# Highlighting new Research Trends on Zirconium-89 Radiopharmaceuticals beyond Antibodies

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# **Abstract** (100 words)

Zirconium-89 ($^{89}$Zr) is a cyclotron-produced positron-emitting radioisotope with a half-life of 3.27 days, which makes delayed or longitudinal imaging possible. It is a superior isotope for tracking particles over several days at a high sensitivity, resolution, and specificity. $^{89}$Zr-monoclonal antibodies ($^{89}$Zr-mAb) have gained significant attention in the field of molecular imaging. However, the past decade has shown an avid increase in research concerning $^{89}$Zr-radiopharmaceuticals apart from $^{89}$Zr-mAb. In this article we highlight and discuss the status and challenges attributed to current preclinical and clinical investigations of $^{89}$Zr-radiopharmaceuticals developed beyond $^{89}$Zr-mAb, e.g., mAb-derived variants and macro-biomolecules, proteins, peptides, nanoparticles, and living cells.

# **Keywords**

Zirconium-89; immuno-PET; $^{89}$Zr-radiopharmaceuticals; nanoparticles, radiolabeled cells; $^{89}$Zr-mAb fragments; $^{89}$Zr-PET imaging and theranostics.

# Introduction

For nuclear medical imaging or therapy, a monoclonal antibody (mAb) may be commonly labeled with various radionuclides such as actinium-225 ($^{225}$Ac), astatine-211 ($^{211}$At), bismuth-213 ($^{213}$Bi), indium-111 ($^{111}$In), iodine-123/-124-131 ($^{123/124/131}$I), lead-212 ($^{212}$Pb), lutetium-177 ($^{177}$Lu), technetium-99m ($^{99m}$Tc), copper-64 ($^{64}$Cu), gallium-68 ($^{68}$Ga), yttrium-86/90 ($^{86/90}$Y), or zirconium-89 ($^{89}$Zr).[1] The ever-growing number of valuable $^{89}$Zr mAbs for personalized medicine has gained significant attention in the field of molecular imaging using mAb-based positron emission tomography (PET), also known as $^{89}$Zr-"immuno-PET". By using metal-chelation, $^{89}$Zr is conjugated to mAbs that demonstrate high affinity to specific antigens expressed on tumor cells. In turn, immuno-PET allows for highly specific targeted imaging where the $^{89}$Zr-labeled mAb can specifically bind to tumor-associated antigens, which will provide detailed images of solid tumor and cancer burden *in vivo* and in real time. Furthermore, immuno-PET may be capable of monitoring treatment response to enable clinicians to evaluate the effectiveness of therapies and follow the uptake of $^{89}$Zr-labeled mAbs over time.

During the last three decades a significant amount of preclinical data has been produced using $^{89}$Zr-PET imaging to facilitate clinical translation of radiopharmaceuticals. As a result, the versatility and potential of immuno-PET has been demonstrated in various clinical settings, mainly in oncology, but also in cardiovascular and neurodegenerative diseases and the imaging of inflammation, infection and autoimmune disorders.[2, 3] The first clinical use of immuno-PET dates back to 2006, involving a $^{89}$Zr -mAb to detect lymph node metastases in patients presenting with head and neck cancer.[4] A well-understood radiochemistry, applicable to numerous $^{89}$Zr-labeled mAbs, now supports a world-wide ease of access, widespread availability and a significant clinical footprint of $^{89}$Zr-immuno-PET.[5]

PET imaging of $^{89}$Zr-radiopharmaceuticals has also become a consistent tool in preclinical and clinical drug development, and patient selection, primarily due to the advantageous physical properties of $^{89}$Zr, which allow for straightforward radiolabeling approaches. The ability of $^{89}$Zr-PET to provide detailed images of tumor biology, predict treatment toxicity and immune responses, positions it as a critical component in the development of personalized cancer therapies. To date, several targeted radionuclide

diagnostic and therapeutic technologies are in development, comprising positron-emitting diagnostic agents for targeted PET imaging and beta- and alpha-emitting agents as radionuclide therapies, or radioimmunotherapy (RIT). Recent suggestions for a combination of $^{89}$Zr-labeled compounds for imaging with other therapeutic isotopes like $^{90}$Y, $^{177}$Lu, $^{227}$Th or $^{225}$Ac for treatment exemplifies their usefulness in a theranostic approach. For example, a study evaluated a glypican-1 targeting $^{89}$Zr-labeled mAb, allowed for precise imaging and subsequent $^{211}$At-targeted alpha therapy to effectively reduce pancreatic tumor growth.[6]

To warrant a significant role, value and high impact of $^{89}$Zr-PET for RIT and theranostics, scientists also began investigating $^{89}$Zr-radiolabeling techniques suited for the various antibody fragments or other relevant compound classes - apart from radiolabeled antibodies. This addition of novel $^{89}$Zr-labeled radioimmune-conjugates and $^{89}$Zr-radiopharmaceuticals is considered an rapidly emerging research field expected to offer both unique diagnostic and therapeutic benefits in a single, streamlined process.[7, 8] Due to the constantly growing attention and demand for $^{89}$Zr-immuno-PET, research and clinical translation for new applications using mAb derivatives or other non-mAb compounds are becoming a rapid reality, but with their own challenges. As the current state of $^{89}$Zr-radiolabeled mAbs has been recently reviewed; in this article we will highlight and discuss the status and challenges attributed to current preclinical and clinical investigations of those $^{89}$Zr-radiopharmaceuticals developed beyond $^{89}$Zr-mAb, e.g., mAb-derived variants and macro-biomolecules, proteins and peptides, nanoparticles, and living cells.[9]

# Zirconium-89 - a broadly utilized radioisotope for Positron Emission Tomography Imaging

Zirconium-89 ($^{89}$Zr) is a long-lived, cyclotron-produced PET radioisotope with a half-life of 78.4 h (3.27 d). Thus, it is a superior isotope for tracking particles (most common are larger bio-molecules like mAbs or cells) over several days to a week at a high sensitivity, resolution, and specificity.[10] Importantly, delayed or longitudinal imaging is thus possible, with $^{89}$Zr via sequential imaging providing excellent *in vivo* data for pharmacokinetics of the same molecule/particle in the same subject.

The $^{89}$Zr benefits are from its relatively low mean positron emission energy of 395.5 keV (23% and maximum positron energy of 0.91 MeV), compared to the commonly used Fluorine-18 (249.8 keV, 97% and maximum 0.63 MeV), which translates into high image resolution.[10] Zirconium-89 decays to yttrium-89m that further decays in 16 s to stable yttrium-89 emitting a high energy γ, 909 keV. This emission, together with the low-abundance positron emission, is a concern for patient and staff radiation burdens, thereby limiting the amount of $^{89}$Zr that may be injected into a patient.[11] From an imaging perspective the 909 keV photons do not interfere with the PET signal (511 keV).

Zirconium-89 can be obtained through the $^{89}$Y(p,n)$^{89}$Zr reaction with high radionuclide purity, high specific activity and at high yields through well described processes from Yttrium (natural) solid targets (yttrium foil or a thin layer of yttrium that has been sputtered onto a niobium backing). Post irradiation processing results in $^{89}$Zr-activity in the form of oxalic acid (1M or lower) which facilitates the chelation to desferrioxamine (DFO) - the most commonly used bifunctional chelator suited for zirconium-89's coordination chemistry (oxidation state + 4).[12] A possible downside of oxalic acid is that it offers limited buffering capacity above pH = 6.0 making physiological pH adjustment difficult. DFO is an approved pharmaceutical, clinically used for aluminum and iron demetallation therapies, allowing for easier clinical approval and can be easily attached to proteins through p-NCS-Bz-DFO - epsilon-N-lysine conjugation.

Production of $^{89}$Zr through liquid cyclotron targetry systems has been reported; however, the comparative quantities of $^{89}$Zr produced are substantially lower.[13] Radiolabeling with [$^{89}$Zr]$ZrCl_4$ is sometimes preferred for better radiochemical yields at lower reaction temperatures; furthermore oxalate has to be removed before administration as it is highly toxic.[14] For incorporation into highly lipophilic molecules, liposomes and living cells that also require neutral reaction conditions [$^{89}$Zr]Zr(oxinate)$_4$ is preferred.[15]

# Discussion of novel $^{89}$Zr-labeled compounds

For this review two authors independently screened publications of the past decade (up to August 2024), and excluded literature that solely investigated mAbs labeled with $^{89}$Zr, and/ or applications of mAb-immuno-PET.

