The current status and future of theranostic Copper-64 radiopharmaceuticals

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ABSTRACT

Copper-64 was produced in large scales and high specific activities in late 1990s' using compact cyclotrons based by $^{64}Ni(p,n)^{64}Cu$ reaction and many radiopharmaceuticals developed since then by various groups based on interesting physicochemical and nuclear properties of the radionuclide. The unique emission of beta particles as well as positron particles offers a spectacular real therapeutic/diagnostic ("*Theranostic*") radionuclide in nuclear medicine. Although the development of copper-64 radiopharmaceuticals continued with a slower rate in 2010s' due to availability of ^{68}Ga-tracers, however recent advances in application of therapeutic doses of ^{64}Cu has emerged a new trend in the radiopharmaceutical development based on coppe-64. In this review, recent advances in the copper-64 theranostic radiopharmaceuticals including introduction of new chelating groups with enhanced stability as well as radiolabelling conditions as well as application of simple $^{64}CuCl_2$ radiopharmaceutical as areal theranostic agent in human subjects are summarized. A proposed strategy for development of peptide based copper-64 radiopharmaceuticals with high and low dose therapeutic applications has been suggested.

Key words: Copper-64; Theranostic; Radiopharmaceutical; Chelates; PET; Therapy

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INTRODUCTION

Copper-64 is a unique radionuclide with interesting properties for imaging and possible therapy [1], decaying via electron capture, beta-emission and positron emission routes. The 18% positron emission has a low energy leading to production of high-resolution images while no abundant gamma emissions are available not impairing the imaging process compared to other positron emitters. Dual existence of positron and beta-emission (39%) imposes high local radiation dose at the micro-environmental levels in cells theoretically suitable for targeted radionuclide therapy. On the other hand the electron capture decay (43%) is followed by the emission of high LET auger electrons increasing the cytotoxic potency, in case the radioisotope is targeted near or within cell nucleus.

According to these properties, copper-64 is described as the archetypal "theranostic" radioisotope, providing excellent PET imaging properties at low doses with least dosimetry or radiobiological concerns, while demonstrating potentials for radionuclide therapy at higher doses (Figure 1).
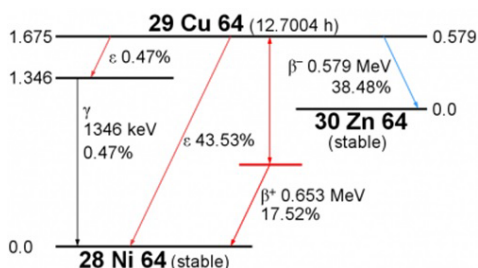

Fig 1. Detailed decay scheme for copper-64 radionuclide [2].

Its rather medium half-life as a PET radionuclide (12.8 h) makes it versatile for both tracers with rapid pharmacokinetics such as small molecules and peptides, as well as slow pharmacokinetic agents such as monoclonal antibodies (mAbs), and stem cell tracking. The medium half-life makes it useful as a surrogate for development of new copper tracers with longer or shorter half-lives (Cu-60, Cu-61, Cu-62 for PET and Cu-67 for therapy).

Copper chemistry is advantageous, although being a less inert than other transition metals, using well-designed macrocyclic chelators forming stable complexes attached to targeting molecules (mAbs, peptides, antibody fragments etc.). During 2-3 decades of design and optimisation of copper-specific chelators, a wide selection of useful bifunctional chelators has been selected for this purpose with approved and straightforward radiolabelling process.

On the other hand, the reduction of Cu(II) to Cu(I) in the biological microenvironments is used as a basis

for molecular imaging while chelated to small molecule such as thiosemicarbazones leading to their application as blood flow and/or hypoxia imaging agents.

Lastly, as a naturally occurring essential element, copper and its pharmacokinetics are tightly controlled in mammal biological systems and its concentration fluctuations leads to abnormalities such as cancer, inflammation, dementia, copper metabolic related diseases and nutritional abnormalities. This suggests unique potentials for application of copper-64 in diagnosis and/or therapy that has not yet been fully recognised and studied.

In this review a rapid overview of all applications of copper-64 tracers and radiopharmaceuticals has been presented.

