Letters to the Editor

CORRESPONDENCE RE: O'SULLIVAN MJ, KYRIAKOS M, ZHU X, WICK MR, SWANSON PE, DEHNER LP, HUMPHREY PA, PFEIFER JD: MALIGNANT PERIPHERAL NERVE SHEATH TUMORS WITH T(X;18). A PATHOLOGIC AND MOLECULAR GENETIC STUDY. MOD PATHOL 2000;13:1336–46.

To the Editor: We read with great interest the recent paper by O'Sullivan *et al.* on the detection by RT-PCR of the *SYT-SSX* fusion transcript resulting from the t(X;18)(p11;q11) of synovial sarcoma in malignant peripheral nerve sheath tumors (MPNSTs) and a variety of other soft tissue tumors (1). There has in recent years been an impressive accumulation of data from multiple groups supporting certain translocation-derived gene fusions as primary, necessary, and tumor-type-specific genetic events in certain sarcomas (reviewed in Refs. 2–4, among others). With the more widespread application of PCR, there also have been papers questioning the specificity of these associations. Often, such reports describe the RT-PCR detection of translocation-derived fusion transcripts in tumors of unexpected histologic types, and generally the claims are neither corroborated by independent methods nor reproducible by others. It is important to critically and systematically review such reports, because they often attract undue attention and serve to confuse casual readers. We believe the paper by O'Sullivan *et al.* is such a paper.

These authors report RT-PCR detection of *SYT-SSX* fusion transcripts in RNA extracted from paraffin blocks in 15 of 20 MPNSTs. Despite this unusual result, in no case did they attempt to confirm the presence of a t(X;18) using techniques other than RT-PCR, for instance, Southern blotting, conventional cytogenetics, or FISH. The latter technique could have been readily applied to their paraffin-embedded material, but it was not. They did verify their RT-PCR products by hybridization and sequencing, but this does not represent an independent confirmatory method. O'Sullivan *et al.* also report that 1 of 4 adult fibrosarcomas, 1 of 10 malignant fibrous histiocytomas, 1 of 7 congenital fibrosarcomas, and 2 of 3 neurofibromas were positive for *SYT-SSX* by RT-PCR. The lack of confirmatory data independent of RT-PCR, in conjunction with this peculiar proportion and variety of positive cases, raises the spectre of contamination-related technical issues, even without knowledge of the considerable existing literature contradicting their results. We will first outline the existing cytogenetic and molecular genetic literature, and then we will provide further unpublished data from our laboratories.

In their Discussion, O'Sullivan *et al.* sidestep a clear presentation of the extensive cytogenetic literature on MPNSTs while raising the suggestion that cytogenetically inapparent or "masked" t(X;18)s in MPNSTs may account for the marked discrepancy with their data. A database of the human cancer cytogenetics literature is now publicly available at the Cancer Genome Anatomy Website (cgap.nci.nih.gov/Chromosomes/Mitelman). A simple search shows abnormal karyotypes from 53 MPNSTs taken from 19 papers. None contain a t(X;18), and indeed there are no cases with any abnormality of Xp11 or 18q11, arguing against the proposal by O'Sullivan *et al.* that masked t(X;18) translocations abound in MPNSTs. Furthermore, masked or cryptic translocations are rare occurrences in those karyotypic rearrangements involving the exchange of cytogenetically distinct chromosome fragments such as the t(X;18)(p11;q11). Thus, we view the possibility of masked t(X;18) in MPNSTs as remote. Although masked translocations never can be excluded as rare events, they certainly cannot be legitimately used to explain the glaring discrepancy between the complete absence of a cytogenetic t(X:18) in 53 MPNSTs available in the literature and the RT-PCR evidence from O'Sullivan *et al.* of the t(X;18) in 15 of 20 their MPNST samples. In addition to the 53 negative MPNSTs in the cancer cytogenetics database, there are more than 29 additional reported MPNSTs not included in this database in which neither cytogenetics (5, 6) nor FISH (7, 8) could detect a t(X;18). We also note that the authors cite some early case reports of t(X;18) (Refs. 38,41,64 in Ref. 1) in which the correct pathologic diagnosis was uncertain. These cannot be used to support the "nonspecificity" of the t(X;18), in as much as the authors of some of these reports later recognized that these tumors were, in fact, synovial sarcomas (5,9).

