Ibrutinib and the chemotactic lymph node choreography

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The introduction of Bruton tyrosine kinase (BTK) inhibitors has revolutionized the therapeutic management of B-cell malignancies such as chronic lymphocytic leukemia (CLL), with ibrutinib being the first covalent inhibitor in its class. BTK plays a major role in B-cell receptor signaling but is also involved in other signaling pathways and can be expressed by other immune cells. Upon starting therapy, BTK inhibitors cause an initial increase in lymphocytosis which is driven by the release of activated CLL cells from lymph nodes.[1] This is therapeutically relevant as CLL cells proliferate exclusively in this compartment, in contrast to CLL cells in blood which are in a resting state. Consequently, subgroups of patients with enhanced homing and retention capacity in lymph nodes under therapy, e.g., due to high expression of the CD49d integrin, are more prone to develop resistance.[2,3]

The extent and kinetics of the treatment-induced lymphocytosis vary among CLL patients and there are still many gaps in understanding how BTK inhibition causes lymphocytosis. In fact, there is surprisingly little knowledge about the mechanisms of malignant B-cell trafficking out of lymph nodes. The study by Rey-Barroso and colleagues,[4] published in this issue of *Haematologica*, addresses some of these gaps by phenotyping the effects of ibrutinib on chemotactic properties of CLL cells and T cells (Figure 1). To this end, the authors employed a combination of *in vitro* flow cytometric phenotyping, chemotaxis assays, and time-lapse motility video imaging of cell motility parameters. They describe important common and differing properties of immediate (*in vitro*) and long-term (*in vivo*, "real-world") effects of ibrutinib.

To understand the dynamics of lymphocyte homeostasis in the lymph nodes, it is essential to consider the mechanisms by which normal B lymphocytes enter and exit the lymph node. Although it is well established that B cells rely on CXCR5 to enter the lymph node from the bloodstream via high endothelial venules, their modes of exit remain unclear. For example, the involvement of the sphingosine 1-phosphate (S1P)/S1P receptor 1 (S1PR1) axis in lymphocyte exit has been well described in the context of T cells but its role in B-cell exit is still under debate.[5] Furthermore, memory B cells are able to use unconventional egress routes, including the subcapsular sinus, the primary area where tissue-derived lymph fluid drains.[6]

Inside lymph nodes, normal B cells engage in continuous dynamic stop-and-go migration to encounter antigens and undergo activation and differentiation. The CXCR5 ligand CXCL13 plays a key role in attracting B cells to antigen-rich follicular dendritic cells and acts in concert with integrin ligands and antigen presented by these cells. The strength of antigen binding to the B-cell receptor and the migratory velocity of the malignant cells towards CXCL13 are reciprocally connected.[8] The chemokine receptor CCR7 directs B cells via its ligands CCL19 and CCL21 to T-cell zones, while CXCR4 and CXCR5 shuttle the B cells between dark and light zones via CXCL12 and CXCL13 during germinal center reactions. Our knowledge of lymphocyte dynamics in the lymph node is mainly based on murine models, and there are few reports addressing the differences from the human situation.[7] The study presented by Rey-Barroso and colleagues is, therefore, crucial in advancing our understanding of lymphocyte dynamics in human lymph nodes under therapy.[4]

It is important to note that in CLL, the lymph node architecture (such as compartmentalization of the B-cell zone and the T-cell zone, along with their zone-specific chemokines) is already disrupted at the start of therapy, with a chaotic positioning of tumor cells simultaneously overexpressing CXCR4, CXCR5, and CCR7. Long-term treatment with ibrutinib gradually restores the architecture to a more normal state. This might explain the differences that Rey-Barroso and colleagues found when comparing samples treated short-term *in vitro* with those collected during a 6-month monitoring of therapy. In particular, the repression of CXCR4- and CXCR5-induced migration in CLL cells *in vivo* was different from the *in vitro* situation and the effect of ibrutinib on the basal and chemokine-evoked migration of T cells was milder. Ibrutinib has off-target activity which affects several other tyrosine kinase pathways beside BTK, including TEC family kinases such as ITK,[9] which affect T-cell responses. This off-target suppression may be particularly pronounced *in vitro*. Another explanation might be that

