IAEA
International Atomic Energy Agency
Vienna International Centre, PO Box 100,
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PROGRAMME OF COORDINATED RESEARCH ACTIVITIES
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PROPOSAL FOR RESEARCH CONTRACT

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Research Contracts are generally awarded to institutions in developing countries or countries in transition insofar as they can effectively carry out the research. The template for Proposal for Research Contract is also used for Doctoral Contract and for Technical Contract.

1. CODE OF THE COORDINATED RESEARCH PROJECT (CRP) UNDER WHICH THE RESEARCH CONTRACT SHOULD BE PLACED:
F22077

2. TITLE OF THE COORDINATED RESEARCH PROJECT (CRP) UNDER WHICH THE RESEARCH CONTRACT SHOULD BE PLACED:
Develop new Technetium-99m Radiopharmaceuticals for Disease Diagnosis

3. TITLE OF PROPOSED RESEARCH CONTRACT (should reflect the proposed research work):
Freeze-Drying Process Design for Radiopharmaceutical Development of Peptide-Based FAPI and PSMA Ready-to-Use Kits for Labeling with Technetium-99m Including Stability Studies on Batch Formulations

4. CONTRACTING INSTITUTION
(The contracting institution can ONLY be an institution with independent legal personality)

Inst. Name: Goce Delcev University, Stip

Street: Krste Misirkov 10A
P.O. Box: 201
Postal Code: 2000
City: Stip
Region/District:
Country: Republic of North Macedonia
Tel.: +389 32 550 400 /+389 32 550 070
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Email: contact-fmn@ugd.edu.mk
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elena.drakalska@ugd.edu.mk
https://fmn.ugd.edu.mk/index.php/mk/
web: http:// www.ugd.edu.mk

5. IMPLEMENTING INSTITUTION:
(Where the research is performed - can be the contracting institution or a sub-institution, a branch of the main institution or a laboratory)
If not the contracting institute, please provide:

Inst. Name: Faculty of Medical Sciences
Goce Delcev University, Stip
Laboratory of Radiopharmacy

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Region/District:
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emilija.janevik@ugd.edu.mk
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https://www.ugd.edu.mk/rflab/en/default.html

2. Ss. Cyril and Methodius University in Skopje, Faculty of Medicine, Institute of Pathophysiology and Nuclear Medicine
Republic of North Macedonia
email: tmakazlieva@medf.ukim.edu.mk

3. REPLEK, LTD
Pharma Industry
Kozle 188, 1000 Skopje,
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4. Ss. Cyril and Methodius University in Skopje, Faculty of Natural Sciences and Mathematics, Institute of Chemistry
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6. SUMMARY OF PROPOSED RESEARCH:

Technetium-99m radiopharmaceuticals are indispensable in modern medical imaging, offering high-quality images with relatively low risk, aiding in diagnosing, staging, and following various diseases, particularly in oncology, cardiology, and neurology. Their unique properties and the continuous development of new Tc-99m labelled compounds expand their potential in medical diagnostics.
Tc-99m is the most frequently used SPECT radionuclide. The optimal energy of emitted photons for imaging, its wide availability, and the low costs of 99Mo/99mTc generators have facilitated its frequent use in diagnostic imaging.
Radioactive peptides that recognize tumor cells are already being used for diagnosis and treatment of malignant diseases, causing image or tumor cell death by radiation toxicity through specific binding to the cancer cells.

In our previous work, we indicated the possibility of introducing available technology for the "ready to use" preparation of a cold kit freeze-dried formulation of conjugated antibodies (rituximab, trastuzumab) and peptide-based radiopharmaceuticals for labeling with different radioisotopes. At the same time, we established and standardized the methods for identification and characterization of the obtained products, suggesting quality control and demonstrating the stability of the final product.
For this project proposal, we aim to design and evaluate novel Technetium-99m peptide based radiopharmaceuticals for selective targeting, focusing on the following pharmacophores:
- Fibroblast activation protein inhibitor (FAPI), a peptide based on the molecular targeting of the FAP, which is known as a serine proteinase highly expressed on the surface in the major cell population in tumor stroma on the subpopulation of activated fibroblasts, termed cancer-associated fibroblasts.
- Prostate cancer-specific membrane antigen (PSMA), type II transmembrane glycoprotein (100 kDa) expressed in prostate epithelial cells and demonstrates glutamic carboxypeptidase (GCP-II) and folic acid hydrolase activity. PSMA is highly overexpressed on the surface of 90% of PCa cells, 1000 times more than in normal tissue. The expression of PSMA is further increased in high-risk PCa, metastatic PCa and castration-resistant prostate cancer (CRPC).

This project proposal will be focused on enhancing cancer imaging by designing selective and targeted radiopharmaceuticals based on the FAPI and the PSMA protein, labeled with Technetioum-99m, for clinical use in malignant diseases. Thorough evaluation of the proposed formulations will facilitate further diagnostic development, promising targeted and effective cancer and metastasis discovering. An important aspect of this project is to make advanced cancer treatments accessible in developing countries, thereby improving global access to cutting-edge medical interventions.
To achieve a stable formulation of a potential radiopharmaceutical using Technetium-99m as a radionuclide, it is crucial to introduce a suitable molecule and chelators specific for Technetium-99m labelling. These steps are executed before the radionuclide is added in the final stage, therefore saving many half-lives of radioactivity and obtain interpretable image.
In this project proposal we suggest using several molecules of FAPI and PSMA, published in the literature and commercially available. This will result in identifying the best composition for the final product and employ procedure for the labeling with Tc-99m. Tc-99m ($t1/2 = 6.0$ hours) is a pure γ–ray emitting radionuclide rendering this isotope suitable for SPECT imaging. Because peptides, as biopharmaceutical formulations are often unstable liquids having a poor shelf-life, freeze-drying is one way to convert these sensitive molecules into stable formats. Acoording to that, we propose to employ previously established and standarized protocols for preparation of home-made freeze-dried radiopharmaceuticals in a stable, shippable after designing the final formulation. The final reeze-dryed product, as potential radiopharmaceuticals will be evaluated using standardized techniques for quality control of nonradioactive ("cold") formulated kit and radioactive form after the labeling with Tc-99m. All kits will be prepared according GMP/GMRP.

The very important segment in our proposed project concerns defining the final formulation that should be ready for use in clinical studies in human medicine, but preferably also in veterinary medicine, considering the similar mechanism of expression of pathological changes. In accordance with this, an important part is the realization of stability studies in the same way as they are carried out for all biological preparations in the pharmaceutical industry. We expect these studies to provide information on the length of use of potential whales for use and at the same time be a guarantee of quality.

The last part of our project proposal will consist of determining the pharmacokinetic parameters of potential radiopharmaceuticals using in silico modeling. These predictions are helpful in optimizing the design and dosing strategies of

radiopharmaceuticals, improving their therapeutic efficacy, minimizing potential adverse effects, and may provide a cost-effective and time-efficient approach to obtain critical information on the behavior of radiopharmaceuticals. This ultimately contributes to more informed decision-making in their clinical development and application.

