leads to increased cytokine production by T cells and hyperphosphorylation of 2 key signaling intermediates downstream of T-cell activation, LAT and ERK. 4.1R colocalizes with LAT at the site of TCR activation (see figure) and mediates its effects on T-cell activation through direct association with LAT. 4.1R binding to LAT inhibits LAT phosphorylation by ZAP-70. These results show that 4.1R is a negative regulator of T-cell function through effects on LAT phosphorylation.

Interestingly, while 4.1R and ERM proteins share significant sequence homology, this report shows that 4.1R and ERM family members play fundamentally opposing roles in their regulation of T-cell activation. ERM proteins move negative regulators to the DPC to positively regulate T-cell activation, while 4.1R localizes to the immunologic synapse to negatively regulate T-cell signaling. ERM proteins differ in the mechanism by which each links the actin cytoskeleton and signaling molecules downstream of the TCR. While the precise mechanism by which 4.1R mediates the inhibition of LAT phosphorylation by ZAP-70 remains to be determined, Kang et al contribute to our broader understanding of how actin cytoskeletal regulators can affect T-cell signaling and function.

**Conflict-of-interest disclosure:** The author declares no competing financial interests.

**REFERENCES**


**LYMPHOID NEOPLASIA**

Comment on Cullion et al, page 6172

**Notch targeting 2.0**

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In this issue of Blood, Cullion and colleagues add an encouraging chapter to the saga of Notch1 as a therapeutic target in T-ALL.

Notch1 is a member of a family of highly conserved receptors that normally signal by way of a series of ligand-induced proteolytic cleavage events. These permit the intracellular portion of Notch1 (ICN1) to gain access to the nucleus, where it forms a short-lived transcriptional activation complex. The final cleavage that liberates ICN1 is carried out by γ-secretase, a multiprotein complex that also implicates the generation of amyloidogenic peptides from β-amyloid precursor protein in the brains of patients with Alzheimer disease. Interest in Notch1 in T-cell acute lymphoblastic leukemia (T-ALL) has been sparked by the recognition that acquired Notch1 mutations leading to elevated levels of ICN1 are found in the majority of human T-ALLs as well as many murine T-ALL models.1 Subsequent studies have shown that ICN1 drives the growth of T-ALL cells, in large part due to its ability to up-regulate c-Myc expression and enhance signaling through the PI3-kinase/AKT/mTOR pathway. The increases in ICN1 levels caused by Notch1 mutations are counteracted by drugs that inhibit γ-secretase, a large number of which are in preclinical development due to the link between γ-secretase and Alzheimer disease. This fortuitous circumstance made Notch1 a very attractive rational therapeutic target, but the first attempt to treat patients with refractory/relapsed T-ALL with an oral γ-secretase inhibitor (GSI) was plagued by both treatment failures and “on-target” gut toxicity.2 The latter probably resulted from goblet cell metaplasia, as in the absence of Notch signaling the differentiation of epithelial cells lining the small bowel and colon is skewed toward goblet cell fate and away from enterocyte fate. Although these were the early days, the disappointing results of this trial raised serious questions about the future of Notch-directed therapeutics.

The tide may have turned, however, based on 2 recent reports. Earlier this year, Ferrando’s group reported that GSI and dexamethasone, long known to be highly active against ALL, have strongly synergistic anti-T-ALL effects in vitro and in murine xenografts.3 This, in and of itself, is not completely surprising, as Notch1 signaling had been shown through retroviral mutagenesis screens conducted more than a decade ago to protect against dexamethasone-mediated killing of murine T-cell lines.4 What was entirely unexpected was that dexamethasone also protected mice against GSI-induced gut toxicity by blocking goblet cell development and shifting differentiation back toward enterocyte fate. One critical uncertainty hangs over this remarkable observation, however. The dose of...
dexamethasone used in these studies was very high (15 mg/kg), well above that which can be tolerated by patients, and it remains to be determined whether clinically achievable doses of dexamethasone will have the same salutary effect on GSI-limiting gut toxicity.

