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REVIEW ARTICLE

Monoclonal Antibodies and Immuno-PET Imaging: An Overview

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SUMMARY

Radiopharmaceuticals are radioactive medicines used for imaging and/or therapeutic purposes, consisting of radionuclidic and pharmaceutical parts. While Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT) methods are commonly used for imaging purposes, the immuno-PET imaging method has gained popularity recently. Immuno-PET imaging method is a combination of PET radionuclides and biomolecules, especially monoclonal antibodies (mAb), proteins, peptides, which are frequently used for the imaging of different types of cancer. Radionuclides with long half-lives are generally used in immuno-PET imaging. Long biological half-lives of mAbs are the most important reason to be preferred for immuno-PET imaging. Today, Zirconium-89 (Zr-89), Iodine-124 (I-124) with long half-lives and Copper-64 (Cu-64) and Yttrium-86 (Y-86) radionuclides with relatively long half-lives are preferred in immuno-PET imaging. In this article, preclinical and clinical studies of Zr-89, Cu-64, I-124 and Y-86-labeled mAbs with a long half-lives were reviewed. Also, these 4 radionuclides, which are frequently used in the labelling of biomolecules (particularly mAbs) are compared.

Key Words: *Immuno-PET, zirconium-89, copper-64, yttrium-86, iodine-124, monoclonal antibodies, imaging.*

Monoklonal Antikorlar ve Immuno-PET Görüntülenmesi: Genel Bakış

ÖZ

Radyofarmasötikler, radyonüklidik ve farmasötik kısımlardan oluşan görüntüleme ve/veya tedavi amaçlı kullanılan radyoaktif ilaçlardır. Görüntüleme amaçlı pozitron emisyon tomografisi (PET) ve tek foton emisyon tomografisi (SPECT) yöntemleri kullanılırken, son zamanlarda immuno-PET görüntüleme yöntemi de popülerlik kazanmıştır. PET radyonüklidleri ve biyomoleküllerin, özellikle monoklonal antikor (mAb), protein, peptit kombinasyonları olan immuno-PET görüntüleme ajanları ve yöntemi özellikle farklı kanser türlerinin görüntülenmesinde sıklıkla kullanılmaktadır. İmmuno-PET görüntülenmesinde sıklıkla uzun yarılanma ömrüne sahip radyonüklidlerden yararlanılmaktadır. Bunun en önemli nedeni ise uzun biyolojik yarılanma ömrüne sahip mAb'ların immuno-PET görüntülenmesinde sıklıkla tercih edilmeleridir. Günümüzde uzun yarılanma ömrüne sahip Zirkonyum-89 (Zr-89), Iyot-124 (I-124) ve nispeten uzun yarılanma ömrüne sahip Bakır-64 (Cu-64) ile İtriyum-86 (Y-86) radyonüklitleri tercih edilmektedir. Bu çalışmada, kullanımı ve popüleritesi giderek artan mAb'ların immuno-PET görüntülenmesinde kullanımları ve uzun yarılanma ömrüne sahip klinik ve preklinik çalışmaları süren Zr-89, Cu-64, I-124 ve Y-86 ile işaretli mAb'ların çalışmaları derlenmiştir, özellikle mAb'ların, işaretlenmesinde sıklıkla kullanılan bu 4 radyonüklidin karşılaştırılmasına yer verilmiştir.

Anahtar Kelimeler: *İmmuno-PET, zirkonyum-89, bakır-64, itriyum-86, iyot-124, monoklonal antikor, görüntüleme*

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INTRODUCTION

Monoclonal antibodies (mAb) have been used for imaging and therapy for more than three decades. Hybridoma technology, discovered by Köhler and Milstein, is a milestone for mAb production and broke fresh ground for using mAbs (Wong et al., 2011). The use of mAbs and mAb-linked products have gradually increased since the first mAb was approved in 1986. Besides their use for therapeutic purposes, they can also be benefited from targeting due to their specificity for the target (Ecker, Jones, & Levine, 2015).

Radiolabelled mAbs have been used for diagnosis and/or therapy for many years. Immuno-imaging was done in the '90s with immuno-Single Photon Emission Computed Tomography (SPECT) (Sarcan & Özer, 2021). SPECT requires radiopharmaceutical radiolabelled with gamma-emitting radionuclides with an ideal gamma energy of 100-250 keV (Wadas, Wong, Weisman, & Anderson, 2010) .

Positron Emission Tomography (PET) is based on positron travels after emission and being annihilated with an electron. After the annihilation of the positron and electron, 2 gamma rays with 511 keV energy are launched. PET imaging methods show better sensitivity, specificity, and higher resolution than SPECT imaging methods (Kaur et al., 2012; Sarcan, Silindir-Gunay, Ozer, & Hartman, 2021). F-18, N-13, O-15, and C-11 are conventional PET radionuclides with short half-lives. In addition to these conventional radionuclides, new PET radionuclides with long half-lives show increasing popularity, such as Zr-89, Cu-64, Y-86, and I-124 in preclinical and clinical stages (Holland, Williamson, & Lewis, 2010; Wadas et al., 2010). These long-lived radionuclides are used in immuno-PET because their half-lives are similar to biomolecules such as mAbs, proteins, etc. (Reddy & Robinson, 2010). Biomolecules can circulate in the body for a long time, and when they are labelled with long half-life radionuclides, monitoring of mAbs can be possible (van Dongen, Visser, Lub-de Hooge, de Vries, & Perk, 2007).

Immuno-PET is based on the "magic bullet" concept, and the idea explains the utilization of radionuclides to biomolecules. Beyond the diagnosis purposes of immuno-PET with Zr-89, Cu-64, Y-86, and I-124 radionuclides, it can also be used for tracking mAbs behavior and monitoring therapy (Sarcan et al., 2021; Van Dongen et al., 2015).

