## MUM1 expression dichotomizes follicular lymphoma into predominantly, MUM1-negative low-grade and MUM1-positive high-grade subtypes

**We investigated the expression of MUM1 (multiple myeloma oncogene 1)/IRF4 (interferon regulatory factor 4) in 46 cases of follicular lymphoma (FL) and correlated this with grade and expression of CD10, Bcl-6 and Ki-67. The analysis suggests that MUM1 expression dichotomizes FL into low-grade FL of CD10$^{+}$/Bcl-6$^{+}$/MUM1$^{-}$/Ki-67$^{low}$ phenotype, and high-grade FL of CD10$^{+/-}$/Bcl-6$^{+/weak}$/MUM1$^{+}$/ Ki-67$^{high}$ phenotype.**

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The MUM1 gene was first identified by cloning the chromosomal breakpoints of a multiple myeloma cell line with translocation t(6;14)(p25;q32).[1] MUM1, a member of the IRF family of transcriptional factors, is induced by antigen receptor mediated stimuli and plays a crucial role in cell proliferation, differentiation and survival.

MUM1 is expresssed in plasma cells and in a small percentage of germinal center (GC) B cells mainly located in the *light zone*. Most importantly, expression of MUM1 and Bcl-6 in GC B cells appears to be mutually exclusive.[2] MUM1 is strongly expressed in multiple myeloma, lymphoplasmacytic lymphoma, classical Hodgkin's lymphoma, nearly one-half of diffuse large B-cell lymphomas (DLBCL), primary effusion lymphoma, immunoblastic lymphoma and plasmablastic lymphoma in the acquired immunodeficiency syndrome setting, post-transplant lymphoproliferative disorders, nearly one-fifth of marginal zone lymphomas and a proportion of small lymphocytic lymphomas. On the other hand it is said to be absent among follicular lymphoma (FL) and mantle cell lymphoma.[3-7]

FL, a prototype GC B-cell lymphoma that expresses the GC markers CD10 and Bcl-6 is composed of centrocytes and centroblasts. FL is graded based on the number of centroblasts per high-power-field (hpf) and on the presence or absence of centrocytes.[8] Grading of FL and counting of centroblasts have high inter-observer variability. Furthermore, the distribution of centroblasts can be heterogeneous. Hence additional ways of classifying FL need to be explored.

For the purposes of this study, 46 patients with FL, who had been evaluated for expression of MUM1, CD10, Bcl-6 and Ki-67 during routine diagnostic work-up, were selected. Twenty of the 46 cases were from the Hammersmith Hospital. The other 26 cases were referrals. Immunohistochemical detection was performed using MUM1 antibody (Santa Cruz Biotechnology Inc., USA). For immunohistochemical evaluation of Bcl-6, CD10 and MUM1, cases had been scored 'positive' when at least 30% of cells showed moderate to strong expression. With regards to Ki-67 expression, the proportion of positive cells within the neoplastic follicles was estimated and expressed as a percentage.

Pearson's $\chi^2$ analysis was performed to correlate MUM1 expression with grade (grades 1 and 2 vs. grades 3a and 3b), expression of CD10 and Bcl-6, and with the presence or absence of a DLBCL component. An independent sample T-test was used to correlate the difference in the mean Ki-67 expression between MUM1 positive and negative groups.

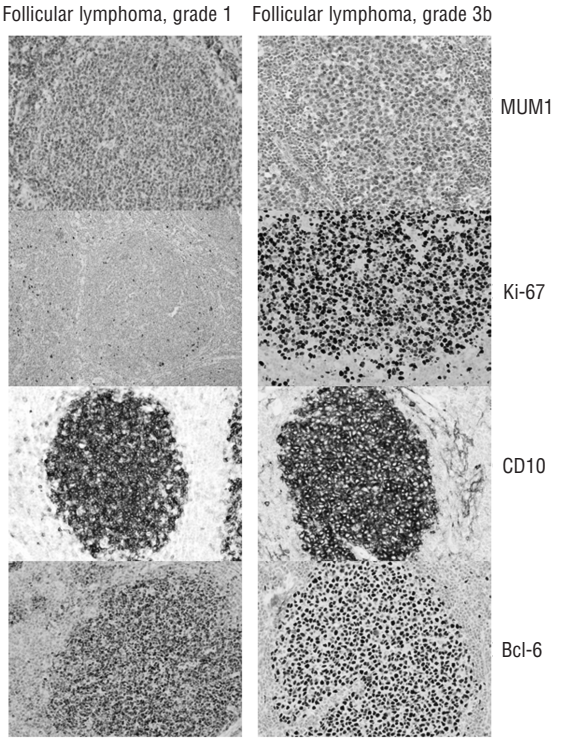


**Figure 1.** A case of grade 1 follicular lymphoma with lack of MUM1 expression and a case of grade 3b follicular lymphoma showing easily appreciable MUM1 expression. The panel also shows differences in Ki-67 expression and a very similar expression of CD10 and Bcl-6 (all X200).