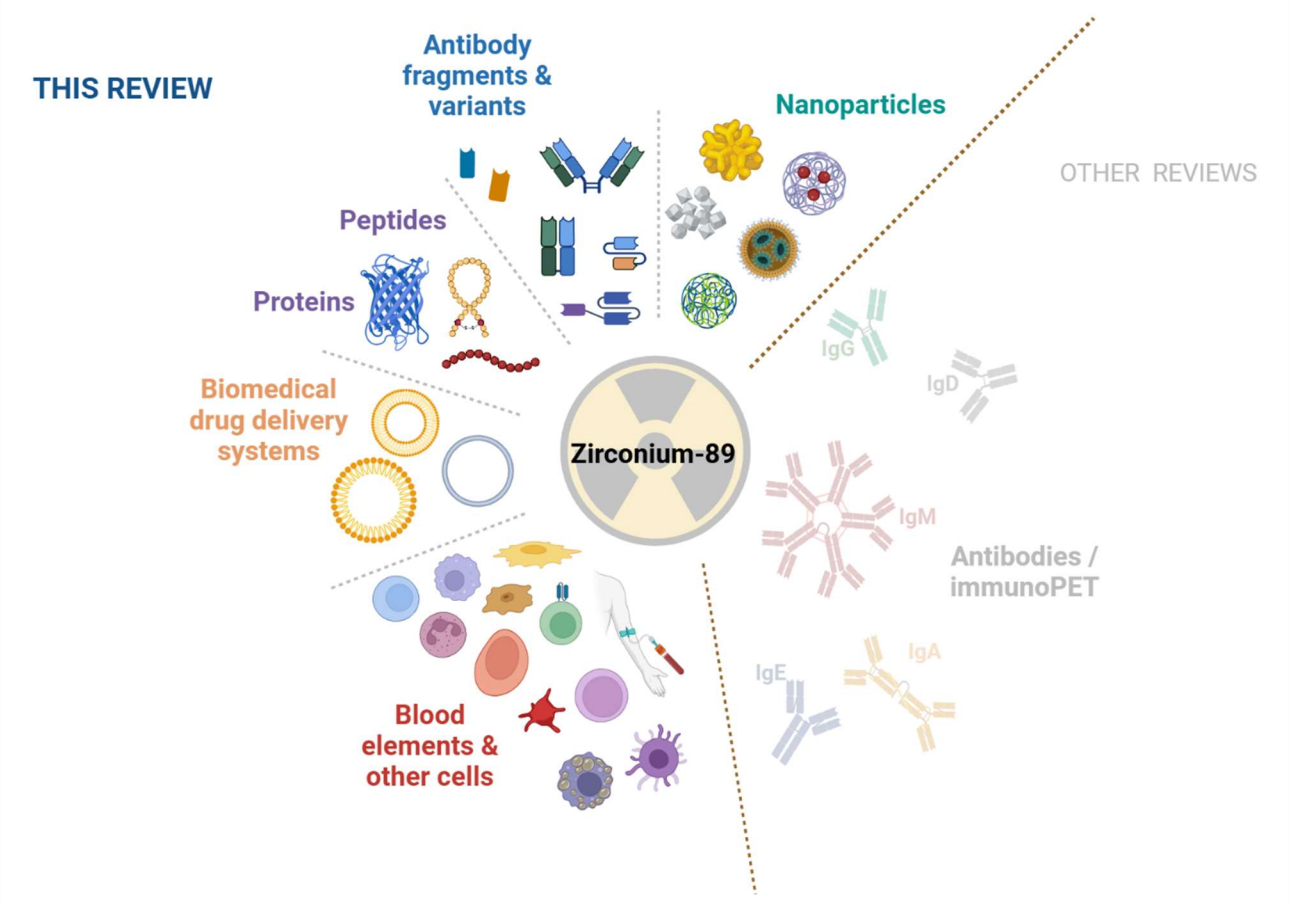


**Figure 1** – Overview of available groups of $^{89}$Zr radiopharmaceuticals, Created in BioRender.com

The classes of $^{89}$Zr-compounds as displayed in **Figure 1** (bold colors) could be identified. Literature on these compounds is discussed below; clinical studies using these $^{89}$Zr-compounds are summarized in **Table 1**.

## $^{89}$Zr-labeled Proteins and Peptides

Bioactive proteins and peptides can be considered suitable molecules for $^{89}$Zr-PET imaging as they may demonstrate a prolonged biodistribution *in vivo*.[16] $^{89}$Zr-labeled proteins have been investigated preclinically for PET imaging in various murine tumor models, including transferrin,[17-19] engineered single-chain vascular endothelial growth factor proteins,[20] anticalin proteins,[21, 22] and thyroid-stimulating hormone receptor-targeting proteins.[23] Furthermore, $^{89}$Zr-labeled human serum albumin has been developed for the early identification of inflammatory foci in a mouse model of atherosclerosis .[24] Various clustered, regularly-interspaced short palindromic repeats (CRISPR) proteins have recently emerged as promising therapeutic agents for a wide range of diseases due to their gene modification capabilities. For example, such a $^{89}$Zr-labeled protein under preclinical investigation is $^{89}$Zr-labeled LbCas12a.[25] The authors successfully performed $^{89}$Zr-labeling of LbCas12a loaded with crRNA, to track it's *in vivo* delivery to targeted cells. They demonstrated that the radiolabel entities ($^{89}$Zr-DFOs) did not alter the therapeutic function of the protein in a mouse model of liver fibrosis and concluded that, in future, radiolabeled CRISPR proteins may be attributed with an important *in vivo* tracking role in various research fields.

[$^{68}$Ga]Ga-PSMA-PET for the detection and localization of prostate cancer recurrence and metastases is well established, often presenting extensive (superscan) tumor uptake shortly after tracer administration.[26] However, the short half-lives of gallium-68 ($^{68}$Ga) and fluorine-18 ($^{18}$F), do not allow late image acquisition. $^{89}$Zr-labeled PSMA has been investigated for its role in low PSMA-expressing prostate cancer cells, where [$^{68}$Ga]Ga-PSMA or [$^{18}$F]F-PSMA fail to detect these foci; delayed imaging may improve tumor-to-background ratios. A long-lived tracer may also more accurately determine the retention time of the ligand in the tumor, which can potentially aid in predicting the response to radionuclide therapy.[27-31] Vázquez *et al*. performed the first-in-human [$^{89}$Zr]Zr-DFO-PSMA-PET[30] imaging. The group published a more recent study enrolling prostate cancer patients with biochemical recurrent cancer and no PSMA-avid lesions detected with [$^{68}$Ga]Ga-PSMA-11- or [$^{18}$F]F-PSMA-007-PET imaging.[27] [$^{89}$Zr]Zr-DFO-PSMA was able to localize 15 PSMA-positive lesions in 8 patients, with a significantly increased SUVmax (11.5 ± 5.8) when compared to the initial PET scans (4.7 ± 2.8).

These promising results confirmed the potential benefit of prolonged PET imaging with [$^{89}$Zr]Zr-DFO-PSMA (i.e., performed 2 to 3 days after administration), to allow ligand internalization for increased tumor-to-background ratios, especially relevant in patients with foci suspected for crucially low PSMA avidity.

In 2022, Privé *et al.* performed first-in-human [$^{89}$Zr]Zr-PSMA-617-PET/CT with two further clinical studies,[29, 31] including a retrospective study which assessed the performance of [$^{89}$Zr]Zr-PSMA-617-PET/CT enrolling patients with negative [$^{68}$Ga]Ga-PSMA-11-PET and biochemical recurrence of prostate cancer (**Table 1**).[31] In 18 of the 23 patients, prostate cancer foci were detected with [$^{89}$Zr]Zr-PSMA-617-PET/CT, with a resultant 36 lesions detected in total, including local recurrence, lymph node metastases, and bone metastases. Prospective clinical studies were recommended.

The radiolabeling of peptides other than PSMA ligands with $^{89}$Zr , for various clinical applications, has been investigated; however, these studies are still confined to the preclinical space. To mention one interesting recent example; a $^{89}$Zr-labeled pH-low insertion peptide, [$^{89}$Zr]Zr-DFO-Cys-Var3 was developed and investigated by Bauer *et al*. for its ability to quantify the acidic tumor environment.[32] Acidic cancer cells are associated with tumor growth and progression and are caused by the increased glycolytic rate, carbonic anhydrase overexpression and the presence of acidic metabolically active tumor-associated macrophages. The authors reported high tumor uptake of [$^{89}$Zr]Zr-DFO-Cys-Var3 in murine late-stage prostate and breast cancer models, with optimal tumor accumulation after 48 h, relative to the degree of acidity of the tumor. The authors were of the opinion that [$^{89}$Zr]Zr-DFO-Cys-Var3 demonstrated potential for clinical translation.[32]

In general, the latter clinical studies, however in small patient cohorts, support the clinical usefulness of $^{89}$Zr-peptides and -proteins and an increasing number of clinical trials may be expected.

