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Review

Emerging Trends for Radioimmunotherapy in Solid Tumors

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Abstract

Due to its ability to target both known and occult lesions, radioimmunotherapy (RIT) is an attractive therapeutic modality for solid tumors. Poor tumor uptake and undesirable pharmacokinetics, however, have precluded the administration of radioimmunoconjugates at therapeutically relevant doses thereby limiting the clinical utility of RIT. In solid tumors, efficacy of RIT is further compromised by heterogeneities in blood flow, tumor stroma, expression of target antigens and radioresistance. As a result significant efforts have been invested toward developing strategies to overcome these impediments. Further, there is an emerging interest in exploiting short-range, high energy α-particle emitting radionuclides for the eradication of minimal residual and micrometastatic disease. As a result several modalities for localized therapy and models of minimal disease have been developed for preclinical evaluation. This review provides a brief update on the recent efforts toward improving the efficacy of RIT for solid tumors, and development of RIT strategies for minimal disease associated with solid tumors. Further, some of promising approaches to improve tumor targeting, which showed promise in the past, but have now been ignored are also discussed.

Key words: animal model, antibody, radioimmunotherapy, radiopharmaceuticals, targeted therapy

Introduction

Radioimmunotherapy (RIT), despite its limited success in solid tumors, remains an attractive concept due to its ability to potentially target both overt and occult lesions in an antigen-specific manner. Poor tumor accretion (that reduces efficacy), unfavorable pharmacokinetics (that cause dose-limiting myelotoxicity) of antibody-based radiopharmaceuticals, and radioresistance are the major impediments that limit the efficacy of RIT. Thus, it is not surprising that much of preclinical research has been centered on improving the pharmacokinetics of radioimmunoconjugates, overcoming the physiological barriers that result in limited delivery, searching for ideal chemistries and radionuclide combinations for enhancing the therapeutic efficacy. Efforts have also been directed toward developing combined modality therapies by including cytotoxic and radiation-response modulating agents in conjunction with RIT to enhance therapeutic response.

We have previously reviewed the utility of antibody engineering to improve the pharmacokinetics and biodistribution of radiolabeled antibodies in solid tumors.[1] Recently, we also reviewed various impediments that limit the delivery of radioimmunoconjugates and discussed the experimental approaches investigated to modulate these barriers.[2] Several articles have reviewed various other aspects of solid tumor RIT, including the role of radiobiology in tumor cell killing, clinical experience with RIT, pretargeting strategies, targeted therapies, optimization of delivery, and the use of various radionuclides for RIT.[3–13]

Development of efficient labeling methodologies has fueled recent interest in exploiting short-range, high energy α-particle emitting radionuclides for treating solid tumors by RIT in a minimal residual disease setting. In addition to the identification and use of new antigenic targets, RIT and radioimmunoimaging strategies are also being developed using monoclonal antibodies (MAbs) that are conventionally approved as unlabeled therapeutic agents. Antiangiogenic agents are being explored to improve the efficacy of both cold and radiolabeled antibodies. On one hand, these new developments provide hope for RIT-based approaches for treating nonhematological malignancies. On the other hand, these developments have introduced novel concepts, concerns, and new models in the field of RIT. One of the

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emerging concepts is the possibility of targeting stem cells with RIT. Some of the concerns include peritoneal toxicity of α-emitter and variable response to antiangiogenic agents. Recent efforts have been directed toward identification of ideal radionuclides for intraperitoneal RIT, and development of appropriate models for minimal residual disease after surgical resection. In this review article we provide an update on the recent developments for radiolabeled antibody-based approaches for solid tumors. The review further discusses some of the promising approaches tested in the past, to improve tumor-to-tissue ratios (extracorporeal elimination) and counter the problem of antigen heterogeneity (use of antibody cocktails) in tumors, but have now been forgotten or ignored.

RIT and Minimal Residual Disease

In solid tumors, external beam radiation therapy (EBRT) remains the method of choice for delivering a high radiation dose to large tumors. There is an emerging consensus that the primary role of RIT in solid tumors will be to treat micrometastatic disseminated disease or minimal residual disease for preventing relapse. This has been reaffirmed with the success of intraperitoneal RIT in the management of ovarian cancer patients after debulking.[14] Similarly, a phase I study involving colorectal cancer patients with liver metastasis demonstrated the usefulness of RIT over chemotherapy for treating disseminated disease.[15] Further, in a phase II trial, RIT with ^{131}I-labetuzumab (anti-CEA) after resection of liver metastasis in colorectal cancer patients improved the overall survival, supporting the utility of RIT in an adjuvant setting.[16,17]

Intraperitoneal RIT: emerging trends

The peritoneal cavity is a preferable metastatic site for several malignancies like ovarian, colorectal, pancreatic cancers and mesotheliomas. Because of their disseminated and microscopic nature, peritoneal metastases are difficult to manage surgically and can benefit from RIT. Intraperitoneal RIT can deliver high absorbed doses to locally confined disease with minimal irradiation to radiosensitive organs because of a slow and incomplete exchange with systemic circulation.[14] Hence, in comparison to systemic RIT, intraperitoneal RIT is more effective and less toxic. Several antibodies conjugated with β-particle emitting radionuclides were initially evaluated in clinical trials (reviewed in Meredith et al.[14]). Several studies have been undertaken to identify the most suitable radionuclides (β-, α- or auger-emitters), antibody formats [F(ab')$_2$ IgG, IgM] and types (internalizing vs. noninternalizing), and evaluating the toxicity of radionuclides on the peritoneal membrane.[18–24] In the context of intraperitoneal RIT involving α-particle emitters, recent studies have also explored their utility in several ways: multiple administrations[25] and the impact of using variable specific activities[26]; compared the efficacy of intraperitoneal RIT with pretargeted intraperitoneal RIT[27]; and investigated the impact of antibody glycosylation on efficacy.[28]

