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Review

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Pretargeting : A concept refraining traditional flaws in tumor targeting

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ABSTRACT

Pretargeting is a two-component strategy often used for tumor targeting to enhance the tumor-to-background ratio in cancer diagnosis as well as therapy. In the multistep strategy, the highly specific unlabeled monoclonal antibodies (mAbs) with the reactive site is allowed to get localized at tumor site first, and then small and fast-clearing radiolabeled chelator with counter reactive site is administered which covalently attaches to mAbs via inverse electron demand Diels-Alder reaction (IEDDA). The catalyst-free IEDDA cycloaddition reaction between 1,2,4,5-tetrazines and strained alkene dienophiles aid with properties like selective bioconjugation, swift and high yielding bioorthogonal reactions are emergent in the development of radiopharmaceutical. Due to its fast pharmacokinetics, the in vivo formed radioimmunoconjugates can be imaged at earlier time points by short-lived radionuclides like ^{18}F and ^{68}Ga; it can also reduce radiation damage to the normal cells. Ultimately, this review elucidates the updated status of pretargeting based on antibodies and IEDDA for tumor diagnosis (PET and SPECT) and therapy.

Key Word: pretargeting, monoclonal antibodies, radioisotopes, tumor targeting, tumor-to-background, tumor accumulation

Introduction

By far now, the pretargeting strategy is eminent in the field of radioimmunotherapy. From mid-1980s to the present, many distinguished researchers have worked on the improvement of this strategy. Prior to the progress of pretargeting, the radiolabeled antibodies was broadly studied in preclinical and clinical trials for delivering radioactivity at tumor sites for vivid visualization, characterization, and for the therapy of tumor counting primary and metastatic cancers (1, 2, 3). The conventional strategy had some setbacks including the detrimental usage of high amount of radiation and its exposure by virtue of prolonged blood clearance of antibodies which results to less tumor-to-background ratio with minimum dose at the tumor site (4, 5). Reardan et al. (1985) because of the excellent binding efficacy of antibodies toward respective antigens and the effectiveness of antibodies against metal ions, and Goodwin et al. (1988) demonstrated a pretargeting strategy using radionuclide and bifunctional haptens (6, 7). Although pretargeting can be referred as in vivo radiolabeling of antibodies, the current improvements in antibody pretargeting and radiolabeling methods along with high response and better resolution PET imaging makes immuno-PET an advantageous approach for both antibody-based diagnosis as well as therapy. The use of short half-life positron-emitting radionuclides that would reduce patient undesired irradiation and improve the performance of immuno-PET is made possible by pretargeting (8).

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In the conventional strategy, direct use of radiolabeled mAbs resulted in slow blood clearance and may accumulate at non targeted tissues (4, 5). However, in pretargeting, the unlabeled mAb is administered 24–72 h initially depending on the affinity of antibody toward its respective antigen. In second phase, the radionuclide is injected after excess antibody blood clearance. One of the strategies introduced by Finn and Sharpless in 2001 and Sleten and Bertozzi revived click chemistry in the inert biological atmosphere which was termed as bioorthogonal click chemistry (9, 10). Bioorthogonality literally means the biocompatibility of the reaction and avoidance of inevitable detrimental effects in living systems. This resulted in bioorthogonal IEDDA click reaction between tetrazine (Tz) and transcyclooctene (TCO) which turned out beneficial in the pretargeted tumor PET imaging, where one of the reactive site TCO is conjugated with antibody and Tz with the radiochelator or vice versa (11). The fastest reaction kinetics (10^6 $M^{-1}s^{-1}$) of reactive groups Tz and TCO in click reaction at biological environment is well reported as compared to other bioorthogonal reactions like strain-promoted azide-alkyne cycloaddition (SPAAC) (12, 13). Pretargeting mainly has four steps: (a) the injection of a TCO-modified antibody, (b) the tolerated accumulation of TCO conjugated antibody at the tumor site and its concomitant clearance from the blood; (c) the injection of a radiolabeled chelator bearing Tz, and (d) the in vivo click reaction between the two components (Figure 1). The small radiolabeled chelator reaches the tumor swiftly and binds with the local mAb conjugates, consequently forming a radioimmunoconjugates in vivo. The excess radiolabeled chelator is rapidly excreted from the blood or healthy tissues, resulting in a higher tumor-to-background ratio which is beneficial for diagnosis and therapy.

