JRMP Journal of Radiopharmaceuticals and Molecular Probes Vol. 5, No.2, 2019
J Radiopharm Mol Prob 2019;5(2): 135-144
ISSN 2384-1583(Print) / ISSN 2508-3848(Online)

Review

https://doi.org/10.22643/JRMP.2019.5.2.135

# Targeted alpha therapy (TAT) for cancer using metallic radioisotopes

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**ABSTRACT**

Targeted alpha therapy (TAT) based on metallic radionuclides has attracted a lot of attention lately due to its impressive therapeutic efficacy displayed in couple of clinical studies for cancer. Representative metallic radionuclides emitting alpha-particle include 225Ac, 213Bi, and 227Th, and there have been variety of TAT formulations based on different targeting moiety and chelating agents. In this review, we introduce strategies to label metallic radioisotopes with biomolecules and look at some of recent preclinical and clinical results of TAT for cancer.

**Key Word:** Targeted alpha therapy (TAT), metal chelators, Ac-225, Bi-213, Th-227

## Introduction

Cancer is the most devastating non-communicable disease responsible for approximately 9.6 million deaths in 2018 worldwide (1, 2). Although its mortality rate has been decreased in developed countries, cancer has become leading cause of death due to increased mortality rate in developing countries for the past couple of decades (3, 4), and cancer is considered as the most important hurdle to overcome for human being to extend life expectancy.

Traditional treatment options for cancer include surgery, radiotherapy, and chemotherapy. Surgery can be applied only on localized solid tumors, and the other regimens suffer from the inevitable side-effects including leukopenia, neutropenia, thrombocytopenia and anemia severely degrading patient's quality of life (5, 6). To overcome such limitations, tumor-specific therapy, for example, antibody-based immunotherapy was implemented, but low efficacy and tumor resistance have been the issue (7). Antibody-drug conjugates (ADCs) composed of an antibody and chemotherapeutics such as trastuzumab emtansine (Kadcyla®) have been developed and showed improved therapeutic efficacy (8), but multidrug resistance also became an issue for ADCs (9, 10).

Another way to improve therapeutic efficacy of cancer treatment is to arm antibodies with radioisotopes. There have been two FDA-approved antibody-based radiopharmaceuticals, which are Baxxar® and Zevalin®, for the treatment of lymphoma, both utilizing radioisotopes with beta radiation. However, their therapeutic efficacy is not satisfactory and consequently one of them (Baxxar®) was withdrawn from the market in 2014. The efforts to

Received: December 12, 2019 / Revised: December 26, 2019 / Accepted: December 28, 2019

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find more effective cancer therapeutics turned researcher's attention to radioisotopes emitting alpha-particles, which are known to have energy around 5~8 MeV that is 100 to 1000 times higher energy than beta radiation and short path length (50~80 μm) consequently providing high linear energy transfer (LET). Therefore, alpha-particles can cause irreversible double-strand DNA breaks on tumors while avoiding unwanted damages on normal tissues provided they are deposited within or close proximity to the cancer cells (11). Recent FDA-approval and success of $^{223}RaCl_2$ (Xofigo®) for castration-resistant prostate cancer with bone metastases indicate the potential of alpha-particle emitting radioisotopes for cancer treatment. To develop targeted alpha-particle emitting radiopharmaceuticals using biomolecules such as peptides and antibodies as a targeting moiety, the radioisotopes should be labeled efficiently in a mild condition to prevent damages on the biomolecules. Furthermore, the labeled complex should be stable enough not to lose the radioisotopes to reduce side-effects caused by radiation exposure on non-targeted tissues. Researchers have worked hard for decades to develop chelators with mild labeling conditions, which satisfy aforementioned prerequisites, and indeed we are starting to see more promising results on clinical trials using targeted alpha therapy (TAT) on cancers lately.

In this paper, we review development of TAT using various metallic radioisotopes for cancer. Radiolabeling strategies as well as preclinical and clinical results are introduced for the most frequently studied alpha-particle emitting metallic radioisotopes.