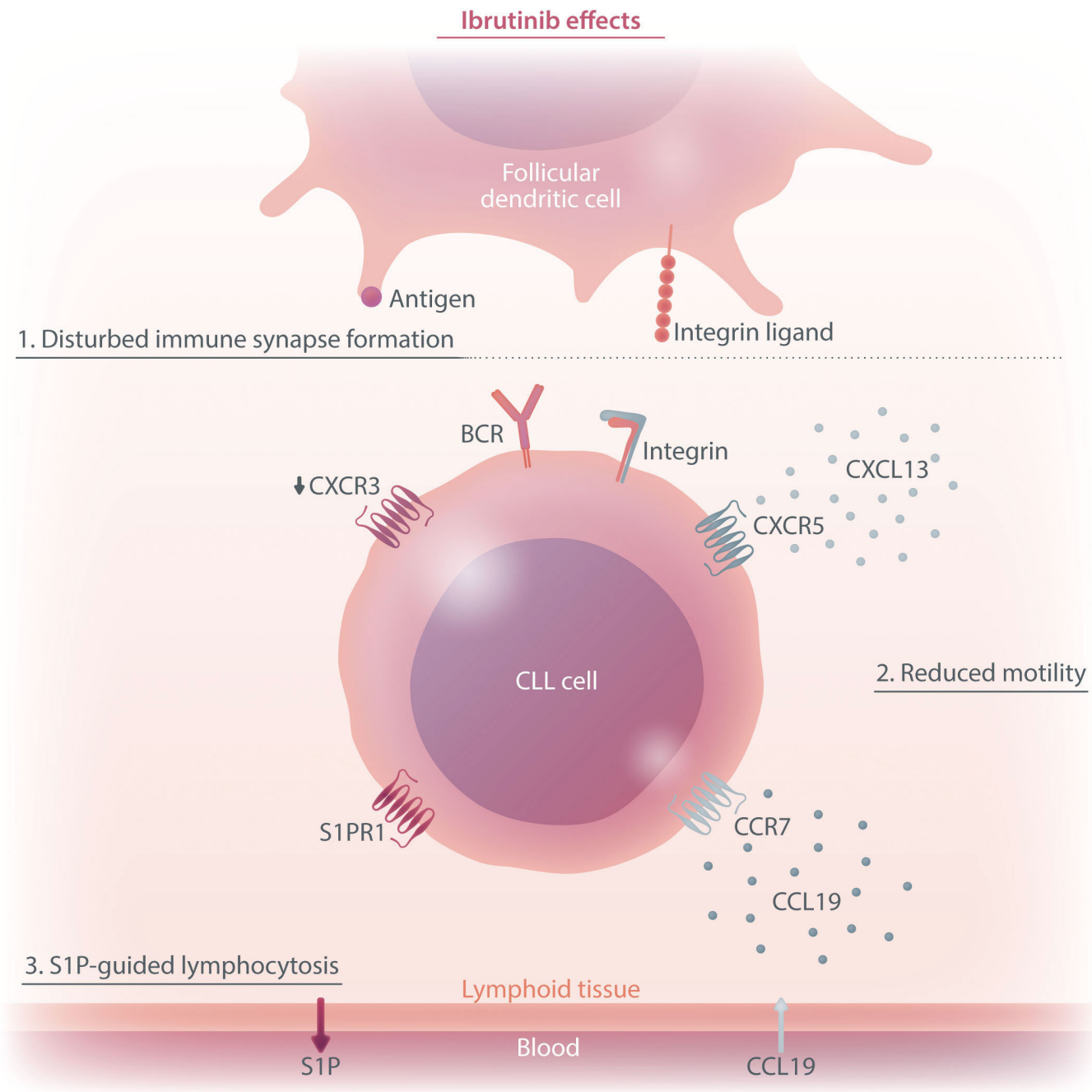

Figure 1. The effects of ibrutinib on the chemotactic properties of chronic lymphocytic leukemia cells. The figure summarizes the chemotactic changes that lead to initial lymphocytosis under ibrutinib treatment. First, the immune synapse between tumor cells and follicular dendritic cells is disrupted, leading to delocalization of the tumor cells. Secondly, delocalization is promoted by reduced tumor cell motility towards CXCR5, CCR7 and CXCR3 ligands, which would direct the cells towards the follicular dendritic cell and activating T cells. Thirdly, increased responsiveness to S1P ultimately shifts the balance towards lymph node exit. BCR: B-cell receptor; CLL: chronic lymphocytic leukemia; S1P: sphingosine 1-phosphate; S1PR1: S1P receptor 1.

the restoration of the lymph node architecture during long-term treatment alters the migratory properties of the T cells. Consistent ibrutinib-dependent modulation was observed for CXCR3 expression in CLL cells, with an almost complete loss of expression after 2 months, while CXCR4 expression did not vary along the 6-month follow-up period. This can be explained by the negative co-operativity of these two receptors in CLL.[10] In other words, CXCR3 engagement by its inflammatory ligands leads to desensitization of the CXCR4 responsiveness to its ligand CXCL12. This is caused by intracellular downstream components and does not require or necessarily translate into an alteration in CXCR4 expression.[10] It must be noted that during the CLL cell cycle, CXCR3 is dynamically expressed in a reciprocal manner to the activation marker CD69. Its loss in early phases of ibrutinib treatment likely reflects the initial mobilization of activated CLL cells from lymph nodes, in line with previous observations.[1]

In normal lymphocytes CD69 and S1PR1 can be considered counteractors. An important aspect of the study by Rey-Barroso and colleagues is the relation of S1PR1 to CCR7 expression as a key determinant of the strength of BTK inhibition-induced lymphocytosis. This suggests that CLL cells use the classical S1P axis for exit, in analogy to B cells upon a T-dependent antigen response, with CCR7 dominance resembling an activated B cell that constantly migrates towards the T-cell zone. It is interesting that the CCR7 responsiveness towards CCL19 stimulation was retained under continuous ibrutinib treatment.

Rey-Barroso *et al.* did not use the validated clinical cutoff of 30% for CD49d low and high cases, and there was a relevant proportion of CD49d (VLA-4)-negative cases in their cohort. It will be beneficial to combine their chemotactic observations with those in validated CD49d-expression subgroups of patients, the highly variable extents of lymphocytosis in individual patients and, most importantly, the

outcome of patients under BTK inhibitor therapy.
In conclusion, the study by Rey-Barroso and colleagues deepens our understanding of the intricate processes underlying lymphocytosis in CLL. By uncovering the dynamics of cell motility and chemotaxis, and by bringing our attention to the differences between *in vitro* and *in vivo* studies, the authors provide a solid basis for further exploration of the mechanisms underlying the clinical efficacy of BTK inhibitors. Further investigations considering different subgroups of patients and outcomes will help unravel the full potential of this therapeutic approach.

Disclosures
No conflicts of interest to disclose.

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