O'Sullivan *et al.* also fail to provide a clear presentation of the published molecular genetic studies where RT-PCR for *SYT-SSX* was performed on MPNSTs. The authors argue that the availability of translocation data leads to a "reclassification bias," *i.e.*, that the diagnosis is revised simply on the basis of the molecular result, regardless of the histopathology. The differential diagnosis of spindle cell sarcomas, and especially of MPNSTs, can be quite

difficult, and cases are routinely "reclassified" based on ancillary data other than molecular tests. It is perhaps futile to argue the merits of individual "reclassified" cases in the literature. To our knowledge, none of these "reclassified" cases have been clear MPNSTs in NF1 patients. Indeed, the entity that may be the most difficult to reliably separate from MPNST is monophasic synovial sarcoma, not only in a routine setting, but also particularly those rare cases that are intraneural. We should note that this entity was described without t(X;18) translocation analysis (see Refs. 15–19 in Ref. 1). Even if one were to entertain the hypothesis of a "reclassification bias," the data in existing molecular genetic studies are statistically incompatible with those of O'Sullivan *et al.* Namely, of at least 31 MPNSTs tested by RT-PCR by 4 groups (10–13), only 3 presumptive "MPNSTs" were positive for *SYT-SSX, versus* 15 of 20 in the data of O'Sullivan *et al.* ($P < .00001$).

We also would like to briefly outline unpublished data from several laboratories, which also contradict the results of O'Sullivan *et al.* We should note that these negative data were obtained using a variety of techniques, including conventional cytogenetics, FISH, and RT-PCR.

1. At Memorial Sloan-Kettering Cancer Center, we tested eight cases of high-grade MPNST by RT-PCR on RNA extracted from frozen tumor tissue (obtained from six MSKCC and two Mayo Clinic patients). Four patients had NF1 and nerve involvement by tumor. Among the other four patients, the tumor directly involved nerve or nerve and ganglion in two and was associated with a neurofibroma in another. RT-PCR for *SYT-SSX1* and *SYT-SSX2* was negative in all eight cases, whereas a control reaction using synovial sarcoma RNA was appropriately positive. RT-PCR for a housekeeping gene transcript was positive in all eight MPNST RNAs, confirming their integrity.
2. At the University of Nebraska Medical Center, 14 MPNSTs (8 from patients with NF1) and 2 Triton tumors have shown clonally abnormal karyotypes by conventional cytogenetics, but none had evidence of a t(X;18). RT-PCR was performed on eight of these karyotypically aberrant specimens in which frozen tissue was available, and no *SYT-SSX1* or *SYT-SSX2* product was identified. RT-PCR also was performed on two MPNSTs and one Triton tumor in which only frozen tissue was available, and these cases were similarly negative for *SYT-SSX1* or *SYT-SSX2*. Cytogenetic studies performed on 46 synovial sarcomas have revealed the X;18 translocation in 90% of the cases.
3. At the Hospital of the University of Pennsylvania, we tested 21 samples signed out as MPNST by RT-PCR on RNA extracted from frozen tumor tissue (obtained from 19 Cleveland Clinic patients). In all 19 patients, the tumor was either reported to arise in a neurofibroma or in the setting of NF1, typically arising in a plexiform neurofibroma. All were found to be negative for *SYT-SSX*, except for one case. The latter case, on review, contained no pathologic evidence of a surrounding neurofibroma, although it clearly arose within a nerve (in a patient without NF1). The case was sent *blindly* to another sarcoma pathologist (C.F.), who made the diagnosis of intraneural synovial sarcoma, with which the referring pathologist (J.R.G.) concurred. This case is instructive because it shows how "reclassification" is not simply the result of "bias" based on knowledge of the translocation data, but typically involves cases with truly problematic histopathology.
4. At Brigham and Women's Hospital (Boston), Children's Hospital (Boston), and University Hospital in Leuven (by P.d.C.), we have karyotyped 96 MPNST and 123 synovial sarcomas. An average of 20 metaphase cells were examined per case, and t(X;18) was evaluated in every cell. None of the MPNST contained cells with identifiable t(X;18), whereas 112 of the synovial sarcomas (91%) had t(X;18). The t(X;18) always was found in at least 50% of the cells from each positive synovial sarcoma and generally was identified in 80 to 100% of the cells. We also have performed dual-color fluorescence *in situ* hybridization (FISH) for t(X;18) in 3 MPNST and in 11 synovial sarcomas. These studies were accomplished using a YAC telomeric to the *SYT* locus and a chromosome X pericentromeric alpha-satellite, with hybridization against intact nuclei disaggregated from 50 micron paraffin sections. None of the MPNSTs had t(X;18), whereas 9 of the synovial sarcomas (82%) had t(X;18), which always was found in at least 80% of the cells.

