

Conjugating Antibodies to Cytotoxic Agents: Getting the Best of Both Worlds?

Anna Azvolinsky

The strength of chemotherapy drugs is their ability to kill tumor cells. Unfortunately, these treatments are nonspecific, destroying normal rapidly dividing cells, making these drugs toxic for cancer patients. Monoclonal antibodies, by contrast, can home in on a target found only on tumor cells. But binding well to a target on a tumor cell does not necessarily mean that the antibody efficiently kills that cell.

Pairing these drug types into what is called an antibody–drug conjugate (ADC) is now an active area of research. Like any successful partnership, playing up the strengths of each partner is key and requires a linker, the Lego-like connector that holds the antibody and toxin together.

The US Food and Drug Administration has approved two ADCs for cancer: brentuximab vedotin for Hodgkin lymphoma and anaplastic large-cell lymphoma in 2011, and T-DM1 (trastuzumab emtansine, the first ADC against a solid tumor) in February of this year for patients with metastatic breast cancer that overexpresses HER2. T-DM1 combines trastuzumab, an antibody already approved to treat these HER2-positive patients, with DM1, a chemotherapy drug. Treating patients with T-DM1 resulted in fewer toxic effects and higher efficacy than standard therapy.

“In some ways, we have been working on ADCs for many decades,” said George J. Weiner, MD, professor of internal medicine at the University of Iowa Carver College of Medicine in Iowa City. According to Weiner, an antibody researcher, radioimmunotherapy (antibody linked to a radioactive isotope) and immunotoxins that combine a peptide with a small-molecule toxin helped lead to more modern ADC constructs.

Drug companies and academic researchers are building up their own toolboxes of different types of linkers along with

toxins and antibodies that can be used to build an optimal ADC, said Hans-Peter Gerber, PhD, who heads a program at Pfizer in Pearl River, NY, to develop new biotherapeutics.

Liquid or Solid?

Traditionally, researchers pursued ADCs for blood cancers rather than solid tumors. “It was thought that because these were large macromolecules, they would not penetrate well beyond the first few layers of a tumor that are exposed to blood vessels,” said Beverly Teicher, PhD, chief of the molecular pharmacology branch at the National Cancer Institute. But with better ways to screen for antibodies and evidence of clinical efficacy for ADCs that target solid tumors, at least half the ADCs in development are for solid tumors, including lung, gastric, colorectal, pancreatic, and prostate. For example, phase I results with DMUC5754A for patients with advanced ovarian cancer have recently shown promise (*American Association for Cancer Research* annual meeting 2013, abstract #LB-290).

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But researchers still see targeting blood cancers as an easier path forward because the potential targets on malignant blood cells are less likely to be found on other tissues, minimizing toxic effects on healthy tissues. Many blood cells also have shorter life spans and have intrinsic signals to undergo apoptosis that ADCs can exploit more readily than with solid tumor, noted Weiner.

Vehicle of Choice: Zeroing In on the Best Targets

The ideal characteristics of a cell surface target for the antibody portion of the ADC are complicated. Abundance of the target on tumor cells is important, as are antigens expressed on components of the tumor microenvironment—researchers are exploring the stroma and vasculature as targets.



Beverly Teicher, PhD

Regular therapeutic antibodies bind to a cell-surface protein on a cell, blocking its activity. By contrast, the antibody for an ADC, essentially a vehicle for getting the cytotoxic drug into the cell, must bind its target and be internalized into specific cellular compartments to release the cytotoxic drug.

Initially, researchers tried using just the antibody component of most ADCs in clinical trials, but this approach failed because blocking the function of the cell-surface protein by the antibody was not crucial for survival of the cancer cell. But as part of an ADC, these antibodies could be used as chauffeurs to deliver the toxin to tumor cells.

Antibodies must be tested empirically to ensure that cells can internalize them. Most toxic effects from ADCs are a result of off-target effects that limit the ability to give the drug at higher doses, making antibody specificity a priority for drug development.

According to Gerber, researchers are focusing on identifying new targets and antibodies with ideal characteristics for ADCs for an optimal therapeutic index. “By selecting better ADC targets, we can bring better programs to the clinic,” said Gerber.

