Rezultate obtinute pe tot parcursul desfasurarii

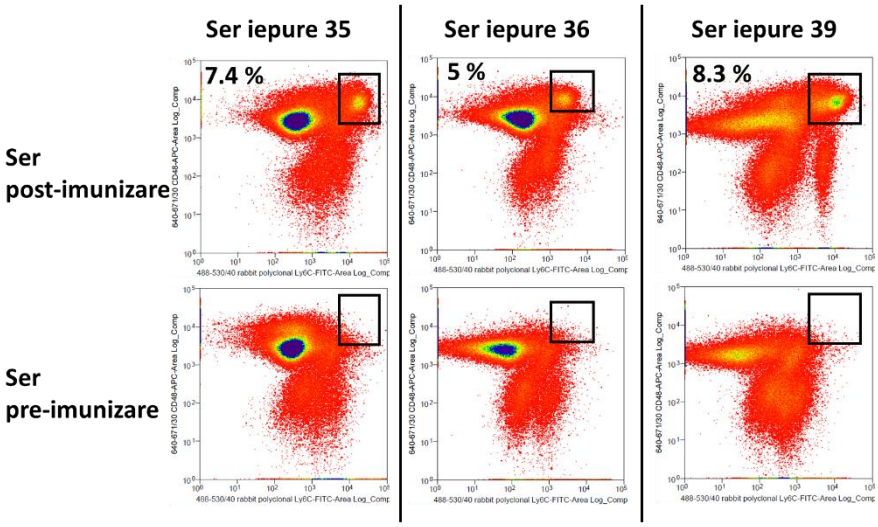
de la inceput 1mai 2018 pana la 30 sep. 2021 FINAL

Proiectul PN-III-P1-1.2-PCCDI-2017-0769 , Contract 64/2018

Dezvoltarea de radiofarmaceutice si tehnici nucleare in oncologie, pentru imagistică si tratament personalizat la nivel molecular **(ONCORAD)**

