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Examination report on Doctoral Dissertation:

Nanoparticle radioconjugates of ^{103}Pd/^{103m}Rh and ^{109}Pd/^{109m}Ag *in-vivo* generators for Auger electron therapy

by Ms. Nasrin Abbasi Gharibkandi

BACKGROUND

In 2025, it will be one hundred years since Pierre Auger published his article 'Sur les rayons β secondaires produits dans un gaz par des rayons X' (*C. R. Acad .Sci.* **1925,** *180*, 65). In that contribution, he reported on the electron emissions from atoms after incident X-ray excitation.[1] Such emissions (Auger effect) have the following explanation. When an inner shell electron is removed from an atom, the atom is in an excited state. The relaxation process to the ground state requires filling the vacancy with electrons from a higher energy level. The excess energy may then be emitted as X-rays or given as kinetic energy to another electron that is ejected out of the atom. This in turn forms new vacancies in higher subshells, which results in a cascade of atomic transitions and a shower of electron ejections and X-rays. The so emitted electrons are collectively called 'Auger electrons' (AEs). Auger effect emissions may be the result not only of the photoelectric effect, but they can also be induced by the decay of radionuclides taking place via electron capture and/or internal conversion.

What is characteristic of Auger electrons is their low energy, deposited over very short distances (in the nanometre/micrometre range), yielding high Linear Energy Transfer values. This property makes AE-emitting radionuclides interesting candidates for the application in radiation therapy of cancers. If precisely delivered into proximity to the key intracellular structures of cancer cells, the AE-emitter provides radiation-induced damage to these structures, which in turn results in blocking cell division and killing the cancer cells. In principle, the combination of precise intracellular targeting and short AE path-length should enable sparing healthy cells, giving therapeutic anticancer effect with little toxicity.

This concept, however promising, still requires further development in order that its potential is fully realised. So far, only a few AE emitter-based radiopharmaceuticals have been subject to clinical trials. None has received regulatory approval. Researchers then continue to explore new avenues, and part of their efforts is related to demonstrating the utility of different AE emitters and the selection of proper targeting platforms.

In the centenary year of Auger's work, I am having the pleasure to examine and review the work that contributes to this exciting area of research, that is the doctoral dissertation of Ms. Nasrin Abbasi Gharibkandi who conducted her research under the supervision of Prof. Aleksander Bilewicz and Dr. Agnieszka Majkowska-Pilip (as auxiliary supervisor) in the Institute of Nuclear Chemistry and Technology in Warsaw.

[1] Independently, the phenomenon had been mentioned a few years earlier by Elise Meitner. Auger had also published preliminary results in 1923.

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RESEARCH TOPIC

The objective of the doctoral research was **to verify the effectiveness of the $^{109}Pd/^{109m}Ag$ radionuclide pair in an *in vivo* generator for combined β^- and Auger electron therapy**. This dual strategy is based on the idea that β^- radiation should enable damaging massive tumours while the AEs would enhance the therapeutic effect by affecting single tumour cells, especially in metastatic sites. Interesting biological results to support the beneficial effects of this combination came from Müller et al., who employed ^{161}Tb, a β^--emitter with additional significant AE emission.[2] The Candidate's group proposed an alternative radionuclide that could be utilised in such a dual approach, that is palladium-109. ^{109}Pd decays through β- transition to form ^{109m}Ag, which, on the other hand, is an effective AE-emitter. As a result, the pair $^{109}Pd/^{109m}Ag$ forms an *in vivo* generator of interesting properties since, in comparison with ^{161}Tb, its β^- particles have higher energies, the generator emits more AEs, and it is easy to produce ^{109}Pd by thermal neutron irradiation of a Pd target.

Successful demonstration of the effectiveness of this $^{109}Pd/^{109m}Ag$ radionuclide pair would be a significant result for the development of AE-based cancer therapies, by adding this pair to the toolbox of interesting emitters worth more advanced preclinical, clinical and translational efforts. Therefore, **I consider the research problem undertaken by the Candidate as important.**

FORMAL ASPECTS OF THE DISSERTATION

The reviewed doctoral dissertation is a collection of four (4) thematically related scientific articles:

Paper # 1: Gharibkandi, N. A.; Gierałtowska, J.; Wawrowicz, K.; Bilewicz, A. Nanostructures as Radionuclide Carriers in Auger Electron Therapy. *Materials* **2022,** *15*, 1143.

