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. However, in CLL, cytotoxicity with the anti-CD20 antibody rituximab has been demonstrated to be dependent on the 

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 examining 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.

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**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. 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.

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
The efficacy of rituximab therapy would be expected to be lost if the malignant cells remain viable after target antigen has been lost from the surface. If antigen down-modulation of CD20 limits the efficacy of rituximab therapy, is all lost with respect to making a better anti-CD20 mAb? Not according to Beers et al. They previously reported that type II (tositumomab) anti-CD20 monoclonal antibody outperforms type I (rituximab-like) reagents in B-cell depletion regardless of complement activation. Blood. 2008;112(10):4170–4177.

There are a number of next-generation anti-CD20 mAb in development including antibodies with enhanced affinity for FcR and enhanced ability to fix complement. Most of these are type I anti-CD20 mAb similar to rituximab. Tositumomab is a type II anti-CD20 mAb, first reported 30 years ago. It is a murine mAb that has been studied as a component of radioimmuno-therapy, but not extensively as a single agent or in a humanized form. A humanized type II anti-CD20 mAb, GA101, is now in early-phase clinical development. It is too early to know whether it will be more effective than rituximab.

So, when it comes to making a better anti-CD20 mAb, is all lost with loss of surface target antigen? Maybe not if the preclinical findings of Beers et al translate into more efficacious therapy in the clinic.

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REFERENCES

Comment on Sacharidou et al, page 5259

3D trumps 2D when studying endothelial cells

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An elegant study by Sacharidou and colleagues in this issue of Blood provides fascinating insights into a signaling complex that is critical for endothelial cells to form lumen and tube structures in a 3-dimensional (3D) culture system, but not for their migration in 2D cultures.
Making a better antibody: all is not lost

George J. Weiner