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Review Article

Pretargeted radioimmunotherapy, a potential cancer treatment

Laura Lam[1], Xiaoyu Liu[2] and Ying Cao[3*]

[1]Department of Pharmaceutical Services, The University of Chicago Hospitals, 5841 South Maryland Ave., Chicago, IL 60637; [2]Eli Lilly and Company, 639S Delaware Rd., Indianapolis, IN 46285; [3]BioMedSci Consulting, 8056 Lincoln Ave., Unit 2SF, Skokie, IL 60077, USA.
[*]Correspondence

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Abstract

Radioimmunotherapy is often limited by radiation toxicity due to the long-circulating radiolabeled antibody. Pretargeted radioimmunotherapy is used to minimize this toxicity by separating the long-circulating antibody and the rapidly cleared radionuclide. Although results from animal studies and clinical trials are promising, they have also raised many questions. Preclinical and clinical experiences will foster an in-depth understanding of pretargeted radioimmunotherapy as an alternative choice for cancer treatment. Breakthroughs in recombinant DNA technology and protein engineering, the discovery of alpha particle radionuclides and their applications and the concerns in designing human clinical trials together will expedite the use of pretargeted radioimmunotherapy in the clinical setting for cancer treatment.

Introduction

Pretargeted radioimmunotherapy (PRIT) is an alternative strategy for conventional radioimmunotherapy. Conventional radioimmunotherapy has had remarkable clinical success recently. ^{90}Y-ibritumomab tiuxetan (Zevalin™) became the first FDA approved pharmaceutical of its kind for patients with relapsed B-cell non-Hodgkin's lymphoma (NHL) (1). However, further progress for such a treatment is largely hindered by dose-limiting bone marrow toxicity due to the long-circulating radiolabeled antibody. Pretargeting therefore has been postulated as an alternative method because of the potential to lower bone marrow toxicity and achieve higher intratumor concentrations of radionuclide as compared to those achieved with conventional radioimmunotherapy (2, 3).

PRIT is a multistep strategy. First, the bispecific antibody or antibody-streptavidin conjugate is administered to target the tumor antigen. Second, after the bispecific antibody or antibody-streptavidin conjugate has been concentrated in the tumor, a polyvalent "chase" is given to rapidly remove the pretargeted antibody or antibody-streptavidin conjugate from the circulation by forming a cross-linked aggregate. Third the radiolabeled small molecule is administered which penetrates the tumor quickly and binds through high-affinity interactions with the early pretargeted bispecific antibody or antibody-streptavidin conjugate. The untargeted radiolabeled molecule is rapidly excreted renally (Fig. 1).

Several experimental models have been explored to interpret the concept of PRIT (4-6). Among them, 3 were well established including antibody-streptavidin/biotin, biotinylated antibody/avidin/biotin and bispecific antibody/bivalent hapten. These systems have all been extensively tested in preclinical and clinical settings with promising outcomes. This review examines the most recent progress of PRIT in animal studies and clinical trials, highlights the critical issues and discusses the future directions for optimizing PRIT.

Hematological malignancies

Hematological malignancies are excellent candidates for radioimmunotherapy due to the sensitivity of the malignant cell to radiation, the accessibility of the tumor to