Many Moving Parts

The linker, a small organic chemical moiety or peptide of several amino acids that secures the antibody to the toxin, is important, said Amit K. Verma, MB, BS, associate professor of medicine at the Albert Einstein College of Medicine in New York. Because the ADC must travel through the bloodstream to reach its target without breaking apart, an ADC is only as good as the linker. Linkers need to be stable in the bloodstream (ADCs can circulate for as long as a few weeks) but must break down once inside the cell, where the linker is cleaved by enzymes or undergoes a change in pH to release the toxin. This is a fine balance. A linker that is too stable can result in minimal antitumor activity and toxic effects.

“You don’t want the toxin portion of the ADC to be hanging around in the circulation for too long,” said Verma. “The toxin in circulation can result in vascular leak and other toxic effects.”

This small peptide needs to be stable also because only a tiny fraction of the ADC molecules—about 1%–5%—will end up binding to tumor cells. The rest are metabolized, preferably to a less toxic version, to minimize potential toxic effects. For T-DM1, “the metabolite that is released into the liver was found to be less toxic than the original toxin that was conjugated,” said Gerber. According to Gerber, the site on

the antibody where the linker is attached also plays a role in the safety profile of these macromolecule drugs.

Toxic Payload

The toxins in current ADCs target either DNA or tubulin, the two main targets of chemotherapy. “ADCs are very selective, but they are not very efficient ways to deliver chemotherapy, so these drugs need to be very potent agents,” said Teicher. Several toxins currently incorporated into ADCs originally failed as stand-alone chemotherapy drugs: They were too toxic.

Much ADC research focuses on developing toxins, either as derivatives of natural compounds or new molecules. The toxins must be able to bind to the linker and be stable in the three-part complex in the blood. ADCs that work on solid tumors can benefit from toxins that, when liberated from inside the dying cell, can slip through the membranes of adjacent tumor cells, which need not express the targeted antigen. Like tinker toys, some antibody, linker, and toxin combinations allow multiple linker–toxin pairs to be attached to an individual antibody, lowering the ADC concentration required for tumor cell death, and probably the toxicity.

Just as for other targeted cancer agents, research efforts to find new ways to identify patients who could benefit from ADCs are

ongoing. Although immunohistochemistry is still the “gold standard” to detect whether a tumor expresses a specific target, detecting the antigen on circulating tumor cells (CTCs) may be a noninvasive approach to assess whether a patient will respond to the ADC. During the clinical development stage, expression on CTCs, associated with patient response, can identify the threshold of expression on CTCs needed for a clinical response.

With many clinical programs to create ADCs, much more is now understood about both the efficacy and safety of these drug types than was true even just a few years ago. “We can now connect the dots better from the preclinical studies to more reliably predict what will or will not be seen in the clinic with these agents,” said Gerber.

The current burst of activity to develop more-effective and better-tolerated ADCs in the pharmaceutical industry is thanks to the research supported by NCI in the last few decades, said Weiner. “We are world leaders in this exciting technology, and the reason is that we invested in basic and applied research,” said Weiner. “This is a great example of how investment in biomedical research can show a great long-term payoff.”

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Genetic Events in Head and Neck Squamous Cell Carcinoma Revealed

Nancy J. Nelson

Researchers at April’s annual meeting of the American Association for Cancer Research in Washington, DC, announced a complete analysis of the genetic alterations of head and neck squamous cell carcinoma (HNSCC). “This is really a parts list for head and neck cancer,” said David Neil Hayes, MD, MPH, an associate professor at the University of North Carolina at Chapel Hill who sees

HNSCC patients at the UNC Lineberger Comprehensive Cancer Center. Hayes is senior author of the February *PLoS ONE* article that highlights some of the findings. “There hasn’t been such a list available until now.”

The study is part of The Cancer Genome Atlas (TCGA) project supported by the National Institutes of Health to catalog genomic changes in cancers with

poor prognosis or a major impact on public health. The data are then made publicly available. Thirty cancers have been selected; about one-third are complete, whereas the others are in various stages of sample collection or data analysis (<https://tcga-data.nci.nih.gov/tcga/>). HNSCC is the sixth-most-common cancer worldwide, with about 600,000 new cases every year, and includes cancer of the nose cavity,