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III patients. This report may lead to a paradigm shift.

These data suggest that somatic FAS mutations constitute the second largest group of known mutations in ALPS, affecting approximately 10% of patients. Accordingly, a large number of patients with clinical features of ALPS do not fulfill current diagnostic criteria. On the basis of the findings by Dowdell et al as well as recent papers highlighting the ability to use biomarkers to diagnose ALPS,[3-5] an international ALPS consensus conference was held at the National Institutes of Health in 2009 with the goal of defining a better diagnostic algorithm. This new algorithm will be published shortly and incorporates genetic information, biomarkers, and histopathology in the diagnostic schema. This is an exciting time in ALPS research, as key insights into the diagnosis, pathophysiology, and treatment of ALPS have been recently published. New insights into signaling pathways, such as mTOR, that may be dysregulated in ALPS have been reported, leading to novel and effective treatments.[6]

ALPS may be a premalignant condition, as 10% to 20% of patients with germline *FAS* mutations develop cancer, most commonly lymphoma. This is consistent with the identification of somatic *FAS* mutations in both Hodgkin and non-Hodgkin lymphoma. The authors mention that no patients with somatic *FAS*-ALPS have been diagnosed with lymphoma and wisely point out that they are probably at risk and should be monitored carefully. Holzeva and colleagues surmised that somatic *FAS*-ALPS is not a premalignant state because TCR gene rearrangements were oligoclonal or polyclonal in the small subset they studied.[2] Whereas TCR gene rearrangement analysis is helpful in establishing clonality, further research is needed to determine whether somatic *FAS*-ALPS is a clonal disease. Clonal early lineage T-cell populations can produce polyclonal circulating T cells. A correlative example is T-lineage myelodysplastic syndrome that can have polyclonal TCR rearrangements, yet have clonal cytogenetic abnormalities. Hypothetically, in somatic FAS-ALPS, the mutation may arise in a T-cell clone that produces polyclonal DNTs. Nevertheless, clonality does not necessarily equate to malignancy, as patients with Epstein-Barr virus infections can develop monoclonal expansion of T cells that resolve spontaneously. Further investigation and prospective monitoring of these patients is needed.

A final lingering question that the study of somatic *FAS*-ALPS may help answer is whether DNTs drive the disease or are merely an epiphenomenon. If the mutation in *FAS* is localized to the DNT compartment, and patients develop the same phenotype as those with germline mutations, it suggests the DNTs drive the disease as they are the only affected cell. Recent work suggests DNTs are the drivers and not bystanders in lupus patients.[7] In summary, Dowdell et al have made a number of key observations in a poorly studied ALPS variant that can potentially change the understanding of ALPS pathophysiology and clinical practice.

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

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● ● ● LYMPHOID NEOPLASIA

Comment on Stein et al, page 5180

HLA-DR meets ERK

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In this issue of *Blood*, Stein and colleagues present a novel mechanism by which MHC class II antibodies induce apoptosis in lymphoid malignancies. These are important findings that provide fresh insight into a common antibody target.

While HLA-DR and other MHC class II molecules are typically known for their role in antigen presentation and elicitation of immune response, their increased expression in lymphoid and other hematologic malignancies has made them a prime target for antibody therapy in these diseases. Much has been published on the ability of HLA-DR directed antibodies to mediate death, but the pathway by which this occurs has remained elusive.

In this issue of *Blood*, Stein et al describe a new humanized monoclonal antibody to HLA-DR, IMMU-114.[1] This antibody has not yet entered the clinic but is in line for development with a promise of being different from other agents such as rituximab, ofatumumab, and alemutuzumab that are currently approved for marketing by the FDA for treatment of lymphoid malignancies. One drawback for current antibody therapies is the dependence on antibody-dependent cellular cytotoxicity or complement-dependent cytotoxicity, which requires competent immune effector cells and functional complement, respectively. Additionally, complement activation may also lead to significant side effects. IMMU-114, however, has been designed specifically to disable the ability to mediate ADCC and CDC, while maintaining direct cytotoxicity toward target cells. In fact, the effect of IMMU-114 direct cytotoxicity is equivalent in several lymphoid cell lines in vitro even in the absence of a secondary cross-linking antibody. This is not the case with monoclonal antibodies to other surface molecules such as CD74 and CD20 that require cross-linking for cytotoxicity, which suggests a novel mechanism of action for IMMU-114.