Optimal radionuclides and antibody characteristics for intraperitoneal RIT

The initial studies with intraperitoneal RIT have evaluated β-particle emitting radionuclides, like ^{131}I, ^{90}Y and ^{177}Lu due to their clinical acceptability and availability.[14,29–32] However, due to their higher linear energy transfer (LET) rates, α-emitters are more potent in eradicating small lesions in the peritoneal cavity. Further, due to their short half-life in conjunction with the slow clearing kinetics of the peritoneal cavity infusions, α-emitters are likely to have less nontarget toxicity as compared to β-emitters. In fact, the first clinical trial with an intraperitoneal administration of ^{211}At-conjugated MX35 F(ab')$_2$ (directed against sodium-dependent phosphate transport protein 2b or NaPi2b) in ovarian cancer patients demonstrated that the absorbed dose to the peritoneum was >100 times more compared with red bone marrow without any noticeable toxicity.[21] In the intraperitoneal RIT model of ovarian cancer, the ^{211}At-conjugated MOv18 (anti-folate MAb) exhibited greater therapeutic efficacy as compared to ^{131}I-MOv18.[24] A direct comparison of ^{213}Bi- and ^{177}Lu-labeled D9 MAb (specific for delta 9-1 mutant E-cadherin) in intraperitoneal RIT indicated a similar therapeutic efficacy against gastric cancer carcinomatosis. However, ^{177}Lu-labeled immunoconjugate was associated with adverse effects, including lymphoblastic lymphoma, proliferative glomerulonephritis, and hepatocarcinoma.[18] The efficacy of RIT regimens, including intraperitoneal RIT, is dependent on the retention of radioimmunoconjugate at the target site. In a recent study, Rauch et al. investigated the impact of the molecular size on the intraperitoneal retention of radiolabeled antibodies.[23] The D9 MAb described previously, was engineered into Fab, IgG and IgM formats and the constructs radiolabeled either with ^{213}Bi or ^{111}In were evaluated for retention and biodistribution. Due to the differences in the half-lives of the radioisotopes, the biodistribution of ^{213}Bi-labeled immunoconjugates was evaluated up to 180 minutes after intraperitoneal administration, while ^{111}In-labeled antibodies were evaluated at 24 and 72 hours postadministration via intraperitoneal and intravenous routes. Rapid accumulation of ^{213}Bi-Fab was observed in the kidneys, while ^{213}Bi-IgM and ^{213}Bi-IgG exhibited the highest accumulations in the liver and blood, respectively.[23] Further, a higher accumulation of ^{111}In-IgM was observed in the liver and spleen 24 hours after i.v. administration. Immunoscintigraphy indicated greater intraperitoneal retention of IgM compared to IgG after intraperitoneal administration. Peritoneal clearance is mediated by the diffusion across peritoneal membrane, which provides greater resistance to high molecular weight IgM as compared to smaller IgG and Fab. However, radiolabeled IgM exhibited a faster blood clearance rate than IgG that resulted in a relatively shorter biological half-life of ^{111}In-labeled IgM (24 hours) in comparison to IgG (165 hours). Thus, due to increased peritoneal retention and rapid blood elimination, IgM appear to be ideally suited for delivering α-emitters via intraperitoneal route.

Although most of the studies with intraperitoneal RIT have involved β- and α-particle emitters, Pouget et al. have demonstrated the utility of Auger electron-emitting radionuclide ^{125}I in conjunction with noninternalizing antibodies.[33] The energy of Auger electrons is about 20–50 keV and can travel up to 2–500 nm in tissues. The cytotoxic effects of α-particles and Auger electrons are attributed to their ability to induce DNA damage. Thus, given their shorter path length and low rates or linear energy transfer in comparison to α-emitters, the application of Auger electrons in combination with noninternalizing antibodies may appear

counterintuitive. However, previous *in vitro* studies from the group demonstrated that in comparison to internalizing radioimmunoconjugates, noninternalizing ^{125}I-labeled antibodies exhibited more pronounced cell death suggesting the sensitivity of the cell membrane to ionizing radiation.[33] Subsequently, the therapeutic superiority of noninternalizing ^{125}I-labeled antibodies in treating peritoneal carcinomatosis was demonstrated *in vivo* using the vulvar squamous carcinoma xenograft model.[20] After intravenous administration, the maximal tumor accretion of ^{125}I-labeled noninternalizing anti-CEA MAb 35A7 was significantly higher than radioiodinated internalizing anti-EGFR MAb m225. Further, ^{125}I-35A7 resulted in a higher dose deposition in the tumor than ^{125}I-m225, produced greater reduction in tumor size and significantly prolonged the median survival. On the other hand, the dose deposition in the normal organs was comparable.[20] Although the enhanced tumor accumulation can be attributed to the resistance of noninternalized antibody to dehalogenation, differences in antigen density and pharmacokinetic properties of the antibodies should also be carefully examined before drawing definitive conclusions. Recently, a "brief intraperitoneal RIT" approach involving high dose administration of noninternalizing ^{125}I-35A7 was described.[19] In contrast to the previous study where 37 MBq ^{125}I-labeled MAbs were administered intravenously, in "brief intraperitoneal RIT" 185 MBq of radioimmunoconjugate was administered intraperitoneally and the unbound antibody was removed 1 hour postadministration by flushing the peritoneal cavity with saline. SPECT imaging indicated that after flushing the radioiodine signal was associated only with the intraperitoneal tumors. The brief intraperitoneal RIT resulted in a better tumor-to-blood ratio of 5 as compared to intravenous RIT for which the tumor-to-blood ratio was 1.7. The mean absorbed dose in tumor by brief intraperitoneal RIT was comparable to intravenous RIT (11.6 Gy and 16.7 Gy, respectively); however, the latter resulted in significantly higher absorbed doses in the normal tissues.

Impact of α-particles on the peritoneum

The localized delivery of radionuclides in intraperitoneal RIT results in reduced toxicity to distant organs and bone marrow. However, irradiation of peritoneal wall and visceral organs can significantly increase toxicity. In clinical studies involving EBRT and β-emitters, kidneys, liver and intestinal crypts exhibit dose-limiting radiosensitivity.[14,29–32,34–37] In contrast, localized administration of α-emitters delivers a high dose to the target site with minimal toxicity to the surrounding tissues. Hence, radiosensitivity of the peritoneum is the most likely anticipated concern in the context of the intraperitoneal administration of α particles. Cederkrantz et al. studied the effect of α radiation on normal mouse peritoneum after intraperitoneal administration of ^{211}At-trastuzumab at varying doses ranging between 0–50 Gy.[22] Peritoneum to plasma clearance was analyzed using ^{51}Cr-EDTA as a tracer, while inflammation was determined by immunohistochemistry. Irradiated mice exhibited a slower clearance than normal mice at a tolerable dose of 35 Gy, indicating a dose-dependent reduction in the peritoneal capacity, whereas lethality was observed at 50 Gy. Immunohistochemical analysis for the plasminogen activator inhibitor (PAI-1; a marker for peritoneal healing) and calprotectin (a marker of inflammation) revealed no differences between various absorbed dose levels.[22] These functional and immunohistochemical findings suggested a limited risk to the peritoneum by high dose localized administration of α-emitters that are being extensively evaluated for intraperitoneal RIT.

