Medical Xpress

New antibody-combination therapy boosts human lymphoma cure rate in mouse models

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(PhysOrg.com) -- More than half of laboratory mice with human non-Hodgkin's lymphoma are cured by a treatment involving just two monoclonal antibodies, according to a new study.

More than half of laboratory mice with human non-Hodgkin's lymphoma are cured by a treatment involving just two monoclonal antibodies, researchers at the Stanford University School of Medicine have found. The therapy combines the activity of rituximab, an antibody currently in use to treat the disorder, with another that blocks a molecule called CD47 on the surface of the cancer cells. Together the two antibodies synergize to trigger the host's own immune system to eliminate the cancer.

"What we're seeing is that we have a potential therapy for non-Hodgkin's lymphoma that can eliminate the disease in mice even without chemotherapy," said the co-first author of the research, MD/PhD student Mark Chao. Currently, about 30 percent of patients with NHL die of the disease.

Because many cancer cells express elevated levels of CD47, the researchers hope that the potential therapeutic benefit shown in this study by the combination therapy will also extend to other types of cancers.

The findings of this study lay the groundwork for trials in humans. Last October, the researchers received a $20 million Disease Team Grant

from the California Institute for Regenerative Medicine to bring the new antibody therapy into clinical trials in human patients with a related cancer — acute myeloid leukemia — within four to five years.

“The goal is to get the immune system to target and kill cancer cells,” said Ravindra Majeti, MD, PhD, an assistant professor of hematology at the medical school and a study co-author. “We found that, although treating the mice with either antibody alone was somewhat beneficial, treating with both antibodies simultaneously cured the mice in over 60 percent of the cases.”

The research is published in the Sept. 3 issue of Cell. Majeti and Irving Weissman, MD, director of Stanford’s Institute for Stem Cell Biology and Regenerative Medicine, are co-senior authors of the study. Chao and acting assistant professor of oncology Ash Alizadeh, MD, PhD, are co-first authors of the work. Weissman and Majeti are co-principal investigators on the CIRM grant and are both members of the Stanford Cancer Center.

“We want to bring this to patients as quickly as we can,” said Chao. The researchers point out that, although the CIRM grant focuses on investigating anti-CD47 therapies for acute myeloid leukemia, the drug development process will result in an antibody that could also be used for other cancers. They focused their preliminary investigations on non-Hodgkin’s lymphoma because they were curious as to how the anti-CD47 antibody would work with rituximab, which also binds to human lymphoma cells.

“Biologically, it makes sense that these two antibodies would work together,” said Majeti. “One, rituximab, binds to the lymphoma cells and serves as an activator for cells of the immune system. The other, anti-CD47, blocks a ‘don’t-eat-me’ signal these blood cancer cells use to evade the immune cells as they move throughout the body. But we were

amazed at the robustness of the synergy between the two."

Rituximab alone does not cure human patients with NHL. It must be combined with chemotherapy — and even then it does not always work. "A major limitation of our current therapeutic approaches is a lack of increasingly active agents for the most aggressive lymphomas," said Alizadeh, who treats lymphoma patients at Stanford Hospital & Clinics. "Rituximab is the biggest advance that's been made in the last 30 years. But even so, we lose about one-third of patients with systemic disease."

CD47 came to the attention of the researchers in 2008 when Chao, Majeti and Weissman found that the molecule protected human leukemia cells from engulfment and destruction by a protective immune cell called a macrophage. Because many cancer cells have higher-than-normal levels of CD47 on their surface, the researchers speculated that an antibody that binds to CD47 and masks its appearance might allow the macrophages to go back to happily munching on the rogue cells.

Indeed, Alizadeh found that people whose lymphoma cells expressed higher levels of CD47 had a worse prognosis than did those whose cancer cells expressed lower levels of CD47. In particular, those with a form of the disease called diffuse large B cell lymphoma were significantly more likely to die of their disease if their cells had more of the molecule on their surface. Interestingly, he found that high CD47 expression correlates with other, previously identified prognostic factors.

"We've known, for example, that the cell-of-origin for these lymphomas is an important indicator of how a patient is likely to respond to therapy," said Alizadeh. "But until now we've had no way to try to address that therapeutically."

The scientists tested their theory in human non-Hodgkin's lymphoma primary cells and cell lines in culture dishes and in laboratory mice. They

first showed that incubating human NHL cells in a culture dish with either mouse or human macrophages in the presence of anti-CD47 significantly increased the ability of the macrophages to eat and kill the cancer cells, and that this killing ability varied according to the levels of CD47 expressed on the cells' surfaces.

Incubating the cells with rituximab had a similar effect. However, using both antibodies together dramatically increased the macrophages' ability to wipe out the lymphoma cells in a way that was more than additive — that is, the activity of the anti-CD47 antibody and rituximab was synergistic.

When the researchers injected mice intravenously with cells from the human NHL cell line, the cells multiplied and the animals developed disseminated lymphoma. The eight mice treated with a control antibody all had to be euthanized due to tumor burden in just over 20 days. Although treating the mice with either rituximab or anti-CD47 decreased the number of tumor cells and prolonged the animals' survival (to about 30 days), they eventually all died of the disease. But when the animals were treated with the combination antibody therapy, five out of eight mice lived for more than 180 days with no evidence of tumor cells.

Similar results were seen when the cells were injected into the flanks of mice, where they formed palpable tumors. Short-term treatment with the combination of the two antibodies allowed six out of seven of the animals to achieve a complete remission that lasted for more than 190 days, when the experiment was stopped.

Finally, because cell lines can accumulate genetic changes over time that differ from primary cells, the researchers repeated the experiments using cells isolated directly from human patients with NHL. They found that eight out of nine mice injected with diffuse large B cell lymphoma and then subsequently treated with the two antibodies lived for more than

four months without evidence of disease. In contrast, all animals treated with the control antibody, or with either antibody alone, had to be euthanized due to progression of their disease.

The researchers are moving forward to conduct tests on other CD47-expressing cancer cells, which include acute leukemia, bladder and several other cancer stem cells. They speculate that they might see a similar synergistic effect between anti-CD47 and other cancer-specific monoclonal antibodies currently in clinical use. They are also moving ahead as quickly as possible to bring the anti-CD47 antibody treatment to trials in human patients.

“We first found this molecule when we compared leukemia stem cells in mice with their normal counterparts,” said Weissman, who is also a professor of pathology. “It is amazing to me that this new approach to cancer stem cells in mice showed us the most important hidden component of how the body is likely to attack all cancers — the macrophage — and how human cancers evade killing by using the ‘don’t-eat-me’ signal. Blocking this signal and adding an ‘eat-me’ signal to the lymphoma cells is the next step in therapy.

“Let’s hope that this treatment that cures lymphoma in mice will cure it in humans, but we must remember that we are still many steps from a clinical trial in humans,” Weissman added. “Many other exciting potential therapies have failed in humans.”

“In many ways this is a labor of love,” said Alizadeh. “It is a very humbling experience to walk into an exam room and tell a patient with lymphoma that you’ve run out of bullets to shoot at their cancer and to prepare them to give up. Hopefully, this work will be a testament to how hard we’re all trying to help such patients.”

Weissman, Majeti, Alizadeh and Chao have filed a patent application

relating to the process for using the CD47 antibody.

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