Now, Cullion et al provide an alternative way forward, again using mouse models. Cullion et al take advantage of the fact that it takes about 5 to 7 days for a stem cell in a gut crypt to give rise to enterocytes and goblet cells and show that an intermittent, 3-day on/4-day off GSI dosing schedule largely avoids gut toxicity while maintaining significant anti-T-ALL effects. They also demonstrate that the efficacy of GSI is enhanced by the addition of rapamycin, an inhibitor of mTOR. This is in line with prior work showing that mTOR is activated by Notch signaling, at least in part through its ability to suppress PTEN, an important brake on the PI3-kinase/AKT/mTOR pathway. Of note, Armstrong's group has shown that rapamycin also synergizes with glucocorticoids to kill ALL cells, raising the possibility that some combination of Notch and mTOR inhibitors and glucocorticoids might ultimately prove to have the most activity against malignant T lymphoblasts. Substantial further improvements in therapeutic index are needed if Notch inhibitors are to move into clinical practice, but with a number of promising paths to pursue, there is new reason for optimism.

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In this issue of Blood, Nervi and colleagues and Zeng and colleagues independently report similar findings in both in vitro and in vivo AML models, showing chemosensitization by blocking CXCR4/CXCL12 (SDF-1α: stromal cell–derived factor 1) signaling using novel CXCR4 antagonist bicyclams, namely AMD3100 (plerixafor) and AMD3465.

CXCR4 and its ligand SDF-1/CXCL12 have been identified as key participants in many pathways including cell movement, HIV entry into permissive cells, leukemic and normal stem cell trafficking, and remodeling of marrow niches. The impact of CXCR4-SDF-1/CXCL12 signaling has recently been linked to cell survival and chemosensitization in several cancer models including breast, lung, prostate cancer, multiple myeloma and chronic lymphocytic leukemia as well. SDF-1/CXCL12 and stem cell factor / kit ligand (SCF/KL) are elaborated by marrow osteoblasts/ stromal cells and specialized endothelial cells and are essential components of hematopoietic marrow niches (see figure). Recently, leukemic cells were shown to suppress normal hematopoiesis by down-regulating CXCL12 within these niches while neutralization of SCF/KL improved migration of normal progenitors within these areas, hinting at new mechanisms of leukemia-mediated suppression of normal hematopoiesis. Similarly, granulocyte colony-stimulating factor (G-CSF)–mediated stem/progenitor cell mobilization involves secretion of proteases, attenuation of integrin adhesive function, and disruption of CXCL12/CXCR4 signaling in the marrow. The same principle applies to other mobilizing cytokines, such as FLT3 ligand and SCF, which down-regulate CXCL12 expression within the marrow, and decrease CXCR4 expression on hematopoietic progenitor cells, which underscores the central role of the CXCR4/CXCL12 axis in mobilization processes. AMD3100 (plerixafor) has now received FDA approval to boost G-CSF–mediated CD34 stem cell mobilization in lymphoma and myeloma.

Using a murine acute promyelocytic leukemia (APL) model, Nervi et al show convincingly that AMD3100 can mobilize leukemic cells from intramedullary (but not intraperitoneal) niches into the peripheral circulation and the spleen. They also show that stromal cells interacting with leukemic cells confer chemoresistance. The dissociation of APL cells from their marrow microenvironment and/or the interruption of CXCR4 signaling by AMD3100 may explain the increased cell kill observed with cytarabine and anthracyclines. Stromal APL protection can be mediated by direct cell-to-cell contact as well as via soluble stromal-derived factors. These events were tracked in vivo using novel imaging techniques and provide the underpinning of a proof of concept clinical trial in which relapsed AML patients are treated with MEC plus escalating doses of plerixafor. While CXCR4/CXCL12 interactions are the focus of these 2 manuscripts, the dominant role of VLA-4/VCAM-1 integrin-mediated adhesion to matrix components in stem cell homing and peripheralization should not be forgotten. A more optimal dislodgement of leukemic cells from their niches might be attained by combining the CXCR4/CXCL12 blockade with agents that disrupt VLA-4/VCAM-1 interactions such as natalizumab (anti-VLA-4 monoclonal antibody) or Bio5192, a small peptide that also blocks VLA-4 mediated adhesion (see figure).

Besides abnormal cytogenetics, recently recognized high-risk features in AML include FLT3-ITD/ mutations and prominent CXCR4 surface expression. In the other paper addressing leukemic niches in this, Zeng et al focus on AML cell lines as well as patient-derived AML cells and determine that the CXCR4 antagonist AMD3465 blocked signaling of the CXCL12/CXCR4 axis by suppressing stroma-activated PI3K/AKT and MEK/
Notch targeting 2.0

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