PRODUCTION

The production of Cu-64 using a low/medium energy cyclotron is relatively straightforward. Most centres use proton bombardment of isotopically enriched Ni-64 at a beam energy of 11−15 MeV (Figure 2). Other routes are feasible, though with additional technical difficulties that limit product purity. The solid targetry requires careful design for adaptation to different cyclotrons but the basic elements of a gold backing electroplated with Ni-64 are widely established and adopted [3]. Thus, in centres with a medical cyclotron on the premises or within a few hours transport time, Cu-64 can be routinely available. Alternative routes of production such as $^{68}Zn(p,\alpha)^{64}Cu$ in cyclotrons is also reported [4] and used in the production of small molecules [5, 6] and mAbs [7, 8], however due to the presence of long half-life radionuclides the shelf life is limited to 24 h post production. Other methods usually lead to low specific activities or carrier added products.
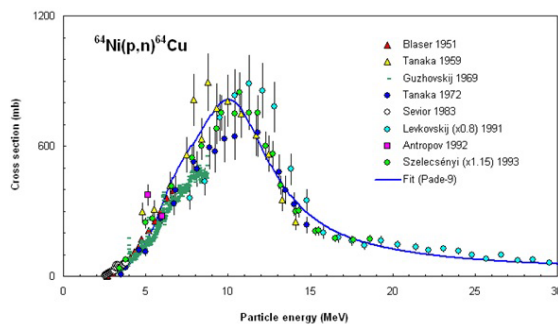

Fig 2. Recommended cross sections for $^{64}Ni(p,n)^{64}Cu$ reaction [9].

LIMITATIONS AND AVAILABILITY

Despite the relatively simple and straightforward production, the availability of Cu-64 remains limited. This caused application of other production routes in many countries as described earlier. The principal

production route relies on a supply of Ni-64, which has low natural abundance and, thus, is extremely costly to purchase in enriched form. Production is also intensive in cyclotron beam time, requiring several hours at beam currents available on most biomedical cyclotrons to produce a batch of Cu-64 sufficient for several human PET imaging studies or a single radionuclide therapy dose. The requirement for solid targetry makes it costly to adapt to wide range of commercial cyclotron designs, and makes processing either labour- (and radiation dose-) intensive, or costly in terms of automated equipment. Consequently, Cu-64 will remain a high-cost radionuclide, and it is unlikely to be available daily in individual centres. Typically, it is produced once a week or once every two weeks.

DEVELOPMENTS OF COPPER-64 RADIOPHARMACEUTICALS

Site-directed targeting vectors or biologically-active conjugates such as peptides, small proteins, antibody fragments, or intact mAbs in radiolabelled form, continue to hold promise for early diagnosis or treatment of human diseases. These molecules consist of: 1) a targeting moiety, 2) a linker that also is a pharmacokinetic modifier (often an aliphatic or amino acid linker/tethering moiety), 3) a chelating agent, and 4) a radionuclide usually in metal form (Figure 3).
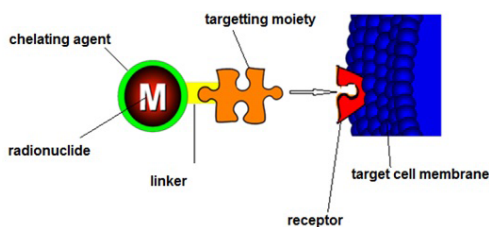
Fig 3. Components of a designed targeting radiopharmaceutical.

Bifunctional chelating agents: During last 3 decades, much effort has been devoted to develop chelators for linking copper to targeting moieties, to overcome kinetic instability that results in loss of the radiolabel *in-vivo*. Macrocycles appear to be essential for Cu-64 chelation, and there are no acyclic chelators that perform adequately. Early success with cyclam and TETA (12-membered tetra(aminocarboxyalate)) was able to demonstrate the use of Cu-64 and Cu-67 [1], but evidence of *in-vivo* dissociation led to further development culminating in a variety of designs, including cross-bridged analogues of 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (TETA), which show greater kinetic stability against dissociation but require harsh radiolabelling condition [10]. The ligand 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) (popular because it is versatile and useful for many radio metals – yttrium, lutetium, gallium and indium) was widely used for a while but the harsh conditions required for radiolabelling combined with doubts regarding *in-vivo* stability led to waning of its popularity. 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) derivatives (*e.g.* NODAGA) containing a 9-membered ring and the hexaamino sarcophagine ligands now appear to be the leading chelators for copper, combining labelling under mild conditions with *in-vivo* stability [11] (Table 1, Figure 4).
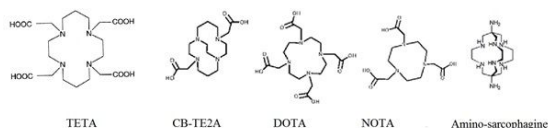
Fig 4. Most applied complexing agents to produce copper-64 biomolecular conjugates.