RIT in animal models of minimal residual disease and metastasis

While minimal residual disease accounts for relapse and metastasis in most patients, there is a paucity of animal models for evaluating therapeutic strategies like RIT in a minimal disease or metastatic setting. Most preclinical studies for RIT have employed subcutaneous xenograft models that neither represent minimal disease nor metastasize to distant sites. However, several recent studies have modeled minimal residual and metastatic disease in rat and mouse models and evaluated the efficacy of RIT.[38–45] Some of the models of minimal disease and the RIT strategies employed in these studies are schematically described in Figure 1.

In a preclinical model and a phase I clinical trial, Behr et al. provided one of the earliest evidences indicating that the efficacy of RIT was comparable to chemotherapy in treating small volume metastatic colorectal cancer with relatively reduced side effects.[15] For the preclinical studies, human colon cancer cells (GW-39) were transplanted by intrasplenic administration in athymic mice to induce multiple liver metastases and treatment (chemotherapy or ^{131}I-labeled anti-CEA antibody) was initiated either on day 10 or 20 post-tumor implantation.[15] The therapeutic efficacy of radiolabeled antibodies in treating metastatic mammary cancer in a HER-2/*neu* transgenic mouse model was evaluated recently by Song et al.[46,47] The transgenic mice expressing rat HER2/*neu* under the control of MMTV promoter were injected with rat HER2/*neu*-transfected mouse mammary tumor cell line NT2.5 via either intracardiac or intravenous routes. While the intracardiac inoculation resulted in metastatic dissemination into the liver, spleen, and bone,[46] the intravenous injection of tumor cells caused lung metastases.[47] In the disseminated metastatic model, RIT with ^{213}Bi-labeled anti-rat HER2/neu MAb was effective in controlling early stage micrometastatic disease. Although the survival improved from 28 days (control untreated animals) to 41 days after RIT, the increase in survival was not statistically significant.[46] In the lung metastasis model, the efficacy of ^{225}Ac-, ^{213}Bi-, and ^{90}Y-labeled anti-rat HER2/neu was compared.[47] RIT was administered either 3 or 18 days after cell implantation to model early and late stage disease, respectively. ^{225}Ac-labeled antibody was more effective in comparison to ^{213}Bi- and ^{90}Y-labeled antibody and significantly improved the survival. In the early disease setting the ^{225}Ac-labeled antibody led to a complete eradication of disease in 67% of animals and with a median survival of 1 year.[47] In late stage disease, however, α-particle RIT was less effective and ^{225}Ac-labeled antibody resulted in a median survival of 51 days.[47] In an elegant study, Pfost et al. demonstrated the utility of localized α-particle RIT for orthotopic bladder cancer.[48] Transurethral resection is the standard of care for patients with urothelial bladder cancer. However, the procedure results in free floating cells, which frequently

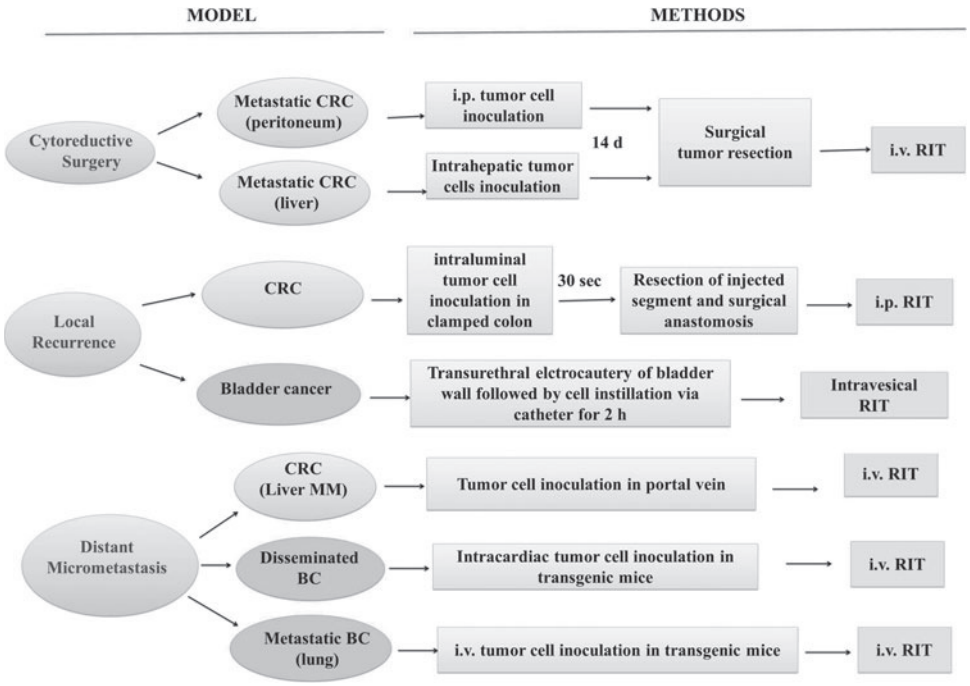

FIG. 1. Schematic representation of various models of minimal disease and metastases tested for evaluating solid tumor RIT. These models have been developed either in mouse (colorectal cancer) or rats (bladder and breast cancer). A brief description of methods for cell inoculation and subsequent procedures, if any, is provided in the boxes. RIT with β- or α-particle emitters was administered by various routes as indicated and the details regarding the antibodies tested and cell lines used is provided in the text. Most of these studies involved administration of RIT both immediately after cell inoculation or surgical procedures and after a delay of 7–14 days to model both minimal disease and established tumors, respectively. CRC, colorectal cancer; BC, breast cancer; MM, micrometastases; i.p., intraperitoneal; i.v., intravenous; RIT, radioimmunotherapy.

contribute to recurrence. To recreate this clinical scenario in a mouse model, Pfost et al. performed electrocauterization of the bladder wall via a catheter to mimic surgical resection. This was followed by transcatheter instillation of luciferase-labeled EJ28 human urothelial carcinoma cells, which were allowed to settle for 2 hours.[48] Intravesical RIT was performed (via catheter) with ^{213}Bi-labeled anti-EGFR MAb (matuzumab) after 1 hour, 7 days, or 14 days postcell instillation. RIT was highly effective in mice receiving radiolabeled antibody either 1 hour or 7 days after cell instillation as compared to mice receiving no treatment (PBS), standard localized chemotherapy (intravesical mitomycin C) or RIT after 14 days.[48] Again these findings demonstrated the usefulness of α-particles in treating minimal disease.