The emergence of using heterospecific antibodies for certain delivery of toxic agents at tumor cells has since been well known (14). However, Reardan et al. reported the initial studies using two-step mechanism to target tumors, and better tumor-to-background ratio was obtained using antibodies against radiolabeled EDTA. By just separating the targeting of a slow-clearing antibody from the small fast-clearing radionuclide, increased tumor-to-background ratio and vivid imaging was achieved (6) . Additionally, O. Boerman et al. optimized pretargeting strategy in renal cell carcinoma NU12 xenografts based on bispecific antibody and ^{111}In-DTPA (15).

Moreover, specific pretargeting of tumors requires the production of mAbs with dual functionality, and it should bind with tumor-associated antigen as well as with the radiolabeled chelator. In 2010, Rossin and coworkers circumvent the immunogenic (strept)avidin systems and

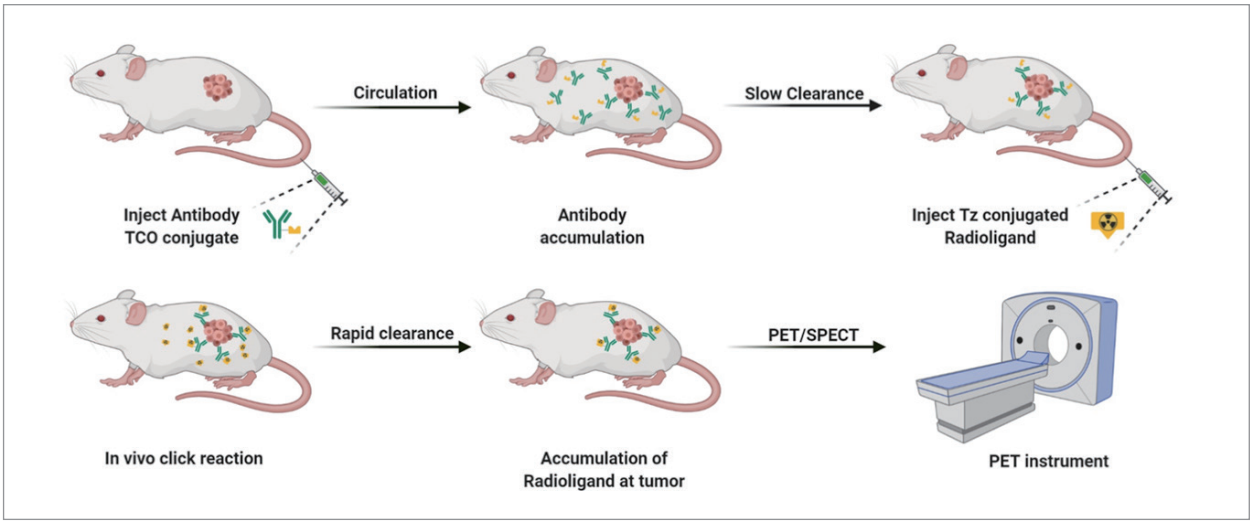

Figure 1. Schematic representation of pretargeting strategy. First, tumors are pretargeted with TCO-antibody conjugate. Secondly, small radiolabeled chelator is intravenously injected that binds to the pretargeted antibodies at the tumor sites.

the protein engineering techniques used for bispecific antibodies by utilizing IEDDA. In this, pretargeting was achieved in colon cancer xenografts by TCO-conjugated mAb CC49 and with ^{111}In-radiolabeled tetrazine-DOTA derivative. The kinetics of ^{111}In-tetrazine-DOTA with CC49-TCO in phosphate-buffered saline (PBS), a second order rate constant of 13090 ± 80 m^{-1} s^{-1} at 37°C within 3 minutes, was obtained (16). Although detailed explanation about antibody and bioorthogonal click reactions of respective PET and SPECT imaging is well reported (9, 10), herein, we will focus on recent promising optimization in pretargeting accompanying IEDDA and other factors like of collating two radiochelator and modifying chelator with multimeric reactive sites in the improvement of reactive sites.