Targeting moieties: Among targeting moieties known to now, mAbs, fragments, nanobodies, peptides and recently decorated nanoparticles have been used as molecule-directed vectors against antigens, receptors, channel proteins *etc*. Radiolabelled mAbs have demonstrated the potential to be used as site-directed compounds for development of new and successful diagnostic and therapeutic radiopharmaceuticals for human cancers [7].

Table 1: The properties of various chelating cores used in development of coppe-64 radiopharmaceuticals

Chelate	Advantage	Disadvantage	Ref
CB-TE2A	High in vivo stability, easy conjugation, High radiolabeling yields	Tedious synthesis	[12]
DOTA	Available, easy conjugation, High radiolabeling yields	Demetallation (in vivo conditions)	[8]
NOTA	Ease of synthesis, ease of conjugation, High radiolabeling yields, sufficient in vivo stability,	Some degree of hepatic accumulation	[13]
Sarcophagine	Ease of conjugation, High radiolabeling yields, sufficient in vivo stability	Tedious synthesis	[14]

Very slow clearance from blood serum/non-target tissue and the less-than-desirable tumour uptake are the major disadvantages of radiolabelled mAbs. In order to overcome this problem, fragment antibodies (such as F(ab)2, Fab', Fsc, *etc.*) were developed with more success. With the help of molecular engineering the sequence of antigen-antibody binding sites has been developed and known as nanobodies.

On the other hand, peptides with rapid clearance from whole blood, ease of penetration into the tumour vascular endothelium, more rapid excretion from the body, and relatively low immunogenicity offer distinct advantages over mAbs and are more economically prepared compared to fragments and nanobodies. They can readily be designed, synthetized, characterized and also modified by the sequence modifications. High-affinity receptors selectively over-expressed on a variety of neoplastic cells have been identified for a number of small peptides making them ideal candidates as new diagnostic and/or therapeutic targeting vectors. Much of the development work and clinical translation of these has been done with Ga-68 [15] but Cu-64 offers an alternative with potential advantages in some applications. Many peptide families such as somatostatins, bombesin, cholecystokinin/gastrin, glucagon-like peptide-1 (GLP-1)/exendin, arginine-glycine-aspartic acid (RGD) *etc.* have been explored during the last few years and quite a number of potential radiolabelled probes have been derived from them [16].

AVAILABLE COPPER-64 RADIOPHARMACEUTICALS

Copper-64 thiosemicarbazones for blood flow and/or hypoxia imaging

Bioreductive *bis*(thiosemicarbazone) copper complexes demonstrate intracellular trapping due to the redox properties of copper and is intrinsic to the targeting mechanism of several Cu-64 radiopharmaceuticals. The well-established tracers such as Cu-64 PTSM (blood flow) [17] and Cu-64 ATSM [5, 18] (for hypoxia) have been studied in vitro and in vivo. The putative trapping mechanism is that the planar, lipophilic complexes diffuse readily into cells, whereupon they are exposed to intracellular reducing agents and are reduced to Cu(I) leading to dissociation [19].

The rate of reduction, reoxidation by molecular oxygen, and dissociation can be controlled by modifying the redox potential *via* altering the alkyl groups of the *bis*(thiosemicarbazone) ligand, leading to three main classes. Complexes with two alkyl groups at the diimine backbone have low redox potential and are hard to reduce, dissociate slowly and can therefore readily be reoxidised by O₂ back to the Cu(II) form allowing them to escape from cells

[20]. This endows them with hypoxia targeting properties that have been evaluated in several clinical trials with Cu-64 ATSM. Although Cu-PTSM became accepted clinically as a blood flow tracer because of its lack of selectivity in cellular uptake, many subsequent studies indicate that in fact it does have a slightly increased trapping in hypoxic cells.