A series of models depicting various scenarios of minimal disease or micrometastatic disease using syngeneic rat colon carcinoma cells CC-531 was described in Wag/Rij rats. MAb MG1 reactive against a 80 kDa antigen on the surface of CC-531 cells was used for RIT. In a model with resected peritoneal carcinomatosis, the ^{177}Lu-MG1 antibody improved survival after cytoreductive surgery as compared to chemotherapy.[44,45] Koppe et al. also demonstrated the efficacy of RIT in peritoneal carcinomatosis after cytoreductive surgery.[49] In these studies, rats were first injected intraperitoneally with 2×10^6 CC-531 cells and tumors were allowed to grow for 14 days. Subsequently, the animals were allowed to undergo cytoreductive surgery or exploratory laparotomy and either left untreated or treated with intraperitoneal RIT or chemotherapy. Increase in overall survival by 88 days and eradication of tumor growth was observed with 74 MBq of ^{177}Lu-labeled MG1 after cytoreductive surgery.[49] Improved survival in rats with microscopic liver metastasis was also demonstrated.[39] CC-531 cells were injected in the portal vein of Wag/Rij rats to induce liver metastasis followed by intravenous RIT with ^{177}Lu-MG1 either at the day of inoculation or 14 days postinoculation. A significant difference in the survival was observed with RIT immediately after surgery as compared to the control and 14 days after surgery, indicating the relative usefulness of RIT for small microscopic metastasis.[39] Another model was tested to demonstrate the utility of intravenous RIT as an adjuvant therapy after the surgical removal of hepatic metastasis.[40] Subcapsular inoculation of the tumor cells in the liver was performed to initiate tumor formation. After 2 weeks, the liver tumors were surgically resected and animals were subjected to intravenous RIT with ^{177}Lu-MG1 either within 3 hours or 7 days after resection. An improved overall survival was observed with immediate administration of ^{177}Lu-MG1 compared to the administration after 7 days, indicating the efficacy of RIT as an adjuvant treatment modality.[40] Utility of RIT in an adjuvant setting to prevent local recurrence was also demonstrated in another variation of a rat model of minimal disease.[38,41] The tumor cells were surgically implanted in the clamped colonic lumen and the tumor cells were allowed to adhere to the walls for 30 seconds after which, a 5 mm section of the colon surrounding the needle puncture was resected and the continuity was restored by suturing.[38,41] The animals were randomized and left untreated (PBS) or subjected to intraperitoneal RIT with ^{177}Lu-

MG1 either on the same day of surgery or 5 days post-surgery.[41] No tumor growth was found in the majority of the animals administered with RIT on the day of surgery, whereas animals receiving the RIT after 5 days developed macroscopic tumors.[41] The same model was also utilized to compare the efficacy of RIT with chemotherapy in preventing local recurrence.[38] While RIT and chemotherapy exhibited comparable efficacy, RIT was associated with fewer signs of acute toxicity as compared to chemotherapy.[38]

Targeting stem cells with radiolabeled antibodies

In various malignancies, cancer stem cells (CSCs) have emerged as critical determinants of disease aggressiveness, chemotherapy resistance and relapse.[50–52] Hence, there is an interest in developing therapeutic strategies targeting CSCs. Radiolabeled antibodies conjugated to β-emitters can, in theory, kill surrounding cells by a bystander "cross-fire" effect and this killing is independent of the expression of the target antigen or the sensitivity of the cells to chemotherapeutic drugs. Thus, RIT can be a potentially promising approach for eradicating CSCs. However, preclinical and clinical studies have not determined the impact of RIT on stem cells. Encouraged by the promising results in a clinical trial with ^{188}Re-labeled anti-Melanin MAb 6D2 (IgM) in patients with metastatic melanomas,[53] Jandl et al. evaluated the effect of ^{188}Re-6D2 on the stem cells in a preclinical xenograft model.[54] As compared to untreated controls, the ^{188}Re-6D2 treated animals had a fourfold decrease in tumor size. Resected tumors were analyzed for melanoma stem cell markers ABCB5 (marker of chemoresistant melanoma stem cells) and JARID1B (marker for slow-cycling melanoma cells) by immunohistochemistry and flow cytometry. Unlike chemotherapy which results in the enrichment of chemoresistant stem cells, no change in the percentage of ABCB5 and JARID1B positive cells was observed in RIT-treated tumors as compared to untreated tumors, indicating a similar rate of killing of melanoma stem cells and bulk tumor cells. These results suggested that chemoresistant and slow-cycling cells are susceptible to β-emitter-based RIT.

While β-emitter-based radioimmunoconjugates can possibly eradicate CSCs via a "cross-fire" effect, a more meaningful approach will be to directly target CSCs in an antigen-specific manner. CSCs are characterized by distinct surface markers.[55] Several studies have established that CD133 and CD44 are the universal surface markers for different cancers, including brain, breast, ovarian, lung, prostate, pancreatic, hepatocellular, colon, and head and neck cancers.[50–52,55]

Two recent studies demonstrated the feasibility of targeting CD133 using antibodies *in vivo*. Tsurumi et al. utilized two sets of isogenic CD133 positive (U251 glioblastoma cells engineered to overexpress CD133 and HCT116 colon carcinoma cells that have endogenous and uniform expression of CD133) and negative cells (parental U251 and HCT116 $p53^{-/-}$ derivative that have undetectable level of CD133) to evaluate the *in vivo* targeting with anti-CD133 MAb AC133.1.[56] The study employed near infrared fluorescence molecular tomography to quantitatively determine CD133-specific tumor uptake of Cy 5.5-tagged antibody in xenograft tumors. CD133 positive tumors could be reliably imaged even after 7 days of systemic MAb administration. In the titration experiments (mixing CD133 positive and negative cells in different ratios), however, quantitative distinction between CD133 positive and negative tumors was lost (statistically insignificant) if the proportion of CD133 positive cells was less than 80%. This was attributed to the decreased expression of CD133 *in vivo* as compared to the cells in culture and also due to some nonspecific uptake of labeled antibody by macrophages via Fc receptors.[56] In a similar study, Jin et al. studied the biodistribution of ^{125}I-labeled anti-CD133 MAb in HCT116 xenograft tumor bearing mice at a single time point postadministration (24 hours).[57] As compared to the control antibody, radioiodinated anti-CD133 MAb ANC9C5 exhibited nearly a twofold higher tumor uptake. However, a significantly higher uptake of the specific antibody was observed in the stomach, while the isotype control antibody exhibited significantly higher accumulation in the liver.[57] Although the tumor uptake of the specific antibody was modest, possibly due to the early time point selected for biodistribution and lower expression of CD133 *in vivo*, the study demonstrated *in vivo* targeting of CD133 by radiolabeled MAb. CD44 is regarded as a CSC marker in several malignancies including breast, prostate, ovarian, colon, and pancreatic cancer. A recent study demonstrated hypoxia-induced upregulation of CD44 in triple negative breast cancer cells.[58] An increase in CD44 expression in the hypoxic regions of MDA-MB-231 xenograft tumors *in vivo* was determined by SPECT imaging after the administration of ^{125}I-labeled anti-CD44 MAb A3D8. The hypoxic regions were imaged by optical imaging of tomato RFP under the control of hypoxia-inducible promoter.[58] Just like studies with CD133, these studies utilized cells with an inherently higher and uniform expression of CD44 ($\sim$80% MDA-MB-231 express CD44). While the *in vivo* imaging failed to address whether the increased uptake of the CD44 antibody was due to enhanced infiltration of CD44 positive cancer stem cells in the hypoxic region or due to the upregulation of CD44 in the cells in the hypoxic region, the feasibility of *in vivo* targeting of CD44 by radiolabeled antibody was established.