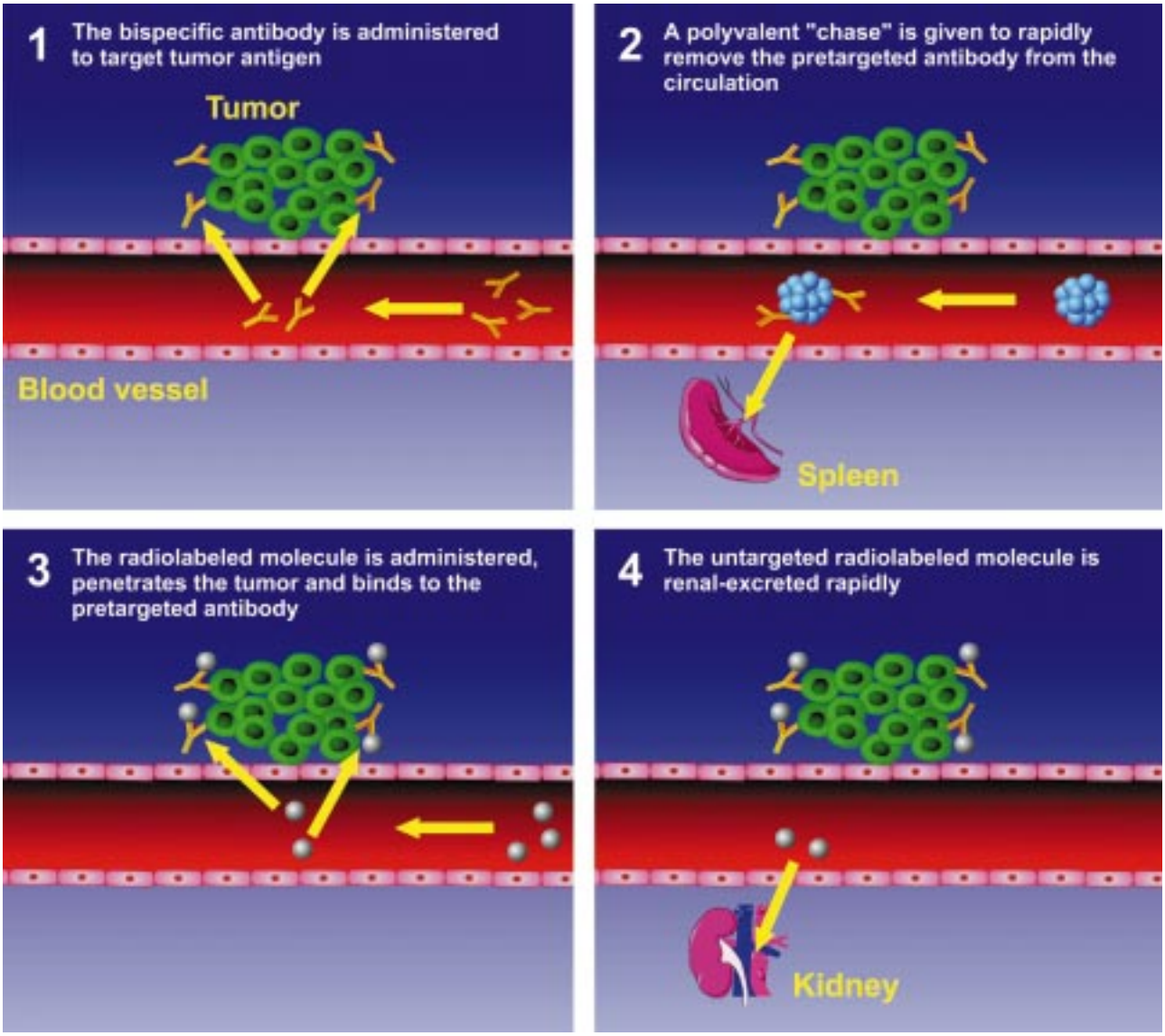

Fig. 1. Schematic representation of multistep pretargeting radioimmunotherapy.

antibodies, and the abundance, homogeneity and specificity of tumor antigens. However, the majority of patients eventually have a relapse because the low tumor-to-blood ratio of absorbed radioactivity limits the dose that can be safely administered without hematopoietic stem cell support. Both animal studies and a small-scale clinical trial have demonstrated that pretargeting can lead to improved therapeutic efficacy and a favorable toxicity profile of radioimmunotherapy for patients with hematological malignancy.

A study using antibody-streptavidin/biotin 3-step PRIT has reported improved tumor-to-blood ratios in mice bearing Ramos lymphoma xenografts (7). In the study, a pretargeted streptavidin-conjugated anti-CD20 antibody was infused, followed 24 h later by a biotinylated *N*-acetyl-galactosamine-containing “clearing agent” and finally 3 h later by ^{90}Y-labeled 1,4,7,10-tetraazacyclododecane-*N*,*N*’,*N*”,*N*”’-tetraacetic acid (DOTA)-biotin. Tumor-to-blood ratios reached 3:1 in PRIT in comparison with 0.5:1 obtained with conventional radioimmunotherapy. The study also found that up to 800 μCi of ^{90}Y-DOTA-biotin could be safely administered with PRIT, resulting in 89% of the mice cured with only minor toxicity. In contrast, conventional radioimmunotherapy required 400 μCi of ^{90}Y-labeled antibody to obtain major tumor responses and resulted in 100% of the mice experiencing lethal toxicity.