Many papers have been published about preclinical and clinical studies of immuno-PET for different aims. In this article, radiolabelled mAbs for immuno-PET are reviewed and discussed comparatively for the advantages and disadvantages of Zr-89, Cu-64, Y-86, and I-124 radionuclides for mAb labelling.

MONOCLONAL ANTIBODIES

MAb production studies started after Köhler and Milstein discovered hybridoma technology in 1975. Briefly, hybridomas are formed by combining B cells of mice immunized with antigen and myeloma cancer cells (Figure 1) (Barbaros & Dikmen, 2015; Templar Smith, 2012). mAbs have an average molecular weight of 150 kDa, containing 2 heavy and 2 light chains, and these chains stay together with disulfide bonds (Figure 2) (Breedveld, 2000a). Murine, chimeric, humanized, and fully human mAbs have been produced with developing Technologies (Breedveld, 2000a). Murine mAbs faced several problems in clinical use because of their immunogenicity of murine mAbs (van Dongen & Vosjan, 2010).

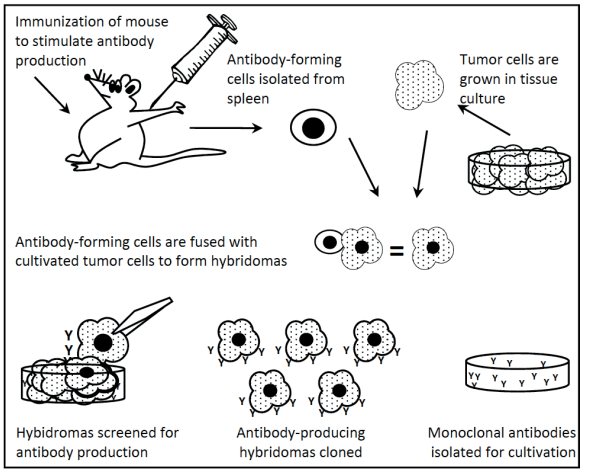

Figure 1. Monoclonal antibody production with hybridoma technology (Templar Smith, 2012).

MAbs consists of 4 regions, i.e., 2 heavy and 2 light chains of Fab and Fc fragments (Figure 2). Fab fragment is the antigen-binding site, while the Fc fragment defines the antibody functions (Breedveld, 2000b). Intact antibodies show slow clearance, high tumor uptake, and background signal while showing nonspecific accumulation (Kobayashi, Choyke, & Ogawa, 2016). Unlike intact antibodies, antibody fragments show rapid renal clearance and high tumor penetration (Kenanova & Wu, 2006).

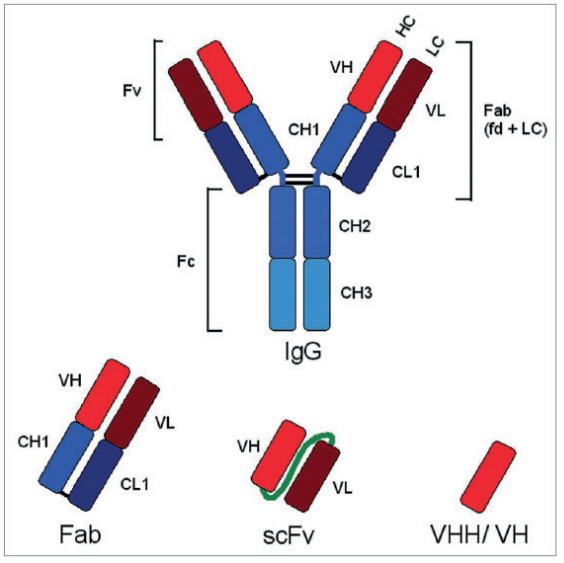

Figure 2. mAb fragments (Nelson, 2010).

MAbs have been used for therapy, diagnosis, etc., with the contribution of hybridoma technology, although the clinical use of mAbs took several decades after the discovery of hybridoma technology by Köhler and Milstein (Breedveld, 2000b; Templar Smith, 2012).

Many mAbs, mAb-drug conjugates, radionuclide labelled mAbs, etc., have been approved by Food and Drug Administration (FDA). Also, many of them are used in clinics as much as in clinical trials (Hollinger & Hudson, 2005). FDA first approved Muromonab-CD3 with the generic name Orthoclone OKT3 in 1986 for kidney transplant rejection (Lu et al., 2020). After that, the approved mAb for cancer treatment was Rituximab with Rituxan generic name in 1997 for B-cell Non-Hodgkin's Lymphoma (NHL) therapy (Dillman, 2006; Schrama, Reisfeld, & Becker, 2006). Trastuzumab (Herceptin) for breast cancer; Alemtuzumab (Campath) for chronic lymphocytic leukemia; Bevacizumab (Avastin) for colorectal cancer were approved by FDA between 1998-2004 (Schrama et al., 2006). 24 antibody-drug conjugates were approved by FDA for cancer therapy until 2016 (McKnight & Viola-Villegas, 2018).

In radiopharmaceutical science, many antibodies have been radiolabelled with various radionuclides, and many are under preclinical and clinical trials. Paul Ehrlich developed the concept of how biomolecules, especially mAbs, affect as a targeting part to transfer the radionuclides called "magic bullet," and radiolabelled biomolecules investigation started with this concept (Cutler, Hennkens, Sisay, Huclier-Markai, & Jurisson, 2013). The high specificity and accumulation on the target area of mAbs make them attractive and suitable for molecular imaging (Kobayashi et al., 2016).

RADIOPHARMACEUTICALS

Radionuclides such as gamma and positron emitters (Table 1) are used for the therapy, diagnosis, and monitoring diseases with SPECT and PET (Drude, Tienken, & Mottaghy, 2017). Multiple cameras of SPECT rotate around the patients to give three-dimensional information about the radioactivity uptake in the body. Radionuclides should emit gamma radiation within the 100-300 keV range to be detected by SPECT camera.

Table 1. Gamma and positron emitting radionuclides.