While none of these studies provided conclusive evidence for successful targeting of rare CD133 or CD44 positive cancer stem cells (as these studies utilized cells that uniformly expressed CD133 or CD44), the therapeutic potential of CD133 or CD44 targeting with radiolabeled antibodies should still be evaluated in clinically relevant experimental models like the models of minimal disease or in combination with chemotherapy. Rather than the absolute tumor uptake (by imaging or biodistribution), stem cell-targeted RIT should evaluate the eradication of CD133 or CD44 positive stem cells (as determined by prolonged survival and/or decreased recurrence) as a viable endpoint to determine effectiveness.

Optimization of RIT: An Update

Antiangiogenic agents in the context of RIT

Poor uptake and heterogeneous intratumoral distribution of therapeutic agents, including radiopharmaceuticals are the direct manifestations of structural and functional abnormalities of tumor vasculature. In contrast to normal vasculature, tumor blood vessels are tortuous, dilated, hyperpermeable and poorly interconnected, resulting in poor perfusion, increased hypoxia, and elevated interstitial fluid pressure (IFP). Encouraging outcomes in clinical studies with the

combination of antiangiogenic agents with chemotherapy were attributed to the so-called "vascular normalization" induced by antiangiogenic agents, presumably resulting in transient improvement of blood flow, as well as delivery and distribution of therapeutic agents. Several subsequent clinical and preclinical studies have yielded contrasting results regarding the impact of antiangiogenic agents on drug delivery and there is an emerging consensus that the dosing and scheduling of antiangiogenic agents should be carefully planned and optimized in combination therapies. There are multiple mechanisms by which antiangiogenic agents can impact the outcome of RIT, both positively and negatively (Fig. 2).

Several antiangiogenic agents have been demonstrated to improve the efficacy of RIT. In a series of studies, Kinuya et al. demonstrated the utility of exploiting the antiangiogenic properties of 2-methoxyestradiol (2-ME) and thalidomide for improving the efficacy of RIT in experimental models of colon cancer.[59–62] Both 2-ME and thalidomide decreased the microvessel density in the tumors. Antiangiogenic therapy improved the therapeutic efficacy of ^{131}I-labeled anticolorectal cancer antibody A7 as determined by a reduction in tumor growth in xenograft tumors and improved survival in the hepatic metastasis model of colon cancer induced by LS180.[59–62] Similar enhancement in the efficacy of anti-CEA directed RIT in combination with antiangiogenic therapy was observed in medullary thyroid carcinoma.[63,64] Pretreatment with bevacizumab (anti-VEGF antibody) before RIT with an ^{131}I-labeled F(ab')$_2$ fragment of anti-CEA MAb F6 significantly prolonged the tumor volume doubling time from 87 ± 25 days to 127 ± 5 days without any significant change in toxicity.[63] A subsequent, more elaborate study from the same group further demonstrated the benefits of thalidomide and COBO11 (a VEGF inhibitor) in improving the efficacy of CEA-directed RIT in both small and large tumors.[64] Enhancement in the efficacy of RIT was only observed when the antiangiogenic agents were administered before RIT, while post-RIT antiangiogenic therapy offered no benefit. After thalidomide pretreatment, biodistribution studies indicated increased tumor uptake of ^{125}I-labeled antibody as compared to the control only at 24 hours, while tumor uptake at 72 hours postadministration was similar. This suggests that thalidomide possibly improved initial tumor uptake because of enhanced tumor perfusion.[64]

Antiangiogenic therapy has also been evaluated in combination with chemotherapy and unlabeled therapeutic antibodies targeting EGFR family receptors in clinical studies with both favorable and adverse outcomes. In HER2 positive breast cancer patients, bevacizumab in combination with trastuzumab and chemotherapy improved the outcome in

