



Gracia Dayton. *Walden Study I*. Watercolor on paper, 15" × 22".

*The absolute B-cell count is a key factor associated with progression of monoclonal B-cell lymphocytosis to CLL.*

## Clinical Aspects of Monoclonal B-Cell Lymphocytosis

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**Background:** Monoclonal B-cell lymphocytosis (MBL) is an asymptomatic precursor condition for chronic lymphocytic leukemia (CLL). It is defined by the presence of small clones of aberrant B cells in the peripheral blood, with a total B-cell count below the threshold for diagnosis of CLL ( $< 5.0 \times 10^9$  cells/L).

**Methods:** The authors review current literature on the prevalence of MBL, and the clinical course of this CLL precursor condition, and recommended management for individuals with MBL.

**Results:** MBL occurs in approximately 4% to 5% of healthy adults. While most cases of CLL are preceded by MBL, progression to leukemia requiring CLL treatment occurs in only 1% to 2% of individuals with MBL per year. The absolute B-cell count is most strongly associated with progression, and patients with low-count MBL identified in population screening studies rarely develop CLL. Studies are ongoing to better define the relationship between MBL and CLL and to identify prognostic indicators that predict which patients will progress to CLL. Given their elevated risk of developing malignancy, individuals with clinical MBL should be monitored at least annually for progressive lymphocytosis and signs or symptoms of CLL.

**Conclusions:** Many of the epidemiologic and genetic factors associated with MBL development and its progression to CLL have not yet been identified. However, ongoing studies by many research groups are aimed at answering these questions to facilitate management of individuals with this premalignant condition. In addition, active investigation of MBL will likely yield new insights into the biology of CLL, potentially identifying new therapeutic targets for this incurable disease.

### Introduction

Monoclonal B-cell lymphocytosis (MBL) is an asymptomatic condition characterized by the circulation of small, clonal B-lymphocyte populations in the peripheral

blood. According to revised guidelines developed at the 2008 International Workshop on Chronic Lymphocytic Leukemia, MBL involves fewer than  $5.0 \times 10^9$  B-cells/L in the peripheral blood, in the absence of clinical signs or symptoms of a B-cell lymphoproliferative disorder (B-LPD).<sup>1</sup> Most MBL clonal B cells express an immunophenotype similar to that observed in chronic lymphocytic leukemia (CLL): CD5+, CD19+, CD20<sup>low</sup>, CD23+, and surface immunoglobulin (sIg)<sup>low</sup>.<sup>2</sup> The absolute B-cell count (B-ALC) threshold distinguishes this CLL-like MBL from CLL. However, progression to leukemia requiring CLL-directed therapy occurs in approximately 1% to 2% of cases per year.<sup>3,4</sup> CLL is the most common leukemia in the Western world, with a lifetime risk of 1 in 210 in the United States.<sup>5</sup> The finding that MBL is a precursor

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condition for CLL has focused research interest on MBL to better understand the pathogenesis of CLL and other B-LPDs. Although CLL remains incurable with conventional therapies, the scientific insights gained through the study of MBL may facilitate the development of more effective treatments through increased understanding of CLL biology.

## Prevalence

Recent studies have reported a wide range in MBL prevalence, dependent largely on the characteristics of the population examined and the detection methods utilized for MBL identification. Prevalence in the general adult population (not including those with a family history of CLL) ranges between 0.1% and 14%, according to a recent systematic review of MBL population studies.<sup>6</sup> The absence of standardized laboratory methods for diagnosis of MBL complicates determination of the true prevalence, as the use of more sensitive flow cytometry methods invariably results in higher estimates. General trends across studies indicate epidemiologic similarities to CLL, with a greater observed risk of MBL among those with increasing age and among men.<sup>2</sup> The majority of MBL cases involve B cells with a CLL-like immunophenotype (CLL-like MBL), although other less common phenotypes have also been detected, namely atypical CLL and non-CLL types of MBL. The B cells of atypical CLL-type MBL express CD5 but, unlike those of CLL, have bright surface expression of CD20 or sIg, or no expression of CD23. The B cells of non-CLL-type MBL are CD5- and show bright expression of CD20 and sIg.<sup>7,8</sup> Both atypical CLL and non-CLL types of MBL together account for 15% to 30% of all MBL cases.<sup>7,9</sup> For the remainder of this review, the term MBL implies a CLL-like immunophenotype.