Complexes with only hydrogen at the diimine backbone are very rapidly reduced and subsequent dissociation is fast, leading to non-selective trapping in all cells and tissues. Cu-GTSM is a member of this class and has been used for non-selective delivery of Cu-64 to tissues but has not been used in humans [21]. Many other related ligands have been used for in-vitro and preclinical in-vivo studies (Figure 5).
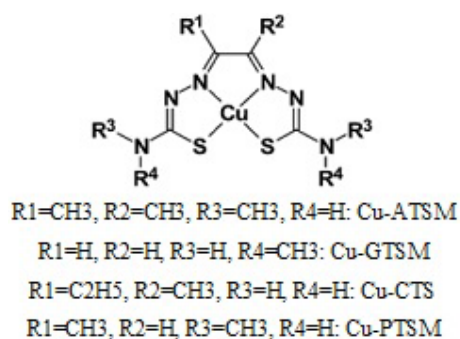

R1=CH3, R2=CH3, R3=CH3, R4=H: Cu-ATSM
R1=H, R2=H, R3=H, R4=CH3: Cu-GTSM
R1=C2H5, R2=CH3, R3=H, R4=H: Cu-CTS
R1=CH3, R2=H, R3=CH3, R4=H: Cu-PTSM

Fig 5. The chemical structures of various copper-bis-thiosemicarbazones developed for blood flow and/or hypoxia imaging. Cu-ATSM; H₂ATSM= diacetyl bis-(methyl-thiosemicarbazone), Cu-GTSM; H₂GTSM= glyoxal bis-(methyl-thiosemicarbazone) Cu-CTS; H₂CTS= 2,3-pentanedione bis-(thiosemicarbazone) Cu-PTSM; H2PTSM= pyruvaldehyde bis-(methy-lthiosemicarbazone).

Although Cu-64 ATSM has shown promise in clinical trials and can be predictive of failure of radiotherapy in some cancers (see below), attempts to validate its hypoxia selectivity in animal tumour models and in humans have been inconclusive and validation remains extremely challenging. Some preclinical and clinical studies support the hypothesis that Cu-64 ATSM is a hypoxia selective tracer, while others suggest that uptake reflects perfusion [22].

New analogues [19, 23] with reduced lipophilicity are now being evaluated preclinicaly to overcome this problem. Suggestions that hypoxia selectivity is specific to tumour types (this is plausible, as it is also for F-18 nitroimidazole based hypoxia imaging agents), and that images may reflect the behaviour of released copper rather than Cu-ATSM (this is implausible at early imaging times, but certainly gives rise to complications in interpreting later images) [24] contribute to a high degree of uncertainty about the clinical value of hypoxia imaging with Cu-64 ATSM at the present time.

Clinical studies of hypoxia imaging with Cu-ATSM (labelled with various Cu radioisotopes) have been reviewed recently [22, 25]. There has been a particular focus on non-small cell lung cancer (NSCLC). Takahashi et al. compared Cu-62-ATSM (20 minutes post-injection) with F-18-FDG in ten NSCLC patients, and with O-15-H_2 PET in four patients with non-small cell lung cancer [26]. Intense uptake of Cu-62-ATSM was found in lung tumours; this uptake did not correlate with F-18-FDG. A negative correlation was found between blood flow and Cu-62-ATSM uptake in three of four patients, suggesting that Cu-62-ATSM is retained in poorly perfused (and hence potentially hypoxic) areas. Lohith et al. also investigated differences in Cu-62-ATSM (20 minutes p.i.) and F-18-FDG uptakes in lung cancer of different lung cancer sub-types (eight squamous cell carcinoma (SCC) and five adenocarcinoma) [27]. In SCC, high Cu-62-ATSM and low F-18-FDG uptake was observed at the tumour periphery with the opposite at the tumour centre. In contrast, adenocarcinomas had similar spatial distribution of both Cu-62-ATSM and F-18-FDG uptake. It was suggested that F-18-FDG uptake is increased in pre-necrotic cells at the tumour centre and Cu-64-ATSM uptake is increased at the periphery where there are active hypoxic cells.

