rely purely on a molecular genetic diagnosis. An extreme example in this regard is the *ETV6-NTRK3* fusion gene which, when expressed in different cell lineages, appears to be the primary transforming event in infantile fibrosarcoma, congenital mesoblastic nephroma, secretory carcinoma of breast and in rare examples of acute myelogenous leukemia. Perhaps a better-known example is the substantial overlap between the *ALK*-related fusion genes in both inflammatory myofibroblastic tumor and anaplastic large cell lymphoma. However, potentially more

confusing examples of such overlap are now being identified even within quite different tumors of mesenchymal type – the best example thus far is the recent demonstration of an identical *EWSRI-CREB1* fusion gene in clear cell sarcoma arising at visceral locations as well as in so-called angiomatoid ‘MFH’. Fascinatingly, a minority of cases of so-called angiomatoid ‘MFH’ appear to show an *EWSRI-ATF1* gene fusion, identical to that seen in clear cell sarcomas arising at somatic locations. This type of overlap, while being of considerable biologic and pathogenetic interest, is quite likely to give rise to confusion among non-specialists dealing with soft tissue sarcomas.

CONCLUSION

Evolution and advances in classification as well as our understanding of soft tissue tumors has been substantial in recent years and there is every sign that this will continue. Such advances will hopefully lead to an ever more logical and reproducible approach to both diagnostic and nosologic classification of these lesions, but there is still very much work to be done in trying to understand the basic biology of certain quite common tumor types and in the meaningful incorporation of molecular genetic data into any new classification scheme. For sure, this continues to be an exciting time to be interested in this field of pathology.