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*Short communication*

# Zirconium-89 labeled antibodies: general considerations towards radioisotope production and labelling strategies

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## Introduction

Radiopharmaceutical preparations based on zirconium-89 ($^{89}$Zr) radioisotope in the last decade have been increasingly used in preclinical and clinical studies for visualisation by positron emission tomography (PET). There are literature data on $^{89}$Zr labelling of nanoparticles, proteins, peptides and cells, but antibody labelling is the main application of this radioisotope. As a long-lived radiometal, with a half-life of 78.4 h, zirconium-89 is suitable for visualising slow biological processes, such as antibody biodistribution (immuno-PET). $^{89}$Zr-immuno-PET imaging is a promising technique for predicting the efficacy of radioimmunotherapy and antibody therapies, imaging target expression, detecting target-expressing tumours, and monitoring anti-cancer chemotherapies. According to ClinicalTrials.gov, there are more than 120 clinical studies, of which already completed studies involve more than 20 antibodies labelled with $^{89}$Zr. The most common antibodies used in these clinical trials are bevacizumab, trastuzumab, IAB2M, cetuximab, pembrolizumab, J591, panitumumab, girentuximab, pertuzumab etc. The purpose of this paper is to present the most common methods of producing zirconium-89 radioisotope and antibody labelling strategies.

## Materials and methods

A search on PubMed and Google Scholar included the keywords: zirconium-89 production, 89Zr production, 89Zr radiochemistry, and 89Zr labeling.

## Results and discussion

*Production of zirconium-89 radioisotope:* $^{89}$Zr is a radioisotope of zirconium with 49 neutrons and 40 protons. It decays to $^{89}$Y via electron capture (77%) and positron emission (23%). Zirconium-89 radioisotope can be obtained in a cyclotron (particle accelerator which propels a beam of charged particles in a circular path) by irradiating a solid target with protons of low energy (10–18 MeV). Yttrium-89, which is 100% naturally present in the earth's crust, is used as the target material. The most commonly used nuclear reaction for the production of $^{89}$Zr is $^{89}$Y(p,n)$^{89}$Zr. Another reaction is $^{89}$Y(d,2n)$^{89}$Zr, but due to the availability of the proton beam in most medical cyclotrons and suitable beam energy coverage, the method of choice is the $^{89}$Y(p,n)$^{89}$Zr reaction.

A few types and techniques for the preparation of yttrium solid target as a starting material are reported: foils, pellets, sputtered layers and electrodeposition. Production of $^{89}$Zr using yttrium liquid target, $Y(NO_3)_3$ solution, was also registered. Given the cross-sections for the production of $^{88}$Zr, $^{89}$Y and $^{89}$Zr by proton irradiation of yttrium, the optimal energy for proton bombardment is considered to be 14 MeV (Jalilian at Osso, 2017; Kasbollah et al., 2013).

In general, the production process of $^{89}$Zr using a solid target includes the following phases: proton irradiation of the target material, dissolution of the irradiated target by hydrochloric acid, and purification. Impurities must be separated from $^{89}$Zr because they could compete with antibodies in labelling. As separation methods are reported: solvent extraction, cation and anion extraction chromatography and separation by solid-phase

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hydroxamate resins. Weak cation exchange chromatography using hydroxamate-modified resin has been introduced as the method of choice because it provides high recovery of $^{89}$Zr and high (≥ 99.9%) radionuclidic and radiochemical purity. In this method, $^{89}$Zr, ionically bonded to the hydroxamate resin column, is eluted using oxalic acid in a concentration of at least 0.5 M. Because of the toxicological aspects of oxalic acid for *in vivo* application, oxalate anions are removed using another strong anion exchange column, which is flushed with a large volume of water, followed by the use of HCl for chloride exchange. The produced zirconium-89 radioisotope is tested for radionuclidic purity, chemical purity, and radiochemical purity (Holland et al., 2009).

*Antibody radiolabelling:* The physical half-life of zirconium-89 ($t_{1/2}$ = 3.3 days) well matches the biological half-life of full-size monoclonal antibodies, allowing optimal biodistribution for studying the pharmacokinetics of antibodies and antibody conjugates. The preparation of radiolabeled monoclonal antibodies usually consists of conjugation, conjugate purification, radiolabelling, radioimmunoconjugate purification, and quality control.

As is the case in radiometal-based radiopharmaceuticals, $^{89}$Zr is bound to the antibody using a bifunctional chelating agent to create a stable covalent bond and ensure stable complexation of $^{89}$Zr *in vivo*. The most prominent chelator for $^{89}$Zr radiolabelling is the hexadentate siderophore desferrioxamine B (DFO). It coordinates $^{89}Zr^{4+}$ through 3 hydroxamate groups, leaving two coordination sites available for coordination with, e.g., water molecules. The primary amine tail can be modified for conjugation to the antibody. Due to concerns regarding the instability of the $^{89}$Zr-DFO complex (the free cation $^{89}Zr^{4+}$ is known to be osteophilic), research has been conducted in the direction of designing new chelators with increased stability of the resulting $^{89}$Zr-DFO-mAb conjugate (Brandt et al., 2017; Severin et al., 2011).

A few conjugation methods have been registered: exploiting thiol linkages, amide couplings, and click chemistry. These techniques are mostly based on the reaction of an activated bifunctional chelator with a lysine or cysteine residue of the protein. The most common approach for developing $^{89}$Zr radioimmunoconjugates is the application of p-SCN-Bz-DFO as a bifunctional ligand for reacting with amino group on lysine amino acid (Deri et al, 2013; Jalilian and Osso, 2017).

In general, the procedure for $^{89}$Zr-labelling includes the following steps: dilution of the $^{89}$Zr solution and adjustment of the pH of the solution to the optimal pH range of 6.8 to 7.2 by buffer addition (both chloride and oxalate chemical forms of $^{89}$Zr can be used in radiolabelling procedure); addition of the DFO-derivatized bioactive compound and reaction for 30-60 min at ambient temperature; and purification of the radiolabeled product via HPLC, size exclusion chromatography or ultrafiltration. Radiolysis can be a problem but can be prevented by adding agents such as gentisic acid. Regarding quality control, $^{89}$Zr-mAbs are tested for radiochemical purity and protein integrity, antigen binding, sterility, and endotoxin levels (Fisher et al., 2013; Knight et al., 2016; Verel et al., 2003).

## Conclusion

Research regarding $^{89}$Zr radioisotope production and $^{89}$Zr radiolabelling of antibodies results in constant progress in this field. The availability of commercial systems for radioisotope production and purification, as well as radiolabelling systems, contributes to the greater application of $^{89}$Zr-labelled antibodies in nonclinical studies and clinical practice.

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