JRMP Journal of Radiopharmaceuticals and Molecular Probes Vol. 5, No. 1, 2019
J Radiopharm Mol Probes 2019;5(1):26-35
ISSN 2384-1583(Print) / ISSN 2508-3848(Online)

ORIGINAL ARTICLE

https://doi.org/10.22643/JRMP.2019.5.1.26

^{188}Re Labeled liver therapeutic drugs for hepatic carcinoma (HCC)

Sudhakara Reddy Seelam[1,2,3], Vinay Kumar Banka[1,2,3], Yun-Sang Lee[1,2*], Jae Min Jeong[1,2,3]

[1] Department of Nuclear Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea
[2] Cancer Research Institute, Seoul National University College of Medicine, Seoul, Republic of Korea
[3] Department of Radiation Applied Life Science, Seoul National University College of Medicine, Seoul, South Korea Department of Nuclear Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea

ABSTRACT

^{188}Re is one of the most readily available generator derived and useful radionuclides for therapy emitting β^- particles (2.12 MeV, 71.1% and 1.965 MeV, 25.6%) and imageable gammas (155 keV, 15.1%). The ^{188}W/^{188}Re generator is an ideal source for the long term (4-6 months) continuous availability of no carrier added (NCA) ^{188}Re suitable for the preparation of radiopharmaceuticals for radionuclide therapy. Rhenium-188 has been used for the preparation of therapeutic radiopharmaceuticals for the management of diseases such as bone metastasis, rheumatoid arthritis and primary cancers. Several early phase clinical studies using radiopharmaceuticals based on ^{188}Re -labeled phosphonates, antibodies, peptides, lipiodol and particulates have been reported. In this review, we addressed the current development status of ^{188}Re radiopharmaceuticals for liver cancer therapy and their applications.

Key Word: ^{188}Re, Rhenium, Hepatic Carcinoma, Lipiodol, N_2S_2, HDD, TDD, HTDD.

Introduction

Various radioisotopes have been used for trans-arterial radiotherapy of hepatocellular carcinoma (HCC) (Table 1), among them rhenium-188 (^{188}Re) is most commonly used. Rhenium-188 is a high energy β-emitting radioisotope obtained from the ^{188}W/^{188}Re- generator (Figure 1), which has shown utility for a variety of therapeutic applications in nuclear medicine, oncology, and interventional radiology/cardiology. The decay of ^{188}Re is accompanied by a 155 keV predominant energy β-emission, which could be detected by γ-cameras, for imaging, biodistribution, or absorbed radiation dose studies. It has an attractive physical properties (Table 1) and its potential low cost associated with a long-lived parent make it an interesting option for clinical use.

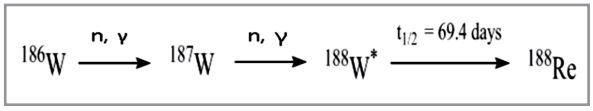

Figure 1. Decay scheme for ^{188}Re production.

Table 1. Examples of radioisotopes used for HCC transarterial metabolic radiotherapy.

Radiele -ments	Half-life (days)	$E\beta^-_{max}$ (MeV)	Maximum range in tissues (mm)	Eγ (KeV)
^{131}I	8.05	6.06	2	364
^{186}Re	3.7	1.7	5	137
^{188}Re	0.7	2.1	10	155
^{90}Y	2.67	2.2	12	None
^{166}Ho	1.1	1.85	8.7	80.6

Received: June 17, 2019 / Revised: July 5, 2019 / Accepted: July 8, 2019

Correspnding Author : Yun-Sang Lee, Department of Nuclear Medicine, Seoul National University Hospital, 101, Daehak-ro, Jongno-gu, Seoul 03080, Republic of korea, Tel: +82-2-3668-8906, Fax: +82-745-7690, E-mail: wonza43@snu.ac.kr.

The clinical efficacy, for several therapeutic applications, of a variety of ^{188}Re-labeled agents is demonstrated. The high energy of the β-emission of ^{188}Re is particularly well suited for effective penetration in solid tumors. Its total radiation dose delivered to tissues is comparable to other radionuclides used in therapy. Furthermore, radiation safety and shielding requirements are an important subject of matter. In the case of bone metastases treatment, therapeutic ratios are presented in order to describe the efficacy of ^{188}Re usage.

Lipiodol

Lipiodol is an iodinated and esterified lipid of poppy seed oil that has been used as a contrast agent for the detection of liver cancer (1-4). When injected through the hepatic artery it accumulates in the liver cancer because of its high viscosity. This property of lipiodol has encouraged many researchers to use it as a radioisotope carrier. Other carriers such as microspheres can also be used. However, lipiodol is the most effective and convenient carrier because of its excellent targeting ability as well as its capacity to be accurately monitored by X-ray.

