Letters to the Editor

CORRESPONDENCE RE: JIMENEZ RE, WALLIS T, TABASCZKA P, VISSCHER DW. DETERMINATION OF HER-2/NEU STATUS IN BREAST CARCINOMA: COMPARATIVE ANALYSIS OF IMMUNOHISTOCHEMISTRY AND FLUORESCENT IN SITU HYBRIDIZATION. MOD PATHOL 2000;13:37-45.

To the Editor: Jimenez *et al.* reported the levels of concordance between FISH and three antibodies for Her-2/*Neu* status in 34 cases of invasive breast carcinoma. The antibodies employed (TAB 250 from Zymed Laboratories, the CB11 from Ventana Laboratories, and the DAKO polyclonal) are all well-described in the literature. The utility of the article stems from a head-to-head comparison between the antibodies using an automated (Ventana) stainer with well-defined protocols.

Their results showed that of the three antibodies, CB11 had the worse sensitivity, detecting only 8 of the 10 cases scored as 3+ by the other two antibodies and determined to be amplified using FISH.

We have been using CB11 (Ventana Laboratories) on the Ventana automated immunostainer for 2 years and have found that antigen retrieval with heat (microwave for 14.0 minutes in a pressure cooker with 0.1 mm EDTA PH 8.0) is essential for optimum performance with this antibody. This also is recognized by the manufacturer, as they include a recommendation for heat retrieval with their antibody.

We have since characterized the performance of the three antibodies with or without antigen retrieval using a panel of nine breast and ovarian cell lines. All of the staining was performed on the Ventana automated stainer. The most sensitive technique was the DAKO polyclonal used as specified in the article by Jimenez *et al.* With retrieval, the CB11 and TAB 250 showed equivalent sensitivities. Without antigen retrieval, both CB11 and the DAKO antibody showed poor reactivity with decreased sensitivity (CB11) or decreased specificity (DAKO).

The need for standardized protocols for Her-2/*Neu* testing is paramount. In formalin fixed paraffin-embedded tissue, we are now recognizing that epitope retrieval is essential for optimizing many of the antibodies (1) and that comparisons between antibodies without such retrieval are meaningless.

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REFERENCE

1. Jacobs TW, Gown AM, Yaziji H, Barnes MJ, Schnitt SJ. HER-2/neu protein expression in breast cancer evaluated by immunohistochemistry: a study of interlaboratory agreement. Am J Clin Pathol 2000;113:251–8.

In reply: As noted in the Materials and Methods section, we employed staining methods as recommended by each vendor, including Ventana. This included antigen retrieval on the stains that employed the CB11 antibody. In addition, the two amplified cases that did not demonstrate 3+ staining were repeated, showing similar results. The reason(s) for lack of correlation with *in situ* hybridization in these two cases is unclear.

We disagree somewhat with the statement by Dr. Hugh *et al.* that the point of our article was a comparison of antibody reagents. Rather, the study was designed as a comparison between immunohistochemistry and *in situ* hybridization for analyzing the status of Her2/Neu.

We also disagree with their implication that antigen retrieval is essential for all immunohistochemical studies. Our experience has shown that antigen retrieval should be investigated as a component of the overall evaluation of immunohistochemical reagents. It is not necessarily essential, or even desirable, for all staining protocols.

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CORRESPONDENCE RE: VISWANATHA DS, FOUCAR K, BERRY BR, GASCOYNE RD, EVANS HL, LEITH CP. BLASTIC MANTLE CELL LEUKEMIA: AN UNUSUAL PRESENTATION OF BLASTIC MANTLE CELL LYMPHOMA. MOD PATHOL 2000;13:825–33.

To the Editor: I read with great interest the article titled "Blastic Mantle Cell Leukemia: An Usual Presentation of Blastic Mantle Cell Lymphoma." The report presents actually four cases of "*de novo*" blastic mantle cell leukemia, because one patient had a previous history of classical nodal mantle cell lymphoma and the other case had no molecular evidence of a mantle cell origin. This latter case may be better termed a CD5+, CD23-blastic B-cell leukemia. Unfortunately, this latter patient was lost

