Advantage of a Residualizing Iodine Radiolabel in the Therapy of a Colon Cancer Xenograft Targeted with an Anticarcinoembryonic Antigen Monoclonal Antibody

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Abstract

Purpose: A disadvantage of conventionally radioiodinated monoclonal antibodies (mAb) for cancer therapy is the short retention time of the radionuclide within target cells. To address this issue, we recently developed a method in which radioiodine is introduced onto antibodies using an adduct consisting of a nonmetabolizable peptide attached to the aminopolycarboxylate diethylenetriaminepentaacetic acid, designated IMP-R4. This adduct causes the radioiodine to become trapped in lysosomes following antibody catabolism. Clinical-scale production of ¹³¹I-IMP-R4-labeled antibodies is possible using a recently developed facile method.

Experimental Design: The properties of ¹³¹I-IMP-R4-labeled anticarcinoembryonic antigen (CEA) humanized mAb hMN-14 were compared with the directly radioiodinated hMN-14 (¹³¹I-hMN-14) in CEA-expressing human colon cancer cell lines, LoVo and LS174T, and in nude mice bearing established LoVo tumor xenografts.

Results: ¹²⁵I-IMP-R4-hMN-14 retention in the cell lines was significantly increased (61.5% after 3 days) compared with ¹²⁵I-hMN-14. In vivo, a significant improvement in tumor accretion of radio-label was obtained using ¹³¹I-IMP-R4-hMN-14, which led to a marked improvement in therapeutic efficacy. Eight weeks post-treatment, mean tumor volumes were 0.16 ± 0.19 and 1.99 ± 1.35 cm³ in mice treated with ¹³¹I-IMP-R4-hMN-14 and ¹³¹I-hMN-14, respectively, with complete remissions observed in 27% of mice treated with ¹³¹I-IMP-R4-hMN-14 and none using ¹³¹I-hMN-14.

Conclusion: ¹³¹I-IMP-R4-hMN-14 provides a significant therapeutic advantage in comparison to the conventionally ¹³¹I-labeled antibody. The ability of this labeling method to lend itself to clinical-scale labeling, the broad applicability of a humanized anti-CEA mAb for CEA-expressing cancers, and the clinical benefits of radioimmunotherapy with anti-CEA mAb shown recently for small-volume and minimal residual disease combine to make ¹³¹I-IMP-R4-hMN-14 a promising new agent for radioimmunotherapy.

With its success in lymphoma patients (1), radioimmunotherapy is evolving into a conventional modality for cancer therapy. Ibritumomab tiuxetan and Tositumomab are two radiolabeled antibody products directed against the CD20 antigen that have been approved by the U.S. Food and Drug Administration for radioimmunotherapy of non-Hodgkin’s lymphoma. Although radioimmunotherapy of hematologic malignancies has exhibited a certain measure of success, solid tumors have been less responsive, especially when large bulky disease is present. Our goal was to make ¹³¹I, a readily available and popular radioisotope with imaging and therapeutic properties, more effective for radioimmunotherapy by a new facile method applicable to virtually any antibody.

