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Beata Brzozowska
University of Warsaw
Faculty of Physics
Institute of Experimental Physics
Biomedical Physics Division

FACULTY OF PHYSICS
UNIVERSITY OF WARSAW

REVIEW OF THE DOCTORAL DISSERTATION
ENTITLED 'NANOPARTICLE RADIOCONJUGATES OF ^{103}PD/^{103m}RH AND ^{109}PD/^{109m}AG *IN-VIVO* GENERATORS FOR AUGER ELECTRON THERAPY'
BY NASRIN ABBASI GHARIBKANDI

The PhD project entitled 'Nanoparticle radioconjugates of ^{103}Pd/^{103m}Rh and ^{109}Pd/^{109m}Ag *in-vivo* generators for Auger electron therapy' was carried out by Nasrin Abbasi Gharibkandi under the supervision of Prof. Aleksander Bilewicz and co-supervision of Dr hab. inż. Agnieszka Majkowska-Pilip, prof. of the Institute, both affiliated with the Institute of Nuclear Chemistry and Technology in Warsaw. The thesis is based on four first-author publications, all linked by studies on combining palladium radionuclides with biological vectors using nanostructures. Based on the statements provided by the co-authors, I conclude that the PhD candidate played a leading role in the research described in the publications forming the basis of the dissertation. The project was funded by the National Science Center Poland through two OPUS grants: 'Bioconjugates of ^{103}Pd nanostructures for targeted Auger electron therapy' and 'Bioconjugates of radioactive platinum nanoparticles for targeted Auger electron therapy'.

This review was prepared at the request of the Discipline Council of the Institute of Nuclear Chemistry and Technology in Warsaw, within the field of natural and exact sciences.

1 Scientific value of the thesis

The objective of the doctoral research was to assess, through experimental studies, the therapeutic potential of ^{109}Pd/^{109m}Ag radioconjugates functioning as an *in vivo* generator for combined β^- and Auger electron emissions. The PhD candidate used a nanotechnology-based method involving the conjugation of monoclonal antibodies to gold nanoparticles coated with a monolayer of ^{109}Pd. This design ensures that the palladium atoms remain on the nanoparticle surface, allowing the ^{109m}Ag atoms, formed during decay, to emit Auger electrons freely. It also enables the study of whether ^{109m}Ag can diffuse from the nanoparticle and potentially exert toxic effects on surrounding healthy cells.

The thesis opens with a comprehensive and systematic review of radiobioconjugates labeled with Auger electron emitters, encompassing both DNA-binding and

non-DNA-binding agents. It acknowledges that DNA intercalation is not an obligatory condition for inducing cellular damage, and accordingly discusses emerging approaches involving receptor-targeted vectors capable of nuclear internalization. The review further addresses radiobioconjugates based on the dual-emission radionuclide ^{161}Tb, notable for its concurrent emission of β^- particles and Auger electrons. Given its favorable decay characteristics, ^{161}Tb is positioned as a promising candidate for combined therapeutic and diagnostic (theranostic) applications, including single-photon emission computed tomography (SPECT). The concept of *in vivo* generators for Auger electron emitters is also discussed, highlighting their potential in optimizing therapy by combining β^- and Auger emitters on the same carrier molecule to ensure intracellular decay of the daughter radionuclide following internalization. Importantly, the author stresses that beyond efficient *in vivo* generation, the key factor is the chemical outcome of the parent radionuclide's degradation, as this critically influences the behavior and localization of the daughter radionuclide. The thesis also provides a detailed overview and characterization of palladium radionuclides relevant to nuclear medicine. Among these, ^{103}Pd, ^{109}Pd, and ^{112}Pd display physical and chemical properties that suggest their potential suitability for medical applications, although their use has not been extensively investigated. Consequently, ^{109}Pd was studied by the PhD candidate, with the results discussed in the project outcomes section.

