

Radioimmunotherapy Against the Tumor Vasculature: A New Target?

The lack of clinical success of antibody-targeted radionuclide therapy in advanced solid tumors has unfortunately created a level of uncertainty for the future of radioimmunotherapy (RAIT) in the more prevalent types of cancer. However, the success of RAIT in non-Hodgkin's lymphoma (NHL) and other hematopoietic neoplasms (1) certainly should instill more confidence that RAIT can be an effective therapeutic modality with relatively little toxicity when compared with standard chemotherapy regimens. Also, the treatment requires about 1 wk for

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completion (less time if imaging is not required), whereas conventional chemotherapy is given in repeated cycles over weeks to months. Although the challenges for RAIT in solid tumors are considerable, it is important to remember that RAIT is still evolving (2,3). The article by Tijink et al. (4) in this issue of *The Journal of Nuclear Medicine* illustrates several promising avenues for advancing the RAIT of solid tumors.

GENERAL OBSTACLES

Perhaps the most formidable problem with RAIT is its inability to deliver a sufficient amount of radioactivity to kill solid tumors, especially when >5 cm, at tolerable doses to other

organs. This problem can be divided into 2 parts: an inability to concentrate enough radioactivity in the tumor, and the problem of distributing the radioactivity within the tumor homogeneously, so that all tumor cells receive a lethal dose of radiation. A variety of physiologic and immunologic factors have been identified as barriers inhibiting antibody-targeted radionuclides from overcoming these obstacles. Rather than trying to breach the tumor vasculature to deliver the radioactivity to the individual tumor cells, Tijink et al. (4) used a recombinantly engineered antibody, L19-SIP, that binds to an isoform of fibronectin found primarily on tumor blood vessels (ovaries and endometrial tissues also express this antigen). Vascular targets have received increasing attention over the past decade, with a variety of agents, including antibodies, being used to inhibit neovascularization (5–7). Some of these agents act on substances found on the endothelial cells of the blood vessels, but others, such as inhibitors of vascular endothelial growth factor, act indirectly by binding to substances required to activate blood vessel formation (6). In the case of L19-SIP, the antibody binds selectively to the blood vessels formed within the tumors, and thus the antibody does not have to overcome the barriers that inhibit migration to the individual tumor cells (8). Therefore, this target represents a promising new opportunity for RAIT.

Because antivasculature agents have shown efficacy in some clinical trials, it is a logical extension to determine whether the selective killing of tumor blood vessels by targeted radioactivity could also have a similar chance for success. Of course, like all targets, the fibronectin isoform must be selectively

expressed in the tumor and in sufficient quantity to allow the radioactivity to concentrate to lethal levels to the target cells before having detrimental effects on normal tissues. The studies by Tijink et al. (4), and others who have used L19-SIP in another model system (8,9), suggest that this fibronectin isoform is accessible and in sufficient quantities to deliver therapeutic radioactivity doses specifically to xenografted tumors. Most often targets used in RAIT have been selected to place the radioactivity in a position where it can act directly on the tumor cells. Most targets are found on the tumor cell surface, in the extracellular space surrounding the tumor cells, or even in necrotic areas of tumors (2,3,10). There have even been studies using an antibody that localizes the stromal tissue surrounding the tumor cells (11). By selecting a vascular target, the main goal would be the strangulation of the tumor, denying it access to essential nutrients found in the blood supply. Thus, the major question is whether the selective destruction of tumor blood vessels by targeted radioactivity alone would suffice to eradicate a tumor or will it be necessary to combine this approach with other treatments that directly (or indirectly) attack the tumor cells? Antivasculature agents, such as bevacizumab, are typically used in combination with other agents (5), and therefore it is likely that a combinational approach will enhance the antitumor effects of this approach.

RADIONUCLIDE SELECTION

Focusing on the RAIT aspect of this treatment, the selection of the most appropriate radionuclide for this approach needs to be addressed. Should the radionuclide have a long pathlength so that the radiation dose might

Received Feb. 15, 2006; revision accepted Mar. 6, 2006.

