have confirmed that the presence of certain genetic alterations is associated with overall prognosis and response to treatment. CLL is characterized by relatively few recurrent somatic mutations, of which those in the TP53 gene are the strongest predictors of chemoresistance and poor survival.\(^7\)

In this study, Rossi et al\(^6\) have applied highly sensitive ultra-deep next-generation sequencing to examine a large cohort of patients (309) with newly diagnosed CLL for the presence of very small TP53-mutated subclones (sensitivity down to 0.3% allele frequency), which would not have been detected by Sanger sequencing (which detects >20% frequency).\(^7\) The 5.8% of patients with such subclones had the same adverse survival as those 9% in whom TP53 mutations were detected by conventional methods, and accounted for a third of all cases with TP53 abnormalities.

The current recommendation is that all patients should be tested for abnormalities of TP53 prior to initiating any line of treatment, in order to select TP53-independent therapy when appropriate. The prevailing dogma is that the size of the TP53-deleted clone is important and that below certain thresholds (variably reported as 10% or 20% using FISH), response to treatment is unaffected. Rossi et al overturn the view that small clones are clinically unimportant by showing that even very small subclones (<1%; present below the threshold of detection using current standard methods but with no apparent cutoff in the size of the clone) have an adverse impact on patient survival. They argue that the effect on outcome of the presence of these subclones is a yes/no determinant independent of clonal size.

Importantly, sequential samples showed that these subclones expanded over time, particularly under the selective pressure of chemotherapy, leading ultimately to chemoresistant disease (see figure). In the 2 patients who did not receive treatment, the clonal size remained unchanged. This would be consistent with other studies showing increasing frequency of TP53 abnormalities in patients with disease progression and refractoriness.\(^7\)

If multiple subclones coexist, what drives any to become dominant? There are many reasons, including limited potential for expansion due to “competition” from other clones as well as the independent effect of the surrounding microenvironment. If these subclones are vying for space and resources, the reduction of some clones may unbalance the status quo. It is therefore unsurprising that failure of chemotherapy to completely eradicate CLL cells can result in expansion of minor, more resistant, and more dangerous subclones. “Selection” can thus be introduced artificially by the use of chemotherapeutic agents. Certain subclones are likely to gain a competitive advantage due to their “fitness” in relation to these selection pressures. It is therefore important to identify low-level molecular lesions that are known to predict for chemoresistance so that treatment can be tailored appropriately. In TP53-mutated CLL, this may involve use of novel targeted therapies (eg, Ibrutinib, ABT199)\(^8\) which have been shown to have promising activity in this subset.

What other strategies might be considered to improve therapeutic efficacy and prevent emergence of resistance? Cytotoxic drugs are likely to select for resistant cells by clearing the ground of more sensitive ones. On the other hand, cytostatic drugs (some small-molecule inhibitors) may cause cells to remain in the tissue space but without either expanding themselves or allowing expansion of other subclones. In addition, early intervention with effective treatment before clonal expansion may be a more effective way to deal with these more clinically adverse subclones. It may also be the case that carefully designed concurrent or sequential combinations of therapies may overcome some of the issues related to clonal diversity. It is important to note, however, that not all chemoresistant CLL is characterized by TP53 mutation and it will be crucial to understand the biology of any other clinically important subclones which may be present in order to prevent their dominance. The underlying principles are likely to be the same but the therapeutic strategies may be different.

Clearly, CLL is not a static disease, but has a clonal architecture that changes over time and is influenced by selection pressures, including treatment. Certain clinically adverse genomic changes appear to be present in the CLL cells from a very early stage of disease. Understanding how this population becomes dominant is crucial for the development of new therapeutic strategies, which will be effective by rendering them the least fit for survival.