7. PROJECT PERSONNEL (if space provided below is insufficient, please attach additional sheets)

A. *Chief Scientific Investigator (CSI)*

Family Name:	First Name:	Gender: M/F	Date of birth: yyyy-mm-dd	Nationality:
Drakalska Sersemova	Elena	F	1986-10-26	Macedonian

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+38977682808	/	elena.drakalska@ugd.edu.mk	Associate Professor on Pharmaceutical Technology

Academic degree:	Subject:	Institution:	From:	To:
Master's Degree	Pharmacy	Ss. Cyril and Methodius University, Skopje, Faculty of Pharmacy	2005	2010
PhD	Pharmaceutical Technology and Biopharmacy	Medical University-Sofia, Faculty of Pharmacy	2011	2014
Health Care Specialty	Pharmaceutical Technology	Goce Delcev University, Stip Faculty of Medicl Sciences	2019	2023

Related scientific experience: 12 years
Recent publications related to the project (within the past 2-3 years):
http://eprints.ugd.edu.mk/view/creators/Drakalska=3AElena=3A=3A.html
https://scholar.google.com/citations?hl=en&user=f0h1l-oAAAAJ
https://orcid.org/0009-0005-0322-0935

% of total working time devoted to the project: 30%

B. *Secondary CSI (if applicable)*

Family Name:	First Name:	Gender: M/F	Date of birth: yyyy-mm-dd	Nationality:
/	/	/		/

Telephone (office):	Fax (office):	Email (office):	Position held:

Academic degree:	Subject:	Institution:	From:	To:

Related scientific experience:
% of total working time devoted to the project:

C. *Main additional Scientific Staff*

Family Name:	First Name:	Gender: M/F	Date of birth: yyyy-mm-dd	Nationality:
Janevik-Ivanovska	Emilija	F	1963-02-11	Macedonian

Telephone (office):	Fax (office):	Email (office):	Position held:
+389 32 550 400 / +38975374805	/	emilija.janevik@ugd.edu.mk	Full Professor on Pharmaceutical Chemistry and Radiopharmacy Head of Laboratory of Radiopharmacy

Academic degree:	Subject:	Institution:	From:	To:
PhD in Biology/Biochemistry	Radiopharmacy/ Radiopharmaceutical `Chemistry	Faculty of Natural Sciences and Mathematics, University Ss Cyril and Methodious, Skopje	2000	2003
MScc in Biology/Biochemistry/	Radiopharmacy/ Radiopharmaceutical `Chemistry	Faculty of Natural Sciences and Mathematics, University	1997	2000

		Ss Cyril and Methodious, Skopje		
Health Care Specialty	Pharmaceutical Technology	Faculty of Pharmacy, University Ss Cyril and Methodious, Skopje	1993	1997
Pharmacist (BSPharm)	Pharmacy	Faculty of Pharmacy, University Ss Cyril and Methodious, Skopje	1981	1986
Specialty in Radiopharmacy	Radiopharmacy	EANM Radiopharmacy training - European School of Radiopharmacy, INSTN, Saclay, France	2000	2001

Related scientific experience: 36 years
http://eprints.ugd.edu.mk/view/creators/Janevik-Ivanovska=3AEmilija=3A=3A.html
https://scholar.google.com/citations?hl=en&user=q7xKvHEAAAAJ
https://orcid.org/my-orcid?orcid=0000-0002-8493-5481
% of total working time devoted to the project: 20%

D. Main additional Scientific Staff

Family Name:	**First Name:**	**Gender: M/F**	**Date of birth: yyyy-mm-dd**	**Nationality:**
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Academic degree:	**Subject:**	**Institution:**	**From:**	**To:**
PhD in Medicine	Epidemiology	Faculty of Medicine, University Ss Cyril and Methodius, Skopje	1999	2002
Master of Science in Medicine,	Epidemiology	Faculty of Medicine, University Ss Cyril and Methodius, Skopje	1996	1999
MD	General Medicine	Faculty of Medicine, University Ss Cyril and Methodius, Skopje	1979	1985

Related scientific experience: 34 years
https://scholar.google.com/citations?hl=en&user=P8X8BocAAAAJ
http://eprints.ugd.edu.mk/view/creators/Zdravkovska=3AMilka=3A=3A.html
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% of total working time devoted to the project: 5%

E. Main Additional Scientific Staff

1. Goce Delcev University Stip, Faculty of Medical Sciences

1.1. PhD student at Goce Delcev University Stip, Faculty of Medical Sciences, Study Program in Pharmacy, Republic of North Macedonia, whose doctoral thesis will be related to the problem outlines in the project proposal.

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Position held: Graduate Teaching Assistant (GTA), Faculty of Medical Sciences, Goce Delcev University-Stip. 2007-2012

Academic degree:
MSCc in Pharmacy, Faculty of Pharmacy, Ss. "Cyril and Methodius" Skopje
Health Care specialist in Control and analysis of drug, Faculty of Medical Sciences, Goce Delcev University-Stip. 2018-2021
PhD studies in Pharmacy, Faculty of Medical Sciences, Goce Delcev University – Stip 2021- in progress
Related scientific experience: 9 years
https://scholar.google.com/citations?hl=en&user=t9YG3o4kG0oC
http://eprints.ugd.edu.mk/view/creators/Apostolova=3APaulina=3A=3A.html
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1.2. Arev Marija F 1984-11-27 Macedonian
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Master of Pharmacy, Pharmacy, Faculty of Pharmacy, University Ss Cyril and Methodius, Skopje - 2003-2008
PhD in Pharmacy, Pharmaceutical Chemistry, Department of Pharmacy, Faculty of Medical Sciences, University in Nis, Serbia - 2013-2021
Related scientific experience: 10 years
https://scholar.google.com/citations?hl=en&user=jVrcPnMAAAAJ
http://eprints.ugd.edu.mk/view/creators/Arev=3AMarija=3A=3A.html
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Health Care Specialist, Drug Quality Control, Goce Delcev University Stip, Faculty of Medical Sciences, 2020 – 2023
PhD, Pharmacy, Goce Delcev University Stip, Faculty of Medical Sciences, 2021 - In progress
Related scientific experience: 6 years
https://scholar.google.com/citations?hl=en&user=WAZgplAAAAAJ
http://eprints.ugd.edu.mk/view/creators/Karpicarov=3ADino=3A=3A.html
https://orcid.org/0009-0009-5832-014X
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1.4. Miceva Dijana F 1992-02-19 Macedonian
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PhD, Pharmacy, Goce Delcev University Stip, Faculty of Medical Sciences, 2021 - In progress
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https://scholar.google.com/citations?hl=en&user=pDJWNsAAAAAJ
http://eprints.ugd.edu.mk/view/creators/Miceva=3ADijana=3A=3A.html
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PhD, Medical Sciences, Ss. Cyril and Methodius University in Skopje, Faculty of Medicine, 2014 - 1017
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PhD, Medical Sciences, Ss. Cyril and Methodius University in Skopje, Faculty of Medicine
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MSc in Pharmaceutical Sciences, Ss. Cyril and Methodius University in Skopje, Faculty of Pharmacy, 1995 - 1900
PhD in Pharmaceutical Sciences, Ss. Cyril and Methodius University in Skopje, Faculty of Pharmacy, 2011-2019
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Related scientific experience:
Projects:
Implementation of Statistical, Mathematical and Computer Simulation Programmes for Development and Improvement of the Processes in Pharmaceutical Technology, financed by Ministry of Education of Republic of Macedonia and Replek Farm Skopje, 2007 – 2008
Influence of Biopolymer Interactions on the Drug Delivery from Chitosan-Alginate Colloidal Carrier Systems, financed by NATO Science for Peace Programme, 2002 – 2006
https://scholar.google.com/citations?hl=en&user=Dw6w9goAAAAJ
http://eprints.ugd.edu.mk/view/creators/Makraduli=3ALiljana=3A=3A.html
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Implementation of Statistical, Mathematical and Computer Simulation Programmes for Development and Improvement of the Processes in Pharmaceutical Technology, financed by Ministry of Education of Republic of Macedonia and Replek Farm Skopje, 2007 – 2008
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3.5. Bakovska Stoimenova Tanja F 1988-05-05 Macedonian
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3.6. Milosevic Krstevska Sanja F 1981-10-06 Macedonian
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% of total working time devoted to the project: 5%