to follow-up and the clinical course could not be compared with the other four cases. Of the four cases, two had splenomegaly and two did not. Of the two patients with splenomegaly, one had an aggressive clinical course (dead of disease [DOD] at 2 weeks) and one was alive with disease at 6 months. The two patients without splenomegaly were DOD at 2 months and 4 months, respectively. In 1999, we reported a case of *de novo* blastic mantle cell leukemia that had not previously been described (1). The patient presented with marked splenomegaly. The diagnosis was established by morphology combined with the classical flow cytometric immunophenotype and intense cyclin D1 staining. At last follow-up, the patient was in complete remission after six cycles of CVP (Cytoxan, Vincristine, Prednisone) over a 5-month period. Thus, our case had a different clinical course than the four present cases described. However, two of the four cases received palliative treatment only and had the most aggressive clinical courses (DOD, 2 weeks and 2 months, respectively). The other two cases received combination chemotherapy. The one patient treated somewhat similarly to our patient was lost to follow-up. In agreement with Viswanatha, *et al.*, additional studies are required to explore the pathophysiologic basis for the marked, predominant leukemic dissemination in such cases and what factors may predict response to therapy.

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REFERENCE

1. Dunphy CH, Hancock JC, Rodriguez JJ, Hilton JG. Blastic mantle cell leukemia: a previously undescribed form. J Clin Lab Analysis 1999;13:112–5.

In reply: We thank Dr. Dunphy for her interest and comments concerning our publication on blastic mantle cell leukemia (1). We also are grateful for her re-emphasis of a case of blastic mantle cell leukemia previously described by her group (2). There are a number of similarities between the case described by Dunphy *et al.* and several of the patients in our series. Dr. Dunphy's analysis of our reported patient series is correct in that at least four patients can be termed "*de novo*" blastic mantle cell leukemia (Case numbers PN1, PN3, PN5, and PN6). One patient described by us (PN2) had an antecedent history of classical nodal mantle cell lymphoma. However, we felt that inclusion of this case, though clearly not a primary tumor, was appropriate in our series, based on the clinical presentation and pathologic features at the time of leukemic transformation. Furthermore, the presence of underlying mantle cell lymphoma in case PN2 lends support to the concept that aggressive, primarily leukemic variants of mantle cell lymphoma are indeed rarely occurring phenomena. Dr. Dunphy notes that one case in our series (PN4), without additional material for molecular study, could be better termed a CD5 positive, CD23 negative blastic B-cell leukemia. This is of course the most prudent pathologic diagnosis without additional diagnostic data; however, this case was again felt to be relevant to our series based on striking similarities in clinical presentation and pathologic findings. The patient reported by Dunphy *et al.* clearly demonstrated a sustained clinical remission, at least for a brief period of time. A satisfactory comparison to the cases presented in our series is not strictly possible with regards to clinical outcome, as the treatment options employed in our patient group were quite variable. We did not intend to imply in our study that blastic mantle cell leukemia is poorly responsive to treatment; rather, given minimal or palliative management, this tumor displays a relatively aggressive clinical course with short interval from diagnosis to death. However, in agreement with the patient reported by Dunphy *et al.*, the longest surviving patient in our series (PN5) also received the most intensive chemotherapy, although examination of the bone marrow after therapeutic induction revealed persistent microscopic blastic mantle cell disease. At least anecdotally then, therapy with an aggressive grade lymphoma protocol may provide longer remission intervals, as also suggested by the study of Singleton *et al.* (3) Nonetheless, given the biological aggressiveness of mantle cell lymphoma generally and the relatively advanced age at presentation for most patients, the ultimate clinical course is likely to be strongly influenced by disease burden and patient performance status. We certainly agree with Dr. Dunphy that further investigation in such cases is necessary to understand the basis for such marked leukemic dissemination in a subset of mantle cell lymphoproliferative disease. Furthermore, from a diagnostic prospective, this entity should be included in the morphologic differential diagnosis in adults presenting with atypical circulating blastic disease.

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REFERENCES

1. Viswanatha DS, Foucar K, Berry BR, Gascoyne RD, Evans HL, Leith CP. Blastic mantle cell leukemia: an unusual presentation of blastic mantle cell lymphoma. Mod Pathol 2000;13:825–33.
2. Dunphy CH, Hancock JC, Rodriguez JJ, Hilton JG. Blastic mantle cell leukemia: a previously undescribed form. J Clin Lab Anal 1999;13:112–5.
3. Singleton TP, Anderson MM, Ross CW, Schnitzer B. Leukemic pahse of mantle cell lymphoma, blastoid variant. AM J Clin Pathol 1999;111:495–500.