are, in CLL, skewed toward a phenotype aimed to evade apoptosis.[7] Blocking BCL-2 protein function by the BH3 mimetic drug venetoclax leads to apoptosis of CLL cells and excellent control of the disease (see figure).[5]

Why was this clinical trial performed? Idelalisib, an oral phosphoinositide 3-kinase (PI3K) isoform δ selective inhibitor, has been approved in combination with rituximab or ofatumumab for the treatment of relapsed/refractory CLL.[3,8] In spite of its significant activity, one-third of patients progressed while on treatment, whereas ~50% discontinued idelalisib owing to adverse events, with the survival after discontinuation being less than 3 months.[9] The ideal salvage therapy for CLL patients failing idelalisib therapy has not been defined. Venetoclax seems a natural choice, because of its different mechanism of action.

What are the lessons learned from this study? Venetoclax showed promising activity after idelalisib failure in patients with CLL with adverse prognostic features; namely, 88% of cases had unmutated *IGHV* genes, or ~30% had *TP53* gene disruption. Notably, with the use of venetoclax it was reported that a substantial number (40%) of patients had undetectable minimal residual disease. All of this activity translated to a prolonged progression-free survival (Coutre et al's Figure 2). Remarkably, discontinuing venetoclax was primarily due to progression of the disease, whereas toxicity was tolerable and mainly hematological. The study has significant limitations, among them the relatively small number of patients reported and short follow-up. Unfortunately, biological and further genetic analyses in this trial were somewhat limited, preventing identification of markers predicting response to venetoclax. Finally, it would have been desirable to report in 1 article the results of venetoclax in patients relapsing to ibrutinib[10] to allow for a direct comparison of treatment efficacy and side effects of venetoclax after BCR inhibitor therapy.

In summary, salvage therapy with venetoclax following idelalisib therapy of CLL has emerged as an excellent option and deserves further study.

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HEMATOPOIESIS AND STEM CELLS

Comment on Duarte et al, page 1712

Can genetics resolve what Notch does in HSCs?

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The effects of Notch signaling in hematopoietic stem and progenitor cells remain controversial. In work presented in this issue of *Blood*, Duarte et al interfere genetically with the Notch transcriptional complex (NTC) in hematopoiesis and demonstrate that canonical Notch signals are dispensable in primitive hematopoietic progenitors, as well as across the myeloid, erythroid (E), and megakaryocyte (Mk) lineages.[1]

Notch signaling is essential during fetal life for hematopoietic stem cell (HSC) emergence from hemogenic endothelium. This carefully regulated process is sensitive to Notch signaling intensity, as high levels of Notch signaling maintain the endothelial fate, whereas intermediate levels are conducive to hematopoietic commitment. Once established, hematopoietic progenitors are shielded from high-intensity Notch signals, although transgenic reporters driven by the *Hes1* Notch target gene have revealed activity in bone marrow HSCs, as well as in subsets of E progenitors.[2] The functional importance of this activity is the subject of intense debate. Genetic blockade of the Notch transcriptional activation complex showed that canonical Notch signaling is dispensable for mouse and human HSC self-renewal in steady-state conditions and after transplantation.[3,4] In contrast, other studies, mostly interfering with microenvironmental Notch ligands or with proximal aspects of Notch activation in HSCs, reported roles for Notch signals in HSC regeneration after myeloablation, in suppressing myelopoiesis, as well as functions in E and Mk development.[2,5-8] To date, these discrepant findings await a cohesive explanation.

To situate the debate, it is useful to review Notch signaling. Notch encodes a transmembrane receptor that functions as an environmental sensor by interacting with Notch ligands (Jagged, Delta-like) on adjacent cells. This interaction initiates serial proteolytic cleavages of the Notch extracellular domain, leading to release of the Notch intracellular domain by γ secretase–dependent mechanisms. The cleaved Notch intracellular domain translocates to the nucleus where it associates with the DNA-binding factor recombination signal-binding protein J κ (Rbpj). The Notch:Rbpj complex recruits a member of the Mastermind family, forming the NTC, which recruits coactivators leading to gene transcription. Without Notch, Rbpj can repress transcription, although the context and functions of Rbpj repression are still being defined. The 4 mammalian Notch receptors (Notch1-4) differ in their affinity for individual Notch ligands, as well as in their ability to activate transcription. To date, the vast majority of well-described Notch functions are mediated through the NTC, termed "canonical" signaling. In canonical Notch signaling, phenotypes obtained with NTC knockout should be congruent with those observed upon Notch receptor or ligand deletion. For example, T-cell development is similarly affected by *Dll4*, *Notch1*, or *Rbpj* inactivation. However, it is not certain that all Notch phenotypes rely on canonical signals, and Notch-dependent but NTC-independent pathways are reported for selected phenotypes, for example, in mouse hair follicles.[9] The molecular mechanisms of such "non-canonical" Notch signaling remain to be described.