3.7. Milanovic Natasha F 1981-10-06 Macedonian
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% of total working time devoted to the project: 5%

3.8. Smiljanovska Nena F 1964 -12- 31 Macedonian
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Health Care Specialty, Pharmaceutical Technology, Ss. Cyril and Methodius University in Skopje, Faculty of Pharmacy, 2004 - 2008
% of total working time devoted to the project: 5%

4. Ss. Cyril and Methodius University in Skopje, Faculty of Natural Sciences and Mathematics, Institute of Chemistry

4.1. Makreski Petre M 1977-11-24 Macedonian
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PhD in Chemistry, Vibrational spectroscopy and Structural chemistry, SS. Cyril and Methodius University, Faculty of Natural Sciences and Mathematics, Institute of Chemistry 2006
Related scientific experience:
https://ih.pmf.ukim.edu.mk/teachers/view/127
https://scholar.google.co.za/citations?user=KT1BV24AAAAJ&hl=en
https://orcid.org/0000-0003-0662-5995
https://www.scopus.com/authid/detail.uri?authorId=8924201100
% of total working time devoted to the project: 5%

8. PROPOSED RESEARCH PROJECT (if space provided below is insufficient, please attach additional sheets)

A. Scientific Background

Diagnostic imaging with Technetium-99m (Tc-99m) represents a cornerstone in modern medical imaging, owing to its optimal physical and chemical properties. Tc-99m is a radioisotope that emits gamma rays, which are ideal for detection by a gamma camera, a device specifically designed to visualise the distribution of gamma-emitting radiopharmaceuticals within the body. This capability enables clinicians to create highly detailed images of internal structures and functions, providing invaluable insights into various medical conditions.

One of the most significant advantages of Tc-99m is its short half-life of approximately 6 hours. This brief half-life means that the radioisotope decays quickly, significantly reducing the duration of radiation exposure for patients. This feature is essential in medical imaging, as it minimises the potential risks associated with radiation, making the procedure safer, especially for repeat examinations or for use in vulnerable populations such as children.

Moreover, the gamma radiation emitted by Tc-99m is of a suitable energy level that facilitates easy detection and results in high-resolution images. This quality is crucial for accurately diagnosing and monitoring a range of medical conditions. The clear images produced by Tc-99m help clinicians in making precise assessments of bodily functions and structures, such as evaluating blood flow, identifying cancerous growths, or detecting bone abnormalities.

Technetium-99m (Tc-99m) showcases remarkable versatility in the field of nuclear medicine, primarily due to its ability to form diverse radiopharmaceuticals by combining with various compounds. Each of these radiopharmaceuticals is tailored to target specific organs or functions, making Tc-99m a pivotal tool in diagnostic imaging.

The Tc-99m used in medical diagnostics has a short, six-hour half-life and does not remain in the body. Technological advancements in medical imaging, particularly in gamma camera technology and hybrid systems like Single Photon Emission Computed Tomography/Computed Tomography (SPECT/CT), have significantly enhanced the applications of Technetium in clinical diagnostics. These advancements have led to more precise and detailed images, thereby improving the accuracy and efficacy of diagnoses.

Modern gamma cameras, equipped with advanced detectors and sophisticated software, have improved the resolution and quality of Tc-99m images. These cameras are more sensitive and capable of producing clearer images, allowing for the detection of smaller lesions and finer anatomical details. This enhancement is crucial in early disease detection, particularly in oncology, cardiology, and neurology.

SPECT/CT provides a better understanding of complex anatomical regions, like the spine or joints, enhancing the diagnosis of orthopaedic and neurologic conditions. It also aids in differentiating between benign and malignant lesions, thereby improving

patient management, and reducing the need for invasive procedures. These technological advancements not only enhance the diagnostic capabilities of Tc-99m but also contribute to more personalised and effective patient care. By providing detailed and accurate diagnostic information, these modern imaging techniques enable clinicians to tailor treatment strategies to individual patient needs, ultimately improving patient treatment outcomes and quality of life.

In recent years, the tumor microenvironment (TME) has emerged as a new paradigm in cancer diagnosis and therapy, owing to its distinctive biological characteristics, particularly the interactions between cancer cells and stromal cells. Among the stromal cells, cancer-associated fibroblasts (CAFs) have been identified as crucial regulators of tumor cell growth, progression, immunosuppression, and metastasis within the TME. CAFs express various biomarkers on their surfaces, including fibroblast activation protein (FAP), which have emerged as a promising target for cancer diagnosis and therapy. Fibroblast activation protein (FAP) is a type II integral membrane serine protease which is abundantly expressed in the stroma of more than 90% of the epithelial cancers, including malignant breast, colorectal, skin, prostate, and pancreatic cancers, while exhibiting a restricted expression in normal adult tissues. Fibroblast activation protein-α (FAPα) is uniquely expressed in activated fibroblasts, including cancer-associated fibroblasts that populate tumor stroma and contribute to proliferation and immunosuppression. One advantage of targeting FAP-expressing CAFs is the absence of FAP in quiescent fibroblasts, allowing for specific targeting of diagnostic and therapeutic compounds to the malignant tumor stromal area using radiolabeled FAP-based ligands. As a result, FAP-based radiopharmaceuticals have been extensively studied for visualizing malignancies and delivering theranostic radiopharmaceuticals to the TME. This approach holds great potential for advancing cancer diagnosis and treatment strategies by selectively targeting the tumor microenvironment.
As FAP is mostly absent in healthy tissue, inhibitors of FAP (FAPIs) can be used in nuclear medicine for imaging. Indeed, a large number of FAPI-based radiopharmaceuticals have been developed for PET/CT imaging, and a promising role for Ga-68 FAPI PET/CT in the diagnosis, staging, and radiotherapy plan- ning of digestive tract cancers has been demonstrated . Due to its lower cost, SPECT/CT with Technetium-99m is a more widely available, and Tc-99m labelled FAPIs are generally applicable tracers that are attractive options for imaging in clinical management. The findings indicate selective uptake of Tc-99m FAPI SPECT/CT and demonstrate a high target-to-background ratio for various types of diges- tive system cancers as well as related metastasis, especially liver metastasis, which contributes to the current literature on FAP inhibitor molecular imaging.

