Double Whammy: Bispecific Antibodies Help Immune Cells Attack Tumors

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With their unique ability to selectively attack tumor cells, monoclonal antibodies represent a major advance in the search for a "magic bullet" against cancer. However, by targeting only tumor cells, these molecules don't fully exploit the body's own cancer-fighting mechanisms. Enter the bispecific molecule, a monoclonal antibody that binds both to tumor cells as well as cells of the immune system, bringing these two archenemies in close contact. Preliminary clinical studies indicate that this new type of antibody can unleash powerful immune mechanisms such as cytotoxic T cells against a tumor. When used in conjunction with existing cancer treatments such as surgery and chemotherapy, "bispecific antibodies could not only help shrink a tumor, but could also trigger cellular and humoral responses," says Lawrence Lum, a clinical immunologist at the Karmanos Cancer Institute in Detroit. "This could induce a long-term immune response to the tumor."

The key to an effective anti-tumor immune response are the body's T lymphocytes. Abundant, ubiquitous, and agile, T cells come equipped with two types of powerful toxins for killing harmful cells: perforins, which pierce cells, and granzymes, which break up cellular proteins. T cells can recognize potential tumor cells by their surface antigens and destroy them right away. They can kill multiple times, recharging their toxins as needed. They can amplify the attack by rapidly proliferating at the tumor site. Finally, by "vaccinating" the immune system with protein fragments from killed tumor cells, they can establish a long-term anti-tumor response. Despite all this, however, we do get cancer. Tumor cells, with their high mutation rates, present a moving and elusive target for T cells. The few that manage to hide from the immune onslaught can grow into a tumor. "Any tumor that you see is essentially a Darwinian product," says Richard Flavell, an immunologist at the Yale University School of Medicine. "It is made of cells that have already succeeded in fighting off the immune system."

Fortunately for us, the tumor's disguise is not perfect. Its cells almost always bear some marker that sets them apart from healthy tissue; much effort now goes into identifying these markers and targeting them using monoclonal antibodies.

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For instance, trastuzumab (Herceptin) binds to and deactivates cells that carry Her2/neu, a protein overexpressed in certain breast cancers. (Indeed, despite the high cost of this drug—about $40,000 per year—some Canadian states now provide it as first-line therapy for eligible patients.) Such targeted remedies typically have milder side effects than the more brute-force drugs used in chemotherapy. However, in many cases their ability to kill or cripple tumor cells is limited. This is where going bispecific could pay off—by grabbing a tumor cell with one arm and roping in an immune cell with the other, the antibody could let the immune cell do the killing. "The dream is to use bispecific targeting to make T cells kill tumors more effectively," says Lum.

In pursuit of this goal, researchers have been developing a wide range of bispecific antibody molecules since the 1980s. Early designs retain the basic "Y" shape of natural antibody molecules: stemming from a constant (Fc) region, two variable arms branch out, and the tip (Fv region) of each arm binds to a different molecule or epitope. (In contrast, a conventional monospecific antibody [mAb] such as trastuzumab has identical Fv regions.) In a bispecific antibody (bAb), one tip might target a tumor-associated antigen and the other a molecule on an immune cell. In 1991, the first molecule of this type to undergo a clinical trial targeted CD19, a protein found on certain non-Hodgkin's lymphoma cells, and CD3, a marker found on T cells. To make this molecule, researchers fused two types of hybridoma cell: one designed to produce an anti-CD3 mAb and the other an anti-CD19 mAb. Another early molecule had the same T cell affinity but targeted the EGP-2 protein found on some epithelial tumors. Two more hybridoma-derived bAbs, anti-CD3 × anti-EPCAM for stomach cancer and anti-CD3 × anti-Her2/neu for advanced breast cancer, are now in advanced clinical trials.

Preclinical data shows that bispecific antibodies do indeed help guide T cells to tumors and may surpass conventional mAbs in their antitumor effects. However, results from clinical trials are mixed. Many bAbs don't work unless the patient's immune system is simultaneously given an immune stimulator such as interleukin-2, which increases the risk of complications. Further, the molecules often attach themselves to circulating immune cells and miss their tumor targets. Finally, immune self-regulating mechanisms such as activation-induced cell death prevent T cells activated by bAbs from surviving long enough to do their job. As a result, many bispecific antibodies work only under unrealistically high ratios of immune cells to tumor cells, ranging from 1:1 to nearly 100:1. "But as any pathologist who has ever looked at a tumor can tell you, the actual ratio is more like 1:10 or even 1:1000," says Louis Weiner, director of the Lombardi Comprehensive Cancer Center at Georgetown University and one of the earliest designers of bispecific molecules.