## $^{89}$Zr-labeled Antibody Fragments and Smaller Variants

As stated initially, various mAbs have been used in nuclear medicine as carriers for radionuclides for imaging and for therapy; however, with the emergence of nuclear medicine radiotheranostics a unique

medical scope has been created. Thus, $^{131}$I-, $^{225}$Ac-, and $^{117}$Lu-labeled mAbs have extensively been studied for their role in radioimmunotherapy, while $^{89}$Zr-labeled mAbs have contributed the required *in vivo* imaging via immuno-PET. Although mAbs play an indispensable role in nuclear medicine, mainly due to their specificity, their limitations cannot be denied. These limitations include difficult synthesis, long biological residence times and high uptake (radiation) in non-specific tissues/organs. The neonatal Fc-receptor is mainly responsible for the recycling and the subsequent long serum half-lives of mAbs. Although this usually leads to high tumor uptake, it also results in increased activity in the blood and other healthy tissues and thereby reduces the image contrast and/or therapeutic value. This challenge can be overcome by antibody engineering where the Fc-region is removed from the full-sized antibodies, yielding antigen-binding fragments (Fabs) and other, even smaller-sized mAb variants (e.g., the single-chain variable fragment (scFv), affibodies, diabodies, minibodies, and nanobodies). Numerous pre-clinical studies have been done to evaluate $^{89}$Zr-labeled smaller mAb variants in nuclear medicine; however, to date, only a few have progressed to clinical trial investigations. There are several mAbs that have been approved as immune checkpoint inhibitors (ICI) by targeting immune checkpoint proteins involved in cancer.[33] Through activation of immune cells to enhance tumor killing, ICIs have shown great efficacy in patients with advanced metastatic cancer, with some ICIs even suggested to be used as first-line treatment. Although proven beneficial to cancer patients and decreasing mortality rates, there has been a wide variability in treatment response among patients, with some experiencing severe adverse effects. Therefore, methods that would allow a predictable response to ICIs would permit evaluation of patients before treatment. Non-invasive *in vivo* imaging modalities would allow a better understanding of the immune responses and thereby assist in development of better ICIs.

IAB22M2C is a humanized 80 kDa minibody genetically engineered from the murine OHT8 Ab which targets CD8-positive (CD8+) T cells. Preclinical studies of [$^{89}$Zr]Zr-IAB22M2C have demonstrated potential of this probe to be used clinically for PET imaging and thereby assess it as a potential drug candidate.[34] In 2020 Pandit-Taskar *et al*. the first-in-human imaging of $^{89}$Zr-IAB22M2C was reported providing preliminary data indicating its favorable pharmacokinetics and targeting ability.[35] A phase I study of [$^{89}$Zr]Zr-IAB22M2C has demonstrated that [$^{89}$Zr]Zr-IAB22M2C-PET imaging has great

potential to visualize whole-body biodistribution of CD8+ leukocytes in tumors and other reference tissues, and thereby could possibly predict early response to immunotherapy.[36] The use of [$^{89}$Zr]Zr-IAB22M2C-PET/MRI has additionally demonstrated the potential of this technique to assess changes in CD8 expression in metastases and primary and secondary lymphatic organs due to immunotherapy.[37] The human epidermal growth factor receptor 2 (HER2) is overexpressed in various cancers, particularly breast cancer (BCa), and linked with increased tumor proliferation, aggressive disease, and poor prognosis. HER2-targeted mAbs have improved the survival rate of BCa patients; however, triple-negative BCa does not express HER2, therefore these mAbs can neither diagnose nor treat these patients.[38] In spite of this, HER2 nuclear imaging tracers have permitted the quantification and localization of BCa expressing this receptor, and thereby identified patients who would not benefit from HER2-specific therapy due to the absence of HER2 expression. $^{89}$Zr-labeled trastuzumab is one of the most studied HER2-positive imaging agents that has made it possible to both visualize and quantify HER2-expressing lesions. Fab fragments usually have faster clearance rates than trastuzumab and therefore they are expected to provide good tumor detection at earlier time points. However, this rapid clearance of Fabs can potentially limit the overall tumor uptake. The proline-alanine-serine (PAS-)ylation of Fab fragments has been proven to increase their biological half-life. In 2020 Richter *et al*. reported the first-in-human nuclear imaging with a PAS-ylated $^{89}$Zr-labeled anti-HER2 Fab in a patient with metastatic BCa.[39] PAS-ylation was achieved by genetically fusing PAS to the C-terminus of the light chain of the trastuzumab Fab (HER2-Fab). Facile $^{89}$Zr-DFO-labeling of this HER2-Fab-PAS$_{200}$ conjugate was achieved, yielding [$^{89}$Zr]Zr-HER2-Fab-PAS$_{200}$. A newly HER2-positive patient with metastatic brain lesions (identified by MRI) also underwent [$^{89}$Zr]Zr-HER2-Fab-PAS$_{200}$-PET/CT imaging to identify the tumor and assist in therapy planning. Tracer accumulation was clearly visible in the previously diagnosed cranial lymph node metastasis in the left axillary. Additionally, an unknown lesion was detected within the dense left breast parenchyma. However, no tracer uptake was observed in the known brain metastasis or the second axillary lymph node (necrosis observed). Numerous other $^{89}$Zr-labeled fragments and their variants are undergoing pre-clinical investigation for cancer detection.[40-45] Additionally, $^{89}$Zr-labeled fragments are also being explored for preclinical imaging of inflammation and infections.[46-52] For example, a recent study by Lai *et al.* demonstrated how

a $^{89}$Zr-labeled Fab that targets β-glucans within the fungal cell wall was able to accurately visualize *Aspergillus* infections.[52] With the $^{89}$Zr-decay matching the physiological half-life of most mAb variants and fragments it is well foreseeable that an increasing number of such compounds will emerge in clinical trials and will also address imaging of various diseases beyond cancer.

## $^{89}$Zr-labeled Biomedical Drug Delivery Systems

To date, clinical investigations using $^{89}$Zr-labeled cell components such as liposomes have not yet emerged, however, extensive focus has been put on development of lipid-based drug delivery systems (LBDDS) due to their valuable impact on drug delivery and efficacy. As part of these efforts, dedicated preclinical investigation addresses $^{89}$Zr-LBDDS to study enhanced drug efficacy through improved solubility, adsorption, penetration, and site-specific delivery.[53] Additionally, LBDDS can reduce the toxicity of drugs. Different LBDDS types include liposomes, extracellular vesicles (EV), and micelles. *In vivo* imaging methods can help to assess the therapeutic potential of LBDDS, and preclinical studies utilizing $^{89}$Zr-PET have played a pivotal role in this aspect.

Patel *et al.* described a robust method for radiolabelling EVs with $^{89}$Zr-DFO.[54] PET imaging was used to explore the biodistribution and fate of $^{89}$Zr-labeled engineered EVs, administered through different routes, across numerous mammalian species. Intravenous administration in mice, rats, and non-human primates revealed rapid kinetics with accumulation in the liver and spleen. Intraperitoneal and subcutaneous administration showed localisation mainly in lymph nodes, while consistent accumulation in meninges occurred after administration into the cerebrospinal fluid. This study highlighted the opportunity that EVs provide for targeted delivery of drugs. In another study, $^{89}$Zr-labeled EVs were achieved through reaction with [$^{89}$Zr]Zr-(oxinate)$_4$.[55] The radiosynthesis did not affect the morphology or damage the EVs, and PET imaging proved that this method can reliably be used in the future for tracking LBDDS.

Liposomes are the most popular nanoparticle drug delivery system (NPDDS) used. Different $^{89}$Zr-labeling strategies of liposomes have been developed and preclinical PET imaging has furthermore

allowed determining the stability, pharmacokinetics, biodistribution and fate of these liposomes.[56-58] Although most of the preclinical evaluation of NPDDS is focused on cancer therapy, there has been movement to other conditions including imaging of atherosclerosis and bacterial infection.[56, 59, 60] For example, in an attempt to increase the safety profile of the antibacterial drug GSK2485680, the effect of liposome formulation of the drug (end product Lipo680) was studied by PET imaging of [$^{89}$Zr]Zr-Lipo680 in *Escherichia coli*-infected mice.[59] PET imaging revealed that [$^{89}$Zr]Zr-Lipo680 localized at the sites of infection, thereby possibly increasing the therapeutic value of the drug. $^{89}$Zr-PET imaging has been utilized for assessing the ability of liposomal nano-reporters to predict the therapeutic value of a nanodrug. A previous study reported the use of a $^{89}$Zr-labeled liposomal nano-reporter ([$^{89}$Zr]Zr-NRep) to determine the effective therapeutic concentration of co-injected doxorubicin via preclinical PET imaging.[61] [$^{89}$Zr]Zr-NRep was additionally successful in proving that reversible electroporation can facilitate nanoparticle delivery.[62]

The development of polymeric micelles as drug carriers has benefited from micelle-$^{89}$Zr-labeling strategies that permitted real time *in vivo* assessment of polymeric micelles by means of preclinical PET imaging.[63-65] In 2018 Sun *et al.* developed multifunctional nanocarrier micelles that combined two therapeutic anticancer drugs.[65] $^{89}$Zr-labeling of these nanocarriers was carried out by simple click chemistry, allowing for preclinical PET imaging to track the co-delivery of drugs. Micelles have also been used as a strategy to overcome radioresistant tumor environments, such as hypoxia. A recent study reported how fluorinated micelles allowed transport and selective delivery of oxygen to tumor tissues and thereby improved the hypoxic environment to enhance radiation efficacy. The subsequent $^{89}$Zr-labeling of such micelles allowed for preclinical imaging of the drug, highlighting that these micelles could be used as theranostic agents.[64] $^{89}$Zr-labeled delivery systems are relatively new in the toolbox of PET imaging, however based on the positive outcome from studies of the past 5 years, this research space is likely to expand and bring forward novel PET imaging strategies.