Progress in Pretargeting

Currently, there are several research groups who have contributed in the development of pretargeting approach to enhance the tumor accumulation of radionuclide while minimizing involvement of normal tissues. In 2015, J. Lewis and B. Zeglis et al. demonstrated pretargeting by featuring short-lived radionuclide ^{18}F and first-of-its-kind Al[18F]-NOTA-labeled tetrazine radioligand with TCO bearing anti-CA19.9 antibody 5B1in pancreatic cancer xenografts. In the ex vivo biodistribution along with fast-cleared radiolabeled chelate in healthy mice (Figure 2a), substantially the uptake in vital organs is gradually decreased respective of increasing time. The radiochelator in the blood reduces over a time from 1 h to 4 h and reaches as low as 0.78 ± 0.08 %ID/g after injection. It also shows dual fecal and renal elimination pathways improving the efficiency in clearance. In honor with healthy mice BxPC3 xenografts bearing nude mice, after injecting 5B1-TCO at 72 h prior to the administration of Al[18F]-NOTA, the data showed increasing tumoral uptake over the course of study from 30 mins to maximum at 4 h was 5.6 ± 0.85 %ID/g., which was justified in the PET images where increased tumor-to-background ratio was achieved (Figure 2b) (17).

As after diagnosis pretargeting mainly aims to provide therapy and optimize the pretargeted radioimmunotherapy (PRIT) in the same BxPC3 tumor xenograft, in 2016, B. Zeglis and J. Lewis et al. compared ^{177}Lu-DOTA-PEG_7-Tz and ^{177}Lu-CHX-A''-DTPA-Tz by utilizing IEDDA (18). The nude tumor mice treated with ^{177}Lu-DOTA-PEG_7-Tz showed better tumor accumulation efficiency as compared to ^{177}Lu-CHX-A''-DTPA-Tz. Therapy was carried in five groups of mice, among which two groups were selected as control experiments. The mice were

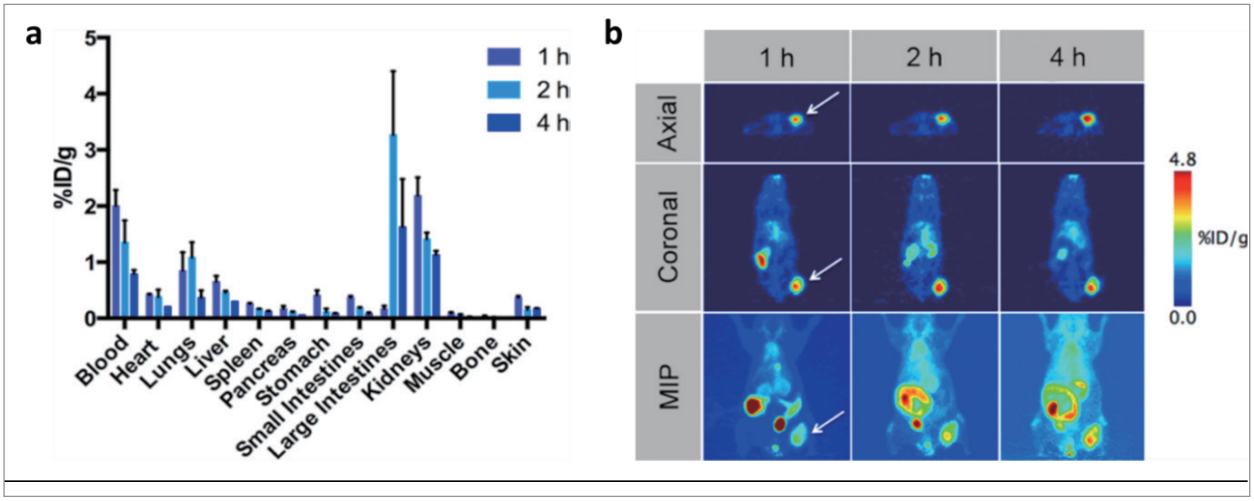

Figure 2. of Al[^{18}F]-NOTA-labeled tetrazine radioligand in healthy nude mice (a); PET images of pretargeting strategy by Tz-PEG11-Al[^{18}F]-NOTA/5B1-TCO in BxPC3 xenografts bearing nude mice (b).