Paper # 2: Gharibkandi, N. A.; Wawrowicz, K.; Majkowska-Pilip, A.; Żelechowska-Matysiak, K.; Wierzbicki, M.; Bilewicz, A. Au@^{109}Pd core–shell nanoparticle conjugated to trastuzumab for the therapy of HER2+ cancers: studies on the applicability of $^{109}Pd/^{109m}Ag$ in vivo generator in combined β^- Auger electron therapy. *EJNMMI Radiopharm. Chem.* **2023**, *8*, 26.

Paper # 3: Gharibkandi, N. A.; Majkowska-Pilip, A; Walczak, R.; Wierzbicki, M.; Bilewicz, A. Au@^{109}Pd core-shell nanoparticle conjugated to panitumumab for the combined β^- - Auger electron therapy of triple-negative breast cancer. *Int. J. Mol. Sci.* **2024**, *25*, 13555.

Paper # 4: Gharibkandi, N. A.; Wawrowicz, K.; Walczak, R.; Majkowska-Pilip, A.; Wierzbicki, M.; Bilewicz, A. $^{109}Pd/^{109m}Ag$ *in vivo* generator in the form of nanoparticles for combined β^- - Auger electron therapy of hepatocellular carcinoma. *EJNMMI Radiopharm. Chem.* **2024**, *9*, 59.

The collection of papers is preceded by:

- an introductory (state-of-the-art review) chapter,
- statement of the research aims (two pages),
- brief commentary on each of the included papers (three to five pages),
- an appendix (three pages) describing unpublished research results,
- 'Summary and conclusions' section (two pages).

The appended papers are followed by the co-authors' statements of their contributions to those publications and a list of the Candidate's published works.

Of the four appended papers, the first is a review article, while the remaining three report on original, experimental results. The thesis is written in English, and includes both English and Polish abstracts (as stipulated by Article 187 of the Law on Higher Education and Science). I therefore conclude that **the dissertation form is consistent with the legal requirements and the typical practices** in the fields of chemistry or biomedical research.

LITERATURE REVIEW

The theoretical (literature review) part of the dissertation consists of the introductory chapter (a 'general' review) and **Paper # 1**, which is a published review paper (a specialised, 'narrow' review).

[2] *E.g.*, Müller et al. EJNMMI **2019**, 46, 1919.

The introductory chapter (of about 20 pages) briefly describes the field of radionuclide therapy and the application of Auger electron emitters therein. Then, the Candidate devotes separate subsections to describing recent research on ^{161}Tb radionuclide (a β^- emitter with substantial AE emission), *in vivo* generators for AE emitters and the recoil effects in these. The introductory chapter concludes with a section on palladium radionuclides in nuclear medicine. The selection of issues and references appears appropriate. After going through the Introduction chapter, the reader is well-equipped to understand the research problems undertaken by the Candidate in the broader context.

The theoretical part in the main body of the thesis is well supplemented by **Paper #1**. This is a review article dealing **with nanostructures as carriers of radionuclides for Auger electron therapy**. In my opinion, the choice of this topic is well-justified considering both the gap in the literature and the aims of the doctoral research of Ms. Gharibkandi. The Candidate walks the reader through the fundamental issues of Auger emitters in therapy, exposes the need for a precise delivery of the emitter to the targeted cellular substructure and then explains the promise that the nanocarriers provide in this problem. A set of recent (primarily after 2010) works relevant to the topic is discussed in detail. The review has separate subsections on organic and inorganic nanocarriers, as well as one on Auger electron generation on high Z-number nanocarriers. The Candidate concludes the paper (and her summary thereof in the thesis' main body) with essential and mature remarks on pharmacokinetics/distribution of the nanocarrier-based radiopharmaceuticals.

Leaving aside, for a moment, the editorial aspects of the introductory chapter (to which I shall come back later) I conclude that both the introductory chapter and Paper # 1 **demonstrate the candidate's general theoretical knowledge in the discipline** (as required by Article 187 of the Law on Higher Education and Science) and **familiarity with key literature** in this research area.

The only substantial critical comment regarding this part is that, regrettably, the Candidate did not mention any of the clinical trials that were performed with radiopharmaceuticals based on Auger electron emitters.[3] This I find a certain drawback of the 'background' chapter, since the clinical trials are the crowning piece in biomedical research and a critical element demonstrating feasibility of the particular approach.