The incidence of prostate cancer is significant, ranking it as the second-highest diagnosis of malignancy after lung cancer in men and the fifth leading cause of death worldwide. For that reason, significant efforts have been made in nuclear medicine to develop PSMA-targeted radiopharmaceuticals for prostate cancer imaging and therapy.
One of the molecular targets used for the visualization of prostate cancer is PSMA (also known as glutamate carboxypeptidase II, folate hydrolase 1, N-acetylated α-linked acidic dipeptidase, or N-acetyl-L-aspartyl-L-glutamate peptidase). PSMA is a type II transmembrane protein containing 750 amino acids (707 extracellular, 24 transmembrane, and 19 intracellular amino acids).
For the development of novel and more efficacious PSMA-targeting radiopharmaceutical with enhanced tumor uptake and minimized off-target uptake, many structural determinants need to be considered in the molecular design. The charge of the agent; the length of the linker between the PSMA ligand and the radionuclide; and its chemical composition, hydrophilicity, or lipophilicity can be investigated. For that reason, 99mTc (T1/2 = 6 h) is a more attractive alternative because of the better imaging properties and better logistics aspects of its application.
In the past two decades several PSMA-targeting radiopharmaceuticals (PSMA I&S, PSMA-T4, and MIP-1404) have been developed for radiolabeling with Tc-99m to provide simply prepared radiotracers for clinical use. These potential radiopharmaceuticals contain different chelators for coupling Tc-99m, maG3(mercaptoacetyl–triglycine), HYNIC (6-hydrazinonicotinic acid), and CIM (2,2′-(2,2′-(azanediylbis(methylene))bis(1H-imidazole-2,1-diyl))diacetic acid). The selection of an optimal chelator is essential for successful clinical translation because chelators influence the uptake of PSMA ligands in normal tissues.
From our perspective, and in order to obtain a radiopharmaceutical that would have access to developing countries and a reproducible production technology, we thought that using commercially available molecules would guarantee a quick result. Therefore, using HYNIC-iPSMA and HYNIC-iPSMA as PSMA ligands for Tc-99m labeling would be a good proposal for work.

Freeze-drying producing stable peptides.
Biopharmaceutical formulations including peptides are often unstable liquids that have a poor shelf-life. This includes raw materials such as peptides and oligonucleotides that are necessary to produce important drugs. Freeze-drying is one way to convert these sensitive molecules into stable formats.
As a result, it is necessary to develop innovative and sustainable solutions to meet all the imposed needs.
Peptides are promising candidates for pharmaceutical drugs, including peptide-based radiopharmaceuticals, and for those reasons, lyophilization of peptides as a technological procedure requires complex production processes and special expertise.
Due to peptide properties, there are numerous potential applications for these small proteins in molecular biology, immunology and (bio)medicine, including nuclear medicine by offering a promising perspective for new drug designs.

Stability study of prepared freeze-dried radiopharmaceuticals ready to use for labelling with Technetium-99m
Protein and peptide-based radiopharmaceuticals are noted as biological products and determination of their stability is important to retain the quality, in terms of physical, chemical, and biological properties within defined limits and thus providing good diagnostic characteristics. These biological products are more labile than small organic or inorganic drug molecules. The stability indication profile of the drug product, or precisely intermediates, should include testing as visual appearance, the potency, purity, structure characterization.

The physical stability of peptide- and protein-based therapeutics remains a huge challenge for the pharmaceutical industry including different types of aggregation processes that can occur that lead to low physical stability.
In contrast to peptides that aggregate and that are associated with disease states, the relative propensity of therapeutic peptides to aggregate may be low. However, the fact that these peptides are frequently used at high concentrations and are required to have long-term physical stability under these conditions makes the problem particularly acute. In addition, therapeutic peptides have to withstand a number of processes during production, formulation and use, that increase their propensity to aggregate, and which exacerbate the problem.
Fundamentally, the basic thermodynamic and kinetic parameters that underpin the aggregation of peptides whether they be disease-associated or therapeutic must be the same. In many cases, the intrinsic and extrinsic factors that affect the physical stability of peptides, such as amino acid sequence, pH etc., will be common to both classes. In other cases, factors will differ. For example, the physical stability of disease-associated peptides depends upon many factors in vivo such as the presence of molecular chaperones, proteostasis and membrane surfaces which are not relevant to therapeutic peptides. In contrast, therapeutic peptides are exposed to a range of surfaces and processes, such as high pressures and freeze-drying, that are not relevant to in vivo systems. Despite this, much knowledge is directly transferable between the different classes from studying the fundamentals of aggregation in vitro in either system, particularly from a mechanistic perspective. It might even be that strategies for increasing the physical stability of therapeutic peptides may come from a deeper understanding of how nature deals with the problem.
Given the large number of different factors that can affect the aggregation of peptides, and which are outlined in this review, it is no great surprise that this remains a challenging area in the development and use of peptide-based therapeutic agents. In some cases, great strides have been made in recent years in our understanding of some of the factors, particularly the sequence of the peptide. In other cases, such as impurities, there is almost no knowledge on which and how these species affect aggregation although it is known to be a significant issue. In other cases, there is a large amount of empirical data available, for example on how post-translation modifications affect oligomer and fibril formation in disease-related peptides. Even in this case though, there is a fundamental lack of understanding of how the modifications affect each step on the aggregation pathway. Without such, it is impossible to rationally predict effects. In addition, there are an increasing number of chemical modifications employed by the pharmaceutical industry to enhance the properties of therapeutic peptides, whose effects on physical stability are not known.
The number of factors that affect aggregation reactions to some degree reflects the intrinsic complexity of the reactions and very subtle changes in the physico-chemical properties of the system can result in large changes in aggregation propensity and rates. There is clearly a significant amount of research that is still required in order for scientists to fully understand the complex energy landscape of aggregation and how this is affected by numerous variables. As well as empirical studies, more fundamental research is needed for this major problem to be fully addressed.
Suggested formulations peptide-based radiopharmaceuticals consist of small peptide molecules. As a reference document for the design of the stability studies will be ICHQ5 guideline: Quality of Biotechnological products: Stability testing of biotechnological/biological products. According to these documents, they can be classified as conjugated products, defined as “A conjugated product made up of an active ingredient (for example, peptide, carbohydrate) bound covalently or noncovalently to a carrier (for example, protein, peptide, inorganic mineral) with the objective of improving the efficacy or stability of the product”. Due to their specific characteristics, this document could not be fully applicable, but useful for establishment of protocol for stability study and analytical methods used. Beside this guideline, stability studies will also be based on ICH Q1A(R2): Stability testing of new drug substance and product and ICH Q1B: Photostability testing of new drug substance and product.
Usually, storage conditions for the lyophilized kits are at 4-8 °C because they are particularly sensitive to environmental factors such as temperature changes, oxidation, or light. Thus, the stability study should include parameters susceptible to change.
The design of this stability study relay on the behavior, properties and stability studies of the drug substance and previously experience from the formulation studies. Firstly, studies under stress conditions will be performed to predict how the possible exposures to different storage conditions can alter the product characteristics.