How then does a monoclonal antibody specifically kill tumor cells independently of antigen expression and ADCC? To address this question, the authors explore downstream

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signaling events following treatment IMMU-114, specifically looking at JNK-MAPK signaling activation. The MAPK pathway is constitutively active in several hematologic malignancies and is generally thought to prevent apoptosis.[2] However, in CLL, cytotoxicity with the anti-CD20 antibody rituximab has been demonstrated to be dependent on the p38-MAPK pathway.[3] In this particular study, while both ERK and JNK were phosphorylated following CD20 cross-linking, these pathways were not required for cell death.

IMMU-114 also induces phosphorylation of both ERK and JNK, which again occurs independently of the presence of secondary cross-linking antibody. Furthermore, the activation occurs quickly and correlates with increased ROS production and mitochondrial membrane depolarization. However, in contrast to previous studies with rituximab, inhibition of ERK, JNK, or both pathways combined using either pharmacologic inhibitors or siRNA completely abrogates the cytotoxic effect of IMMU-114. These studies establish a novel death pathway activated by IMMU-114 and provide justification for its differentiation from other therapeutic antibodies utilized for the treatment of lymphoid malignancies.

Overall, this article from David Goldenberg's laboratory presents exciting data, indicating that the induction of ERK and JNK signaling pathways play an important role in the cytotoxicity of the HLA-DR monoclonal antibody IMMU-114, providing a novel mechanism for this class of antibodies. More importantly, this manuscript takes the importance of signal transduction mediated by therapeutic antibodies to another level of significance by examing how these agents ultimately produce clinical response in patients with CLL and related B-cell malignancies. Given the importance of signal transduction through the ERK pathway by IMMU-114 to mediate cytotoxic effect in B-cell tumors, Stein et al have established this as a potential pharmacodynamic marker to utilize in clinical trials to predict who will respond to treatment. Additionally, this paper provides the basis to justify studies of what is different about the ERK signaling pathway in those who fail to respond to IMMU-114. An important question not addressed by the study is the influence of the microenvironment present in patients that also can contribute to activation or inhibition of specific signal transduction pathways. As IMMU-114 moves forward through preclinical studies necessary for introduction into clinical trials, it will be important to examine whether this same signaling pathway is relevant to primary tumor cells when placed under the protective auspices of stromal or cytokine microenvironment.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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● ● ● LYMPHOID NEOPLASIA

Comment on Beers et al, page 5191

Making a better antibody: all is not lost

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Effective anticancer therapy requires effective targeting of the malignant cells. In this issue of *Blood*, Beers and colleagues demonstrate that rituximab-induced loss of CD20 from the surface of B cells may explain why rituximab is more effective in some B-cell malignancies, such as follicular lymphoma, than in others like CLL.[1] They also provide evidence that anti-CD20 mAb, designated type II anti-CD20 mAb, induce considerably less down-modulation of CD20 than rituximab, and therefore could be more effective therapeutically.

Positive clinical trials reported almost yearly have led to expanded clinical indications for rituximab. Most of these trials have been empiric in design. Even with these broadening clinical indications, it remains enigmatic why rituximab-based therapy works better in some subjects than in others, and resistance often develops. The mechanistic explanations for primary or secondary resistance to rituximab remain unclear.

It has generally been accepted that an advantage of CD20 as a target antigen is that it does not down-modulate significantly when bound by monoclonal antibodies (mAb), that is, the anti-CD20/CD20 complex remains on the cell surface long enough for effector mechanisms to kill the target cell. This assumption is based on in vitro observations with a limited number of cell lines using short incubation times. In vivo, there is evidence that this may be different in select scenarios. Beum et al have described a "shaving reaction" in which mAb-CD20 complexes are "shaved" off chronic lymphocytic leukemia (CLL) cells by phagocytes as the malignant cells circulate.[2]

In this issue of *Blood*, Beers et al demonstrate in vivo in a mouse model and in vitro using malignant human B cells that rituximab can indeed induce down-modulation of CD20. The mechanism that Beers et al identifies—internalization of the rituximab/CD20 complex into the target cell itself—is distinct from that described by Beum and colleagues as it does not require phagocytes. Beers et al also demonstrate considerable variability in down-modulation based on the target cell type. They find considerable variability even within a given histology. In general, CLL and mantle cell lymphoma showed greater down-modulation of CD20 in response to rituximab than did follicular lymphoma and diffuse large B-cell lymphoma—a pattern that mirrors clinical response to rituximab.

It remains unclear which of the primary mechanisms of action that have been identified in preclinical and correlative studies (target cell killing induced by interaction between rituximab Fc and FcR, complement-induced lysis, or signaling-induced cell death) is responsible for clinical response to rituximab. Indeed, each of these mechanisms may be important in different scenarios based on the type of B-cell malignancy, location of the malignant cells, level of mAb achieved, and use of concomitant therapy such as chemotherapy. Irrespective of which of these mechanisms of action is important in a given scenario, the

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