New axis in lymphoma

By Kai-Jye Lou, Staff Writer

Researchers at the **National Cancer Institute** have found that mutant forms of an adaptor protein called MYD88 could drive prosurvival pathways in the most aggressive subtype of diffuse large B cell lymphoma.¹ The findings lay the foundation for developing new targeted therapies that could potentially improve the cure rate for this blood cancer.

The only targeted therapy approved for diffuse large B cell lymphoma (DLBCL) is Rituxan rituximab, a chimeric anti-CD20 mAb from **Biogen Idec Inc.** and the **Genentech Inc.** unit of **Roche**. Rituxan plus CHOP, a chemotherapy regimen consisting of cyclophosphamide, hydroxydaunorubicin (doxorubicin), oncovin (vincristine) and prednisone or prednisolone, is the standard of care for DLBCL, which is the most common type of non-Hodgkin's lymphoma (NHL) in adults. The combination therapy, dubbed R-CHOP, has a cure rate of about 50% in the overall DLBCL patient population. But the cure rate is less than 40% for the most aggressive subtype, called activated B cell-like DLBCL (ABC-DLBCL).²

Previously, the NCI group and other labs published studies linking the aberrant transcriptional activities of NF-κB and signal transducer and activator of transcription 3 (STAT3) to the survival and proliferation of ABC-DLBCL cells.³⁻⁵

However, those two targets also mediate many normal cellular processes. As a result, directly targeting NF-κB and STAT3 could cause side effects.

Thus, a team led by NCI's Louis Staudt, head of the Molecular Biology of Lymphoid Malignancies Section in the Metabolism Branch, has been looking for the precise causes of aberrant signaling in the NF-κB- and STAT3-mediated pathways. The goal is to selectively disrupt the abnormal signaling activity upon which lymphoma cells depend and minimize the impact on the normal functions of the signaling factors. According to the group's new paper in *Nature*, the MYD88 (myeloid differentiation primary response gene 88) pathway could fit the bill.

In 382 biopsies from patients with various types of lymphoma, the researchers found that about 37% of ABC-DLBCL samples had *MYD88* mutations. In contrast, *MYD88* mutations occurred in less than 10% of other evaluated lymphoma subtypes.

In a panel of human lymphoma cell lines, small hairpin RNA-mediated knockdown of *MYD88* or a downstream target, *IL-1 receptor-associated kinase 4* (IRAK4), killed ABC-DLBCL cells and had little effect against other lymphoma subtypes.

Immunoprecipitation and gene profiling studies showed that a gain-of-function mutation in *MYD88* drives the formation of a protein complex containing IRAK1 and IRAK4. That complex then acti-

Figure 1. MYD88-mediated modulation of prosurvival pathways in ABC-DLBCL. Activated B cell-like diffuse large B cell lymphoma (ABC-DLBCL) is the most aggressive form of lymphoma and has the lowest cure rate. Identifying new targets and pathways critical to the survival of these cells could aid the development of targeted therapies that improve cure rates in this subtype.

In *Nature*, researchers from the **National Cancer Institute** showed that mutant forms of the myeloid differentiation primary response gene 88 (MYD88) are associated with increased activity in NF-κB and signal transducer and activator of transcription 3 (STAT3) signaling pathways. The NCI group and other labs have previously linked aberrant NF-κB and STAT3 signaling to the proliferation and survival of ABC-DLBCL cells.

The researchers showed that the most commonly identified MYD88 mutant in ABC-DLBCL samples promoted the assembly of a protein complex containing IL-1 receptor-associated kinase 1 (IRAK1) and IRAK4 [a(1)]. This results in greater IRAK4 activity, which leads to increased IRAK1 phosphorylation (P) and NF-κB signaling [a(2)]. NF-κB signaling is associated with increased survival and proliferation of lymphoma cells and greater production of IL-6 and IL-10.

IL-6 and IL-10 can signal through their respective receptors and promote Janus kinase (JAK) activity, which leads to increased STAT3 phosphorylation and transcriptional activity [b(1)]. Like NF-κB, STAT3 signaling is associated with greater survival and proliferation in this lymphoma subtype and also stimulates the production of IL-6 and IL-10 [b(2)].

The researchers showed that small hairpin RNA-mediated knockdown at the level of MYD88 and IRAK4 inhibited NF-κB and STAT3 signaling and was selectively toxic against human ABC-DLBCL cells. In contrast, shRNA-mediated knockdown of MYD88 and IRAK4 had minimal effect in other lymphoma subtypes.

vates cell-survival signaling pathways through the NF- κ B and STAT3 pathways (see Figure 1, “MYD88-mediated modulation of pro-survival pathways in ABC-DLBCL”). Mutant forms of MYD88 caused stronger activation of NF- κ B signaling than wild-type MYD88. The mutants also increased production of cytokines that promote STAT3 signaling.

“This work is important from a genetic perspective, as the mutations we identified in MYD88 are, to my knowledge, among the most frequent to have ever been described in this type of lymphoma,” said Staudt, corresponding author on the paper. “We also show that the most prominent gain-of-function MYD88 mutant engages at least two previously identified survival pathways in this disease.”