Recently, the same animal model was used to compare anti-CD20 and anti-CD45 antibodies for conventional and pretargeted radioimmunotherapy of B-cell lymphomas (8). The data confirmed the previous finding and

showed that PRIT using either the anti-CD20 or anti-CD45 conjugate generated improved tumor-to-blood ratios and response rates as compared to conventional radioimmunotherapy. The study also suggested that better results were achieved with anti-CD45 due to its prolonged retention time on the tumor surface as compared to anti-CD20. For example, pretargeting with anti-CD45 can cure 100% mice at 400 μCi of ^{90}Y-DOTA-biotin and 70% mice at 200 μCi of ^{90}Y-DOTA-biotin. In contrast, pretargeting with anti-CD20 cured 90% mice at 400 μCi ^{90}Y-DOTA-biotin and none at 200 μCi ^{90}Y-DOTA-biotin. In addition, complete remissions occurred 8-10 days sooner in mice receiving anti-CD45 as compared to those given anti-CD20.

A small-scale clinical trial of PRIT in patients with refractory NHL has been reported (9). A total of 10 patients with relapsed or refractory NHL participated in the study, with 7 administered 30 or 50 mCi/m^2 ^{90}Y-DOTA-biotin. A chimeric anti-CD20 antibody (rituximab) conjugated to streptavidin was used in the first step. Thirty-four hours later, a clearing agent, synthetic biotin-*N*-acetyl-galactosamine, was administered. Finally, ^{90}Y-DOTA-biotin was administered for therapy. The mean tumor dose calculated was 29 ± 23 cGy/mCi ^{90}Y and the mean tumor to whole body dose ratio was 38:1. Six of seven patients who received 30 or 50 mCi/m^2 ^{90}Y-DOTA-biotin achieved objective tumor regression, including 3 complete responses and 1 partial response. Only grade 1/2 nonhematological and transient grade 3 hematological toxicity were observed. Although 6 of 10 patients developed humoral immune responses to the streptavidin, these were delayed and transient and hence may not preclude retreatment.

Solid tumors

PRIT has produced optimal outcomes for treating solid tumors in various animal models by producing positive response and overcoming dose-limiting bone marrow toxicity. Yao *et al.* performed PRIT in A431 xenografted mice with pretargeted B3 antibody-streptavidin and ^{90}Y-DOTA-biotin (10). A431 is the human epidermoid carcinoma cell line and B3 antibody recognizes Lewis^y tumor antigen. All of the normal tissues had less than 2.6% ID/g of the injected dose per gram, whereas tumor uptake reached approximately 15% ID/g. In the first study, the median survival of the control group was 8 days, whereas it increased to more than 163 days in the treatment group. In a second study, 7 of 10 mice in the treatment group were cured and remained healthy for more than 180 days after therapy as compared to control groups that exhibited a mean survival of less than 29.2 days.

Axworthy *et al.* also demonstrated the antitumor efficacy of a single dose of pretargeted antibody-streptavidin and ^{90}Y-DOTA-biotin in mice with human carcinoma xenografts (11). NR-LU-10 is a monoclonal antibody (MAb) that binds an antigen expressed on the surface of most human carcinomas. A single dose of 600-800 μCi of ^{90}Y-DOTA-biotin produced cures in 100% of the mice with human small cell lung or colon cancer xenografts and in 80% of the mice with human breast cancer xenografts; no significant toxicity was observed.

PRIT was shown in clinical trials to have a positive effect in treating minimal residual carcinomas. Kraeber *et al.* reported preliminary results of a phase I/II clinical trial of PRIT in treating medullary thyroid cancer using the bispecific antibody, hMN-14 (humanized anti-CEA) x 734 (murine anti-DTPA-In) and bivalent ^{131}I-di-DTPA-In hapten (^{131}I-hapten). In this trial, toxicity and therapeutic efficacy were evaluated in a total of 26 patients with recurrent medullary thyroid cancer (12). The bispecific antibody (20-50 mg) and approximately 40-100 mCi of ^{131}I-hapten were injected 4 days apart. Grade 3/4 hematological toxicity was observed in 7 patients, most of whom had bone metastases. Among the 17 evaluable patients, 4 reported pain relief, 5 had minor tumor responses and 4 had biological responses with a decrease in thyrocalcitonin. The therapeutic responses were mainly observed in patients with a small tumor burden.

