Scientific report on the implementation of the project
PN-III-P1-1.1-PD-2016-1642 (contract no. 110／2018) entitled *Development of a multifunctional radiolabelled hybrid magnetic nanoparticle agent for dual-modality (SPECT and MRI) medical imaging*
(Acronim: NANOIMAG)" Phase 2-2019

Phase 2:
- Develop hybrid magnetic nanoparticle 99mTc radiolabeling procedure
- *In vitro* evaluation of hybrid magnetic nanoparticles on normal and different pathological (tumoral and nontumoral) cells
- *In vivo* biodistribution with quantitative evaluation of the new radiotracer on small animals (normal and tumoral tissues)

Related delivered activities:

Activity 2.1 - Design and optimize hybrid magnetic nanoparticles radiolabeling. Selection of the optimum parameters
Activity 2.2 - Quality control of labeling process
Activity 2.3 - In vitro radiochemical stability evaluation of ^{99m}Tc hybrid magnetic nanoparticle
Activity 2.4 – Cell culture studies
Activity 2.5 – *In vitro* stability
Activity 2.6 – Saturation binding assay and cell uptake study
Activity 2.7 – Experimental animals procedure
Activity 2.8 – General toxicity/health screening. Histology studies
Activity 2.9 – *Ex vivo* biodistribution studies. *In vivo* stability of the labeled complexes
Activity 2.10 – Statistical analysis

During the phase 2-2019, 3 types of hybrid nanoparticles were prepared, namely:
- magnetite nanoparticles obtained by co-precipitation of iron ions, subsequently coated with 3-aminopropyl triethoxysilane (batch GD 17);
- magnetite nanoparticles obtained by co-precipitation of iron ions, subsequently coated with tetraethyl orthosilicate (batch GD 22);

- magnetite nanoparticles obtained by co-precipitation of iron ions, subsequently coated with tetraethyl orthosilicate and 3-aminopropyl triethoxysilane medium (batch GD 26);

The optimum hybrid magnetic nanoparticles were the ones prepared using magnetic core (iron oxide) and multifunctional silica shell chain (with 3-aminopropyltriethoxysilane) using very simple methodology (Figure 1).

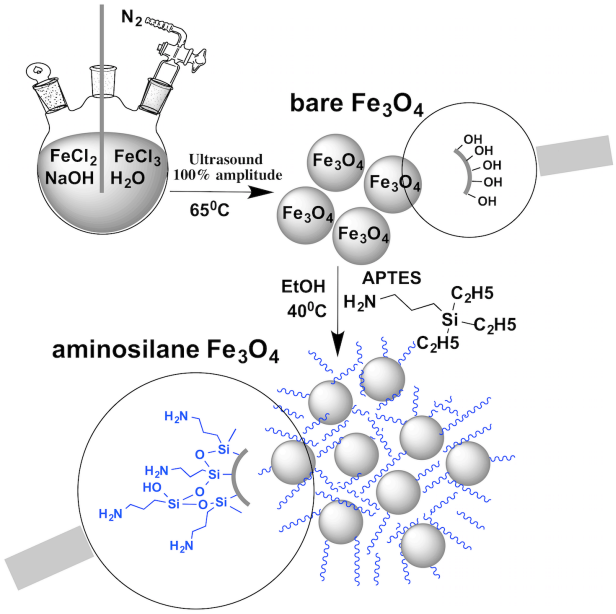

Figure 1. Reaction method

The nanoparticles were radiolabelled with $^{99m}TcO^{-}_{4}$ Na (sodium pertechnetate) chosen for labelling because it is the most commonly used emitting radioisotope in nuclear medicine having a convenient half-life of approximately 6 h, appropriate energy (140 keV) for imaging on a standard gamma camera and less attenuation by soft tissue. For optimum labelling yield, many factors were studied in details such as, the variation of the reducing agent (sodium borohydride and/or stannous chloride) and concentration, the variation of the Na^{+} $^{99m}TcO_4^{-}$ dose used and reaction time. The radiolabelling efficiency was assessed by using instant thin layer chromatography (ITLC). The coupling stability of the radiolabeled hybrid magnetic nanoparticles was verified in both sterile water and human serum at room temperature at different intervals (1, 2, 3, 4, 5 and 6 hours) as presented in Figure 2.