Among the 46 cases, 12 had grade 1; 15 had grade 2; 9 had grade 3a and 10 had grade 3b disease. Ten cases had an associated DLBCL component. Seventeen cases (37%) were scored MUM1-positive. These cases showed moderate to strong expression in the nuclei in more than 30% of the cells within the follicles. Thirty-five cases (76%) showed moderate to strong cytoplasmic membrane expression of CD10. Forty-two cases (91.3%) showed moderate to strong Bcl-6 expression. In the other four cases, Bcl-6 expression was weak (three cases) or focal (one case). None of the cases was completely negative for Bcl-6 (Figure 1).

While 78.9% of FL of grades 3a and 3b were MUM1-positive, only 7.4% of FL of grades 1 and 2 were MUM1-positive ($p<0.0001$). Nine of ten FL grade 3b FL (90%) were MUM1-positive. MUM1 expression showed a significant inverse correlation with CD10 and Bcl-6 expression. Nine of 11 CD10-negative cases and all four cases in which Bcl-6 expression was weak were MUM1-positive ($p<0.0001$ and $p=0.006$, respectively). Furthermore, MUM1-positive FL had a significantly higher proliferation / Ki-67 expression (56% in MUM1-positive cases vs. 26% in MUM1-negative cases; $p<0.0001$).

The first two papers on MUM1 expression in lymphomas suggested that FL are MUM1-negative.[2,3] However, in the study by Falini *et al.*, MUM1 expression was noted in 1/15 FL and the case was in fact a grade 3 FL.[2] In a later publication, involving 50 FL, 8% of grades 1 and 2 FL and 40% of grade 3 FL were found to be MUM1-positive.[9]

**Table 1.** Correlation of MUM1 expression with grade, expression of CD10, Bcl-6 and Ki-67, and presence of a DLBCL component.

| | *MUM1 Positive (n=17)* | *MUM Negative (n=29)* | *Significance* |
|---|---|---|---|
| Grade | | | |
| Grades 1 and 2 (n=27) | 7.4% | 92.6% | <0.0001 |
| Grade 3a and 3b (n=19) | 78.9% | 21.1% | |
| Grade | | | <0.0001 |
| Grade 1 (n=12) | 8.3% | 91.7% | |
| Grade 2 (n=15) | 6.7% | 93.3% | |
| Grade 3a n=(9) | 66.7% | 33.3% | |
| Grade 3b (n=10) | 90% | 10% | |
| CD10 | | | <0.0001 |
| Positive (n=35) | 22.9% | 77.1% | |
| Weak/negative (n=11) | 81.8% | 18.2% | |
| Bcl-6 | | | 0.006 |
| Positive (n=42) | 31% | 69% | |
| Weak (n=4) | 100% | 0% | |
| Presence of a DLBCL component | | | 0.014 |
| Absent (n=36) | 27.8% | 72.2% | |
| Present (n=10) | 70% | 30% | |
| Ki-67 expression | 55.88±9.8 | 25.69±5.6 | <0.0001 |

Many studies have shown that tumor cells in DLBCL can co-express MUM1 and Bcl-6.[10] Based on CD10, Bcl-6 and MUM1 expression, DLBCL has been sub-classified into GC B-cell like and non-GC subtypes. A high proportion of the non-GC subtype expresses MUM1 and this subtype is associated with a poor prognosis. The current study suggests a biological dichotomy among FL there being a low-grade FL with a $CD10^{+}/Bcl\text{-}6^{+}/MUM1^{-}/Ki\text{-}67^{low}$ phenotype and a high-grade FL with a $CD10^{+/-}/Bcl\text{-}6^{+/weak}/MUM1^{+}/Ki\text{-}67^{high}$ phenotype.

*Kikkeri N. Naresh*
*Department of Histopathology, Hammersmith Hospital & Imperial College, London, UK*

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*Key words: follicular lymphoma, MUM1, IRF4, Bcl-6, Bcl-2, CD10, Ki-67, germinal center, B-cell.*

*Correspondence: Kikkeri N Naresh, Haematopathologist, Department of Histopathology, Hammersmith Hospital & Imperial College, Du Cane Road, W12 0HS, London UK.*
*Phone: international +44.20.83833969. Fax: international +44.20.83838140. E-mail: k.naresh@imperial.ac.uk*

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