These negative results on a total of 145 cases of MPNST raise further concerns regarding the data of O'Sullivan *et al.* It is indeed unfortunate that the authors did not seek to test the validity of their own results more critically, before publication. Instead, they provided the readers with a lengthy and well-crafted Discussion, in which the results of isolated reports akin to their own are accepted uncritically but the bulk of the data in the literature, which are inconsistent with their results, are downplayed. Furthermore, we disagree strongly with the authors' somewhat glib final comment that the nonspeci-

ficity of translocation-based molecular diagnostic markers implied by their results is not unexpected given the experience with immunohistochemical markers. That assertion does not reflect the fundamental biological difference between the expression of a normal antigen by a tumor and the detection of a specific abnormal gene fusion implicated in the development of that tumor.

Tumors do not exist primarily as morphologic entities; they are also biological and genetic entities. In the case of sarcomas with chromosomal translocations, these specific gene fusions represent a pivotal element of their genetic profile that can be readily detected by simple molecular methods. We believe most pathologists view the excellent one-to-one correlation of specific morphologic entities with new translocation-based molecular diagnostic markers not as a challenge to the primacy of histopathology but rather as gratifying confirmation of its validity in originally defining these many different entities. In our experience, the specificity of the association of the t(X;18) with tumors morphologically classified as synovial sarcoma is a graphic demonstration of this correlation.

Marc Ladanyi, M.D.
James M. Woodruff, M.D.
Memorial Sloan-Kettering Cancer Center, New York, New York

Bernd W. Scheithauer, M.D.
Mayo Clinic, Rochester, Minnesota

Julia A. Bridge, M.D.
University of Nebraska Medical Center, Omaha, Nebraska

Frederic G. Barr, M.D., Ph.D.
Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania

John R. Goldblum, M.D.
Cleveland Clinic Foundation, Cleveland, Ohio

Cyril Fisher, M.D.
The Royal Marsden NHS Trust, London, UK

Antonio Perez-Atayde, M.D.
Children's Hospital, Boston, Massachusetts

Paola Dal Cin, Ph.D.
Christopher D. M. Fletcher, M.D.
Jonathan A. Fletcher, M.D.
Brigham and Women's Hospital, Boston, MA