## Actinium-225 ($^{225}Ac$)

Ac-225 is one of the most studied alpha-particle emitting radioisotopes in the application of targeted cancer therapy due to its ideal physical characteristics including relatively long decay half-life (9.9 d), which matches with biological half-life of typical antibodies, and high energy (6.0 MeV alpha-particle) that can effectively damage cancer cells (12). Various different chelators were developed and tested for the fast and stable labeling of $^{225}Ac$ (Figure 1), but 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) has been used almost exclusively as the $^{225}Ac$ chelator in preclinical and clinical studies (13-15).

Radiolabeling of $^{225}Ac$ using DOTA as a chelator for the development of radiopharmaceuticals normally takes place in two steps. In the first step, DOTA with functional group is labeled with $^{225}Ac$ at around 55-60°C, and the labeled DOTA is conjugated with biomolecules in the second step to avoid degradation of the biomolecules in the harsh labeling condition (Figure 2).

Although DOTA has been almost exclusively utilized as a $^{225}Ac$ chelator in preclinical and clinical studies, its labeling conditions, efficiency, and the stability of the labeled complex are far from perfect. Since thermodynamic

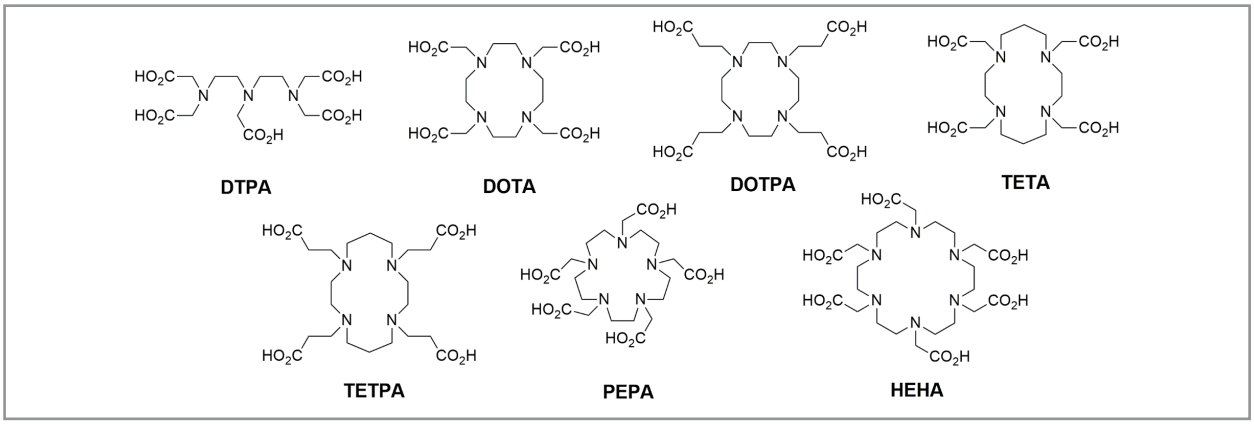


**Figure 1.** Ac-225 chelators in preclinical and clinical studies.

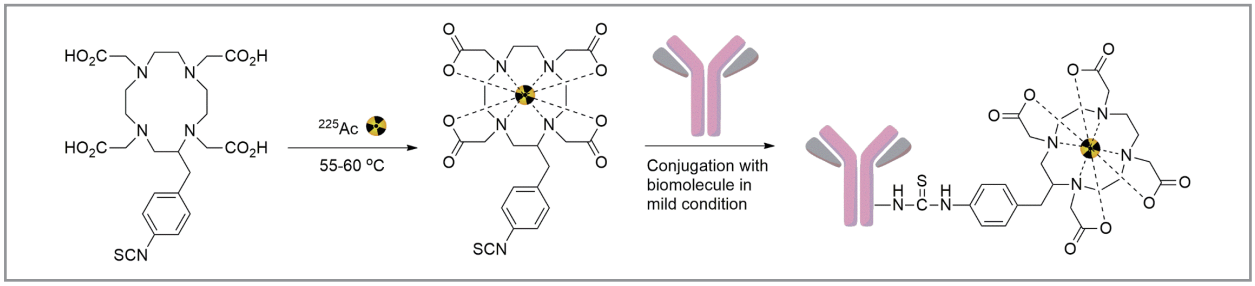


**Figure 2.** Typical $^{225}$Ac labeling procedure for biomolecule-based radiopharmaceuticals.

stability of DOTA-metal complex is inversely proportional to the ionic radius of metal ion, the stability of a complex with a large metal is normally low. For this reason, $^{225}$Ac-DOTA complex might not be stable enough because $^{225}$Ac is the largest one among trivalent metals (16). Furthermore, kinetic stability of a metal complex would be reduced in diluted condition *in vivo*, the effort to develop better $^{225}$Ac chelators continue (16). Figure 3 shows recently developed $^{225}$Ac chelators.