Stability studies data

Stability Study	Conditions	Minimum Time Period of Submission	Frequency of testing
Normal (Long Term)	25°C ± 2°C/60% RH ± 5% RH	12 months	*3, 6, 9, 12,18, 24 months
Intermediate	30°C ± 2°C/65% RH ± 5% RH	6 months	3, 6, 9, 12 months
Accelerated	40°C ± 2°C/75% RH ± 5% RH	6 months	3, 6 months

* Stability for a duration of 2 years - the stability study can be extended up to 3, 4 or 5 years depending on the need. In that case it is possible to continue with a frequency of testing the receipts of once or twice a year. The number of samples for one analysis, and thus the total number of samples, should be determined depending on the design and needs of the project.

Lyophilized kit of immunoconjugates will be submitted on thermal, oxidative, and acid/base degradation studies. After the design of this study, in terms of the time of exposure, and different reagents, different analytical methods will be employed.
Visual appearance of the product is the first mandatory parameter for quality control of the formulated immunoconjugates. This includes determination of color, opacity after reconstitution, visible particles in solution after reconstitution, pH, and moisture level of the lyophilized products. These characteristics give the physical stability of the kit, and the reconstructed solution does not include any quantification. Microscopic examination can be included as method for counting particles and possible evaluation of size and shape (Light scattering method).

One of the main parameters that should be determined in the predefined time points is the potency, which is dependent upon the conjugation of the active ingredient. The potency will be determined after labeling with radioactive isotope, at different time points, previously established in the stability study.
The purity will be evaluated with methods such as SDS-PAGE electrophoresis and gel filtration chromatography with spectrophotometric detection, which are methods of choice.
According to our previous work we are planning to use as un complementary analysis FTIR Spectrometry for identification of chemical bonds and profile of the sample of final peptide- based formulation and Micro RAMAN Spectrometry as well for the analysis and identification of peptides, their secondary structure and functional groups.
The last parameter established in the stability study will be the stability after reconstitution of freeze-dried product. With this analysis the maximum storage period in the containers will be demonstrated, or to determine in-use stability. Thus, our point of interest will be only physico-chemical stability, but not microbiological.

Pharmacokinetic/pharmacodynamic (PK/PD) modeling of peptide based radiopharmaceuticals
Pharmacokinetic/pharmacodynamic (PK/PD) modeling and physiologically based pharmacokinetic (PBPK) modeling are both essential tools used in the development of new therapeutic radiopharmaceuticals. While they share some similarities in their aims of understanding drug behavior in the body, they differ in their approaches and applications.
Pharmacokinetic/Pharmacodynamic (PK/PD) Modeling focuses on the relationship between drug concentrations in the body (pharmacokinetics) and the resulting pharmacological effects (pharmacodynamics). In the context of therapeutic radiopharmaceuticals, PK/PD modeling helps to understand how the drug is distributed, metabolized, and eliminated (pharmacokinetics) and how these processes relate to its therapeutic effects (pharmacodynamics).
Applications of PK/PD modeling in radiopharmaceutical development:
– Determining the optimal dosage and dosing schedule to achieve the desired therapeutic effect.
– Assessing the therapeutic efficacy and safety of the radiopharmaceutical.
– Predicting treatment response in different patient populations.
– Identifying potential drug interactions and optimizing combination therapies.
– Physiologically Based Pharmacokinetic (PBPK) Modeling is a more comprehensive and mechanistic approach that takes into account physiological, anatomical, and biochemical data to simulate the behavior of drugs, including radiopharmaceuticals, in the body. PBPK models are built on a system of differential equations that describe the movement of the drug throughout various tissues and organs.
Applications of PBPK modeling in radiopharmaceutical development:
– Predicting drug distribution in different tissues and organs, including the tumor site, to achieve targeted delivery.
– Incorporating individual variability in physiological parameters to predict personalized drug responses.
– Assessing the impact of organ function and disease conditions on drug disposition.
– Estimating radiation dose delivered to tumors and normal tissues for therapeutic efficacy and safety assessment.
Integration of PK/PD and PBPK Modeling in Radiopharmaceutical Development:
PK/PD and PBPK modeling can be used together to provide a comprehensive understanding of a radiopharmaceutical's behavior in the body. PK/PD modeling can inform the pharmacological effects of the drug, while PBPK modeling can provide insights into its distribution, metabolism, and elimination.
By combining PK/PD and PBPK modeling, researchers can optimize dosing regimens, predict treatment outcomes, and identify potential safety concerns in the development of new therapeutic radiopharmaceuticals. These modeling approaches contribute to more informed decision-making, efficient drug development, and the advancement of precision medicine in cancer therapy and other applications of radiopharmaceuticals.
Overall, the design, radiolabeling, and nonclinical evaluation of potential Tc-99m radiopharmaceuticals are crucial steps in their development as targeted and effective diagnosis for various diseases, particularly cancer. Nonclinical studies provide essential data to support the decision to move forward with clinical trials in humans.

B. Scientific Scope of the Project (Scientific problems to be addressed with overall and specific objectives)

The main goal of this project is to improve the potential of malignant disease diagnostics and therapy monitoring by designing highly selective and specifically targeted pharmacophores in the form of new radiopharmaceuticals. These radiopharmaceuticals will be based on protein-based molecules such as FAPI and PSMA, labeled with Tc-99m and intended for clinical application. The proposed formulations will undergo thorough evaluation to facilitate further development, providing promising avenues for targeted and effective cancer diagnosis. Importantly, the project aims to make these advanced diagnostic radiopharmaceuticals available for use in developing countries, thereby improving global access to cutting-edge medical tools, including effective and early diagnostics.

The specific objectives are as follows:
Following the primary objective that is to develop, formulate, and characterize radiopharmaceuticals utilizing Tc-99m peptides such as FAPI, targeting specific malignant tumors, and PSMA. The proposed methodology intends to enhance the stability, efficiency, and safety of these radiopharmaceuticals, thereby mitigating potential issues and ensuring their reliability for clinical use.
- To use commercially available molecules, peptides, and ligands to compare their performance in proposed radiopharmaceutical formulations. The aim is to identify the most suitable option allowing efficient radiolabeling with Tc-99m. This step is crucial to