REFERENCES

1. O'Sullivan MJ, Kyriakos M, Zhu X, Wick MR, Swanson PE, Dehner LP, *et al.* Malignant peripheral nerve sheath tumors with t(X;18). A pathologic and molecular genetic study. Mod Pathol 2000;13:1336–46.
2. Fletcher CD. Soft tissue tumours: the impact of cytogenetics and molecular genetics. Verh Dtsch Ges Pathol 1997;81:318–26.
3. Bennicelli JL, Barr FG. Genetics and the biologic basis of sarcomas. Curr Opin Oncol 1999;11:267–74.
4. Ladanyi M, Bridge JA. Contribution of molecular genetic data to the classification of sarcomas. Hum Pathol 2000;31: 532–8.
5. Fletcher CD, Dal Cin P, de Wever I, Mandahl N, Mertens F, Mitelman F, *et al.* Correlation between clinicopathological features and karyotype in spindle cell sarcomas. A report of 130 cases from the CHAMP study group. Am J Pathol 1999; 154:1841–7.
6. Mertens F, Dal Cin P, de Wever I, Fletcher CD, Mandahl N, Mitelman F, *et al.* Cytogenetic characterization of peripheral nerve sheath tumours: a report of the CHAMP study group. J Pathol 2000;190:31–8.
7. Nagao K, Ito H, Yoshida H. Chromosomal translocation t(X; 18) in human synovial sarcomas analyzed by fluorescence in situ hybridization using paraffin-embedded tissue. Am J Pathol 1996;148:601–9.
8. Yang P, Hirose T, Hasegawa T, Hizawa K, Sano T. Dual-colour fluorescence in situ hybridization analysis of synovial sarcoma. J Pathol 1998;184:7–13.
9. Turc-Carel C, Dal Cin P, Limon J, Li F, Sandberg AA. Translocation X;18 in synovial sarcoma. Cancer Genet Cytogenet 1986;23:93.
10. Hiraga H, Nojima T, Abe S, Sawa H, Yamashiro K, Yamawaki S, *et al.* Diagnosis of synovial sarcoma with reverse transcriptase-polymerase chain reaction: analyses of 84 soft tissue and bone tumors. Diag Mol Pathol 1998;7:102–10.
11. Willeke F, Mechtersheimer G, Schwarzbach M, Weitz J, Zimmer D, Lehnert T, *et al.* Detection of SYT-SSX1/2 fusion transcripts by reverse transcriptase-polymerase chain reaction (RT-PCR) is a valuable diagnostic tool in synovial sarcoma. Eur J Cancer 1998;34:2087–93.
12. van de Rijn M, Barr FG, Collins MH, Xiong Q-B, Fisher C. Absence of SYT-SSX fusion products in soft tissue tumors other than synovial sarcoma. Am J Clin Pathol 1999;112: 43–9.
13. Naito N, Kawai A, Ouchida M, Dan'ura T, Morimoto Y, Ozaki T, *et al.* A reverse transcriptase-polymerase chain reaction assay in the diagnosis of soft tissue sarcomas. Cancer 2000; 89:1992–8.

Letters to the Editor

In reply: We thank Ladanyi and associates for highlighting several issues that continue to provoke lively discussion among interested pathologists, and we are first to agree that our paper should be thoughtfully scrutinized as it was initially by us, and subsequently by peer reviewers. Not unexpectedly, we do not share the concern of Ladanyi *et al.* that our paper will have an untoward effect on the "casual reader," who, after all, may be as discerning as they are. In as much as the readership of *Modern Pathology* consists of educated and interested pathologists, geneticists, and molecular biologists fully capable of independently evaluating our experimental design, methods, and data, we are not worried that the presentation of unexpected findings will have an adverse effect on our specialty, nor even tincture the scientific process.

We emphasize that we are as enthusiastic as Ladanyi and colleagues about the potential of molecular diagnostic testing. For the past several years, we have incorporated molecular testing into our practice and have found it useful in the context of our other studies. However, as with any laboratory test, the additional experience that comes from everyday application identifies limitations that were not initially recognized, and it is in this context that we reported the results of our study, designed specifically to focus on PCR-based testing of malignant peripheral nerve sheath tumors (MPNST) harboring t(X;18) (1). We chose to focus on MPNSTs because this entity is commonly included in our differential diagnosis of synovial sarcoma (SS). Our results indicate that examples of MPNST can harbor t(X;18), an observation that is certainly not unique, as a recent report documents t(X;18) in a case diagnosed as MPNST, a result that was confirmed by PCR, cytogenetics, and FISH (2). And we note that in their correspondence, Ladanyi and his colleagues mention but do not challenge three presumptive MPNSTs that were positive for the SYT-SSX fusion transcript in studies compiled from other investigators (3–6). We agree that the percentage of spindle cell neoplasms other than SS that harbor t(X;18) is remarkably low; however, the published data do not support the view that there is an absolute correlation between SS and the presence of t(X;18).