Joint research team from Germany and Canada recently developed a new $^{225}$Ac chelator named Bispa2, which is composed of two picolinic acids, and it was found that 10-4 of Bispa2 had 100% labeling efficiency within 30 min when reacted with 40 kBq of $^{225}$Ac at room temperature, and 225Ac-Bispa2 maintained stable complex in human serum for 7 days (17). Another research group from the USA and Canada developed a new $^{225}$Ac chelator called Macropa consisting of diaza-18-crown-6 with two picolinic acids. Macropa also showed fast complexation kinetics (100% labeling within 5 min) when 59 μM of Macropa was reacted with 26 kBq of $^{225}$Ac at room temperature. Furthermore, $^{225}$Ac-Macropa was stable in human serum for 7 days and in vivo (mouse) for 5 h (18). Based on the results from aforementioned researches, it could be concluded that the labeling efficiency and stability of $^{225}$Ac complex would improve with increasing number of coordination group. However, this is not always the case as Kelly JM et al. found out (19). They prepared prostate specific membrane antigen (PSMA) targeting peptide conjugated with DOTA-based chelator named EuK-106, which has twelve coordination groups (Figure 3). It was found that labeling efficiency of EuK-106 with $^{225}$Ac was less than 10% even at high temperature (95°C). Therefore, it seems a lot of research should be done to develop optimal $^{225}$Ac chelators. Nonetheless, many successful preclinical results have been reported using $^{225}$Ac for the treatment of various cancers. For instance, a research group from Technical university of Munich in Germany recently developed a conjugate composed of a DOTA and a peptide called F3, which can targets nucleolin highly expressed in some cancer cells. They found a $^{225}$Ac-DOTA-F3 treated group had extended median survival duration (95 days) compared to a PBS treated control group (60 days) using subcutaneous peritoneal carcinomatosis mouse models

