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PROGRAMME OF COORDINATED RESEARCH ACTIVITIES
Webpage: cra.iaea.org

REQUEST FOR RENEWAL OF RESEARCH CONTRACT No. 16651

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ONLY DULY FILLED AND SIGNED RENEWAL REQUESTS WILL BE PROCESSED.

1. CODE OF THE COORDINATED RESEARCH PROJECT (CRP):
Contract 16651/R2 - 2014

2. TITLE OF THE COORDINATED RESEARCH PROJECT (CRP):
Development and preclinical evaluation of therapeutic radiopharmaceuticals based on Lu-177 and Y-90 labeled monoclonal antibodies and peptides

3. TITLE OF RESEARCH CONTRACT:
Establishment and standardization of a technology for ready to use production of cold kit formulation of DOTA-Rituximab and peptide based radiopharmaceuticals for labeling with lu-177 and Y-90

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

Inst. Name: **University "Goce Delcev"**
Faculty of Medical Sciences
Study program of Pharmacy

Street: Krste Misirkov " b.b.
P.O. Box: 201
Postal Code: 2000
City : Stip
Region/District : Republic of Macedonia
Country: Republic of Macedonia
Tel.: + 389 32 550 014/+38975374805
Fax: + 389 32 390 700
Email: emilija.janevik@ugd.edu.mk
Does the institution have an independent legal personality
☒ Yes ☐ No

5. IMPLEMENTING INSTITUTION:
(Where the research is performed - contracting institution, sub-institution, branch of the main institution or laboratory)

Inst. Name: **University "Goce Delcev"**
Faculty of Medical Sciences
Study program of Pharmacy

Street: Krste Misirkov " b.b.
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6. DETAILED WORK PLAN FOR COMING YEAR (including proposed methods or techniques):

The detailed work plan for the last year of the project include:

1. Standardization of proposed procedure for freeze drying of the conjugated MAb (Rituximab) and comparison of different chelating agents:
- Characterizarion of obtained freeze dried products
- Stability of the freeze dried products
- Dissolution of the freeze dried products
- Integrity of antibody in the freeze dried products
- Immunoreactivity of the freeze dried products containing antibody

2. Quality control of obtained conjugated Rituximab using different conjugating agents
- Stress testing of products

- HPLC profile of the cold conjugates
- SDS-PAGE electrophoresis of cold conjugates

3. Chemical analyses on bifunctional ligand labeled with cold Lutetium and Ythrium and determination of the structure of obtained products
- Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES)
- RAMAN spectroscopy
- IR spectroscopy

4. Chemical analyses of conjugated antibodies labeled with cold Lutetium and Ythrium
- Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES)
- RAMAN spectroscopy
- IR spectroscopy

5. Standardization of the final protocol for freeze-draying of conjugated -Rituximab
- Uniformity test of the obtained freeze-dried products
- Dissolution of the obtained freeze-dried products

6. Standardization of the procedures for Quality Control of radiolabeled product
- Radiolabeling yield
- Radiochemical purity (Quality control performed using ITLC, HPLC, Gel-filtration, SEP-PAK)
- Stability of radiolabeled product on room temperature
- In vitro stability of radiolabeled kit in saline
- In vitro stability of radiolabeled kit in plasma

7. Biodistribution studies in animal model
- Imaging studies of animal models after application of radiolabeled conjugates
- Biodistribution of radiolabeled conjugates in critical organs after application