In this issue, Duarte et al provide new information by genetically inactivating *Rbpj* in mouse hematopoietic progenitors, and carefully studying the consequences of this intervention on HSC maintenance and myeloid, E, and Mk differentiation. In a series of rigorous studies, the authors showed that *Rbpj* was dispensable for HSC homeostasis and did not affect myeloid, E, or Mk development, both in steady-state and stress conditions. Furthermore, the authors investigated the effect of *Rbpj* inactivation on expression of several canonical NTC targets, and the only impact was increased *Hes1/Hes5* expression, which may reflect Rbpj-mediated repression in the setting of low Notch signaling intensity. These data confirm and extend previous studies, bolstering the evidence that cell-autonomous canonical Notch signals are nonessential for HSC and myeloid homeostasis.

How do the findings of Duarte et al relate to studies that identified important roles for Notch signaling in myelopoiesis? Using approaches that depend on a dominant-negative version of Mastermind or by inactivating the upstream Notch regulator, Nicastrin, other investigators reported defects in megakaryopoiesis and/or erythropoiesis, and enhanced myelopoiesis, respectively.[2,5,6] It is possible that these approaches influence pathways that are unrelated to Notch or that the effects may involve yet-to-be-defined noncanonical Notch signals. Consistent with the latter, *Notch1-3* inactivation also resulted in E defects and myeloid expansion.[2,6] Although noncanonical Notch signaling is an attractive model, another possibility is that these approaches led to markedly reduced Notch signaling that was optimal for producing these phenotypes, whereas *Rbpj* deletion incompletely abrogated downstream consequences of Notch signaling due to loss of Rbpj-mediated repression. In contrast to loss-of-function studies, enhanced Notch signaling can expand both human and mouse HSCs and myeloid progenitor cells. Thus, Notch signaling dose, timing, and context are critical. It is also possible that non–cell-autonomous effects contributed to the phenotypes that were reported previously upon interference with Notch signaling in hematopoiesis.[10] Such mechanisms could play important roles when Notch ligands are blocked or inactivated in the hematopoietic microenvironment, for example, in endothelial cells, or when genetic systems and experimental transplantation strategies fail to strictly restrict Notch loss of function to hematopoietic progenitors.

Although the findings of Duarte et al do not support an essential cell-autonomous role for canonical Notch signaling in myelopoiesis, questions remain about the disparate results obtained using different models. Is there a way to achieve consensus? One approach that may clarify the discrepancies is to combine the different genetic models. This is not a task for the timid; however, a recent study from Turkoz et al showed that taking a rigorous genetic approach in hair follicle development can reveal the roles of both canonical and noncanonical Notch signaling, and identify potential contributions from Rbpj-mediated target gene repression.[9] In this study, the authors sought to understand why *Rbpj* inactivation in utero resulted in milder skin phenotypes than blocking Notch receptor activity. By combining expression of a loss-of-function *Notch1* allele with *Rbpj* deletion, they could identify Rbpj-independent functions of Notch while ruling out a contribution of Rbpj-mediated repression to the phenotype. This example shows the power of combining multiple genetic models and suggests a path forward for resolving current controversies related to Notch signaling and hematopoiesis.

In summary, the recent work of Duarte et al raises the bar for studying canonical Notch signaling in hematopoietic progenitors. However, resolving the overall functions of Notch in this process awaits definitive experiments directly comparing complementary genetic approaches that interrogate the Notch pathway in defined cellular compartments.

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IMMUNOBIOLOGY AND IMMUNOTHERAPY