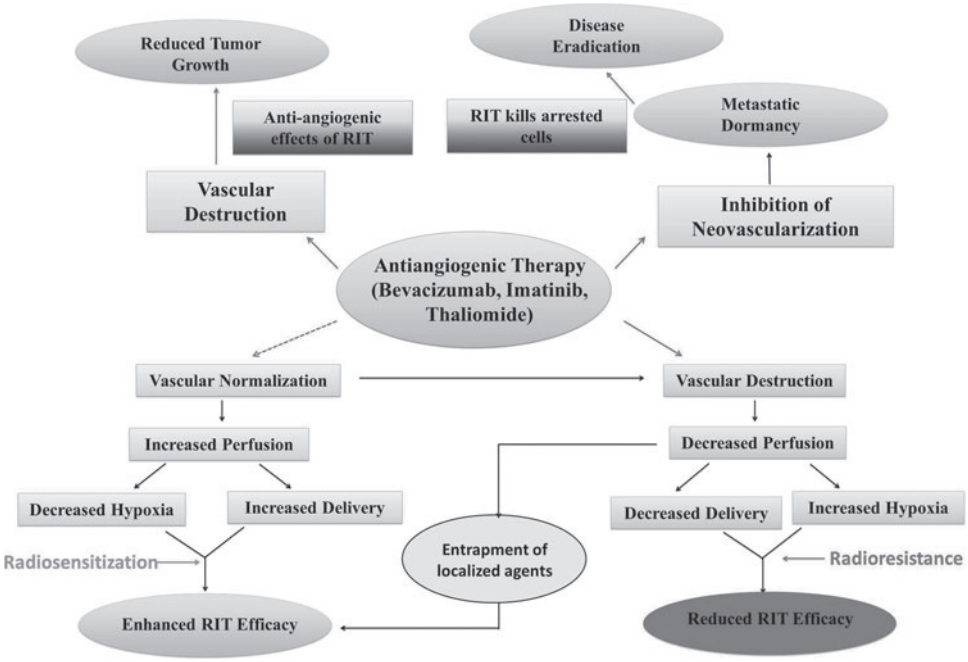

FIG. 2. Impact of antiangiogenic therapy on the efficacy of RIT. Antiangiogenic agents lead to destruction of tumor vasculature resulting in decreased delivery of radiopharmaceuticals and increased hypoxia thereby decreasing the overall efficacy of RIT. However, antiangiogenic agents also result in transient normalization of tumor vessels that can improve delivery and decrease hypoxia that can improve the efficacy of RIT. Further, antiangiogenic agents can also result in the entrapment of radiopharmaceuticals/chemotherapeutic agents that might have localized in the tumor before vessel destruction and this enhanced residence time can have a positive impact on therapeutic efficacy. Antiangiogenic agents can also inhibit neovascularization of metastatic foci; thus, resulting in their growth arrest or dormancy. RIT can target these arrested/dormant cancer cells in an antigen-specific manner and can potentially lead to complete eradication of metastatic disease in combination with antiangiogenic therapy. RIT also induces antiangiogenic effects and can synergistically enhance the efficacy of antiangiogenic agents. The complex interplay between antiangiogenic agents and RIT can result in distinct therapeutic outcomes. Hence, it is absolutely critical to optimize the dosing and scheduling of antiangiogenic agents when used in combination with RIT.

Phase II trials.[65–67] In contrast, in advanced colorectal cancers, the combination of chemotherapy and bevacizumab with anti-EGFR antibodies (cetuximab and panitumumab) failed to improve patient survival[68–70] and exhibited adverse effects to an extent of significantly reducing survival.[70] To determine the underlying cause of these diverse clinical observations, two recent studies determined the impact of anti-VEGF antibodies on the tumor uptake of radiolabeled antibodies in experimental breast cancer models.[71] Pastuskovas et al. comprehensively studied the impact of anti-VEGF MAb B20-4.1 (cross-reactive with both murine and human VEGF) on the pharmacokinetics, biodistribution, and tumor penetration of ^{111}In- and ^{125}I-labeled trastuzumab in a HER2 expressing KPL-4 xenograft model.[71] Pretreatment with B20-4.1 resulted in decreased tumor uptake of tracer radiolabeled trastuzumab in a dose dependent manner, while the uptake in the nontarget tissues was unaffected. Autoradiographic analysis indicated a more homogenous intratumoral distribution of ^{111}In-trastuzumab in untreated animals as compared to B20-4.1 pretreated animals, where the tracer antibody was restricted to the periphery. Anti-VEGF treatment was found to significantly reduce the blood flow in the tumor, while that in the normal tissue was unaffected.[71] Similarly, Heskamp et al. demonstrated that bevacizumab reduced the uptake of ^{111}In-labeled cetuximab and R1507 (anti-EGFR and anti-IGF-1R antibodies, respectively) in SUM149 (breast cancer) xenograft tumors.[72] Quantitative SPECT/CT indicated that bevacizumab reduced tumor uptake of ^{111}In-cetuximab and ^{111}In-R1507 by 40% and 35%, respectively.[72] In the biodistribution studies, after bevacizumab pretreatment, tumor targeting of radiolabeled cetuximab and R1507 was reduced by 44% and 29%, respectively, while the uptake in normal tissues was unaltered. Further, this decrease was associated with decreased microvessel density in the tumor, while there was no change in the expression of the target antigens (EGFR and IGF-1R).[72]

The utility of PDGFR inhibitor imatinib mesylate to improve the efficacy of anti-TAG72 was previously demonstrated using LS174T colorectal cancer xenografts.[73] This effect was accompanied by decreased tumor IFP that was attributed to the effect of imatinib on stromal fibroblasts. In contrast, in prostate cancer xenografts, the inhibition of PDGFR-β signaling did not result in significant lowering of tumor IFP but still appeared to improve the efficacy of RIT possibly by modulating HIF1-α and reducing hypoxia.[74] However, recent evidence suggests that imatinib can directly impact tumor vasculature.[75] Rajkumar et al. investigated the impact of imatinib on the antibody uptake in LS174T and SW1222 bearing xenograft models of colorectal cancer. The LS174T and SW1222 xenografts exhibit distinct vascular arrangements.[75] While vasculature in LS174T tumors is sparse and heterogeneous, SW1222 tumors have perfuse and homogenous vascular supply. Imatinib treatment resulted in a more pronounced enhancement in tumor uptake and improved intratumoral distribution of ^{125}I-A5B7 antibody (anti-CEA) only in LS174T tumors, while no significant changes were observed in SW1222 tumors. However, reduced microvessel and pericyte density was observed in both LS174T and SW1222 xenografts.[75] Confocal analysis demonstrated that imatinib treatment reduced pericyte attachment to endothelial cells in SW1222 tumors but not in LS174T tumors. The impact of agents like PDGFR inhibitors on the uptake of radiopharmaceuticals in a given tumor-type can be dictated by various factors (architecture of tumor vasculature and differential response of cells of tumor microenvironment) and may involve distinct mechanisms. Therefore, caution should be exercised in generalizing these criteria and extrapolating the findings to other tumor-types and sub-types.

Extracorporeal Elimination: An Underexplored Avenue to Reduce Myelotoxicity

Slow elimination of unbound radiolabeled immunoglobulins is one of the primary causes of dose-limiting myelotoxicity and poor tumor to normal tissue ratios of antibody-based radiopharmaceuticals. It is also one of the major impediments for RIT of solid tumors. Extracorporeal elimination represents an attractive yet underexplored avenue for the removal of unbound radioimmunoconjugates for reducing their myelotoxicity and for administration of higher doses. Extracorporeal elimination involves hemapheresis to remove the undesired substances and the purified blood is returned back to the patient. In clinical settings, extracorporeal elimination is employed for the management of autoimmune diseases (removal of autoantibodies and immune complexes), cases of poisoning, sepsis, and organ transplantation.[76–81]