ORIGINAL RESEARCH

Now I shall pass on to the original research by the Candidate. Its aim was **to verify the effectiveness of the ^{109}Pd/^{109m}Ag radionuclide pair in an *in vivo* generator for combined β^- and Auger electron therapy**.

The effective therapeutic use of this in vivo generator requires an appropriate platform through which the radioactivity can be delivered to the target site. As discussed by the Candidate, chelating moieties, which are a mainstay of most clinical and experimental approaches in targeted radionuclide therapy, might not necessarily be most suitable in this case. First, this is due to a lack of an appropriate chelating moiety for palladium, and second, it is because of the possible liberation of the daughter ^{109m}Ag. In light of these considerations, the Candidate decided to work with nanoparticles as ^{109}Pd/^{109m}Ag-carriers. The potential anti-cancer effects of the researched radionuclide pair were then tested with three model agents bearing this generator in three different model cancer cell lines:

- **Agent # 1:** Au@^{109}PdNP-PEG-trastuzumab (**Paper # 2**)
  ^{109}Pd deposited on 15-nm gold nanoparticles that were PEGylated and conjugated to trastuzumab, a monoclonal antibody with affinity for HER2 receptors; these were tested *in vitro* in SKOV-3 ovarian cancer cells and MDA-MB-231 breast cancer cells,
- **Agent # 2:** Au@^{109}PdNP-PEG-panitumumab (**Paper #3**)
  ^{109}Pd was deposited on 15-nm gold nanoparticles that were PEGylated and conjugated to panitumumab, a monoclonal antibody with affinity for EGFR protein; these were tested *in vitro* in MDA-MB-231 cancer cells,

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[3] For example: 1. Vallis K.A. et al. *Am. J. Nucl. Med. Mol. Imag.*. **2014,** *4,* 181. 2. Macapinlac H. A. et al. *J Nucl Med.* **1996,** *37(4 Suppl)*, 25S. 3. Rebischung C. et al. *Int. J. Radiat. Biol.* **2008**, *84,* 1123. 4. Krenning E.P. et al. *Ann. Oncol.* **1999**, *10(Suppl 2),* S23. 5. Valkema R. et al. *Semin. Nucl. Med.* **2002**, *32*, 110. 6. Limouris G. S. et al. *Eur. J. Nucl. Med. Mol. Imag.* **2008**, *35*,1827. 7. Li L. et al. *J. Neurosurg.* **2010**, *113*, 192.

- **Agent # 3:** ^{109}PdNP-PEG (**Paper # 4**)
  ^{109}Pd was used for the synthesis of 5nm ^{109}Pd-nanoparticles and PEGylated; these were tested in the hepatocellular carcinoma cell line HepG2.

The research problem required a skilful application of a broad range of research techniques, including those related to radiochemistry, nanoparticles, bioconjugate chemistry, cell culture, molecular biology and biochemistry. This makes the reviewed research an interdisciplinary endeavour. For the purposes of this review, the work can be divided into a chemical and biological part.

In the chemical part, the ^{109}Pd radionuclide was obtained via thermal neutron irradiation of natural or ^{108}Pd-enriched palladium metal targets. After typical processing, the radionuclide purity was determined with γ-ray spectrometry. The Candidate synthesised gold nanoparticles (AuNP, **Papers # 2** and **# 3**) onto which Pd (cold or ^{109}Pd) was deposited, yielding core-shell Au@Pd nanoparticles. Palladium nanoparticles (PdNP) were also synthesised with typical methods (**Paper # 4**). The NPs were PEGylated (**Papers # 2**, **# 3** and **# 4**) and, if applicable, conjugated to the monoclonal antibodies (**Papers # 2** and **# 3**) employing NHS-active esters and disulfide/thiol chemistry. Radioiodination of antibodies (**Papers # 2** and **# 3**) and nanoparticles (**Paper # 4**) was applied to generate iodinated control agents aimed at separating the influence of particular types of radiation. The synthesised NPs and their conjugates were characterised with transmission electron microscopy (TEM) and dynamic light scattering (DLS) measurements. Mean numbers of attached monoclonal antibodies molecules were determined indirectly using ^{131}I-labelled antibodies attached to the Au@NPs (**Papers # 2** and **# 3**). The Candidate confirmed colloidal (no tendency to agglomeration), chemical (no disintegration) and radiochemical stabilities of the research agents by DLS or γ-spectroscopy measurements in relevant media (PBS buffer, saline) and/or human serum (**Papers # 2**, **# 3** and **# 4**).