^{64}Cu-DOTATATE

Due to favourable pharmacokinetics, stability and easy handling of peptide radiopharmaceuticals, some Cu-64 peptide radiopharmaceuticals have been applied in imaging human diseases. The first Cu-64 clinically evaluated peptide radiopharmaceutical was the well-known somatostatin ligand DOTATATE for neuroendocrine tumours. ^{64}Cu-DOTATATE is also used in some on-going clinical trials. The image quality was superior to In-111 analogue (Figure 6).
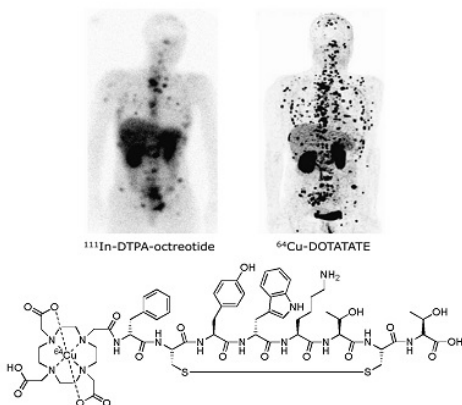

Fig. 6. Comparison of images of a neuroendocrine patient using the somatostatin ligand DOTATATE labelled with In-111 and Cu-64 (above) and structure of ^{64}Cu-DOTATATE (below) [28].

Until recently, clinical evaluation in human patients has been limited to SSTR-targeting agonist ligands, which are internalized upon binding to the receptor. Antagonist ligands, on the other hand, are not internalized but recent studies with SSTR-targeting antagonist ligands have indicated that they may have advantages notwithstanding.

Gastrin releasing peptide receptor antagonist

Another interesting Cu-64 peptide tracer, based on bombesin, was developed following early work on the Tc-99m tracer for imaging of prostate cancer. In 3 of 4 patients, prostate tumours were identified by PET imaging. Of note was clear differentiation of bladder and prostate, demonstrating the potential diagnostic use of the GRPR-targeting agent. Other tissues with radioactivity above background were liver, intestine, bladder, pancreas and kidneys (Figure 7) [29].
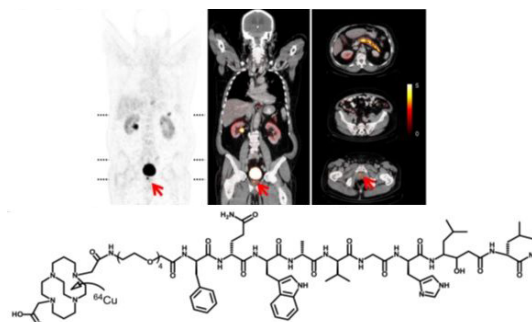

Fig. 7. Comparison of PET and PET/CT images of a patient with prostate cancer (above) using a Cu-64 GRPR-targeting peptide (below) [29].

^{64}Cu-Anti-colorectal carcinoma monoclonal antibody (^{64}Cu-MAb 1A3)

An anti-colorectal carcinoma mAbs (MAb 1A3) labelled with ^{64}Cu, by use of bromo-acetamidobenzyl-TETA as bifunctional agent was evaluated in patients with suspected advanced primary or metastatic colorectal cancer while studied with [^{18}F]FDG as standard. The Phase I/II results suggested that immunoPET using ^{64}Cu-MAb 1A3 has important applications in clinical oncology, particularly for detecting smaller colorectal tumour foci in the abdomen or pelvis and for determining accurate dosimetry (Figure 8) [30].

^{64}Cu-DOTA-AE105

Urokinase-type plasminogen activator receptor (uPAR) is expressed in many types of human cancers and the expression is predictive of invasion, metastasis and indicates poor prognosis. uPAR PET imaging therefore holds promise to be a new and innovative method for improved cancer diagnosis,

staging and individual risk stratification. A DOTA based uPAR ^{64}Cu-DOTA-AE105 complex has been developed and used in human clinical trial recently (Figure 9). However considering the unstable copper-DOTA complex and release of copper cation from the ligand at all post injection accumulation of activity mainly in the liver and bowel was obvious [31].
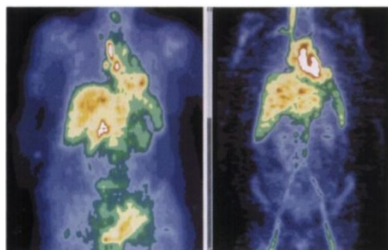