Early population studies identified MBL by systematically screening for B-cell clones with 1- to 3-color flow cytometric analysis (Table 1).<sup>2,3,7-15</sup> In these studies, screening of otherwise healthy adults indicated an MBL prevalence of 0.1% to 3%.<sup>16</sup> Studies using 4-color protocols reported a higher MBL prevalence, ranging from 3% to 6%.<sup>3,8</sup> The recent use of 4- to 8-color flow cytometry has predictably resulted in even greater detection of MBL. For example, Dagklis et al,<sup>7</sup> utilizing 5-color flow cytometric analysis, diagnosed MBL in more than 6% of 1,322 normal subjects aged 40 years and older. In the most sensitive study published to date, Nieto et al,<sup>9</sup> employing 8-color flow cytometry and analyzing  $5 \times 10^6$  B cells per subject, reported an MBL prevalence of 14% among 608 healthy adults older than 40 years of age. In addition to the increased sensitivity of multicolor flow cytometry for detecting cell surface markers, the analysis of greater numbers of B cells per subject has been a major factor in the increase, over time, in reported MBL rates.

The utilization of increasingly sensitive detection methods has led to the detection of smaller and smaller MBL clones. For example, the great majority of B-cell

clones identified by Nieto et al<sup>9</sup> constitutes a minor proportion (median 0.38%) of the overall B-cell population, with a mean CLL-like cell count of  $0.17 \times 10^9$  cells/L. These cases of low-count MBL should be distinguished from clinical MBL, with the former generally detected in population screening studies among healthy individuals with a normal absolute lymphocyte count (ALC) and/or a normal sIg kappa:lambda ratio. Low-count MBL typically involves fewer than  $50 \times 10^6$  aberrant B-cells/L.<sup>17</sup> In contrast, clinical MBL is most often identified during follow-up diagnostic studies to evaluate an absolute lymphocytosis. Individuals with clinical MBL often have more than  $1.5 \times 10^9$  abnormal B-cells/L and are more prone to progression to CLL than those with low-count MBL.<sup>17,18</sup> MBL is detectable in approximately 4% to 5% of adults in the general population when flow cytometric techniques typical of clinical laboratories (4-color with a detection sensitivity of 1:10,000 events) are used. However, it is important to recognize that the overwhelming majority of CLL-like MBL cases detected with techniques of greater sensitivity are low-count MBL and thus have little malignant potential.

## Risk Factors

MBL is more common in families with 2 or more individuals with CLL. The prevalence of MBL among first-degree relatives of patients with familial CLL ranges from 12% to 18%.<sup>13-15,19,20</sup> Elevated MBL risk, relative to that of the general population, is particularly evident in younger age groups, which otherwise have low incidences of MBL.<sup>15,19</sup> Genome-wide association studies designed to determine genetic risk in CLL have identified several risk loci.<sup>21-23</sup> A recent report has shown that some of these alleles also confer risk for the development of MBL, suggesting that MBL and CLL have shared genetic risk and potentially explaining the observed increased prevalence of MBL in CLL kindreds.<sup>24</sup>

A recent investigation by Matos et al<sup>12</sup> of relatives of patients with sporadic (nonfamilial) CLL demonstrated that overall MBL prevalence (approximately 4%) among all patients in the group was similar to that detected in the general population when methods of similar sensitivity were employed. In contrast, the authors of this study suggested that older individuals (> 60 years of age) from families with sporadic CLL have an MBL risk similar to that of relatives of familial CLL cases in this age group (approximately 16%). However, only 2 of 135 subjects (1.5%) who were  $\leq 60$  years of age were found to have MBL. Thus the major difference between MBL observed in relatives of sporadic CLL patients and that in relatives of familial CLL patients may be related to the age of MBL onset. This observation is in alignment with the clinical observation that the median age of CLL onset is approximately 10 years earlier in familial CLL than in sporadic CLL cases.<sup>25,26</sup> The manifestation of MBL at a younger age in high-risk CLL families reinforces the importance