The key problem of the chemical part of the reviewed research was to determine **whether the daughter ^{109m}Ag radionuclide is liberated from the investigated agents**. The Candidate demonstrated that the **daughter AE-emitter is completely retained** both in the case of Au@^{109}PdNPs (**Papers # 2** and **# 3**) and ^{109}PdNPs (**Paper # 4**). This is in contrast to the situation with ^{109}Pd-cyclam complex, where she showed a substantial release of the daughter radionuclide into the test medium.

Another critical experiment in the chemical part was meant to evaluate whether PdNPs (**Agent # 3**) would be dissolved in a highly oxidative environment. Such a property could be beneficial for therapeutic efficacy in cancer cells with elevated H_2O_2 levels. Contrary to the team's expectation, **no dissolution was observed**.

To sum up, I consider the chemical part sound in terms of the methodology and techniques applied. The Candidate was able to prepare and characterise a set of novel potential active agents and respective control counterparts for biological testing. The findings on the ^{109m}Ag liberation/retention and no dissolution of PdNPs are significant.

I would like to raise a few questions concerning this part:

Question # 1: *Do you see any room for optimisation of the synthetic process for the Au@Pd core-shell nanoparticles and their bioconjugates?*

Question # 2: *Was the polydispersity index calculated for the tested agents from DLS measurements?*

Question # 3: *Do you think that the liberation of the daughter ^{109m}Ag from the cyclam is always disadvantageous for therapeutic purposes?*

Now, I shall comment on the biological part of the Candidate's research. In this part, several subproblems were addressed by the Candidate with respect to each or some of the three novel agents bearing the *in vivo* generator:

- does it bind to the desired receptor on the cell surface? (**Papers # 2** and **# 3**)
- is it efficiently internalised into the cell? (**Papers # 2, # 3** and **#4**)
- is it cytotoxic *in vitro* against selected cancer cell lines? (**Papers # 2, # 3** and **#4**)
- is the toxicity of the *in vivo* generator (combined β^- and AE radiation) greater than that of β^- radiation or AEs alone? (**Papers # 2, # 3** and **#4**)

- does the radiotoxicity add anything to the effect of chemo- and immunotoxicities of the metal-nanoparticles and monoclonal antibodies? (**Papers # 2, # 3** and **#4**)
- does it induce DNA double-strand breaks? (**Papers # 3** and **#4**)
- is its toxicity detectable in a 3D cell culture? (**Paper # 4**)

The Candidate showed *via* radioligand binding assays in the cellular material that **Agents # 1** and **# 2** recognise and specifically bind their target receptors, that is HER2 receptors on HER2-positive cancer SKOV-3 for **Agent # 1** (**Paper # 2**) and EGFR on MDA-MB-231 cells for **Agent # 2** (**Paper # 3**). **Agent # 1** did not show specific binding to HER2-negative MDA-MB-231 cells (Supporting Information to **Paper # 2**). This indicates that nanoparticle conjugation does not impair the targeting ability of the monoclonal antibodies. Interestingly, and perhaps typically for NP-based conjugates **Agent # 2** exhibits very high non-specific binding to MDA-MB-231. In light of this, I want to ask my **Question # 4**: *What were the levels of total and non-specific binding of **Agent # 1** on SKOV-3 cells?*

Internalisation of the tested agents was assessed by radiometric uptake assays (**Papers # 2, # 3** and **#4**) and confocal imaging (**Papers # 2** and **# 3**). **Agent # 1** undergoes complete internalisation into SKOV-3 cells after 1h (radiometry and confocal imaging, **Paper # 2**). **Agent # 2** is internalised to MDA-MB-231 in > 90% (**Paper # 3**). The non-targeted **Agent # 3** (^{109}PdNP-PEG) can enter HepG2 cells to a similar high extent in 18h (**Paper # 4**).

That the non-targeted control counterpart of **Agent # 1** and **Agent # 2**, Au@PdNP-PEG-COOH is not internalised (according to confocal imaging) in either MDA-MB-231 (**Paper # 3**) or SKOV-3 (**Paper # 2**) cells suggests that the internalisation of the studied radiobioconjugates is receptor-dependent. A more robust conclusion would be, however, possible if Au@^{109}PdNP-PEG-COOH had been tested in the more sensitive radiometric uptake assay.