In a series of studies, Tennvall et al. have demonstrated the utility of extracorporeal affinity adsorption using avidin columns to deplete biotinylated radiolabeled antibodies from circulation.[82–92] In one of the earliest demonstrations of the approach using athymic rats bearing human melanoma and lung cancer xenografts, removal of biotinylated ^{125}I-labeled antimelanoma (MAb 96.5) and anticarcinoma (MAb L6) antibodies improved the tumor to normal tissue ratios by a factor of four or five.[91] Subsequent studies using a syngeneic model of colorectal cancer in rats with chimeric anti-Lewis Y antibody BR96 demonstrated the benefits of immunoadsorption from the whole blood.[85,88] A comparative analysis of ^{111}In- and ^{125}I-labeled BR96 indicated that a more pronounced improvement in tumor to normal tissue ratios with ^{111}In-BR96, following extracorporeal affinity adsorption; although the magnitude of reduction in the whole body (39%–55%) and blood (79%–94%) was similar for both ^{111}In-BR96 and ^{125}I-BR96.[85] The group further demonstrated the utility of extracorporeal affinity adsorption as a clearing step in a two-step pretargeting approach involving biotinylated BR96 and ^{111}In-DOTA-streptavidin for improving the tumor uptake and reducing nontarget tissue exposure.[83] Improvement in tumor-to-tissue ratios and reduced circulating radioimmunoconjugates can potentially allow safer administration of higher doses. Direct experimental evidence for the utility of extracorporeal affinity adsorption for increasing the maximum tolerated dose (MTD) of the ^{90}Y- and ^{177}Lu-labeled BR96 was recently reported.[82] Antibodies were radiolabeled with a trifunctional chelating agent 1033, which contains DOTA and biotin. While the MTDs for ^{177}Lu-1033-BR96 and ^{90}Y-1033-BR96 are close to 600 and 350 MBq/kg, respectively, extracorporeal affinity adsorption allowed for administration of 1200 MBq/kg ^{177}Lu-1033-BR96 and 525 MBq/kg of ^{90}Y-1033-BR96 without any noticeable increase in the toxicity.[82]

The feasibility and utility of extracorporeal elimination of radioimmunoconjugates has also been demonstrated in

human subjects. In lung and breast carcinoma patients, Lear et al. tested the utility of extracorporeal immunoadsorption of ^{111}In-labeled murine antibody using a goat anti-mouse antibody column. Imaging analysis indicated a 59% decrease in the blood pool activity, while tumor activity was reduced by only 10% thereby improving the tumor to blood ratios.[93] In refractory B-cell lymphoma, extracorporeal elimination by avidin affinity adsorption resulted in depletion of biotinylated ^{90}Y-rituximab from blood (96%), lungs (65%) whole body (49%), liver (40%) and kidneys (40%).[94] Importantly, no adverse effects of avidin adsorption were observed on the cellular or soluble components (complement activation, bradykinin) of patient blood.[95]

Despite the evidence supporting the utility and feasibility, extracorporeal elimination approaches have not gained prominence in RIT. One of the primary reasons might be the complexity of the approach itself. Because of the variability in the pharmacokinetics of the antibodies and half-lives of various radioisotopes, the scheduling and duration of extracorporeal elimination has to be carefully planned and optimized. The optimized elimination window should allow sufficient accretion of radioimmunoconjugates in the tumor for effective therapy, while simultaneously limiting the exposure to bone marrow to reduce myelotoxicity. Unfortunately, no suitable animal models are available for such optimizations. Preclinical testing and optimization of cancer therapies, including RIT has largely relied on mouse models. However, the small blood volume of mice precludes the possibility of accurately modeling hemaphoresis approaches. Thus, it is no surprise that almost all the experimental evidence for extracorporeal depletion was obtained using a rat model. Yet the rat models for cancer are not well characterized and rodents, in general, due to their small size and relatively radio-resistant bone marrow, are not ideally suited to determine a dose-response relationship.[82] Recently, the utility of extracorporeal elimination for removing biotinylated radiolabeled ^{111}In- and ^{177}Lu-labeled rituximab was evaluated in a macaque model.[96] The use of nonhuman primates for evaluating and optimizing cancer therapies, particularly RIT, is difficult due to the unavailability of well characterized tumor models and cross-reactive antibodies; a lack of information regarding target antigen expression; logistical challenges like cost, and requirement for shielded animal housing.

Antigenic Heterogeneity and Antibody Cocktails

Tumors are characterized by physiological, genetic, spatiotemporal and antigenic heterogeneities.[97] Using a cocktail of antibodies directed against multiple antigens expressed in tumors can be a useful approach not only to improve the tumor uptake but also to enhance tumor specificity. Several preclinical and clinical studies have evaluated the utility of antibody mixtures for improved tumor targeting.

In one of the earliest clinical studies involving antibody cocktails, Chatal et al. evaluated the efficiency of ^{131}I-labeled MAbs 17-1A (anti-EpCAM) and 19-9 (anti-Sialyl Le^a) in enhancing diagnostic sensitivity in a patient cohort predominantly comprised of GI malignancies.[98] First, the antibodies were evaluated individually (administered either as intact MAb or F(ab')$_2$ fragments) in 90 cancer patients. In the next phase radioiodinated 19-9 was administered, either in combination with ^{131}I-17-1A or anti-CEA in 24 patients. Antibody cocktail exhibited enhanced sensitivity (77%) as compared to the individual antibodies (59% and 66% for 19-9 and 17-1, respectively) in detecting the tumor sites.[98] A subsequent clinical study also demonstrated the utility combined targeting of CEA and TAG-72 with ^{131}I-labeled antibodies (COL-1 and CC49, respectively) in colorectal cancer patients.[99] Similarly, an additive or synergistic increase in tumor cell reactivity *in vitro* and enhancement in tumor contrast *in vivo* was observed with a mixture of F(ab')$_2$ fragments of MAbs GA 73-3 (reactive against 29, 30 and 37 kDa protein antigens in carcinomas) and CO 29.11 (anti-Sialyl Le^a) in mice bearing colon carcinoma xenografts.[100,101] Hay et al. demonstrated the feasibility of targeting a receptor-ligand pair by a radiolabeled antibody mixture.[102] A tumor xenograft model derived from transformed NIH3T3 cells expressing c-met and its ligand hepatocyte growth factor (HGF) either in a paracrine or autocrine setting, was utilized. A mixture of five ^{125}I-labeled antibodies (one anti-met + four anti-HGF) was administered and evaluated for tumor targeting by gamma camera imaging.[102] Similarly, combined targeting of EGFR and CD44 with ^{99m}Tc-labeled antibody mixture resulted in increased tumor uptake and improved tumor-to-normal tissue ratios in the lung cancer xenograft model. The tumor to nontarget ratio for cocktail was found to be 5.59 ± 0.42 as compared to 2.78 ± 0.20 and 2.28 ± 0.16 for ^{99m}Tc-EGFR-mAb and ^{99m}Tc-CD44-mAb, respectively.[103] Targeting dual molecules by α-radiation *in vivo* was recently demonstrated by Milenic et al. using trastuzumab and humanized CC49 (HuCC49DCH2) directed against HER2 and TAG72, respectively.[104] Intraperitoneal coadministration of ^{213}Bi-labeled antibodies in athymic mice bearing disseminated intraperitoneal tumors (derived from LS174 colorectal carcinoma cells) resulted in enhanced therapeutic efficacy and improved survival by 4.3–4.7-fold compared to either agent alone.[104]

