similar in N-803 and IL-2 cohorts, other factors could have contributed to the low clinical response. Indeed, the frequency and proliferation of host $CD8^+$ T cells was increased in patients with N-803 support. In vitro mixed lymphocyte reaction experiments demonstrated that IL-15/N-803 induced higher T-cell proliferation and activation than IL-2 and resulted in enhanced killing of allogeneic NK target cells. These data indicate that N-803 administration induces recipient $CD8^+$ T-cell alloresponses that may limit the persistence of the infused NK cells (see figure).

This report shares the results of clinical trials from two medical centers and provides an explanation of why IL-15/N-803 negated the expectations of positively impacting adoptive haplo-NK cell therapy. The sustained serum levels of IL-15 generated by subcutaneous N-803 administration provide optimal support not only for the donor-derived activated NK cells but also for the host alloreactive $CD8^+$ T cells. Therefore, the therapeutic NK cells infused were rejected, leading to a worse clinical outcome compared with IL-2. Thus, IL-15/N-803 is a double-edged sword. The high degree of HLA incompatibility in the haploidentical setting can be an advantage for NK cell antitumor activity but is extremely treacherous for promoting T-cell alloreactivity. Because haploidentical donors can be readily available for providing cellular therapy to patients with hematological malignancies, this is a disappointment. Haploidentical HSCT (haplo-HSCT) provides life-saving therapy for high-risk patients with leukemia who do not have HLA-matched donors. However, strategies have been developed to avoid patient and donor reciprocal T-cell alloreactivity, which is mainly responsible for graft rejection and graft-versus-host disease (GVHD).[9] NK cells quickly reconstitute and can directly kill leukemia blasts without prior sensitization, thereby exerting a graft-versus-leukemia effect, which is dissociated from GVHD. NK cells can be alloreactive, according to the expression of inhibitory KIR(s) specific for HLA allotypes present in the donor and absent in the patient cells.[9] In a haplo-HSCT setting, the adoptive immunotherapy using ML-NK cells, derived from the same donor and supported by N-803, may be well tolerated and characterized by efficient and persistent antileukemia activity (NCT02782546). In a nontransplantation setting, adoptive transfer of haplo-NK cells requires careful attention to avoid high doses of systemic IL-15/N-803 and/or to possibly use a more intense lymphodepleting chemotherapy.

Approaches of adoptive NK cell immunotherapy need to be tailored for optimal NK cell activity and reduced host T-cell–mediated allorejection to preserve NK cell expansion and persistence. Important factors to take into consideration include the type of allogeneic donor, the HLA-matched or HLA-mismatched donor/recipient pair, the cytokine(s) used in vitro to preactivate NK cells, and the cytokine protocols in vivo to sustain NK cells. In addition, the development of safer and more selective preparatory conditioning regimens should be pursued to improve and expand the clinical use of adoptive NK cell therapy.