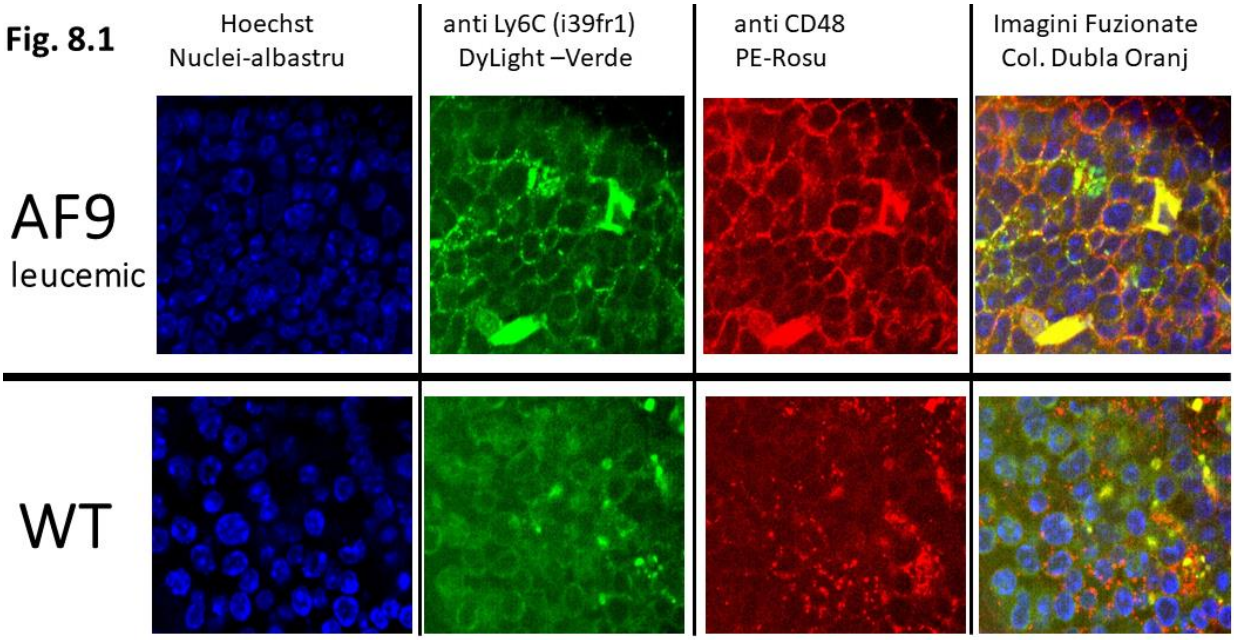
Proiect 1

S-au realizat cu succes obiectivele planificate incepand cu producerea de antigen pur necesara definitivarii ultimelor rapeluri adresate animalelor imunizate si sacrificarea lor. Serurile si celulele B reactive obtinute de la aceste animale au fost caracterizate si confirma reactivitatea fata de cele doua antigene evolutive de diferentiere mielocitara prevazute Ly6C si ScaI. Serurile obtinute de la animalele imune au fost testate in timp pe parcursul imunizarii prin ELISA si titrurile releva maturarea lor afina. Am obtinut cu succes colorarea FACS diferentiat pozitiva(fata de referinta ser pre-imun) anti Ly6C a celulelor ex-vivo murine CD48+ folosind serul obtinut de la animalele imunizate. Figura de mai jos arata clar reactivitatea comparative a serurilor inainte si dupa imunizare de la 3 iepuri (35,36 si 39)dublu pozitiv CD48/Ly6C+. (Figura)



Au fost sortate celule medulare de soarece in diferentiere, preliminar pentru investigandu-se caracteristicile intracelulare si de suprafata necesare discriminarii caracteristicilor necesare liniei mielocitare nisei patologice leucemice (MLL-AF9). Remarcabil intracelular detectia prin RT-PCR la populatia sortata MAC(CD11b) indica cu mare probabilitate prezenta transcriptului MYC 201 (exprimat pe toti cei 3 exoni cMYC) asociat cu dinamica de diviziune marcata fie in stadiile timpurii de diferentiere fiziologica mieloida fie in degenerarea leucemica mielocitara.

Si in 2020 s-au realizat cu succes obiectivele planificate, si anume definitivarea recoltarii ultimelor probe immune (ser si material biologic) de la iepurii imunizati cu ScaI , s-au izolat 4 fractii de anticorpi reactive anti Ly6C care au fost testate pentru afinitatea fata de Ag solubil si la nivel tisular prin imunofluorescenta pe tesut splenic de la soareci cu leucemie mielocitara MLL-AF9(AF9) si sanatosi(WT), si s-au pus in evidenta un set de biomarkeri moleculari pentru caracterizarea evolutiva a modelului murin de leucemie MLL-AF9. Subliniem rezultatul de punere in evidenta si colocalizare pe sectiunile de splina a Ly6C si CD48+. Un set de sectiuni splenice sunt colorate



prin imunofluorescenta (Fig.8.1de la stanga la dreapta)cu : Hoechst pentru profilul nuclear (albastru), cu anticorpii fragmente imune purificate de noi anti-Ly6C colorate cu DyLight-verde, cu Anti CD48 marker de filiatie monocitar -macrofagica matura coloratie rosie PE, si ultima imagine fuzionarea tuturor imaginilor pentru determinarea colocalizarii Ly6C si CD48. Imaginile sunt luate cu fluorescenta la obiectiv 100x. In mod remarcabil subliniem contururile dense portocalii sub forma de coroane perinucleare in imaginile fuzionate de la splenocitele leucemice (AF9 imagine dreapta sus) spre deosebire de aspectul punctiform la tesutul splenic al soarecilor normali(WT dreapta jos). Acest set de coloratii a fost verificat pentru toate cele 4 fragmente imune. Fragmentul i39fr1 cu care s-au obtinut coloratiile din Fig. 8.1 este cel mai reprezentativ pentru identificarea antigenului de suprafata Ly6C celular. Acest rezultat incununeaza efortul nostru de 2

demonstrand atat atributul de biomarker in leucemia acuta pe celule mieloide monocitare MLL-AF9 al Ly6C cat si faptul ca am putut obtine un anticorp cu recunoastere selectiva a acestui receptor celular in context tisular.

Folosindu-se extrase celulare de la celule medulare in diferentiere de soarece normal si leucemic MLL-AF9, s-a pus in evidenta comparative prin RT-PCR la populatiile sortate MAC(CD11b+) si CD48+ nivelul de expresie a transcriptului MYC 201 (exprimat pe toti cei 3 exoni cMYC) asociat cu dinamica de diviziune marcata, fie in stadiile timpurii de diferentiere fiziologica mieloida fie in degenerarea leucemica mielocitara, boala manifesta 25 sapt. Alternativ s-au testat prin imunobloting la aceleasi populatii de provenienta descrisa anterior 6 markeri legati in transductie de cMYC; Ly6C, SOX2, CDK4, CyclinD2, p53 si TIP60.

