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Cancer radioimmunotherapy

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Abstract

Targeting of radionuclides with antibodies, or radioimmunotherapy, has been an active field of research spanning nearly 50 years, evolving with advancing technologies in molecular biology and chemistry, and with many important preclinical and clinical studies illustrating the benefits, but also the challenges, which all forms of targeted therapies face. There are currently two radiolabeled antibodies approved for the treatment of non-Hodgkin lymphoma, but radioimmunotherapy of solid tumors remains a challenge. Novel antibody constructs, focusing on treatment of localized and minimal disease, and pretargeting are all promising new approaches that are currently under investigation.

Keywords

bispecific antibody; cancer; monoclonal antibody; pretargeting; radioimmunotherapy; radionuclide

Antibodies are integral agents of our immune system, primarily used to identify and aid in the clearing of foreign pathogens. While antibodies have been examined for many years as possible cancer therapeutics, early trials with murine monoclonal antibodies were disappointing [1–3]. However, as many as 22 antibodies are now approved for clinical use in a variety of indications, with many more under investigation [4–6]. While antibody-dependent cellular cytotoxicity (ADCC) and complement activation play a role in the success of some unconjugated antibodies, identification of pathways required for cell growth has opened new possibilities for using antibodies as relatively nontoxic agents that can alter these processes and control tumor progression. Still, on their own, relatively few antibodies alter patient survival significantly, but are becoming increasingly important adjuncts, being administered along with standard chemotherapy to enhance the overall response/survival. Thus, interest in developing antibody conjugates to enhance efficacy continues. In this article, we review the use of antibodies conjugated with radionuclides, known as radioimmunotherapy (RAIT), for the treatment of cancer.

Investigations on the use of antibody- conjugated radionuclides began in the early 1950s [7], but it took nearly 25 more years before these feasibility studies came to clinical fruition, demonstrating first that antibodies could selectively localize cancer [8,9], and then illustrating their therapeutic potential [10]. Clinical studies began with the evaluation

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of ^{131}I-labeled antibodies, but in combination with chemotherapy [11,12]. This effort drew criticism because the efficacy of the ^{131}I-labeled antibody alone had not been established and, therefore, future clinical trials focused on monotherapy with radiolabeled antibodies [13]. The initial clinical trials focused on using radioiodinated antibodies but, over time, advances in chelation chemistry have allowed many new therapeutic radionuclides to be explored (Figure 1 & Table 1) [14,15].

Hematological malignancies

The first major advance in RAIT occurred with hematological malignancies, starting with a report that a fractionated dosing regimen using ^{131}I-labeled anti-HLA-DR antibody (Lym-1) achieved remarkable regressions of bulky masses, primarily in patients with non-Hodgkin lymphoma (NHL) [16,17]. Subsequent trials reported success with ^{131}I-labeled anti-CD37 IgG, anti-CD20 IgG, and anti-CD22 IgG in lymphoma [18–22]. In all instances, RAIT was limited by hematologic toxicity, because the radiolabeled antibody cleared slowly from the blood, exposing the radiosensitive bone marrow to a continuous source of low-dose radiation. When the dose was escalated to myeloablative levels with help of bone marrow grafting, a significant number of patients achieved complete objective responses for extended durations [22]. Excellent responses were also reported with nonmyeloablative doses of an ^{131}I-labeled anti-CD20 antibody [21].

These studies claimed that adding unlabeled antibody to the radioimmunoconjugate resulted in a more ‘favorable biodistribution’ [18,21–23]. At low protein doses, the radiolabeled antibody cleared into the spleen quickly, where a sizeable number of normal B cells reside, which also express these antigens, but patients with bulky disease also cleared the antibody quickly. Predosing with unconjugated antibody blocked the rapid uptake in this antigen sink and slowed the radiolabeled antibody’s blood clearance, which, in turn, gave the radioimmunoconjugate more time to localize to more tumor sites. A similar finding was confirmed in clinical investigations with a ^{90}Y-labeled anti-CD20 antibody [24], selecting 250 mg/m^2 as the preferred predose of unlabeled antibody [25]. While examining the optimal protein dose for an ^{131}I-anti-CD20 IgG, Kaminski *et al.* noted that some patients given a predose of 685 mg of anti-B1 (tositumomab) actually experienced tumor shrinkage after receiving only an imaging dose of the ^{131}I-labeled anti-CD20 antibody [21,26]. Preclinical studies also noted antitumor activity with the unlabeled antibody in xenograft models [27]. Shortly thereafter, clinical studies confirmed the antitumor effects by another unlabeled, chimeric anti-CD20 antibody, later known as rituximab [28].

The ^{90}Y- and ^{131}I-labeled anti-CD20 antibodies, now known as ^{90}Y-ibritumomab tiuxetan (Zevalin™, Spectrum Pharmaceuticals, CA, USA) and ^{131}I-tositumomab (Bexxar®, GlaxoSmithKline, NC, USA), respectively, are the only radiolabeled antibodies approved for cancer therapy in the USA, being registered to treat chemotherapy-refractive, follicular NHL, with or without transformation. ^{131}I-tositumomab requires a pretherapy imaging study in order to establish the prescribed therapy dose. ^{90}Y-ibritumomab is administered at a fixed dose of 0.4 mCi/kg, but requires a dose reduction to 0.3 mCi/kg if platelet counts are below 150,000. Neither treatment is recommended for patients with more than 25% bone marrow involvement, since these patients have an increased risk of more severe hematologic toxicity.

Antitumor responses in NHL occur at relatively low radiation-absorbed doses (e.g., much less than 1000 cGy) [29–32]. Although clear evidence for a dose-response relationship is lacking, recent data using 3D dosimetry and radiobiological modeling have provided reasonable positive predictive values for response versus mean absorbed dose and equivalent uniform dose [33]. Radiation dose–response relationships are probably confounded by the

fact that each of these treatments uses substantial amounts of their respective unconjugated anti-CD20 IgG, which are therapeutically active, yet the radioimmunoconjugate is able to enhance the response. For example, a randomized trial comparing the efficacy of rituximab (Genentech, CA, USA) (375 mg/m^2 four-times weekly) to ^{90}Y-ibritumomab tiuxetan (250 mg/m^2 chimeric rituximab two-times weekly with the radiolabeled murine anti-CD20) found superior objective response with RAIT [34]. ^{131}I-tositumomab was also more active than unlabeled murine anti-B1 antibody [35]. Research also has shown that these antibodies can enhance a tumor cell's sensitivity to radiation and chemotherapy [36–40], and thus in these treatments, responses probably represent a combination of the unconjugated antibody and targeted radiation. Unfortunately, the data from the randomized trial did not find significant difference in the duration of response or time to progression for RAIT over the unconjugated antibody therapy, so clinical acceptance for this treatment has been tempered, even though durable responses have been reported for patients who achieved a complete response following RAIT [41,42].

