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Combining antibodies to eliminate NHL

By Lauren Martz, Staff Writer

A **Stanford University School of Medicine** team has found a way to boost the efficacy of blockbuster non-Hodgkin's lymphoma drug Rituxan rituximab by combining the anti-CD20 antibody with mAbs against CD47.[1] The group thinks the combination could elicit fewer side effects than Rituxan plus chemotherapy, which is standard care for the blood cancer.

Rituxan is a chimeric antibody marketed by **Biogen Idec Inc.** and the **Genentech Inc.** unit of **Roche** to treat non-Hodgkin's lymphoma (NHL), chronic lymphocytic leukemia (CLL), rheumatoid arthritis (RA) and multiple sclerosis (MS). In cancer, the antibody induces cell death via apoptosis and antibody-dependent cellular cytotoxicity (ADCC), which helps promote phagocytosis.[2]

Rituxan alone is not curative for most B cell lymphomas, and some cancers have developed resistance to the antibody, which has led to adoption of a first-line therapy combining it with chemotherapy.[3]

In hopes of taking chemo out of the equation, Irving Weissman and Ravindra Majeti at Stanford have been looking to combine Rituxan with antibodies against CD47, an NHL surface protein.

Weissman is director of the Institute of Stem Cell and Regenerative Medicine at the school of medicine, professor of pathology at the Institute and professor of developmental biology at Stanford; Majeti is assistant professor of medicine at Stanford.

In previous studies, the Stanford group showed that forced expression of CD47 in a human leukemia cell line helped the cancer engraft in mice by allowing the cells to evade phagocytosis.[4] The group also reported that blocking CD47 in acute myeloid leukemia (AML) cells enabled phagocytosis and helped eliminate cancer cells, including cancer stem cells.[5]

Given that inhibiting CD47 prevents cancer cells from evading phagocytosis, Mark Chao, a corresponding author on the paper and a **Howard Hughes Medical Institute** fellow at Stanford, and colleagues hypothesized that antibodies against the target should be synergistic with drugs such as Rituxan that promote cell death at least in part through phagocytosis.

Now, in a paper published in *Cell*, the Stanford team showed that a half dose of both Rituxan and an anti-CD47 antibody was more effective at inducing NHL cell death than a full dose of either antibody alone.

In immunodeficient mice, injection of the antibody combination eliminated disease in 60% and 86% of localized and disseminated xenograft models of NHL, respectively. No mice treated with either antibody alone survived.

The researchers also suggested that CD47 levels could be a prognostic marker for NHL. On primary NHL samples, the protein's expression was greater than that on normal B cells, and high CD47 expression correlated with poor prognosis.

Chao said the team is continuing with preclinical toxicity, efficacy and pharmacokinetic studies of an anti-CD47 antibody under a grant from the **California Institute for Regenerative Medicine**.

Combination benefits

Chao told *SciBX* that Stanford is working toward an IND filing for an anti-CD47 antibody as monotherapy for AML and expects it to enter Phase I testing within four years. He said that they will expand the therapeutic application of the anti-CD47 antibody to lymphoma, both alone and in combination with rituximab, in rapid successive Phase I/II trials.

"We are currently in the process of making chimerized and humanized versions of an anti-CD47 antibody so that it can be administered to patients," he said.

"Combining CD20 and CD47 antibodies in treating patients with NHL has potential to decrease or avoid the use of cytotoxic therapies and their accompanying debilitating side effects. Combination therapies are definitely the way forward," said David Roberts, head of the biochemical pathology section of the laboratory of pathology at the **National Cancer Institute**'s Center for Cancer Research.

"The strategy could be a new and exciting paradigm of treatment that enhances the immune responses against cancer cells," said Yann Echelard, VP of corporate and technology development at **GTC Biotherapeutics Inc.** "The potential advantage of this work is that blocking CD47 not only could help enhance efficacy, but it could also be used when patients relapse after treatment with rituximab. An anti-CD47 antibody could be a third-, fourth- or fifth-line therapy."

Echelard also said a combination of antibodies might help increase the immune memory response, which should lead to improved results for maintenance therapy with single rituximab infusions every three months.

Under a deal with **LFB S.A.**, GTC is developing an antibody that targets CD20. The molecule is in Phase I testing, and Echelard said that it provides better cytotoxic activity than rituximab.

