To the Editor:

I found the recent report by Raza et al.1 quite interesting; however, I believe some comments on the technique described and its implications could benefit those interested in plastic embedding and immunohistology of bone marrow biopsy sections.

1. Looking at the Fig 1A and B and Fig 6A through C it appears that the staining reaction for transforming growth factor-β (TGF-β) exhibits a pattern very similar to that observed when the sections are stained for reticulin (collagen). Were consecutive sections also stained for reticulin? What were the results? My interest is further enhanced because the description of TGF-β expression in the interstitial areas (Fig 1A) and around the blood vessels (Fig 1B) in the acute promyelocytic leukemia (APL) cases noted is almost exactly the same as I observed in some of my acute myeloid leukemia (AML) cases with marrow fibrosis.2 Obviously, the relationship of reticulin fiber distribution and TGF-β localization should be explored. It would be interesting to see the interstitial TGF-β staining reaction in some of these APL cases without concomitant marrow fibrosis.

2. Their report states that “pronase digestion is routinely used to remove plastic before the application of any reagents on coverslips containing biopsy sections.” Raza et al. failed to provide any reference to this statement. In my view, this opinion is incorrect or
mislabeled. To date, we have not found a reagent/technique that will remove the plastic (glycol methacrylate [GMA], which Raza et al have used for their study) from the tissue in sections. In my experience, the plastic matrix does not interfere with enzyme staining4, or immunostaining5,6 or staining by basic and acidic water-soluble dyes.7 Although the basic dyes will stain the matrix to some extent, it is recognized that the treatment of GMA sections with trypsin8 or pronase9 may be necessary to perform the immunohistochemical reactions. However, their role in "unmasking" the antigens in fixed tissue has not been defined. Nevertheless, after such treatment, the specimen (tissue and its embedding medium) remains undissolved and affixed to the slide.

Regarding the processing of bone marrow biopsies, Raza et al cited a GMA embedding method9 that is old, tedious, complicated, and no longer used. Like most current investigators, I believe that Raza et al have probably used a commercial kit (eg, Polysciences) in their methodology. Anyone reading this report should be given the actual techniques used to undertake the study.

ANWARUL ISLAM
Division of Hematologic Oncology
Department of Medicine
Roswell Park Cancer Institute
Buffalo, NY

REFERENCES

RESPONSE

We appreciate the comments made by Dr Islam on our recently published observations1 in patients with acute promyelocytic leukemia (APL). Dr Islam's question regarding the possible relationship between reticulin fiber distribution and the localization of transforming growth factor-β (TGF-β) in plastic embedded bone marrow biopsies were answered in studies performed but not described in our report. Biopsy sections from 14 of 19 patients were examined for the presence of fibrosis, which included an evaluation of the Golomori-stained sections. Of the 14 cases examined, 6 showed an increase in reticulin (diffuse marked fibrosis in 4 and partial in 2 cases), whereas 8 patients showed no increase. Indeed, there were four cases (patients 1205, 1209, 1375, and 12099, Table 3 of our original report1) who had 3+, 3+, 2+, and 4+ interstitial TGF-β expression, respectively, in day 0 biopsies, but showed absolutely no increase in reticulin. On the other hand, of the two patients with no TGF-β expression on day 0, one had a grade III diffuse increase in reticulin, whereas the other did not. Additionally, because the monoclonal antibody is highly specific for TGF-β, it is apparent that there is no relationship between high TGF-β expression and the presence of fibrosis in this group of APL patients. The application of pronase is extremely important for obtaining excellent immunohistochemistry in plastic-embedded biopsies and the effects of pronase digestion on the intensity of TGF-β staining were thoroughly examined and reported.1 We agree with Dr Islam that because glycol methacrylate is miscible with water, there is no need for treatment with xylene or alcohol before placing the sections directly in staining solutions.2 Such enzyme histochemical techniques have been well reviewed by Burns and Yook.2 If further questions remain, Dr Islam is most welcome to contact us directly.

AZRA RAZA
HARVEY PREISLER
University of Cincinnati Medical Center
Cincinnati, OH
BEATRICE LAMPKIN
Children's Hospital Medical Center
Cincinnati, OH
JOHN BENNETT
University of Rochester
Rochester, NY

REFERENCES
High expression of transforming growth factor-beta long cell cycle times and a unique clustering of S-phase cells in patients with acute promyelocytic leukemia [letter; comment]

A Islam