Radioimmunotherapy, a therapeutic modality in which radiolabeled monoclonal antibodies (mAb) are used to selectively target ionizing radiation to tumor sites, has principally involved the β emitters ¹³¹I and ⁹⁰⁰Y. Each of these radiolabels has advantages and disadvantages warranting the use of each, possibly for different applications. ⁹⁰⁰Y mAbs exhibit greater accretion to tumor targets than ¹³¹I mAbs due to the trapping of chelated radiometals within target cells after protein catabolism. In contrast, the catabolic product of radioiodinated antibody, ¹³¹I-iodotyrosine, is able to diffuse out of target cells leading to shortened residence times and correspondingly lower radiation doses delivered to the tumor target (2). To make ¹³¹I more effective in radioimmunotherapy, there has been an ongoing effort to develop new methods of radioiodination designed to trap radiiodine inside a tumor cell following delivery by a labeled mAb. Such intracellularly retained labels are called residualizing radiolabels. The
underlying principle behind these approaches is the inclusion of $^{131}$I as a component of a nonmetabolizable moiety, chemically designed to be lysosomally trapped after catabolism of the carrier mAb. Early work was based on the use of nonmetabolizable carbohydrates as linking agents. Dilactitol-tyramine (3, 4) and tyramine cellobiose (5) are two substrates examine for this purpose. Using dilactitoltyramine, radioiodine accretion in tumor cells increased over 3-fold in vivo (4), and pronounced dosimetric and therapeutic advantages were obtained in animal experiments using nude mice bearing human lung tumor (6) and lymphoma xenografts (7, 8). Using tyramine cellobiose, radioiodine accretion was over twice greater than that of conventionally iodinated antibody, and plasma clearance and uptake in normal tissues was not changed (5). An alternative technique for preparing residualizing labels involved the use of positively charged moieties, including pyridine-based N-succinimidyl 5-[$^{131}$I/$^{125}$I]iodo-3-pyridinecarboxylate (9) and N-succinimidyl 4-guanidinomethyl-3-iodobenzoate (10). Another approach involves the use of nonmetabolizable peptide adducts. We described the use of diethylenetriaminepentaacetic acid–appendaged radioiodinated peptides containing D-amino acids as a class of residualizing $^{131}$I label (11–13). Foulon et al. reported the use of a radioiodinated basic pentapeptide (Lys-Arg-Tyr-Arg-Arg), containing all D-amino acids, as a residualizing label for anti-EGFRVIII mAb, LBA4 (14). A recent report by Shankar et al. (15) described the use of a negatively charged acylating agent, N-succinimidyl 3-[$^{131}$I]iodo-4-phosphonomethylbenzoate (131I-SIPMB), for labeling peptides and mAbs.

All the above approaches for the production of residualizing iodine have yielded enhanced tumor accretion and, where evaluated, substantial improvements in therapeutic efficacy. In comparison with the radiometal-labeled mAbs, the residualizing iodine-labeled mAbs examined by us yielded similar tumor uptake, retention, and efficacy in human tumor xenografts (4, 12, 16, 17). Given that most of the $\beta$-energy of $^{131}$I is absorbed within a small radius, $^{131}$I may be more cytotoxic for small and micrometastatic tumors than a higher energy $\beta$-emitting isotope such as $^{90}$Y, in which a higher proportion of the dose would be dissipated outside the tumor, possibly in neighboring normal tissues. In addition, the longer half-life of $^{131}$I should be an advantage for radioimmunotherapy, where there is relatively slow uptake and accumulation of the radiolabel within tumors. The 2.7-day half-life of $^{90}$Y may not be long enough to take full advantage of the retention of the label at the tumor site. Another difference between $^{131}$I and $^{90}$Y is that $^{131}$I has a $\gamma$ emission permitting localization and quantitation of radioactivity in tissues by imaging. Finally, $^{131}$I has an $\sim 10$-fold cost advantage over $^{90}$Y, assuming the cost of the manufacture of the radiolabeled mAb to be the same for both nuclides, for respective levels of radioactivity approximately corresponding to nonmyeloablative doses used in the clinical radioimmunotherapy of hematologic malignancies.

Despite the compelling advantages of residualizing iodine for radioimmunotherapy these agents have not met with widespread acceptance for clinical use, mainly because of limitations imposed by the chemical syntheses. Low conjugation efficiency and unacceptable antibody aggregation precluded the clinical use of dilactitoltyramine (4) and tyramine cellobiose (5), respectively. In addition, production of the residualizing agents described above involved multistep procedures of radioiodination, activation, conjugation to mAb, and purification. For example, with the pentapeptide of Foulon et al., an intermediate purification step at the radioiodination stage was reported as well as purification of the radioiodinated ligand-mAb conjugate on a size exclusion column done at the end of the process (to remove unincorporated radioiodide and unconjugated $^{131}$I-radioiodinated small molecular mass moieties; ref. 14). The use of $^{131}$I-SIPMB necessitated a high-performance liquid chromatography purification at the radioiodination stage and a PD10 column purification after mAb conjugation (15).