A number of other issues have hindered the acceptance of RAIT. For example, the incidence of secondary cancers and of myelodysplastic syndrome (MDS) is a concern, but there is considerable evidence suggesting that the overall risk for RAIT is no higher than chemotherapy [43–45]. There were concerns that the hematologic toxicity associated with RAIT would reduce a patient's tolerance to chemotherapy, yet clinical studies contradicted this [46]. Kaminski *et al.* reported encouraging response rates and durations with ^{131}I-tositumomab given as a front line treatment [47]. In addition, 28 patients were able to receive a second ^{131}I-tositumomab treatment with no additional toxicity, with all of the 18 patients who responded to the first treatment responding to the second treatment [48,49]. While RAIT as a stand-alone treatment for follicular lymphoma has not garnered much attention, an increasing number of clinical trials are incorporating RAIT successfully with chemotherapy or, occasionally, external beam therapy [50–63]. There is also interest in using RAIT in high-dose therapy regimens with chemotherapy and external beam radiation (or possibly as a replacement for whole-body radiation) in cytoreductive marrow conditioning regimens [43,64–76].

Several other radioantibody conjugates have been tested clinically in hematologic malignancies. A few centers reported success with ^{131}I-rituximab [77,78]. One recent study administered two doses of ^{131}I-rituximab, given approximately 6 weeks apart, commencing 6 weeks after completion of a standard unconjugated rituximab therapy [78]. They reported a 90% overall response with a 50% complete response and a median time to progression of 20 months, which was significantly longer than the last chemotherapy regimen the patients had received prior to qualifying for this study. Lym-1, a murine anti-HLA-DR10 antibody, was examined as an ^{131}I-labeled (Oncolym, Peregrine Pharmaceuticals, CA, USA) and ^{90}Y-labeled conjugate with promising activity in initial clinical testing [79,80], but is no longer being pursued for commercial development. A ^{131}I-labeled anti-CD45 antibody is being tested in patients who will undergo a myeloablative procedure because CD45 is expressed on many different types of white blood cells [81]. A Phase I/II trial for ^{90}Y-labeled epratuzumab (humanized anti-CD22 IgG; Immunomedics, Inc., NJ, USA), administered as fractionated two- or three-weekly injections, found that 11 out of 12 (92%) of the follicular lymphoma patients receiving a total dose of more than 30 mCi/m^2 had a complete response, with a median duration of 24.6 months [82]. In addition, ten patients with follicular lymphomas who were previously refractory to an anti-CD20-containing therapeutic regimen achieved a median progression-free survival of 21.5 months, with nine of these patients (90%) obtaining a complete response. Encouraging responses were also noted in patients with aggressive forms of NHL.

Preclinical studies suggest that further improvements could be achieved by combining the ^{90}Y-labeled anti-CD22 IgG with unconjugated anti-CD20 antibody therapy [83]. As mentioned previously, both of the currently approved anti-CD20 RAIT agents administer a substantial amount of unlabeled anti-CD20 IgG prior to the radiolabeled anti-CD20 (as much as 900 mg is administered prior to the therapeutic radioimmunoconjugate) [84]. This procedure was reported to enhance tumor visualization and tumor dosimetry, derived in small, separate groups of patients, suggesting that the radiation-absorbed dose was not affected [23,24]. However, preclinical studies have indicated that unlabeled anti-CD20 can reduce tumor uptake of radiolabeled IgG [83,85]. By using a radioimmunoconjugate that binds to a different, yet B-cell-specific, antigen, both agents would have an equal ability to localize in the tumor without competing for binding sites. If the unconjugated anti-CD20 IgG is administered sometime before the radiolabeled anti-CD22 (e.g., 1–3 weeks), it also would reduce the normal B-cell sink that could affect the radiolabeled anti-CD22 targeting. Animal studies have also suggested that a consolidation course of unlabeled anti-CD20 antibody therapy after RAIT could amplify the response and its duration [86].

Radioimmunotherapy with β-emitting radionuclides increases the risk for severe myelosuppression, because the β-emissions, which can travel several millimeters, cause collateral damage to nontargeted normal tissues (Table 1). However, shorter-range radionuclides, such as α-emitters, travel less than 0.1 mm (i.e., a few cells deep) and, thus, would be a better choice in treating cancer in the blood or bone marrow. Early clinical investigations focused on ^{213}Bi-labeled anti-CD33 IgG in myeloid leukemia [87–91]; however, even though leukemic cells were rapidly localized, ^{213}Bi's short physical half-life (46 min) presents logistical issues for conjugate preparation and is not ideally suited for an IgG when tumors are less accessible. Investigators have speculated that a more rapid targeting system might be necessary [92,93]. Longer-lived α-emitters, such as ^{211}At (7.2 h), and ^{225}Ac, which has a 10-day half-life and emits four daughter α-particles during its decay, are also being explored as a better match for an IgG. However, issues with renal toxicity need to be addressed [94–101]. Targeted α-particle therapy is being explored in more easily accessible settings, such as the peritoneal cavity or the cavity left following primary brain cancer surgery [102–106], or to the tumor vasculature [107–111], where the radionuclide would be more quickly localized to the disease.

Auger-emitters, such as ^{125}I, ^{67}Ga and ^{111}In, have such a limited range that they are only effective when deposited very near or within the nucleus [112]. Therefore, they are active only against single cells, but could be suitable for micrometastatic disease, even in the bone marrow, because of the limited potential for collateral damage [113,114]. One of the few systems where Auger-emitters have been used successfully has been with an anti-CD74 antibody [115]. In this system, even though there are a small number of antigen sites on the cell surface, the antigen is constantly recycling, thereby transporting and emptying the antibody with its radioactive payload inside the cell and then returning to cell surface, where it is available to bind additional antibody. Activity was also reported for ^{111}In-labeled antibodies to the EGF receptor (EGFR) and HER-2 [116].