There have been many attempts to label lipiodol with therapeutic radioisotopes, including ^{166}Ho, ^{131}I, ^{90}Y, ^{186}Re and ^{188}Re (5-9).

^{131}I-labelled lipiodol is commercially available and is currently used in many countries. However, its high cost and high external radiation dose due to its high energy gamma radiation limits its general use (6, 10-12). Among the metallic beta emitters, ^{188}Re became the most important candidate for labelling lipiodol because of its convenience and economy. Early attempts to label lipiodol with ^{188}Re were not very successful, since ^{188}Re is obtained as an aqueous solution and lipiodol is available as an oil. Direct chemical reaction is precluded because they are not miscible. If a bichelating agent were to be linked with lipiodol in order to facilitate labelling with ^{188}Re, the chemical properties of the resulting conjugate would be changed. In addition, its chemistry would be difficult to characterize since lipiodol is not composed of a single substance.

Bifunctional chelating agents for ^{188}Re Labeling

Several N_2S_2 based bifunctional chelating agents, 2,2,9,9-tetramethyl-4,7-diaza-1,10-decanedithiol (TDD) (1), 4-Octyl-2,2,9,9-tetramethyl-4,7-diaza-1,10-decanedithiol (ODD) (13), 4-Dodecyl-2,2,9,9-tetramethyl-4,7-diaza-1,10-decanedithiol (DDD) (13), 4-hexadecyl-2,2,9,9-tetramethyl-4,7-diaza-1,10-decanedithiol (HTDD) (14), 4-hexadecyl-2,2,9,9-tetramethyl-4,7-diaza-1,10-decanedithioacetate (HTDD-A) (2) and 4-hexadecyl-4,7-diaza-1,10-decanedithiol (HDD) (15), have been developed for ^{188}Re labeling (Figure 2).

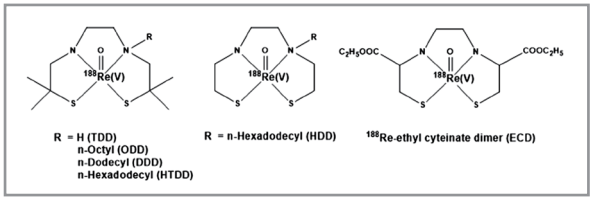

Figure 2. Chemical structures of ^{188}Re labeled N_2S_2 based bifunctional chelating agents.

^{188}Re–lipiodol for Hepatic Carcinoma (HCC)

Hepatic carcinoma is a most prevalent cancer worldwide and although most cases still occur in developing countries in South-East Asia and Africa

(16). Almost 700,000 new cases are diagnosed yearly throughout the world and unfortunately the prognosis remains poor since more than 500,000 deaths are ascribed to HCC each year (16). Thus the development of therapeutic radiopharmaceuticals for its management if intercepted early, is an active field of radiopharmaceuticals research for many researchers. ^{131}I labelled lipiodol is a commercially available radiopharmaceutical for the treatment of hepatocarcinoma (17). Lipiodol or ethiodized oil, contains iodine combined with ethyl esters of fatty acids and is used as a contrast agent in myelography. The inactive iodine of lipiodol are exchanged with ^{131}I, followed by solvent extraction of the labeled product for preparation of the radiopharmaceutical.

There are several reports describing the preparation of ^{188}Re-lipiodol starting with Kim et al. suspending ^{188}Re sulphur colloid in lipiodol (18). A N2S2 (diaminedithiol) based chelating agent 2,2,9,9-tetramethyl-4,7-diaza-1,10-decane dithiol (TDD, Figure 2) was synthesized and labeled with technetium (^{99m}Tc-TDD) or rhenium (^{188}Re-TDD) in high yields (72.0 ± 5.0 %), and found to be stable in room temperature and in human serum at 37ºC for up to 48 h (13). ^{188}Re-TDD showed high accumulation in the hepatoma (5 min: 33.1±24.1; 60 min: 13.5±10.6; 24 h: 3.02 ±2.78) was observed in hepatoma-bearing Sprague-Dawley (SD) rats after injection through the hepatic artery (13). However, ^{188}Re-TDD tumor retention is not enough to treat liver cancer. Therefore a new form of TDD, 4-hexadecyl-2,2,9,9-tetramethyl-4,7-diaza-1,10-decanedithiol (HTDD) was developed to improve tumor retention by introducing a long alkyl chain and it is labeled with ^{188}Re in high yields (65.0 ± 7.0 %). A comparative study between ^{188}Re-TDD and ^{188}Re-HTDD were performed in VX2 carcinoma (liver) bearing rabbits.