Radionuclides	Half-life	Main Energy (kEv)	Imaging Method
Tc-99m	6.06 h	140.51 (gamma)	SPECT
In-111	67.9 h	245 (gamma)	SPECT
I-123	100.8 h	159 (gamma)	SPECT
I-124	4.2. day	2149 (β^+)	PET
Ga-68	68 min	1,899 (β^+)	PET
F-18	110 min	633 (β^+)	PET
Zr-89	78.4 h	395 (β^+)	PET
Y-86	14.7 h	1248 (β^+)	PET
Cu-64	12.7 h	653 (β^+)	PET

SPECT imaging with different types of collimators and detectors provides individualization of diagnosis and therapy of patients with other biomarkers (Figure 3) (Wallberg & Stahl, 2013). PET imaging provides higher resolution and sensitivity with lower radiotracer than SPECT imaging. PET imaging method is based on the positron-emitting radionuclides, and the positron produces two gamma-photons with 511 keV while traveling in the body. These two photons are directed in 180° opposite direction, and annihilation is placed (Cutler et al., 2013; Wallberg & Stahl, 2013).

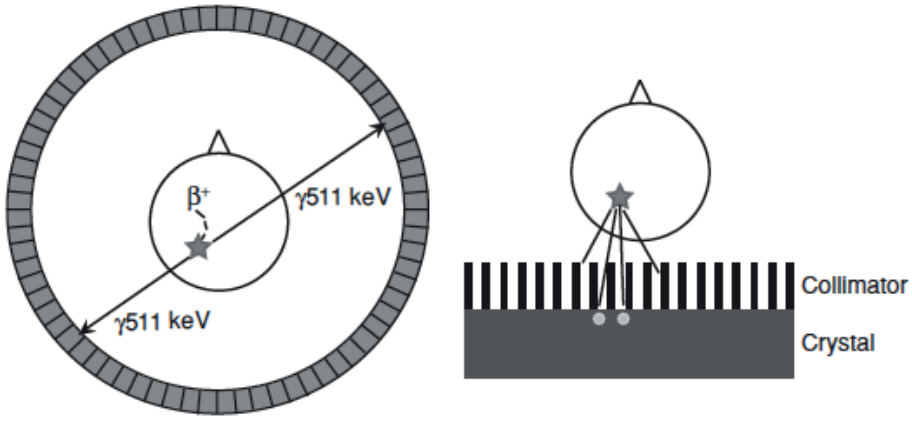

Figure 3. PET (left) and SPECT (right) schema (Wallberg & Stahl, 2013).

Radiometals have some advantages, such as simple labelling procedures and half-life, which are more suitable for antibody labelling, circulation, and accumulation studies in tumor tissue rather than non-radiometal tracers (Morais & Ma, 2018). Germanium-67/Gallium-68 (^{67}Ge/^{68}Ga) is one of the most widely used generators of PET radionuclides which is evaluated as a better alternative to some of the other radionuclides like ^{18}F, ^{124}I, ^{64}Cu, ^{89}Zr due to easy accessibility in radiopharmacy labs (Drude et al., 2017). However, considering the half-life of radionuclides, ^{89}Zr, ^{64}Cu, ^{86}Y, and ^{124}I are more suitable radiotracers for mAb radiolabelling instead of ^{68}Ga, ^{18}F (Morais & Ma, 2018).

^{89}Zr, a radiometal with a 78.4 h half-life, is generally produced by ^{89}Y(p,n)^{89}Zr reaction in cyclotrons. ^{89}Zr is widely used in mAb radiolabelling due to its long half-life (Aluicio-Sarduy et al., 2018). ^{89}Zr also helps to understand the tumor targeting properties and pharmacokinetics of mAbs (Heskamp et al., 2017). The high resolution, sensitivity, and accurate image quantification are more frequently used properties for the labelling of mAbs. Also, the residualized

features of ^{89}Zr provide improved tumor retention and tumor/normal tissue ratios due to internalization (Verel et al., 2003).

^{64}Cu, another commonly used radiotracer for radiolabelling of mAbs and proteins, has a 12.7 h half-life and is generally used by ^{64}N(p,n)^{64}Cu reaction (Avila-Rodriguez, Nye, & Nickles, 2007). ^{64}Cu has been used for radiolabelling of many mAbs (anti-Epidermal growth factor receptor (EGFR), anti-CD20 mAbs, etc.), but most of them are limited in preclinical studies due to the half-life (12.7 h) of ^{64}Cu; intermediate half-life limits the clinical imaging studies (Aluicio-Sarduy et al., 2018; Ferreira et al., 2008; Sihver et al., 2014).

^{86}Y is another promising radionuclide as an immuno-PET radiotracer. ^{86}Y can be produced by ^{86}Sr(p,n)^{86}Y reaction in a small cyclotron with high radionuclidic purity. It has a 14.7 h half-life. However, due to the decay properties of ^{86}Y, production rates of ^{86}Y are lower than ^{89}Zr and ^{64}Cu (Lubberink & Herzog, 2011; Wadas et al., 2010).

^{124}I is a radionuclide with a long half-life (4.18 days) used for imaging and therapy. ^{124}I radiolabelled mAbs show high potential as an immuno-PET imaging agent (Crisan et al., 2022).

Many radiolabelled mAbs with ^{64}Cu, ^{89}Zr, ^{124}I, and ^{86}Y have been investigated for many years in both preclinical and clinical studies. Researches on ^{86}Y and ^{64}Cu radiolabelled mAbs are limited due to the relatively short half-lives of ^{86}Y and ^{64}Cu compared with the mAbs' biological half-life. ^{89}Zr has a long half-life, making it a suitable radionuclide for mAbs radiolabelling; hence, it has been successfully used in clinical and preclinical studies. ^{124}I shows lower residualized features compared with ^{89}Zr, which means ^{124}I shows lower liver uptake (Carrasquillo et al., 2018).