**Table 1. — Prevalence of Monoclonal B-Cell Lymphocytosis**

Study		Flow Cytometry Parameters			Prevalence	
Study	Population Characteristics	No. of Colors	Markers Assessed	No. of Events	CLL-Like MBL	Atypical CLL and/or Non-CLL-Type MBL
Shim et al <sup>10</sup> 2007	N = 1,926 General population, US Ages: 40–76 yrs	2	CD19 or CD20, CD45, CD3, CD5	NS	0.6%	NS
Rachel et al <sup>11</sup> 2007	N = 5,141 Blood donors, US Ages: 17–70+ yrs	2	CD19, CD5; extended panel on select samples: CD2, CD3, CD4, CD7, CD8, CD10, CD14, CD20, CD23, κ, λ	NS	0.14%, all ages; 0.93%, ≥ 65 yrs; 1.47%, ≥ 70 yrs	NS
Rawstron et al <sup>3</sup> 2008	N = 1,520 Healthy outpatients, UK Ages: 62–80 yrs	4	CD19, CD5, κ, λ	5 × 10 <sup>5</sup>	5.1%	1.8%, non-CLL
Ghia et al <sup>9</sup> 2004	N = 500 Healthy outpatients, Italy Ages: > 65 yrs	4	CD5, CD19, κ, λ or CD5, CD20, CD79b, CD19	> 2 × 10 <sup>5</sup>	4.4%	0.6%, atypical CLL; 1.4%, non-CLL
Dagklis et al <sup>7</sup> 2009	N = 1,725 General population, Italy Ages: 18–102 yrs	5	CD5, CD19, CD20, κ, λ	< 5 × 10 <sup>5</sup>	5.2% 6.7%, ≥ 40 yrs	1.1%, atypical CLL; 1.2 %, non-CLL
Nieto et al <sup>9</sup> 2009	N = 608 Healthy outpatients, Spain Ages: 40–97 yrs	8	CD3, CD5, CD8, CD10, CD19, CD20, CD23, CD45, CD56, κ, λ, Bcl2	≥ 5 × 10 <sup>6</sup>	12%	2.3%, non-CLL
Rawstron et al <sup>2</sup> 2002	N = 910 Outpatients or ER patients with normal blood counts, UK Ages: > 40 yrs	4	CD5, CD19, CD20, CD79b or CD5, CD19, κ, λ	≥ 2 × 10 <sup>5</sup>	3.5%, all ages; 5.0%, > 60 yrs	1.0%, non-CLL
Rawstron et al <sup>3</sup> 2008	N = 2,228 Outpatients with lymphocytosis, UK Ages: 39–99 yrs	4	CD19, CD5, κ, λ	5 × 10 <sup>5</sup>	13.9%	NS
Matos et al <sup>12</sup> 2009	N = 167 First-degree relatives of patients with nonfamilial CLL, Brazil Ages: < 18–80+ yrs	4	CD5, CD19, CD20, CD79b, κ, λ	3 × 10 <sup>5</sup>	3.6%, all ages 0%, < 40 1.3%, 40–60 yrs 15.6%, > 60 yrs	0.6%, atypical CLL
Marti et al <sup>13</sup> 2003	N = 33 First-degree relatives of patients with familial CLL, US Ages: NS	3	CD3, CD4, CD5, CD8, CD14, CD16, CD19, CD20, CD22, CD34, CD56, κ, λ	NS	18%	NS
Rawstron et al <sup>14</sup> 2002	N = 59 First-degree relatives of patients with familial CLL, UK Ages: 23–86 yrs	4	CD5, CD19, CD20, CD22, CD79b, κ, λ, FMC7	NS	13.6%	1.7%
Goldin et al <sup>15</sup> 2010	N = 505 First-degree relatives of patients with familial CLL, US Ages: < 40–90+ yrs	6	CD5, C19, CD20, CD23 or CD45, κ, λ	1–5 × 10 <sup>5</sup>	14%	1.4%, atypical CLL 1.0%, non-CLL

NS = not specified, MBL = monoclonal B-cell lymphocytosis, CLL = chronic lymphocytic leukemia.

of genetic predisposition to this precursor condition.<sup>15,19</sup> Since familial CLL accounts for approximately 5% of all CLL cases,<sup>27,28</sup> MBL in these high-risk families accounts for a small but significant percentage of all MBL cases among the general population.<sup>15,29,30</sup>

Few epidemiologic studies have investigated the association of environmental factors with MBL development. However, Shim et al<sup>10</sup> observed that MBL occurred more frequently in people living near a hazardous waste site than in those residing in a control location (6.2 age-adjusted odds ratio). Chemical exposures from the waste sites included volatile organic compounds and polychlorinated organic compounds. Further investigation is required to confirm the association between these contaminants and MBL, as this study involved a limited number of participants (n = 46 in the target area) and individual exposure measurements were not recorded. Given the epidemiologic association between CLL and organic solvents and herbicides, these types of studies inform our understanding of environmental risks for CLL.

Hepatitis C virus (HCV) infection has been recently reported as a host factor potentially involved in MBL development. MBL was detected in 28.5% (35 of 123) of HCV patients evaluated, with a trend toward increased frequency among subjects with more advanced disease.<sup>31</sup> Interestingly, the distribution of cases among the subtypes of MBL differed in HCV-infected patients relative to the general population. All three phenotypes (CLL-like, atypical CLL, and non-CLL MBL) occurred at a higher frequency in the HCV-infected individuals than in the uninfected control group. While CLL-like MBL accounts for approximately 70% of cases in the general population, only 37% of the HCV patients with MBL had this phenotype. Atypical CLL and non-CLL types of MBL constituted 46% and 17% of the cases, respectively. The underlying cause for the association between HCV and MBL is not yet understood; however, HCV infection has also been associated with a variety of other lymphoproliferative disorders. Chronic antigen stimulation, potentially in response to an infectious agent such as HCV, has been proposed to play a role in CLL pathogenesis.<sup>32,33</sup> Landgren et al<sup>34</sup> further supported this in a recent study demonstrating an increased risk of CLL in individuals with a personal history of pneumonia (odds ratio 1.4; 95% confidence interval, 1.2–1.8), suggesting that B-cell stimulation by particular respiratory pathogens may play a role in CLL development. The authors also suggested a plausible alternative hypothesis: that individuals with pneumonia predating their CLL diagnosis were more susceptible to infection due to decreased immunoglobulin levels while in an undiagnosed precursor CLL state of MBL. While epidemiologic data for MBL are currently limited, further study of environmental and host factors associated with this condition will likely provide insights into the immunologic triggers for expansion of B-cell clones.