Table 2. Physical properties of ^{188}Re.

Property	^{188}Re
Physical half-life	16.98 h
E_{β^-max} (abundance)	2120.4 keV (71.1%) 1965.4 keV (25.6%)
Eγ (abundance)	155.0 keV (15%)
Tissue penetration $_{max}$	10 mm (average 3.1 mm)
Availability	^{188}W/^{188}Re generator
Specific activity	Carrier-free [6.8 TBq (184 Ci)/μmol]
Chemistry	VII B transition metal, chemistry is Similar to technetium.

The residences times of radioactivity in the liver were 10.2 ± 1.0 h in the ^{188}Re-TDD and 17.6 ± 0.8 h in the ^{188}Re-HTDD (p = 0.034) (1). A comparative study between ^{188}Re-HTDD and ^{131}I-lipodol was conducted in patients (Ghent University hospital, Belgium). This study showed that ^{188}Re-HDD/lipiodol yielded smaller cytotoxic effect and a lower radiation exposure for an expected higher tumor-killing effect. Dosimetry-guided transarterial radionuclide therapy with ^{188}Re-HTDD/lipiodol in a patient with unresectable hepatocellular carcinoma was evaluated. This study showed that transarterial radionuclide therapy with ^{188}Re-HTDD/lipiodol appears to be a safe, effective and promising therapeutic option in cases of unresectable hepatocellular carcinoma with portal vein thrombosis (19, 20). The maximum tolerated activity to be safely injected in the patient was calculated to be about 8.325 GBq (225 mCi) with the lungs being the dose limiting organ. Two doses of the radiopharmaceutical resulted in the complete disappearance of a large volume tumor and the patient was disease free for 18 months. A large scale clinical trial involving 93 patients in India and Vietnam using ^{188}Re-HDD-lipiodol were conducted (21). Similarly, 35 treatments in 28 patients with ^{188}Re-HDD-lipiodol activity ranging from 4.81-7.03 GBq(130-190 mCi) has been reported (22, 23). The

Table 3. Reported conditions of Rhenium chelating agents for optimal radiolabeling efficiencies.

Conditions	TDD	ODD	DDD	HTDD	HTDD-A	HDD	ECD
Precursor Weight (mg)	1	1	1	1	3	1	1
$SnCl_2 \cdot 2H_2O$ (mg)	10	3	3	10	10	6	15
Tartaric acid (mg)	200	30	30	40	20	20	10
Mannitol (mg)				20	10	10	-
$^{188}ReO_4^-$ (mL)	3	3	3	3	3	3	1-2
Heating Time (min)	15 ~ 30	60	60	60	60	60	30
Lipiodol (mL)	1-3	3	3	3	3	3	3
Labeling efficiency (%)	88	60-80	60-80	83.4±3.2	60-80	98.8±0.2	79.7
Final yield (%)	72.0±5.0	50-70	50-70	82.1±0.6	50-70	90.2±2.6	
Reference	(1)	(13)	(13)	(2) (36)	(2)	(36)	(24)

studies confirmed that the patients tolerated the dose and no severe complications were reported. Response assessment showed partial response in 1, stable disease in 28 and disease progression in 2 treatments. There was a significant reduction in AFP (An alpha-fetoprotein) levels measured in patients six weeks after treatment. Radiolabeling procedure and radiolabeling conditions of ^{188}Re-N_2S_2 based derivatives were presented in Table 3.

^{188}Re–EDD/Lipiodol

^{99m}Tc-ECD (Ethyl cyteinate dimer) is a known brain perfusion imaging agents and it is an approved drug. Ethyl cyteinate dimer (ECD) was labelled with ^{188}Re instead of ^{99m}Tc and then extracted by Lipiodol to form a new agent ^{188}Re-ECD/Lipiodol (79.77±3.0), and it found to be stable in human serum at 37ºC for at least 2 days (Figure 2) (24). Radiolabeling conditions of ^{188}Re-ECD/lipiodol were presented in Table 3. ^{188}Re-ECD/Lipiodol showed high accumulation in the hepatoma (1 h: 11.19±4.11; 24 h: 7.30±2.20; 48 h: 3.55 ±1.03) was observed in hepatoma-bearing Sprague-Dawley (SD) rats after injection through the hepatic artery.