Pr1.Etapa 4/2021 (Et4).Au fost izolate genele anticorpilor de la iepurii imunizati cu ScaI, si s-au obtinut si caracterizat prin BLI anticorpi puri anti ScaI cu Kd=216,3± 55nM(i40_2.3). Cu acesti anticorpi si cu cei anti Ly6C(i39fr1) s-au caracterizat comparativ profilul monocitar din maduva osoasa provenita de la soareci

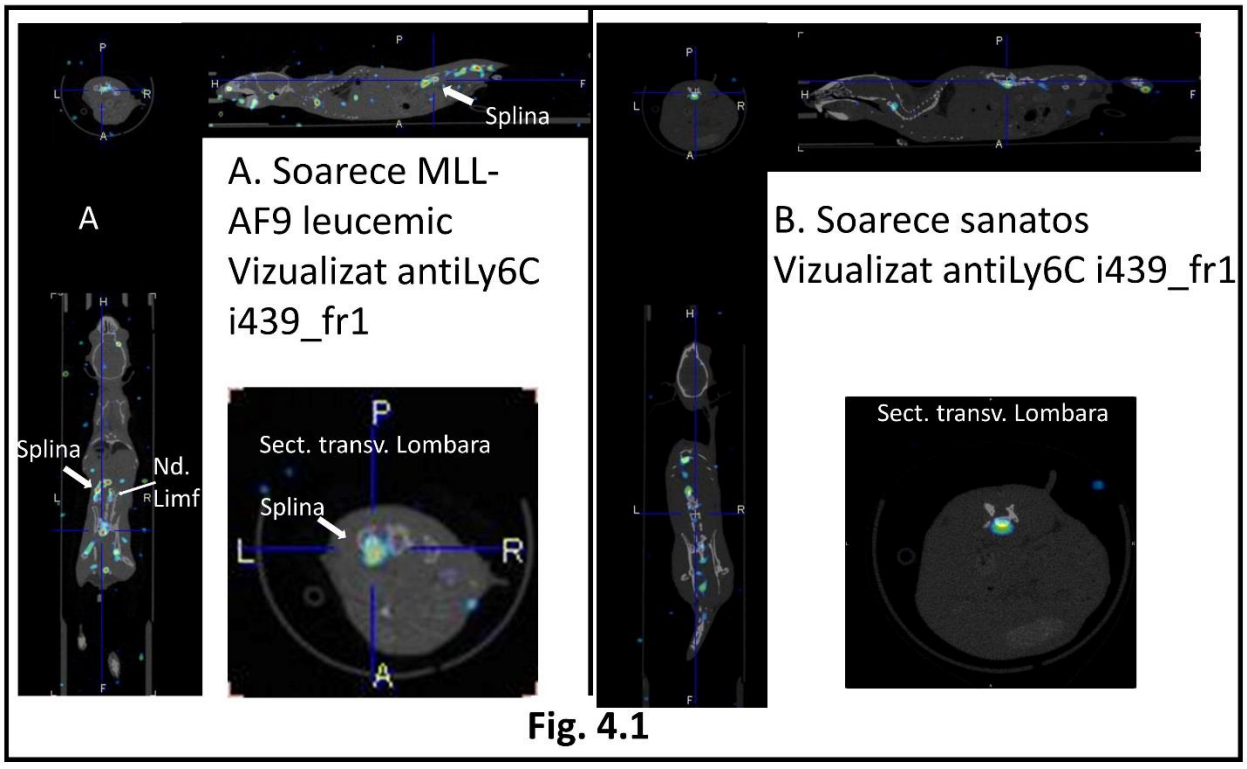


Fig. 4.1

recipienti transplant maduva MLL-AF9 cat si maduva soareci sanatosi, prin citometrie in flux cat si imunohistologic Act4.1.2 indeplinita RST_Et4_2021 pg 1-3. Aceeiasi anticorpi purificati marcati cu ^{89}Zr au fost folositi pentru imagistica PET pe soareci normali sau cu transplant maduva osoasa MLL-AF9 aflati atat in stadiu incipient cat si de leucemie manifesta(Act4.3). Imaginile PET/CT definesc profilul splenic

afectat leucemic pentru biomarker-ul anti Ly6C(Fig. 4.1A) , absent la soareci recipient sanatosi(Fig. 4.1B) si anticorelativ reducerea substantiala a populatiei suse mielocitara din maduva osoasa la soareci leucemici prin biomarker anti ScaI (spre deosebire de cei sanatosi Fig. 5.1). Acest lucru confirma folosirea cu succes a anticorpilor anti-Ly6C ca biomarker in leucemia acuta. (Act4.1. indeplinita RST_Et4_2021 pg 3-5).