Agents # 2 and **# 3** (but not **Agent # 1**) were found to be transported into the nucleus. This statement is supported by the confocal microscopy images (Figure 5 in **Paper # 3**) but also by some quantitative measurement (Figure 4 on the right in **Paper # 3**; Figure 6c in **Paper # 4**), whose procedure is however not clearly described in these papers (Hence my **Question # 5**: *What was the procedure used to determine intranuclear uptake of radioconjugate in **Papers # 3** and **# 4**?*).

The central component of biological evaluation in the Candidate's research was cytotoxicity (cell viability) testing on appropriate cancer cell lines.

Agent # 1 was found to be highly toxic to SKOV-3 cells. Through appropriate controls (non-radioactive and/or non-targeted control counterparts), the Candidate demonstrated that this activity is primarily radiotoxicity, with no significant chemo- and immunotoxicity. Additionally, by employing MDA-MB-231 (HER2-negative) and radioactive but non-targeted control counterparts in SKOV-3 cells, she was able to show that this toxicity is receptor-dependent. Finally, she made a preliminary demonstration of the benefit of the combined β$^-$/AE activity (Table 2, **Paper # 2**) by comparing the SKOV-3 toxicity of the **Agent # 1** and Au@Pd-^{125}I-trastuzumab (AE radiation alone) or ^{198}AuNP-trastuzumab (β$^-$ radiation alone). Commenting upon this comparison, the Candidate gave a mature remark on the approximate nature of such comparisons. Interestingly, the benefit of β$^-$/AE-combination is visible despite **Agent # 1** not being localised in the nucleus but in the perinuclear area.

In the case of **Agent # 2**, the toxicity testing showed that it is highly effective against EGFR-positive MDA-MB-231 cells, but in this case, in addition to the radiotoxicity of the generator, the immunotoxicity of the monoclonal antibody is at play, too (Figures 6b and 6a, **Paper # 3**), which can be explained by intranuclear localisation.

With regard to **Agent # 3,** it was found to be highly toxic to HepG2 cells. Comparison of the activity data for ^{cold}PdNP-PEG *vs* ^{109}PdNP-PEG *vs* ^{125}I-PdNP-PEG *vs* ^{131}I-PdNP-PEG enabled the conclusion that the combined radiotoxicity of β$^-$/AE generator is greater than that of isolated types of radio-nanoparticles.

Additional mechanistic insight into the toxicity of **Agents # 2** and **# 3** was provided by proving that they induce DNA double-strand breaks (DSBs). For **Agent # 3** in HepG2 cells, the Candidate showed that this double-mechanism agent is a more efficient DSB-inducer than a single mechanism agent, ^{131}I-PdNP. **Agent # 3** was also studied with respect to radiotoxicity on a 3D-

tumour spheroid model which is a more realistic model of cancer tissue. The Agent was found to inhibit tumour growth, and at higher doses, to decrease the tumour volume.

Overall, these results constitute a valuable contribution to the field. The set of subproblems addressed and methods for their examination were chosen correctly (though it is not exhaustive, as remarked below). The Candidate's findings prove that the $^{109}Pd/^{109m}Ag$ *in vivo* generator is worth further development for combined β⁻/AE-therapy and that nanoparticles (either alone or as part of bioconjugates) are reasonable carriers for this generator.

On the other hand, after going through these — admittedly very interesting — biological results, there remains a certain sense of incompleteness. The Candidate could have obtained additional, important results if she had applied cell cycle testing using flow cytometry, apoptosis assays, measurements of reactive oxygen species levels, or other tests related to toxicity mechanisms. One wonders why toxicity in 3D-spheroids was measured only for **Agent # 3** in HepG2 cells and not for the other two agents in SKOV-3 and MDA-MB-231 spheroids. A critical input enhancing our understanding of the utility of the $^{109}Pd/^{109m}Ag$ generator on nanoparticles could come from testing the toxicity of these agents against normal, non-cancer cells (especially in light of high non-specific binding of the radiobioconjugates, NPs and even internalisation of non-targeted **Agent # 3**). Of course, I do perfectly understand that the doctoral projects are always executed in non-ideal circumstances of limited resources. Here then, let me ask my **Question # 6:** *What would you suggest as a development plan for your agents? What experiments and with which hypotheses in mind?*