^{188}Re(III)–SSS–lipiodol

SSS-lipiodol [SSS = (S2CPh)(S3CPh)2] is an another N_2S_2 based bifunctional chelating agent developed and labeled with ^{188}Re with a very high radiochemical yields (87 ± 9.1%), showed a clear advantage over the previously mentioned radiolabeling techniques for ^{188}Re (Figure 3). Radiolabeling conditions of ^{188}Re-SSS lipiodol were presented in Table 4. The radiochemical purity (93 ± 3.4 %), is satisfactory and the radiolabeling is stable for at least 48 h *in-vitro*. ^{188}Re-SSS lipiodol was injected to the hepatic artery of healthy pigs and they were sacrificed at 1, 24 and 48 h post-injection, for ex-vivo γ-counting. *Ex-vivo* γ-counting confirmed the predominantly hepatic uptake and revealed weak lung and intestinal uptake. There was very weak urinary elimination (2.3 ± 0.5% at 48 h) and a slightly higher level of intestinal elimination (4.8%±1.9% at 48 h). The

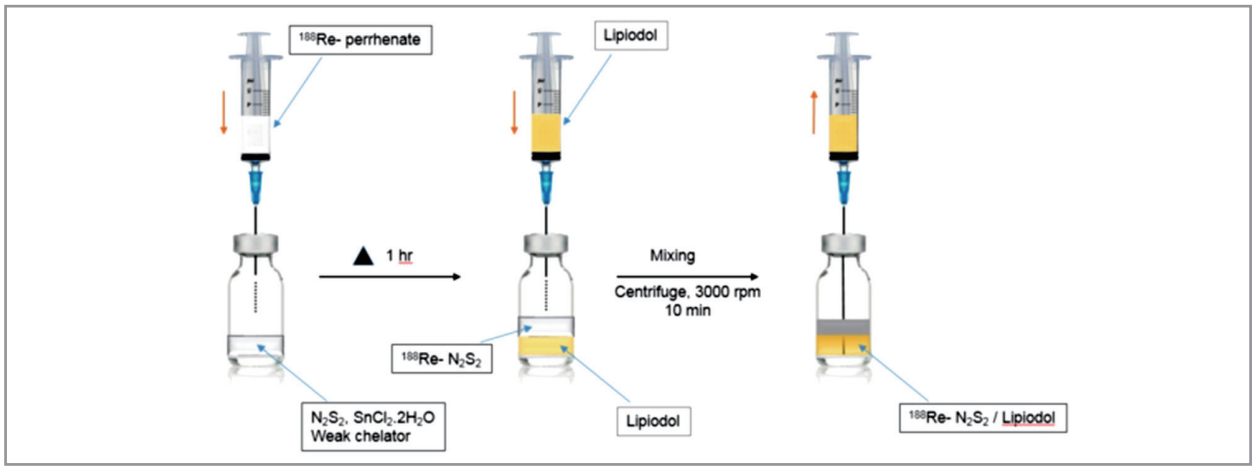

Figure 3. Procedure for the preparation of a lipiodol solution of ^{188}Re-labeled agents

Table 4. Reported conditions of ^{188}Re-SSS/lipiodol for optimal radiolabeling efficiencies.

Materials	Conditions
Ligand sodium dithiobenzoate (mg)	20
Sodium gluconate (mg)	30
Ascorbic acid (mg)	40
Potassium oxalate (mg)	0.8
Physiological serum (mL)	0.5
Perrhenate activity (mL)	0.5
RT stirring (min)	15
Heating (°C)/ time (min)	100/30
Final yield (%)	87% ± 9.1
Radiochemical purity (%)	93% ± 3.4
Reference	(25)

autoradiographic studies showed ^{188}Re-SSS lipiodol to be located mainly in sinu-soids, like ^{131}I-lipiodol. The main difference is represented by very weak urinary elimination and more marked intestinal elimination (25).

A comparative evaluation of ^{188}Re-SSS-lipodol with ^{131}I-lipiodol in rats bearing HCC, was performed. This study shows that, in HCC-bearing rats, treatment by ^{131}I-lipiodol is more effective compared with the ^{188}Re-SSS lipiodol/^{131}I-lipiodol mixture or ^{188}Re-SSS lipiodol alone, because it is the only treatment that makes it possible to obtain a prolonged improvement of survival. This study also shows that treatment by ^{188}Re-SSS lipiodol alone is ineffective with the HCC-tumour model used here, which is built up of a single, small tumour (26).

However, this may not be extendable to HCC in humans as the tumors are fairly large. The International Atomic Energy Agency (IAEA) conducted a multi-country Phase I/II clinical trial involving 185 patients in eight countries using ^{188}Re-HTDD/Lipiodol in which three complete responses and 19 partial responses were reported (27, 28). The overall results of the clinical studies with ^{188}Re-HTDD/Lipiodol demonstrated that it is a clinically useful agent (27, 28)

However, potential liver leakage of activity has been a concern and there are more developments to improve the radiolabeling yields and the *in vivo* stability of the ^{188}Re-SSS lipiodol radiopharmaceutical.