treated 72 h prior with 5B1-TCO immunoconjugate for pretargeting, and then different amounts of radioactivity (400 μCi, 800 μCi, and 1200 μCi) were given to mice groups; pancreatic tumor volume was monitored every 3 to 7 days until 51 days. In the ex vivo experiments, the activity concentrations observed in the tumor tissue were high, reaching 16.8 ± 3.87% ID/g at 120 hours postinjection, and 51 days posttreatment, 75% of the mice from the therapy groups receiving high amount as 1,200 μCi of ^{177}Lu-DOTA-PEG7-Tz had tumors that were smaller than on day one. As a principal proof, the PET images and ex vivo experiments carried out by treating selective tumor mice with ^{89}Zr-5B1 showed marginally high nonuniform tumoral uptake at 120 h postinjection. However, the explanation of nonuniform tumoral uptake is given in original article (18).

In 2018, the study of D. Clemens et al. demonstrated that an increase in the number of tetrazine moieties will benefit the enhancement of PET imaging as it increases reactive sites toward protein binding. To form the multimeric tetrazine ligand as mono-, di-, and trimeric conjugates, the macrocyclic siderophore Fusarinine C (FSC) was chosen as it allows conjugation of up to three Tz residues due to three primary amines available for site-specific modification. The non-internalizing anti-CD20 antibody rituximab (RTX) modified with TCO was chosen as a targeting vector against tumor surrogates. The [^{68}Ga]Ga-FSC-Tz multimeric conjugates showed a higher binding capacity toward CD20-expressing Raji cells with increasing number of Tz motifs attached to the chelator. The cell-binding studies on Raji cells pretreated with RTX or RTX-TCO prior to incubation with ^{68}Ga-labeled Tz-ligand conjugates showed highly specific targeting properties as the amount of unspecific bound radioligand to RTX pretreated Raji cells was negligibly low (<1%). The binding of ^{68}Ga-labeled Tz ligands on RTX-TCO-bound Raji cells increased with the grade of multimerization and was 4.01 ± 0.24%, 7.35 ± 0.77% and 15.93 ± 0.88 for monomer, dimer, and trimer, respectively. The results were confirmed by micro-PET imaging and the tumoral uptake was higher for trimer, moderate for dimer and comparatively less for monomer (Figure 3). As a conclusion, the protein binding is increased by multimeric tetrazine as compared to monomers (19).

Recently, in 2020, Koen Augustyns and coworkers reported pretargeting based by modifying radiolabeling strategy toward TCO group. Herein, they synthesized novel dTCO-amide probe and further treated with tetra-n-butylammonium fluoride and 6-fluoronicotinoyl chloride to afford MICA-212 and MICA-213 two chelators for radiolabeling (Figure 4a). These succeeding novel dTCO-amide PET probes in were radiolabeled with short-lived radionuclide ^{18}F and in vivo and ex vivo studies were carried out. The in vivo micro-PET studies