We recently reported the development of $^{131}$I-IMP-R4, an improved residualizing form of $^{131}$I that overcomes many of the limitations that have impeded the development of residualizing iodine for clinical use (17, 18). Importantly, a practical method for producing residualizing $^{131}$I-IMP-R4-labeled mAb at levels needed clinically, which does not involve column purifications and is not more complex than that used in direct radioiodinations, has been developed (19). IMP-R4 contains D-amino acids in the peptide to confer proteolytic stability in lysosomes, D-tyrosine to provide a radioiodination site, diethylenetriaminepentaacetic acid moieties to increase hydrophilicity and aid in intracellular retention, and two maleimide groups for protein binding. Using this adduct to label hRS7, a humanized mAb recognizing EGP-1, an antigen highly expressed in carcinomas of breast, lung, ovary and prostate, marked improvement in tumor uptake and retention and therapeutic efficacy was observed in preclinical models of human lung and breast cancers as compared with conventional radioiodine labeling (17, 18).

In this article, we show the potential of $^{131}$I-IMP-R4-labeled mAb for use in the therapy of carcinoembryonic antigen (CEA)–expressing cancers using colon cancer models. The humanized high-affinity anti-CEA monoclonal antibody hmN-14 was used for radioimmunoconjugate production. Although CEA is well known as a secreted tumor-associated antigen, present in the circulation, and used as a marker of disease progression, it is also present on the surface of many tumor cells, and there have been reports of internalization of anti-CEA antibodies (20–22). We show that the residualizing adduct IMP-R4 can improve radioiodine retention in colon cancer cells, leading to increased radioiodine accretion in human tumor xenografts and marked improvement in therapeutic efficacy in the model system. With its advantage established previously using a rapidly internalizing mAb in two other cancer models, IMP-R4-based $^{131}$I-labeling technology is now evolving into a general method for improving therapeutic outcomes with $^{131}$I-radioimmunotherapy.

Materials and Methods

Monoclonal antibodies and cell lines. Human colon carcinoma cell lines, LoVo and LS174T, were purchased from the American Type Culture Collection (Rockville, MD). The cells were grown in DMEM (Life Technologies, Gaithersburg, MD) supplemented with 10% fetal bovine serum, penicillin (100 units/mL), streptomycin (100 µg/mL), and l-glutamine (2 mmol/L). The cells were routinely passaged after detachment with trypsin, 0.2% EDTA.

MN-14 is a class-III anti-CEA (CD66c, CEACAM5) mAb, reacting with CEA and unreactive with the normal cross-reactive antigen, NCA, biliary antigen, and miconium antigen (23). The construction and characterization of the humanized form of MN-14 (hMN-14, labeltu-zumab) has been described previously (24). hMN-14 was provided by Immunomedics, Inc. (Morris Plains, NJ).
Radiiodination. The peptide IMP-R4 {4-(N-maleimidomethyl)-
cyclohexane-1-carbonyl-Lys[4-(N-maleimidomethyl)-cyclohexane-1-
carbonyl-Lys[1-[[p-thiocarbamylamino]benzyl]diethylenetriaminepen-
taetacetic acid]-0-Tyr-D-Lys[1-[[p-thiocarbamylamino]benzyl]diethyle-
netriaminepentaetacetic acid]}-OH was used for preparation of radiodiode labeled mAb. Preparation of the IMP-R4 peptide, and the procedures for radioiodination of IMP-R4 and conjugation to reduced mAb were as described previously (11, 17). Direct radioiodination of hMN-14 was carried out by the chloramine-
T method (25). Assessment of immunoreactivity was done by size
exclusion high-performance liquid chromatography analysis following incubation of the radioiodinated antibody with the antigen. CEA
(Scripps Laboratory, Inc., San Diego, CA).

Binding and processing of labeled hMN-14 by colon cancer cell
lines. In vitro processing experiments were carried out using the
published procedure (26). Cells were plated at 50,000 cells per well in a
96-well tissue culture plate. After 24 hours, cells were incubated in triplicate with 5 \times 10^5 cpm of radioiodinated antibody (125I-IMP-R4-
hMN-14 or conventionally iodinated 125I-hMN-14) in 0.1 mL of tissue
culture medium for 2 hours at 37°C, washed four times to remove
unbound antibody, and the radioactivity associated with the cells was
counted. This was the total cell-bound radioactivity at “zero” time,
which was taken as 100%. Media was added to all other wells, and the
incubation was continued for 69 hours. Samples were taken at 4, 21,
45, and 69 hours. At each time point, the supernatant was removed and
counted in a γ counter to determine radioactivity released to the
medium. After counting, trichloroacetic acid was added to the
supernatant in the presence of a carrier protein. Samples were
centrifuged, and the radioactivity associated with the pellet was counted
to determine the level of undegraded antibody in the supernatant.
Cells were solubilized in 2 mol/L NaOH, and the cell-bound radioactivity
was determined. All data were expressed as percent of “zero” time
cell-bound radioactivity. Nonspecific binding was determined by adding an
excess of unlabeled hMN-14 before the addition of the radiolabeled
antibodies.