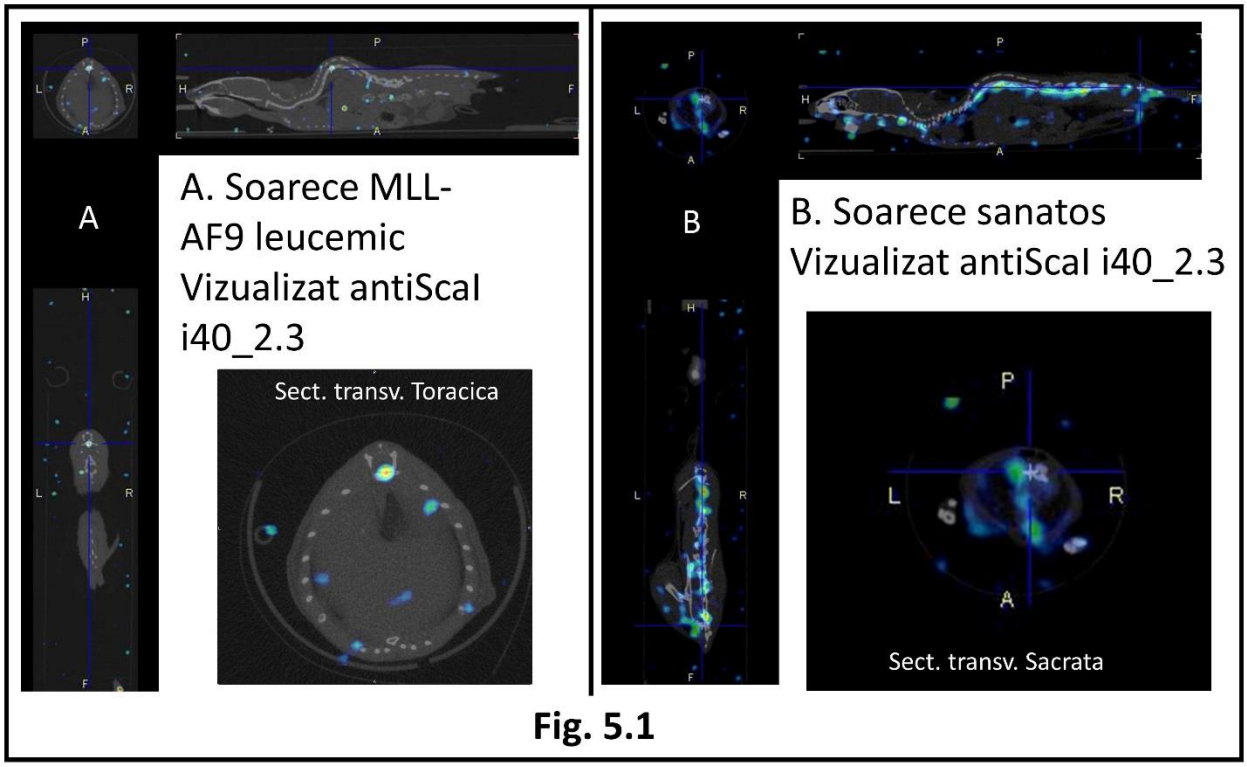


Fig. 5.1

Toti partenerii proiectului *IFIN-HH, IBAR, IC, INCD Victor Babes* au participat la implinirea obiectivelor de faza.