## MBL Progression to CLL

MBL is at least 100 times more prevalent than CLL, indicating that only a small subset of these cases progress to leukemia (Table 2).<sup>3,4,10,17,35-37</sup> A recent retrospective study using biospecimens from the prostate, lung, colon, and ovary (PLCO) observational cohort demonstrated that essentially all subjects who developed CLL (45 of 46) over a 6-year period previously had a detectable MBL clone.<sup>38</sup> Thus, MBL is a premalignant state for CLL, akin to monoclonal gammopathy of undetermined significance (MGUS) as a precursor for multiple myeloma.<sup>39</sup> In most cases, the B-ALC in MBL remains stable over the short-term; however, 1% to 2% of individuals with MBL, detected during a workup for lymphocytosis, progress annually to CLL requiring treatment.<sup>3,17,18</sup> Research is ongoing to identify factors that predict which patients with MBL will advance to CLL. As described earlier, current data indicate CLL is more likely to develop in individuals with clinical MBL than in those with low-count MBL. In fact, data from the United Kingdom, the United States, and Italy show that the B-ALC is the most important determinant of progression to CLL.<sup>3,4,17</sup>

Investigations into the natural history of MBL have indicated that disease progression is strongly associated with B-ALC. In one recent study of individuals with clinical MBL, the annual risk of developing progressive lymphocytosis was 4.1%. B-ALC was the only independent predictor of progressive disease; a subset of these subjects went on to develop CLL requiring treatment (1.1% per year).<sup>3</sup> A Mayo Clinic study performed by Shanafelt et al<sup>40</sup> found that a threshold of  $11 \times 10^9$  B-cells/L at diagnosis is predictive of treatment-free survival and overall survival times in individuals with CLL-like MBL. Molica et al<sup>41</sup> recently reported a similar cut-off ( $10 \times 10^9$  B-cells/L) as a predictor for time to first therapy. Rossi et al<sup>35</sup> observed a median time to progression of 55 months among 123 individuals with clinical MBL, with 10% advancing to CLL or small lymphocytic lymphoma (SLL) annually for the first 6 years, followed by 3% per year thereafter. Time to progression was significantly associated with the absolute number of CLL-phenotype cells, with a B-ALC greater than  $3.7 \times 10^9$  B-cells/L predicting the highest risk of developing CLL or SLL. Interestingly, progression to CLL/SLL did not plateau over the > 18-year follow-up, indicating that clinical MBL inevitably evolves to a malignancy if given sufficient time.

Several research groups have investigated the biologic characteristics of the clonal B cells circulating in MBL and whether these factors predict risk of MBL progression to CLL. B-ALC remains independently associated with treatment-free survival in MBL when controlling for ZAP70 and CD38 expression, immunoglobulin heavy chain variable region (IgVH) mutation status, and genomic aberrations of the CLL-type cells.<sup>40</sup> Data are limited regarding the utility of CLL prognostic factors for predicting MBL progression. However, a few stud-

ies have indicated that certain biologic properties may provide additional prognostic information in MBL. In two reports,<sup>35,42</sup> individuals with a high proportion of CD38+ clonal B cells were more likely than those with a low proportion of CD38+ cells to progress from MBL to CLL requiring treatment. Results from these two studies are consistent with CD38 expression as a negative prognostic indicator in CLL. In a third study,<sup>3</sup> CD38 expression was not associated with disease progression. In general, however, CLL-phenotype cells in individuals with MBL have properties that are associated with favorable prognosis in CLL. For example, 70% to 95% of MBL cases involve B cells with IgVH somatic hypermutation (> 2% mutation relative to germline sequence).<sup>3,4,7,35,43-45</sup> In addition, fluorescence in situ hybridization (FISH) analysis of CLL-type cells from individuals with MBL has demonstrated a high rate of 13q14 deletion, a genomic aberration indicating favorable prognosis in CLL.<sup>3,4,9,35,43,46</sup> In contrast, the poor prognostic chromosomal deletions (17p and 11q) that are observed in approximately 25% of CLL patients are rarely detected in purified MBL B cells.<sup>3,35,44,47</sup>

Limited data are available regarding the utility of IgVH mutation status and FISH karyotype as predictors for MBL progression to CLL. At least one research group has observed no association between IgVH mutation

status and disease progression among 40 individuals with CLL-like MBL.<sup>45</sup> A study of a different cohort of 128 patients with clinical MBL reported that IgVH status, as well as CD38 expression and genomic aberrations, did predict progression to CLL/SLL requiring treatment.<sup>35</sup> When controlling for IgVH status and CD38 expression, FISH karyotype was the only independent predictor of treatment-free survival. Further investigation is required to determine which properties of CLL-type cells have prognostic importance in MBL and whether the clinical relevance of these biologic markers differs between clinical MBL and low-count MBL. These findings will likely contribute to increased understanding of CLL pathogenesis and aid in the identification of those MBL patients who should be followed closely for progression.

