is a member of the tumor necrosis receptor superfamily and binds to a proliferation-inducing ligand (APRIL) and B-cell–activating factor (BAFF) with, as net effect, promotion of plasma cell proliferation and induction of antiapoptotic proteins.

Others have previously reported the targeting of BCMA with nonengineered mAbs. BCMA is highly and homogeneously expressed in virtually all myeloma patients, with little or no expression in normal tissues including human CD34+ cells, which should limit any mAb-mediated organ and hematopoietic toxicity. GSK2857916 is of particular interest because it displays multiple mechanisms of action and the potency of the native mAb is enhanced in several ways. First, defucosylation of the Fc region carbohydrates of the antibody increases the binding affinity to FcyRIII receptors and potentiates antibody-dependent cell-mediated cytotoxicity (ADCC). Similar glycoengineering helps to explain the enhanced efficacy of the novel anti-CD20 mAb, obinutuzumab. Second, the mAb is conjugated via a noncleavable linker to its cytotoxic cargo, monomethyl auristatin F, which binds to tubulin and inhibits polymerization, thus disrupting mitosis through G2/M arrest with induction of apoptosis. The use of a noncleavable linker has the advantage that GSK2857916 should be more stable in the blood with minimal spontaneous release of the cytotoxic conjugate. The experiments by Tai et al suggested that GSK2857916 is efficiently internalized and spares bone marrow stromal and effector cells. Further mechanisms of action include macrophage-mediated phagocytosis and the interruption of the BCMA/BAFF/APRIL pathway leading to inhibition of nuclear factor-κB signaling.

High levels of soluble BCMA (sBCMA) have been reported in the serum of myeloma patients and have been correlated with progressive disease and worse outcome. Tai et al added MM1s cell supernatants (a source of sBCMA) to ADCC assays and noted some reduction in lysis of myeloma cell lines which was partly reversible by addition of lenalidomide. Clinical studies will have to establish whether a sBCMA “sink” could potentially interfere with the efficacy of GSK2857916. BCMA is expressed by plasma cells and B-cell subsets and anti-BCMA mAb therapy may affect these lineages. However, this potential toxicity is not likely to preclude clinical application. Two other nonglycoengineered ADCs, nBT062 (indatuximab ravtansine) and IMGN901 (lorvotuzumab mertansine), respectively, targeting CD138 and CD36, are presently in phase 1 clinical trial for myeloma. Dose-limiting toxicity of nBT02 was skin and gastrointestinal-related, and objective responses were observed in 2 of 20 patients. IMGN901 elicited a partial response in 1 of 25 patients treated.

BCMA is an interesting molecule from an immunotherapy perspective. Anti-BCMA antibodies have been detected as part of the graft-versus-myeloma response following donor lymphocyte infusion after allogeneic transplant, and patient-derived serum killed primary myeloma cells. BCMA-derived peptides can generate antigen-specific T-cell responses and are candidates for future vaccination strategies. T cells transduced with anti-BCMA chimeric antigen receptors have been reported to kill primary myeloma cells in vitro and in a mouse model, and will likely be tested in clinical trial. GSK2857916 will be both the first defucosylated ADC compound tested in multiple myeloma and the first BCMA-based immunotherapy entering the clinical arena.

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**MYELOID NEOPLASIA**

Comment on Nelson et al, page 3152

**A(nother) RAF mutation in LCH**

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In this issue of Blood, Nelson et al1 describe a novel somatic ARAF mutation in a child with Langerhans cell histiocytosis (LCH) and demonstrate that the encoded protein has strong gain-of-function properties. Importantly, this mutant A-Raf molecule is sensitive to inhibition by vemurafenib, a potent and selective Raf kinase inhibitor that is Food and Drug Administration (FDA)-approved for the treatment of advanced melanoma.2,3 This work thus identifies a new driver mutation in LCH that is potentially actionable in the clinic.

LCH is a rare hematologic disorder that is classified as a unified disease entity based on common histopathologic features and the proliferation of cells with phenotypic and cell surface marker expression characteristic of Langerhans cells.4 However, the clinical presentation is highly variable, ranging from generally benign single system lesions to life-threatening multisystem disease with organ dysfunction. The antigen-presenting function of Langerhan’s cells, mixed population of infiltrating leukocytes in pathologic specimens,
and protein clinical manifestations naturally raised questions regarding whether LCH is primarily an immunologic or neoplastic disease. Studies demonstrating that LCH lesions are clonal followed by genetic analysis demonstrating somatic \(BRAF^{V600E}\) mutations in >50\% of cases provided compelling evidence that LCH is a hematologic neoplasm.\(^4,5\) Although \(BRAF^{V600E}\) mutations do not define clinical risk groups,\(^5,6\) they are associated with a higher incidence of disease recurrence. Interestingly, Beres et al\(^7\) recently reported that \(BRAF^{V600E}\) mutations are restricted to lesional CD207\(^+\) cells in low-risk patients. However, high-risk patients carried \(BRAF^{V600E}\) mutations in CD207\(^-\) lesional cells, circulating CD11c\(^+\) and CD14\(^+\) cells and bone marrow CD34\(^+\) hematopoietic cell progenitors. These data suggest that high-risk LCH arises from somatic mutation of hematopoietic stem/progenitor cells, whereas low-risk LCH arises in tissue-restricted precursor dendritic cells.\(^6\) In addition to advancing our understanding of LCH pathogenesis, the presence of \(BRAF^{V600E}\) mutations in many patient specimens has immediate therapeutic implications, as the high prevalence of this mutation in melanoma and other cancers stimulated the development of small molecule inhibitors that induced genotype-specific responses in clinical trials.\(^2,3,7\)

