Nuclear Localizing Sequences: An Innovative Way to Improve Targeted Radiotherapy

Systemic targeted radiotherapy is an evolving and promising modality of cancer treatment. The goal of systemic targeted radiotherapy is to be efficacious, yet with minimal normal tissue toxicity. The key characteristic of systemic targeted radiotherapy is that a molecule (antibody, antibody fragment, or peptide) can deliver higher amounts of a radionuclide to cancer cells than to normal tissue. Unlike chemotherapy, systemic targeted radiotherapy is cancer cell specific.

The radiopharmaceutical used by Chen et al. (*1*) in this issue of *The Journal of Nuclear Medicine* is ^{111}In-DTPA-NLS-HuM195 (DTPA is diethylenetriaminepentaacetic acid; NLS is nuclear localizing sequence). HuM195 is a humanized IgG1 monoclonal antibody (mAb) that targets the CD33 antigen on acute myeloid leukemia cells. By itself, HuM195 is not toxic but it effectuates cell killing as a toxin carrier. HuM195 is in current clinical use as Mylotarg (Wyeth-Ayerst; HuM195 conjugated with gemtuzumab ozogamicin, a chemotherapy agent). Despite the enticing ability to target leukemia cells with Mylotarg, the complete response rate is only 26% and patients who do respond ultimately relapse (*2*). Another part of ^{111}In-DTPA-NLS-HuM195 is the NLS, a peptide (CPYGPKKKRKVGG) derived from the simian virus 40 large T-antigen. This is a unique use of biology; the peptide sequence of a virus (which facilitates viral genome entry into its target cell) is now being used in a positive way, to allow entry of a radiopharmaceutical into the malignant cell nucleus. DTPA is used to chelate ^{111}In, the therapeutic radionuclide.

See page 827

^{131}I and ^{90}Y are commonly used therapeutic radionuclides (*3*). The β-particles of these radionuclides are responsible for their efficacy as well as toxicity. Despite specificity, systemic radiation therapy as usually delivered does have toxicity, with myelotoxicity being predominant. Nonhematologic toxicity is usually minimal, thereby giving hope that this form of therapy can be improved to create a truly specific, effective, yet nontoxic, therapy applicable to patients with different kinds of cancer. It is this hope that makes the publication by Chen et al. (*1*) encouraging.

The article by Chen et al. (*1*) is exciting not only because of the data that it presents but also because of its concepts. The basic strategy starts with the HuM195 anti-CD33 mAb to specifically target myeloid leukemia cells. HuM195 is rapidly internalized into targeted cells; internalization is one of the first requirements for this strategy to be successful. Many other antibodies are also rapidly internalized into the cytoplasm after cell-surface receptor binding. The innovative part of this new strategy is that NLS have been conjugated to the antibody. Thus, what is transported into the cell cytoplasm is a drug that has secondary targeting ability—namely, it targets the cancer cell nucleus and is transported through the nuclear membrane. In that way, HuM195 will be in close contact with the DNA. This provides a great opportunity for a new kind of targeted therapy. Because the antibody has been able to enter the nucleus, drugs that work by contact with DNA have an opportunity to be effective. In this case, ^{111}In was used to kill malignant cells through direct DNA damage. This was a logical choice of radionuclides because the Auger electrons have only a nanometer-to-micrometer range that is long enough to damage DNA in the malignant cells, but not so long as to damage surrounding normal cells. In addition, the decay product of ^{111}In is cadmium, which is stable, unlike the decay products of other potentially useful radionuclides. This is different than standard systemic targeted radiotherapy where "normal bystander" cells along with the cancer are killed by β-particles.

It is easy to see the therapeutic possibilities of cancer cell–nuclear targeting as it is developed into drugs for patients. For example, for a therapy to be curative, virtually all malignant cells must be killed. However, it is difficult to completely eliminate cancer because of the therapy's toxicity. ^{111}In-NLS-mAb could be a way to eradicate minimal residual disease. Because it would be specific and because the accompanying toxic drug would itself not be very destructive outside of the cell nucleus, cancer-specific nuclear targeting would be able to effectively target and kill residual cancer cells. This is similar in some ways to traditional radioimmunotherapy because that also involves the use of a specific antibody carrying a radionuclide. However, with traditional radioimmunotherapy, the typical radionuclides (^{90}Y, ^{131}I, ^{67}Cu) have significant toxicity in the amounts used. Relatively long-range β-emissions that can damage normal cells are a particular problem with hematologic

Received Jan. 18, 2006; revision accepted Jan. 23, 2006.