In vitro experiments. For targeting, 5 μCi 125I-hMN-14 (CT) and 12.5
μCi of 131I-IMP-R4-hMN-14 were combined in a final injectate volume
of 0.15 mL/animal, and injected i.v. into 20 tumor-bearing mice.
Groups of five animals were sacrificed at indicated times. Tumor and
normal tissues were counted for radioactivity in a γ counter, and
corrections were made for backscatter of 131I counts into the 125I
counting window. The results were expressed as percent injected dose
per gram of tissue. Radiation dose estimates were determined from
131I-IMP-R4-hMN-14 and 125I-hMN-14 (131I surrogate) biodistribution
data. Calculations were carried out by first integrating the trapezoidal
regions (for tumors) or exponential regions (for normal organs)
defined by the time-activity data corrected for physical decay. To avoid
overestimation of the tumor cumulative dose, a zero-time value of zero
was assumed for the trapezoidal fit of the tumor. For other tissues, the
zero time point was extrapolated according to the exponential curve.
The resulting integral for each organ was converted to cGy/mCi using
values appropriate for organ weight and calculated by assuming
uniformly distributed activity in small unit-density spheres (27).
For therapy, seven groups of 11 mice each, consisting of the following,
were used: untreated group, 250, 275, and 300 μCi 131I-hMN-14 (CT)
dose groups and 225, 250, and 275 μCi of 131I-IMP-R4-hMN-14 dose
groups. Protein doses in the therapy groups were adjusted with unlabeled hMN-14 to account for the different specific activities of the
products of the IMP-R4 and chloramine-T methods. Protein doses
given were all within the range of 60 to 90 μg/dose. The groups were
randomized to contain similar assortment of initial tumor volumes.
Baseline data were compared with weekly measurements of body
weight, tumor volumes, and WBC counts. Animals with progressive
tumor growth reaching 3 cm were euthanized. All animal experiments
were carried out in accord with Institutional Animal Care and Use
Committee-approved protocols.

Results

In vitro studies

Cellular retention of radionuclide, after labeled mAbs were
bound to the cell surface, was studied in colon cancer cell lines using an in vitro assay. Retention of hMN-14 labeled with 125I
using IMP-R4 was compared with that of directly labeled hMN-
14 by measuring the percent of originally bound radionuclide still
bound to the cells at indicated times after removal of excess
labeled mAb. Data are shown for LS174T and LoVo colon carcinoma cell lines in Table 1 and Fig. 1. In both cell lines, a
greater percentage of the radioiodine remained associated with
cells at the later time points when the residualizing label, 125I-
IMP-R4, was used. At 45 and 69 hours 125I-IMP-R4-hMN-14 showed 48.3% greater (P = 0.002) and 61.5% greater (P =
0.0003) levels of cell-bound radioactivity than the directly
labeled antibody in LoVo. In the LS174T cell line, 125I-IMP-R4-
hMN-14 also yielded higher levels of cell-bound radioactivity
compared with the directly labeled antibody at 45 and 69 hours
(P = 0.02 and 0.03, respectively). The percent of intact
antibody, as measured by trichloroacetic acid–precipitable
radioactivity in the supernatant, was the same for both
radiolabels, indicating that the differences observed were due
to internalization and catabolism of the antibody, rather than
loss of intact mAb. Nonspecific binding was <10% for both
LoVo and LS174T cell lines.