ensure the successful development of targeted radiopharmaceuticals with high radiochemical purity and stability, crucial for safe and effective clinical application in cancer diagnosis.
- To establish and implement technology for the ready-to-use preparation of freeze-dried kit formulations following GMP/GMRP and related protocols applicable to peptide-based radiopharmaceuticals for Tc-99m labeling. The intention is to improve stability, efficacy, safety, reliability, consistency, and practical accessibility of these radiopharmaceuticals for clinical use.
- To develop protocols and standardize methods for the identification and extensive quality control of manufactured "ready-to-use" dried and labeled radiopharmaceuticals using existing techniques and equipment.
-To perform stability studies according to ICH guidelines used in the pharmaceutical industry to define shelf life, storage methods, and potential risks of degradation and aggregation during use.
-To implement in silico simulations to predict the pharmacokinetic (PK) and pharmacodynamic (PD) models of the developed radiopharmaceuticals. This approach aims to optimize design and dosing strategies, improve therapeutic efficacy, minimize adverse effects, and provide cost-effective and time-efficient insights into radiopharmaceutical behavior for informed decision-making in clinical development and application.
- To form a cohesive working group comprising experts in the field to standardize protocols and procedures related to the techniques and proposed methods used in developing and evaluating new radiopharmaceutical products. Standardizing protocols ensures consistency, reproducibility, and quality in research processes, fostering efficient collaboration and effective decision-making.
Through achieving these objectives, the project aims to advance malignant disease therapy by developing and clinically applying new radiopharmaceutical protein-based preparations utilizing Technetium-99m. These preparations target specific malignant cells and enhance diagnostic outcomes in human patients.

C. Overall programme of work for the whole duration of the Contract, including proposed methods or techniques

Designing and standardizing the process for the production of ready-to-use kits for FAPI and PSMA-based radiopharmaceuticals, intended for labeling with Tc-99m, requires a systematic and rigorous approach. The objective is to ensure reproducibility, quality, and safety of the radiopharmaceuticals for comparative preclinical studies.

The goal is to ensure reproducibility, quality and safety of radiopharmaceuticals for comparative preclinical studies
Below is the overall program of work and the key steps for his implementation:
1. Target Selection and Conjugation Strategy
   a. Identify the appropriate targets for comparative oncology studies, such as tumor-associated antigens for the FAPI and PSMA based peptides.
   b. Design conjugation strategies to link Tc-99m chelators to FAPI and PSMA based peptides while preserving their targeting capabilities.
2. Chelator Selection and Radiolabeling Optimization:
   a. Select appropriate chelators for Tc-99m that can form stable protein complexes with high radiochemical yield and purity.
   b. Optimize the radiolabeling process for both peptides to achieve consistent and efficient labeling.
3. Kit Development:
   a. Develop ready-to-use kits containing all necessary components for radiolabeling, including pre-optimized amounts of chelator, stabilizers, and buffers.
   b. Perform stability studies on the kits to assess their shelf life and suitability for distribution.
4. Quality Control (QC) Procedures:
   a. Establish robust QC procedures to test the kits' components and the final radiolabeled products, including radiochemical purity, radionuclidic purity, and sterility all in vitro analysis employed to define the obtained products
   b. Define acceptance criteria based on regulatory guidelines and established standards.
5. Standardize the production procedures across different sites to enable multicenter studies and facilitate data comparison.
6. Radiopharmaceutical Validation:
   a. Validate the radiolabeled FAPI and PSMA based protein in vitro and in vivo to demonstrate their specificity, stability, and targeting efficacy.
7. Stability Studies Evaluation:
8. Regulatory Compliance:
   a. Ensure adherence to relevant regulatory guidelines and requirements for investigational radiopharmaceuticals.
   b. Prepare necessary documentation for regulatory submissions as part of the proof of concept for clinical translation.
9. Collaboration and Knowledge Sharing:
   a. Foster collaboration with academic institutions, research centers, and pharmaceutical companies to leverage expertise and resources.
   b. Engage in knowledge sharing and data exchange within the research community to advance comparative oncology research.
10. Clinical Translation:
   a. If nonclinical studies demonstrate promising results and safety profiles, proceed to clinical trials to evaluate the radiopharmaceuticals in human patients.
   b. Continuously monitor and assess safety and efficacy in clinical settings, further contributing to comparative oncology research.

Designing and standardizing such radiopharmaceutical kits requires interdisciplinary collaboration among chemists, radiopharmacists, biologists, and medical doctors to ensure a successful approach in oncology research.
All in vitro analysis employed to define the obtained molecules including during the project duration we are proposing following methods and techniques:
1. Purity check of commercially procured molecules – FAPI and PSMA and if necessary additional purification process Identification and for characterization of FAPI and PSMA precursors to be used
2. Preparation of products using different chelating peptides - (including all available methods for Quality Control. The conjugation of proteins with different cleating agents will be performed using the established and validated method, including purification steps. The adjusted concentrations in the solutions will be lyophilized to solid state.
3. Standardization of the protocol for freeze drying of peptides / Labconco Free Zone Stoppering Tray Dryer including medium in which the peptides will be and use of cryoprotectants
4. Definition of the concentration determination of peptides / UV spectrophotometer (Jenway UV/VIS spectrophotometer 6715) and semi-micro-UV polypropylene tubes with 0,1M PBS pH=8.0, at 280nm
5. Determination of presence of aggregates
6. Protein Characterization
7. SDS-PAGE electrophoresis
8. Analysis of freeze-dried non-labeled and labeled immunoconjugates (before and after freeze-drying) using SDS-PAGE electrophoresis
9. FT-IR and Raman spectroscopy to liquid and lyophilized samples and conducted in the spectral range 2000–500 cm-
10. Scanning electronic microscopy of freeze drying immunocnjugate conjugates
11. Labelling of the freeze-dried kits of peptide formulation /ready to use formulations with radioactive Technetium-99m
12. Determination of radiochemical purity of the radioimmunoconjugate with ITLC with the autoradiography technique with Cyclone Plus Phosphor Imager (Perkin Elmer)
13. Determination of radiochemical purity of the radioimmunoconjugates with HPLC
14. Stability study of the freeze-dried conjugates - stability for a duration of 2 years - the stability study can be extended up to 3, 4 or 5 years depending on the need. In that case it is possible to continue with a frequency of testing the receipts of once or twice a year. The number of samples for one analysis, and thus the total number of samples, should be determined depending on the design and needs of the project.
15. Characterization of obtained peptide preparations including all available methods for Quality Control
16. FTIR spectrometry - to obtain an infrared spectrum of absorption/emission of a solid (freeze-dried) and liquid form of kit formulation
17. Micro-Raman spectrometry for determination of secondary structure of liuid and freeze-dried peptides
18. Pharmacokinetic- pharmacodynamic computer modelling/in silico for the evaluation of conjugated radiopharmaceuticals
19. In silico simulation to predict PK / PD - optimization, estimation of half-life, determination of effective dose, and dosing regimen, in humans including dosimetry
All obtained data we are planning to treat statistically using suitable parameters
All proposed methods and techniques will be performed in the institutions declared in the project proposal.