Juergen Hess, manager of scientific affairs at **Trion Pharma GmbH**, told *SciBX* that it may be premature to assume the antibody combination could be effective against resistant and recurring cancers. He wanted to see whether the combination works in other NHL cancer cell lines, including those resistant to rituximab and those with varying levels of CD20 expression.

Trion is developing Lymphomun (FBTA05), an anti-CD20 and anti-CD3 antibody that is in Phase I/II testing to treat B cell lymphoma. The compound is partnered with **Fresenius SE**.

Off-target activity

Because the data in the *Cell* paper came from immunodeficient mice, other researchers said it's still unclear whether combining anti-CD20 and anti-CD47 antibodies could overstimulate the immune system and destroy healthy cells.

"The key limitation of the current study is that xenograft NHL is being studied in a severely immunocompromised mouse model. In this model, the anti-human CD47 antibody can only interact with the human NHL cells. None of the expected side effects in humans are possible in this context," Roberts said.

In addition, Roberts noted that CD47 has a dramatically different distribution profile than CD20.

"The CD20 antibody is a viable therapeutic because its expression is limited to one normal cell type: B cells," he said. "CD47 is expressed by every cell in circulation. Therefore, it is unclear that sufficient antibody loading of NHL cells in a human patient could ever be achieved to see a therapeutic benefit."

According to Roberts, "such extensive loading with this CD47 antibody would most likely have significant cardiovascular side effects in terms of blood pressure dysregulation and thrombosis."

He also noted that CD47 is expressed on red blood cells, "and its level of expression is critical for the rate of clearance of aging red blood cells. If a patient is loaded with the anti-CD47 antibody at a sufficient dose to enhance macrophage phagocytosis of NHL, one would expect to see increased clearance of red blood cells by the same mechanism. This would result in acute anemia and potentially an acute hemolytic crisis."

Roberts said the best next step would be showing efficacy without side effects in mouse syngeneic lymphoma models.

In addition to the inability of anti-CD47 antibodies to discriminate between healthy cells and cancer cells in the circulatory system, it is possible that the combination of antibodies could overstimulate the immune system.

"There is a fine line between stimulating the immune response and overstimulating the immune response, which could lead to a bad autoimmune or inflammatory side effect. In extreme cases, it could even lead to widespread inflammation such as shock," said Echelard.

"The problem is that mice don't do a very good job of replicating the human immune system," he said, "so it might not be until clinical trials are performed that we understand how this immune stimulation might affect the human immune response."

"In this model, the anti-human CD47 antibody can only interact with the human NHL cells. None of the expected side effects in humans are possible in this context."

—David Roberts, National Cancer Institute

Chao said the Stanford team recognized the safety concerns. "CD47 is expressed on many normal tissues, including most cells in the hematopoietic system, so as such there is a significant possibility for off-target effects," said Chao.

But, he added, "we have shown *in vitro* that the anti-CD47 antibody selectively eliminates tumor cells by phagocytosis while sparing normal cell counterparts including normal bone marrow and peripheral blood. *In vivo* we have also demonstrated that the anti-CD47 antibody has minimal toxicity when administered to normal wild-type mice."

Even so, Chao told *SciBX* that the team is looking into developing a bispecific antibody that targets both CD47 and an antigen highly specific for the cancer cells to help avoid off-target effects.

Chao said Stanford has filed for a patent covering the use of an anti-CD47 antibody to treat several cancers and for a patent covering an anti-CD47 antibody in combination with other antibodies to treat hematological malignancies. The IP is not available for licensing.

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COMPANIES AND INSTITUTIONS MENTIONED

Biogen Idec Inc. (NASDAQ:BIIB), Weston, Mass.
California Institute for Regenerative Medicine, San Francisco, Calif.
Fresenius SE (Xetra:FRE), Bad Homburg, Germany
Genentech Inc., South San Francisco, Calif.
GTC Biotherapeutics Inc. (OTCBB:GTCB), Framingham, Mass.
Howard Hughes Medical Institute, Chevy Chase, Md.
LFB S.A., Les Ulis, France
National Cancer Institute, Bethesda, Md.
Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland
Stanford University School of Medicine, Stanford, Calif.
Trion Pharma GmbH, Munich, Germany