The report of Nelson et al adds a new piece to the LCH puzzle. The current work was stimulated by the provocative observation that LCH lesions with and without \(BRAF\) mutations exhibit high levels of phosphorylated extracellular signal-regulated kinase (ERK), an important effector kinase of Raf in the Ras/Raf/mitogen-activated protein kinase kinase (MEK)/ERK signaling cascade\(^5\) (see figure). This suggests that mutations in other genes cause ERK activation in LCH patients lacking \(BRAF\) mutations. The authors address this question by performing whole exome sequencing of DNA isolated from the LCH lesions and normal tissues of 3 children. They uncovered a \(ARAF^{K351E}\) mutation in 1 case and, unexpectedly, a mutation in the related \(ARAF\) gene in lesional DNA from a second patient. Importantly, the \(BRAF\) and \(ARAF\) mutant allele frequencies were high in each case (45\% and 63\%, respectively), suggesting a growth advantage for mutant cells. Whereas \(ARAF\) mutations were thought to be uncommon in human cancer, a recent paper suggested that it is an oncogenic driver in ~1\% of lung adenocarcinomas.\(^8\) Given the potential significance of \(ARAF\) as a bona fide LCH oncogene and the complex mutation detected in this case (the mutant allele contains a sequence alteration that results in an amino acid substitution at codon 351 [F351L] and a 6-nucleotide in-frame deletion that removes amino acids 347 and 348), Nelson et al perform elegant functional analyses in which they demonstrate that the mutant A-Raf protein aberrantly activates recombinant MEK, a direct substrate of activated Raf (see figure). They further showed that the relative kinase activity of this mutant A-Raf molecule is comparable to B-Raf\(^{V600E}\) and that it can transform fibroblasts in a classic 3T3 soft agar assay. Finally and importantly, the authors show that this constitutively activated mutant A-Raf kinase is sensitive to inhibition by vemurafenib.

The identification and functional characterization of this novel \(ARAF\) mutation has biological and clinical implications. First, the discovery of a new somatic mutation altering the Ras/Raf/MEK/ERK signaling cascade reinforces the central role of this pathway in LCH pathogenesis and provides further impetus for comprehensive genomic analysis of additional cases lacking \(BRAF\) mutations. The unusual nature of the \(ARAF\) mutation described by Nelson et al suggested that it would be uncommon. Indeed, sequencing 23 other LCH specimens with normal \(BRAF\) failed to uncover additional \(ARAF\) mutations. Performing whole exome sequencing of additional patients without \(RAF\) gene mutations is a logical next step toward unraveling the pathogenesis of LCH. Second,
this study has implications for establishing a diagnosis of LCH and for identifying patients who might benefit from targeted therapies. Although ARAF mutations appear to be uncommon in LCH, they are straightforward to test for and are clinically actionable. As mutations in other genes are identified and functionally validated, it should be feasible to develop a targeted molecular diagnostics panel for LCH that includes BRAF, ARAF, and other driver genes. Finally, this new study raises questions regarding optimal approaches for implementing pathway-directed treatments for LCH. In addition to vemurafenib, the FDA-approved MEK inhibitor trametinib⁹ is a rational therapeutic strategy for patients with LCH with mutations that aberrantly activate Raf/MEK/ERK signaling (see figure). However, because the long-term risks and benefits of these agents are unknown and other effective treatments exist for many patients with LCH, the optimal indications for administering a tyrosine kinase inhibitor needs broad target blockade in BRAF-mutated melanoma. N Engl J Med. 2012;367(2):107-114.


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Comment on Kasirer-Friede et al, page 3156

Platelet αIIbβ3 activation: filling in the pieces

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In this issue of Blood, Kasirer-Friede and colleagues show that the adhesion and degranulation promoter protein (ADAP) promotes αIIbβ3 activation by presenting the cytoplasmic proteins talin and kindlin to the β3 cytoplasmic tail.¹

The integrin αIIbβ3 on circulating platelets is constrained in an inactive conformation by intramolecular interactions involving the stalk, transmembrane, and cytoplasmic domains of its αIIb and β3 subunits to prevent spontaneous platelet aggregation in the

Talin and kindlin bind to ADAP in resting platelets and are transferred to the β3 cytoplasmic tail after platelet stimulation. Whether intermediary proteins are involved in the associations of ADAP with the platelet membrane and with talin and kindlin is not clear. L αIIbβ3 ligand; GFFKR, conserved membrane proximal region of the αIIb cytoplasmic tail that helps to maintain inactive αIIbβ3. Adapted with permission from Figure B in O'Toole et al.¹¹ © 1994 Rockefeller University Press. Originally published in Journal of Cell Biology. 124:1047-1059. doi:10.1083/jcb.124.6.1047.

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