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kill malignant cells deeper within the tumor or should the pathlength be short so that the endothelial cells of the blood vessels are optimally dosed to ensure their destruction? Tijink et al. (4) compared the biodistribution of ^{131}I - and ^{177}Lu -labeled L19-SIP. These radionuclides have a similar β -emission that would likely be highly effective in killing the blood vessels as well as killing tumor cells within an ~ 0.5 -mm radius. Not surprisingly, the renal retention of the small-molecular-weight recombinant L19-SIP protein prepared with a residualizing radionuclide, such as ^{177}Lu , was severalfold higher than that of the tumor, making this particular construct suitable for use only with nonresidualizing radioiodine. Thus, the properties of this molecular construct restrict its use seemingly to radioiodine, limiting a closer examination of whether radionuclides with a longer pathlength would be more beneficial for this type of target. With radioiodine being the prime radionuclide candidate, the next question is whether radioiodine will have sufficient residence time in the tumor, because if the target is internalized, when prepared by conventional tyrosine-labeling methods, radioiodine would be rapidly expelled from the tumor. Although there was a gradual decrease of L19-SIP binding in the 2 xenografted tumor cell lines over time, this was most likely associated with the rapid blood clearance of the antibody rather than an internalization process. Thus, it would appear that, though limited, ^{131}I as a radionuclide for L19-SIP would be a reasonable choice for delivering a lethal radiation dose to the blood vessels, with the added benefit of being able to kill tumor cells residing within ~ 0.5 mm of these vessels. In this regard, autoradiographic data illustrating the tumoral distribution of the radioactivity would have been useful in the 2 cell lines.

Because treatment was initiated in these studies when tumors were ~ 0.15 cm^3 , their small size was ideally suited for an ^{131}I -labeled product. Indeed, with the disappointing clinical results

in advanced solid tumors using antibodies radiolabeled with the long-range β -emitter ^{90}Y , the specific clinical indications where RAIT should be applied in solid tumors needs to be reevaluated. In contrast to advanced cancers, there have been some encouraging results from clinical studies in patients with less-advanced disease (12,13) or when given in a regional manner (2,3,13–16). From this perspective, the choice of ^{131}I as a therapeutic should not be viewed as a deterrent with the L19-SIP construct. In addition to reevaluating the conditions where RAIT should be best applied, careful consideration also needs to be given to choosing solid tumors that, like NHL, will be more susceptible to radiation. In this regard, head and neck cancers are noted for their relative sensitivity to radiation, and therefore this would be an excellent indication for the development of a targeted radionuclide therapy.

VASCULAR TARGETING: DÉJÀ VU ALL OVER AGAIN?

Acknowledging that targeting the tumor's blood vessels is an attractive approach for radionuclide therapy, it invites the question of whether tumor blood vessel destruction has in fact already played an important role in RAIT using radionuclides targeting cancer cells. For example, several autoradiography studies have shown the heaviest accumulation of radiolabeled antibodies on the tumor cells residing in the perivascular space surrounding the tumor blood vessels (17,18). This pattern of tumor localization arises because an antibody's movement into the tumor is impeded by the "binding-site barrier," and there are unfavorable interstitial pressures within the tumor as a consequence of the tumor's inability to build a fully functional vascular system that inhibit the migration of IgG within the tumor (19–22). Despite the nonuniform distribution pattern for almost all directly targeted antitumor antibodies, effective therapy has been achieved in several preclinical models. Might it