The main objective of the work envisaged to be carried out under the doctoral CRP will be development of I77Lu and 90Y-labeled monoclonal antibodies (RituximabR) and peptide-DOTA (DOTATATE and DOTA-Substance P) as radiopharmaceuticals for radionuclide therapy.
The main goal of the next stages of the project will be to standardize already established procedures for preparation of MoAb conjugates and radiolabeling them with 1 77Lu or 90Y, with reproducible results, optimally in a kit formulation could constitute a relevant subject of investigation. The work would include preparation of Antibody (Rituximab)-DOTA conjugate, optimisation of the radiolabeling of the antibody DOTA , DTPA and 1B4M conjugateS with 90Y and I 77Lu and preclinical evaluation in in vitro and in vivo systems.
The other part of the study will be focused on the chemical identification of the not radioactive conjugate, conjugate labeled with not radioactive Lutetium and Ythrium. As a very important part of our work wor the next year is to see the aviability of the final freez dryed kit for the maximum amount of the radioactivity appropriate for the therapeutical aplication.
Peptide receptor radionuclide therapy (PRRT) can be further explored by identiifing suitable targets and carrier peptides and developing them to 1 77Lu radiopharmaceuticals for targeted therapy.
The biological evaluation of some of the radiolabeled conjugates will be carried out in suitable in vitro and in vivo systems to evaluate their potential applicability as tumour targeting molecules.
The biodistribution studies will be performed using mice and rats as a normal animal distribution and animal model.
The normal rats will be trerated with the cold conjugates and labeled with non radioactive lutetium and ythrium to follow the biochemical parameters as result of the maximim injected dose.
The radioactive conjucates will be used to see the biodistribution in animal model. We are planing to have imaging studies and biodistribution in critical organs after application.

Similar synthetic modifications of the unsymmetrically substituted water soluble porphyrins with DOTA as the BFCA can be envisaged. The porphyrin-DOTA conjugates will be radiolabeled with 1 77Lu and 90Y and the tumour targeting potential can be evaluated for further development as targeted agents for radiotherapy of tumour.

The expected outcomes of the Ph.D. work will be in accordance with the primary objective of the CRP and will be constituted by the following aspects.

1. Preparation of ready-to-use kit formulations for labelling RituximabR with I77Lu and 90Y and preclinical data that will help in its clinical use.
2. Technology for the production of 177LuJ9OY-DOTA-substance P and preclinical evaluation data using in vitro and in vivo models.
3. Technology developed for labelling RituximabR to be extended to other antibodies such as anti-CD22, anti-PSMA and antitenascin MoAbs.
4. Preparation of other radiolabeled tumour targeting carrier molecules such as porphyrins and ROD peptides.

A final PhD thesis comprehensively describing all results and achievements obtained from the experimental work carried out during the PhD will be also prepared and submitted.

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:
Janevik - Ivanovska	Emilija	F	1963-02-11	Macedonian

Telephone (office):	Fax (office):	Email (office):	Position held:
+ 389 32 550 014 /+38975374805	+ 389 32 390 700	emilija.janevik@ugd.edu.mk	University Professor

B. Secondary CSI (if applicable)

Family Name :	First Name:	Gender: M/F	Date of birth: yyyy-mm-dd	Nationality:
Gjorgoski	Icko	M	1950-10-10	Macedonian
Stafilov	Trajce	M		Macedonian

Telephone (office):	Fax (office):	Email (office):	Position held:
+38970273384		icko@pmf.ukim.mk	University Professor
+389 70350756		trajcest@pmf.ukim.mk	University Professor

C. Main additional Scientific Staff

Family Name :	First Name:	Gender: M/F	Date of birth: yyyy-mm-dd	Nationality:
Gorgieva	Darinka	F	1978-11-08	Macedonian

D. Main additional Scientific Staff

Family Name :	First Name:	Gender: M/F	Date of birth: yyyy-mm-dd	Nationality:
Smilkov	Katarina	F	1979-05-16	Macedonian

8. BUDGET - Estimate for coming project year (please show all amounts in EUR €):

Total Project budget in €	25 000

A. Equipment, materials, supplies to be purchased by the IAEA on behalf of the Institute:

Items	Estimated project costs in €
Ph D student	15 000
Laboratory chemicals	5 000
Disposable lab material	5 000
Sub-total:	

B. Miscellaneous (including transport*):

Item	Estimated project costs in €
Sub-Total:	

*** If funds for travel/transportation have been included in the budget, please indicate specific purpose:**

C. Total - All Costs (Budget Items A - B)

Total budget requested from the IAEA in €	25 000

9. WILL RESEARCH BE COMPLETED IN COMING YEAR?

☐ Yes ☒ No

10. SIGNATURES

CHIEF SCIENTIFIC INVESTIGATOR

Name (in capitals)
Prof.
EMILIJA JANEVIK-IVANOVSKA

Signature

Date 15.09.2014

HEAD OF INSTITUTE

Name (in capitals)
Prof.
SASA MITREV

Signature

Date 15.09.2014