### MBL vs Rai Stage 0 CLL

The revision of CLL diagnostic criteria in 2008 resulted in a significant number of patients being reclassified from Rai stage 0 CLL to CLL-like MBL. Since earlier guidelines were based on ALC rather than B-ALC, an absolute lymphocytosis with  $< 5.0 \times 10^9$  B-cells/L was previously regarded as CLL if light chain restriction indicated B-cell clonality.<sup>4,48</sup> By definition, Rai stage 0 CLL differs from CLL-like MBL only with respect to its higher B-ALC, as both diagnoses stipulate the absence of any signs or

**Table 2. — Frequency of Chronic Lymphocytic Leukemia-Type Monoclonal B-Cell Lymphocytosis Progression to Progressive Lymphocytosis or Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma Requiring Treatment**

Study	Patients Population Characteristics	Follow-up (yrs)		Progression of CLL-Like MBL	
		Median	Range	Progressive Lymphocytosis <sup>a</sup>	CLL/SLL Requiring Treatment
Rawstron et al <sup>3</sup> 2008	N = 185 MBL in setting of lymphocytosis, UK	6.7	0.2–11.8	4.1% per yr	1.1% per yr
Shanafelt et al <sup>4</sup> 2009	N = 210 Newly diagnosed MBL, US	1.5	0.3–7.9	36% (over a period of 0.25–7.9 yrs post-diagnosis)	1.4% per yr
Rossi et al <sup>35</sup> 2009	N = 210 Clinical MBL, Italy	3.6	NS	7% per yr	10% per yr in first 6 yrs 3% per yr in following yrs
Fung et al <sup>36</sup> 2007	N = 46 Clinical MBL, Canada	2.5	0.1–10	NS	0%
Xu et al <sup>37</sup> 2009	N = 20 Clinical MBL, China	3.8	1.5–11.3	NS	4% per yr <sup>b</sup>
Shim et al <sup>10</sup> 2007	N = 8 Clinical MBL, US	NS	9–12	NS	12.5% (1/8) died of CLL 12.5% (1/8) diagnosed with Waldenström's macroglobulinemia
Ghia et al <sup>17</sup> 2010	N = 53 Low-count MBL, Italy	2.8	0.9–4.2	< 2% per yr (estimated from data)	0%

<sup>a</sup> Progressive lymphocytosis is defined here as a doubling of the lymphocyte count relative to the initial count at diagnosis.  
<sup>b</sup> Patients were described as progressing to CLL, but requirement of treatment was not specified.  
 NS = Not specified.

symptoms of a B-LPD. Given the high degree of similarity between MBL and Rai stage 0 CLL, several research groups have compared the outcomes of patients with these clinical entities. Fung et al<sup>36</sup> observed similar overall survival rates in these two groups, with a trend toward longer progression-free survival for MBL patients during a median of 2.5 years of follow-up. Faguet et al<sup>49</sup> showed equivalent disease progression in individuals with lymphocyte counts below  $10^{10}$  cells/L, regardless of whether the patients had MBL or Rai stage 0 CLL. A recent Mayo Clinic study<sup>4</sup> also reported no significant difference in survival rates between individuals with MBL and Rai stage 0 CLL, over a 4.5-year period. However, significantly fewer MBL patients (1.4%) progressed to the point of requiring treatment. Time to treatment was correlated with B-ALC, while the clonal B-cell percentage was not associated with disease progression among individuals with  $< 1.5 \times 10^9$  B-cells/L. Similarly, Rawstron et al<sup>3</sup> observed a 1.1% per year progression to CLL requiring treatment among subjects with lymphocytosis and CLL-like MBL. As in the Mayo Clinic study, B-ALC was useful as a predictor for progression. Rossi et al<sup>35</sup> also recently reported longer treatment-free survival in subjects with MBL than in those with Rai stage 0 CLL (68.7% vs 51.3% at 10 years, respectively). The observed difference in time to treatment between Rai stage 0 CLL and CLL-like MBL supports the clinical utility of the B-ALC, rather than the ALC, to differentiate these entities. On the other hand, a provocative interpretation of these studies relates to the diagnostic threshold between MBL and CLL. Results of studies by Shanafelt et al<sup>40</sup> and Molica et al<sup>41</sup> suggest that the B-ALC of  $10\text{--}11 \times 10^9$  B-cells/L provides better performance as a predictor of clinical outcome, suggesting that the current diagnostic value of  $5.0 \times 10^9$  B-cells/L may be too low.