In a broader context, it is puzzling that Ladanyi *et al.* have focused their criticisms on the presumed absolute specificity of the SYT-SSX gene fusion given the many other well-established examples of less controversial gene fusions that are not tumor specific. As illustrations, TPM3-ALK and Clatharin-ALK gene fusions have been identified in both inflammatory myofibroblastic tumors (IMT) and anaplastic large cell lymphomas (ALCL) (7–11); are we to assume that Ladanyi and associates, on this discovery, have reclassified their examples of IMT as ALCL variants? Given that ETV6-NTRK3 fusions have been documented by several genetic methods in an acute myeloid leukemia (AML) as well as congenital fibrosarcoma/cellular mesoblastic nephroma (CFS/CMN) (12), would Ladanyi *et al.* diagnose a case of AML harboring t(12;15) as just a hematogenous round cell variant of CFS/CMN? The existence of common oncogenic mechanisms in tumors of different lineages is well established and has even been the subject of a recent editorial by Ladanyi himself (13).

Ladanyi *et al.* propose that cases in which an unexpected result is obtained by PCR-based testing be subjected to additional analysis before the result is considered valid. There is some merit to this proposal, but it raises several issues. First, this approach ignores the realities of clinical practice, where frozen tissue is often not available, cytogenetics is often not performed, and the quantity of even formalin-fixed tissue may be limited. Second, we are concerned that by this approach the same level of scrutiny is not applied to all cases. Specifically, by their proposal, the result of PCR-based testing is accepted when it yields the expected result (*e.g.*, an SS in which the presence of t(X;18) is demonstrated); on the other hand, when the result of PCR-based testing yields a result that is unexpected (*e.g.*, an MPNST shown to harbor t(X;18)), additional *ad hoc* genetic tests must be performed before the result is considered valid. Additional *ad hoc* testing on only a subset of cases introduces an obvious experimental bias and can only serve to skew the results. Objective evaluation of the sensitivity and specificity of any laboratory test (in this case, PCR-based testing) requires the same protocol for every case and does not provide the opportunity to test and retest only selected cases by various methodologies until a preconceived result is obtained. Third, we note that the method of testing Ladanyi *et al.* propose is not consistently applied in even their own published studies. As one example, in their recent report on the unexpected finding of *EWS-FLI1* fusion transcripts in adamantinomas, only one case was confirmed by additional genetic tests, whereas all the other cases were tested by RT-PCR alone (14). At least in this instance it seems that Ladanyi and colleagues are proposing a testing regimen for unexpected results that other groups

must follow but from which they themselves are exempt.

It should be noted that Ladanyi *et al.* make no challenge to either our histologic diagnoses or the specific protocols employed in the study, but rather attribute our results to vague "contamination-related technical issues." We agree that contamination is an ever-present concern when performing PCR-based assays, but Ladanyi propose a highly selective form of contamination, found repeatedly on retesting (even in subsequent specimens from some patients) but absent in 40 other soft tissue tumors that were retested several times. This is an improbable way for contamination to manifest itself, and, in fact, formal statistical testing of our data using the χ^2 test rejects the null hypothesis that the results are due to random contamination with $P < .0001$.

In our view, at the present time, the result of testing for a single genetic abnormality should be integrated with all the other relevant clinical and pathologic information of a particular case, including the clinical setting, light microscopic features, immunophenotype, and even electron microscopic findings (15). Consequently, we would render the diagnosis of MPNST for a malignant spindle cell tumor arising in a nerve in a patient with NF-1 that had the histologic, immunohistochemical, and electron microscopic features of an MPNST, even if the tumor were shown to harbor t(X;18). Faced with the same case, what diagnosis do Ladanyi and his colleagues propose?

Maureen J. O'Sullivan, MB, M.D., MRCPath
Department of Pathology
Edinburgh University Medical School
Edinburgh, Scotland

Mark R. Wick, M.D.
Division of Surgical Pathology
University of Virginia Medical Center
Charlottesville, Virginia

Michael Kyriakos, M.D.
Xiaopei Zhu, M.D.
Paul E. Swanson, M.D.
Louis P. Dehner, M.D.
Peter A. Humphrey, M.D., Ph.D.
John D. Pfeifer, M.D., Ph.D.
Lauren V. Ackerman Laboratory of Surgical Pathology
Washington University Medical Center
St. Louis, Missouri