D. Detailed programme of work for the coming year (used as reference for the annual Progress Report)

For the coming year, we propose the following detailed program:
– Procurement of all necessary materials for the work, including FAPI and PSMA proteins, precursors, ligands and a comparison of their performance in the proposed radiopharmaceutical formulations.
– Identification of the most suitable preparation that allows efficient radiolabeling with Tc-99m.
– Establishment and validation of protocols for the ready-to-use preparation of freeze-dried kit formulations following GMP/GMRP and related protocols applicable to antibody/protein-based TC-99m radiopharmaceuticals.
– Establishment of protocols for radiolabelling process: detail the procedure for radiolabeling the molecules with TC-99m, including the reaction conditions, purification steps, and radiolabeling efficiency determination.
– Establishment and standardization of methods for identification and extensive quality control of the produced "ready-to-use" freeze-dried and labeled radiopharmaceuticals, utilizing existing techniques and equipment.
– Establishment and standardization of methods for quality control of radiolabeled formulations.
– Selection of an appropriate program for computational modeling and simulation techniques to predict the pharmacokinetic (PK) and pharmacodynamic (PD) patterns of the developed radiopharmaceuticals.
– Establishment of master files for collecting data for statistical analysis of the obtained results and to validate the efficacy of the proposed concept.
– Formation of a cohesive working group comprising experts in the field, responsible for standardizing the protocols and procedures related to the techniques and proposed methods used in the development and evaluation of these new radiopharmaceuticals.
Overall, the text clearly outlines the proposed program for the development and evaluation of radiopharmaceuticals and emphasizes the importance of establishing protocols, standardizing methods, and forming a cohesive working group of experts in the field.

E. Expected Outputs

The expected outputs from the project related to the design and standardization of the technology for the production of ready-to-use kits for peptide FAPI and PSMA -based radiopharmaceuticals, intended for labeling with TC-99m, with a proof of concept for comparative oncology, include:

– Optimized and Standardized Radiopharmaceutical Formulations: The project should yield optimized and standardized formulations for peptide FAPI and PSMA based radiopharmaceuticals, ensuring their stability, reproducibility, and consistency.

– Ready-to-Use Kits: Development of ready-to-use kits containing all necessary components for radiolabeling, making it easier for researchers and clinicians to prepare the radiopharmaceuticals for use.

– Validated Protocols: Establishment and validation of protocols for the preparation, radiolabeling, and quality control of the radiopharmaceuticals, adhering to GMP/GMRP and relevant regulatory guidelines.

– Computational Modeling and Simulation Results: Utilization of computational modeling and simulation techniques to predict the pharmacokinetic (PK) and pharmacodynamic (PD) patterns of the developed radiopharmaceuticals, providing valuable insights into their behavior.

– Master Files and Data Collection: Establishment of master files for collecting data on the radiopharmaceuticals' efficacy and statistical analysis of the results obtained during the project.

– Expert Working Group and Collaborative Network: Formation of a cohesive working group comprising experts in the field, fostering collaboration with academic institutions, research centers, and pharmaceutical companies to leverage expertise and resources.

– Define the necessary qualifications and training requirements for personnel involved in the preparation, handling, and administration of TC-99m radiopharmaceuticals

– Developing and disseminating protocols and guidelines for the safe and effective home preparation of TC-99m radiopharmaceuticals across various healthcare facilities and countries, which will ultimately benefit patients and advance the field of targeted radionuclide therapy, as well as guidance for internal and external audits or inspections to assess compliance with

GRP principles and identify areas for improvement.

Overall, these outputs will lay the foundation for the successful translation of the radiopharmaceutical technology from preclinical studies to potential clinical applications, enhancing cancer diagnosis and treatment in both human and veterinary patients.

9. RELATED WORK ALREADY PERFORMED OR IN PROGRESS AT INSTITUTE (including work performed in connection with the IAEA through Technical Cooperation projects):

Projects related to International Atomic Energy Agency

1. Technical Cooperation - TC:

1995-1997 Preparation of Radioimmunoassay kits and Radiopharmaceuticals
1997-1999 Upgrading of Instrumentation
1999-2000 Local Production of Radiopharmaceuticals
2000-2003 Introducing of 99mTc-labelled antibodies and peptide-based radiopharmaceuticals
2003-2005 Introduction of Radio-guided Lymphatic Surgery
2009 -2017 Implementation of positron emission tomography in Republic of Macedonia
2012 – 2018 Establishing Nuclear Medicine to Improve Health Care of Patients Affected by Chronic Disease

2. Coordinated research Projects – CRP:

2004-2008 Standardisation and quality control of in-house prepared radiopharmaceuticals for nuclear oncology
2010 – 2014 Development and preclinical evaluations of therapeutic radiopharmaceuticals based on Lu-177 and Y-90 labelled monoclonal antibodies and peptides
2023 - 2028 - Design and standardization of the technology for the production of ready-to-use kits for conjugated antibody Daratumumab and protein FAPI-based radiopharmaceuticals, intended for labeling with Lu-177 - proof of concept for comparative oncology in a One Health approach

3. 2017 – 2023 IAEA - RFP No. 34923 - Developing, Testing and Installing E-learning System for African Member States (RAF6049). (main coordinator)
4. 2020 – current - COST Action CA19114 - Network for Optimized Astatine labeled Radiopharmaceuticals/ (Science Communication Manager)
5. University project:

2014 – 2018 Establishment and standardization of a technology for the production of ready to use kit formulation of antibody and peptide-based radiopharmaceuticals
2015 – 2018 Synthesis and Quality Control of new imaging radiopharmaceuticals

Publications relevant to the project proposal from the group:

1. Standardization of a method for freeze-drying of antibodies as ready to use therapeutic radiopharmaceutical, Mwanza Wanjeh, David and Aschalew Alemu, Marie and Arev, Marija and Arsova-Sarafinovska, Zorica and Apostolova, Paulina and Janevik-Ivanovska, Emilija (2020) . Farmacia. ISSN 0014-8237

2. Electrophoresis and Raman spectroscopy characterization of integrity and secondary structure of p-SCN-Bn-DTPA- and p-SCN-Bn-1B4M-DTPA-conjugated trastuzumab. Arev, Marija and Dzodic, Predrag and Makreski, Petre and Zivkovic, Jelena and Janevik-Ivanovska, Emilija (2019Farmacia, 67 (4). ISSN 0014-8237
3. Preparation and integrity examination of freeze dried kit of trastuzumab-immunoconjugates and cold labeled immunoconjugates by applying SDS-PAGE electrophoresis. Arev, Marija and Dzodic, Predrag and Ruskovska, Tatjana and Apostolova, Paulina and Risteski, Milan and Janevik-Ivanovska, Emilija (2019) Acta Medica Medianae. ISSN 1821-2794
4. Rituximab-immunoconjugate kit-formulations for NHL radioimmunotherapy Smilkov, Katarina and Gjorgieva Ackova, Darinka and Gjorgoski, Icko and Janevik-Ivanovska, Emilija (2014). Physioacta, 8 (2). pp. 113-120. ISSN 1857-5587
Relevant presentations:

1. Lu-177 labelled Rituximab - new approach to have suitable radiopharmaceutical. Smilkov, Katarina and Gjorgieva Ackova, Darinka and Janevik-Ivanovska, Emilija. 2nd Balkan Congress of Nuclear Medicine, 8-12 May 2013, Belgrade, Serbia.
2. Assessment of changes in freeze-dried protein pharmaceuticals. Smilkov, Katarina and Gjorgieva Ackova, Darinka and Janevik-Ivanovska, Emilija and Stafilov, Trajče and Arsova-Sarafinovska, Zorica and Makreski, Petre and Gjorgoski, Icko. 17th CEEPUS Symposium and Summer School on Bioanalysis, 2-8 July 2017, Ohrid, R. Macedonia.
3. Freeze-drying approach to enhance antibody stability. Smilkov, Katarina and Gjorgieva Ackova, Darinka and Janevik-Ivanovska, Emilija. International Symposium at Faculty of Medical Sciences "Current achievements and future perspectives in medical and biomedical research", 24 Nov 2015, Stip, Macedonia.