In addition to these long-lived PET radionuclides, several radiolabelled mAbs have been approved by FDA and European Medicine Agency (EMA) (Table 2): ^{111}In radiolabelled Capromab Pendetide (ProntaScint); ^{90}Y radiolabelled Ibritumomab Tiuxetan (Zevalin); ^{99m}Tc radiolabelled Sulesomab (LeukoScan); ^{111}In radiolabelled Satumomab Pendetide (OncoScint); ^{131}I radiolabelled Tositumomab (Bexxar) for different purposes. ^{111}In-ProstaScint was approved in 1996 for prostate cancer diagnosis; ^{90}Y-Zevalin was the first radioimmunoconjugate approved as an anticancer agent in 2002 (Dilworth & Pascu, 2018).

Table 2. Some of the FDA and EMA approved radiolabelled mAbs.

Commercial Name	Radiolabelled-mAb	Year
OctreoScan	^{111}In-Octreotide	1994
ProntaScint	^{111}In-Capromab Pendetide	1996
	^{99m}Tc-Apcitide	1997
LeukoScan	^{99m}Tc-Sulesomab	1997
OncoScint	^{111}In-Satumomab Pendetide	1998
Zevalin	^{90}Y-Ibritumomab Tiuxetan	2002
Bexxar	^{131}I-Tositumomab	2003
Scintimun	^{99m}Tc-Besilesomab	2010

IMMUNO-PET with RADIOLABELLED MONOCLONAL ANTIBODIES

Immuno-imaging studies were started at the beginning of the '90s with SPECT with radiolabelled antibodies or fragments (Knowles & Wu, 2012). Although SPECT is a very sensitive imaging method, PET provides better quantification and higher sensitivity. Also, it is possible to determine the pharmacokinetics and biodistribution of pharmaceuticals with immuno-PET (Kumar & Ghosh, 2021). Radiopharmaceuticals of Immuno-PET development have several principles: appropriate radionuclide and chelator selection, suitable biological and chemical properties of mAbs, and the stability between mAbs, chelators,

and radionuclides (McKnight & Viola-Villegas, 2018).

PET radionuclides (radiometals and non-radiometals) are frequently used in preclinical and clinical stages. Especially, long-lived PET radionuclides are commonly utilized for immuno-imaging due to their several advantages. ^{89}Zr, ^{64}Cu, and ^{124}I PET radionuclides show appropriate half-lives to antibodies and/or proteins/peptides (Knowles & Wu, 2012). The similarity of physical and biological half-lives of mAbs and PET radionuclides provides proper accumulation in the tumor before radioactive decay and also provides enough clearance from normal tissues (McKnight & Viola-Villegas, 2018). Although long biological and physical half-lives ensure these advantages, long waiting times for imaging can be considered as a disadvantage (Kaur et al., 2012). In this part, ^{89}Zr, ^{64}Cu, ^{86}Y and ^{124}I radiolabelled mAb studies have been reviewed (Table 3).

Table 3. Summary of the studies on radiolabelled mAbs mentioned in this article.

Radionuclide	Compared Radionuclide	mAb	References
Zr-89		U36	(Borjesson et al., 2006; Börjesson et al., 2009)
	In-111	Trastuzumab	(E. Dijkers et al., 2007; E. C. Dijkers et al., 2009)
		MMOT0530A	(Lamberts et al., 2016)
		J591	(J. P. Holland, Divilov, et al., 2010)
		anti-PD-L1 mAb	(Kikuchi et al., 2017)
Cu-64		Cetuximab Abegrin	(Achmad et al., 2012; T. P. Nayak & Brechbiel, 2009; Zeng et al., 2014)
		Trastuzumab	(Parakh, Lee, Gan, & Scott, 2022; Tamura et al., 2013)
		Trastuzumab	(Kurihara et al., 2015; Tamura et al., 2013)
		3/F11 mAbs	(Maier et al., 2019)
		Rituximab	(Xie et al., 2017)
		PSMA-617	(Grubmuller et al., 2016)
Y-86	In-111	hu3S193	(Lövqvist et al., 2001)
	In-111	Trastuzumab	(Palm et al., 2003)
		Bevacizumab	(T. K. Nayak, Garmestani, Baidoo, Milenic, & Brechbiel, 2011)
I-124		Codrituzumab	(Carrasquillo et al., 2018)
		U36	(Verel et al., 2004)
		uA33	(Carrasquillo et al., 2011)
		mu1G8	(Olafsen et al., 2007)
		cG250	(Divgi et al., 2007; Povoski et al., 2013)

REGULATIONS

The preparation and use of radiopharmaceuticals are regulated by directives, regulations and rules in European Union (EU) (Gillings et al., 2021) and United States (U.S.) and many other countries. Radiopharmaceuticals are regulated by the Center for Drug Evaluation and Research (CDER) under the FDA in the USA; by European Medicine Agency in European Union; by The Australian Radiation Protection and Nuclear Safety Agency (ARPANSA) and Radiation Health Committee in Australia; by Health Product and Food Branch (HPFB) in Canada; by Atomic Energy Regulatory Board (AERB) in India (Saharma, Baldi, Singh, & Sharma, 2018).

Regulations of radiopharmaceuticals are associated with radiopharmaceutical monographs in pharmacopeias such as United States Pharmacopeias, European Pharmacopeias. The guidelines on cGMPs and current Good Radiopharmacy Practice (cGRPP) are the main guidelines for radiopharmaceuticals from development to release. In addition, the requirements are specified in the relevant monographs of the phar-

macopeias (USP, EP, JP, etc.) and in the general information section. For example, USP general chapter indicates the regulations about PET radiopharmaceuticals and how they should produce, prepare and the information given in USP meets the cGMP regulations ((CDER), 2011; Gillings et al., 2021; U.S.Pharmacopoeia, 2009). In the EU, adoptions and interpretations of regulations and directives may differ depending on the countries and these differences may occur only if the members provide the general scope and limits of directives (Gillings et al., 2021).