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LYMPHOID NEOPLASIA

Comment on Blombery et al, page 1198

A BAX door to venetoclax resistance

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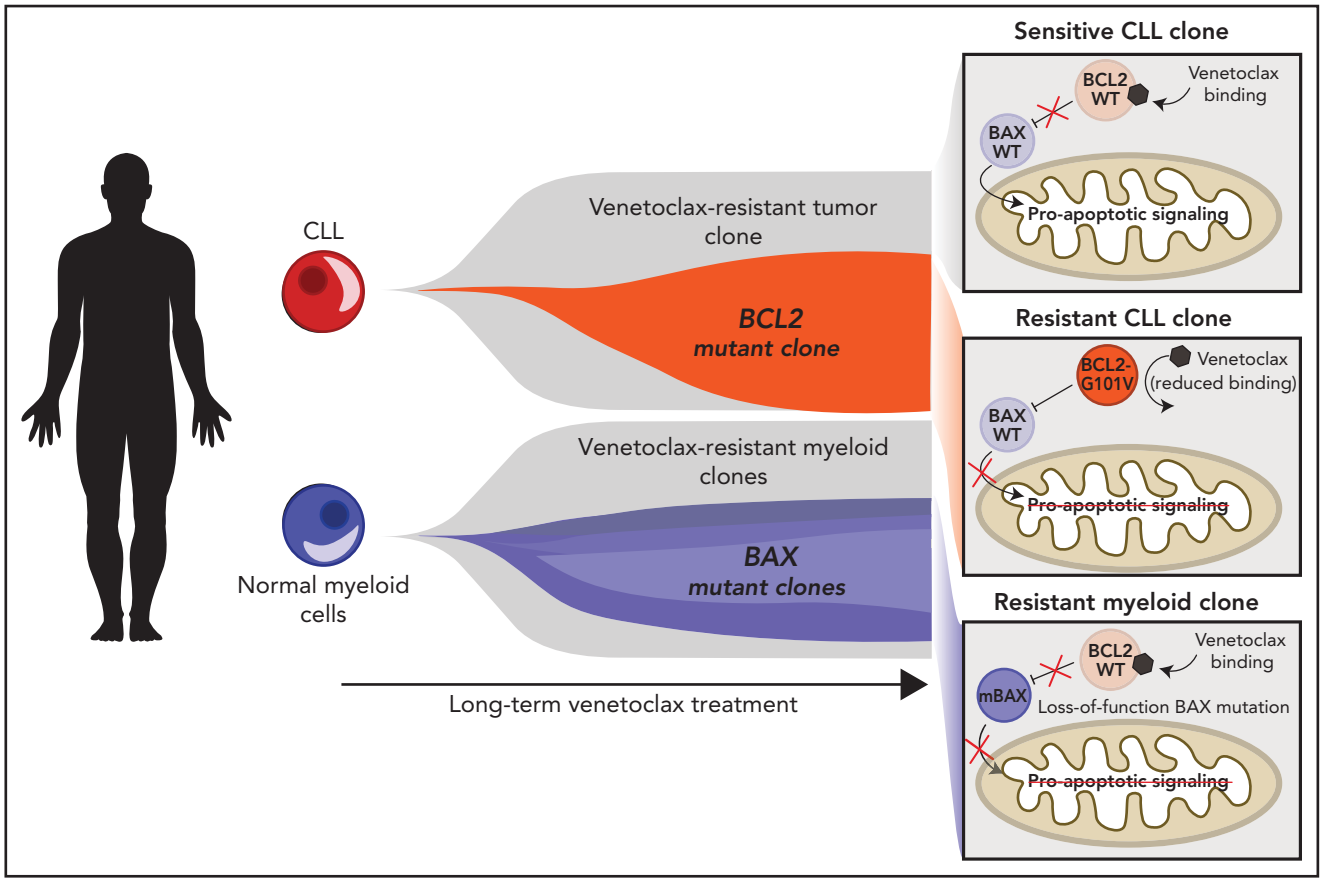
In this issue of *Blood*, Blombery et al[1] identify cytopenias and clonal hematopoiesis in patients with chronic lymphocytic leukemia (CLL) undergoing long-term venetoclax treatment, with unexpected concomitant clonal selection of *BAX* mutations in normal myeloid cells (see figure).

One of the most exciting discoveries in human biology of recent years is that, in fact, we are all genetic mosaics. Vast age-associated clonal mosaicism is observed across healthy tissues,[2,3] linked with a wide array of autoimmune, inflammatory, degenerative, and malignant adverse health outcomes.[4] Likewise, in the hematopoietic system, somatic mutations previously associated with myeloid malignancies are commonly observed in otherwise healthy individuals.[5]

Previous work has shown that chemotherapy can select hematopoietic clones harboring *PPM1D*, *TP53*, and *CHEK2*



Schematic representation of clonal expansions in CLL and normal myeloid cells. On long-term venetoclax treatment, CLL cells undergo clonal selection resulting in expansion of the resistant clones harboring mutations in the *BCL2* gene, for example, BCL2-G101V. Concomitantly, in the normal myeloid compartment, venetoclax results in clonal selection of BAX loss-of-function mutations (mBAX). Thus, venetoclax results in lineage-specific clonal expansions harboring alternative resistance mechanisms.

mutations.[6] However, the question of whether strong selection pressure from targeted cancer therapy impact clonal dynamics in healthy tissues remained largely unexplored.

Venetoclax is a potent agent used in blood malignancies, which targets the antiapoptotic protein BCL2. BCL2 inhibition in CLL results in up to a 79% response rate and is effective even in subgroups with adverse prognostic factors, including fludarabine-resistant and TP53-mutated CLL.[7] However, long-term treatment with venetoclax can eventually result in expansion of resistant clones, driven by clonal evolution. As is often the case with targeted therapy, mutations arise in the target itself. With venetoclax, mutations in BCL2 that reduce venetoclax binding have been observed in relapsed disease.[8] Interestingly, cytopenias may be seen with venetoclax treatment, suggesting that it exerts a selection pressure on the normal hematopoietic system as well.