10. FACILITIES and EQUIPMENT

A. Please list facilities (building, equipment - including type and name of manufacturer, and materials) presently available which would be used for the project:

1. University Radiopharmacy laboratory, Faculty of Medical Sciences, Goce Delcev University:

- Safety cabinet for work and installation of scales
- Miniflo 2 horizontal airflow laminar chamber
- Free Zone 6 Split Compartment Cantilever Lyophilizer, Labconco
- Perkin Elmer Phosphor/Instant Imager Visualization and Quantification System
- Centrifuge HuMax 4K, Human
- Mrc ABX-110-X 5 Decimal Analytical Scale
- KERN EW/EG- N)/EWB 3-Decimal Analytical Scale
- Thermostat (furnace for heating and maintaining temperature) MRC
- Humaterm dry sterilizer
- Autoclave MRC
- Comecer dose calibrator
- Personal radiation detector
- Comecer Radioactive Isotope Identifier
- Ambient (room) dosimeter Atomtex® AT1125
- Biometra vertical electrophoresis system
- Oakton® Acorn™ pH meter
- Mrc TOS-4030P/D Rotary Platform
- Heating element for test tubes (heat block)
- Multi purpose rotator
- Mrc MOS-188/2525 Magnetic Stirrer
- HPLC system for liquid chromatography under high pressure Waters alliance e2295/ Uv/Vis detector and radioactivity detector Gabi Star
- Laminar flow with FASTER BIO 48M base
- Comecer radioactive isotope separation isolator
- Electrophoresis system with Western block transfer system Enduro PAGE system E2010-PB
- GLite 900BW Gel Docu System gel scanning camera
- Accu Reader M965/965+ Microplate Reader
- System for obtaining ultra-pure water (type 1) TKA, MicroPure-ST
- Centrifuges for microtubes eppendorf Centrifuge 5415 D
- HuMax 4K bench top centrifuges with maintenance free induction motors from small to medium size capacity. Maximum speed 5,000 rpm,
- V-1200 and UV-1600PC are basic Visible and UV/Vis spectrophotometerAll other laboratory eqiopment necessairy for preparation of radiopharmaceuticals (safe cabinet, laminar flow cabinet with horizontal flow, owen, incubator, pH meter, et. all).

2. Institute of Pathophysiology and Nuclear Medicine - Radiopharmacy laboratory

- 99m/99mTc Generator - 15 GBq
- Equipment for ITLC analysis

3. REPLEK

- Stability chambers:
KBF 720, "Binder GmbH", Germany

4. Faculty of Natural Sciences and Mathemetics, Institute of Chemistry

- FTIR spectrometers - PerkinElmer FTIR spectrometer 2000 (covering mid-IR region, 400-4000 cm-1) and PerkinElmer Spectrum3 spectrometer (covering far-IR, mid-IR and near-IR region, 100-10000 cm-1) and various accessories:
- Golden Gate diamond ATR module, Specac (20-200 C); - Variable Temperature transmission cell holder P/N 21525, Specac (-190 to 250 C);
- Fixed angle specular reflecting accessory with a KRS-5 grid polarizer, PerkinElmer;
- Transmission cells for liquids and gases suitable for far- and mid-IR measurements (100-5700 cm-1)];
- Near Infrared Reflectance Accessory (NIRA) coupled to the PerkinElmer Spectrum3 model (4000-10000 cm-1)
- Micro-Raman spectrometer - Horiba Jobinyvon Labram 300 (Spectrograph: 300 mm focal length; Gratings with 1800 gr/mm and 600 gr/mm; Low Frequency Measurement: from 30 cm-1; Internal laser: HeNe 17 mW (632.817 nm); External laser: Nd:YAG frequency doubled 50 mW (532 nm); High stability microscope: BX41; Objectives: Air (10x, 50x, 100x, 50x LWD) and Water Immersion (40x), Manuel stage; Automatic XY microscope stage for point by point imaging/mapping)

B. Equipment needed for the project which is not available under 10.A.:

Items	Estimated project costs in €		
	To be provided by the institution	**To be provided by Other (non-IAEA)**	**Requested contribution from the IAEA**
PSMA	2 000	/	
FAPI	1 000	/	
Technetium-99m	2 000	/	
Additional cost - chemicals	/	/	5 000
Additional cost - consumables	/	/	5 000
Sub-total:			15 000

If equipment contribution from the IAEA is requested,

a) will this equipment be purchased by the institute, using the cash award? ☐ **OR**

b) shall the IAEA purchase the requested equipment on behalf of the institute? ☐

Please specify: /

11. BUDGET ESTIMATE of the project by year (please show all amounts in EUR €)

Project Year	1. Staff Costs	2. Equipment	3. Miscellaneous*	Project Total (= 1+2+3) or (= 4+5+6)	4. Contribution by Institute	5. Contribution by Other (non-IAEA)	6. Requested contribution from the IAEA
1st	5 000	/	5 000	10 000	15.000	/	10 000
2nd	5 000	/	5 000	10 000	15.000	/	10 000
3rd	5 000	/	5 000	10 000	15.000	/	10 000
4th	5 000	/	5 000	10 000	15.000	/	10 000
5th	5 000	/	5 000	10 000	15.000	/	10 000
6th	5 000	/	5 000	10 000	15.000	/	10 000
Total	30 000	/	30 000	60 000	90 000		60 000

****If 'Miscellaneous' costs are entered in the table, please elaborate here:***

PSMA
FAPI
Chemicals / material for synthesis of chelators
Laboratory chemicals
Disposable lab material
Consumables
- MLPA kit, PCR reagents, cappilary electrophoresis gels/capilaries, DNA extraction kits, plasticware, sequencing kits, ITLC, SE-HPLC column
Publishing papers,
Additional material cost for three PhD students

NB.: Travel cost to Research Coordination Meetings (RCMs) should not be included in the Budget Estimate.

12. PROPOSED PROJECT COMMENCEMENT DATE:
01.06.2024

13. ADDITIONNAL INFORMATION (if required):
Time schedule for distribution of the activities by every year will be performed and submit after receiving project acceptance including obtained duration and IAEA contribution

14. SIGNATURES

CHIEF SCIENTIFIC INVESTIGATOR:	**HEAD OF INSTITUTE:**
(Signature)	(Signature)
Prof. Dr. Elena Drakalska Sersemova	Prof. Dr. Dejan Mirakovski
(Name and Title)	(Name and Title)
Stip, 26.03.2024	Stip, 26.03.2024
(Place and Date)	(Place and Date)