In vivo studies

Paired-label biodistribution. Targeting of hMN-14 labeled with
131I-IMP-R4 was compared with that of directly radio-
odinated hMN-14 in paired labeled biodistribution studies.
Figure 2A summarizes the accretion of radioiodine in tumor and
normal tissues over a 14-day time period. 131I-IMP-R4-hMN-14
yielded a progressively enhanced retention in tumor compared
with the directly radioiodinated antibody prepared using the
conventional chloramine-T method [125I-hMN-14 (CT)]. For
example, % ID/g values in LoVo human colon cancer xenografts
7 days post-injection of dual-labeled hMN-14 were 33.1 ± 6.3
and 26.6 ± 4.8 (P = 0.005) for 131I-IMP-R4-hMN-14 and 125I-
hMN-14 (CT), respectively. The percent injected dose (ID)/g in
tumor and blood for the two labels are shown for individual
mice in Fig. 2B. In each animal, IMP-R4 labeling yielded an
increase %ID/g in tumor in the range of 16% to 36% (mean = 25

| Table 1. In vitro processing by LoVo and LS174T cell lines |
|----------------|----------------|----------------|
| Cell line      | Radiolabel     | % Cell-bound cpm (mean ± SD) |
|                |                | 45 h            | 69 h            |
| LoVo           | 125I           | 48.2 ± 5.0      | 40.3 ± 3.3      |
| 125I-IMP-R4    | 71.5 ± 6.6     | 65.1 ± 1.8      |
| LS174T         | 125I           | 57.7 ± 7.5      | 51.6 ± 6.1      |
| 125I-IMP-R4    | 73.2 ± 1.9     | 66.1 ± 4.5      |

Statistical analyses. For in vitro processing and in vivo
targeting experiments, statistical significance was determined by Student’s t test.
For therapy experiments, statistical analyses on different treatment
groups were done by the Student’s t test on the area under the growth
curves. Two-sided tests were used.
on day 7 and 23% to 31% (mean = 26 ± 3%) on day 14 (data not shown). In addition, the residualizing label cleared from blood more quickly (range = 26-30%, mean = 28 ± 1%) on day 7 and 18% to 22% (mean = 20 ± 1%) on day 14 (data not shown). Resulting mean tumor/blood ratios on day 7 were 4.78 ± 0.88 (range = 3.96-6.19) and 2.76 ± 0.42 (range = 2.32-3.29) for the residualizing and conventional labels, respectively (P < 0.001), a mean increase of 73 ± 10%.

Cumulative absorbed radiation doses were calculated from the biodistribution data shown in Fig. 2. Cumulative absorbed doses for 131I-hMN-14 (CT) were calculated using the 125I-hMN-14 (CT) distribution data. Doses were normalized to blood and are presented in Fig. 3. As shown in Fig. 3, the residualizing label, 131I-IMP-R4-hMN-14, is estimated to increase the dose to tumor dose by 47% in comparison to the conventional label, 131I-hMN-14 (CT). Although doses to organs involved in protein processing, including liver, spleen, and kidney, are also elevated for the residualizing label compared with the direct label, the absolute doses are expected to be in the nontoxic range at the estimated MTD. Toxic levels for these organs are taken to be above 2,000 cGy for spleen and kidney and above 3,000 cGy for liver (28).
Radioimmunotherapy. The therapeutic efficacy of $^{131}$I-IMP-R4-hMN-14 was compared with that of directly labeled $^{131}$I-hMN-14 in nude mice bearing LoVo human colon cancer xenografts. Three escalating doses of each agent were given to groups of 11 mice with established tumors. Initial tumor volumes were $0.220 \pm 0.098 \text{ cm}^3$ (range = 0.041-0.388 cm$^3$). Doses given were 225, 250, and 275 μCi in the $^{131}$I-IMP-R4-hMN-14 dose groups and 250, 275, and 300 μCi in the $^{131}$I-hMN-14 (CT) groups. The doses selected for therapy thus had μCi match in the 250 and 275 μCi categories of the two labels but a higher 300 μCi dose in the conventionally treated group, because historical data indicated that the directly iodinated mAb could be tolerated up to this dose. Figure 4A shows the tumor volumes of individual mice in each group over a 10-week period. The results are also shown in terms of mean tumor volumes, in Fig. 4B. Radioimmunotherapy with any radiolabeled hMN-14 preparations used in the study produced significant growth control compared with the untreated group ($P$ for area under the curve = 0.006 at 4 weeks). However, the residualizing $^{131}$I-IMP-R4-hMN-14 label at all doses was markedly better than any of the conventional $^{131}$I-hMN-14 doses ($P$ for area under the curve < 0.0004 versus the 300 μCi conventional $^{131}$I dose at 8 weeks). Overall, the mean tumor volume in the $^{131}$I-IMP-R4-hMN-14-treated mice was $0.16 \pm 0.19$ versus $1.99 \pm 1.35 \text{ cm}^3$ in the $^{131}$I-hMN-14-treated mice at 8 weeks. Complete remissions were observed in six mice in the residualizing $^{131}$I groups compared with none in the conventional $^{131}$I groups.