These encouraging results prompted further study to define the optimal dosing and timing scheme of this approach and to assess immunogenicity and tumor response (13). A total of 25 patients were enrolled in another study. The first part of the study indicated that a dose of 75 mg/m^2 of the bispecific antibody might be optimal with an interval of 5 days between injections of bispecific antibody and ^{131}I-hapten. This was used in the second part of the study to evaluate the optimal dose (mass, activity) of ^{131}I-hapten. Among the 23 assessed patients, dosimetric calculations showed variable results depending on tumor size. Mean tumor absorbed doses were higher for the pretargeted ^{131}I-hapten (16.0 cGy/mCi) than after injection of directly radiolabeled bispecific antibody (6.7 cGy/mCi). HAMA (human antimouse antibody) and HAHA (human anti-human antibody) tests were clearly positive in 1 patient and weakly positive in 3 patients. No objective responses were observed, but most patients had a high tumor burden. In 1 patient with a small recurrence of medullary thyroid carcinoma, a marked drop (80%) in serum thyrocalcitonin levels was observed 1-3 months after treatment.

Phase I/II trial demonstrated promise for PRIT as a treatment for small cell lung carcinoma (14). A total of 14 patients with small cell lung carcinoma who had relapsed after chemotherapy were first injected with a bispecific antibody (anti-CEA/anti-DTPA) and, 4 days later, with di-DTPA-tyrosyl-lysine hapten labeled with approximately 40-180 mCi of ^{131}I. Toxicity was mainly hematological, with 2 cases of grade 2 leukopenia and 3 cases of grade 3 or 4 thrombocytopenia. Among the 12 patients evaluated, 9 progressions, 2 partial responses (1 almost complete for 3 months) and 1 stabilization of more than 24 months were observed. Efficacy and toxicity were dose-related. The maximum tolerated dose (MTD) without hematological rescue was 150 mCi.

Paganelli *et al.* have developed an avidin-biotin pretargeting approach for delivering therapeutic radionuclides to gliomas using anti-tenascin MAbs. A phase I/II study was conducted and included 48 patients with histologically confirmed grade 3 or 4 glioma and documented residual disease or recurrence after conventional treatment. A 3-step radionuclide therapy protocol was performed including i.v. administration of 35 mg/m^2 of biotinylated anti-tenascin MAb (1st step), followed 36 h later by 30 mg of avidin and 50 mg of streptavidin (2nd step), and 18-24 h later by 1-2 mg of ^{90}Y-labeled biotin (3rd step). ^{90}Y doses of 2.22-2.96 GBq/m^2 were administered and the MTD was determined to be 2.96 GBq/m^2. A positive response occurred in 12/48 patients (25%), with 8/48 having a duration of response of at least 12 months.

The encouraging results obtained in this phase I/II study prompted the authors to apply the same approach in an adjuvant setting to evaluate time to relapse and overall survival (16). This controlled open, nonrandomized study enrolled 37 high-grade glioma patients, 17 with grade 3 glioma and 20 with glioblastoma; 19 patients received adjuvant treatment *versus* 18 control patients. In the 8 treated glioblastoma patients, the median disease-free interval was 28 months and the median survival interval was 33.5 months. In contrast, all 12 control glioblastoma patients died after a median survival interval of 8 months. In treated grade 3 glioma patients, the median disease-free interval was 56 months; the survival interval could not be calculated since only 2 died within this group.

Although the clinical trials mentioned above reported promising results, the severe toxicity observed in a phase II clinical trial of PRIT as a treatment for patients with metastatic colon cancer has allowed little room for optimism (6). In this study, 25 patients were treated with a single dose of 110 mCi/m^2 of ^{90}Y-DOTA-biotin and the overall response rate was 8%. Two patients experiencing partial responses were free from progression for 16 weeks and 4 patients (16%) had stable disease and were progression-free for 10-20 weeks. Grade 3 and 4 diarrhea was observed as the most frequent nonhematological toxicity. The hematological toxicities reported included grade 3 and 4 neutropenia and grade 3 and 4 thrombocytopenia. Moreover, all patients generated an antibody response to the mouse antibody, streptavidin and the conjugate. The severe toxicity seen was concluded to be due to cross-reactivity of the NR-LU-10 antibody with the bowel epithelium.

Issues and future directions

Results from animal studies and clinical trials have indicated that PRIT is a promising strategy for treating both hematological malignancy and solid tumors. To maximize the potential benefit of PRIT, the following issues have to be resolved: immunogenicity of the antibody and streptavidin, low solid tumor penetration, lack of tumor specific antigen, low level or heterogeneous expression of tumor antigens, radioresistance of tumor, low antibody affinity or avidity, and interference of endogenous biotin. Recent technological advances and emerging new concepts will help to overcome these hurdles.