Further, many patients treated with bispecific antibodies suffer toxicities that force investigators to use a less-than-effective dose. In a 1995 clinical trial led by Weiner, an anti-CD16 × anti-HER2/neu bAb used to treat advanced breast cancer triggered a cytokine storm in some patients. The molecule's Fc region is often to blame in such cases, as it can activate a wide range of unwanted immune cells. Consequently, this region is absent in some of the newer molecules such as MDX-H210, another bAb for breast cancer; in a Phase I trial of this Fc-less antibody completed in 2001, the investigators found no dose-limiting toxicities. Despite these improvements, adverse immune effects remain a major concern, says Weiner. ''Until someone can figure out how to deliver the bispecific antibody to the tumor site without encountering and activating leukocytes on the way, this will remain a problem,'' he says.

To design safer and more effective bAbs, some researchers have turned to recombinant DNA technology. While most of these designs are in the preclinical stage, one format, termed BiTE (bispecific T cell engager molecule), has advanced further. In a recently completed Phase I dose-escalation study of an anti-CD3 × anti-CD19 BiTE for advanced non-Hodgkin's lymphoma, the investigators reported that all six patients receiving the highest dose responded, including two that had a complete response. Made by Bethesda, Maryland-based Micromet, a BiTE molecule is basically just a pair of Fv regions joined together by a short, flexible linker. Thanks to its small size and flexibility, a BiTE can bring a T cell and a tumor cell into close contact; once this so-called immunological synapse forms, the T cell can unleash its toxins on its target. Unlike the case with earlier formats, a T cell recruited by a BiTE molecule needs no costimulation, according to Christian Itin, chief executive of Micromet. Further, the T cell doesn't die after a single kill, but goes on a ''serial killing'' spree, he says. As a result, the molecule works even at extremely low concentrations. ''It causes enormously potent redirected lysis even in the low picomolar range,'' says Itin.

Despite these advances, working with recombinant formats offers some challenges. The loss of the Fc domain could reduce efficacy. Foreign peptides used in the linkers could trigger an immune reaction. Some molecules are unstable or form insoluble aggregates in vivo. The smaller ones typically circulate only for a short time in the body – sometimes just an hour or less, compared to several weeks for whole antibodies. And toxicity remains a major concern, even if animal studies suggest a molecule is safe. ''Mice are notoriously resistant to cytokine storms,'' says Weiner. ''You can be fooled into believing that something is a lot safer for people than it actually is.''

Many safety and efficacy issues with bispecific antibodies arise because the molecules either miss their target or hit the wrong one. One way to improve both safety and efficacy is to not administer the bAb directly, but to use it to arm T cells ex vivo and infuse them instead. Studies by Lum and others show that this method needs much lower antibody doses than direct infusion. Since the bAb molecules can't make unwanted bindings ex vivo, the threat of cytokine storm diminishes. Further, it turns out that arming T cells ex vivo prolongs their effective life; this extra time allows them to proliferate at the tumor site. ''We discovered, to our total surprise, that ex vivo armed T cells did not die when they engaged a tumor,'' says Lum. ''This not only creates more effector cells to kill the tumor but also vaccinates the endogenous immune system against it.''

Ex vivo arming of T cells, however, requires greater expertise and more advanced facilities than direct bAb infusion. As a result, few large studies have employed this strategy so far. In a 1995 Phase II trial, 28 advanced ovarian cancer patients were treated with T cells armed with an anti-CD3 × anti-OC/TR bAb. Four patients had a complete response, while three others had a partial response. Side effects were mild to moderate and transient, according to the researchers. In ongoing clinical trials led by Lum, investigators are using T cells armed with anti-CD3 × anti-HER2/neu and anti-CD3 × anti-CD20 bAbs to treat advanced breast cancer and non-Hodgkin's lymphoma, respectively. Infusions of billions of armed T cells seem to provoke only mild side effects such as chills and fever, says Lum. In many patients, blood profiles indicate that cancer cells are being killed and the immune system is being primed to attack the tumor, he adds.

As researchers continue to explore the potential of bispecific antibodies, Lum and Weiner caution against expecting any bAb format or strategy to make tumors vanish. Indeed, they question the relevance of tumor shrinkage as a metric in the case of immune-based therapies such as bispecific antibodies. They argue that such a therapy needs to kill only a fraction of tumor cells to initiate antigen processing and presentation, triggering a long-term immune response. Clinical trials that relate efficacy to tumor shrinkage may thus undervalue immune-based therapies. ''The ultimate value of these therapies may need to be measured not by how much tumor is destroyed, but by how long it's controlled,'' says Weiner. ''In this regard, a little bit of killing may be just as effective as a lot of killing.''

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