A key component of the dissertation is the detailed presentation of the results from four peer-reviewed publications, which form the core of the doctoral project. The first publication discusses the potential of combining Auger electron emitters with both organic and inorganic nanostructures. Special emphasis is placed on metallic nanoparticles, which enhance therapeutic efficacy without compromising targeting specificity, thereby justifying the PhD candidate's choice to employ gold nanoparticles in the study. When the parent radionuclide is immobilized on a metallic surface, such as a gold nanoparticle, delocalized electrons in the metal facilitate charge neutralization after decay. The positively charged daughter nuclide draws electrons from nearby atoms, distributing the charge across the nanoparticle. As a result, the release of daugher nuclide from the surface is unlikely. The integration of high-Z materials, precise targeting mechanisms, and combination therapies is highlighted as essential for maximizing therapeutic outcomes while minimizing side effects.

Publication No. 2 presents promising results for the use of Au@^{109}Pd core–shell nanoparticles conjugated to trastuzumab in the treatment of HER2+ cancers. The unique combination of β^- and Auger electron emissions from the ^{109}Pd/^{109m}Ag *in vivo* generator offers a potent therapeutic strategy, with significant cytotoxic effects observed *in vitro*. Gold nanoparticles (15 nm) were coated with a monolayer of ^{109}Pd, achieving over 95% efficiency in deposition. The bioconjugate was synthesized by attaching polyethylene glycol (PEG) chains and trastuzumab to the Au@^{109}Pd nanoparticles and characterized using transmission electron microscopy (TEM) and dynamic light scattering (DLS). This bioconjugate demonstrated specific binding to HER2 receptors on human ovarian cancer cells (SKOV-3), with 96% internalization observed, as reported in the thesis (although the publication indicates internalization exceeding 99% after 1 hour). Confocal imaging confirmed the accumulation of the bioconjugate in the perinuclear region, which is critical for the cytotoxic effect observed in both SKOV-3 ovarian cancer cells and MDA-MB-231 human breast cancer cells.

Publication No. 3 focuses on the further development and application of pre-

viously studied nanoparticles, this time conjugated with panitumumab for targeted treatment of triple-negative breast cancer (TNBC). The Au@^{109}Pd-PEG-panitumumab nanoparticle conjugates demonstrated dual toxicity against cancer cells, combining radiotoxic effects from β^- and Auger electron emission with immunotoxicity mediated by the panitumumab antibody. Auger electron radiotoxicity predominated due to the efficient nuclear internalization of the radiobioconjugate mediated by panitumumab.

Publication No. 4 presents studies on the cytotoxicity of 5 nm diameter ^{109}Pd-PEG nanoparticles against HCC cells. Significant cytotoxicity was observed even at low radioactivity levels, attributed to high uptake and accumulation within the cells, leading to substantial cell death. Pd-PEG nanoparticles exhibited marked toxicity due to strong cytoplasmic uptake in HepG2 cells, with over 50% accumulating in the nucleus. The cytotoxic effect was significantly greater than that of nanoparticles labeled with either pure β^- or pure Auger electron emitters. Therefore, ^{109}Pd nanoparticles should be considered for small metastatic cancers, where Auger electron radiation becomes crucial.

The final part of the research description presents unpublished results obtained using ^{103}Pd produced at the high-flux reactor at the ILL in Grenoble. These investigations sought to quantify the release, or confirm the retention, of Auger electron-emitting ^{103m}Rh daughter nuclides from ^{103}Pd conjugates, including cyclam complex and gold nanoparticles. The data convincingly demonstrate that metallic nanoparticles serve as effective carriers that securely retain decay products, thereby precluding their release.

2 Editorial correctness of the dissertation

The dissertation is organized into several parts: a list of symbols, an abstract, an introduction, research objectives, a comprehensive presentation of the PhD project results primarily through a discussion of the publications, and unpublished data (referred to—somewhat confusingly—as an appendix), followed by a summary and a bibliography containing 112 references. Additionally, the dissertation contains the full texts of the publications, co-author statements clarifying individual contributions, and a complete list of the PhD candidate's publications.