Proiect 2

S-a pus accent pe prepararea si formularea farmaceutica a agentilor destinati imagisticii moleculare prin PET si testarea biologica preclinica in celule tumorale si modelelor animale purtatoare de tumori. S-au furnizat compusi radiomarcati pentru testare; producerea de radioizotopi(Cu-64 si Ga-68) medicali, analize fizico-chimice si furnizarea acestora marcarea radiochimica; prepararea radiofarmaceuticelor si furnizarea acestora pentru evaluarea biologica comparativa; Formularea farmaceutica si caracterizarea analitica a radiofarmaceuticelor tip peptida-chelator-radionuclid compusi marcati si radiofarmaceutice: (^{64}Cu-DOTA-NT, ^{68}Ga-DOTA-PEG(4)-BBN(7-14), ^{68}Ga-DOTA-NeuromedinB.). S-au initiat culturi de linii celulare tumorale standardizate si pe ele s-au testat in vitro a caracteristicilor biologice ale peptidelor radiomarcate pentru validarea acestora privind evaluarea legarii specific mediate de receptori.

Deasemenea, s-au efectuat si testari ale radiofarmaceuticelor mentionate pe modele animale tumorale pentru validarea acestora ca agenti imagistici, studii farmacocinetice, imagistica PET-CT. S-au indeplinit activitati categorie de activitate D3- Activitati suport – vizite de lucru):

Instruirea tinerilor cercetatori si a noilor angajati, Activitati suport pentru instruirea tinerilor

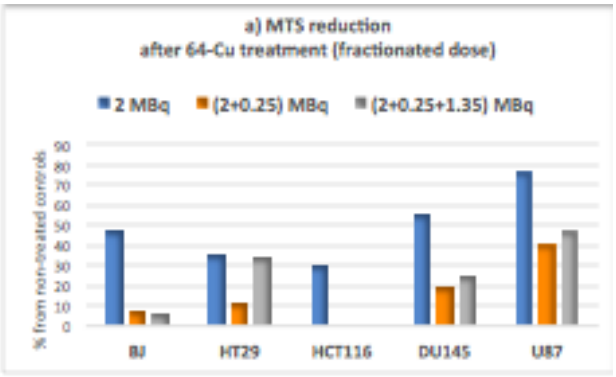
- Stagii de pregatire tineri cercetatori (2) Tehnici de preparare XRF microscopie electronica, XPS si IR
- Vizite de lucru cercetatori cu experienta (2) Determinari microscopie XRF/XPS/IR
- Formarea resursei umane nou angajate si pentru intelegerea de noi tehnici si tehnologii (1): Participare la curs pentru specialisti privind bunele practici de productie (GMP) in industria farmaceutica

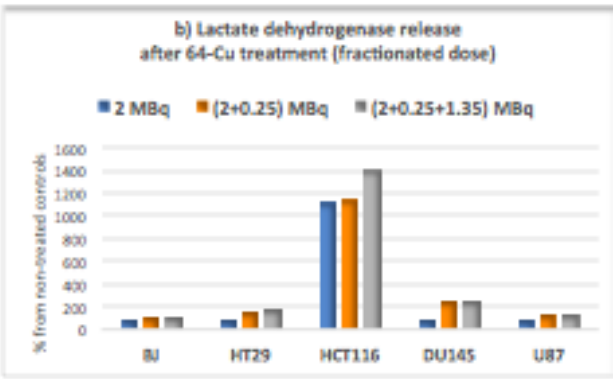
Optimizarea proceselor de iradiere, sinteza radiochimica, formulare farmaceutica si control a agentilor destinati endoradioterapiei (terapiei sistemice cu radioizotopi) si testarea biologica preclinica in diverse celule tumorale si modele animale purtatoare de tumori induse

Rezultate asteptate: Consolidarea competentelor stiintifice si tehnice ale partenerilor prin corelarea si coordonarea activitatilor de cercetare si a resurselor in abordarea experimentala a endoradioterapiei (terapiei sistemice cu radioizotopi) in imagistica PET-CT si terapia personalizata a cancerului.

1- ***Producerea radioizotopului medical Cu-64, separare radiochimica si analize fizico-chimice, marcarea radiochimica*** . A fost produs la ciclotronul TR-19 radioizotopul medical Cu-64, pornind de la tehnologia dezvoltată/optimizată în etapa anterioară și îmbunătățită (validată) în această etapă a proiectului. Astfel, ^{64}Cu pentru utilizare la obținerea de medicamente radiofarmaceutice se realizează în cadrul Centrului de Cercetare Radiofarmaceutică prin iradierea la ciclotronul TR-19 (ACSI Canada), cu protoni accelerați, a țintelor solide de ^{64}Ni, prin reacția nucleară ^{64}Ni(p,n)^{64}Cu. S-au marcat cu ^{64}Cu urmatoarele peptide : secretoare de gastrină (GRP) și din clasa peptidelor tip glucagon (GLP-like) s-au selectat pentru testare analogi ai bombezinei (BBN 1-14 si BBN 7-14) respectiv NeuromedinB.