REFERENCES

1. O'Sullivan MJ, Kyriakos M, Zhu X, Wick MR, Swanson PE, Dehner LP, *et al.* Malignant peripheral nerve sheath tumors with t(X;18). A pathologic and molecular genetic study. Mod Pathol 2000;13:1336–46.
2. Vang R, Biddle DA, Harrison WR, Heck K, Cooley LD. Malignant peripheral nerve sheath tumor with t(X;18). A synovial sarcoma variant? Arch Pathol Lab Med 2000;124:864–7.
3. Hiraga H, Nojima T, Abe S, Sawa H, Yamashiro K, Yamawaki S, *et al.* Diagnosis of synovial sarcoma with the reverse transcriptase-polymerase chain reaction: analyses of 84 soft tissue and bone tumors. Diag Mol Pathol 1998;7:102–10.
4. Willeke F, Mechtersheimer G, Schwarzbach M, Weitz J, Zimmer D, Lehnert T, *et al.* Detection of *SYT-SSX1/2* fusion transcripts by reverse transcriptase-polymerase chain reaction (RT-PCR) is a valuable diagnostic tool in synovial sarcoma. Eur J Cancer 1998;34:2087–93.
5. van de Rijn M, Barr FG, Collins MH, Xiong Q-B, Fisher C. Absence of SYT-SSX fusion products in soft tissue tumors other than synovial sarcoma. Am J Clin Pathol 1999;112: 43–9.
6. Naito N, Kawai A, Ouchida M, Dan'ura T, Morimoto Y, Ozaki T, *et al.* A reverse transcriptase-polymerase chain reaction assay in the diagnosis of soft tissue sarcomas. Cancer 2000; 89:1992–8.
7. Lawrence B, Perez-Atayde A, Hibbard MK, Rubin BP, Dal Cin P, Pinkus JL, *et al.* TPM3-ALK and TPM4-ALK oncogenes in inflammatory myofibroblastic tumors. Am J Pathol 2000;57: 377–84.
8. Lamant L, Dastugue N, Pulford K, Del Sol G, Mariame B. A new fusion gene TPM3-ALK in anaplastic large cell lymphoma created by a (1;2)(q25;p23) translocation. Blood 1999; 93:3088–95.
9. Siebert R, Gesk S, Harder L, Steinemann D, Grote W, Schlegelberger B, *et al.* Complex variant translocation t(1;2) with TPM3-ALK fusion due to cryptic ALK gene rearrangement in anaplastic large-cell lymphoma. Blood 1999;94:3614–7.
10. Ma Z, Hill A, Collins M, Witte DP, Bridge J, Ladanyi M, *et al.* ALK fusion genes in the pathogenesis of a nonhematopoietic malignancy—inflammatory myofibroblastic tumor (IMT) [abstract 377]. Blood 2000;96(Suppl. 1):88a.
11. Touriol C, Greenland C, Lamant L, Pulford K, Bernard F, Rousset T, *et al.* Further demonstration of the diversity of chromosomal changes involving 2p23 in ALK-positive lymphoma: 2 cases expressing ALK kinase fused to CLTCL (clathrin chain polypeptide-like). Blood 2000;95:3204–7.
12. Eguchi M, Eguchi-Ishimae M, Tojo A, Morishita K, Suzuki K, Sato Y, *et al.* Fusion of ETV6 to neurotrophin-3 receptor TRKC in acute myeloid leukemia with t(12;15)(p13;q25). Blood 1999;93:1355–63.
13. Ladanyi M. Aberrant ALK tyrosine kinase signaling. Different cellular lineages, common oncogenic mechanisms. Am J Pathol 2000;157:341–5.
14. Bridge JA, Fidler ME, Neff JR, Degenhardt J, Wang M, Walker C, *et al.* Adamantinoma-like Ewing's sarcoma; genotypic confirmation, phenotypic drift. Am J Surg Pathol 1999;23: 159–65.
15. Pfeifer JD, Hill DA, O'Sullivan MJ, Dehner LP. Diagnostic gold standard for soft tissue tumors: morphology or molecular genetics? 2000 Histopathology;37:485–500.