Comment on Manček-Keber et al, page 1720

Lymphoma exosomes reprogram the bone marrow

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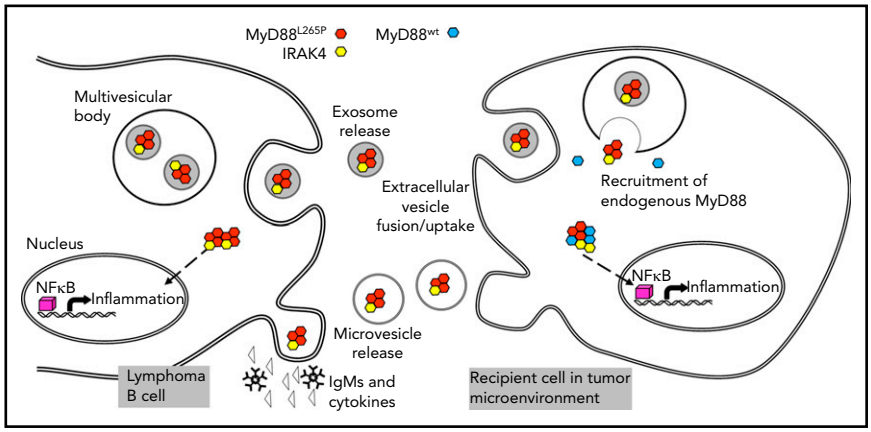
In this issue of *Blood*, Manček-Keber et al report that exosomes and microvesicles (MVs) produced by lymphoma B cells and carrying MyD88^{L265P} reprogram the bone marrow environment (BME) by activating endogenous proinflammatory signaling pathways in recipient nonmalignant cells.[1]

The report by Manček-Keber et al highlights the key role of extracellular vesicles (EVs) derived from malignant B cells in the conversion of mast cells and macrophages into cells that promote lymphoma progression. In 90% of patients with Waldenström macroglobulinemia (WM), B cells harbor a mutation in the innate immune signaling adapter MyD88.[2] MyD88^{L265P} is a gain-of-function mutation encoding the membrane-associated protein with a propensity to aggregate into the myddosome. Manček-Keber and colleagues found that EVs produced by WM tumor cell lines or those isolated from bone marrow (BM) aspirates of WM patients carried the signaling-competent MyD88^{L265P} and delivered the myddosome to mast cells and macrophages in coincubation experiments as well as in vivo in mice injected intravenously or intraperitoneally with PKH67-labeled EVs carrying MyD88^{L265P}.

What are EVs and how do they manage to transfer signaling complexes from one cell to another? When EVs were first discovered in the 1980s,[3] they were thought to be responsible for removing cellular waste. Today, EVs emerge as a universal communication system that conveys molecular and genetic signals to near or distant target cells and reprograms their functions.[4] EVs are membrane-bound vesicles of diverse cellular origins present in all body fluids. EVs comprise exosomes, MVs, and apoptotic bodies.[5] Exosomes are the smallest subset of EVs (30-150 nm) that originate from the endocytic compartment of parent cells. MVs are larger and are formed by budding of the parent cell membrane.[6] EVs are coated with a wide variety of membrane-associated biologically active molecules, including cell adhesion molecules necessary for the uptake of EVs by recipient cells via phagocytosis, endocytosis, fusion with the membrane, or receptor-mediated uptake.[7] The EV lumen contains various soluble molecules and nucleic acids (messenger RNA, microRNA [miRNA], and DNA) that EVs shuttle from parent to recipient cells. Small (femtomolar) quantities of cellular contents in EVs delivered to recipient cells need to be amplified to achieve physiological effects; hence, EVs carry their own amplification machinery in the form of enzymes, transcription factors, and cytokines.[5] Isolation of EVs from cell line supernatants or body fluids by ultracentrifugation at >100 000*g* for 2 hours is still the most widely used collection method, although the recovered vesicles are mixtures of EV subsets. Methods for isolating EV from body fluids that discriminate between the EV subsets are being developed.[8] Tumor-derived EVs are reported to be associated with cancer progression and downregulation of antitumor immune responses.[9] EVs are also involved in chronic inflammation associated with the development of cancer.[10] For these reasons, tumor-derived EVs have recently been of great interest.



Lymphoma B-cell crosstalk with a recipient cell in the WM BM is mediated by MVs or exosomes carrying the MyD88^{265P} complex (myddosome). In the parent B cell, the mutated MyD88 protein activates the NF-κB pathway inducing proinflammatory cytokine and immunoglobulin M (IgM) release. MyD88^{265P} is also packaged into exosomes or is enclosed into MVs budding off the surface of the parent cell. These vesicles transport it to recipient cells such as mast cells or macrophages in the BME. Once taken up into the cytoplasm, the MyD88^{265P} complex recruits endogenous MyD88^{wt}, activates the NF-κB pathway, and reprograms the recipient cell to produce proinflammatory cytokines. See Figure 6 in the article by Manček-Keber et al that begins on page 1720.

The mechanisms EVs use for information transfer may vary, depending on the identity of a parent cell, the type of EV released, and the nature of recipient cells. Advantages of information transfer by EVs are that they circulate freely in body fluids, that intraluminal materials are protected from degradation en route, and that signaling molecules are delivered embedded in the membrane, which enhances their biological activity. Once EVs are delivered, they initiate signaling at the target cell membrane or reorganize transcription using miRNAs or both, and they initiate signaling cascades that activate endogenous molecular pathways. In