2- ***Teste preclinice in vitro*** Cultivarea si caracterizarea liniilor celulare tumorale (umane si murine) si furnizarea acestora pentru realizarea experimentelor in vitro. Testarea in vitro a caracteristicilor biologice ale peptidelor radiomarcate cu emitatori α/β- pentru investigarea si cuantificarea efectelor terapeutice(Vezi Figura alaturata). Efectul expunerii la $^{64}CuCl_2$ asupra dinamicii numarului de celule metabolic active in cultura a fost investigat prin tesul reducerii MTS in celule umane tumorale (carcinom de colon HT29 si HCT116, carcinom de prostata DU145 si glioblastom U87 MG) si in celule umane normale (fibroblasti normali din linia BJ). Datele prezentate au aratat ca expunerea celulelor la ^{64}Cu induce scaderea reducerii MTS la 24h de la initierea tratamentului, cele mai reactive celule fiind cele de carcinom de colon din linia HCT116.





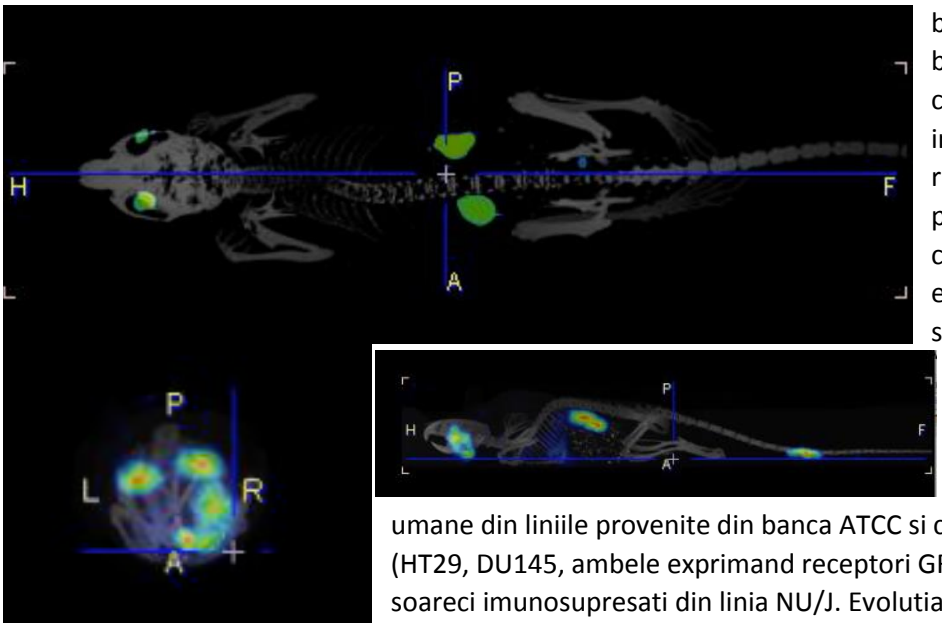
3-Efectul exercitat de ^{64}Cu asupra profilului de expresie a genelor de stress in celulele tumorale sau normale Prin qRT-PCR au fost analizate modificarile induse de expunerea celulelor timp de 24h la ^{64}Cu (doza initiala de 2 MBq/mL) la nivelul a 84 gene critic implicate in raspunsul celulelor la stress. Lista genelor analizate (Human Stress & Toxicity PathwayFinder, Qiagen):

