Brief report
Immunohistochemistry accurately predicts FGFR3 aberrant expression and t(4;14) in multiple myeloma
Hong Chang, A. Keith Stewart, Xiao Ying Qi, Zhi Hua Li, Qi Long Yi, and Suzanne Trudel

The t(4;14) translocation detected by fluorescence in situ hybridization (FISH) is an independent prognostic factor for an adverse outcome of multiple myeloma (MM). Because t(4;14) uniquely results in fibroblast growth factor receptor 3 (FGFR3) expression, decalcified, paraffin-embedded bone marrow biopsies were immunostained for FGFR3, and its expression was correlated with the t(4;14) status. FISH detected t(4;14) in 16 (19%) of 85 MM patient specimens, and immunocytochemistry detected aberrant FGFR3 expression in 13 (15%). Twelve (75%) t(4;14)-positive cases expressed FGFR3, and 12 (92%) FGFR3-positive cases harbored a t(4;14). FGFR3 expression and t(4;14) were strongly correlated (P < .001). FGFR3 expression by immunohistochemistry was associated with the immunoglobulin A (IgA) isotype (P < .001), a shorter progression-free survival (median, 11.5 versus 25.8 months; P < .001), and a shorter overall survival (median, 19.2 versus 46.3 months; P < .001). (Blood. 2005;106:353-355)

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Differences between survival curves were analyzed by the log-rank test. Progression-free survival (PFS) and overall survival (OS) were calculated from the transplantation date by the Kaplan-Meier method. Wilcoxon test. The t(4;14) is an independent prognostic factor in MM and, because it is predictive for an adverse outcome, a simple, robust, and rapid method to detect t(4;14) would be clinically useful. In this study, we found IHC can detect FGFR3 expression in myeloma cells and is strongly correlated with t(4;14) detected by interphase clg-FISH. To our knowledge, this is the first report of aberrant expression of FGFR3 detected by IHC in a large MM series. Furthermore, FGFR3 expression in MM is associated with the IgA isotype and a shorter OS or PFS, consistent with the features of t(4;14) detected by interphase FISH. In this series, 80% of the MM cases were negative for both FGFR3 expression and t(4;14); 14% were positive for both FGFR3 and t(4;14); 4.7% were negative for FGFR3 but were t(4;14) positive; and 1.5% were positive for FGFR3 but were t(4;14) negative. Therefore, the sensitivity of IHC is 75% and the specificity is 98% in predicting t(4;14). We found that 25% of the t(4;14)-positive cases are FGFR3 negative by IHC, in agreement with reports by Keats et al6 and Santra et al16 who identified that 26% and 32% of their MM cases, respectively, lacked FGFR3 with reports by Keats et al6 and Santra et al16 who identified that 26% and 32% of their MM cases, respectively, lacked FGFR3 expression. Other

### Results

**Immunocytochemistry**

Anti-CD138 stained all myeloma cells in the evaluated cases. In 13 (15%) of 85 cases, more than 50% of the myeloma cells had moderate to strong membrane and/or cytoplasmic FGFR3 immunoreactivity (Figure 1A). The bone marrow hematopoietic cells were FGFR3 negative as were all 10 normal and 10 CLB bone marrow controls. Plasma cells (less than 5%) from normal marrows or CLL marrow was CD138 positive but negative for FGFR3.

**Correlation of FGFR3 expression, t(4;14), and clinical features**

In 16 (19%) of 85 cases, clg-FISH detected t(4;14) fusion signals in 54% to 98% of the clonal plasma cells. Twelve (92%) of the 13 FGFR3 IHC-positive cases had a t(4;14), and 12 (75%) of the 16 t(4;14)-positive cases overexpressed FGFR3. The t(4;14) and FGFR3 expression were strongly correlated (P < .001). FGFR3 expression was associated with the IgA isotype (P < .001), but there was no correlation with age, sex, disease stage, lytic bone lesions, bone marrow plasmacytosis, albumin, creatinine, or β2-microglobulin levels (Table 1). Patients with FGFR3 expression by IHC had a significantly shorter OS than those without expression (median, 19.2 versus 46.3 months; P < .001) (Figure 1B). The PFS of FGFR3 IHC-positive patients was also significantly shorter (median, 11.5 versus 25.8 months; P < .001).

**Discussion**

The t(4;14) is an independent prognostic factor in MM and, because it is predictive for an adverse outcome, a simple, robust, and rapid method to detect t(4;14) would be clinically useful. In
Cryptic mechanisms that block FGFR3 expression at transcriptional or translational levels may account for this discrepancy.

That 1 (7.7%) of our 13 cases with FGFR3 expression was t(4;14)-negative is unexpected and contrasts with a previous study in which t(4;14) was detected in all cases expressing FGFR3 at the mRNA level. Although rare, it is possible that FGFR3 activation may be due to gene mutations19 or other mechanisms not involving the IgH locus. A recent study by gene expression profiling to further analyze t(4;14)-positive and t(4;14)-negative MM cases found 25% of the IgH locus. But paraffin immunohistochemistry reliably detects for aberrant expression of FGFR3 in MM and is strongly correlated with the presence of the t(4;14) translocation and an adverse clinical outcome. Because paraffin immunohistochemistry is routinely available, robust, and inexpensive, we suggest FGFR3 immunocytochemistry of myeloma cells become a panel of routine evaluation because, if present, FGFR3 represents a potential target for kinase inhibitor therapy.

Acknowledgments
The authors thank K. So for technical advice and B. Patterson for helpful comments.

References
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