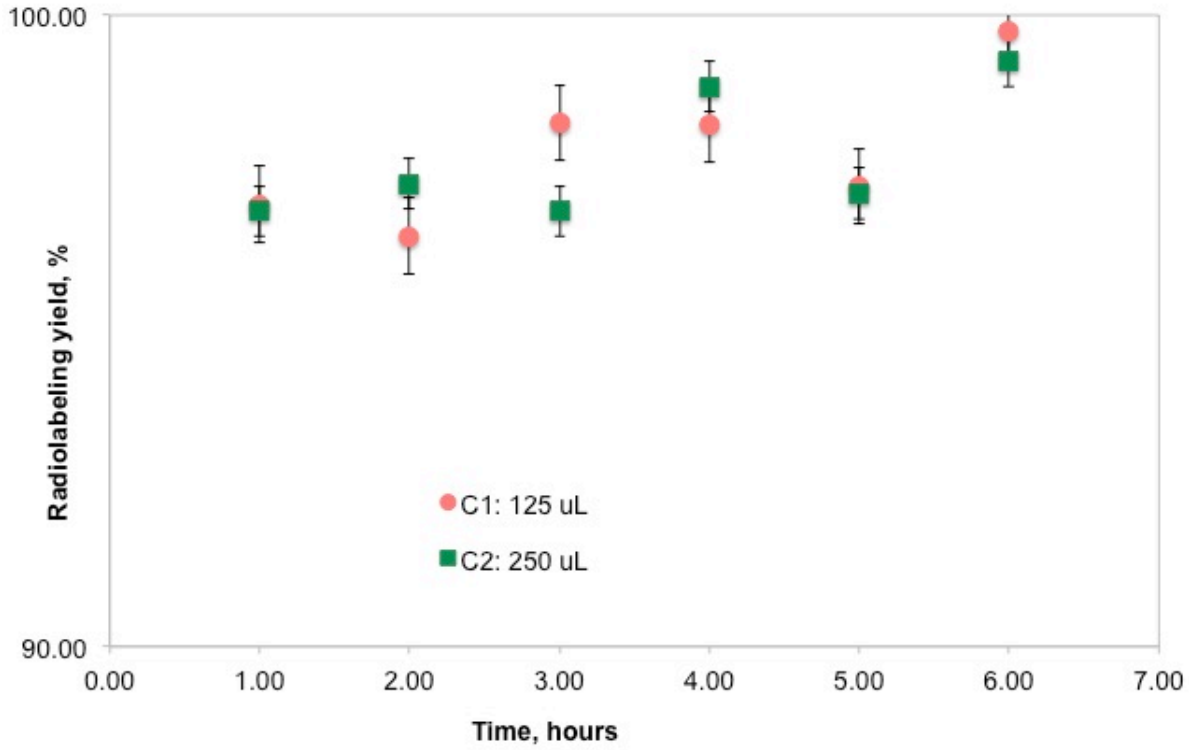

Figure 2. Radiolabelling yield for magnetic amine nanoparticles (batch GD17).

The cytotoxicity induced by hybrid nanoparticles was investigated by MTT assay at 6, 12, 24 and 48 hours.

All animals experimental procedures employed in the present study are strictly in accordance with the European Community Guidelines regarding ethics and approved by ‘‘Grigore T. Popa’’ University of Medicine and Pharmacy animal care and use committee.

Toxicity: The agents under study were administered and then, carefully observed for obvious signs of toxicity, such as convulsions or body weight effects or by macroscopic evidence of organ and tissue damage or dysfunction.

Pharmacokinetic studies: distribution studies on normal tissues using gamma counter. Estimation of the possible separation of free ^{99m}Tc/degradation after administration of labelled complexes by ITLC strips blood analysis (Figure 3).