Blombery et al study a cohort of 92 patients with CLL treated with venetoclax alone or in combination with rituximab. Of note, this cohort was heavily pretreated with fludarabine-alkylator combination chemotherapy. Cytopenias were frequently observed, with a cumulative 5-year incidence of 10.4% of patients progressing to therapy-related myeloid neoplasm (tMN) after venetoclax initiation. As expected, fludarabine-alkylator treatment remained the strongest predictor of tMN progression, as previously described.[9] To characterize the clonal dynamics underlying these clinical observations, the authors focused on non-CLL cells and applied error-corrected deep-targeted sequencing of a panel of genes recurrently mutated in clonal hematopoiesis. Mutations were identified in the normal hematopoietic compartment in 83% of the subset of patients with adequate follow-up. Despite an association between clonal hematopoiesis-related mutations and idiopathic cytopenia, the data did not support an association with progression to tMN. In an elegant twist, the authors posited that, given the specific selection pressure of venetoclax, clonal investigation should not be limited to established age-related clonal hematopoiesis drivers. To more closely link hematopoietic clonal dynamics with the therapeutic pressure of venetoclax, they applied targeted sequencing of BCL2 family genes (*BCL2*, *MCL1*, *BCL2L1*, *BAK1*, and *BAX*) to samples where the CLL fraction was known to be minimal. Unexpectedly, the authors identified 20 different loss-of-function mutations specifically in *BAX* across 13 patients. In contrast, *BCL2* mutations, which were previously observed in resistant CLL, were not seen in the normal hematopoietic compartment. The observation that recurrent *BAX* loss of function variants arise in the normal myeloid compartment under the same selection pressure is perhaps the first example of parallel clonal evolution in healthy tissues with targeted cancer therapy. The authors used an in vitro re-expression system to functionally

validate that the observed *BAX* mutations impede venetoclax-induced cell death. Four of 5 variants tested endowed cells with the capacity to resist venetoclax. Therefore, the clonal expansion of BAX mutants in the myeloid compartment can be attributed, at least in part, to increased fitness because of the loss of BAX proapoptotic function.

In their final experiment, the authors perform a longitudinal study of 2 patients who received 7 years of continuous venetoclax treatment. Both patients showed steadily increasing allelic frequencies of *BAX* mutations over time. The authors then leveraged single-cell sequencing to show that *BAX* mutations present in a single patient are mutually exclusive at the single cell level, representing 2 independent clonal expansions within the same healthy tissue. This finding is a powerful testimony both to the remarkable genetic diversity that fuels clonal mosaicism and to the strong selection pressure exerted by venetocalx on normal hematopoietic cells. Interestingly, when multiple BAX-mutated clones are observed, the dominant clone was found to be carrying co-occurring clonal hematopoiesis mutations (DNMT3A in one patient and ASXL1 in another patient), suggesting increased fitness. Thus, the strong venetoclax selection pressure in healthy tissue drives BAX convergent clonal evolution, potentially cooperating with canonical clonal hematopoiesis mutations.

Overall, the study by Blombery et al provides robust evidence for the presence of clonal evolution in healthy tissues receiving targeted therapy with venetoclax. This exciting observation raises interesting questions. First, given that the observed *BAX* mutations are predicted to be loss-of-function, is biallelic loss required for functional impact? The authors infer loss of heterozygosity in high variant allele fraction samples, supporting homozygous *BAX* loss. Biallelic loss was also the model used in vitro. However, other cases were compatible with heterozygous loss, suggesting that it may be sufficient to impart a fitness advantage. Further studies are needed to definitively link gene dosage with resistance phenotypes. Second, perhaps one of the most exciting observations in this work is the diverging disruption of *BCL2* vs *BAX* in the malignant lymphoid vs the normal myeloid compartments, respectively. What lineage-intrinsic dependencies in the apoptosis pathway drive this observation remains to be determined and may also aid in therapeutic development of apoptosis targeting across blood cancers.

More broadly, this study shows that a strong targeted therapy can select for resistant mutations not only in malignant populations but also in normal tissue clonal mosaicism. As somatic evolution occurs in both normal and malignant cells,[2–6,8] the exquisitely focused selection pressure of targeted therapies is likely to impact both. Future studies will reveal if this observation is unique to venetoclax or will be found with other targeted interventions.

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MYELOID NEOPLASIA

Comment on Yang et al, page 1208

And the germline beat (AML) goes on

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In this issue of *Blood*, Yang et al[1] report on the germline screening of 391 patients from the BEAT acute myeloid leukemia (AML) study, finding that 13.6% of patients unselected by family history have pathogenic or likely pathogenic variants of known cancer-predisposition genes.

Germline predisposition to hematological malignancies (HMs) is a topic of increasing interest to the research community and increasing concern to clinicians, particularly with regard to the selection of hematopoietic stem cell donors from family members.[2] Over the past 2 decades, family studies of the germline predisposition to myeloid neoplasms have increased our understanding of the genes known to underlie this predisposition. The *2016 Revision to the World Health Organization Classification of Myeloid Neoplasms and Acute Leukemia* included a new category for myeloid neoplasms with germline predisposition.[3-5] Despite these advances, genetic analysis of well-curated families who are predisposed to myeloid malignancy identifies known or potential predisposition candidates at ~50%, suggesting that additional predisposition genes are yet to be identified.[5] Likewise, the frequency of germline predisposition mutations in patients in a typical leukemia