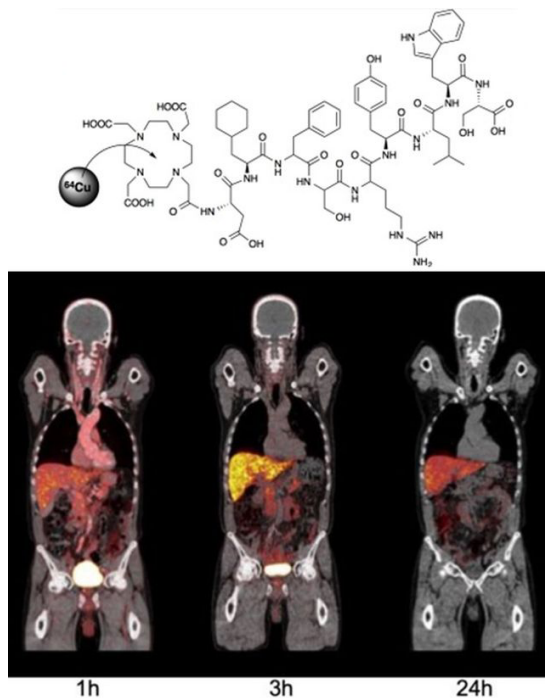**Fig 8.** PET images of a colorectal carcinoma patient using ^{64}Cu-MAb 1A3 monoclonal antibody [30].

Fig 9. Chemical structure (above) and PET/CT coronal whole-body images of ^{64}Cu-DOTA-AE105 at 1, 3 and 24 hours post injection (below) [32].

^{64}Cu-dichloride

The elevated Cu concentration in cancer cells may potentially be used to differentiate healthy from transformed cells. Thus, Cu-64 dichloride has been applied to perform diagnostic PET/CT e.g. in staging of prostate cancer and detection of recurrent disease. High hepatic concentration of the

radiopharmaceutical is an issue for use of Cu-64 dichloride PET/CT in clinical routine, but interesting results have been obtained in a recent application in Hodgkin lymphoma. In this case report two patients affected by therapy-refractory progressive Hodgkin lymphoma after radiochemotherapy and allogenic transplantation underwent to a diagnostic Cu-64 dichloride PET/CT within two weeks after a FDG PET/CT scan. All the avid FDG lesions showed high concentration of Cu-64 in both patients. FDG PET/CT showed in one of them a hypermetabolic lesion in the bone, which was not visualized with Cu-64 dichloride. However, an MRI scan showed a benign pathology in this area. These data suggest the feasibility of Cu-64 dichloride PET/CT scanning in patients with progressive Hodgkin lymphoma and its potential for tailored radionuclide therapy [32]. In a recent study [33] a high uptake of ^{64}CuCl$_2$ in prostate cancer and involved regional lymph nodes was observed (Figure 10).

Some metallic nutrients like iron, zinc, Cu and calcium seem to play a role in the pathologic processes of the human body, presumably due to their role as co-factors in enzymes [34]. In particular, copper is required for cell proliferation, angiogenesis and other cellular functions, since it is an essential component of numerous enzymes. Paradoxically, excess of Cu is cytotoxic, therefore copper homeostasis is tightly regulated by a delicate network of influx Cu transporter (hCtr1), efflux Cu transporters (ATP7A and ATP7B), Cu chaperones (ATOX1, Cox17, CCS) and other Cu binding molecules.
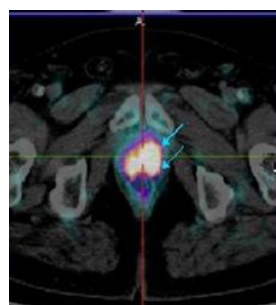
Fig 10. PET/CT images of ^{64}CuCl$_2$ in prostate cancer and involved regional lymph nodes [33].

Copper-64 dichloride can be used to study copper metabolism in conditions with Cu deficit or excess and as a tracer to identify tumour localization [35]. In contrast to the biodistribution of Cu-64-DOTATATE, Cu-64 dichloride does not show ureteral and bladder uptake, either in early or late images [34], which indicates the diagnostic advantage of this tracer for diagnosis of recurrent prostate cancer and may be beneficial for therapeutic purposes.