## $^{89}$Zr-labeled Nanoparticles

$^{89}$Zr-labeled nanoparticles (NP) are gaining attention in the field of medical imaging and cancer treatment. Overall, NP demonstrate good stability in biological environments, which is suited for application in long-term imaging studies. However, although the half-life of $^{89}$Zr is ideal to track the *in vivo* behavior of NP, the integrity of $^{89}$Zr within the NP-radiopharmaceuticals is crucial to prevent its unwanted release. Therefore, addressing either prolonged residence times or non-specific accumulation in non-targeted organs is a current research focus that is expected to improve the safety, tolerability and also effectiveness of $^{89}$Zr-NP *in vivo*.[66] For $^{89}$Zr-PET image-guided diagnostic procedures a sufficient (but safe) amount of $^{89}$Zr-labeled NPs needs to be administered into the body (non-intravenous routes may be required). The non-invasive signal and time point of optimal imaging may thereby depend on the NP's (zeta) potential, size, as well as its in vivo kinetics and targeting abilities. The clinical usefulness of $^{89}$Zr for NP-based anticancer drug delivery was first reported in 2019.[67] Briefly, the nanoparticle entrapping docetaxel CPC634, designed to improve tumor accumulation and tolerability compared to conventionally administered docetaxel, has been $^{89}$Zr-radiolabeled and studied first-in-human in five subjects with different solid tumors. The biodistribution of [$^{89}$Zr]Zr-DFO-CPC634 was consistent with a prolonged exposure of NPs containing docetaxel. [$^{89}$Zr]Zr-DFO-CPC634-PET/CT hereby visualized a high retention in tumors confirming the enhanced permeability and retention effect of CPC634 in humans. In 2022, nine patients with adenocarcinoma or squamous cell carcinoma were enrolled to validate a new $^{89}$Zr-labeled high-density lipoprotein (HDL) NP. [$^{89}$Zr]Zr-HDL-PET imaging and quantification of the delivered HDL amount to the cancer foci performed. The authors were confident that the positive results support the development of HDL NP as a clinical delivery platform for drug agents.[68] A meeting report highlighted the readiness of the $^{89}$Zr labeled iron oxide nanoparticle drug ferumoxytol ([$^{89}$Zr]Zr-FMX) for prospective measurement of SARS-CoV-2 infection-associated inflammation and "Long Covid" symptomatic. An acceptable radiation dosimetry in non-human primates and outcome from preliminary GMP production support the safety for administration of [$^{89}$Zr]Zr-FMX for first-in-human studies.[69]

Since 2015, the preclinical research landscape has shown a continuously increased use of $^{89}$Zr for radiolabeling a variety of NPs (**Table 2**). $^{89}$Zr-labeled co-polymers, clusters, diamonds, co-carrier and quantum dots are examples for a broad variety of possible applications; however, most of the $^{89}$Zr-NP are currently tested first-in-animals for administration safety and *in vivo* tolerability. An interesting investigation was published by Winter *et al.* using serum albumin coated nanodiamonds (ND), which were functionalized with DFO-PEG groups to allow for either $^{89}$Zr or $^{68}$Ga. Their biodistribution was assessed in two different mouse strains. The [$^{89}$Zr]Zr-DFO-PEG-ND-PET/MRI imaging thereby allowed further evaluation of the ND's physiological properties *in vivo*.[70] By attaching drugs to $^{89}$Zr-nanoparticles, it is possible to deliver the drugs directly to the tumor site, minimizing the impact on healthy tissues and reducing side effects. For example doxorubicin could be targeted to triple negative breast cancer, when it was loaded onto, the nanoscale carrier [$^{89}$Zr]Zr-P1@PEG-RGD which showed the required *in vivo* behavior to serve as a biocompatible nanoplatform for fluorescence and PET image-guided cargo delivery.[71] In another investigation bone targeting [$^{89}$Zr]Zr-$TiO_2$-TF-NPs has shown treatment efficacy towards multiple myeloma cells, a disease of plasma cells originating in the bone marrow, with [$^{89}$Zr]Zr-$TiO_2$-TF generating sufficient amounts of cytotoxic reactive oxygen species to induce cancer cell apoptosis.[72] PET/CT imaging and tissue biodistribution studies hereby revealed that *in vivo* administration of [$^{89}$Zr]Zr-$TiO_2$-TF-NPs in mice leveraged the osteotropic effect of $^{89}$Zr selectivity by localizing about 70% of the injected radioactivity in mouse bone tissue.

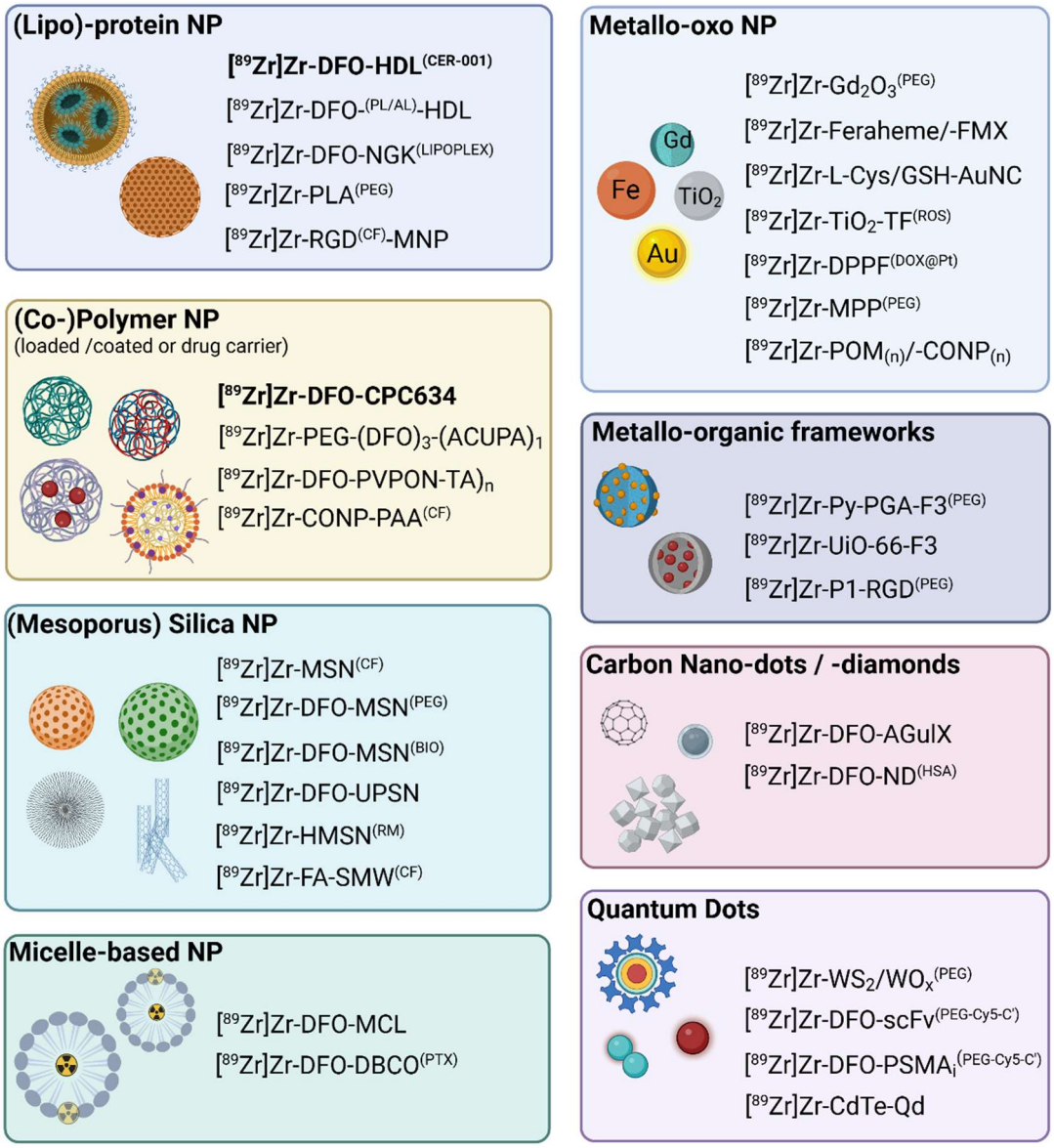


**Figure 2** – Overview of different types of $^{89}$Zr-labeled NP tested in clinical trials (**bold font**) and preclinical evaluations. Created with Biorender.com.