Toxicity of the radioimmunotherapy treatments was monitored by weekly WBC counts and body weight measurement. All of the tested doses were tolerated, with no resulting treatment-related deaths. The WBC counts for the various groups are shown in Fig. 4C. The nadirs for the residualizing radiiodine-treated mice were lower than that for conventional groups, although for the 225 μCi $^{131}$I-IMP-R4-hMN-14 group, differences in WBC counts from the directly iodinated...
131I-hMN-14 groups were not statistical significantly at most time points. The WBC counts recovered, albeit more slowly in the case of residualizing iodine labeled groups. None of the treatments caused significant body weight loss.

**Discussion**

In this study, the superiority of the IMP-R4 residualizing labeled mAb over directly radioiodinated antibody was shown by in vitro assays and in vivo animal biodistribution and therapy studies. In vitro, after 3 days, cellular retention of radioiodine was increased by 61.5% using 131I-IMP-R4-anti-CEA mAb hMN-14 compared with conventionally iodinated hMN-14. In biodistribution studies in nude mice bearing colon cancer xenografts, 131I-IMP-R4-hMN-14 yielded a mean increase in %ID/g in tumor of 25%, a significant enhancement of retention in tumor compared with the conventionally iodinated antibody. Although this increase is modest compared with the enhancement of radionuclide accretion obtained with the more rapidly internalizing anti-EGP-1 mAb, RS7, previously evaluated in human lung and breast cancer models (where the %ID/g in tumor on day 7 with the IMP-R4-labeled mAbs were 4- and 7-fold the values of directly iodinated mAb), a marked difference in therapeutic efficacy was obtained. At 8 weeks post-treatment, mean tumor volumes were 0.16 ± 0.19 and 1.99 ± 1.35 cm³ in human colon cancer-bearing nude mice treated with 131I-IMP-R4-labeled and directly iodinated-hMN-14, respectively, with complete remissions observed in 27% of mice treated with 131I-IMP-R4-hMN-14 and none using the directly iodinated antibody. The marked tumor growth control achieved using the residualizing 131I radiolabel was not anticipated from dosimetry calculations, which estimated that the radiation dose increase due to the residualizing iodine labeled mAb (47%) was more modest than that observed with the more rapidly internalizing anti-EGP-1 mAb. Apparently, even a small portion of the label internalized and residualized, as a result of membrane turnover, for example, can result in a significant difference in the therapeutic potentials of the two labels.

Colorectal cancer was among the first diseases for which radiolabeled antibodies were investigated as targeting agents. This early application stemmed from the identification of CEA as a suitable target antigen. CEA, also known as CD66e or CEACAM5 (29, 30), was first described in 1965 as a gastrointestinal oncofetal antigen (31) but is now known to be overexpressed in the majority of carcinomas including those of the gastrointestinal tract, the respiratory and genitourinary systems, and breast cancer (32–36). Radiolabeled antibodies to CEA were first applied to the detection of cancer using purified polyclonal anti-CEA immunoglobulin G labeled with 131I (37). Despite this long history, successful radioimmunotherapy of colon cancer, as well as of other solid tumors, is still limited (38–45), probably due to the low specific accretion of the radiolabeled antibody in the tumor target compared with normal tissues and the relative radioresistance of these tumors.

Nevertheless, because tumor uptake, and thus the radiation dose to the tumor, increases with decreasing tumor size, it is likely that radioimmunotherapy can be applied with greater success in small volume and minimal residual disease, as has recently been shown in clinical studies with 131I-labeled humanized anti-CEA antibody hMN-14 (46, 47). An overall response rate of nearly 60% (~20% objective, 40% minor responses) was obtained in patients with known metastatic disease. In addition, seven of nine patients treated in an adjuvant setting after resection of liver metastases remained disease free for up to 36 months, whereas the relapse rate in a corresponding historical control group receiving chemotherapy was 67% over the same time frame. Thus, it is likely that the benefits of radioimmunotherapy can be optimized by using 131I-labeled mAbs in small-volume and minimal residual disease.