The publications cover topics including the development of nanotechnology-based radioconjugates with Auger electron emitters, targeted cancer therapies using radionuclide-labeled nanoparticles, and investigations into the radiochemical stability and cytotoxic effects of palladium-based nanoconstructs. The information presented in each section is thorough, and the conclusions are appropriately formulated with careful consideration of uncertainties in data interpretation, reflecting thoughtfulness and scientific rigor. The overall structure, depth of analysis, and inclusion of up-to-date literature demonstrate a high level of scientific maturity.

The structure of the work is clear and logical, with minor formal shortcomings described below. The readability of the thesis would be significantly improved by organizing the content into clearly defined, numbered chapters and subsections, rather than presenting the entire text as a continuous narrative with only bolded headings. Such structure would enhance clarity and make navigation through the document easier for the reader.

3 Critical remarks

The work has been edited appropriately; however, a few minor editorial issues and questions have been identified, which are listed below for the sake of clarity.

1. In the section titled 'Beta-emitters in Radionuclide Therapy' (page 11), the description of beta particle characteristics is somewhat imprecise. Beta particles, in general, can be either positive (positrons) or negative (electrons). Given that the dissertation focuses specifically on beta- emitters, it can be inferred that the emphasis is on electrons. However, it would be beneficial to explicitly state that the discussion pertains solely to beta- particles. Furthermore, the provided Linear Energy Transfer (LET) value for electrons is presented as a generalization, whereas the LET is known to vary depending on the energy of the electrons—specifically, high-energy electrons exhibit different LET values compared to low-energy ones. Another assumption pertains to the medium in which the electrons deposit their energy, as this will differ depending on whether the medium is water or air, for instance.

2. I noticed a couple of typographical errors that might need attention. For example, in Table 1 (Thallium), 'TI-201' should be corrected to 'Tl-201'. Additionally, on page 41, the notation ^{109}Pd/^{109}mAg is incorrect; the 'm' should be written as a superscript to properly indicate the metastable state of silver. The correct notation would be ^{109}Pd/^{109m}Ag. These small adjustments would improve the accuracy and clarity of the text.

3. As a general rule, abbreviations should be introduced with their full term upon first use, followed by the abbreviation in parentheses. On page 18, the abbreviation 'NLS' is introduced before the full term 'Nuclear Localization Sequence/Signal' is given (page 26). Additionally, the abbreviation 'ROS' is introduced at the end of page 18, whereas it would be more appropriate to introduce it at the beginning of the page for clarity and readability.

4. The description of the decay scheme of palladium and the energy characteristics of its decay products appears to be redundant, as this information is presented both in the general overview section (page 30) and again in the discussion of Publication No. 2 (page 37).

5. The caption of Figure 5 is unclear. It would be helpful to label the figure with panels A and B for each cell line, and then specify which data correspond to the left and right sides of the graph (with or without trastuzumab). This would improve clarity and help the reader interpret the figure more easily. In Figure 11, panels B and C are smaller in size, making them difficult to read and interpret clearly, especially since the y-axis range differs from that of panel A.

6. It would be beneficial to discuss in the section 'Biological Action of Auger Electrons' how double-strand breaks (DSBs) are detected using the gamma-H2AX assay, the results of which were presented in Publications No. 3 and 4. This would provide further insight into the methods used to assess DNA damage in the context of Auger electron radiation. Also, are there any other radiobiological assays that could be used to study DNA damage and cytotoxicity in relation to Auger electron radiation?

7. Could you please provide a comment on why a metabolic viability analysis was not presented for MDA-MB-231 cells with ^{109}Pd-PEG without trastuzumab, similar to the analysis conducted for SKOV-3 cells?

8. Could you please elaborate on the motivation behind choosing Dynamic Light Scattering (DLS) over Nanoparticle Tracking Analysis (NTA) for characterizing gold nanoparticles? Additionally, could you outline the advantages and disadvantages of both techniques in this context?

These minor shortcomings do not affect the scientific merit or substantive value of the dissertation.