Nonhematologic malignancies

Clinical trials in advanced solid tumors rarely progress beyond Phase I/II because, even though there is occasional evidence for tumor shrinkage, most fail to meet accepted criteria of an objective response [117–121]. However, two agents did proceed to more advanced clinical testing in solid tumors. The first was pemtumomab (R1549; Antisoma PLC, UK), a ^{90}Y-labeled murine anti-HMFG1 (MUC-1) murine antibody that was administered intraperitoneally to ovarian cancer patients with measurable and occult disease. Promising data were first reported from a Phase II trial, where this agent (18 mCi/m^2) was administered

to 21 women with stage Ic–IV ovarian cancer, who had no detectable disease after completing surgery and a platinum-based chemotherapy regimen [122]. Unfortunately, this experience was not confirmed in a large, Phase III, randomized study [123]. A pivotal trial performed in China showed more promising results in 107 lung cancer patients that were given an ^{131}I-labeled chimeric antitumor necrosis therapy IgG (Shanghai MediPharm Biotech Co. Ltd, China) [124]. A total of 62 patients received two intravenous injections spaced 2–4 weeks apart (0.8 mCi/kg), while another 45 patients received an intratumoral injection of 0.8 mCi/kg. Not surprisingly, patients receiving the intratumoral administration had less hematologic toxicity than the systemically administered group. However, the overall response rates were similar (~35%), with most having partial responses. Because the trial was designed to evaluate response only at 10 weeks post-treatment, the full duration of these responses, and the impact on survival, are not known. This agent is not approved for this indication in the USA, but early clinical trials in the USA using this antibody labeled with ^{131}I (Cotara, Peregrine Pharmaceuticals Inc., CA, USA) for the treatment of brain and colorectal cancers have been reported [125–127].

Perhaps one of the more obvious issues with RAIT in solid tumors is that, unlike lymphoma, most of the antibodies used, are themselves, unable to affect tumor growth. Preclinical and clinical studies have been examining the possibility of using trastuzumab, cetuximab or panitumumab [95,128–138], but their binding specificity might limit their utility. For example, imaging studies have indicated that trastuzumab has enhanced uptake in the heart wall, but dosimetry estimates suggested this might not be dose limiting [137]. Encouraging clinical responses, using a trastuzumab–drug conjugate, may provide additional incentive to evaluate the radioimmunoconjugate [139]. Improved responses with ^{90}Y-labeled panitumumab in a head and neck cancer model was also reported recently [140]. In addition to ensuring tumors express antigen, cetuximab or panitumumab radioimmunoconjugates should also require patient selection based on wild-type *KRAS* status in order to enhance the prospects that the unconjugated antibody will provide additional therapeutic response [141].

Owing to a lack of evidence for significant benefit in patients with advanced and disseminated solid tumors, efforts are being undertaken to find ways to: improve antibody uptake (both quantitatively and in a more uniform distribution); enhance the tumor's sensitization to radiation; or administer RAIT when less disease is present.

Meredith *et al.* combined an ^{131}I-labeled anti-carcinoembryonic antigen (CEA) antibody (COL-1) with ^{131}I-CC49 anti-tumor-associated glycoprotein (TAG)-72 IgG, because immunohistology demonstrated that the combination gave a more homogeneous distribution within the tumor than either antibody alone [142]. In addition, patients received IFN-α, which had been reported to enhance CEA and TAG-72 expression in gastrointestinal tumors. Tumor imaging was judged to be excellent in most cases, and in comparison with other trials that used only ^{131}I-CC49, the combination of anti-CEA and anti-TAG-72 antibodies, together with interferon, appeared to result in a modest increase in the absorbed dose to the tumor; however, no objective responses were reported. When using antibody combinations, one needs to be cognizant that while this might enhance the uniformity of distribution, the absolute uptake of the antibody combination may be less than that of the single antibody with the highest uptake, which was illustrated well by Pagel *et al.* using three non-cross-reactive antilymphoma antibodies [143].

Enhancing uptake by modifying the tumor's vascular properties through hyperthermia, radiation, or biologically active compounds has been widely investigated [144–150], but these manipulations usually have only a modest effect on antibody uptake. Locoregional administration, such as intraperitoneal and intracranial, is another example of modifying the route of administration in order to give an antibody a 'first-pass' opportunity to bind to the

tumor, enhancing uptake [151]. Early preclinical data demonstrated an advantage for intraperitoneal injection, particularly, but not conditionally, when there is malignant ascites [152,153]. One of the earliest clinical studies to examine route of administration coinjected $^{131}I/^{125}I$-B72.3 anti-TAG-72 IgG intraperitoneally and intravenously. It was concluded that peritoneal implants were more likely to benefit from the intraperitoneal injection, whereas nonimplants (i.e., those metastases in the peritoneal cavity derived from hematogeneous spread) were more likely to have higher uptake by intravenous injection [154]. A clinical trial using only intraperitoneally administered ^{177}Lu-CC49 anti-TAG-72 IgG in ovarian cancer noted a trend for improved responses only in patients with minimal disease, which is expected based on the short-range β-emission of ^{177}Lu [155]. This group also examined the combination of intraperitoneally administered ^{90}Y-CC49 with IFN-α and paxlitaxel (for upregulation of TAG-72 and radiosensitization, respectively) [156]. Two partial responses were noted in patients who had measurable disease at the time of treatment, while four out of of 11 patients who had no measurable disease were disease-free from 9 to 24 months following therapy.

As mentioned previously, a Phase III trial of intraperitoneal RAIT for ovarian cancer with a ^{90}Y-antibody failed to improve survival in patients who had been surgically debulked and had received primary chemotherapy [123]. Whether this failure was related to using the long-range-emitting ^{90}Y in a potentially suboptimal setting of minimal or microscopic disease could be debated, particularly because a retrospective analysis showed relapse was less likely to occur in the peritoneal cavity in the RAIT-treated patients, and primarily in a subgroup of patients who had residual disease following surgery [157]. These results are very important since they reflect a very common finding for regional therapies of all kinds; namely, cancer is a systemic disease that, when treated locally, can lead to impressive local responses, but often needs to be coupled with a potent systemic treatment to affect survival. Thus, while a number of investigators are considering shorter-ranged β-emitters or even α-emitters for less toxic and more directed localized treatment [158–162], improved survival may be achieved only when the treatment includes an effective systemic counterpart.