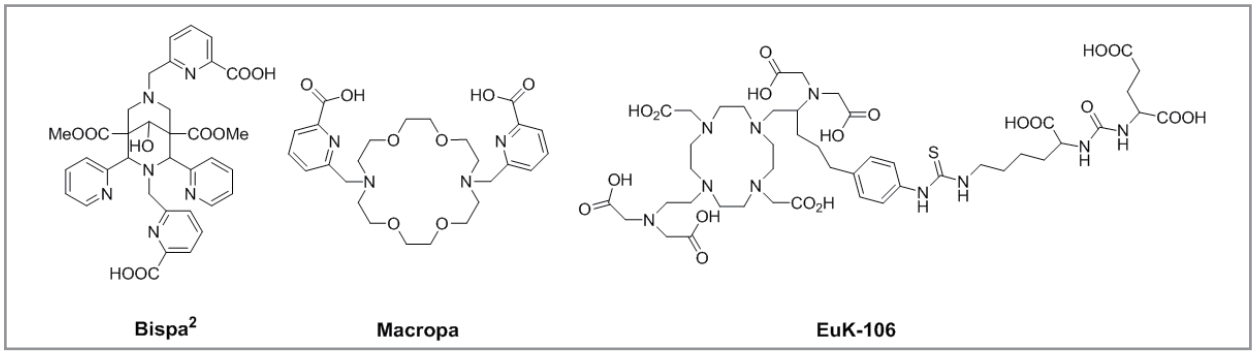


**Figure 3.** New $^{225}$Ac chelators in preclinical studies.

(20). Another group from Germany directly compared therapeutic efficacy of beta and alpha-particle emitting radionuclides using pancreatic cancer xenografted mouse models. They prepared $^{225}$Ac- or $^{177}$Lu-labeled DOTATOC targeting somatostatin receptor overexpressed in pancreatic cancers and showed $^{225}$Ac-DOTATOC exhibited better tumor growth inhibition resulting in prolonged survival of mouse models compared to the ones treated with $^{177}$Lu-DOTATOC (21). The researchers also provided experimental proof that alpha-particle resulted in more DNA double-strand breaks (DSB) than beta radiation. A joint research from Wake forest school of Medicine in the U.S. and the People's hospital of Zhengzhou university in China recently showed the great therapeutic efficacy of $^{225}$Ac using glioblastoma mouse models (22). They synthesized $^{225}$Ac-labeled peptide ($^{225}$Ac-DOTA-Pep-IL) which selectively binds to glioblastoma and showed treatment of $^{225}$Ac-DOTA-Pep-IL on orthotopic mouse models resulted in much longer median survival time (41 days) compared to the control (23 days). Almost all of the preclinical and clinical studies using 225Ac have exploited DOTA as a chelator. However, there is a risk for radiation toxicity on normal tissues caused by dissociated daughter radionuclides, such as $^{221}$Fr and $^{213}$Bi. One way to prevent such a risk is to trap all the possible daughter radionuclides in a nanoparticle, and a group from Delft University of Technology in Netherlands showed possibility of trapping $^{221}$Fr (69% retention) and $^{213}$Bi (53% retention) from $^{225}$Ac in polymersomes (23). However, more study should be done to improve retention percentage and to resolve rapid clearance of the large polymersomes from blood circulation.

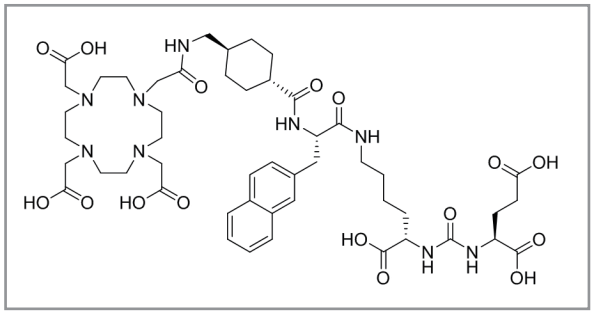

**Figure 4.** Structure of PSMA-617.

While a lot of successful preclinical studies have come out, an outstanding clinical result on the treatment of patients with metastatic prostate cancer using $^{225}$Ac-labeled PSMA-617 ($^{225}$Ac-PSMA-617) was reported recently.

PSMA-617 is composed of DOTA conjugated with PSMA-targeting peptide unit (Figure 4). PSMA is an enzyme located in a cell's membrane, and it consists of protease, apical, and C-terminal domains where apical domain forms binding pocket and shows high binding affinity with amino acid sequence of glutamate-urea-lysine (24). It is known the existence of aromatic group in PSMA-617 increases the binding affinity to PSMA and helps internalization (25). Recent clinical trial using $^{225}$Ac-PSMA-617 on metastatic prostate cancers showed encouraging outcome for targeted therapy based on alpha-particle emitting radionuclides and favorably compared with targeted therapy using beta radiation. The patients who did not have any therapeutic benefits with treatment of $^{177}$Lu-PSMA-617 showed complete response on imaging and dramatic decline of prostate specific antigen (PSA) level after treatment of $^{225}$Ac-PSMA-617 (26). There is also couple of clinical trials being prepared for the treatment of acute myeloid leukemia and refractory multiple myeloma using $^{225}$Ac-lintuzumab.

## Bismuth-213 ($^{213}$Bi)

Bi-213 also can be chelated by traditional chelators such as DOTA and diethylenetriaminepentaacetic acid (DTPA). Especially DTPA analogue CHX-A″-DTPA (Figure 5) was shown to have great labeling efficiency and complex stability with $^{213}$Bi, so it has been used in many preclinical studies. However, the efforts to develop $^{213}$Bi chelators with better performance continues, and a research group from Los Alamos National Laboratory in the U.S. recently developed series of $^{213}$Bi chelators (Figure 5) with