Immuno-PET radiopharmaceuticals, like other radiopharmaceuticals, should be produced according to the guidelines. Gillings and co-workers (2021), prepared the *"Guideline on cGRPP for Small-Scale Preparation Radiopharmaceuticals"* in the 3 sections: general aspects; radiopharmaceutical preparations with licensed generators and kits; and radiopharmaceutical preparations with non-licenced materials (Gillings et al., 2021). Some of the immune-PET radiopharmaceuticals can be evaluated under Section 2 and Section 3. However, all processes should meet the cGRRP rules.

Quality Control

Radiolabelled mAb formulations quality control tests are done right after radiolabelling procedures (purification, formulation preparation). Radiochemical purity, antigen binding, internalization and stability tests should be performed as clinical and preclinical quality control tests of radiolabelled mAbs. Radiochemical purities are mostly analyzed by Radio-High Pressure Liquid Chromatography (RHPLC); however, Radio-Thin Layer Chromatography (RTLC) and spin filter are also used as radiochemical purity analysis based on the molecular weight separation. Immuno-reactivity tests and/or binding assays are performed for the determination of antigen binding (Parakh et al., 2022). More quality control tests should be done for clinical applications. All formulations and radiolabelling steps should be performed under cGMP (current Good Manufacture Practise) rules and cGMP grade requires: 1) radiochemical purity 2) appearance 3) immunoreactivity and/or binding assay and/or ELISA 4) apyrogenicity 5) sterility 6) stability (Vugts, Visser, & van Dongen, 2013).

Radiochemical purity: Any analytical method can be mentioned in monographs depending on the radiopharmaceuticals. These methods may be paper chromatography, TLC, ITLC, size exclusion chromatography, liquid and gas chromatography with radioactivity detector. Bacterial endotoxin/pyrogen test are done after product release for radiopharmaceuticals with a short half-life. Recently, a kinetic LAL test taking 20 mins and provides the opportunity complete pyrogen tests before releasing for the radiopharmaceuticals with a longer half-life than 30 mins. Limits of bacterial endotoxins are mentioned in each monograph. Sterility tests are carried out according to the general methods. Also, radiopharmaceuticals can be released for patient use before the results of sterility tests are available ("Guideline on Radiopharmaceuticals, CHMP, EMEA/CHMP/QWP/306970/2007 (draft released for consultation)," 2007; WHO, 2017).

^{89}Zr Radiolabelled Monoclonal Antibodies

Many preclinical and clinical trials started after Immuno-PET technology with ^{89}Zr (Table 2) (E.T. Sarcan & Özer, 2021; van Dongen & Vosjan, 2010). In 2006, the first clinical ^{89}Zr radiolabelled mAb, ^{89}Zr-U36, was investigated for primary head and neck squamous cell carcinomas (HNSCC), especially lymph nodes, as reported by Börjesson and co-workers in 2006 (Borjesson et al., 2006). 20 patients with HNSCC have been included in this study, and 74 MBq ^{89}Zr-U36 was applied as a safe dose and no adverse reactions were observed. All primary tumor lesions were detected by ^{89}Zr-U36 immuno-PET. These results could prove that ^{89}Zr-U36 is a suitable agent which can be used for tumor detection, although it did not show any significant improvement compared to the traditional agent, ^{18}F-fluorodeoxyglucose (FDG) (Jauw et al., 2016). Biodistribution study was performed by Borjesson et al. in 2009, as a following

study. After injection, the uptake increased gradually in tumor while the uptake in blood pool, lungs, liver, kidneys and spleen decreased (Börjesson et al., 2009).

Before the clinical study of ^{89}Zr-trastuzumab, several preclinical studies showed that ^{89}Zr-trastuzumab could be a promising agent for HER2 overexpressed breast cancer imaging and therapy (Dijkers et al., 2009). Dijkers et al. (2009), radiolabelled trastuzumab with ^{89}Zr and ^{111}In (for comparison), and animal PET/CT imaging and biodistribution studies were performed. ^{89}Zr labelling procedures were performed with Verel et al. (2003) method (Verel et al., 2003) and quality control tests showed that trastuzumab was labelled with ^{89}Zr with high chemical purity and specific activity. In-vivo studies were performed for 6 days in 15 mice. In this stage, the optimal amount of trastuzumab was also calculated and 100 μg of trastuzumab was evaluated as optimal. During the *in vivo* studies between day 1 and 6, tumor uptake was significantly increased while blood-pool uptake decreased. But the liver and spleen radioactivity uptakes were found slightly higher than expected. When comparing to the ^{89}Zr-trastuzumab uptake of HER2/neu positive and negative groups, tumor uptakes were not different on day 1. However, significantly different tumor uptake was found on day 6. As a result of this study, ^{89}Zr-trastuzumab can be considered for clinical testing due to labelling with high chemical purity, high stability, and appropriate tumor uptake with high Tumor to Normal Tissue ratios. Also, biodistribution studies showed similarity to ^{111}In-trastuzumab used in clinical breast cancer studies (Dijkers et al., 2009).

Dijkers and co-workers reported a study of ^{89}Zr radiolabelled trastuzumab in 14 patients who have HER2-positive metastatic breast cancer (Dijkers et al., 2007). This study showed trastuzumab dose-dependent relation with imaging dose and biodistribution (Dijkers et al., 2007). ^{64}Cu-trastuzumab was also successfully labelled and found to be a comparable agent with ^{89}Zr-trastuzumab due to tumor/tissue ratios and the ability to identify primary and metastatic tumors with low uptake (Parakh et al., 2022; Tamura et al., 2013).