### Prognosis of Low-Count MBL

Most individuals from the general population who meet the diagnostic criteria for MBL have low-count MBL and a normal lymphocyte count. In contrast to clinical MBL, low-count MBL rarely develops into CLL, and affected individuals have equivalent survival to age-matched controls. In a study of 40 adults with MBL and normal blood counts, Bennett et al<sup>50</sup> observed no difference in overall survival between subjects with low-count CLL-like MBL and the control group (4.8% vs 6.8% per year mortality, respectively). None of the individuals with CLL-like MBL progressed to CLL requiring treatment during a 5-year follow-up. In a recent evaluation of an Italian cohort with MBL, only 1 of 54 CLL-like cases had clinical MBL, and 50 of 54 subjects (93%) had  $< 50 \times 10^6$  monoclonal B-cells/L.<sup>17</sup> Among the low-count MBL cases, B-cell numbers decreased or remained unchanged, with none of these individuals progressing to leukemia over a period of 34 months. Cytogenetic analysis of the aberrant B cells for a subset of these subjects demonstrated a frequency

of 13q deletions (8 of 17) similar to that observed among CLL patients; 17p deletion was observed in only 1 case, while the other common genomic aberrations in CLL — trisomy 12 and 11q deletion — were not detected. A similar distribution of cytogenetic abnormalities was observed in a different study of low-count CLL-like MBL.<sup>43</sup> Ghia et al<sup>17</sup> hypothesized that the 13q deletion is an early event involved in B-cell development of the CLL-like phenotype that does not affect progression to leukemia.

Another study by this Italian group recently examined IgVH sequences in subjects with low-count MBL.<sup>7</sup> They discovered extensive differences between the immunoglobulin gene repertoires of the B-cell clones in low-count MBL and those in CLL. For example, IgVH4-59 and IgVH4-61 were the most frequently expressed genes among individuals with low-count MBL, whereas these genes were relatively infrequent among CLL patients. In contrast, immunoglobulin genes commonly expressed in CLL (IGVH1-69 and IGVH4-34) and clinical MBL (IGVH4-34 and IGVH3-23) are rarely observed in low-count MBL. These data suggest that a biologic difference between the aberrant B-cells in low-count MBL and those in CLL may account for the low risk of progression from low-count MBL to leukemia.<sup>7</sup>

### Management of Clinical MBL

The application of highly sensitive diagnostic flow cytometry to evaluate even mild cases of lymphocytosis is identifying an ever-increasing group of individuals with clinical MBL. Therefore, standardized clinical approaches to this patient population are needed. Individuals with clinical MBL should be monitored periodically for progression to CLL or SLL. Conversely, no follow-up is necessary for low-count MBL since B-cell clones smaller than  $0.1 \times 10^9$  cells/L rarely, if ever, advance to malignancy.<sup>3,17,18</sup>

### Initial Evaluation

Prior to employing a watch-and-wait approach for clinical MBL, it is essential that clinicians rule out other B-LPDs that can present with monoclonal B cells in the peripheral blood. If the cells have a CLL immunophenotype and the B-ALC exceeds  $5.0 \times 10^9$  cells/L, then the patient has CLL. Again, it is important to make the distinction that the current diagnostic criteria for CLL utilize the B-ALC, not the absolute CLL B-cell count. According to the current International Workshop on Chronic Lymphocytic Leukemia formulation,<sup>1</sup> the diagnosis is SLL if CLL phenotype cells are present, if either lymphadenopathy or hepatosplenomegaly is detected upon physical examination, and if the B-ALC is below  $5.0 \times 10^9$ /L. The presence of any constitutional symptoms such as fever, weight loss, night sweats, or fatigue can also distinguish SLL from MBL since the latter is defined as an asymptomatic condition. As discussed below, further testing is necessary to rule out non-Hodgkin lymphoma (NHL), only if the aberrant cells are atypical CLL type or non-CLL type.

Evaluation of CLL-like MBL requires only physical examination, patient history, B-ALC, and flow cytometric analysis of the peripheral blood to establish the presence and immunophenotype of the monoclonal B cells. In light of the well-established association between MBL and CLL, a detailed family history is needed in order to identify any first-degree relatives with CLL. Studies are ongoing to determine whether CLL is more likely to develop in individuals with MBL from high-risk CLL families than in those without a genetic predisposition to MBL. Patients should also be questioned about prior exposures to volatile organic compounds, certain herbicides such as Agent Orange, and high levels of ionizing radiation since these factors are associated with increased risk of developing CLL.<sup>51-55</sup>