In contrast to peritoneal involvement, brain cancer does not usually spread to other organs, making localized treatment ideal, particularly when there is a solitary lesion at diagnosis. Clinical studies have examined treatments where RAIT is injected directly into the tumor, but more often treatment is administered to eradicate locally advanced disease after a solitary mass is surgically removed [104]. A real advantage for the latter approach is that, unlike the peritoneal cavity, radiolabeled antibodies placed directly in the surgical cavity reside almost entirely in this cavity, sparing exposure of other organs. Clinical studies with an ^{131}I-labeled antitenascin antibody for the treatment of glioblastoma multiforme (GBM; Neuradiab, Bradmer Pharmaceuticals, Inc., ON, Canada) have been encouraging, with the median survival rivaling that of brachytherapy or steriotactic radiosurgery, but with a lower rate of radionecrosis [163–166]. A correlation between the radiation-absorbed dose delivered to the rim of the cavity, where the radioantibody was deposited, and tumor recurrence or radionecrosis was also reported, including various parameters that reflected a positive outcome for the patient [167]. Encouraging survival results were also reported for the same agent when administered locally in newly diagnosed patients, so as to boost radiation exposure to the surgical margins in combination with conventional external beam radiation and chemotherapy [168]. Early clinical testing of ^{188}Re-labeled humanized anti-EGFR antibody (nimotuzumab, Biocon, Bangalore, India) and an ^{211}At-antitenascin antibody has also been reported [106,169,170]. In addition to intracranial RAIT, radiolabeled antibodies are being administered intrathecally to treat disease in the CNS [138,171–175]. Although not given compartmentally, Quang *et al.* reported positive outcomes using intravenous or intra-arterial injections of ^{125}I-labeled 425, an anti-EGFR antibody, to patients following surgical resection of their primary brain cancer and in

concert with external beam therapy [176]. In a recent overview of a Phase II experience of patients who received three weekly intravenous treatments of ^{125}I-anti-EGFR (~50 mCi/dose) following surgery and radiation therapy or with temozolomide therapy, the median survival was 15.7 months, which was improved over a contemporaneous control group that did not receive RAIT, but since the study was not randomized, the control group was not well balanced [177]. Indeed, the median survival for GBM patients following surgical resection with subsequent radiotherapy and temozolomide is 14.6 months [178]. With additional improvements being reported for the standard treatment of recurrent GBM by the addition of bevacizumab [179], and with new clinical trials now examining this combination in first-line therapy, RAIT will need to do better if it is to be integrated in these currently approved regimens. However, because of its good safety profile, it may still become an important part of GBM treatment, particularly when administered compartmentally.

Early preclinical studies had demonstrated that RAIT rarely eradicated large tumors, but cures could be achieved when tumors of minimal or microscopic size were treated, and, just as importantly, effective treatment against microscopic metastatic disease could be erased if the animals also had bulky tumors at the time of treatment [180,181]. Thus, it is reasonable for clinical trials to turn to indications where the extent of disease is minimal or microscopic (i.e., adjuvant). Liersch *et al.* reported trial results using an ^{131}I-humanized anti-CEACAM5 IgG in 23 patients who had undergone liver resection for metastatic colorectal cancer [182]. At the median follow-up time of 91 months, 19 assessable patients had a median survival of 58 months, while in a contemporaneous group of similar patients who had not received RAIT after liver resection, the median survival was only 31 months [183].

Radioimmunotherapy is also being combined with other agents to enhance therapeutic prospects in solid tumors. Most efforts in solid tumors have focused on adding a sub-therapeutic, rather than a therapeutic dose of drugs, such as paclitaxel, docetaxel, topotecan and gemcitabine, as demonstrated in models for breast, colorectal, pancreatic and prostate cancers, enhancing responses to RAIT [184–189]. As with any combination modality, there are numerous dosing and scheduling issues required to optimize the therapy [190,191]. Panijdeh *et al.* showed the feasibility of cotargeting RAIT with chemotherapy through antibody-directed enzyme-prodrug therapy (ADEPT), using an engineered single chain (scFv) of the A33 anti-gpA33 antigen fused with cytosine deaminase, a prodrug-converting enzyme [192]. We recently reported enhanced antitumor activity by combining an effective antibody–drug conjugate with RAIT [193]. In this approach, the targeting was performed with antibodies identifying different antigens coexpressed in pancreatic cancer xenografts, but effective therapy could also be achieve using antibodies against the same target. Interestingly, the antibody–drug conjugate (IgG-SN-38) could be administered at a dose that was effective alone, but also with the maximum tolerated dose of RAIT without appreciably altering toxicity. Clinically, a number of feasibility trials combining RAIT with chemotherapy have been reported [120,194–196]. Most recently, a Phase Ib clinical trial was reported to have promising results with a ^{90}Y-labeled antibody (clivatuzumab tetraxetan; Immunomedics, NJ, USA) directed against a pancreatic cancer mucin used in combination with low-dose gem-citabine as a front line treatment for patients with metastatic pancreatic cancer [197,198]. In this protocol, patients are administered three weekly doses of the ^{90}Y-antibody, each dose followed by 200 mg/m^2 of gemcitabine. Interestingly, the fractionated dosing regimen has been tolerated at a cumulative dose of 45 mCi/m^2 (3 × 15 mCi/m^2), whereas as single-dose treatment, the maximum tolerated dose was just 20 mCi/m^2 [199]. Investigators reported that 68% of the patients had disease control (i.e., stable and partial responses) across all dose levels, starting with 3 × 6.5 mCi/m^2 of the ^{90}Y-antibody [198]. While this and other reports have demonstrated the safety of these combinations, it is too early to determine if any of these approaches will provide the benefit necessary for RAIT to become an acceptable treatment for solid tumors.

Most therapy trials have used whole IgG, not only because it can be easily manufactured and handled, but preclinical models found that IgG has the highest tumor uptake when compared to other forms, such as enzymatically cleaved $F(ab')_2$ or Fab′ fragments or various engineered forms (Table 2) [200–202]. IgG's slow blood clearance encourages high tumor uptake, but this also exposes the highly radiation-sensitive red marrow to a continuous source of low-dose radiation, causing myelosuppression well before a tumoricidal dose is achieved (except in radiosensitive hematopoietic diseases). Tumor uptake is also affected by others factors. For example, tumors have an abnormal vascular system that is 'leaky', allowing macromolecules to pass more easily, which is why even a nonspecific antibody will have a higher concentration in the tumor than in most normal tissues [203–205]. However, tumors can have reduced lymphatics, and so internal pressure builds within the tumor, which impedes the free flow of molecules throughout the tumor mass, with larger molecules being affected more than smaller ones. Movement of targeted molecules (i.e., not just antibodies) is also affected most significantly by the 'binding-site barrier' (i.e., when a molecule binds to its target, it will be retained at this location, usually being concentrated in the perivascular space) [206–208]. Movement of molecules with higher affinity within the tumor is more affected than ones with lower affinity, albeit higher-affinity antibody may have enhanced uptake [206,208,209]. This restricted localization can be overcome to some degree by increasing the protein dose [210,211], but excessive unlabeled product could compete and reduce the amount of radiolabeled product in the tumor. Nevertheless, concentrating radiation in the perivascular space may cause the local destruction of the blood vessels, which, in turn, could affect tumor progression.