Many researchers have been carried out on EGFR, HER2, PSMA, CD20 and VEGF-A, PSMA, and PD-L1 antigens (Heskamp et al., 2017; Holland, Sheh, Smith-Jones, & Lewis, 2009). ^{89}Zr-cetuximab, ^{89}Zr-pertuzumab, ^{89}Zr ibritumomab tiuxetan, ^{89}Zr huJ591, ^{89}Zr-bevacizumab, ^{89}Zr-nimotuzumab, ^{89}Zr-pembrolizumab have also been investigated for various cancer diagnosis (Table 4).

Lamberts and co-workers (2016) used ^{89}Zr radiolabelled MMOT0530A (mesothelin antibody) to follow DMOT4039A (antibody-drug conjugate) treatment of pancreatic and ovarian cancer lesions in Phase 1 studies. The main purpose of ^{89}Zr-MMOT0530A used was to understand the mesothelin expression, antibody tumor uptake and distribution, relation between the uptake and treatment response (Lamberts et al., 2016). This was the first-in-human study for anti-mesothelin antibody tumor uptake and body distribution. ^{89}Zr- MMOT0530A provided the visualization of antibody distribution and provided the imaging of pancreatic and ovarian cancer lesions. Researchers mentioned that this immuno-PET imaging technique with ^{89}Zr- MMOT0530A can potentially guide the treatment (Lamberts et al., 2016).

In 1997, J451, J533, J591, and E99 mAbs, specific to epitopes on PSMA, were produced by Liu et al. (Liu et al., 1997; Liu et al., 1998). J591 was found to be a promising one for diagnostic and therapeutic immuno-conjugates for PSMA targeting among of these 4 PSMA targeting mAbs. After this finding, preclinical and clinical studies started and have been reported for radiolabelled J591 with various radionuclides for treatment and diagnostic purposes (Holland, Divilov, et al., 2010).

Holland et al. (2010) studied the production of ^{89}Zr-J591 and evaluated the in vitro studies and preclinical data of ^{89}Zr-J591 in prostate-specific membrane antigen (PSMA) (+) tumor (LNCaP cells). In this study, researchers completed the radiochemistry

part with a high yield of radiolabelling (77%) and very high radiochemical purity (99%). In vivo studies proved that ^{89}Zr-J591 shows a high tumor/background tissue ratio. All results in radiochemistry for in vitro and preclinical studies showed that ^{89}Zr-J591 is a promising radiopharmaceutical for clinical studies in PSMA (+) tumor types (Holland, Divilov, et al., 2010).

Kikuchi and co-workers (2017) studied PD-L1-based radiotherapy and mAb therapy and measured PD-L1 expression with ^{89}Zr radiolabelled anti-PD-L1 mAb. Because of the difficulties in monitoring the radiotherapy with mAbs, ^{89}Zr radiolabelled anti-PD-L1 mAb was planned to overcome this difficulty. In this study, researchers investigated the treatment of head and neck squamous cell carcinoma (HNSCC). In the end, PET/CT images with ^{89}Zr-anti PD-L1 mAbs showed therapeutic efficiency and proved increased uptake in irradiated tumors, although it could not be monitored non-invasively. This report is the first data indicating that ^{89}Zr-radiolabelled anti-PD-L1 can be employed to monitor of radiotherapy in PD-L1 positive tumors (Kikuchi et al., 2017).

Table 4. ^{89}Zr radiolabelled mAbs and their clinical trial situation (McKnight & Viola-Villegas, 2018).

mAb	Types	Target	Cancer Type	Clinical Trial Status	Responsible Party
Rituximab	**Chimeric**				
Trastuzumab	**Humanized**	HER 2	Breast Cancer	Completed, 2017	Dr. Géraldine Gebhart et. al.
Pertuzumab	**Humanized**	HER 2	HER 2 (+) malignancy	Completed, 2018	Memorial Sloan Kettering Cancer Center
U36	**Chimeric**	CD 44	Head and neck cancer	Completed	
Ibritumomab Tiuxetan	**Mouse**	CD 20	NHL	Completed	
huJ591	**Humanized**	PSMA	Prostate cancer	Completed, 2020	Weill Medical Colloge of Cornell University
Bevacizumab	**Humanized**	VEGF	Breast cancer	Completed, 2019	Heather A. Jacene
Cetuximab	**Chimeric**	EGFR	Colorectal cancer	Completed, 2020	C. Menke- van der Houven van Oordt
Pembrolizumab	**Humanized**	PD-1	Non small cell lung cancer	Phase 2	E. F. Smith

^{64}Cu Radiolabelled Monoclonal Antibodies

Various ^{64}Cu radiolabelled mAbs have been reported for immuno-imaging and radioimmunotherapy. Cetuximab and Abegrin (FDA-approved mAbs) have been radiolabelled and studied in preclinical models (Nayak & Brechbiel, 2009; Zeng et al., 2014).

Cai et al. reported the quantitative PET imaging of EGFR expression with ^{64}Cu-cetuximab in mice. In this study, biodistribution results of ^{64}Cu-cetuximab in various EGFR overexpressed tumor models were given. The study showed that the highest tumor uptake was found at 48th h after radiopharmaceutical injection (Battal & Özer, 2021; Cai et al., 2007).

Ping Li and co-workers (2008) reported ^{64}Cu radiolabelled cetuximab for PET/CT imaging of EGFR overexpressed tumor types and evaluated the ^{64}Cu-cetuximab formulations for EGFR overexpressed tumors. Researchers studied receptor binding of ^{64}Cu-cetuximab to EGFR through in vitro and in vivo studies. In vivo studies were performed in A431 and MDA-MB-435 tumor-bearing mice. The uptake in A431 tumors was increased until 48 h after the administration and significant differences were found between EGFR-positive tumor uptake and blocked

uptake at 24 h. Micro PET/CT imaging was performed at 20 and 48 h after injection of ^{64}Cu-cetuximab in A431 and MDA-MB-435 tumor-bearing mice. Overall, ^{64}Cu-cetuximab was found as a promising agent for tumor imaging and also has excellent potential for predicting the cetuximab therapy response and helping the dose calculation in tumors (Anderson & Ferdani, 2009; Ping Li, Meyer, Capretto, Sherman, & Anderson, 2008).