Bone marrow biopsy is not required during the diagnostic assessment of MBL unless the patient has an unexplained cytopenia. Clinical MBL typically involves 10% to 20% of the bone marrow, and this extent of bone marrow infiltration does not significantly affect hematopoietic potential.<sup>35</sup> Therefore, unexplained cytopenias should be investigated further for a separate cause that may require treatment.<sup>56</sup> In the absence of cytopenia, bone marrow biopsy provides no relevant information; the degree of bone marrow infiltration is not associated with MBL progression to CLL.<sup>35,57</sup> Since the only consistent predictor of MBL progression is B-ALC, we recommend that biologic risk stratification testing (IGHV mutation analysis, interphase cytogenetics, ZAP70) *not* be performed in patients with clinical MBL. The role of these prognostic markers in clinical MBL requires validation in larger datasets before their use should become a standard of care. Furthermore, the low number of CLL-phenotype cells reduces the diagnostic yield of these assays, unless samples undergo the technically challenging enrichment for CLL-type cells prior to analysis.

### **Periodic Monitoring**

Given our current inability to predict MBL progression to CLL, evaluation every 6 to 12 months is recommended for all individuals with CLL-type clinical MBL. This assessment should include a review of B-type symptoms (fever, night sweats, unintentional weight loss) and a physical examination, with particular focus on palpation for lymphadenopathy. In addition, the complete blood count with differential should be determined. If available, flow cytometric quantification of the B- and T-cell subsets could be performed upon initial evaluation as these parameters provide more prognostic information than the ALC.<sup>56</sup> A recent study by Shanafelt et al<sup>40</sup> indicated that elevated T-cell and natural killer (NK)-cell counts suggest a favorable prognosis. Since the ALC includes B cells, T cells, and NK cells, it can mask informative changes in the counts of these cell subsets. For example, the ALC may be normal in the presence of a high B-ALC and low T-cell count (poor prognosis) or a low B-ALC and high T-

cell count (good prognosis). Furthermore, distinguishing MBL from CLL requires measurement of the B-ALC, as the 2008 CLL diagnostic criteria are based on B-ALC rather than ALC.<sup>1</sup> However, lymphocyte subset enumeration by flow cytometry is not necessary after the initial diagnosis of MBL unless progressive absolute lymphocytosis or patient symptoms indicate progression to CLL.

A current area of debate is whether a B-cell cutoff should be used to determine which individuals with MBL require annual monitoring. Rawstron<sup>56</sup> recommends annual follow-up only for individuals presenting with  $> 1.9 \times 10^9$  CLL-like B-cells/L, as the Leeds research group has observed limited occurrence of progressive lymphocytosis among individuals with B-cell counts below this threshold. For those with a lower count, the recommendation is at least one follow-up assessment after 6 months to a year in order to confirm a stable B-cell count. No consensus guideline has been set at this time, so annual monitoring is the standard of care for individuals with clinical CLL-like MBL.

The annual evaluation of individuals with MBL is generally performed by a hematologist-oncologist.<sup>18</sup> However, Rawstron and Hillmen<sup>58</sup> have recently proposed an alternative management plan in which the physical examination and patient history are performed by a primary care provider, utilizing a standardized questionnaire to ensure thorough assessment of B-symptoms. In this model, peripheral blood samples are analyzed by flow cytometry at a central diagnostic facility, and the results are reviewed by a hematologist-oncologist. Patients are then referred to a specialist only if laboratory parameters and/or symptoms suggest disease progression. A pilot study in the United Kingdom indicated that patients preferred this more convenient, community-based monitoring over attending a specialty clinic for MBL follow-up.<sup>59</sup> In addition, this method decreased the burden on hematology-oncology clinics so that they were better able to treat patients with overt leukemia or other malignancies. This approach has not yet been tested in a noncentralized health care system such as in the United States. However, given the relatively low malignant potential of MBL, it deserves consideration as an alternative monitoring arrangement with potential benefits for both patients and clinicians.

### **Patient Education and Counseling**

Patient education is a major focus in MBL management, whether it is performed by a primary care provider or a hematologist-oncologist. Individuals reclassified from Rai stage 0 CLL to MBL due to recent changes in the CLL diagnostic criteria should be informed of this reclassification and its implications, particularly given the emerging data suggesting that individuals identified as being in an MBL phase have superior clinical outcomes compared with outcomes of patients diagnosed with Rai stage 0 CLL. Physicians should educate individuals

with MBL to watch for symptoms that indicate disease progression, including fever, night sweats, weight loss, fatigue, and swollen lymph nodes. The low likelihood of annual progression to CLL should be emphasized to reduce patient anxiety arising from the preleukemic label of MBL. However, clinicians should be cognizant of the risk that patients will perceive these reassurances as indicating that physicians view MBL as unimportant. This occurs commonly among CLL patients and has a negative emotional impact. In addition, individuals with MBL are likely to experience apprehension about the uncertainty of their disease course and the lack of treatment, which has also been widely recognized among patients with CLL.<sup>60</sup> Physicians should be sensitive to these concerns as they discuss the risks of MBL and explain the management plan with patients.