Another point of criticism concerns the statistical elaboration of the results. Often in the text, both in the papers and in the dissertation, the Candidate states that some X is **significantly** more toxic than some Y. These comparisons are (usually) not accompanied by any sort of statistical significance testing. While a 'bare-eye testing' of these differences (visual inspection of means and dispersion measures) indeed suggests that the differences are significant, I think it would be more appropriate if such statements were accompanied by statistical testing. In fact, statistical testing was performed only for different conditions for a particular agent (comparisons between doses and incubation times). However, more interesting are the comparisons between the Agents and the non-radioactive and/or non-targeted control counterparts. For these comparisons, no statistical testing has been done.

Another problem is that the Candidate provides no information on how many independent experiments (*n*) in how many technical replicates were performed for MTS cytotoxicity testing, radiometric uptake assays, DSBs quantifications, tumour area measurements *etc.* This information **should be provided** in the methods sections, as well as in the figure captions.

Furthermore, I cannot see any mention of corrections for multiple comparisons. Hence, my **Question # 7**: *Was any sort of correction for multiple comparisons applied? If not, on what grounds were such corrections omitted?*

Figures 6 and 7 in **Paper # 2** and **Figures 6, 7, 8 and 9** in **Paper # 4** lack details on either the statistical testing used or the symbols indicating statistical significance. Figure 6 in **Paper # 3** has a caption with the description of the statistical significance markers, but no mention of the test. Nor are these explanations provided in the reproductions of the figures in the dissertation.

The key Table 2 in **Paper # 2** does not provide standard deviations and sample size (*n*) for the results of cell viability testing. Nor are the dosage and incubation time at which the comparison is made provided.

Error bars in Figures 2, 3, 4 in **Paper # 3** and in Figures 2, 6, 10 in **Paper # 4** are not explained. Sample size (*n*) is not provided.

Plots in Figure 8 in **Paper # 3** and Figure 11 in **Paper # 4** show **average** numbers of foci per cell. The points in the plots should then be accompanied by some indication of the variability of the values (e.g. standard deviation?) and the mention of the number of samples from which the averages were calculated. It is also not clear how the value was obtained. This raises **Question # 8**: *What does this mean that the values are given as 'average number … **in reference to** untreated control' Are these values ratios?*

Another point of critique pertains to the presentation of the toxicity results. For the reader, it would facilitate interpretation of radio-, immuno- and chemotoxicities if the axis legends in Figures

6-right and 7-right in **Paper # 2,** Figure 6b **Paper # 3,** as well as Figures 8 and 9 in **Paper # 4** had conjugate mass concentration in addition to the activity.

Alongside the research presented in the published articles, the Candidate has also included certain results in the **Appendix**. This section provides a concise account of the initial stages of the doctoral project, during which the Candidate had planned to examine a different *in vivo* generator, ^{103}Pd/^{103m}Rh radionuclide pair (AE-emitter). Due to problematic access to ^{103}Pd and scarcity of the available ^{103}Pd material at her disposal, the Candidate was not able to employ this generator in any biological/cellular testing. What she did manage was to examine the issue of the release/retention of the daughter ^{103m}Rh from ^{103}Pd-cyclam complexes and Au@^{103}Pd core-shell nanoparticles after the ^{103}Pd decay. She demonstrated that the daughter radionuclide dissociates from the complex with C-carboxylic acid-cyclam, while the core-shell nanoparticles do not release the daughter radionuclide. Altogether, this is another example demonstrating that metallic nanoparticles can effectively prevent the release of the decay product. Here, I permit myself a minor critical remark. This result, though interesting, does not seem to be that important (in light of the rest of the Candidate's research) to justify the inclusion of ^{103}Pd/^{103m}Rh generator in the title of the dissertation.

Overall, notwithstanding several critical comments above, **the quality of the scientific material contained in the examined dissertation is high**. Without any doubt, the presented research is **an original solution to a scientific problem** (as stipulated by Article 187 of the Law on Higher Education and Science).

