were taken before and after RTX treatment and then analyzed by flow cytometry using an array of probes. The methods and results are clearly presented, and the experiments are well controlled; there was no tumor cell elimination in the absence of RTX. NK cells were found to be essential to promote RTX-mediated elimination of targeted Raji cells, and NK cell–mediated ADCC was considerably better in the presence of the $CD4^+$ T cells. The positive effect of the $CD4^+$ T cells was also evident based on considerably slower tumor growth and enhanced survival curves of RTX-treated mice that had $CD4^+$ T cells in the TME. As expected, after NK cell–mediated ADCC due to RTX, the NK-cell levels ($CD56^+$ cells) in the TME decreased and the remaining NK cells manifested downregulation of both CD16 and CD25, indicative of an exhausted state. However, when the TME included $CD4^+$ T cells, NK-cell levels increased, and the cells retained and expressed considerably higher levels of CD16 and CD25, indicating they were far from exhausted and could be ready to engage in additional ADCC. Thus, addition of $CD4^+$ T cells into the TME appears to be an innovative strategy to enhance and restore ADCC of RTX opsonized target B cells by NK cells.

The provocative findings in the mouse model are reinforced by parallel clinical correlative studies that included analyses of FNA of tumors from patients with B-cell lymphoma treated with RTX and bendamustine. FNAs of patient tumors were taken before and after RTX treatment and analyzed by flow cytometry using the same cocktails of interrogating monoclonal antibodies (mAbs) as used in the mouse model studies. Projection of the mouse model studies to the clinical study suggests that higher levels of $CD4^+$ T cells in the TME of the patients should lead to better outcomes. Indeed, in those patients with the highest levels of $CD4^+$ T cells in the TME, there were substantial increases in expression of CD25 and CD16 in the NK cells, and elimination of B cells (based on the CD19 marker) tended to be more effective in tumors that had higher levels of $CD4^+$ cells. The investigators infer that these positive findings are likely a consequence of the action of IL-2 secreted by the $CD4^+$ T cells.

The implications of these results in the 2 models are considerable because they presage development of enhanced therapies that are based on RTX infusion. Simple use of infused IL-2 along with RTX may not be at all straightforward because of several problems,[9] but as noted by the investigators, RTX-based strategies, including bispecific mAbs that can engage CD3 on T cells and CD19 on B cells, would appear to be among the promising possible new approaches. Selection of the subsets of patients for RTX therapy based on high levels of $CD4^+$ cells in the TME would be reasonable.

This interesting and important report appears to have its origins based on a basic science article published almost 20 years ago.[4] Successful extension and testing of this work in the clinic is a likely next step.

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LYMPHOID NEOPLASIA

Comment on Liu et al, page 1825

BCL2 inhibition: back to the future!

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In this issue of *Blood*, Liu and colleagues report the preliminary characterization of a new BCL2-specific inhibitor (BCL2i), sonrotoclax, which not only binds with higher affinity to BCL2 than the first-in-class BCL2i venetoclax, but also induces apoptosis in models bearing venetoclax-induced *BCL2* mutations.[1] These important data suggest that sonrotoclax may find clinical applications in BCL2-dependent malignancies.

That BCL2 might be a therapeutic target for malignancy was evident from the demonstration of its direct involvement in the chromosomal translocation t(14;18)(q32.3;q21.3) in mature B-cell malignancies. The problem lay in how to target a protein lacking enzymatic activity; the solution came from a combination of functional and structural analyses. Most BCL2 protein is located at the outer mitochondrial membrane, where it suppresses programmed cell death (apoptosis), controlling membrane permeabilization and release of

cytochrome c mediated by oligomerization of the apoptosis effectors, BAX and BAK. BCL2 suppresses apoptosis by binding the proapoptotic molecule BIM. BCL2 binds BIM via a 20-Å-long hydrophobic groove (reviewed by Vogler et al[2]).

Inhibition of the antiapoptotic functions of BCL2 therefore required targeting of protein-protein interactions. This was first achieved by Rosenberg and colleagues in Chicago in a beautiful series of experiments, initially using high-throughput nuclear magnetic resonance screening and structure-based design.[3] The first inhibitor, navitoclax, bound not only to BCL2 but also to BCLxL and BCLw, due to the very close structural similarities of the hydrophobic grooves of BCL2 and BCLxL. Clinically, binding of navitoclax to BCLxL resulted in mechanism-based thrombocytopenia.[4] Therefore, to avoid this toxicity, navitoclax was carefully reverse-engineered, with alterations primarily involving 2 specific areas of interaction within the groove, termed the P2 and P4 hydrophobic pockets, with the aim of producing a BCL2-specific BCL2i. Remarkably, the derived molecule, venetoclax, has subnanomolar affinity for BCL2, with a thousandfold reduction in binding to BCLxL and BCLw.[5] These features underpin the transformational clinical success of venetoclax in chronic lymphocytic leukemia and other malignancies.[6]

Could a more potent BCL2i be produced? Given the available BCL2-venetoclax structures and venetoclax's activities in vivo, this seemed unlikely. But as the architect Mies van der Rohe said, "God is in the details." The "detail" here lies in the flexibility of the hydrophobic groove of BCL2 and its ability to accommodate different molecules with high affinity. From the data presented by Liu and colleagues, it appears that they have produced a novel BCL2i with some very interesting, perhaps unanticipated properties.

Precisely how sonrotoclax was derived is not clear from this article; we await the accompanying medicinal chemistry publication. However, sonrotoclax is of considerable interest structurally and biochemically. The crystal structures of BCL2 bound to sonrotoclax reveal very different modes of interaction within the P2 pocket resulting in a 20-fold increase in binding affinity in comparison with venetoclax. Improved binding of sonrotoclax to BCL2 translated into increased cytotoxicity in a limited number of cell line models. Importantly, sonrotoclax also exhibited activity against venetoclax-induced *BCL2* resistance mutations, notably *BCL2G101V,* therefore having potentially important clinical implications for sequencing and addressing one of the limitations of venetoclax.

Like all good studies, many questions emerge. Clinically, and perhaps most importantly, is whether such additional BCL2is are required in the era of venetoclax-based combinations and where these fit into the current treatment paradigms. Will BCL2is of enhanced potency result in deeper remissions?[7] Another question is whether BCL2is of enhanced potency will result in increased toxicity in normal BCL2-expressing cells. Reassuringly, no additional toxicity signals were reported in clinical trial data presented by Tedeschi et al.[8] Finally, more potent BCL2is may result in more profound apoptotic blocks as a cause of acquired resistance. *BAX* mutations and/or downregulation have been reported in response to both sonrotoclax and venetoclax in both malignant and normal cells; the long-term significance of these requires assessment.[9] BAX-null cells will be resistant to multiple therapeutic agents. Why *BAX* mutations occur only rarely outside of BCL2i therapy is not clear. Despite these potential caveats, sonrotoclax marks an exciting new development in BCL2i research and may have clinical implications beyond the current roles of venetoclax.

Venetoclax, either as a single agent or in combination with other precision medicines, has become a standard of care in several hematological malignancies. Several other BCL2is have been developed and are currently in early phase clinical trial.[10] The data presented by Liu and colleagues suggest that further interrogation of the flexibility of BCL2 may allow the derivation of more BCL2is of enhanced potency and selectivity, which bind to BCL2 in different modes. Rather than venetoclax being an end, it now seems likely that BCL2is are only at the beginning of a pharmacological journey.

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