Although NP are considered excellent delivery systems; however, as recently reviewed, various types of challenges are attributed to nanoparticulate systems that are considered for clinical application.[73] Whilst the use of $^{89}$Zr-labeled NP seems generally feasible, continued research and development in this area are essential to fully realize their relevance and clinical potential.

## $^{89}$Zr-labeled Blood Elements and Other Cells

Over the past five years $^{89}$Zr has been investigated extensively for the radiolabeling of a variety of cells, with the focus on immune cells, as well as stem- and progenitor cells. The relatively long radioactive half-life of $^{89}$Zr matches well with the biological half-life and slow trafficking pace or tissue dissemination of activated cells, thus the longitudinal $^{89}$Zr-PET imaging (i.e., weeks after re-administration), potentially results in a more accurate representation of *in vivo* tracking of radiolabeled cells.[74-77] The most common approaches to direct $^{89}$Zr-cell labeling include the incorporation of the lipophilic radiolabel within the cell, as is the case with [$^{89}$Zr]Zr-oxine, as well as cell surface labeling, most often via a deferoxamine-based bifunctional chelating agent, e.g., activated DFO-esters.[77] In 2022, a first-in-human study with [$^{89}$Zr]Zr-oxine-labeled autologous leukocytes was performed by Lapi *et al.* who reported *in vivo* tracking of the radiolabeled cells (7.4 – 18.5 MBq) to the spleen, bone marrow and liver with PET/CT imaging (up to 144 h p.i.). PET/CT imaging of four healthy volunteers did not reveal significant accumulation of radioactivity in the bone matrix, which illustrated the *in vivo* stability of the radiolabel.[78] The authors stated that these promising preliminary results will be applied to design larger studies with the specific focus on the tracking of [$^{89}$Zr]Zr-oxine-labeled autologous leukocytes into the brain and the localization of systemic infections and inflammatory processes. In 2024, Gultekin *et al.* performed a dosimetry study on four healthy female volunteers utilizing the same dose (7.4 - 18.5 MBq) and radiolabel ([$^{89}$Zr]Zr-oxine-labeled autologous leukocytes) as the Lapi research group. The authors found that the average effective dose of [$^{89}$Zr]Zr-oxine-labeled autologous leukocytes was similar to that reported for [$^{111}$In]In-oxine-labeled autologous leukocytes, i.e. 1.06 ± 0.24 mSv/MBq vs 1.14 mSv/MBq, for healthy adult females.[79] Kahts *et al.* utilized the cell surface labeling approach to perform a first-in-human PET/CT infection imaging study with [$^{89}$Zr]Zr-DFO-labeled mononuclear cells in seven patients with clinically or radiologically confirmed infection.[74] Although the authors achieved notable radiolabeling success, the performance of [$^{89}$Zr]Zr-DFO-labeled mononuclear cells were generally comparable to [$^{99m}$Tc]Tc-HMPAO-labeled mononuclear cells, with potentially improved pathological uptake of [$^{89}$Zr]Zr-DFO-labeled mononuclear cells in only two cases of pulmonary disease. The major challenge reported by the

authors was the pulmonary trapping of the radiolabeled cells, which reduced localization to and the detection of radioactive signals in target tissues. The authors recommended further studies utilizing lower concentrations of [$^{89}$Zr]Zr-DFO-Bz-NCS as the conjugate for cell labeling to potentially decrease accumulation of the radiolabeled cells in healthy lung tissues. The inclusion of a wider variety of infectious diseases was also recommended, as the majority of patients that could be recruited for this study had pulmonary pathology.[74]

Many preclinical investigations with $^{89}$Zr-labeled cells have been reported over the past decade, A recent, interesting nonhuman primate study reported by Young *et al.* tracked [$^{89}$Zr]Zr-oxine labeled autologous B cells in two healthy rhesus macaques with the use of PET/CT. The authors concluded a promising outcome for clinical development of engineered B cell medicines, which has emerged as a topic of interest for the potential treatment of various conditions - including metabolic disorders and cancer.[80] Another example of an interesting preclinical study reported recently, is the radiolabeling of *Escherichia coli* (*E.coli*) cells with [$^{89}$Zr]Zr-DFO-Bz-NCS for selective tumor-targeting in a CT-26 xenograft mouse model, hinged on the anaerobic properties of *E. coli*.[81] The authors reported the potential of the *E.coli*-based drug delivery systems to reduce toxic drug concentrations in major organs. Although rapid and prolonged accumulation of [$^{89}$Zr]Zr-DFO-*E.coli* in the tumor was observed, increased uptake by the reticuloendothelial system (especially the liver and spleen) warrants further investigation.

Although it is evident that the radiolabeling of cells with zirconium-89 is a developing field, it is noteworthy that current cell labeling approaches with technetium-99m and indium-111 may be deemed sufficient by many clinical practices, especially those that do not have the necessary infrastructure for PET, and major investment into $^{89}$Zr-cell labeling approaches may therefore not realize.

**Table 1**: Overview of clinical investigation using novel $^{89}$Zr-radiopharmaceuticals**

| Year | Tracer | Type | Clinical indication | Study population | |
|---|---|---|---|---|---|
| 2022 | [$^{89}$Zr]Zr-oxine-WBC | Cells | FIH, RD | HV, n=4* | 78 |
| 2024 | [$^{89}$Zr]Zr-oxine-WBC | Cells | FIH, RD | HV (♀), n= 4* | 79 |
| 2024 | [$^{89}$Zr]Zr-DFO-PBMC | Cells | suspected infection | n= 8 | 74 |
| 2016 | [$^{89}$Zr]Zr-DFO-IAB2M | MiB | metastatic PC | n=18 | 82 |
| 2016 | [$^{89}$Zr]Zr-DFO-IAB2M | MiB | metastatic PC | n= 38 (Phase I/IIa -NCT01923727) | 83 |
| 2019 | [$^{89}$Zr]Zr-DFO-IAB2M | MiB | metastatic PC | n= 9* (Phase II -NCT03675451) | 84 |
| 2022 | [$^{89}$Zr]Zr-DFO-IAB2M | MiB | localized PC | n=20 | 85 |
| 2020 | [$^{89}$Zr]Zr-DFO-IAB22M2C | MiB | metastatic cancers | n= 6 (Phase I -NCT03107663) | 35 |
| 2020 | [$^{89}$Zr]Zr-DFO-IAB22M2C | MiB | FIH, melanoma | n= 2* | 86 |
| 2022 | [$^{89}$Zr]Zr-DFO-IAB22M2C | MiB | metastatic cancers | n=15 (Phase I -NCT03107663) | 36 |
| 2023 | [$^{89}$Zr]Zr-DFO-IAB22M2C | MiB | metastatic cancers | n= 8 | 37 |
| 2020 | [$^{89}$Zr]Zr-HER2-Fab-PAS$_{200}$ | Fab | FIH, metastatic BCa | n= 1 | 39 |
| 2022 | [$^{89}$Zr]Zr-DFO-PSMA | Pep | FIH; PC (BCR) | n= 1 | 30 |
| 2022 | [$^{89}$Zr]Zr-DFO-PSMA | Pep | PC (BCR) | n=14 | 27 |
| 2022 | [$^{89}$Zr]Zr-DOTA-PSMA-617 | Pep | PC (BCR) | n= 7 | 29 |
| 2022 | [$^{89}$Zr]Zr-DOTA-PSMA-617 | Pep | FIH; dosimetry | n= 1 | 28 |
| 2023 | [$^{89}$Zr]Zr-DOTA-PSMA-617 | Pep | PC (BCR) | n=23 | 31 |
| 2019 | [$^{89}$Zr]Zr-DFO-CPC634 | NP | localized cancer types | n= 5 | 67 |
| 2022 | [$^{89}$Zr]Zr-DFO-HDL$_{(CER-001)}$ | NP | oesophageal cancer | n= 9 | 68 |

***Footnotes and Abbreviations:*** *) preliminary meeting report; **) studies using full-sized antibody are excluded; RD) radiation dosimetry investigation; HV) healthy volunteers; FIH) first-in -human; PBMC) peripheral blood mononuclear cells; WBC) white blood cells/ leucocytes; PC) prostate cancer, BCa) breast cancer; BCR) biochemical recurrence; MiB) minibody; Pep) peptide; Fab) antibody fragment antigen binding region; NP) nanoparticles