EDITORIAL ASPECT OF THE DISSERTATION

The editorial aspect of the dissertation leaves something to be desired. Perhaps, for the reader, the most problematic editorial flaw of the thesis is the absence of a complete list of abbreviations. The list presented on pp. 6 and 7 does not contain all the abbreviations and symbols used throughout the body of the thesis. What is more, the symbols are presented in a random order. The abbreviations missing from the table include, for example IC (internal conversion, p. 13), mAbs (monoclonal antibodies, p. 13), SPECT (Single Photon Emission Computer Tomography, p. 15), NLS (nuclear localization signal, p. 18), mtDNA (mitochondrial DNA, p. 19), miRNA (micro RNA, p. 19), RIBE (radiation induced bystander effect, p. 19), DOTA (tetraxetan, p. 20), DTPA (p. 20), NOTA (p. 20), MIBG (*meta*-iodobenzylguanidine, p. 22), NET (neuroendocrine tumours, p. 23), SSTR (somatostatin receptors, p. 23), ER (oestrogen receptors, p. 23), PR (progesterone receptors, p. 23), AR (androgen receptors, p.23), GR (glucocorticoid receptor, p. 23), THP (p. 25 not explained in any place), BCM (block copolymer micelles, p. 35), etc.

Throughout the text, many of the abbreviations used are not defined at their first occurrence. For example, DOTA (p. 13), mAbs (p. 13), NLS (appears on p. 18, explained on p. 35), PSMA (p. 19), THP (p. 25, not explained in any place). DOTA and NOTA are explained twice (p. 20 and p. 30, none of these upon their first occurrence; both times with incorrect systematic names, see my remark below). ROS is defined in the list on p. 6 as 'reactive oxygen species', while in the text (p. 18) as 'reactive oxide species'.

This fragment appears twice in the exactly same wording, on pp. 10 and 11. *The selection of an optimum radionuclide for therapy requires finding an ideal radioisotope with properties that meet the therapeutic requirements of both the provider and the patient. This issue is complex, considering the particular disease, the required treatment duration, treatment objectives, patient tolerance during treatment, and overall treatment efficiency compared to the disease pathophysiology.*

On p. 48, two lines are marked in grey, perhaps a remnant of the writing and polishing process. On pp. 51 and 52, the figure captions contain a minor typo: cpm stands for 'count**s** per minute' and not 'count per minute'.

The author alternates between using 'alpha', 'beta', 'gamma' and the Greek letters α, β, γ in phrases such as 'beta particles', 'beta radiation' etc. To ensure consistency, it would be preferable to use either the word or the symbol throughout.

There are several nomenclature defects. According to the preferred IUPAC nomenclature, the DOTA chelator is **2,2',2'',2'''**-(1,4,7,10-tetraazacyclododecane-**1,4,7,10-tetrayl**)tetraacetic acid and not 1,4,7,10-tetraazacyclododecane 1,4,7,10-tetraacetic acid (p. 20). Similarly, NOTA and DTPA chelators are assigned incorrect systematic names (p. 20).

Thallium-201 in Table 1 (p. 16) is written TI (capital 't', capital 'i'), while it should be Tl (capital 't', low-case 'l') [the thesis is printed in a serif font, where capital 'i' and low-case 'l' letters are visually different]

In the section on cisplatin (p. 21) the Candidate refers to this agent as either 'cis-platinum' or 'cisplatin', while for consistency, it would be preferable to use either version, perhaps the 'cisplatin' which seems to be a prevailing usage in the modern writing.

The dissertation consistently assumes the American spelling and punctuation, but I noted one instance of British spelling (*tumour*, p. 48). Other spelling issues include:

- 'anthracyclins' (p. 22) is a misspelling of 'anthracyclin**e**s',
- 'radionuclie' (Table 1, p. 16) should be 'radionucli**d**e',
- '^{99m}Tc-N_2S_2-Tat-(49-57)-Lys**3**-bombezin**e**' (p. 24) should be '^{99m}Tc-N_2S_2-Tat-(49-57)-Lys^3-bombes**i**n',
- *meta* in 'meta-iodobenzylguanidine' (p. 22) should be in italics.