be possible that the concentration of radioactivity on the tumor cells immediately outside the blood vessel actually leads to the death of tumors by destroying the tumor blood vessels rather than having a direct impact on the tumor cells? If vessel destruction alone could have a profound impact on tumor growth, then how important is it for the radionuclide to be distributed uniformly within the tumor as compared with heavily concentrated in or around the blood vessels? Boerman et al. (18) found more favorable therapy with radiolabeled antibodies at a low protein dose than at higher protein doses, suggesting that having a more restricted distribution in the tumor around the blood vessels might be more beneficial. Blumenthal et al. (23,24) found profound changes occurred to the vascular volume and permeability of tumors treated with radiolabeled antibodies that primarily localized in the perivascular space. This disruption of vascular function also had a direct impact on the ability of a second dose of radiolabeled antibody to target the tumor. In a follow-up study, Blumenthal et al. (25) expanded their studies to include an evaluation of changes in the vascular permeability of several different tumor cell lines, finding it decreasing in some tumors after receiving a dose of 1,500 cGy with an ^{131}I -labeled antibody, whereas others showed no effect, and still others even had an increase in vascular permeability. These findings are not surprising since, as with all biologic systems, considerable diversity is encountered, with multiple variables likely accounting for these results. Earlier studies with modest doses of external beam radiation described a transient increase in vascular permeability, which has been used in an attempt to enhance the localization of radiolabeled antibodies in tumors (26). However, with enough radiation dose directed to the blood vessel, its destruction will occur, resulting in a significant decrease in accessibility. Thus, we know that antibody-targeted radionuclides binding to tumor cells will deposit radiation that impairs

tumor vascularization, but we do not know to what extent these effects—as compared with direct effects on the tumor blood vessels—contribute to the observed antitumor effects.

Our group also examined the treatment of micrometastatic colon cancer growing in the lungs of nude mice given radiolabeled antibodies that directly bound to the tumor foci compared with an antibody that almost exclusively bound to the lung endothelial cells (27). Despite the higher concentration of radioactivity delivered to the lungs with the radiolabeled antibody targeting the lung vasculature (i.e., percentage injected dose per gram tumor-bearing lung tissue), significantly improved therapeutic responses and cures were found with the radiolabeled antitumor antibodies. Autoradiographic studies had shown the antitumor antibodies localizing primarily in the perimeter of these millimeter-sized tumor nodules, which would suggest a more concentrated delivery of activity selectively to the region of those blood vessels feeding the tumor nodules; however, because the tumors were also small enough to be within the range of the ^{131}I -labeled antibody, it was impossible to determine whether it might have been the destruction of the blood vessels feeding the tumors or irradiation of the tumor cells that caused the antitumor effect. Although the antilung vasculature antibody did have some therapeutic benefit compared with untreated animals, its ineffectiveness might have been related to the fact that the radioactivity was more dispersed throughout the lungs, thereby failing to concentrate sufficient activity against the blood vessels specifically feeding the tumors. Had the activity of the antiendothelial cell antibody been increased to a level where it would have affected the blood vessels, it most certainly would have resulted in pulmonary failure. These studies also illustrated that, despite calculating an exceptional high radiation dose to the lungs with the antilung endothelial cell antibody, this generalized radiation was not very effective against tumors

seeding the lungs. L19-SIP's specificity for the tumor's blood vessels could concentrate the radioactivity selectively and perhaps be as effective as the antitumor antibodies. However, the tumor nodules in the lungs were exceptionally small and did not have a vascular supply within the mass but, rather, seemed to have small vessels located in a capsular structure surrounding the tumor. It would be interesting to determine if blood vessels surrounding such small nodules, which were perhaps not developed by the tumor, express the unique fibronectin isoform.

ANTIBODY ENGINEERING

The use of L19-SIP is also important because it represents another example of how recombinant engineering has, over the past decade, produced several different antibody forms that may ultimately prove to be more effective targeting agents than IgG or its enzymatically generated fragments. Notwithstanding the improvements that recombinant engineering has made to the enhanced manufacturing capability of antibodies, antibodies now come in a variety of shapes and sizes, with some modifications specifically directed at affecting the pharmacokinetic behavior of the construct (28). One of the difficulties in using targeted radionuclides is that it is generally desirable to minimize the length of time that the radionuclide spends in the blood, as this impacts both imaging and therapeutic procedures. Hepatobiliary/gastrointestinal and renal/urinary clearance are the 2 prominent methods of filtering the radioactivity bound to antibodies from the blood. This physiologic paradigm directly impacts on the choice of radionuclide since, as was shown with the L19-SIP construct, residualizing radionuclides bound to agents that rapidly clear through the kidneys lead to enhanced renal uptake that exceeds the amount delivered to tumor, whereas excessive hepatobiliary clearance is also an unfavorable outcome for residualizing