^{188}Re–DEDC–lipiodol

Radiolabeling yields (> 95%) have been considerably improved by labeling lipiodol through rhenium(V)-Nitride-bis(diethylthiocarbamate) (DEDC) complex (29). This method involves the reaction of [^{188}Re] [ReO_4]$^-$ with N-methyl S-methyl dithiocarbazate

(DTCZ), as donor of nitrido nitrogen atoms, sodium oxalate and $SnCl_2$ to afford a mixture of two intermediate compounds. When this mixture is reacted with the sodium salt of a dithiocarbamate ligand (L) of the type $Na[R_2N\text{-}C(=S)S]$ (R = CH_3, CH_3CH_2, $CH_3CH_2CH_2$), the formation of the bis-substituted, neutral complexes [^{188}Re][Re(N)(L)2] is easily obtained in high yield (> 95%). The Re(V) nitrido precursor was prepared in high yields using a lyophilized kit formulation and the resultant complex being highly lipophilic is quantitatively extracted into lipiodol. It was found that the ^{188}Re-N-DEDC-lipiodol was highly stable in physiological solution and in rat's blood. It selectively gets accumulated in the tumor with high target to non-target ratios (30). Furthermore, results of transchelation experiments showed that these compounds were inert toward transchelation by cysteine and glutathione (29).

The results of the initial clinical trials showed that this could be a useful radiopharmaceutical for the therapy of unresectable hepatocellular carcinoma.

Automated preparation of ^{188}Re–DEDC–lipodol

An automated synthesis of ^{188}Re-lipodol was first reported by Uccelli et al. (Figure 4) (31). Automated modular preparation of ^{188}Re-DEDC-lipodol allows easy preparation of sterile and pyrogen-free samples of ^{188}Re-lipiodol ready to be administered to the patient. Important advantages include the possibility to incorporate high ^{188}Re activity into the lipiodol hydrophobic phase and a sharp reduction of radiation exposure of the operator assisting the labelling procedure. The flowchart of the automated synthesis is described in Fig. 6. Briefly, Generator-eluted [^{188}Re][ReO_4]Na was first collected from the ^{188}W/^{188}Re generator onto a vial (collecting vial), and initial activity (5.1±1.2 GBq; n=10) counted in a β-counter. This vial was, then, inserted into the automated system. Activity was transferred from the collecting vial to a tandem cation-anion concentration system by mean of a peristaltic pump (P). This system was made by three alumina cationic exchange cartridges placed in series (SCE) followed by one anionic exchange cartridge (QMA). After the [^{188}Re]perrhenate solution passed through the tandem concentration system, activity remained quantitatively trapped into the anionic column (32-34). The accumulation of [^{188}Re][ReO_4]Na onto the anionic column was monitored by a small radiation-sensitive photodiode detector (Det1). This concentration system was used only once and discarded after use. The [^{188}Re]perrhenate adsorbed onto the QMA cartridge was then subsequently eluted with 3.0 ml of saline (V2) and transferred to vial A. Before insertion of vial A into the automated system, 0.1 ml of glacial acetic acid were added under sterile conditions using an insulin-type syringe. The resulting solution was heated at 80°C for 5 min and then transferred to the conic-bottom glass reactor vial (R). The content of vial B was dissolved by transferring 1.5 ml of sterile water (V3). The resulting solution was, then, introduced into the reactor vial (R), and the mixture heated at 80°C for 20 min to afford the final complex ^{188}ReN-DEDC. After cooling, the reaction solution was passed through a Sep-Pak C18 cartridge onto which the radioactive complex was retained. Accumulation of the radioactive product on the C18 column was monitored using another small radiation-sensitive photodiode detector (Det2). The cartridge was first washed with 20 ml of water (V4) followed by 1.5 ml ethanol/water (50:50 v/v) (V5). The product was recovered by washing the cartridge with 2.0 ml of ethanol (V6), and the resulting solution passed through a sterile 0.22-μm membrane filter and collected in vial C. Residual ethanol was removed by heating vial C at 100°C, under a nitrogen stream. Lipiodol (2.0 ml) was finally added to vial C to dissolve the radioactive compound. The total time for completing

the whole procedure was 80 min. Application of this modular reaction system could be also extended to the preparation of other ^{188}Re- radiopharmaceuticals and to compound labelled with different β-emitting therapeutic radionuclides.