#### **Use of Blood Products From Individuals With MBL**

The increasingly frequent recognition of MBL among normal blood donors poses a new problem regarding the suitability of these blood products for use. Since MBL involves an abnormality among lymphocytes, leukocyte-depleted blood products should pose minimal risk of transmitting the monoclonal B cells.<sup>58</sup> Because up to 12% of the adult population has low-count MBL, we believe it would be impractical to exclude all of these individuals from voluntary blood donation.

On the other hand, for the much smaller group of patients with clinical MBL, exclusion from donation may be warranted given the significantly higher number of peripherally circulating clonal B cells and thus greater risk of inadequate depletion of leukocytes. Concerns regarding clonal B-cell transfer via blood products from individuals with MBL are substantiated by recent reports suggesting increased risk for CLL and NHL among individuals who have received allogeneic blood products, including leukodepleted red blood cell transfusions.<sup>61,62</sup>

The transplantation of hematopoietic stem cells from an individual with MBL to an immunocompromised individual warrants significant consideration, particularly if the stem cell recipient has CLL. Given the increased prevalence of MBL among first-degree relatives of familial CLL patients, this clinical situation is not uncommon among potential sibling donors.<sup>20</sup> While cases of clonal transfer of MBL have been reported,<sup>63</sup> the risks of transfer and progression to leukemia have not yet been established. Given that stem cell recipients are severely immunosuppressed, the likelihood of MBL progression to CLL may be higher in this setting than among healthy individuals with MBL.<sup>64</sup> In general, we recommend exclusion of donors with clinical MBL to minimize the risk of clonal B-cell transfer. However, in the absence of other therapeutic options or an alternate matched donor, a risk-benefit analysis suggests that it is ethical to use stem cells from an MBL donor.<sup>65</sup> Until further data become available regarding the suitability of individuals

with MBL for stem cell donation, decisions must be made on a case-by-case basis.

#### **Atypical CLL Type and Non-CLL Type of MBL**

Clinical outcome data are limited regarding individuals with atypical CLL or non-CLL types of MBL. A few small studies have reported no progression of lymphocytosis among subjects with these subtypes of MBL.<sup>17,66,67</sup> However, current evidence suggests that individuals with monoclonal B-cell clones with these immunophenotypes are more likely to have an underlying malignancy than those with CLL-like MBL.<sup>58</sup> Therefore, further clinical testing is required to rule out NHL before classifying these patients as having MBL. Since NHL may involve bone marrow infiltration, a bone marrow biopsy should be examined for the presence of clonal cells matching those present in the peripheral blood. In most cases, computed tomography (CT) scans of the chest, abdomen, and pelvis should also be performed to detect enlarged lymph nodes or extranodal disease involvement. If present, a lymph node biopsy should be strongly considered, given the high diagnostic yield in NHL. If imaging studies and/or bone marrow biopsy demonstrate evidence of disease, then the diagnosis is NHL and the patient should be treated accordingly. Even if these studies are negative, the clonal B cells should be assessed by flow cytometry, cytogenetic analysis, and immunohistochemical staining to determine if the phenotype matches an aggressive NHL. For example, the chromosomal translocation t(11:14) and positive cyclin D1 staining would indicate a mantle cell lymphoma phenotype, which warrants closer follow-up than a phenotype consistent with an indolent NHL subtype. Follow-up recommendations for atypical CLL and non-CLL types of MBL are based on treatment guidelines for asymptomatic NHL according to subtype. If findings are consistent with an aggressive NHL subtype, then treatment could be considered or, at minimum, patients should be monitored every 3 to 6 months, with a CT scan at least every 6 months.<sup>68,69</sup> In contrast, individuals with an MBL clone with an indolent NHL phenotype need follow-up every 12 months.<sup>70,71</sup> Further investigation of atypical CLL and non-CLL types of MBL is required to determine whether such frequent monitoring is necessary.

#### **Conclusions**

Abnormal B-cell clones are detected with increasing frequency among the general population due to highly sensitive flow cytometric analysis. While up to 12% of adults have MBL, most of these are low-count cases that will remain stable over time. In contrast, individuals with clinical MBL, characterized by lymphocytosis and a larger population of aberrant B cells, are more likely to progress to CLL. Each year, approximately 1% to 2% of clinical MBL patients advance to CLL requiring treatment, but no prognostic indicators are available at this

time to predict which patients will develop malignancy. Consequently, all individuals with clinical CLL-like MBL should be monitored at least annually for progressive lymphocytosis and any signs or symptoms of CLL/SLL. At this time, data are limited regarding genetic or environmental factors involved in the development of MBL and its progression to frank leukemia. Research is ongoing to identify epidemiologic associations with MBL and characteristics of the aberrant B cells that indicate likely development of CLL. These studies should enhance our understanding of the pathogenesis of CLL and thus may contribute to the identification of new therapeutic targets for this disease.

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