These encouraging results in solid tumors can most likely be improved by increasing radioiodine accretion in the tumor by using a residualizing methodology as described here. Although the rate and extent of antibody internalization differs for various mAbs, both rapidly and slowly internalizing mAbs are taken into the cell and catabolized. The rapidly internalizing mAbs presumably enter their target cells by way of receptor-mediated mechanisms, whereas membrane turnover apparently accounts for the slower internalization. Thus, residualizing radiolabels affect the radiation dose delivered to target cells for slowly internalizing mAbs, such as anti-CEA and anti-epithelial glycoprotein-2 (EGP-2) mAb, RS11 (4), as well as the more rapidly internalizing mAbs, such as anti-epithelial glycoprotein-1 (EGP-1) mAb, RS7 (2, 4, 26), CD22 mAb (8), and anti-HER-2/new mAbs (48).

Additional methods aimed at improving tumor accretion of radionuclides are under active investigation. Among the approaches taken are pretargeting methods (49), the use of genetically engineered immunoconstructs with molecular mass intermediate between that of intact immunoglobulin G and Fab' fragments (50, 51), and combination of radioimmuno-therapy with other therapeutic modalities such as external beam irradiation or chemotherapy (52–54). Chelated radiometals represent an alternate source of residualizing labels available for radioimmunotherapy, remaining trapped in lysosomes in the form of lysine adducts of the respective metal chelates (55). Our previous studies predicted an increase in absorbed dose to tumor using residualizing 131I-labeled mAbs in comparison to 90Y, due to the longer physical half-life of 131I (4). This advantage would also be predicted for radiometals of longer half-life, assuming that they are trapped inside target cells as well as the residualizing iodine and 90Y. 177Lu is one such radiometal, with radiophysical properties resembling those of 131I, having a 6.7-day half-life, moderate 496-keV maximum β emissions, and low abundance γ emissions. However, in a direct comparison of 90Y, 177Lu, and residualizing 131I-labeled RS7 in a nude mouse-human lung cancer xenograft model, tumor targeting, therapeutic efficacy, and toxicity of the three radio- nuclides were found to be similar (16). 131I-IMP-R1 was used in that study, an earlier version of the residualizing 131I adduct differing from IMP-R4 in labeling efficiency due to its having only one maleimide and one benzyl-diethylenetriaminepenta-acetic acid rather than two of each group in IMP-R4. Similar results were also observed in a comparison of 90Y and residualizing 131I-IMP-R4 labeled RS7 in the lung cancer xenograft model (17). The similarity of therapeutic efficiencies of 90Y and residualizing 131I in these may be due to the s.c. tumor models used, wherein the tumor sizes are relatively large. It would be interesting to compare these radionuclides in micrometastatic tumor models or in adjuvant settings after debulking surgery, situations which are considered particularly amenable to radioimmunotherapy and which may provide a particular advantage for 131I with its shorter tissue range of...
β emission. A direct comparison of this new method with other β-emitting isotopes, including 90Y and 177Lu, in additional tumor models would also be of interest in future studies. In summary, at the present time for solid tumor therapy, radioimmunotherapy is expected to have the greatest potential when applied to patients with small tumor volume or as an adjuvant therapy. Based on its β-particle energy, tissue range, half-life, and ready availability, 131I is the radionuclide of choice for this application. The development of a practical resiliuizing 131I method, in the form of 131I-IMP-R4, overcomes the problem of reduced tumor dose due to a directly radioiodinated mAb. Using the anti-CEA antibody, hMN-14, in an in vivo tumor model of colon carcinoma, we showed a marked therapeutic advantage of resiliuating radioiodine. It is noteworthy that this effect was observed although the anti-CEA mAb is not considered to be a rapidly internalizing mAb and the dosimetric improvement was not as large as seen in the lung and breast cancer models using rapidly internalizing antibody. Thus, the benefit of resiliuating labels has now been shown for rapidly internalizing and nonrapidly internalizing mAbs and in three cancer models, and is expected to be applicable to other cancer types.

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References


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