# Limitations and Challenges

Whilst $^{89}$Zr-radiopharmaceuticals offer significant benefits for PET imaging, particularly in oncology, literature also reports on various study limitations and challenges. Positively, the production and radiochemical processing of $^{89}$Zr is now well-understood, with several commercial suppliers making $^{89}$Zr in industrial-level quantities, so a worldwide availability at a reasonable price may be possible. However, the production of $^{89}$Zr involves complex radiochemical processes that require stringent safety protocols to prevent contamination and ensure the stability of the radiolabeled compounds. Improper handling can lead to radiation exposure for healthcare workers and contamination of the environment. In addition, regulatory hurdles and safety concerns, as with any radiopharmaceutical, are particularly associated with the use of $^{89}$Zr-NP in humans. Ensuring compliance with safety standards and obtaining regulatory approvals can be time-consuming and may delay the clinical application of these imaging agents. Technical limitations in access to imaging technology can also pose challenges. The need for specialized equipment and expertise to perform $^{89}$Zr-immuno-PET imaging can limit its accessibility in clinical practice. Additionally, the complexity of the imaging protocols may require extensive training for healthcare professionals. Measurement sensitivity, e.g., noise interference is another reported challenge that complicates the imaging process. The presence of noise may obscure the true signal, especially from $^{89}$Zr-NP or other $^{89}$Zr-labeled macromolecules, making it difficult to distinguish between healthy and diseased tissues. This interference can lead to false positives or negatives in tumor detection, which is particularly concerning in clinical settings where accurate diagnosis is critical. Addressing any secondary (off-target) pharmacology and undesired organ radiation is often underrepresented within the preclinical settings and may be limiting exploratory clinical investigations. For example, despite the promising applications of $^{89}$Zr-NP, challenges remain, including the potential for non-specific accumulation in certain tissues and the need for improved targeting strategies. Future research may also focus on enhancing the specificity of $^{89}$Zr-labeled agents through advanced conjugation techniques and the development of novel chelators that improve biodistribution profiles.

# Conclusive Statements

The past decade has shown an avid increase in research concerning $^{89}$Zr-radiopharmaceuticals aside from $^{89}$Zr-mAb. 89-Zirconium's steadily expanding role may be directly attributed to the globally growing cyclotron landscape, the successful development of highly efficient $^{89}$Zr-production processes and technological advancement made in PET imaging both preclinically and in the clinics. Overall, $^{89}$Zr 's unique properties and its integration into advanced imaging techniques make it a valuable tool in nuclear medicine, particularly for the diagnosis and treatment of complex diseases. Considering $^{89}$Zr as one of the most versatile radioisotopes, it is likely that more compound classes will soon be joining $^{89}$Zr-radiopharmaceutical development. As highlighted in this review, a large variety of new compounds is currently being investigated in this research space. By their demonstrated diagnostic potential, some novel $^{89}$Zr-PET tracers are clinically tested and increasingly used to monitor cancer therapies, evaluate the biodistribution of therapeutic compounds or drugs (delivered or shuttled to the target) and assist in patient selection for targeted treatments.[87] For instance, filling the gap in clinical investigations using [$^{89}$Zr]Zr-PSMA-PET/CT imaging for patients with low-density PSMA expression, exemplifies the value and significance of a late-imaging option to "detect the undetected" prostate cancer lesions through enhanced sensitivity and diagnostic accuracy. Although this aspect is not being covered in this review, the development of new chelators and radiolabeling techniques has improved the stability and effectiveness of $^{89}$Zr-radiopharmaceuticals.[88] This move has led to more reliable and accurate imaging results, further driving their adoption in both preclinical and clinical research. In addition, tremendous research efforts are ongoing driving the development of humanized animal models such as cancer xenografts, or PDX as well as animals developing infection, inflammation or autoimmune disease.[89-91] To improve the relevance of animal models to better translate results to human diseases, researchers must develop more sophisticated humanized models that better mimic human physiology, and standardizie protocols and statistical analyses to enhance reproducibility.[92] In addition, in radiopharmaceutical development sometimes the complexity of diseases may not be sufficiently represented in animals.

In conclusion, the studies discussed in this review are testament to a future warranting expedited

research efforts on novel $^{89}$Zr-labeled compounds in diverse cancer types and to refine their application in clinical settings. Hence, the integration of $^{89}$Zr-radiopharmaceuticals into routine diagnostic workflows could significantly enhance patient outcomes through earlier and more accurate detection of malignancies.

# Declaration of Competing Interest

All authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

# CRediT Authorship Contribution Statement

Janie Duvenhage: Investigation, Visualization, Writing original draft, Reviewing. Maryke Kahts: Investigation, Visualization, Writing original draft, Reviewing. Beverley Summers: Conceptualization, Supervision, Visualization, Review & Editing. Jan Rijn Zeevaart: Project administration, Supervision, Writing original draft, Review & Editing. Thomas Ebenhan: Conceptualization, Project administration, Supervision, Writing original draft, Review & Editing.

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# References

1. Harsini S and Rezaei N Cancer imaging with radiolabeled monoclonal antibodies. Cancer Immunology: A Translational Medicine Context 739-760, 2020

2. De Feo MS, Pontico M, Frantellizzi V, et al: 89Zr-PET imaging in humans: a systematic review. Clin Transl Imaging 10:23-36, 2022

3. Lugat A, Bailly C, Chérel M, et al: Immuno-PET: Design options and clinical proof-of-concept. Front Med 9:1026083, 2022

4. Börjesson PK, Jauw YW, Boellaard R, et al: Performance of immuno–positron emission tomography with zirconium-89-labeled chimeric monoclonal antibody U36 in the detection of lymph node metastases in head and neck cancer patients. Clin Cancer Res 12:2133-2140, 2006

5. Vosjan MJWD, Perk LR, Visser GWM, et al: Conjugation and radiolabeling of monoclonal antibodies with zirconium-89 for PET imaging using the bifunctional chelate p-isothiocyanatobenzyl-desferrioxamine. Nat Protoc 5:739-743, 2010

6. Watabe T, Kabayama K, Naka S, et al: Immuno-PET and Targeted α-Therapy Using Anti–Glypican-1 Antibody Labeled with 89Zr or 211At: A Theranostic Approach for Pancreatic Ductal Adenocarcinoma. J Nucl Med 64:1949-1955, 2023

7. Abou DS, Longtine M, Fears A, et al: Evaluation of candidate theranostics for 227Th/89Zr paired radioimmunotherapy of lymphoma. J Nucl Med 64:1062-1068, 2023

8. van Dongen GA, Beaino W, Windhorst AD, et al: The role of 89Zr-immuno-PET in navigating and derisking the development of biopharmaceuticals. J Nucl Med 62:438-445, 2021

9. Parakh S, Lee ST, Gan HK, et al: Radiolabeled antibodies for cancer imaging and therapy. Cancers 14:1454, 2022

10. Deri MA, Zeglis BM, Francesconi LC, et al: PET imaging with 89Zr: from radiochemistry to the clinic. Nucl Med Biol 40:3-14, 2013

11. VanBrocklin HF Chapter 25 - PET Radiochemistry. Molecular Imaging (Second Edition) 445-478, 2021

12. Wuensche TE, Lyashchenko S, van Dongen GAMS, et al: Good practices for 89Zr radiopharmaceutical production and quality control. Eur J Nucl Med Mol Imaging Radiopharmacy and Chemistry 9:40, 2024

13. Pandey MK, Engelbrecht HP, Byrne JF, et al: Production of 89Zr via the 89Y (p, n) 89Zr reaction in aqueous solution: effect of solution composition on in-target chemistry. Nucl Med Biol 41:309-316, 2014

14. Noor A, Roselt PD, McGowan ER, et al: Automated synthesis of [89Zr]ZrCl4, [89Zr]ZrDFOSquaramide-bisPh(PSMA) and [89Zr]ZrDFOSquaramide-TATE. Eur J Nucl Med Mol Imaging Radiopharmacy and Chemistry 9:39, 2024

15. Polyak A, Bankstahl JP, Besecke KFW, et al: Simplified (89)Zr-Labeling Protocol of Oxine (8-Hydroxyquinoline) Enabling Prolonged Tracking of Liposome-Based Nanomedicines and Cells. Pharm 13:2021

16. Fischer G, Seibold U, Schirrmacher R, et al: 89Zr, a radiometal nuclide with high potential for molecular imaging with PET: chemistry, applications and remaining challenges. Molecules 18:6469-6490, 2013

17. Truillet C, Cunningham JT, Parker MFL, et al: Noninvasive Measurement of mTORC1 Signaling with 89Zr-Transferrin. Clin Cancer Res 23:3045-3052, 2017