Immunogenicity

Repeated doses to maximize the antitumor effect without eliciting an immune response would be ideal for PRIT. However, the immunogenicity of the murine-derived antibody and bacterial protein streptavidin preclude repeated dosing, thus jeopardizing the potential efficacy of PRIT. In contrast to patients with carcinoma, the NHL patient population has a reduced antibody response due to inherent immunosuppression. Therefore, the immunogenicity issue in NHL patients would not be as severe as in patients with carcinoma as was demonstrated in clinical trials (6, 9).

Until recently, the immunogenicity issue of the antibody has been largely resolved through antibody humanization technologies, including chimeric antibody production, CDR-grafting, phage display and transgenic mouse models. However, the quest for nonimmunogenic forms of streptavidin continues. Mutating surface residues of streptavidin has been explored as a method to reduce immunogenicity (17). Data suggested that substitution of charged, aromatic or large hydrophobic residues on the surface of streptavidin with smaller neutral residues reduced the molecule's ability to elicit an immune response in rabbits.

In addition to modifying streptavidin, a research effort was made toward constructing a humanized single-chain Fv-streptavidin (scFvSA) fusion protein (18). The scFvSA construct (MW 172,000), specific to the EGP40 antigen, was expressed as a soluble, tetrameric species in the *E. coli* periplasm at 110-140 mg/l. The purified protein with 3 biotin-binding sites per molecule, had a biotin dissociation rate identical to recombinant streptavidin. In normal mice, the radiolabeled fusion protein showed a faster blood clearance rate than the corresponding whole antibody-streptavidin conjugate. A single dose of 800 μCi of ^{90}Y-DOTA-biotin produced cures in mice with s.c. human small cell lung or colon cancer xenografts.

Interestingly, a large, naive human single-chain Fv (scFv) library was established to provide human antichelate scFv for 2 metal chelates (Cu-TETA and Y-DOTA) (19). These antichelate scFvs were intended to serve as one arm of bispecific pretargeting molecules and to bind radiochelates given subsequently as ^{67}Cu-TETA or ^{90}Y-DOTA. Selections against metal chelated antibodies provided a wealth of scFvs with diverse binding affinities useful for engineering molecules for PRIT.

Recombinant fusion proteins

Modern recombinant DNA technology has paved the way for creating ideal protein therapeutic candidates with

multiple functional groups, flexible size control and a capacity for large-scale production. Single-chain molecules from the variable domains of antibody light and heavy chains offer an exciting future for PRIT. Schultz *et al.* has reported construction of an anti-CD20 single-chain Fv-streptavidin (scFvSA) fusion protein and the feasibility of mass production of functional end product (20). The scFvSA constructs (MW 173,600) were expressed as soluble, tetrameric fusion protein with 250-300 mg/l in *E. coli*. The affinity of the scFvSA fusion protein to CD20-positive Ramos cells was comparable to that of the parent antibody. The fusion protein, with an average of 3.6 biotin binding sites, had a dissociation rate identical to recombinant streptavidin. The labeled fusion protein had a serum half-life of 16 h in the blood of BALB/c mice. In nude mice bearing Ramos xenografts, the fusion protein demonstrated sufficient tumor localization of functional streptavidin to enable efficient, tumor-specific targeting of a subsequently administered radiolabeled biotin molecule.

Bispecific antibodies have been found to have various applications in PRIT. Bispecific antibodies are routinely generated by chemical cross-linking or by the hybrid hybridoma technology. However, both methods produce a substantial number of undesired nonfunctional molecules. Diabodies are bivalent or bispecific molecules generated by dimerization of 2 VH-VL fragments. A PCR-based primer system was created to easily convert scFv genes into a diabody gene format, once they have been placed into a readily available vector. The primer system for this diabody gene platform was developed and tested by constructing a bispecific diabody (anti-HLA-DR/anti-DOTA). This modular gene design platform provides an easy and rapid method of creating diabody molecules from diverse scFv libraries. Diabodies from various scFv can be produced easily, thereby facilitating comparative preclinical studies en route to development of new tumor targeting molecules (21).

Although the majority of the work on recombinant fusion proteins remains in its infancy, a recent multiinstitutional nonmyeloablative pretargeting phase I clinical trial using the anti-CD20-streptavidin fusion protein was conducted demonstrating the safety and feasibility of the pretargeting approach (22). Recombinant fusion proteins can be designed flexibly to fit the best pharmacokinetic need, *i.e.*, adjusting the circulating half-life, enhancing tumor accessibility and targeting. However, the normal processing of these modified proteins generally has resulted in decreased tumor binding efficiency and increased renal uptake which may produce delayed radiation nephritis. Recombinant fusion proteins will have tremendous impact on future PRIT provided that technological advances can overcome these hurdles.