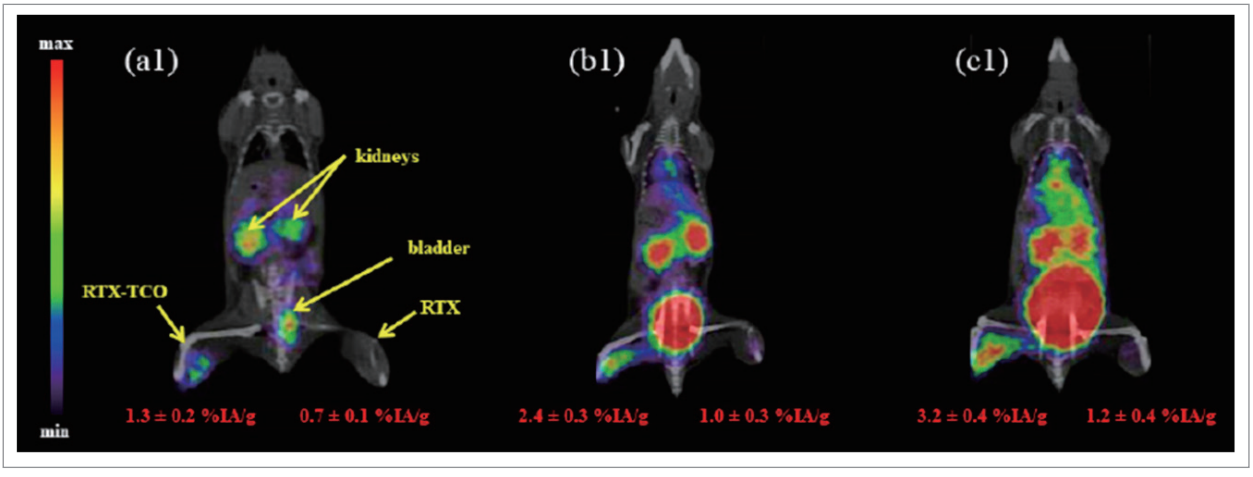

Figure 3. Static μPET/CT image of i.m. RTX(-TCO) pretreated normal BALB/c mice 5 h after treatment and 90 min p.i. of the ^{68}Ga-labeled Tz-monomer (a), Tz-dimer (b), and Tz-trimer (c) (1, coronal slice; 2, 3D volume rendered projections; both in prone position.

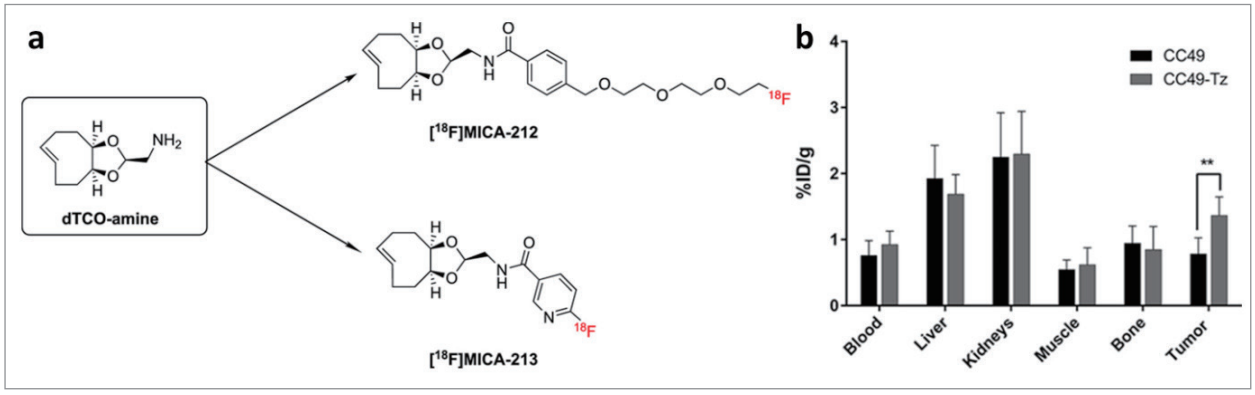

Figure 4. Chemical structures of the new dTCO amine scaffold and its ^{18}F-labeled derivative PET probes (a), Ex vivo biodistribution of [^{18}F]MICA-213 in LS174T tumor-bearing mice 60 min post tracer injection (mean ± SD, n = 5/group, $p < 0.054$) (b).

for both tracers in the normal mice demonstrated same hepatobiliary and renal clearance. Nevertheless, the [^{18}F] MICA-213 was selected due to remarkable kinetic rate for in vivo representative micro-PET/CT studies of the LS174T tumor-bearing mice. The CC49 or CC49-Tz was injected 24 h prior to the administration of radiolabeled chelator and low background image was observed at 60 min postinjection. In ex vivo biodistribution studies comparing non tumor mice, the clearance through kidneys and liver showed nearly same trend, whereas CC49-Tz showed tumoral uptake 1.36 ± 0.28% ID/g than in the control group 0.78 ± 0.24% ID/g (Figure 4b) (20). However, this strategy still needs further optimizations by utilizing clearing agents in order to improve background interference.

In summary, the pretargeting strategy is developed to have superior targeting abilities as well as to obtain better imaging unlike directly radiolabeled antibodies. We believe among other pretargeting strategy, the antibody-based pretargeting aided with IEDDA reactions in the upcoming years will become the foundation in the development of radiopharmaceuticals in diagnosis as well as therapy.

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