Reducing bone marrow exposure by hastening the clearance of the antibody from the blood, but with the same tumor retention as the IgG, has been attempted using anti-antibodies or extracorporeal adsorption procedures [212,213]. The extracorporeal method avoids the formation of immune complexes in the body that will inevitably redirect the radioimmunoconjugate to the liver and spleen, greatly increasing their radiation exposure. This method has been promising in preclinical testing, but whether sufficient escalation in the administered dose will bring about a significant improvement in responses, clinically, is still to be determined.

Single chains (~25,000 Da) and diabodies (~50,000 Da), which are smaller monovalent and divalent binding proteins, respectively, and a variety of other types of constructs, are gaining interest as possible alternatives to intact IgG. Constructs with divalent binding are generally preferred over monovalent ones, because they usually have a longer retention time in the tumor [208,214,215]. These constructs invariably have a faster clearance from the blood than intact IgG, because of their physical size, but also because most lack the Fc portion of the IgG that has regions capable of binding the neonatal Fc receptor, which is used by an intact IgG to traverse vascular endothelium, allowing it to stay in the blood for long periods [216]. Smaller antibody forms are better able to traverse the vascular channels, yielding a more rapid tumor uptake, superior tumor/nontumor ratios and potentially more uniform distribution as compared with IgG, but they too are susceptible to the binding-site barrier effect. In addition, the faster a protein clears from the blood, the less time is allotted for target interaction; therefore, tumor uptake for these constructs is lower than an IgG. Faster clearance reduces red marrow exposure, allowing the total administered activity to be increased. While this increase does not often permit higher doses to the tumor, rapid tumor uptake delivers the radiation at a higher dose rate, which is more cytocidal than a low dose rate [217]. With the advent of molecular engineering, a variety of constructs with more favorable pharmacokinetics and tumor uptake, such as CH2-deleted antibodies and point-mutated scFv–Fc fusion proteins that avoid neonatal Fc receptor binding, have been described [216,218].

The primary deterrent for using molecules less than 50,000 Da is that they are cleared through the kidneys, which raises concern for renal toxicity, particularly when a radionuclide that is reabsorbed and retained by the kidney, such as radiometals, is used. Behr *et al.* reported that a high predose of cationic amino acids could significantly reduce renal tubular reabsorption of radiometal-labeled Fab′, and demonstrated that higher doses could be administered to mice with less renal toxicity [219]. This, and other approaches, have been used to reduce the risk for renal toxicity [220], but since renal uptake often far exceeds tumor uptake, the compensation afforded by these methods, which can be as much as a twofold reduction, may not be sufficient to yield positive tumor:kidney ratios. Because indications of renal toxicity are not manifested for many months, or even years, after treatment, careful monitoring is required for extended periods.

With all the different antibody forms made possible through molecular engineering, it is important to keep in mind that radionuclide selection may vary based on the antibody's biodistribution, tumor retention and blood clearance properties [221,222]. For example, ^{131}I remains an isotope of interest for compounds that clear quickly with elimination primarily through the kidneys, since it does not have as long a residence time as a radiometal conjugated to an antibody. However, smaller antibody constructs that have a shorter retention time in the tumor than an IgG (e.g., ≤1 day vs >3 days, respectively) would not benefit from the longer physical half-life of ^{131}I, namely approximately 8 days. The long physical half-life of ^{131}I is also of little use for antibodies that rapidly internalize, with the cells not retaining the radionuclide sufficiently to take advantage of the longer half-life. In this situation, ^{177}Lu seems to be a reasonable replacement for ^{131}I, since it has similar half-life and β-energy, albeit ^{131}I can be coupled to antibodies that would allow it to be retained in cells [223]. Some conjugation processes can affect antibody binding, which can affect radionuclide selection, and even after radiolabeling, stability and retention of binding must be diligently tested. Thus, there are a number of factors that contribute to the selection of the most appropriate radionuclide for a radioimmunoconjugate, and while there are many radionuclides in nature to choose from, availability and cost have limited the choices for clinical use.

Pretargeted radioimmunotherapy

In the mid-1980s, pretargeting was introduced as a way to improve tumor:blood ratios (Figure 2). The initial concept envisioned a bispecific (bs) monoclonal antibody (mAb) capable of binding a tumor antigen and a chelate [224]. The bs mAb would not be radiolabeled, and thus no exposure would occur while it localized to the tumor. Since there was ample clinical experience with radiolabeled chelates, such as diethylene triamine pentaacetic acid (DTPA) or ethylene-diaminetetraacetic acid (EDTA), demonstrating these agents cleared very quickly from the blood and tissues with minimal residual activity remaining in the body, the radioactivity would be carried by the chelate loaded with a radionuclide. Thus, the antichelate binding arm of the bs mAb that had been pretargeted to the tumor would serve as a receptor for the radiolabeled chelate. The small size and inert properties of the radiolabeled chelate allowed it to traverse blood vessels and distribute in the fluidic volume within minutes, and then just as quickly be eliminated, thereby minimizing nontarget tissue exposure. A significant improvement to bs mAb pretargeting was made by Le Doussal *et al.*, who determined that radiolabeled compounds bearing two haptens were more stably bound in the tumor than a monovalent compound, since they can cross-link two adjacent bs mAbs on the tumor cell surface (Figure 2). This is a concept known as the affinity enhancement system (AES) [225].