The English language in the thesis is generally correct. Still, some minor grammar, style errors or typos can be found. For example:

- 'recent **researches**' (p. 11) is not correct since 'research' is an uncountable noun, and in modern English it does not typically occur in plural form,
- 'it is inappropriate [...] **since** its short range in bone' (p. 11) should have 'due to' instead of 'since',
- 'radiopharmaceuticals with **great** molar-specific activity ...' (p. 17) should read 'high' instead of 'great',
- 'ligands [...] have been **evolved** in preclinical studies' (p. 22) should have either 'developed' or 'evaluated' instead of 'evolved' (depending on what the author meant),
- 'derivatives are currently **no of** practical use' (p. 24) should read 'of no' instead of 'no of',
- 'there are four different radionuclides ... which **are being used** in SPECT' (p. 26) should rather have 'which **are used**',
- 'dislocation of the daughter radionuclide from the **targeted** molecule ...' (p. 28) should read 'targeting' instead of 'targeted',
- 'in vivo generator, which is beneficial **against** 161Tb with a greater number of Auger electrons' (p. 33) should have 'compared to' instead of 'against',
- 'the use of .... is much **greater**' (p. 35) should read 'much more frequent' or similar instead of 'greater',
- 'the studies... aimed to **synthesis** and characterize' (p. 37) should have 'synthesize' instead of 'synthesis'.

Formatting might also have been done more carefully. The Candidate uses first-line no-indent style for most paragraphs throughout the thesis, which is inconvenient for the reader but could be accepted. She is though inconsistent in doing so, because on pp. 19, 32 and 33 the paragraphs have first-line indents. Different pages have different spacings between the paragraphs.

The editorial handling of Figures and Tables could be improved. The dissertation has no Table of Figures. Nor has it a List of Tables. Furthermore:

- Table 1 (p. 42) is in fact Table 3,
- Figure 1 seems to be reproduced from some printed source, but this fact is not properly acknowledged,
- Figures 2-12, which are reproduced from the Candidate's articles should have a note that they come from this and that paper, and if applicable, that they were reproduced with permission; in some cases, a note that they were adapted should be in place,
- Figure 5 lacks a legend allowing the reader to understand the colours and patterns in the bar plot,
- Table 2 caption is on p. 40 while the table itself appears on p. 41,
- the captions of Figures 5, 6, 7, 10, 11 in the dissertation lack an explanation for the statistical significance marks used; missing is also an information of which test was used.
- similarly, Figures 6, 7 in **Paper # 2** and Figures 6, 7, 8, 9 in **Paper # 4** do also lack an explanation for the statistical significance marks used and the testing applied.

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To my personal taste, as a reader of organic medicinal chemistry background, I would expect to have the structural formulas of the compounds/fragments discussed in the introductory chapter (e.g., iododeoxyuridine, DCIBzl, DOTA, NOTA, DTPA chelators etc.).

Some of the entries in the references list are not presented correctly. For example, Reference 5 has a title missing; in Reference 12 is inconsistent as to the use of bolds and dots, Reference 2 has some upper script '1' number; Reference 14 has a publisher information missing; References 2 and 9 (both book chapters) have a different style of citation, *etc.*

The selection of the examples discussed in the part reviewing the radiobioconjugates labelled with Auger electron emitters (pp. 20-25) or the listing of the desired characteristics for Auger electron emitters (p. 14) are clearly inspired by the Polish review paper by the Candidate's Supervisor and Dr Wawrowicz (*Wiad. Chem.* **2020**, *74*, 699). In my opinion, it should be acknowledged by, for example, adding phrases like 'according to Wawrowicz and Bilewicz' or 'the discussed examples were previously selected in a review paper from my group'.

Supplementary information of **Paper # 2** was not appended to the dissertation.

Having listed these editorial flaws, I hasten to stress that **they do not affect the readability of the text to a significant extent**. On the contrary, I read the text with much interest and found it readable and logical, neatly guiding the reader both through the state-of-the-art background and through the research conducted by the Candidate.

FINAL ASSESSMENT

In summary, I highly value the selection of the scientific problem and the methodology applied, and I consider that within the framework of her interdisciplinary research, the Candidate has obtained many interesting results. Certain reservations regarding the absence of some experiments, shortcomings in the analyses, or editorial aspects of the dissertation do not outweigh the overall positive impression. I am convinced that the results obtained may have a stimulating impact on the further development of research on Auger electron therapy. I state that the legal and customary requirements regarding the form of the dissertation, the Candidate's theoretical knowledge in the discipline, her ability to conduct independent scientific research, and the subject of the dissertation (an original solution to a scientific problem) have been fulfilled. **Therefore, I recommend that the Scientific Council admit the Candidate to the subsequent stages of the doctoral procedure.**

Yours sincerely,