In contrast to the antibodies directed against different antigens, administration of the mixture of radiolabeled antibodies directed against distinct epitopes of the same antigen were found to be of limited value in preclinical and clinical studies in melanomas.[105,106] However, a mixture of three scFVs (possibly targeting on different antigens) demonstrated enhanced tumor localization compared to individual scFvs in melanoma xenografts. Tumor uptake of the combination was higher (%ID/g = 24.22%) compared to individual scFvs (%ID/g = 2.854%, 2.263%, and 1.355%).[107] Petronzelli et al. also demonstrated improved tumor targeting with a mixture of two antitenascin antibodies in a colon carcinoma xenograft model.[108] Coadministration of ^{125}I-labeled and biotinylated antitenascin antibodies ST2146 and ST2485 (directed against distinct epitopes adequately spaced to rule out stearic hindrance) exhibited additive accumulation in HT29 xenografts as compared to the individual antibodies.[108]

Summary and Perspectives

Inadequate tumor delivery of radiolabeled antibodies and their prolonged circulation have been the biggest roadblocks for successful RIT of large solid tumors. However, promising preclinical and clinical studies, particularly in the minimal residual or micrometastatic disease settings, have raised

hopes of clinical acceptability of RIT as an adjuvant therapy. Recent research along these lines has been fueled by the ease of availability of α-particle emitting radionuclides, possibility of alternative routes of administration and development of animal models depicting minimal disease. Yet certain challenges remain. To be effective, localized administration of high dose of α-particle-based radiopharmaceuticals is desirable. However, there is still limited understanding regarding the nontarget toxicity of α-particles and studies need to be undertaken to determine the radiosensitivity of candidate sites for localized administration (peritoneum, bladder, pleural cavity, and lungs). Further, while efficacy of RIT for minimal disease can be determined by survival analysis, accurate determination of tumor absorbed radiation dose remains a challenge. Similarly, most animal models of minimal disease that have been utilized for evaluating the efficacy of RIT have remained in the confines of individual laboratories. While the feasibility of targeting stem cell markers with radiolabeled antibodies has been established, actual *in vivo* targeting of CSCs with specific radiolabeled antibodies remains to be demonstrated. Future studies should also test the effectiveness of RIT in controlling or eradicating tumors seeded by CSCs.

Heterogeneity in tumor stroma and vasculature contribute to heterogeneous distribution of radiopharmaceuticals in the tumors. Efforts to modulate tumor stroma, particularly tumor vasculature, for improving the delivery of radiopharmaceuticals and radiosensitivity of tumors have shown promise. However, the interactions between RIT and stromal modulators, like antiangiogenic agents, are complex and mandate careful optimization of dosing and scheduling, use of right models, and multiple cell lines before making generalizations and predictions. In addition to antiangiogenic agents, other avenues to remodel tumor stroma to make it conducive to delivery of macromolecules need to be explored. Heterogeneous distribution of radioimmunoconjugates has also been attributed to the heterogeneity in target antigen expression within the tumor. While the use of antibody cocktails in preclinical and clinical studies has been demonstrated to improve tumor targeting, most RIT research still involves use of single antigen-antibody pair. With the advent of personalized medicine and advances in sequencing technologies, a more rapid and robust genetic and molecular characterization of tumors has become a reality. Such analysis can more accurately predict the antigenic make-up of the tumors and can facilitate in the formulating a tumor-type, and patient-specific cocktail of radiopharmaceuticals.

Approaches to improve the pharmacokinetics of antibody-based radiopharmaceuticals can significantly improve administration of higher doses. While approaches like antibody engineering and pretargeting have been explored extensively to improve pharmacokinetics, approaches which are more practical clinically, like extracorporeal elimination, have received limited attention. One of the primary reasons could be the lack of suitable animal models. While extracorporeal elimination is more practical in humans and large animals, it is challenging to model such an approach in mice due to their small blood volumes. Preclinical RIT predominantly relies on subcutaneous xenograft tumors grown in immunocompromised athymic or severe combined immunodeficiency (SCID) mice. Subcutaneous tumors do not recapitulate the complexities of orthotopic tumors growing in the host organ. The lack of intact immune system precludes the evaluation of radiotoxicity on the immune system. The use of immunocompromised animals also eliminates the possibility of determining the contribution of the immune system on the therapeutic effects of radiopharmaceuticals. In the last decade, various genetically engineered mouse models (GEMMs) have been developed for various cancers. In contrast to xenograft models, the autochthonous tumors that develop in GEMMs, more closely recapitulate the pathophysiology of human cancers. Despite this promise, GEMMs have not been utilized to evaluate RIT. One of the major impediments for the use of GEMMs is their high cost. While the cost of animals has increased alarmingly, the cost GEMMs increases with the extent of engineering involved. Ready-to-use GEMMs are difficult to obtain in appropriate genetic background, while the maintenance of breeding colonies of GEMMs is cost and labor intensive and often requires specialized expertise in genetic engineering, breeding and genotyping. Further, it is challenging to synchronize tumor development and size in GEMMs that can potentially hinder meaningful execution of RIT since the usability of radiopharmaceuticals is dictated by the half-life of the radioisotopes. Another reason for the limited use of GEMMs in RIT could be the predominance of antibodies reactive with human tumor antigens and the available antibodies may not always cross-react with mouse antigens. Further, the status of widely studied RIT target antigens like CEA, TAG-72 and PSMA in the GEMM tumors remains poorly characterized. Yet some transgenic mouse models for human tumor associated antigens like MUC1 and HER2/*neu* have been developed. These models would be the ideal starting points to evaluate the efficacy of RIT in autochthonous tumors.

In conclusion, although there are several impediments in the development of radiolabeled antibody-based therapeutic strategies for solid tumors, recent publications have identified several avenues to overcome the limitations of RIT. The greater understanding of the factors that limit the delivery and efficacy, availability of technologies for tailoring antibodies and development of pertinent animal models will surely guide the increased clinical acceptability of RIT for solid tumors.

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