18. Henry KE, Dacek MM, Dilling TR, et al: A PET imaging strategy for interrogating target engagement and oncogene status in pancreatic cancer. Clin Cancer Res 25:166-176, 2019

19. Henry KE, Dilling TR, Abdel-Atti D, et al: Noninvasive 89Zr-transferrin PET shows improved tumor targeting compared with 18F-FDG PET in MYC-overexpressing human triple-negative breast cancer. J Nucl Med 59:51-57, 2018

20. Meyer J-P, Edwards KJ, Kozlowski P, et al: Selective imaging of VEGFR-1 and VEGFR-2 using 89Zr-labeled single-chain VEGF mutants. J Nucl Med 57:1811-1816, 2016

21. van Scheltinga AGT, Lub-de Hooge MN, Hinner MJ, et al: In vivo visualization of MET tumor expression and anticalin biodistribution with the MET-specific anticalin 89Zr-PRS-110 PET tracer. J Nucl Med 55:665-671, 2014

22. Morath V, Brandt C, Deuschle F-C, et al: Molecular Design of 68Ga-and 89Zr-Labeled Anticalin Radioligands for PET-Imaging of PSMA-Positive Tumors. Mol Pharm 20:2490-2501, 2023

23. Gimblet GR, Houson HA, Whitt J, et al: PET Imaging of Differentiated Thyroid Cancer with TSHR-Targeted [89Zr] Zr-TR1402. Mol Pharm 21:3889-3896, 2024

24. Ahmed M, Tegnebratt T, Tran TA, et al: Molecular imaging of inflammation in a mouse model of atherosclerosis using a zirconium-89-labeled probe. Int J Nanomed 6137-6152, 2020

25. Hwang I, Lee JY, Kim T-H, et al: A radioactive CRISPR interference system using 89Zr-labeled LbCas12a. J Control Release 365:703-715, 2024

26. Lawal I, Vorster M, Boshomane T, et al: Metastatic prostate carcinoma presenting as a superscan on 68Ga-PSMA PET/CT. Clin Nucl Med 40:755-756, 2015

27. Dietlein F, Kobe C, Vázquez SM, et al: An 89Zr-labeled PSMA tracer for PET/CT imaging of prostate cancer patients. J Nucl Med 63:573-583, 2022

28. Privé BM, Derks YH, Rosar F, et al: 89 Zr-labeled PSMA ligands for pharmacokinetic PET imaging and dosimetry of PSMA-617 and PSMA-I&T: a preclinical evaluation and first in man. Eur J Nucl Med Mol Imaging 1-13, 2022

29. Rosar F, Schaefer-Schuler A, Bartholomä M, et al: [89Zr] Zr-PSMA-617 PET/CT in biochemical recurrence of prostate cancer: first clinical experience from a pilot study including biodistribution and dose estimates. Eur J Nucl Med Mol Imaging 49:4736-4747, 2022

30. Vázquez SM, Endepols H, Fischer T, et al: Translational development of a Zr-89-labeled inhibitor of prostate-specific membrane antigen for PET imaging in prostate cancer. Mol Imaging Biol 24:115-125, 2022

31. Rosar F, Khreish F, Marlowe RJ, et al: Detection efficacy of [89Zr] Zr-PSMA-617 PET/CT in [68Ga] Ga-PSMA-11 PET/CT-negative biochemical recurrence of prostate cancer. Eur J Nucl Med Mol Imaging 50:2899-2909, 2023

32. Bauer D, Visca H, Weerakkody A, et al: PET imaging of acidic tumor environment with 89Zr-labeled pHLIP probes. Front Oncol 12:882541, 2022

33. Shiravand Y, Khodadadi F, Kashani SMA, et al: Immune Checkpoint Inhibitors in Cancer Therapy. Curr Oncol 29:3044-3060, 2022

34. Griessinger CM, Olafsen T, Mascioni A, et al: The PET-tracer 89Zr-Df-IAB22M2C enables monitoring of intratumoral CD8 T-cell infiltrates in tumor-bearing humanized mice after T-cell bispecific antibody treatment. Cancer Res 80:2903-2913, 2020

35. Pandit-Taskar N, Postow MA, Hellmann MD, et al: First-in-humans imaging with 89Zr-Df-IAB22M2C anti-CD8 minibody in patients with solid malignancies: preliminary pharmacokinetics, biodistribution, and lesion targeting. J Nucl Med 61:512-519, 2020

36. Farwell MD, Gamache RF, Babazada H, et al: CD8-targeted PET imaging of tumor-infiltrating T cells in patients with cancer: a phase I first-in-humans study of 89Zr-Df-IAB22M2C, a radiolabeled anti-CD8 minibody. J Nucl Med 63:720-726, 2022

37. Schwenck J, Sonanini D, Seyfried D, et al: In vivo imaging of CD8+ T cells in metastatic cancer patients: first clinical experience with simultaneous [89Zr] Zr-Df-IAB22M2C PET/MRI. Theranostics 13:2408, 2023

38. Massicano AV, Marquez-Nostra BV and Lapi SE Targeting HER2 in nuclear medicine for imaging and therapy. Mol Imaging 17:1536012117745386, 2018

39. Richter A, Knorr K, Schlapschy M, et al: First in-human medical imaging with a PASylated 89 Zr-labeled anti-HER2 fab-fragment in a patient with metastatic breast cancer. Nucl Med Mol Imaging 54:114-119, 2020

40. Garousi J, Andersson KG, Mitran B, et al: PET imaging of epidermal growth factor receptor expression in tumours using 89Zr-labelled ZEGFR: 2377 affibody molecules. Int J Oncol 48:1325-1332, 2016

41. Rudd SE, Van Zuylekom JK, Raicevic A, et al: Enzyme mediated incorporation of zirconium-89 or copper-64 into a fragment antibody for same day imaging of epidermal growth factor receptor. Chem Sci 12:9004-9016, 2021

42. Alizadeh E, Behlol Ayaz Ahmed K, Raja Solomon V, et al: 89Zr-Labeled Domain II-Specific ScFv-Fc ImmunoPET Probe for Imaging Epidermal Growth Factor Receptor In Vivo. Cancers 13:560, 2021

43. Alizadeh E, Solomon VR, Zuluaga C, et al: 89Zr-labeled domain II specific anti-EGFR ScFv-Fc antibody fragment for imaging and monitoring of response to anti-EGFR treatment by PET. J Nucl Med 59:464, 2018

44. Ho K-V, Tatum DS, Watkinson L, et al: Single Chelator–Minibody Theranostic Agents for 89Zr PET Imaging and 177Lu Radiopharmaceutical Therapy of PSMA-Expressing Prostate Cancer. J Nucl Med 65:1435-1442, 2024

45. Summer D, Garousi J, Oroujeni M, et al: Cyclic versus noncyclic chelating scaffold for 89Zr-labeled ZEGFR: 2377 affibody bioconjugates targeting epidermal growth factor receptor overexpression. Mol Pharm 15:175-185, 2018

46. Kasten BB, Houson HA, Coleman JM, et al: Positron emission tomography imaging with 89Zr-labeled anti-CD8 cys-diabody reveals CD8+ cell infiltration during oncolytic virus therapy in a glioma murine model. Sci Rep 11:15384, 2021

47. Freise AC, Zettlitz KA, Salazar FB, et al: Immuno-PET in inflammatory bowel disease: imaging CD4-positive T cells in a murine model of colitis. J Nucl Med 59:980-985, 2018

48. Tavaré R, McCracken MN, Zettlitz KA, et al: Immuno-PET of murine T cell reconstitution postadoptive stem cell transplantation using anti-CD4 and anti-CD8 cys-diabodies. J Nucl Med 56:1258-1264, 2015

49. Freise AC, Zettlitz KA, Salazar FB, et al: ImmunoPET imaging of murine CD4+ T cells using anti-CD4 cys-diabody: effects of protein dose on T cell function and imaging. Mol Imaging Biol 19:599-609, 2017

50. Varasteh Z, De Rose F, Mohanta S, et al: Imaging atherosclerotic plaques by targeting Galectin-3 and activated macrophages using (89Zr)-DFO-Galectin3-F (ab') 2 mAb. Theranostics 11:1864, 2021

51. Muns JA, Schooten E, van Dasselaar RD, et al: Preclinical targeting of liver fibrosis with a 89Zr-labeled Fibrobody® directed against platelet derived growth factor receptor-β. Eur J Nucl Med Mol Imaging 1-14, 2024

52. Lai J, Shah S, Martinez-Orengo N, et al: PET imaging of Aspergillus infection using Zirconium-89 labeled anti-β-glucan antibody fragments. Eur J Nucl Med Mol Imaging 1-12, 2024