^{188}Re(N)(cys) (PNP)

Synthesis, characterization and biological evaluation of a ^{188}Re(N)(cys) (PNP) mixed ligand complex for the preparation of ^{188}Re-lipiodol have been reported (Figure 5) (35). Radiolabeling conditions of ^{188}Re-PPP derivatives were presented in Table 5. [^{188}Re(N)(cys ~)(PNP)]$^{+/0}$ mixed-ligand compounds were efficiently prepared in aqueous solution from perrhenate using a multistep procedure based on the preliminary formation of the labile $^{188}Re^{III}$–EDTA species, which easily undergo oxidation/ligand exchange reaction to afford the [$^{188}Re^{V}\equiv N]^{2+}$ core in the presence of dithiocarbazate. The final mixed-ligand compounds were obtained, at 100°C, by adding the two bidentate ligands to the buffered [$^{188}Re^{V}\equiv N]^{2+}$ solution (pH 3.2–3.6). However, a relatively high amount of cys~ ligand was required to obtain a quantitative radiochemical yield. The complexes were stable toward reoxidation to perrhenate and ligand exchange reactions. In vivo studies showed rapid distribution and elimination of the complexes from the body. No specific uptakes in sensitive tissues/organs were detected (35). The continued developments in the preparation and production of ^{188}Re-lipiodol show the interest in this product as it addresses the management of a widely prevalent cancer.

Table 5. Reported conditions of various ^{188}Re-PNP derivatives for optimal radiolabeling efficiencies.

Conditions	^{188}Re-1 and ^{188}Re-2		^{188}Re-3 to ^{188}Re-9	
	Method A	Method B	Method A	Method B
EDTA	5	5	5	5
Mannitol (mg)	5	5	5	-
SnCl2 (mg)	1	1	1	1
0.1M HCl (mL)	0.6	0.6	0.6	0.6
Na[$^{188}ReO_4$] (mL)	1	1	1	0.5
RT stirring (min)	10	10	10	10
DTCZ (mg)	2	2	2	0.1
RT stirring (min)	45	-	45	-
phosphate buffer(mL)	-	-	0.75	0.75
Cysteine derivative ligand (mg)	-	-	5	5
PNP3/ PNP5 (mg)	1	1	1	1
Temp/ Heating time (°C/min)	100/30	100/30	100/45	100/30
^{188}Re RCY (%)	99	98	~78 to 88	~85 to 95
Reference		(35)		

Conclusion

Rhenium-188 is produced by an ^{188}W/^{188}Re on-site generator in a convenient and inexpensive way in the majority of hospitals. Usage of Re-188 radiopharmaceuticals could also enhance therapeutic efficacy to other malignancies (e.g., nonresepctable liver cancer, nonmelanoma skin cancer, and breast cancer) as well as treatment of arthritis and inhibition of arterial restenosis. Therapeutic ratios in these cases could provide a powerful and reliable tool for the estimation of treatment benefits. Therefore, ^{188}Re has a great potential for radionuclide therapy in field of nuclear medicine and will be needed researchers great efforts.

Acknowledgments

This work was carried out by the research fund supported by Radiation Technology R&D program (NRF-2017M2A2A7A01021401) through the National Research Foundation of Korea (NRF). The authors declare no conflict of interest.