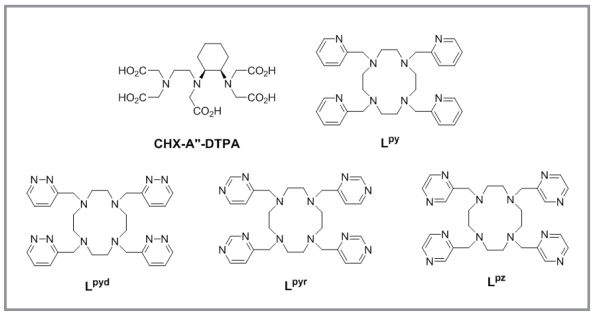


**Figure 5.** Chelators used for $^{213}$Bi labeling.

increased number of nitrogen compared to typical chelators and showed the potential to be utilized for $^{213}$Bi-specific chelators (27). Development of radiopharmaceuticals using $^{213}$Bi is actively progressing, and they are proven to be effective in preclinical studies for many different kinds of cancers. For example, CHX-A″-DTPA conjugates of antibodies labeled with $^{213}$Bi were shown to have great therapeutic effect on mouse tumor models of multiple myeloma, pancreatic cancer, and ovarian cancer (28-30). Clinical studies using $^{213}$Bi are also in progress, and some produced promising results. Kratochwil C et al. used $^{213}$Bi-DOTATOC, which specifically binds to somatostatin receptor, for the treatment of neuroendocrine cancer patients with liver metastases who had no response when treated with $^{90}$Y/$^{177}$Lu-DOTATOC. It was found the treatment of $^{213}$Bi-DOTATOC resulted in enduring responses with low acute hematotoxicity and chronic kidney toxicity (31). In another study, $^{213}$Bi-labeled Substance P, which specifically binds to neurokinin type I receptor highly expressed in glioblastoma multiforme (GBM), was evaluated in GBM patients. It was shown the patients treated with $^{213}$Bi-Substance P had prolonged overall survival time demonstrating the therapeutic efficacy of $^{213}$Bi (32). More recently, Autenrieth ME et al. performed a pilot study using $^{213}$Bi-labeled cetuximab in patients with bladder cancer and found there were no significant side-effects caused by the treatment (33).

Similar to $^{213}$Bi, $^{212}$Bi could be chelated using DOTA and DTPA. However, due to high energy gamma ray (2.6 MeV) produced by 208Tl, a daughter nuclide of $^{212}$Bi, development of radiopharmaceuticals using $^{212}$Bi is not practical considering high radiation exposure to the patients.

## Terbium-149 ($^{149}$Tb)

Tb-149 has no daughter radionuclides that emit alpha-particles, so there is no concern about radiotoxicity as long as $^{149}$Tb itself is not dissociated from the labeled complex. Therefore, $^{149}$Tb has great potential to be utilized in TAT. However, $^{149}$Tb has relatively short half-life (4.1 h) compared to other alpha-particle emitting radioisotopes, and 83% of the energy is emitted by electron capture while only 16.7% is emitted by alpha-particle when it decays. Furthermore, high energy (1.4 GeV) proton beam is required to produce $^{149}$Tb by irradiation on tantalum (Ta) target. Therefore, $^{149}$Tb is not easily accessed radioisotope and consequently it is not frequently studied for the development of radiopharmaceuticals. Anyhow, it can be chelated by the traditional chelators like DOTA or DTPA. Only handful number of preclinical studies has been done, but with promising results, for the evaluation of $^{149}$Tb-applied radiopharmaceuticals. For example, treatment of $^{149}$Tb-labeled rituximab on lymphoma-xenografted mouse model resulted in prolonged tumor-free survival of more than 120 days among 85% of the experimental group while the untreated control mice had to be sacrificed within 37 days due to the clear development of lymphoma (34). In another study, 149Tb-labeled folate was treated in cervical carcinoma-xenografted mice, and it was found the treatment inhibited tumor growth and resulted in increased average survival time of mice (43 days) compared to untreated control mice (21 days) (35). There has been no clinical study using $^{149}$Tb.