High and MYD

The findings could pave the way for developing new targeted therapies that modulate the MYD88 pathway to selectively block aberrant signaling activity in the NF- κ B and STAT3 pro-survival pathways with minimal effect on the normal function of these transcription factors.

“In the last few years, we found that inhibiting the NF- κ B pathway can kill lymphoma cells, but we didn’t know the upstream targets that drove this pathway,” Staudt told *SciBX*. “Our current study uncovers a major new pathway that promotes the malignant survival of these lymphoma cells and also reveals some potentially juicy targets like IRAK4.”

Richard Davis, an associate professor in the Department of Lymphoma and Myeloma at **The University of Texas M.D. Anderson Cancer Center**, liked that the NCI group employed multiple techniques, including shRNA and a small molecule inhibitor, to show that IRAK4 kinase activity was essential to the survival of this form of DLBCL. “The immediate implication of this study is to determine whether an inhibitor of the IRAK4 kinase might be practical and then to develop one that will be suitable as a drug,” he added.

Jürgen Ruland, head of the Department of Molecular Immunology at **Technical University Munich**, added that in addition to fleshing out the safety profile and potential side effects of targeting the MYD88-mediated pathway, it will be important to develop a diagnostic that can pinpoint cases of ABC-DLBCL that are strictly dependent on the MYD88 pathway.

Moving to the source

Researchers contacted by *SciBX* agreed that the targets in the MYD88 pathway could potentially provide a safer entry point than directly targeting the transcription factor-mediated pro-survival pathways in ABC-DLBCL.

Ruland noted that NF- κ B signaling is important for generating normal lymphoid tissues and that targeting the NF- κ B pathway directly is known to cause significant toxicity in animal models.⁶

“In contrast, targeting the NF- κ B pathway at the level of the mutant MYD88 pathway could result in selective toxicity against the lymphoma cells and not significantly affect NF- κ B function in normal cells,” he told *SciBX*.

“This work is important from a genetic perspective, as the mutations we identified in MYD88 are, to my knowledge, among the most frequent to have ever been described in this type of lymphoma.”

**—Louis Staudt,
National Cancer Institute**

Indeed, the NCI group and others already have been pursuing treatments for ABC-DLBCL that indirectly inhibit NF- κ B signaling via other pathways.

In 2009, the NCI group published the results of a Phase II trial showing that chemotherapy plus Velcade bortezomib, a proteasome inhibitor that indirectly blocks NF- κ B signaling, increased response rates and median overall survival in patients with the aggressive subtype of DLBCL compared with patients that had a different form of DLBCL that was not driven by NF- κ B.⁷ The study also

showed that Velcade alone had little therapeutic effect against the disease.

Millennium Pharmaceuticals Inc., a unit of **Takeda Pharmaceutical Co. Ltd.**, markets Velcade to treat multiple myeloma (MM) and mantle cell myeloma. **Johnson & Johnson** has rights outside the U.S.

Last year, Millennium published a preclinical study showing that its MLN4924 blocked NF- κ B signaling and caused tumor regression in multiple DLBCL models, including those with the activated B cell-like subtype.⁸

MLN4924, a small molecule inhibitor of NEDD8 activating enzyme (NAE), is in Phase I testing for myelodysplastic syndrome (MDS) and multiple types of cancer, including lymphoma.

Ruland did caution that “MYD88 is known to have a role in the innate immune system. If you target the MYD88 signaling pathway directly, you could potentially cause short-term suppression of innate immunity.”

“In the short term, I think it could be possible to combine a treatment targeting the MYD88 pathway with chemotherapy,” Staudt told *SciBX*. “I would imagine that such a treatment would have synergy with chemotherapy and increase cure rates, as NF- κ B signaling is known to promote resistance to chemotherapy. While IRAK4 is known to play an important role in immune functions, it is conceivable in cancer to give a short-term treatment that will selectively kill the malignant cells.”

Going forward, Staudt’s group is continuing to look for signaling transduction inhibitors that modulate the MYD88 signaling pathway in ABC-DLBCL. The team also is studying how the MYD88 pathway is functionally linked to B cell receptor signaling, which is another pathway known to drive the aggressive DLBCL subtype.

The work described in *Nature* is unpatented.

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COMPANIES AND INSTITUTIONS MENTIONED

Biogen Idec Inc. (NASDAQ:BIIB), Weston, Mass.
Genentech Inc., South San Francisco, Calif.

Johnson & Johnson (NYSE:JNJ), New Brunswick, N.J.
Millennium Pharmaceuticals Inc. (NASDAQ:MLNM), Cambridge, Mass.
National Cancer Institute, Bethesda, Md.
Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland
Takeda Pharmaceutical Co. Ltd. (Tokyo:4502), Osaka, Japan
Technical University Munich, Munich, Germany
The University of Texas M.D. Anderson Cancer Center, Houston, Texas