Chronic lymphocytic leukemia (CLL) is the most frequent adult leukemia in Western countries. Clinically, the tumor is highly heterogeneous, ranging from cases with stable disease with an almost normal life expectancy to cases in which the disease evolves rapidly. Hence, the prognosis is driven by the heterogeneity in the disease biology that is influenced by many factors, such as the presence of mutations in the variable region of the immunoglobulin heavy-chain genes. Recently, the use of next-generation sequencing technologies has expanded the knowledge of the genomic alterations in CLL and provided new tools for analyzing leukemic clonal architecture. The mutational profile of CLL can be characterized by a relatively low number of somatic mutations per case, few recurrent mutations at moderate frequency (5%–15%), and a long tail of recurrent low-frequency somatic mutations. The clonal architecture of CLL shows striking heterogeneity between patients that could have important clinical implications (1). At the biologic level, CLL is characterized by the progressive accumulation of monoclonal CD5+ B cells in blood, bone marrow (BM), and secondary lymphoid organs (SLO). In BM and SLOs, CLL lymphocytes can interact with a set of accessory cells, collectively known as stromal cells, which constitute the leukemia microenvironment (2). This microenvironment provides proliferation, homing, and survival signals to the malignant B cell clones that seem to limit the efficacy of current therapies. Accordingly, patients with CLL usually relapse after treatment. In vitro, stromal cells protect CLL lymphocytes from spontaneous and drug-induced apoptosis in a contact-dependent manner, but also through the secretion of a variety of chemokines. CLL cells also secrete chemokines (CCL3 and CCL22) that can attract accessory cells, such as T cells and monocytes, suggesting that CLL cells are not simply seeded in a supportive soil, the microenvironment, but instead are actively involved in a complex cross-talk that establishes and maintains the characteristic microenvironment of proliferation centers. Historically, BM has been considered the major tissue of the CLL microenvironment; however, increasing evidence suggests that the lymph node (LN) microenvironment is relevant.

Circulating CLL cells become attracted into the BM and SLOs by chemokine gradients established by tissue stromal cells, in a process also regulated by adhesion molecules and their ligands. It has been reported that bone marrow stromal cells (BMSC) secrete high levels of CXCL12 and that the interaction between CXCL12 and the corresponding chemokine receptor CXCR4 is the predominant factor for BM homing. For homing to SLOs, the critical chemokines are CXCL12, CXCL13, and CCL19/21. CLL cells express high levels of CXCR4, CXCR5, and CCR7 (3), which allow them to sense and follow CXCL12, CXCL13, and CCL19/21 chemokine gradients, respectively. In normal lymphocytes, CXCR5 regulates cell homing and distribution within LN follicles (4). Follicular dendritic cells (FDC) cluster in the B follicles of SLOs, where they capture and present unprocessed antigens to B cells to activate them. FDCs also produce CXCL13, which directs B lymphocytes to the “light zone” of the germinal center, whereas interactions with CXCL12-expressing stromal cells occur in the “dark zone” (5). In CLL, the CXCL12-CXCR4 axis has been extensively studied and already therapeutically targeted (2). However, the role of CXCR5 and the potential of CXCL13-CXCR5-targeted therapies in CLL have been much less explored despite the known functions of CXCR5 in tissue homing, adhesion, and survival of tumor lymphocytes.

In this issue of Cancer Discovery, Heinig and colleagues (6) provide some answers to the unresolved role of CXCR5 in the pathogenesis of CLL. They use the Eμ-Tcl1 transgenic mouse that resembles the aggressive form of CLL and displays less complexity than the human disease. However, this model has been extensively used in the dissection of CLL pathogenesis, is considered one of the most robust for CLL, and is suitable for the identification of microenvironmental factors that promote CLL cell growth (7).
Using CXCR5-defective Eμ-TCL1 mice, the authors first showed that CXCR5 depletion reduces Eμ-TCL1 leukemogenesis. Importantly, this chemokine receptor is indispensable for the recruitment of CLL cells into the germinal center in close proximity to FDCs, because CXCR5-defective cells localized in the marginal zone of the B-cell follicle. Accordingly, the authors also demonstrated that CXCR5-expressing Eμ-TCL1 leukemia has a proliferative advantage compared with the CXCR5-defective model. They reported that CXCR4 cannot compensate for CXCR5 deficiency, as Eμ-TCL1 leukemia cells localized exclusively in the germinal center light zone that is independent of CXCR4 (6).

Using intravital imaging, the authors also elegantly showed that Eμ-TCL1 leukemia cells reach the B-cell follicle faster than normal B cells, and the CXCL13–CXCR5 axis is crucial for regulating and guiding this process. The functional relevance of these findings was then addressed by the identification of a network of FDCs that locally interact with Eμ-TCL1 leukemic cells. Furthermore, to decipher whether tumor cells might support or initiate stroma cell network differentiation, they assessed the stroma-inducing capacity of tumor Eμ-TCL1 cells in Rag2−/− mice lacking FDCs, and they observed that tumoral cells are able to induce the formation of follicular-like CXCL13-expressing FDC networks. They also demonstrated using irradiated recipients that tumor Eμ-TCL1 cells are recruited to mesenchymal stromal cells. Thus, the authors propose an elegant model in which this interaction, controlled by CXCR5, provides the CLL cells with the stimuli that they need to proliferate in vivo through B-cell receptor (BCR) stimulation and paracrine secretion of cytokines.

Heinig and colleagues (6) then go a step further in dissecting the molecular mechanisms of maintaining these FDC structures. They inhibited lymphotoxin (LTαβ)/lymphotoxin-β receptor (LTβR) signaling in Eμ-TCL1 mice and in an adoptive transfer model of the human CLL line MEC-1 expressing an LTβR-Ig fusion protein and found that targeting this pathway profoundly retarded tumor CLL growth and abrogated the FDC network as well as CXCL13 expression. LTβR is mainly produced by T and B lymphocytes and by natural killer cells following cellular activation (8), and high expression has also been reported in CLL cells (9). In addition, FDC stimulation with cell-bound LTαβ can induce the expression of CXCL13 and other protumor cytokines (10). In this context, the results of the present study suggest that CLL cells reciprocally stimulate stromal cells through LTβR activation, resulting in CXCL13 secretion and stromal compartment remodeling.

Overall, the data presented by Heinig and colleagues (6) provide critical insights into understanding the dominant role of the chemokine receptor CXCR5 in the follicular localization of CLL cells, and identify FDCs as a crucial stromal cell population that supports CLL activation and proliferation (Fig. 1). Although the use of a mouse model that resembles the aggressive form of human unmutated CLL prevents us from drawing definitive conclusions, the present study has improved knowledge about the relationship between CLL cells and their microenvironment. The model presented by Heinig and colleagues (6) needs to be further explored and confirmed in human primary CLL samples, but strongly suggests that the development of compounds aimed to target the CXCR5–CXCL13 axis could represent a novel therapeutic strategy to block the cross-talk between CLL cells and the microenvironment.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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CXCR5-Mediated Shaping of the Lymphoid Follicle in Chronic Lymphocytic Leukemia

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