radionuclides. In this regard, many of these new constructs have their best biodistribution properties when labeled with radioiodine. This is where pretargeting methods have made the most significant contribution for targeted radionuclides, as these methods most often result in tumor uptake that can rival a directly radiolabeled IgG but have very rapid blood clearance with minimal renal retention (29). Tumor uptake is also extremely rapid, with maximum accretion seen within 1 h of the radionuclide injection (30), whereas with the rapidly clearing, 80-kDa L19-SIP, maximum tumor accretion was delayed until 6 h after its injection. Though there is no argument that a single injection of a directly radiolabeled compound has a certain appeal, the added flexibility in radionuclide choice and improved targeting properties found with pretargeting procedures would seem to be a fair compensation for the added step(s) (31).

RATIONAL COMBINATIONS

As mentioned earlier, most antivasculature therapies require a combination with chemotherapy to evoke a therapeutic response. Tijink et al. (4) also examined the combination of ^{131}I -L19-SIP with cetuximab, the anti-EGFR (epidermal growth factor receptor) antibody. Cetuximab has received Food and Drug Administration (FDA) approval for use in colorectal cancer in combination with chemotherapy and just recently received FDA approval for use in combination with radiation therapy of head and neck cancers, because a relatively high percentage of these tumors express EGFR (32,33). Inhibitors of the EGF pathways have been shown to enhance the effects of local radiation therapy, and inhibiting EGFR has also been associated with antiangiogenic activity (34). With non-overlapping toxicities, these 2 treatment modalities could be combined at their full potency without concern for excessive toxicity. The results showed the enhanced efficacy of combining a single maximum tolerated dose of ^{131}I -L19-SIP (74 MBq) with a standard

dosing regimen of cetuximab given over 4 wk, but this regimen was shown to be far more effective in one cell line than another. Immunohistology studies suggested that each cell line qualitatively expressed both antigens in a somewhat similar manner, yet significantly improved responses were found with each agent alone and with the combination in the one cell line, HNX-OE, which had about a 1.5-fold lower tumor uptake of the ^{131}I -L19-SIP than the other cell line. Differences in antitumor responses among various cell lines irrespective of their antigen expression are not an uncommon finding, but these results should serve to emphasize the importance of being able to better identify candidates for a given treatment or treatment combinations as cancer therapeutics evolve.

Combination strategies, particularly those that involve the selective destruction of the tumor's blood supply, need to be considered carefully. If, by destroying the tumor's blood vessels, the other agent is no longer able to localize to sites where it would be able to exert its activity, then a different dosing strategy may need to be considered. If an approach is designed to kill selectively only the tumor blood vessels, additional follow-up treatments would likely be required to either inhibit neovascularization, thereby ultimately deprive the tumor of securing new nutrient supply lines, or to otherwise selectively kill the surviving tumor cells. If the blood supply to the tumor were destroyed, retargeting L19 would not be an option unless some blood vessels remain or new ones are formed by the time the second treatment is given. This also raises the question of how well subsequent treatments would be able to reach the surviving tumor cells or otherwise impact the tumor's microenvironment to impede its ability to survive. Admittedly, our knowledge of the tumor or even tissue microenvironments is limited (22,35), and thus we can only address these questions with investigations to evaluate the efficacy of single and multiple treatments, including an as-

essment of temporal relationships with combinational approaches. The importance of examining temporal relationships, particularly with RAIT, has been underscored previously (36,37).

In some respects, RAIT of NHL might be viewed as a combinational approach because the anti-CD20 antibody alone is an effective therapeutic, whose effects are then amplified by the addition of the radioactivity. Unfortunately, in solid tumors most antibodies used for RAIT are themselves not therapeutic, and thus other agents with complementary toxicities capable of amplifying the effects of RAIT will be required. Alternatively, RAIT can supplement other effective treatments, with the combination providing improved responses (38). Pretargeting procedures are also showing more promise than directly radiolabeled antibodies, being able to deliver a significantly higher total dose and increase the dose rate with improved antitumor effects (29,31). Therefore, progress is gradually being made, but it will take more time to assess each new strategy. The promising therapeutic results using the ^{131}I -L19-SIP vascular targeting presented here provide a new stimulus to this field, thus inviting clinical evaluation.

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