## Radium-223 ($^{223}$Ra)

Ra-223 is the first alpha-particle emitting radioisotope that obtained FDA-approval for the treatment of cancer in the form of $^{223}RaCl_2$ (Xofigo®). Since Xofigo® does not have actual targeting biomolecule in the formulation, it is difficult to say the treatment of Xofigo® is a TAT. However, due to similar chemical property of $^{223}$Ra with calcium, its uptake in the bone is very high. Furthermore, bone marrow toxicity by the radiation is known to be lower compared to other beta-emitting radiopharmaceuticals because of the short emission range of alpha-particle (36). Ra-$^{223}Cl_2$ treatment in several phase I and II studies has been shown that administration of multiple doses of 100 kBq/kg or single dose of 250 kBq/kg were well tolerated in the bone metastatic patient with castration-resistant prostate cancer or breast cancer (37-39). Moreover, it was found $^{223}RaCl_2$ treatment resulted in prolonged overall survival time (14.9 months) of patients with castration-resistant prostate cancer and bone metastases compared to placebo group (11.3 months) (40). It seems that alpha therapy using $^{223}RaCl_2$ is a promising option for the patients with bone metastatic castration-resistant prostate cancer to relive bone pain and extend survival time. However, larger clinical studies should be done to concretely confirm the therapeutic effect of $^{223}RaCl_2$ treatment.

## Thorium-227 ($^{227}$Th)

Th-227 is the parent radionuclide of $^{223}$Ra, and it also emits alpha-particle which could be applied in the TAT. Th-227 has much longer half-life (18.7 days) than other alpha-particle emitting radionuclides frequently investigated in the therapeutic applications for cancer such as $^{213}$Bi (46 min) and $^{211}$At (7.2 hrs). Therefore, it is more feasible to be exploited in clinical settings. Furthermore, production of $^{227}$Th is practically unlimited because $^{227}$Th generator could be prepared easily where $^{227}$Th is produced by thermal neutron irradiation of $^{226}$Ra (41). Consequently, $^{227}$Th attracted a lot of attention from researchers recently, and many preclinical studies have been done for years. In the early stage of investigations, DOTA was used as the chelator for $^{227}$Th, and its antibody conjugates using rituximab and trastuzumab exhibited promising in vitro and in vivo results (42-45). However, it was found octadentate chelator 3,2 hydroxypyridinone (3,2-HOPO) was better in labeling with $^{227}$Th and maintaining more stable complex compared to DOTA, and recent investigations using $^{227}$Th have done with 3,2-HOPO. For example, Hagemann UB et al. recently prepared CD70-binding and mesothelin-targeting antibodies labeled with $^{227}$Th using 3,2-HOPO and showed promising anti-cancer effects using mouse xenograft models of renal cell carcinoma (46) and cancers expressing mesothelin (47), respectively. With successful preclinical results, a few phase I clinical studies using $^{227}$Th-labeled antibody conjugates targeting prostate cancer, non-Hodgkin's lymphoma, ovarian cancer, and mesothelioma are in progress or in the stage of recruiting patients.

## Conclusion

Although mortality rate of cancer in developed countries has decreased for decades, it is still considered one of the most difficult diseases to overcome. Therapeutic strategies beyond the traditional treatment options have been employed to improve therapeutic efficacy as well as to reduce side-effects. Such new strategies include treatments using antibody drug conjugates and targeted radiation therapy using radionuclides with beta (β) radiation. These treatment options somehow showed improved therapeutic efficacy in the clinic, but the results was far from satisfaction. In search for better therapeutic strategies to treat cancer, researchers have turned their attention to alpha-particle emitting radionuclides, which have much

higher energy and shorter path-length compared to beta radiation that could maximize therapeutic efficacy and minimize side-effects. Targeted radiation therapy using alpha-particle emitting metallic radionuclides including $^{225}$Ac, $^{213}$Bi, and $^{227}$Th has shown impressive results in preclinical as well as in clinical studies on various cancers. Especially the clinical result reported by Kratochwil C et al. on the treatment of metastatic prostate cancer patients using peptide labeled $^{225}$Ac (26) was quite impressive and attracted a lot of attention. Furthermore, new metal chelators for prompt labeling and increased stability are being developed actively to resolve radiation toxicity problem caused by dissociated radionuclides. Provided by chelation chemistry for such large metals is well understood and appropriate chelators are developed, TAT using metallic radionuclides will be major option for cancer treatment in the near future.

## Acknowledgments

This study was supported by a grant of the Korea Institute of Radiological and Medical Sciences (KIRAMS), funded by Ministry of Science and ICT (MSIT), Korea (No. 50461-2019, No. 50537-2019). The authors declare no conflict of interest.

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