Achmad et al. (2012) studied the ^{64}Cu-cetuximab for predicting cetuximab accumulation in Kirsten rat sarcoma viral oncogene homolog (KRAD) mutant colorectal cancer. In vivo study results were found promising in the end with radioimmunoimaging with ^{64}Cu-cetuximab (Achmad et al., 2012).

Tamura et al. (2012) studied the safety, distribution, and dosimetry of ^{64}Cu-trastuzumab in human HER2 (+) breast cancer. This study was performed on 6 patients with primary or metastatic breast cancer. 130 MBq doses of ^{64}Cu-trastuzumab were injected in 6 patients, and after 1, 24, and 48 h, PET images were taken. Results showed that the best imaging time was 48 h after the administration time of ^{64}Cu-trastuzumab. Also, brain metastases were imaged by PET in 2 patients. Results indicated that ^{64}Cu-trastuzumab is a suitable agent for diagnosing of HER2 (+) breast cancer and likely brain metastases the HER2 (+) breast cancer. Also, researchers mentioned dosimetry and pharmacologic safety results were within acceptable limits (Tamura et al., 2013). After this study, researchers also investigated the same agent (^{64}Cu-trastuzumab) for metastatic brain lesions of HER2(+) breast cancer.

Kurihara and co-workers (2015) studied ^{64}Cu-trastuzumab for metastatic brain cancer imaging and its specificity on HER2. PET studies were performed at 1, 24, and 48 hs after 500 MBq ^{64}Cu-trastuzumab injection on 5 patients suffering metastatic brain cancer from HER2 (+) breast cancer. As a result, researchers found that ^{64}Cu-trastuzumab could visualize metastatic brain cancer in all 5 patients. Overall, researchers indicated that ^{64}Cu-trastuzumab PET imaging is a safe technique and could be used to visualize metastatic brain lesions in HER2(+) breast cancer patients (Kurihara et al., 2015).

Zaheer and co-workers (2019) used ^{64}Cu radiolabelled trastuzumab to investigate the optimal therapeutic administration and micro distribution of mAb in radioimmunotherapy imaging. ^{64}Cu-trastuzumab was found an effective agent in the gastric cancer mouse model for diagnosis (Zaheer, Kim, Lee, Lim, & Kim, 2019).

In another study, immuno-PET and immuno-Cerenkov luminescence imaging (immuno-CLI) were compared for PSMA (+) tumor detection in mice by using ^{64}Cu radiolabelled 3/F11 mAbs. Immuno-CLI was found cheaper and showed fast acquisition times as advantage. However; the overall, sensitivity was lower than PET imaging, and also tissue penetration depth was found lower. In the end, PET imaging with ^{64}Cu-3/F11 was found as a better technique that is already used in clinical and preclinical use. In addition, this new method could be promising in vivo imaging tool (Maier et al., 2019).

Xie and co-workers studied ^{64}Cu radionuclides from production to the imaging process. In this study, rituximab was radiolabelled with ^{64}Cu, and an in-vivo study was performed on Ramos cells (lymphoma). In the end, the production of ^{64}Cu and radiolabelling processes were completed with high radiochemical and radionuclidic purity, and immuno-PET was successfully done (Xie et al., 2017).

Grubmüller et al. (2016) investigated the diagnostic potential of ^{64}Cu radiolabelled PSMA-617 in patients with PSMA (+) prostate cancer. This study was carried out at 2 different centers with 29 patients. The whole body PET images were taken in 1 h after injection and pelvis PET images were taken in 2 h post-injection. Primary prostate lesions were detected in 23 patients out of 29. Even with low prostate specific antigen levels in some patients, lesions were detected with high contrast after 1 h of injection. This study proved that ^{64}Cu radiolabelled mAbs could be

used in patients in centers where ^{68}Ga radionuclide is not available. ^{64}Cu-PSMA-617 showed high imaging quality and contrast (Grubmuller et al., 2016).

^{86}Y Radiolabelled Monoclonal Antibodies

^{86}Y has been used for imaging and monitoring therapy with ^{90}Y and getting more attraction for imaging and investing in ^{90}Y studies due to its relatively long half-life (Mikolajczak, van der Meulen, & Lapi, 2019; Nayak & Brechbiel, 2009). Also, the chelate-bioconjugate reaction could be easier than ^{89}Zr because ^{86}Y and ^{90}Y show the same chemistry (Ramogida & Orvig, 2013). However, ^{86}Y faced some limitations, one of which is the availability of ^{86}Y (Herrero Alvarez, Bauer, Hernandez-Gil, & Lewis, 2021).

Lövqvist et al. (2001) studied the ^{86}Y radiolabelled hu3S193 mAb and compared it with ^{111}In radiolabelled hu3S193 mAb to provide a better understanding of ^{86}Y radiolabelled mAbs for ^{90}Y radioimmunotherapy. 2 days after injections of ^{111}In-hu3S193 and ^{86}Y- hu3S193, uptakes of them were found similar at many tissues and organs, however, after 4 days, uptake and activity of ^{86}Y- hu3S193 were found higher than ^{111}In-hu3S193 in many tissues, tumors and bones. As a result, ^{86}Y- hu3S193 was as a better alternative for observing ^{90}Y biodistribution and therapy (Lövqvist et al., 2001).

Palm and co-workers (2003) studied ^{86}Y radiolabelled trastuzumab for estimating ^{90}Y radiolabelled trastuzumab biodistribution and dosimetry. Before ^{86}Y studies, ^{111}In has been used; however, several differences were detected between ^{111}In and ^{90}Y. ^{86}Y has been investigated due to very similar chemistry with ^{90}Y. ^{86}Y-trastuzumab PET images were confirmed by Magnetic Resonance (MR) images (Palm et al., 2003).