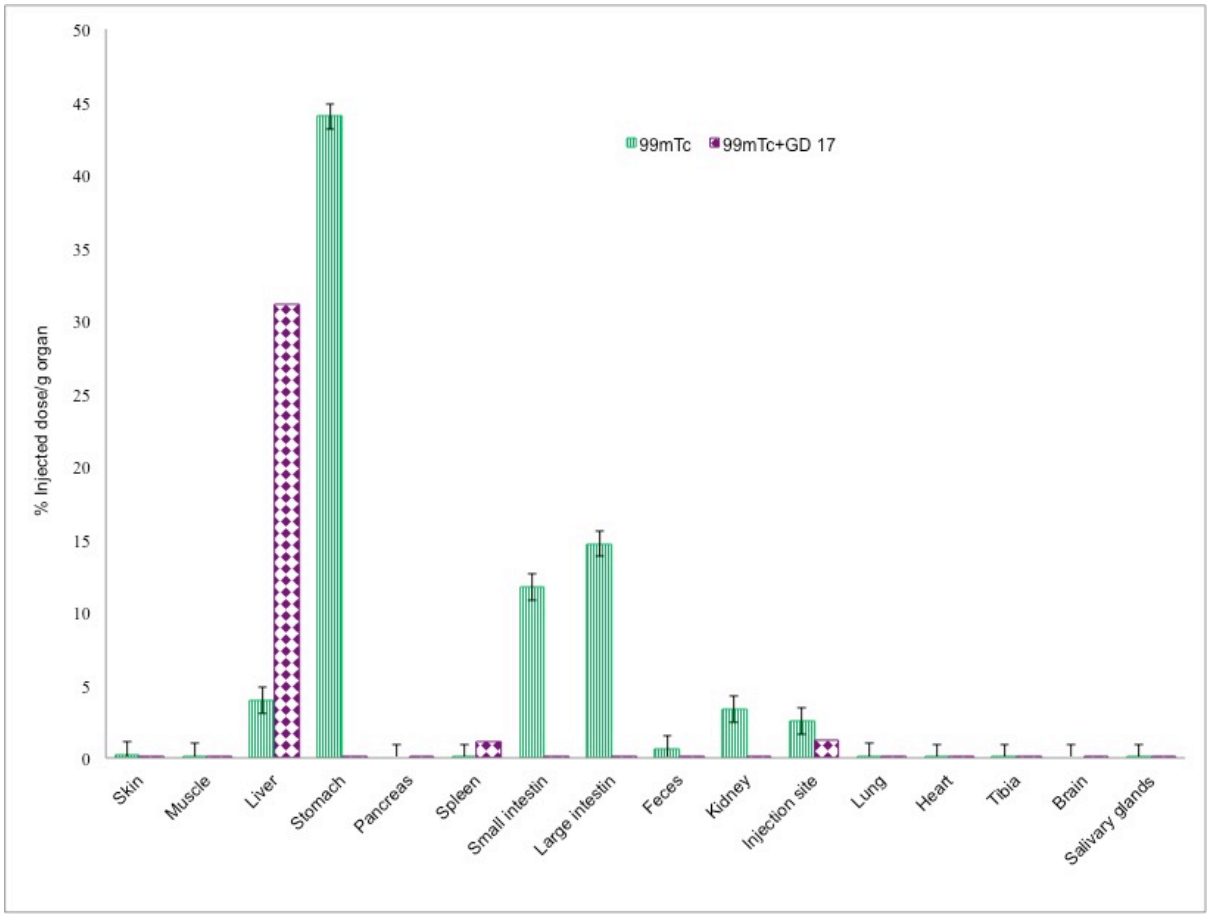

Figure 3. Ex vivo biodistribution of radioactivity after the injection of ^{99m}Tc and radiolabeled aminosilane nanoparticles (^{99m}Tc+GD17 batch) at 6 h.

Conclusions phase 2

The deliverables associated with each activity that were obtained:

- Multifunctional radiolabeled hybrid magnetic nanoparticles adequate for *in vitro/in vivo* studies;

- Optimum parameters for *in vivo* administration of radiolabeled hybrid magnetic molecule:

- Biodistibution map analysis after administration of labeled complexes based on statistical results;

- Oral presentation at 26th Young Research Fellows meeting, 20-22nd February 2019 at Faculté de Pharmacie de Paris, Université Paris-Descartes, France: Synthesis and characterisation of multifunctional hybrid magnetic nanoparticles designed for multimodal imaging by G. Dodi, C.M. Uritu, V. Balan, F. Cojocaru, B.I. Tamba and C. Ștefănescu;

- Poster at NANOTEXNOLOGY International Conferences & Exhibition on

Nanotechnologies – Organic Electronics & Nanomedicine 2019 —ISSON19-13th International Summer Schools on Nanosciences & Nanotechnologies, Organic Electronics and Nanomedicine and NN19– 16th International Conference on Nanosciences& Nanotechnologies, 29 June-6 July 2019 at Porto Palace Conference Centre, Thessaloniki, Greece: Multifunctional hybrid magnetic nanoparticles as multimodal imaging agents: design and in vitro evaluation by G. Dodi, C.M. Uritu, F. Cojocaru, V. Balan, I.L. Serban, B.I. Tamba, C.T. Mihai;

- Poster no. EP-0754 at 32nd Annual Congress of the European Association of Nuclear Medicine – EANM'19, 12-16 October 2019 at Barcelona, Spain: Radiolabelled multifunctional hybrid magnetic nanoparticle probe for dual-modality (SPECT and MRI) medical in vivo imaging by G. Dodi, C.M. Uritu, I. Gardikiotis, C.T. Mihai, L. Agrigoroaie, M. Furcea, B.I. Tamba, C. Stefanescu;
- Review paper: Balan, V.; Mihai, C.-T.; Cojocaru, F.-D.; Uritu, C.-M.; Dodi, G.; Botezat, D.; Gardikiotis, I. Vibrational Spectroscopy Fingerprinting in Medicine: from Molecular to Clinical Practice. Materials 2019, 12, 2884;
- ISI indexed extended abstract: Dodi, G.; Uritu, C.M.; Gardikiotis, I.; Mihai, C.T.; Agrigoroaie, L.; Furcea, M.; Tamba, B.I.; Stefanescu, C. Radiolabelled multifunctional hybrid magnetic nanoparticle probe for dual-modality (SPECT and MRI) medical in vivo imaging. European Journal of Nuclear Medicine and Molecular Imaging (2019) 46 (Suppl 1): S731;
- 1 participation at School 3: Nanomedicine part of ISSON19-13th International Summer Schools on Nanosciences & Nanotechnologies, Organic Electronics and Nanomedicine, 29 June-6 July 2019, Thessaloniki, Greece;
- 1 activity report (WP2-3-4).