Table 2: Copper-64 radiopharmaceuticals in clinical trials [36].

| No.	Title	Status
1	Evaluation of ^{64}Cu-DOTA-U3-1287 in subjects with advanced solid tumors	Terminated
2	Imaging CXCR4 expression in subjects with cancer using ^{64}Cu-Plerixafor	Recruiting
3	Evaluation of a new radiotracer (64Cu-DOTA-AE105) for diagnosing aggressive cancer with positron emission tomography	Completed
4	^{64}Cu-ATSM and hypoxia in stage IV non-small cell lung cancer with carboplatin, paclitaxel, and bevacizumab	Withdrawn
5	^{64}Cu-DOTA-Trastuzumab PET/CT in studying patients with gastric cancer	Recruiting
6	Assessing response to treatment in non-Hodgkin's Lymphoma patients using ^{64}Cu-DOTA-Rituximab PET/CT	Suspended
7	Positron emission tomography in women with advanced HER2-positive breast cancer	Completed
8	^{64}Cu-DOTA-Trastuzumab PET in predicting response to treatment with Ado-Trastuzumab emtansine in patients with metastatic HER2 positive breast cancer	Recruiting
9	PET imaging With ^{64}Cu-labeled Trastuzumab in HER2+ metastatic breast cancer	Completed
10	^{64}Cu-ATSM and pet/ct scan in predicting disease progression in patients with newly-diagnosed stage IB, stage II, stage III, or stage IVA cervical cancer who are undergoing chemoradiotherapy per NCCN guidelines	Active, not recruiting
11	^{64}Cu-Anti-CEA mAbs M5A PET in diagnosing patients with CEA positive cancer	Recruiting
12	Image-derived Prediction of response to chemo-radiation in glioblastoma (^{64}Cu-ATSM)	Recruiting

Dosimetry of the labelled radiopharmaceutical depends on the pharmacokinetics of the labelled carrier. European Medicine Agency (EMA) has published dosimetry data on Cu-64 dichloride, which were reproduced by Capasso et *al.* According to these data the liver is the organ with the highest normalized cumulative activity (6.18 MBq h/MBq) and absorbed dose (2.94×10^{-1} mSv/MBq) [34], thus suggesting that it will be necessary to look for possible hepatic side effects and perhaps make provision to reduce the inappropriate irradiation of liver. Further studies are required to evaluate the critical organ dosimetry.

Table 2 shows the latest status of various clinical trials on copper-64 radiopharmaceuticals around the world. All of the clinical studies were diagnostic PET studies. No clinical trials of therapeutic use of Cu-64 have been published, and there are no therapeutic trials currently in progress. This shows the lack of any experience on the application of copper-64 radiopharmaceuticals as real theranostic agents.

POTENTIAL FUTURE RADIOPHARMACEUTICALS

[64]Cu-uPAR PET (^{64}Cu-NOTA-AE105)

As observed in last section, due to unsatisfactory human results for ^{64}Cu-DOTA-AE105 and availability of more stable copper complexes such as NOTA (Figure 11) and sarcophagine, **r**ecently, a Cu-64 labelled peptide based on uPAR biomarker has been developed and administered to humans. ^{64}Cu-NOTA-AE105 successfully showed promising results in glioblastoma, and further clinical trials are underway [37].
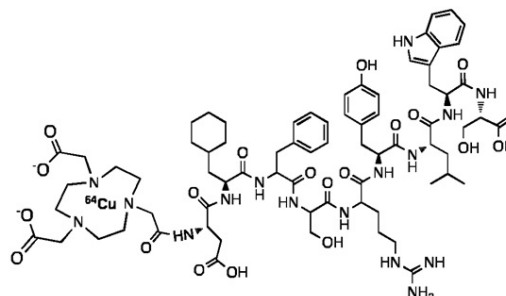
Fig 11. Chemical structure of ^{64}Cu-NOTA-AE105.

[64]Cu-GTSM for cell tracking

The medium half-life of Cu-64 makes it suitable in principle for tracking cell migration (e.g. imaging inflammation with autologous leukocytes, cell-based therapies). Because of its easier reduction and lack of selectivity for hypoxic cells, Cu-64 GTSM is expected to be more suitable than Cu-64 PTSM, and *in-vitro* experiments confirm this: very high labelling efficiencies are achieved very rapidly (i.e. superior to Cu-64 PTSM). A similar bioreductive trapping mechanism was assumed in evaluating lipophilic Cu-64-dithiocarbamate complexes, with similar results. Thus it is easy using this approach to achieve rapid and high uptake of Cu-64 in any cell type [38].