References

1. Jeong JM, Kim YJ, Lee YS, Ko JI, Son M, Lee DS, et al. Lipiodol solution of a lipophilic agent, $^{(188)}$Re-TDD, for the treatment of liver cancer. *Nucl Med Biol* 2001;28(2):197-204.
2. Lee YS, Jeong JM, Kim YJ, Chang YS, Lee HJ, Son M, et al. Development of acetylated HDD kit for preparation of ^{188}Re-HDD/lipiodol. *Appl Radiat Isot* 2007;65(1):64-69.
3. Nakakuma K, Tashiro S, Hiraoka T, Uemura K, Konno T, Miyauchi Y, et al. Studies on anticancer treatment with an oily anticancer drug injected into the ligated feeding hepatic artery for liver cancer. *Cancer* 1983;52(12):2193-2200.
4. Paeng JC, Jeong JM, Yoon CJ, Lee YS, Suh YG, Chung JW, et al. Lipiodol solution of ^{188}Re-HDD as a new therapeutic agent for transhepatic arterial embolization in liver cancer: preclinical study in a rabbit liver cancer model. *J Nucl Med* 2003;44(12):2033-2038.
5. Madsen MT, Park CH, Thakur ML. Dosimetry of Iodine-131 Ethiodol in the Treatment of Hepatoma. *J Nucl Med* 1988;29(6):1038-1044.
6. Nakajo M, Kobayashi H, Shimabukuro K, Shirono K, Sakata H, Taguchi M, et al. Biodistribution and in vivo kinetics of iodine-131 lipiodol infused via the hepatic artery of patients with hepatic cancer. *J Nucl Med* 1988;29(6):1066-1077.
7. Turner JH, Claringbold PG, Klemp PF, Cameron PJ, Martindale AA, Glancy RJ, et al. ^{166}Ho-microsphere liver radiotherapy: a preclinical SPECT dosimetry study in the pig. *Nucl Med Comm* 1994;15(7):545-553.
8. Wang SJ, Lin WY, Chen MN, Hsieh BT, Shen LH, Tsai ZT, et al. Biodistribution of rhenium-^{188} Lipiodol infused via the hepatic artery of rats with hepatic tumours. *Eur J Nucl Med* 1996;23(1):13-17.
9. Wang SJ, Lin WY, Lui WY, Chen MN, Tsai ZT, Ting G. Hepatic artery injection of Yttrium-90-lipiodol: biodistribution in rats with hepatoma. *J Nucl Med* 1996;37(2):332-335.
10. Park CH, Suh JH, Yoo HS, Lee JT, Kim DI, Lee KS. Iodine-131-labeled lipiodol retention within hepatic cavernous hemangioma. *Radiology* 1987;163(1):283-284.
11. Raoul JL, Guyader D, Bretagne JF, Heautot JF, Duvauferrier R, Bourguet P, et al. Prospective randomized trial of chemoembolization versus intra-arterial injection of ^{131}I-labeled-iodized oil in the treatment of hepatocellular carcinoma. *Hepatology* 1997;26(5):1156-1161.
12. Yoo HS, Suh JH, Lee JT, Kim DI, Park CY, Kim BS, et al. Therapeutic Feasibility Study and Clinical Trial of Intrahepatic ^{131} I-Lipiodol on Patients with Hepatocellular Carcinoma. *Korean J of Nucl Med* 1986;20(2):61-71.
13. Lee YS, Jeong JM, Kim YJ, Chung JW, Park JH, Suh YG, et al. Synthesis of ^{188}Re-labelled long chain alkyl diaminedithiol for therapy of liver cancer. *Nucl Med Comm* 2002;23(3):237-242.
14. Paeng JC, Jeong JM, Yoon CJ, Lee Y-S, Suh Y-G, Chung JW, et al. Lipiodol Solution of ^{188}Re-HDD as a New Therapeutic Agent for Transhepatic Arterial Embolization in Liver Cancer: Preclinical Study in a Rabbit Liver Cancer Model. *J Nucl Med* 2003;44(12):2033-2038.
15. Banka VK, Moon S-H, Jeong JM, Seelam SR, Lee Y-S, Kim YJ, et al. Development of 4-hexadecyl-4,

7-diaza-1, 10-decanedithiol (HDD) kit for the preparation of the liver cancer therapeutic agent Re-188-HDD/lipiodol. *Nucl Med Biol* 2015;42(3):317-322.

