Novel approaches to CLL treatment are needed to prevent disease relapse. KIs targeting Bruton’s tyrosine kinase (BTK) and the phosphoinositide 3'-kinase δ (PI3Kδ) are showing very impressive early clinical responses in CLL by inhibiting kinases that are essential for B-cell receptor (BCR) signaling and tumor microenvironment (TME) protective signaling. However, there is emerging evidence that the use of such targeted drugs in some CLL patients may lead to the emergence of resistant tumor cell variants that harbor mutations in kinase genes. It is also possible that leukemic cells could circumvent BCR-targeted therapies by co-opting alternative oncogenic signaling pathways, but these heterogenic signaling pathways in the TME remain to be fully delineated. CLL tumor cells are known to engage in incompletely defined cellular interactions with different stromal cells that reside in the complex TME that include activated CLL Ag-specific CD4+ helper T-cells (contrasting with functionally suppressed CD8+ cytotoxic T-cells in CLL patients). Clearly, identifying new membrane-associated receptors and their ligands in CLL will be essential to design new therapeutic targets that could complement KIs to combat therapy resistance. Clinical scientists are particularly interested in using agents with a mechanism-of-action distinct from the KIs. Immunotherapy aimed at harnessing endogenous anti-tumor immunity could help effectuate long-term tumor control or clearance. The combination of targeted therapy with immunotherapy has the potential to represent a next generation treatment strategy for CLL and other hematologic cancers. A major goal is the identification of TAA to harness the full potential of immunotherapy.

This article by Dubovsky and colleagues describes a novel strategy that exploits the specificity of the cancer patient’s anti-tumor humoral immunity to identify LCP1 (also...
known as β1-plasmin) as a CLL membrane target Ag that should have relevance for understanding disease pathophysiology and the use of biological-immuno-therapies in future trials. Their methodology allows the interrogation of purified CLL cell extracts (biotinylated separation) with autologous patient serum to isolate immunoreactive membrane Ags. Subsequent mass spectrometry analysis allows the identification of candidate proteins for further laboratory studies (see figure). The authors then go on to validate that LCP1 is a highly reactive CLL Ag in multiple CLL patients using quantitative enzyme-linked immunosorbert assay with recombinant LCP1 protein. Their results suggest that LCP1-specific humoral immunity in CLL is elevated compared with healthy donor serum samples (even though healthy B-cells also appear to express strong LCP1). An important caveat for this study and others in the field relates to the use of age-matched healthy donor samples that can help contribute to our understanding of the role of aging and its associated deterioration of immune and inflammatory control systems. A striking finding from this study is that CLL patients possess the ability to form anti-LCP1 immunoglobulin (IgG) despite the lack of IgG-based immunity to strong vaccine Ag (tetanus). This suggests that a strong LCP1 autoimmune response is active in spite of the profound immune dysfunction that is a hallmark of CLL (that impacts on T-cells and B-cell effector functions). A mechanistic explanation for this immune discrepancy is provided by results showing that LCP1 protein is located in the cell nucleus, cytoplasm, and is secreted within exosomes of both CLL and healthy B-cells. Exosomes released from transformed cells have been shown to stimulate CD4+ T-cells in an Ag-specific manner. The significant accumulation of CLL cells during disease progression could cause elevated production of exosomes and increase their ability to present Ag (representing chronic “exosome-antigen presentation”). It is tempting to speculate that this mechanism may contribute to the generation of activated CLL Ag-specific CD4+ helper T-cells that appear to confer pro-tumor survival activity within tissue proliferation centers. Next, the authors demonstrate that LCP1 has potential pathogenic functional significance with short interfering RNA knockdown assays demonstrating impaired in vitro CLL transwell migration to the critical TME chemokine CXCL12. In vivo xenotransplant leukemia studies have great potential as laboratory tools to study the complex and heterogeneous genomic landscape of primary human patient samples and their impact on disease pathophysiology. In vivo characterization, with subsequent flow cytometric and immunohistochemistry read-out assays, showed that LCP1 plays an important role in leukemia homing/migration to the bone marrow niche. Notably, the KIsibrutinib and idelalisib blocked LCP1 activation downstream of BCR ligation on leukemic cells (by inhibiting phosphorylation of the serine-5 residue on LCP1 that regulates filamentous [F]-actin binding and subsequent lymphocyte migration). LCP1 appears to be a highly relevant molecule in CLL, and the authors speculate that it may act as a critical molecular scaffold during protein kinase C-β-driven oncogenic signaling in CLL. The role of LCP1 in regulating CLL B-cell dynamics and homing in the TME warrants further preclinical studies, including study of the effect of KIs in vivo that was not determined in this initial study.

How does LCP1 link to future combinational therapy in CLL? This is an important translational research question and could involve the development of monoclonal antibodies (mAb) that could act as biological therapies interfering with biological homing and critical disease pathogenesis events, including the promotion of immune-mediated tumor cell killing. The immunomodulatory activity (iMiD) lenalidomide has shown impressive clinical trial results in CLL by enhancing both anti-tumor specific granzyme-B+ T-cells and B-cell immunity (humoral autoreactivity against CLL-membrane Ags, such as receptor tyrosine kinase-like orphan receptor, ROR1). Taken together, targeted KIs that inhibit pro-tumor TME signaling may show synergistic activity with biological therapies (future use of clinical anti-LCP1 mAb) or immunotherapy (lenalidomide or programmed-cell death-1, PD-1 immune checkpoint blocking mAbs). This combinatorial approach could inhibit CLL homing to the TME (blocking BCR-signaling and LCP1 activation), while activating anti-tumor immunity against residual tumor membrane-exosome-associated Ags including LCP1. Another potential implication of this study could be the analysis of purified exosomes from patient sera and their associated tumor-Ag cargo (e.g., LCP1) that could act as a response to therapy biomarkers for new agent correlative science studies.
Identifying CLL antigens for future combinational therapy

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