**Conflict-of-interest disclosure:** The author declares no competing financial interests.

---

**REFERENCES**


---

**LYMPHOID NEOPLASIA**

Comment on Dubois et al, page 2199

**An E3 ubiquitin ligase-independent role of LUBAC**

**Rudi Beyaert\(^1,2\) 1 Ghent University; 2 VIB**

In this issue of Blood, Dubois et al show a catalytic-independent role of the linear ubiquitin chain assembly complex (LUBAC) in lymphocyte activation and B-cell malignancy.\(^1\) These data add a new layer of versatility to the recently established role of LUBAC in nuclear factor-κB (NF-κB) signaling.
and B cells sense antigens via specific receptors that induce signaling cascades leading to the activation of multiple transcription factors such as those of the NF-κB family. NF-κB controls the expression of multiple genes essential for the immunogenic response and cell survival. Hyperactivation of NF-κB is involved in many autoimmune and inflammatory diseases and constitutive NF-κB activation is a characteristic of certain lymphoma types such as activated B-cell–like diffuse large B-cell lymphoma (ABC-DLBCL), where NF-κB signaling drives proliferation and cell survival. Understanding the early molecular events leading to NF-κB activation is an active area of research and much progress has been made during the last decade. Several molecules that specifically connect the T-cell receptor (TCR) and B-cell receptor (BCR) proximal adaptors to the IKK complex is still largely unclear. Dubois et al demonstrate that TCR- and BCR-induced NF-κB activation in response to antigen receptor stimulation by enabling the interaction of speciﬁc signaling proteins with multiple ubiquitin modules that are linked to each other via their N and C termini. This type of polyubiquitination is known as linear or tail-linked (linear) ubiquitin modules. However, the study of Dubois et al shows a catalytic-independent role of LUBAC in the CBM complex after antigen receptor stimulation. LUBAC is known as an E3 ubiquitin ligase whose catalytic activity is necessary for the RBNA3–mediated NF-κB signaling by assessing the outcome of small interfering RNA–mediated silencing of each of the 3 LUBAC components. Deﬁciency of HOIP and SHARPIN, but not HOIL-1, signiﬁcantly reduced NF-κB activation and the binding of BCL10/MALT1 to the IKK adaptor protein NEMO. These data indicate that LUBAC contributes to optimal NF-κB activation in response to antigen receptor stimulation by enabling the interaction between CBM and IKK complexes.
M1-linked polyubiquitination and is the focus of intense research. Surprisingly, Dubois and colleagues found that expression of a catalytically inactive HOIP mutant is able to restore reduced NF-κB signaling in HOIP-deficient cells to normal levels, indicating that HOIP mediates TCR signaling independent of its catalytic activity. This is further supported by the observation that silencing of OTULIN, a negative regulator of linear polyubiquitination, did not change TCR-induced NF-κB signaling. It should be mentioned that some modest linear polyubiquitination is detected in TCR-stimulated cells, indicating a possible role for linear ubiquitination in other TCR signaling pathways than NF-κB signaling.

Dubois et al show that LUBAC is also part of the preassembled CBM complex in ABC-DLBCL cell lines and that combined silencing of all 3 LUBAC components inhibits constitutive NF-κB activation in these cells. Consistent with these findings and the known anti-apoptotic function of NF-κB, they show that LUBAC silencing also reduces cell survival. Together, these data indicate that LUBAC guarantees cell proliferation and survival of ABC-DLBCL by maintaining constitutive NF-κB activity.

The results of Dubois et al suggest a novel catalytic-independent role of LUBAC in lymphocytes and B-cell lymphoma. The underlying molecular mechanism is still unclear but the finding that HOIP is necessary for the association between CBM and IKK complexes is indicative for an adaptor function. The exact mechanism could, however, be more complex as many ill-defined components compose the CBM complex. The data of Dubois et al complement the recent demonstration that BCR-mediated NF-κB activation does not require LUBAC catalytic activity in splenocytes. In addition, another parallel study also reports that LUBAC associates with the CBM complex in ABC-DLBCL and is required for cell viability. However, the latter study shows that LUBAC mediates constitutive linear polyubiquitination of the IKK adaptor protein NEMO in ABC-DLBCL, and describes 2 rare HOIP germline mutations that promote LUBAC E3 ubiquitin ligase activity and activate NF-κB in ABC-DLBCL. At first look, these findings do not fit the catalytic-independent role of LUBAC that is proposed by Dubois et al in this issue. However, it should be mentioned that they only analyzed the dependency on HOIP catalytic activity in T cells and not in ABC-DLBCL cell lines. Nevertheless, the finding that LUBAC is part of the CBM complex and mediates NF-κB signaling and cell survival is of high importance for our understanding of the regulation of physiological and pathological signaling in adaptive immunity. The CBM complex is an attractive therapeutic target for diseases associated with aberrant lymphocyte activation and B-cell lymphomas, and recent developments using MALT1 protease inhibitors are very promising. A better knowledge of the function and regulation of LUBAC in the CBM complex may provide additional ways for therapeutic targeting.

Conflict-of-interest disclosure: The author declares no competing financial interests.

REFERENCES

© 2014 by The American Society of Hematology

MYELOID NEOPLASIA

Comment on Lundberg et al, page 2220

Many roads lead to MPN

Heike L. Pahl1 1UNIVERSITY MEDICAL CENTER FREIBURG

In this issue of Blood, Lundberg et al correlate the presence of known mutations in patients with myeloproliferative neoplasms (MPNs) with clinical outcome, thereby proposing a molecular risk stratification.

The clinical presentation of patients with MPNs is heterogeneous, and the individual disease course is difficult to predict at diagnosis. Although in some patients the disorder remains indolent for many years, others experience multiple complications and rapid disease progression. It is therefore gratifying to read that Lundberg et al can corroborate this clinical heterogeneity at the molecular level. The authors investigated the “clonal architecture” of MPNs, that is the nature of different mutations detected in individual patients and the order in which they appear.

Because the authors selected known cancer genes for analysis, many of which have been previously shown to be affected in MPNs, the message of this study is less in the nature of the mutations found but rather in the variable pattern of their acquisition, which this study demonstrates. However, it is noteworthy that in this cohort, mutations in some novel genes, such as p33 and NF-E2, appear to be more frequent than others, such as c-Kit or c-Mpl, which have been known for several years.4 A model presented in Figure 5 of the Lundberg et al paper depicts the many different constellations observed and uses them to stratify patients by risk of leukemic transformation. As is the case in other myeloid neoplasias, such as acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS), a higher number of mutations is associated with poorer outcome. In MDS, the number of mutations is likewise correlated with the time to leukemic transformation. The question remains, however, whether the acquisition of additional
An E3 ubiquitin ligase-independent role of LUBAC

Rudi Beyaert