16. Gallicchio R, Nardelli A, Mainenti P, Nappi A, Capacchione D, Simeon V, et al. Therapeutic Strategies in HCC: Radiation Modalities. *BioMed Research International* 2016;2016:11.
17. Chua TC, Chu F, Butler SP, Quinn RJ, Glenn D, Liauw W, et al. Intra-arterial iodine-131-lipiodol for unresectable hepatocellular carcinoma. *Cancer* 2010;116(17):4069-4077.
18. Kim Y, Jeong J, Kim S, Lee D, Chung J, Lee M, et al.. Rhenium-188-sulfur colloid suspended in Lipiodol: a capillary-blocking radiopharmaceutical for targeting liver cancer. J Nucl Med 1998;39:S51
19. Kumar A, Bal C, Srivastava DN, Acharya SK, Thulkar SP, Sharma S, et al. Transarterial radionuclide therapy with Re-188-HDD-lipiodol in case of unresectable hepatocellular carcinoma with extensive portal vein thrombosis. *European J of Radiology Extra* 2005;56(2):55-59.
20. Kumar A, Bal C, Srivastava DN, Thulkar SP, Sharma S, Acharya SK, et al. Management of multiple intrahepatic recurrences after radiofrequency ablation of hepatocellular carcinoma with rhenium-188-HDD-lipiodol. *European J of gastroenterology & hepatology* 2006;18(2):219-223.
21. Kumar A, Srivastava DN, Chau TT, Long HD, Bal C, Chandra P, et al. Inoperable hepatocellular carcinoma: transarterial ^{188}Re HDD-labeled iodized oil for treatment--prospective multicenter clinical trial. *Radiology* 2007;243(2):509-519.
22. Lambert B, Bacher K, De Keukeleire K, Smeets P, Colle I, Jeong JM, et al. ^{188}Re-HDD/lipiodol for treatment of hepatocellular carcinoma: a feasibility study in patients with advanced cirrhosis. *J Nucl Med* 2005;46(8):1326-1332.
23. Lambert B, Bacher K, Defreyne L, Van Vlierberghe H, Jeong JM, Wang RF, et al. $^{(188)}$Re-HDD/lipiodol therapy for hepatocellular carcinoma: an activity escalation study. *Eur J Nucl Med Mol* Imaging 2006;33(3):344-352.
24. Luo T-Y, Hsieh B-T, Wang S-J, Lin W-Y, Lee T-W, Shen L-H, et al. Preparation and biodistribution of rhenium-188 ECD/Lipiodol in rats following hepatic arterial injection. *Nucl Med Biol* 2004;31(5):671-677.
25. Garin E, Noiret N, Malbert C, Lepareur N, Roucoux A, Caulet-Maugendre S, et al. Development and biodistribution of ^{188}Re-SSS lipiodol following injection into the hepatic artery of healthy pigs. Eur *J Nucl Med Mol Imaging* 2004;31(4):542-546.
26. Garin E, Rakotonirina H, Lejeune F, Denizot B, Roux J, Noiret N, et al. Effect of a ^{188}Re-SSS lipiodol/^{131}I-lipiodol mixture, ^{188}Re-SSS lipiodol alone or ^{131}I-lipiodol alone on the survival of rats with hepatocellular carcinoma. *Nucl Med Comm* 2006;27(4):363-369.
27. Bernal P, Raoul JL, Vidmar G, Sereegotov E, Sundram FX, Kumar A, et al. Intra-arterial rhenium-188 lipiodol in the treatment of inoperable hepatocellular carcinoma: results of an IAEA-sponsored multination study. International J of Radiation Oncology, Biology, Physics 2007;69(5):1448-1455.
28. Sundram F, Chau TC, Onkhuudai P, Bernal P, Padhy AK. Preliminary results of transarterial rhenium-188 HDD lipiodol in the treatment of inoperable primary hepatocellular carcinoma. *Eur J Nucl Med Mol Imaging* 2004;31(2):250-257.
29. Boschi A, Bolzati C, Uccelli L, Duatti A. High-yield synthesis of the terminal ^{188}Re≡N multiple bond from generator-produced $[^{188}ReO_4]^-$. *Nucl Med Biol* 2003;30(4):381-387.
30. Boschi A, Uccelli L, Duatti A, Colamussi P, Cittanti C, Filice A, et al. A kit formulation for the preparation of ^{188}Re-lipiodol: preclinical studies and preliminary therapeutic evaluation in patients

with unresectable hepatocellular carcinoma. *Nucl Med Comm* 2004;25(7):691-699.

31. Uccelli L, Pasquali M, Boschi A, Giganti M, Duatti A. Automated preparation of Re-188 lipiodol for the treatment of hepatocellular carcinoma. *Nucl Med Biol* 2011;38(2):207-213.
32. Guhlke S, Beets AL, Oetjen K, Mirzadeh S, Biersack HJ, Knapp FF, Jr. Simple new method for effective concentration of ^{188}Re solutions from alumina-based ^{188}W-^{188}Re generator. *J Nucl Med* 2000;41(7):1271-1278.
33. Jeong JM, Knapp Jr FF. Use of the Oak Ridge National Laboratory Tungsten-188/Rhenium-188 Generator for Preparation of the Rhenium-188 HDD/Lipiodol Complex for Trans-Arterial Liver Cancer Therapy. *Seminars in Nucl Med* 2008;38(2):S19-S29.
34. Sarkar SK, Venkatesh M, Ramamoorthy N. Evaluation of two methods for concentrating perrhenate (^{188}Re) eluates obtained from ^{188}W-^{188}Re generator. *Appl Radiat Isot* 2009;67(2):234-239.
35. Thieme S, Agostini S, Bergmann R, Pietzsch J, Pietzsch H-J, Carta D, et al. Synthesis, characterization and biological evaluation of [^{188}Re(N)(cys~)(PNP)]+/0 mixed-ligand complexes as prototypes for the development of ^{188}Re(N)-based target-specific radiopharmaceuticals. *Nucl Med Biol* 2011;38(3):399-415.
36. Banka VK, Moon SH, Jeong JM, Seelam SR, Lee YS, Kim YJ, et al. Development of 4-hexadecyl-4,7-diaza-1,10-decanedithiol (HDD) kit for the preparation of the liver cancer therapeutic agent Re-188-HDD/lipiodol. *Nucl Med Biol* 2015;42(3):317-322.