Alpha-particle radionuclides

Radioimmunotherapy has primarily utilized high-energy beta-particles, *e.g.*, ^{131}I and ^{90}Y which are intended to kill large tumor masses. Such conjugates do not kill single cells or micrometastases efficiently. To exterminate single cells, it may be preferable to use radiation with a much shorter path length, such as alpha-particles (^{212}Bi, ^{213}Bi and ^{211}At). With short half-lives, alpha-particle emitters seem more suitable for use in the PRIT system than in conventional radioimmunotherapy.

The first animal study of PRIT using the alpha-particle ^{213}Bi on adult T-cell leukemia was recently reported (23). An anti-Tac antibody-streptavidin conjugate that recognizes CD25 was administered followed by ^{213}Bi-DOTA-biotin (250 μCi; 9.25 MBq). In this study, tumor growth was significantly inhibited in the treatment groups as compared with the control groups. No prolongation of survival resulted from administration of ^{213}Bi directly linked to the antibody. In addition, no prolongation of survival was observed when the beta-emitting particle ^{90}Y was administered instead of ^{213}Bi. The clinical impact of alpha-particles on PRIT will be largely unknown until human trials have been conducted.

Design of clinical trials

The design of clinical trials is very crucial for the success of potential anticancer agents. In particular, phase II evaluation is an important component of development, attempting to estimate efficacy and toxicity of a new candidate. The primary endpoint for phase II evaluation has been tumor response rates, *i.e.*, the percentage of patients whose tumors shrink by more than 50%. Biotechnology has led to promising new anticancer agents that are cytostatic. In contrast to cytotoxic, these agents are expected to delay tumor growth. Mick *et al.* suggested that phase II evaluation of such agents may instead focus on failure-time endpoints, such as time to disease progression (24).

The results from clinical trials involving PRIT as a treatment for metastatic colon cancer patients indicated a discrepancy in response (6). This may be attributed in part to the histology and associated natural history of the disease types studied, since only 22% of the patients in this phase I study had colorectal cancer (29% had prostate cancer and 22% had ovarian cancer, with the remaining 27% with breast, kidney, lung, cervix and endometrial cancers). In addition, few phase III investigations show a benefit for experimental treatments when compared to a standard therapy or placebo. This reflects the need for more reliable estimates of treatment effects for phase transition. Alternatives have been suggested regarding the following 4 aspects of clinical trials design (25): (i) selecting points; (ii) defining the patient population for evaluation; (iii) determining a level of activity that would justify a phase III trial; and (iv) estimating sample sizes. For the future design of clinical trials for PRIT, special attention should also be given to dosing and timing due to the multiple step strategy.

Endogenous biotin

A potential barrier to PRIT is the presence of endogenous biotin in serum which can irreversibly block the biotin binding sites of the antibody-streptavidin conjugate before the administration of radiolabeled biotin. Although biotin-free diets prior to therapy can mitigate significant amounts of endogenous biotin and allow for successful targeting of radiolabeled biotin, compliance may limit their efficacy. One research group found that employing a mutant streptavidin in conjunction with a bivalent biotin can eliminate the impact of endogenous biotin by allowing exchange of bound free biotin with bivalent biotin dimer (26). They tested a streptavidin mutant (SAv-Y43A), which has a 67-fold lower affinity for biotin than wild-type streptavidin, and 3 bivalent bis-biotin constructs. Biotin dimers were engineered with certain parameters including water solubility, biotinidase resistance and linker lengths long enough to span the distance between 2 biotin-binding sites of streptavidin. The faster off-rate of SAv-Y43A allowed efficient exchange of prebound biotin by the biotin dimers. In fluorescent competition experiments, the biotin dimer ligands displayed high avidity binding and essentially irreversible retention with SAv-Y43A.

Conclusions

PRIT is a promising strategy and can provide hope for cancer patients, especially those who are refractory to conventional treatment. At its current stage of development, PRIT seems more applicable for treating hematological malignancies and minimal residual tumors. In the future, research efforts will be largely focused on the development of new targeting conjugates that are nonimmunogenic and have high binding avidity, a controllable pharmacokinetic profile, less renal retention and less interference with endogenous biotin. The first promising report of alpha-particle radionuclides use in PRIT will encourage further exploration. The careful design of clinical trials will provide us with more reliable estimates of the treatment effects of PRIT, allowing successful phase transition. PRIT could become an important antitumor therapy option, giving numerous cancer patients the opportunity to extend and improve their quality of life.

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