Several other pretargeting methods have been investigated, but they all strive to overcome the limitation of the slow blood clearance of a directly radiolabeled IgG by separating

antibody targeting from the delivery of the radionuclide [226]. The first was based on avidin (mammalian) or streptavidin (bacterial) in conjunction with biotin in a variety of configurations [227]. This system appeared to be ideally suited for pretargeting, since the avidins could bind as many as four biotin molecules with an exceptionally high binding constant (10^{-15} M), the reagents could be produced in plentiful amounts, were nontoxic and amenable to chemical modification for coupling to antibodies or radiolabeling. Two avidin/biotin-based pretargeting configurations were examined clinically. One system consisted of three agents, starting with a streptavidin-conjugated antibody (later replaced with a recombinant streptavidin–scFv) that was administered for 1–3 days to localize the tumor [228–231]. A clearing agent then was given to remove residual streptavidin antibody from the blood. The clearing step was essential because the ultrahigh affinity of biotin for avidin would have caused the subsequently administered radiolabeled biotin to bind primarily to the conjugate in the blood, reducing its ability to reach the tumor. The other approach uses four agents, beginning with a biotinylated antibody [232]. Avidin, a glycosylated protein, is used as a clearing agent, but it is administered along with streptavidin, a nonglycosylated bacterial protein. The avidins will bind to the tumor-localized biotinylated antibody, serving as a bridge to link it to radiolabeled biotin that is administered 1 day later. The primary issue with pretargeting methods that rely on avidin/streptavidin is the immunogenicity of these foreign proteins [233,234]. A third type of pretargeting system is also under development, which uses complementary synthetic DNA analogs, known as morpholinos, as bridging agents [235,236]. These morpholino compounds should have a low immunogenicity and therefore, when this system is fully optimized, it could also lead to further improvements in radionuclide targeting.

Since the pretargeting agent acts as a surrogate receptor that will bind the radiolabeled compound, it must remain accessible, either on the cell surface or within the interstitial space of the tumor. Therefore, antigens known to internalize might not be best for pretargeting [237]. However, we have evaluated an anti-Trop-2 bs mAb that can be used effectively in pretargeting even though the bs mAb internalizes [238]. Thus, antigens that internalize should not be dismissed as potential agents for pretargeting.

The initial antihapten antibodies used in a bs mAb pretargeting approach bound with high specificity to metal-binding chelates, but chelates are often so specific for certain metals that an antibody raised against one chelate–metal complex might not bind as well when loaded with a different radiometal. To avoid this complication, the chelate would first need to be loaded with its appropriate metal so it would bind well to the antichelate–metal complex, and then the peptide portion of the divalent hapten complex could be radiolabeled. For example, tyrosine in the peptide core can be radioiodinated. A different ligand capable of binding ^{99m}Tc/^{188}Re has been coupled to a di(In)DTPA-peptide [225,239,240]. However, a more practical approach is to use an antibody against a synthetic compound that is not involved in the binding of the radionuclide, allowing radionuclide binding to be controlled by another constituent within the hapten–peptide complex. The approach has been successfully used with an antibody to histamine-succinyl-glycine (HSG) [241]. Di-HSG-peptide structures have been developed for binding radioiodine, ^{90}Y, ^{177}Lu, ^{111}In, $^{67/68}$Ga, ^{18}F, ^{99m}Tc and ^{188}Re [242–245].

Regardless of the pretargeting approach taken, data have consistently shown that pre-targeting delivers as much, or more, radioactivity to a tumor as a directly radiolabeled antibody, but with much less exposure to the red marrow [228,246]. In addition, radiolabeled biotin or hapten/peptide localizes in the tumor very quickly, maximizing the number of decays occurring in the tumor rather than elsewhere in the body. Thus, pretargeting increases the dose rate to the tumor as compared with a directly radiolabeled IgG that can take 1–2 days to reach maximum accretion. Radiolabeled biotin and hapten/

peptides also have far less renal accretion than a radiometal-labeled antibody fragment, providing favorable tumor:kidney ratios. Pretargeting systems might also avoid problems when circulating antigen is present. With a directly radiolabeled antibody, the antibody–antigen complexes formed in the blood are processed primarily by the liver, increasing hepatic exposure. In pretargeting, if the complexes are cleared and processed before the radiolabeled compound is administered, hepatic uptake would be minimized. Pretargeting approaches might be more amenable to combination therapies, particularly with agents that cause severe hematologic toxicities. Several studies have shown combinations with chemotherapeutic radiosensitizing agents and antiangiogenic agents improve responses [149,247–249].

Most clinical experience has been limited to Phase I trials designed to determine optimal targeting conditions, with subsequent accrual to determine the maximum tolerated dose. One Phase II trial examined a dose of 110 mCi/m^2 of ^{90}Y-DOTA-biotin pretargeted with a NR-LU-10, an anti-EpCam (or EGP-2) IgG–streptavidin conjugate in advanced colorectal cancer [230]. Disappointingly, no significant responses were observed and radiation-absorbed tumor doses reported in just two patients were only approximately 500 and 2900 cGy, respectively. Hematologic toxicity was mostly mild to moderate, but because the anti-EpCam antibody bound to the normal colon, a substantial fraction of the radiation was delivered to the intestine, causing severe diarrhea that was dose-limiting. With most of the radioactivity being removed from the body by renal filtration, it was not surprising that some evidence of renal toxicity was observed in this trial. More recently, a recombinant protein of streptavidin with four CC49 (anti-TAG-72) single chains has been tested in patients [250]. Dosimetry from this study suggests that if 110 mCi/m^2 of ^{90}Y-biotin was tolerated, colorectal tumors could receive 5000 cGy or more in some patients.

Hematologic toxicity was dose limiting in clinical trials using a bs mAb pretargeting approach [251], as well as avidin/biotin approach [252,253]. Investigators found medullary thyroid cancer patients developed hematologic toxicity at lower doses than other cancer patients. Other studies revealed that these patients often have tumor involvement in the bone and bone marrow that is not appreciated at the time of enrollment, and this might explain the lower tolerance [254]. Despite this limitation, a retrospective analysis of the efficacy of the pretargeting procedure in medullary thyroid cancer patients found a statistically significant increase in the survival of a subset of patients who had a calcitonin doubling time of 2 years or less [255]. Investigators speculated that enhanced survival might have been attributed by the control of micrometastatic bone marrow disease by the pretargeted therapy that used an ^{131}I-labeled hapten-peptide.

