Small Protein Engineered to Target PD-L1 is More Potent Than Anti-PD-L1 Antibody Immunotherapeutic

NEW YORK — An engineered high-affinity PD-1 small protein that can bind to PD-L1 on tumors was found to be a more effective anticancer immunotherapeutic than conventional anti-PD-L1 antibodies, and this small protein was more effective in synergizing with other immunotherapies compared with conventional anti-PD-L1 antibodies, according to preclinical data presented at the CRI-CIMT-EATI-AACR International Cancer Immunotherapy Conference, held Sept. 16–19.

“We now have many antibody drugs [such as pembrolizumab and ipilimumab] that target immune checkpoints such as PD-1, PD-L1, CTLA4, and other immune-regulatory pathways. However, these types of drugs have some overlooked drawbacks that can limit their effectiveness,” said Sydney Gordon, a graduate student in the laboratory of Irving Weissman, MD, at the Ludwig Center at Stanford University School of Medicine in California.

“First, it would be ideal if drugs that target PD-L1 could get inside tumors. However, antibodies, because of their large size, cannot get there effectively, which prevents them from working to their full potential,” Gordon said. “Second, antibodies against PD-1 and PD-L1 can result in counterproductive collateral damage, depleting some of the very antitumor immune cells they are supposed to activate.”

Gordon; Aaron Ring, who is an MD/PhD student from Weissman’s lab and will shortly be assistant professor in the Department of Immunobiology at Yale University School of Medicine; and colleagues hypothesized that a small engineered protein that targets PD-L1 and is capable of blocking PD-1/PD-L1 signaling could potentially overcome these setbacks and function as a superior immunotherapy.

The researchers designed a small-protein antagonist of PD-L1 by using a process called directed evolution. “In the end, our best PD-1 variants bound to PD-L1 about 50,000 times more strongly than the natural PD-1 protein,” Ring said. The small-protein, high-affinity PD-1 variant they developed was one-tenth the size of an antibody.

First, the researchers tested whether the high-affinity PD-1 protein could penetrate solid tissues more effectively than anti-PD-L1 antibodies. To do this, they labeled the high-affinity PD-1 protein and an anti-PD-L1 antibody with two differently colored fluorescent dyes and injected tumor-bearing mice with both fluorescent proteins at the same time. When they surgically removed the tumors and looked at them under a microscope, they found that the antibody was
mostly found near blood vessels (outside the tumor), whereas the high-affinity PD-1 protein was able to distribute much more deeply into the tumors.

Next, the researchers tested what impact the high-affinity PD-1 protein had on immune cells. For this, they took blood samples and lymph nodes from mice treated with the PD-1 protein and those treated with the PD-L1 antibody, and estimated the number of T cells. They found that the high-affinity PD-1 protein did not affect T-cell numbers in the blood or lymph nodes, but the anti-PD-L1 antibody caused significant depletion of T cells. Further, they found that this depletion was worsened when they combined the antibody with other immunotherapies. “This may have important implications when considering how to optimize immunotherapeutic combinations for patients,” Gordon said.

Finally, they tested the antitumor activity of the high-affinity PD-1 protein and found that when mice had tumors about the size of a grain of rice (50 mm$^3$), both PD-1 protein and anti-PD-L1 antibodies were effective in shrinking the tumors. However, when the mice had tumors about the size of a pea (150 mm$^3$), the anti-PD-L1 antibodies were entirely ineffective, even when they were combined with anti-CTLA4 antibodies. The high-affinity PD-1 protein was able to slow the growth of big tumors, and it was more effective when combined with anti-CTLA4 antibodies.

“Given how well anti-PD-1 and anti-PD-L1 antibodies are working for cancer patients, it may seem like a reasonable assumption that we have adequately targeted the pathway at this point. However, our results show that significantly more antitumor activity is possible using a small-protein therapeutic compared with a conventional antibody,” Ring said. “The broader implication is that small-protein therapeutics could present the same advantages in additional immunotherapeutic pathways outside of PD-1 and PD-L1.”

Because of their small size, the high-affinity PD-1 proteins may get excreted from the body more quickly, which means that they would have to be administered more frequently than antibodies. Further, they may be “immunogenic,” meaning patients could develop an immune response to the drug itself. “Both issues would need to be addressed for high-affinity PD-1 or a similar small-protein therapeutic to be developed for clinical use,” Gordon said.