Nayak et al. (2011) investigated ^{86}Y-bevacizumab as a potential immuno-PET imaging agent and also the agent for a surrogate marker of ^{90}Y radioimmunotherapy in metastatic colorectal carcinoma. Results showed that ^{86}Y-bevacizumab has great potential as an immuno-PET agent to determine bevacizumab uptake and localization. Additionally, ^{86}Y-bevacizumab also can used as a marker for ^{90}Y-bevacizumab immunotherapy (Nayak et al., 2011).

^{124}I Radiolabelled Monoclonal Antibodies

Carrasquillo and co-workers (2018) performed PET imaging research and biodistribution of ^{124}I-codrituzumab in 14 patients with hepatocellular carcinoma. Different immunotherapy processes were also applied in some patients before and after immuno-PET. In the end, tumor localization was found in 13 patients, and tumor uptake of ^{124}I-codrituzumab in patients undergoing immunotherapy was found to decrease. In conclusion, ^{124}I-codrituzumab successfully detected tumor localization in most patients' suffering from hepatocellular carcinoma (Carrasquillo et al., 2018).

Verel et al. (2004) studied ^{124}I-U36 mAb from the labelling process to PET imaging before ^{131}I-radioimmunotherapy. Radiolabelling was successfully done with more than 95% radiochemical purity, and PET imaging was performed in nude mice. The main focus of this study was the scouting with PET imaging before radioimmunotherapy. Because ^{124}I was thought as an ideal radionuclide for the scouting procedure. In the end, tumor uptake was detected with ^{124}I-U36 mAb, and ^{124}I radiolabelled mAbs were found to be a suitable agent for PET imaging (Verel et al., 2004).

Carrasquillo et al. (2011) studied on huA33 mAbs and radiolabelled with ^{124}I to understand better targeting, biodistribution and safety for patients with colorectal cancer. The study was performed on 25 patients with primary or metastatic colorectal cancer and 19 patients who had surgical exploration and injected with 343 MBq ^{124}I-huA33 solution. Results showed good localization without any toxicity in patients. ^{124}I-huA33 mAbs showed high uptake in tumors and adequate tumor imaging in all patients (Carrasquillo et al., 2011). In another clinical study in 2011, huA33 was radiolabelled with ^{124}I and applied in 15 patients with colorectal cancer by O'Donoghue et al. This study results also supported Carrasquillo and co-workers' study (O'Donoghue et al., 2011).

Olafsen and co-workers (2007) radiolabelled mu1G8 mAbs with ^{124}I to determine the specific activity and tumor uptake in prostate cancer xenografts. Specific tumor targeting was observed by ^{124}I-mu1G8 mAbs in PET imaging (Olafsen et al., 2007).

Povoski et al. (2013) explained the new multimodal imaging and detection methods with ^{124}I radiolabelled cG250 mAbs to confirm preoperative and intraoperative localization for open surgical resection of renal cell carcinoma (Povoski et al., 2013). Divgi and co-workers reported a phase 1 trial of ^{124}I-cG250 mAb administration to assess preoperative PET localization in patients in 2007 (Divgi et al., 2007).

CONCLUSION

PET imaging shows several advantages, especially for deep tissues, such as sensitivity, and contrast resolution compared with SPECT imaging. These advantages provide significant superiority for radioimmunoimaging (Holland, Divilov, et al., 2010).

MAbs have been used for therapy and imaging for several decades because of their selectivity to specific targets (Wong et al., 2011). Radiolabelled mAbs have been shown increasing popularity for many years, and immuno-PET, which combines mAbs and PET radiometals, has great advantages due to the sensitivity of PET radionuclides and the specificity of mAbs. Radiolabelled mAbs are commonly used in applications: diagnose and imaging of diseases, monitoring of tumor response during therapy, metastatic lesion detection, therapy and dosimetric calculations (Wong et al., 2011). Moreover, immune-PET can also be used the determine antigen expression, antibody biodistribution, and organ pharmacokinetics. However, this approach is not yet used as much as immuno-PET is used for disease diagnosis and imaging, and it is seen as a deficiency in many phase 1 studies. (Lamberts et al., 2016).

The PET radionuclides, ^{89}Zr, ^{64}Cu, ^{124}I and ^{86}Y, are the most common radionuclides used for mAb radiolabelling; however, each radionuclide has advantages and disadvantages compared with each others. ^{89}Zr is the most suitable radionuclide for mAb radiolabelling, and it has already been used in clinical studies. Long half-life and half-life compatibility with mAbs's body clearance are the advantages of ^{89}Zr used for mAb radiolabelling, although its high positron energy and residualized features cause disadvantages. ^{124}I, which also has a long half-life, is also a promising radionuclide for mAb radiolabelling and shows lower residualized features when compared with ^{89}Zr. It makes ^{124}I also suitable and promising radionuclides for immuno-PET. ^{64}Cu and ^{86}Y have relatively short half-lives compared with ^{89}Zr and ^{124}I, and their short half-lives limit the studies for mAb radiolabelling.

Besides all these advantages, mAbs show relatively poor tumor uptake. Therefore, labelling of mAb fragments and obtaining immuno-PET images with mAb fragments are also getting popular recently. Fab fragments can also provide several advantages such as reducing non-specific distribution, different blood clearance and tumor localization, faster clearance and better penetration and may be reducing immunogenicity (Wong et al., 2011).

In conclusion, immuno-PET, combined with mAbs and these radionuclides which are relatively long half-life, show great potential for diagnosing diseases, monitoring therapies, and monitoring the mAbs behavior in the body, but various challenges should be overcome before clinical stages.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

Determination of the Subject (AYO; ETS), Literature Review, Preparation of the Text (ETS), Evaluation of the Text (AYO)

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