Pretargeting will also most likely have its greatest impact in patients with more radiosensitive tumors, with minimal disease, or in locoregional applications. In one trial, previously treated glioblastoma (n = 16) or astrocytoma (n = 8) patients, who, after having a second debulking, were given two intracranial treatments of a biotinylated antitenascin antibody followed by ^{90}Y-DOTA-biotin. Two patients had a partial response and four had a mixed response (25% overall response rate) [256]. In another trial, 37 patients with grade III gliomas or glioblastoma (grade IV) were divided into two groups. All had no radiological evidence of disease following surgical debulking and radiotherapy with or without chemotherapy, but one group of 19 patients also received an intravenous adjuvant pretargeted therapy. The median survival of the glioblastoma patients who did not receive the additional pretargeting procedure was 8 months (n = 12), while the median survival in the group given pretargeted therapy was 33.5 months (n = 8). The median survival for six patients with grade III glioma was 33 months, but the median survival for the 11 grade III glioma patients who received adjuvant-pretargeted therapy could not be estimated, since only two had died at the time the report was filed [253].

Encouraging preclinical studies using a streptavidin- or a bs mAb-pretargeting procedure, targeting CD20 in NHL xenografts, have been reported [257]. It appears that an anti-CD45–streptavidin fusion protein is also being developed, primarily in a transplant setting, since this antigen is more broadly expressed in blood cells than CD20s restricted presence on B cells [258]. Our group has also demonstrated that administering an anti-CD20 antibody therapy after RAIT, or pretargeted RAIT, improves responses [86]. Thus, with its ability to exert better responses with much less hematologic toxicity, pretargeting could represent a significant improvement over the existing approved, directly radiolabeled, anti-CD20 IgG agents.

We are now embarking on clinical studies with newly engineered, humanized, recombinant tri-Fab bs mAbs that bind divalently to a tumor antigen, and with the added specificity for binding the HSG hapten (Figure 3) [259]. Preclinical studies have found that these recombinant bs mAbs, prepared using a unique dock and lock technique, to be excellent pretargeting agents. For example, for imaging, small animal imaging studies showed pretargeting with an ^{124}I-labeled di-HSG-peptide revealed very small (0.3 mm) nodules of a human colonic tumor disseminated in the lungs of nude mice, while ^{18}F-fluor-deoxyglucose (^{18}F-FDG) failed to disclose these lesions [245]. Specificity is also improved, with animal studies showing a bs mAb pretargeting procedure does not localize an inflammatory lesion, but will target a tumor in the same animal, while ^{18}F-FDG shows uptake in both lesions [243]. Pretargeting's low background activity is also better suited for specific detection of tumors in the peritoneal cavity [260]. As previously mentioned, therapeutically we found pretargeting has an advantage over direct targeting in models of colorectal and pancreatic cancers, as well as NHL [246,249,261]. Initial clinical studies found that these novel tri-Fab bs mAbs are cleared very quickly from the blood, despite their similar molecular size to an IgG [262]. Data suggested the bs mAb was stable *in vivo*, so their rapid clearance is probably related to lacking the Fc portion of the antibody. The bs mAb localized in the tumor, and initial clinical results with the radiolabeled hapten-peptide have shown targeting of known tumor masses even though the procedure has not yet been optimized.

Conclusion & future perspective

Radioimmunotherapy is a promising modality, with well-documented preclinical studies attesting to its clinical prospects in numerous indications. Clinical studies have been encouraging in NHL, but the slow adoption of the first RAIT products is disconcerting, although there are encouraging studies from Europe indicating that RAIT can be effectively integrated in a multimodality approach. There are also encouraging developments with new therapeutics for lymphoma that combine RAIT and effective antibody therapy in a manner that could enhance the overall response and duration without affecting toxicity. New therapies with α-emitters may still provide greatly needed improvement in the treatment of leukemias.

Integrating RAIT into the treatment of solid tumors will be more challenging, but there are a number of promising developments. Using antibodies that have therapeutic activity could improve efficacy, but preclinical data have emphasized that RAIT would be best utilized in situations where the extent of disease is minimal, even micrometastatic, or localized. RAIT is well suited for these situations since, except for manageable hematologic toxicity, it is well tolerated, and the treatment is not given over a protracted period of time, as are most chemotherapy regimens. Programs evaluating localized treatment of brain cancers are well advanced and have shown encouraging results. However, there have been equally encouraging results in using RAIT as an adjuvant treatment following surgical resection of hepatic metastases in colorectal cancer, but early results from clinical studies with

fractionated RAIT combined with gemcitabine have shown surprising responses even in patients with advanced pancreatic cancer.

While there continues to be some questions concerning long-term safety issues for RAIT, data in NHL patients have upheld the view that RAIT has no higher risks than currently used chemotherapy regimens. Thus, in our view, RAIT remains a viable treatment option for cancer, and there are even interesting new developments for treating various forms of infectious disease that should be considered [263–269].

Radioimmunotherapy is one of the few treatment modalities where the deposition of the therapeutic can be readily detected and measured in tumors and tissues. Thus, molecular imaging to select and monitor patients can be adapted for most agents. While tumors are often readily detected, there are still difficulties in predicting responses based on dosimetry and radiobiological models. Improving these basic understandings would provide RAIT with an essential advantage if these models could better predict toxicity and efficacy. We need to develop a better understanding of how to best administer these treatments (single or fractionated), how often they can be administered (multiple cycles) and how can they be best integrated with other agents to enhance the overall response. This undertaking is formidable, requiring considerable preclinical and clinical testing to refine these models, which would probably vary for different cancers and treatments.

New molecularly engineered antibodies may provide a better balance in achieving high tumor accretion with reduced tissue exposure. Currently, preclinical studies have indicated that pretargeting strategies can boost efficacy by optimizing tumor uptake, while greatly minimizing normal tissue exposure, which could enhance responses in hematologic and nonhematologic malignancies. By reducing radiation exposure to the normal tissues, pretargeting may be better poised to be combined with other treatment modalities. Whether the added complexity will be worth the effort is an issue resolved only by additional clinical testing.

Radioimmunotherapy is truly a multidisciplinary technology, requiring the skills of medical, surgical and radiation oncologists, nuclear medicine physicians and physicists to coordinate and manage patients undergoing these treatments. While the experience in building a user base for the approved radiolabeled therapeutics in lymphoma has highlighted some difficulties, particularly in the USA, these hurdles can be overcome as treatment outcomes improve. Thus, even though RAIT might be considered the grandfather of many of the advances in molecularly targeted therapeutics over the past 10 years, it remains a promising technology with new opportunities to advance cancer treatment.

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Executive summary

Background

- The targeting of therapeutic radionuclides conjugated to antibody (radioimmunotherapy) has a 50-year history for enhancing response over unconjugated antibodies.
- Therapeutic radionuclides have different properties that can be used to optimize the response in various clinical situations.