[64]Cu-dichloride applications in metabolic diseases

Copper-64 presents an obvious opportunity to use PET to study the *in-vivo* trafficking of copper, and how it is affected by disease. This approach should yield insight into the biological handling of this essential element and may also yield useful

diagnostic applications of Cu-64 imaging. This potential has hardly been explored to date, but recently a few studies have emerged that identify changes in copper uptake and distribution, mainly assumed to be related to the activity of the Cu transporters Ctr-1, in some animal models of tumours [39], Alzheimer's disease [40-42], Niemann-Pick disease [43] Menkes disease [44] and clinical studies in Wilson's disease. Much of this has involved injecting Cu-64 chloride or acetate intravenously, but this approach does not mimic the normal biological route of copper intake, which is oral. To fully understand biological copper trafficking, it will not be adequate only to inject ionic Cu-64 intravenously. It will be necessary to evaluate alternative routes of administration (especially oral), and to administer copper in different biological forms such as bound to albumin, amino acids, ceruloplasmin etc.

^{64}Cu-PSMA for prostate cancer detection

With respect to recent development and initial interesting PET/CT imaging of prostate lesions using ^{68}Ga-PSMA ligands, and existing preclinical and clinical experiences [45, 46], the development of a possible ^{64}Cu-PSMA ligand based on appropriate stable chelates such as NOTA and/or sarcophagine seems feasible for upcoming years. Regarding the copper-64 theranostic applications doses up to 80-100 mCi of the complex can lead to therapeutic effects.

THERAPY WITH COPPER-64 IN HUMANS

Although there are several preclinical studies (in vitro and animal models) on therapeutic effects of copper-64 radiopharmaceuticals [47, 48], however very few data in humans are available. Preliminary results with therapeutic application of Cu-64 dichloride in selected patients [32] demonstrated the therapeutic potential of this radionuclide in patients with prostate cancer and recurrent therapy-resistant glioblastoma. The authors described significant reduction in tPSA in a patient with prostate cancer and clinical improvement in a patient with glioblastoma after administration of Cu-64 dichloride. Improvement in quality of life of the treated patients was achieved. These preliminary results indicate the potential of Cu-64 dichloride for use in cancer patients with progressive disease. A case report in 2014 showed significant reduction of lesions size in a patient with prostate and uterine cancer patient following single cycle of treatment with 3700 MBq of ^{64}CuCl$_2$ [49].

FUTURE RESEARCH AREAS ON COPPER-64

A high incidence of receptor expression in either early- or late-stage/metastatic cancers creates potential for development of new and innovative monovalent and bivalent Cu-64 radio-ligands to tailor receptor-specific uptake, optimize localization in cancerous tissues, and minimize uptake in normal tissues to produce high-quality, high-contrast PET images for early diagnosis and staging of human cancers. In addition, these agents offer the added opportunity to be used for targeted therapy due to ideal three decay modes particle emissions. The application of selected targeting vectors can be peptides (GRPR targeting, integrin targeting, somatostatin receptor targeting, etc.) and small molecules (e.g. PSMA inhibitors) rather than nanoparticles and immunoconjugates. The choice of complexing agents to be used to stabilize the metal centre based on report from numerous research groups around the world could be NOTA- or sarcophagine-based complexing agents and should be further evaluated to conjugate the targeting vectors in order to complex and stabilize the Cu-64 metal centre. The development of new complexing agents for Cu-64 is not encouraged. In addition, the results are based upon earlier promising preclinical/clinical work on Cu-64 peptide radionuclide therapy. Given the uncertainties and problems with Cu-64-ATSM, and the imminent biological evaluation of new analogues, new clinical trials with Cu-ATSM, are not promising at this time. Based on the interesting preliminary data on the application of Cu-64 dichloride radiopharmaceutical in human cancers for diagnosis and therapy, it is proposed that research must continue thorough preclinical investigations in various genetically modified mice, tumour models (prostate cancer, glioblastoma, lymphoma, breast cancer and melanoma) and human cell lines to better understand, and exploit diagnostic and therapeutic applications, and endogenous Cu transport mechanisms. Non-oncological applications of the Cu-64 dichloride radiopharmaceutical could include, inter alia, Alzheimer's disease and other dementias, atherosclerosis and inherited genetic diseases affecting Cu metabolism.

In addition, because significant uptake of Cu-64 in the liver would implicate a challenge for clinical therapeutic use, further work is needed to look for possible hepatic side effect and provisions to reduce hepatic accumulation for example by using chelating agents or competing metals to administer before/after the injection of the Cu-64 dichloride. Finally, applying dosimetry calculations for Cu-64 radiopharmaceuticals with emphasis on Cu-64 chloride is highly mandated.

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