This study was funded by the Ludwig Center at Stanford. Several authors are inventors on a patent filing describing the high-affinity PD-1 agents. Sydney Gordon provides consulting services for Ab Initio Biotherapeutics. Several authors, including Aaron Ring, are founders of Ab Initio Biotherapeutics and serve on its scientific advisory board.

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About the CRI-CIMT-EATI-AAACR International Cancer Immunotherapy Conference
The International Cancer Immunotherapy Conference covers all areas of inquiry in cancer immunology and immunotherapy. Launched in 2015, the meeting is jointly sponsored by the Cancer Research Institute (CRI), the Association for Cancer Immunotherapy (CIMT), the European Academy of Tumor Immunology (EATI), and the American Association for Cancer Research (AACR). The meeting is held
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every other year, in lieu of each organization’s individual meeting, and alternates between the United States and Europe. Through this collaboration, the organizations aim to more effectively and efficiently disseminate the latest cutting-edge information and research in cancer immunology and immunotherapy, and to provide an opportunity for teaching, learning, and networking among all stakeholders in the field. Additional support for this year’s meeting comes from Bristol-Myers Squibb (Diamond level), Roche (Platinum level), Amgen, Genentech, Miltenyi Biotec, and Regeneron (Silver level), among others. For more information about the meeting, please visit www.cancerimmunotherapyconference.org.

B101 Engineered PD-1 variants as immunotherapies for cancer.

Sydney R. Gordon¹, Roy Maute¹, Aaron Mayer¹, Melissa McCracken¹, Arutselvan Natarajan¹, Nan Guo¹, Richard Kimura¹, Jonathan M. Tsai¹, Aashish Manglik¹, Andrew Kruse², Sanjiv Gambhir¹, Irving L. Weissman¹, Aaron M. Ring³. ¹Stanford University, Stanford, CA, ²Harvard University, Boston, MA, ³Yale University, New Haven, CT.

Immunotherapies represent the next generation of treatments for cancer. The recent clinical successes of immunotherapies have highlighted the potential to achieve stunning efficacy against otherwise intractable cancers. One such clinically validated immunotherapy target is the T-cell surface receptor PD-1 (programmed cell death protein 1), which inhibits T-cell cytotoxicity upon ligation with PD-L1 (programmed death-ligand 1) on a target cell. Antibody therapies targeting PD-1/PD-L1 can be strikingly effective cancer treatments, as demonstrated in the clinic by the recently approved pembrolizumab and nivolumab. However, antibody therapies have significant drawbacks as agents against this pathway. Due to their large size (~150 kDa), antibodies have limited tissue penetrance. Furthermore, due to the presence of the prophagocytic Fc domain, antibodies bound to PD-1 or PD-L1 on the T-cell cause counterproductive lymphocyte depletion. We hypothesized that a small engineered protein capable of blocking PD-1:PD-L1 signaling could avoid these issues and function as a superior immunotherapy. To accomplish this goal, we used yeast display-based directed evolution to affinity-mature a soluble form of the PD-1 ectodomain to increase its affinity for human PD-L1. After several rounds of selection from two generations of mutant libraries, we identified a high-affinity PD-1 variant. By surface plasmon resonance, high-affinity PD-1 binds to human PD-L1 with 110 pM affinity and a half-life of binding of >40 minutes, a dramatic improvement from wild-type PD-1. Direct in vivo binding assays demonstrate that this small protein penetrates solid tissues much more effectively than an anti-PD-L1 monoclonal antibody. Unlike anti-PD-L1 antibodies, high-affinity PD-1 does not lead to the depletion of effector T-cells. Consistent with these advantages, in syngeneic CT26 tumor models, high affinity PD-1 was effective in treating both small (~50 mm3) and large tumors (>150 mm3), whereas the activity of anti-PD-L1 antibodies was completely abrogated against large tumors. Furthermore, radiolabeling of high-affinity PD-1 enabled us to utilize our reagent as a non-invasive tracer for PET imaging. Taken together, our data demonstrates the benefits of using small, non-antibody therapeutics as cancer immunotherapy and immune diagnostic agents.