For correspondence or reprints contact: Robert T. O'Donnell MD, PhD, University of California Davis Cancer Center, Room 3016, 4501 X St., Sacramento, CA 95817 and Northern California Veteran's Healthcare System, 10535 Hospital Way, Mather, CA 95655.

E-mail: robert.odonnell@ucdmc.ucdavis.edu

malignancies, where the bone marrow may harbor malignant cells. The "normal bystander effect" (death of normal cells near targeted malignant cells) is a problem solved with the strategy described in the publication by Chen et al. (*1*). Radiation safety is also an important issue for patients receiving radioimmunotherapy. This study uses ^{111}In, which does not have some of the radiation safety issues that are encountered with ^{131}I-containing radiopharmaceuticals. In addition, there is little danger to the thyroid from ^{111}In as there is with ^{131}I. Clearance of a radionuclide is always an important consideration with radiopharmaceuticals. ^{131}I is predominantly cleared from the body in the urine; with ^{90}Y-containing radiolabeled antibodies, much of the radionuclide is retained in the body. If modest doses of ^{111}In-NLS-mAb are used, differences in radiopharmaceutical clearance between patients are likely to have less impact on the development of clinical side effects. The lower toxicity with this strategy is due to the use of ^{111}In and the fact that it is likely that the radiopharmaceutical will be retained within the nucleus of the targeted cell. In addition, because a targeting construct and NLS will be able to enter the nuclei of malignant cells, several other interesting agents could be attached to kill cells—for example, drugs that could target specific genes and chemotherapy drugs.

The accompanying publication conclusively showed that the conjugation of 4–8 NLS peptides to the ^{111}In-DTPA-HuM195 mAb increased uptake of ^{111}In into the nucleus, retained specificity, killed leukemia cells, and did not produce toxicity in mice. Of course, almost anything is toxic if enough is administered; that is the point of developing the sort of radiopharmaceuticals described by Chen et al. (*1*)—they enter the nucleus of malignant cells so high doses may not be needed. The radiation-absorbed dose of ^{111}In is 34 times higher when it decays in the nucleus than on the cell surface (*4*). The Auger electrons of ^{111}In are potent and cytotoxic when they decay in the cell nucleus (*5*). The uptake of radiopharmaceutical into the nucleus was increased by increasing the numbers of conjugated NLS, but the affinity of the mAb for cellular targeting was not significantly diminished by up to 12 NLS.

In conclusion, advances in therapy frequently arise one step at a time. A mAb, radionuclide, chelator, and NLS are all part of ^{111}In-DTPA-NLS-HuM195. This may develop into an important new drug and concept that will be translated into better cancer therapy.

Robert T. O'Donnell
University of California Davis Cancer Center
Sacramento, California

Northern California Veteran's Healthcare System
Mather, California

REFERENCES

1. Chen P, Wang J, Hope K, et al. Nuclear localizing sequences promote nuclear translocation and enhance the radiotoxicity of the anti-CD33 monoclonal antibody HuM195 labeled with ^{111}In in human myeloid leukemia cells. *J Nucl Med.* 2006; 47:827–836.
2. Larson RA, Sievers EL, Stadtmauer EA, et al. Final report of the efficacy and safety of gemtuzumab ozogamicin (Mylotarg) in patients with CD33-positive acute myeloid leukemia in first recurrence. *Cancer.* 2005;104:1442–1452.
3. Leonard JP. *Targeting CD20 in Follicular NHL: Novel anti-CD20 Therapies, Antibody Engineering, and the Use of Radioimmunoconjugates.* Hematology (American Society of Hematology Education Program Book). Atlanta, GA; December 10–13, 2005:335–339.
4. Goddu SM, Howell RW, Rao DV. Cellular dosimetry: absorbed fractions for monoenergetic electron and alpha particle sources and S-values for radionuclides uniformly distributed in different cell compartments. *J Nucl Med.* 1994;35:303–316.
5. Kassis A. The amazing world of Auger electrons. *Int J Radiat Biol.* 2004;80:789–803.