Hematologic malignancies

- High sensitivity to radiation has allowed for maximum therapeutic benefit in patients with follicular non-Hodgkin lymphoma.
- Therapeutically active unconjugated antibodies probably enhance overall response.
- Two radiolabeled antibody products are approved for human use; several other agents are under investigation.
- New trials are finding additional advantages when radioimmunotherapy is given for consolidation therapy.

Nonhematologic malignancies

- Nonhematologic malignancies are more challenging to treat owing to higher resistance to radiation.
- Localized delivery (e.g., intracranial for localized brain cancer or intraperitoneal for ovarian cancer), treatment of residual disease and combination with radiosensitizing chemotherapeutics are promising areas of investigation.
- Engineered antibodies are providing new opportunities.

Pretargeted radioimmunotherapy

- Pretargeting is a multistep process that separates antibody targeting from the radionuclide targeting.
- Tumor uptake that can sometimes rival that of an IgG but is capable of reaching maximum accretion within 1 h, while substantially minimizing tissue and red marrow exposure, is a hallmark of all pretargeting procedures.
- Bispecific antibodies with radiolabeled peptides and streptavidin conjugates/fusion proteins with radiolabeled biotin have been examined clinically with promising initial results.

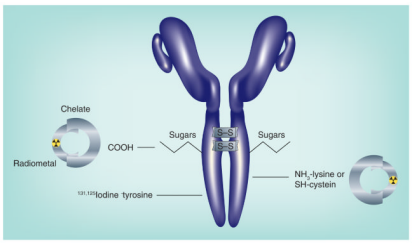

Figure 1. Radionuclides are attached to antibodies principally by two methods

Radioiodine is bound to aromatic rings, primarily to tyrosine, in the presence of a mild oxidative agent, such as iodogen or chloramine-T. The ε amino group of lysine can be modified to accept a metal-binding chelate, which is then loaded with a radiometal. Exposing IgG to a mild reducing agent can split disulfide bonds, allowing the coupling of chelate or other compounds to the reactive sulfhydryl. To ensure amino acids within the antigen-binding sites of the antibody are not altered, carbohydrates, commonly found on the IgG's C_H2 domain, can be modified to accept a chelate.

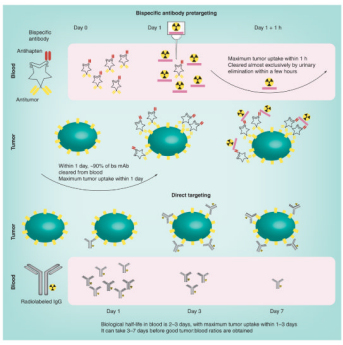

Figure 2. Comparison of bispecific pretargeting with direct targeting

For pretargeting, an unlabeled bs mAb is used (shown is a tri-Fab recombinant structure described in Figure 3). In patients, 90% of this antibody is cleared from the blood, and animal studies have demonstrated that maximum tumor uptake occurs within approximately 6 h. The radioactivity is introduced on a small peptide (~four amino acids) that contains two haptens to help stabilize binding within the tumor (affinity enhancement). The peptide also contains structures that will bind the radionuclide. The radiolabeled hapten–peptide is administered when the bs mAb is sufficiently cleared from the blood, and clears exceptionally fast from the blood and body by urinary excretion, reaching peak tumor uptake within 1 h. Tumor:blood ratios are highly favorable within a few hours. In direct targeting, the isotope is on the antibody. Shown here is an IgG that will clear very slowly from the blood, gradually building its concentration in the tumor, so tumor:blood ratios remain relatively low over the first few days.

bs mAb: Bispecific monoclonal antibody.

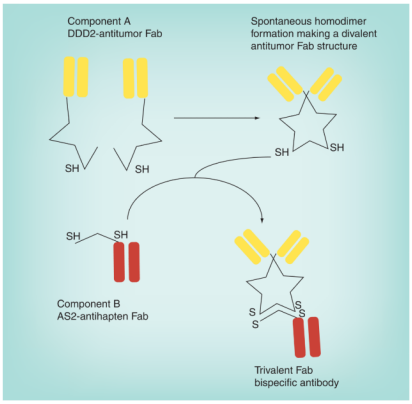

Figure 3. A humanized recombinant bispecific antibody formed by the 'dock and lock' method

The bs mAb consists of two proteins. The first is a Fab, specific for a tumor antigen, modified with a peptide structure known as the DDD, which was modified to introduce a cysteine residue in a strategic location (DDD2). The DDD2 residues have a natural affinity for binding to each other to form homodimers, resulting in a divalent Fab structure. The second protein is the Fab modified with an anchoring domain (AD) that will specifically dock within the dimerized DDD2 structure. The AD segment also had cysteine residues strategically placed so that when the docking occurs, the cysteine residues on the DDD2 and AD2 will lock into place.

bs mAb: Bispecific monoclonal antibody; DDD: Dimerization docking domain. Adapted from [259].

Table 1

Therapeutic radionuclides for radioimmunotherapy.

Radionuclide	Energy (MeV_{max})†	Range†	Half-life
β			
^{90}Yttrium	2.28	11.3 mm	2.7 days
^{131}Iodine	0.61	2.3 mm	8.0 days
^{177}Lutetium	0.50	1.8 mm	6.7 days
^{188}Rhenium	2.12	10.4 mm	0.7 days
^{67}Copper	0.58	2.1 mm	2.6 days
α			
^{213}Bismuth	8.3	60–85 μm	0.8 h
^{211}Astatine	6.8		7.2 h
^{225}Actinium	6.8		10 days
Auger-electron			
^{125}Iodine		2–500 nm	60.5 days

†As reported by Kassis [270].

MeV_{max}: Maximum range of particulate energy in tissue.

Table 2

Targeting properties of various antibody forms.

	IgG	$F(ab')_2$	Fab′	scFv
Physical properties				
Estimated molecular weight (kDa)	150	100	50	25
Biological properties				
Relative biological half-life in blood†	1 day	2 days	3 h	4 min
Target organ	Liver	Liver	Kidneys	Kidneys
Tumor-binding properties				
Relative uptake‡	1	2	3	4
Relative duration‡	1	2	3	4
Time to optimum accretion	Day(s)	Day(s)	Hour(s)	Hour(s)

†Antibody half-life in blood.

‡Highest = 1; lowest = 4.

scFv: Single-chain Fv fragment.