53. Gbian DL and Omri A Lipid-based drug delivery systems for diseases managements. Biomedicines 10:2137, 2022

54. Patel S, Schmidt KF, Farhoud M, et al: In vivo tracking of [89Zr] Zr-labeled engineered extracellular vesicles by PET reveals organ-specific biodistribution based upon the route of administration. Nucl Med Biol 112:20-30, 2022

55. Liu W, Ma H, Li F, et al: PET imaging of VEGFR and integrins in glioma tumor xenografts using 89Zr labelled heterodimeric peptide. Bioorg Med Chem 59:116677, 2022

56. Edmonds S, Volpe A, Shmeeda H, et al: Exploiting the metal-chelating properties of the drug cargo for in vivo positron emission tomography imaging of liposomal nanomedicines. ACS Nano 10:10294-10307, 2016

57. Li N, Yu Z, Pham TT, et al: A generic 89Zr labeling method to quantify the in vivo pharmacokinetics of liposomal nanoparticles with positron emission tomography. Int J Nanomedicine 3281-3294, 2017

58. Pérez-Medina C, Abdel-Atti D, Zhang Y, et al: A modular labeling strategy for in vivo PET and near-infrared fluorescence imaging of nanoparticle tumor targeting. J Nucl Med 55:1706-1711, 2014

59. Cheng S-H, Groseclose R, Mininger C, et al: A Multimodal Imaging Approach to Assess the Quantitative Biodistribution of a Liposomal Nanoformulation with the antibiotic GSK2485680 (Lipo680). 2022

60. Lobatto ME, Binderup T, Robson PM, et al: Multimodal positron emission tomography imaging to quantify uptake of 89Zr-labeled liposomes in the atherosclerotic vessel wall. Bioconjugate Chem 31:360-368, 2019

61. Pérez-Medina C, Abdel-Atti D, Tang J, et al: Nanoreporter PET predicts the efficacy of anti-cancer nanotherapy. Nat Commun 7:11838, 2016

62. Srimathveeravalli G, Abdel-Atti D, Pérez-Medina C, et al: Reversible electroporation–mediated liposomal doxorubicin delivery to tumors can be monitored with 89Zr-labeled reporter nanoparticles. Mol Imaging 17:1536012117749726, 2018

63. Starmans LW, Hummelink MA, Rossin R, et al: 89Zr-and Fe-Labeled Polymeric Micelles for Dual Modality PET and T1-Weighted MR Imaging. Adv Healthc Mater 4:2137-2145, 2015

64. Godel-Pastre S, Porcel E, Pinna G, et al: Tumor-Targeted Perfluorinated Micelles as Efficient Theranostic Agents Combining Positron Emission Tomography and Radiosensitization. ACS Appl Mater Interfaces 16:21557-21570, 2024

65. Sun J, Sun L, Li J, et al: A multi-functional polymeric carrier for simultaneous positron emission tomography imaging and combination therapy. Acta Biomater 75:312-322, 2018

66. Choi PS, Lee JY, Yang SD, et al: Biological behavior of nanoparticles with Zr-89 for cancer targeting based on their distinct surface composition. J Mater Chem B 9:8237-8245, 2021

67. Miedema IH, Zwezerijnen GJ, Oprea-Lager DE, et al: First-in-human imaging of nanoparticle entrapped docetaxel (CPC634) in patients with advanced solid tumors using 89Zr-Df-CPC634 PET/CT. J Clin Oncol 2019

68. Zheng KH, Kroon J, Schoormans J, et al: 89Zr-labeled high-density lipoprotein nanoparticle PET imaging reveals tumor uptake in patients with esophageal cancer. J Nucl Med 63:1880-1886, 2022

69. Wilks M, Yuan H, Wang F, et al: Radiation Dosimetry in Non-Human Primates and Preliminary GMP Production Methods: Progress Towards First-in-Human Studies with [89Zr] Zr-Ferumoxytol. J Nucl Med 64:672, 2023

70. Winter G, Eberhardt N, Löffler J, et al: Preclinical PET and MR Evaluation of 89Zr-and 68Ga-Labeled Nanodiamonds in Mice over Different Time Scales. Nanomat 12:4471, 2022

71. Li Y-P, Pan Z-W, Jiang Y-J, et al: Zirconium-containing nanoscale coordination polymers for positron emission tomography and fluorescence-guided cargo delivery to triple-negative breast tumors. Acta Biomat 179:313-324, 2024

72. Tang R, Zheleznyak A, Mixdorf M, et al: Osteotropic radiolabeled nanophotosensitizer for imaging and treating multiple myeloma. ACS Nano 14:4255-4264, 2020

73. Kleynhans J, Sathekge M and Ebenhan T Obstacles and recommendations for clinical translation of nanoparticle system-based targeted alpha-particle therapy. Materials 14:4784, 2021

74. Kahts M, Summers B, Ndlela AN, et al: First-in-human infection imaging with 89Zr-labelled leukocytes and comparison of scan quality with [99mTc] Tc-HMPAO-labelled leukocytes. Front Nucl Med 4:1426650, 2024

75. Pham TT, Chenoweth A, Patel N, et al: In Vivo PET Imaging of 89Zr-Labeled Natural Killer Cells and the Modulating Effects of a Therapeutic Antibody. J Nucl Med 2024

76. Xu Q, Wang X, Mu Z, et al: Repurposing iron chelators for accurate positron emission tomography imaging tracking of radiometal-labeled cell transplants. MedComm 5:e473, 2024

77. Gawne PJ, Man F, Blower PJ, et al: Direct Cell Radiolabeling for in Vivo Cell Tracking with PET and SPECT Imaging. Chem Rev 122:10266-10318, 2022

78. Lapi S, McConathy J, Jeffers C, et al: First-in-human imaging of 89Zr-oxine labelled autologous leukocytes in healthy volunteers. J Nucl Med 63:2447, 2022

79. Gultekin K, Cardenas C, Jones CL, et al: Measurement of Human Dosimetry of [89Zr] oxine-labeled White Blood Cells with PET/CT using CT-based segmentation. J Nucl Med 65:242377, 2024

80. Young DJ, Edwards AJ, Quiroz Caceda KG, et al: In vivo tracking of ex vivo generated 89Zr-oxine labeled plasma cells by PET in a non-human primate model. bioRxiv, 2024

81. Kim GG, Lee H, Jeong DB, et al: Long-Term Tumor-Targeting Effect of E. coli as a Drug Delivery System. Pharm 17:421, 2024

82. Pandit-Taskar N, O'Donoghue JA, Ruan S, et al: First-in-human imaging with 89Zr-Df-IAB2M anti-PSMA minibody in patients with metastatic prostate cancer: pharmacokinetics, biodistribution, dosimetry, and lesion uptake. J Nucl Med 57:1858-1864, 2016

83. Morris MJ, Martinez DF, Durack JC, et al: A phase I/IIa trial of prostate specific membrane antigen (PSMA) positron emission tomography (PET) imaging with 89Zr-Df-IAB2M in metastatic prostate cancer (PCa). J Clin Oncol, 2016

84. Niaz MJ, Jhanwar Y, Flynn T, et al: Pilot study of dual imaging with 89Zr-IAB2M and 68Ga-PSMA-11 PET/CT prior to prostatectomy. J Clin Oncol 37:90-90, 2019

85. Vlachostergios PJ, Niaz MJ, Thomas C, et al: Pilot study of the diagnostic utility of 89Zr-df-IAB2M and 68Ga-PSMA-11 PET imaging and multiparametric MRI in localized prostate cancer. The Prostate 82:483-492, 2022

86. Daube-Witherspoon M, Farwell M, Sunderland J, et al: Optimization of 89Zr PET imaging using both phantom and clinical studies with [89Zr]-Df-IAB22M2C, an anti-CD8 minibody. J Nucl Med 62, 2021

87. Yoon J-K, Park B-N, Ryu E-K, et al: Current perspectives on 89Zr-PET imaging. Int J Mol Sci 21:4309, 2020

88. Kang CS, Zhang S, Wang H, et al: Novel chelating agents for zirconium-89-positron emission tomography (PET) imaging: Synthesis, DFT calculation, radiolabeling, and in vitro and in vivo complex stability. ACS Omega 7:37229-37236, 2022

89. Oyen W, Boerman O and Corstens F Animal models of infection and inflammation and their role in experimental nuclear medicine. J Microbiol Methods 47:151-157, 2001

90. Mather S, Maina T and de Jong M Animal Models for Developing Radiopharmaceuticals for Therapy. Therapeutic Nuclear Medicine 807-815, 2014

91. Wekerle H, Flügel A, Fugger L, et al: Autoimmunity's next top models. Nat Med 18:66-70, 2012

92. Kleynhans J, Ebenhan T, Cleeren F, et al: Can current preclinical strategies for radiopharmaceutical development meet the needs of targeted alpha therapy? Eur J Nucl Med Mol Imag 51:1965-1980, 2024