4 Final evaluation

The synthesis of nanoparticle carriers, their functionalization with PEG molecules and monoclonal antibodies, as well as the investigation of the biological properties of both non-radioactive and radioactive Au@^{109}Pd-PEG-trastuzumab and Au@^{109}Pd-PEG-panitumumab constructs, represents a significant scientific achievement by Nasrin Abbasi Gharibkandi and fulfills a key criterion for the award of a doctoral degree. The research outcomes presented in the dissertation clearly demonstrate the candidate's capacity for independent scientific work and her strong understanding of methodologies for designing and analyzing the structure and molecular dynamics of chemical systems. Furthermore, the thesis showcases her proficiency in applying experimental approaches to study the biological consequences of ionizing radiation interactions with cells.

The achievements of Nasrin Abbasi Gharibkandi reflect a high level of experimental skill, perseverance, and determination—particularly in overcoming challenges associated with complex chemical syntheses and in conducting labor-intensive cytotoxicity analyses across three cancer cell lines (breast, ovarian, and hepatocellular carcinoma). The impressive output of 11 publications, resulting not only from her own research project but also from active involvement in collaborative studies, highlights both her scientific commitment and her strong ability to work effectively within a research team.

I, the undersigned, confirm that the reviewed doctoral dissertation of Nasrin Abbasi Gharibkandi meets the requirements specified in the Act of March 14, 2003 on Scientific Degrees and Academic Title and Degrees and Title in Art (consolidated text Dz. U. of 2017, item 1789 as amended) with consideration of Art. 179 paragraphs 1 and 2 of the Act of July 3, 2018 pre-writing the Law on Higher Education and Science (Journal of Laws 2018, item 1669, as amended), and I request the Discipline Council of the Institute of Nuclear Chemistry and Technology in Warsaw, within the field of natural and exact sciences, to admit Nasrin Abbasi Gharibkandi to further stages of the doctoral proceedings and public defense.

Given the exceptional quality of the dissertation, I strongly recommend awarding the doctoral degree with distinction.

Sincerely,

Beata Brzozowska

Warsaw, July 18, 2025

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NOMINATION FOR THE DISTINCTION OF THE DOCTORAL DISSERTATION OF NASRIN ABBASI GHARIBKANDI

I hereby submit the nomination for the distinction of the doctoral dissertation entitled "Nanoparticle Radioconjugates of ^{103}Pd/^{103m}Rh and ^{109}Pd/^{109m}Ag *in-vivo* Generators for Auger Electron Therapy," authored by Nasrin Abbasi Gharibkandi. The research was conducted under the supervision of Prof. Aleksander Bilewicz and co-supervision of Dr hab. inż. Agnieszka Majkowska-Pilip, both professors at the Institute of Nuclear Chemistry and Technology in Warsaw.

Nasrin Abbasi Gharibkandi's dissertation is distinguished by its innovative experimental approaches, particularly in the synthesis of Au@^{109}Pd core–shell nanoparticles conjugated with trastuzumab and panitumumab. These nanoconstructs were subsequently applied to investigate DNA damage in ovarian and breast cancer cells. The work represents a significant contribution to the development of new therapeutic modalities using Auger electron therapy, particularly in the realm of targeted cancer treatments. The results presented in the dissertation have been disseminated in high-impact scientific journals, further attesting to the substantial scientific value of the research.

This doctoral project constitutes a notable scientific achievement, with the candidate demonstrating exceptional proficiency in integrating advanced methodologies for nanoparticle synthesis, functionalization, and the investigation of their cytotoxic properties, including DNA damage in cells. The work is both comprehensive and systematic, incorporating comparative analyses with reference studies, thereby strengthening the validity of the findings. Furthermore, the research has not only advanced the field of targeted Auger electron therapy but also opened new avenues for the application of nanoparticle-based radioconjugates in cancer therapy.

Given the outstanding quality of the dissertation and the PhD candidate's research, I wholeheartedly recommend that the doctoral dissertation of Nasrin Abbasi Gharibkandi be considered for the award of distinction.

Sincerely,

Beata Brzozowska