Stres	**Gene**
Stres oxidativ	FTH1, GCLC, GCLM, GSR, GSTP1, HMOX1, NQO1, PRDX1, SQSTM1, TXN, TXNRD1, AKR1B1
Stres hipoxic	ADM, ARNT, BNIP3L, CA9, EPO, HMOX1, LDHA, MMP9, SERPINE1 (PAI-1), SLC2A1, VEGFA
Stress osmotic	AQP1, AQP2, AQP4, CFTR, EDN1, HSPA4L (OSP94), NFAT5, SLC5A3
Stres genotoxic	
- Modularea ciclului celular	CDKN1A (p21CIP1, WAF1), CHEK1, CHEK2 (RAD53), DDIT3 (GADD153, CHOP), HUS1, MRE11, NBN, RAD17, RAD9A, GADD45A
- Alte raspunsuri la alterarea ADN	ATM, ATR, DDB2, GADD45A, GADD45G, RAD51, TP53 (p53), XPC
Stres proteotoxic	ATF4, ATF6, ATF6B, BBC3 (PUMA), BID, CALR, DDIT3 (GADD153, CHOP), DNAJC3, HSP90AA1, HSP90B1, HSPA4 (HSP70), HSPA5 (GRP78)
Stres inflamator	CCL2 (MCP-1), CD40LG, CRP, CXCL8 (IL8), IFNG, IL1A, IL1B, IL6, TLR4, TNF
Moarte celulara	
- apoptoza	CASP1 (ICE), FAS, MCL1, TNFRSF10A (TRAIL-R), TNFRSF10B (DR5), TNFRSF1A (TNFR1)
- necroza	FAS, GRB2, PARP1 (ADPRT1), PVR, RIPK1, TNFRSF10A (TRAIL-R), TNFRSF1A (TNFR1), TXNL4B
- autofagie	ATG12, ATG5, ATG7, BECN1, FAS, ULK1

Rezultatele experimentale au evidentiat diferente intre profilul de expresie a genelor de stres in celulele de carcinom uman de colon din linia HCT116 si cele de carcinom uman de prostata din linia DU145, care sustin diferentele inregistrate privitor la impactul expunerii celulelor la ^{64}Cu asupra viabilitatii/proliferarii acestor celule tumorale. Se remarca faptul ca celulele HCT116 sunt mult mai sensibile la actiunea citotoxica a ^{64}Cu comparativ cu celulele DU145.

4- ***Teste preclinice in vivo*** – Evaluarea comparativa a compusilor marcati cu beta emitatori/electroni Auger (Cu-64). Studii microPET-CT pentru evaluarea imagistica a efectului terapeutic si monitorizarea in timp real a compusilor marcati, evaluarea comparativa a compusilor radiofarmaceutici ^{64}Cu-DOTA-BBN(7-14); ^{64}Cu-DOTA-NeuromedinB, ^{64}Cu-DOTA-NT.

a raport de validare a modelelor animale pentru oncologia translationala,



b raport de evaluare biologica realizata in consortiu, abordata interdisciplinar,1 produs radiofarmaceutic selectat, propus pentru studii clinice, destinat endoradioterapiei (terapie sistemica cu radioizotopi): ^{64}Cu-DOTA-BBN(7-14). *Parteneri: IFIN-HH, IC, UB, INCD Victor Babes, UMFCD*Celule tumorale umane din liniile provenite din banca ATCC si cultivate la IFIN-HH si IVB (HT29, DU145, ambele exprimand receptori GRPR) au fost implantate in soareci imunosupresati din linia NU/J. Evolutia si aspectul tumorilor se observa in figurile de mai jos.

Toti partenerii proiectului *IFIN-HH*, *UB*, *IC*, *INCD Victor Babes*, *UMFCD*, au participat la implinirea obiectivelor de faza.

Proiectul component 3
Dezvoltarea de solutii inovative pentru eficientizarea tehnicilor de tratament cu radioizotopi

Obiectivul stiintific: dezvoltarea de solutii inovative pentru eficientizarea tehnicilor de imagistica si tratament cu radioizotopi, prin:
1) nanoformularea noilor radiofarmaceutice tintite;
2) modularea farmacologica a raspunsului antioxidant endogen al celulelor tumorale prin modularea farmacologica a factorul de transcriptie NRF2.

Noutatea stiintifica si tehnica

a) investigarea modificarilor moleculare din reteau de semnalizare redox a celulelor normale si tumorale pentru monitorizarea endoradioterapiei;
b) utilizarea factorului de transcriptie NRF2 ca tinta terapeutica in radioendoterapia tumorilor solide; **c)** utilizarea conceptelor biologiei de sistem si a tehnologiilor „omice" pentru investigatii avansate in radioendoterapia tumorilor (transcriptomica functionala, profil proteic MALDI).

Consortiu
Coordonator: Institutul National de Cercetare-Dezvoltare in Domeniul Patologiei si Stiintelor Biomedicale "Victor Babes" (IVB, CSI Dr. Gina Manda)

Parteneri
Institutul National de Cercetare-Dezvoltare pentru Fizica si Inginerie Nucleara "Horia Hulubei" (IFIN-HH, CSII Dr. Dana Niculae)
Institutul Național de Cercetare Dezvoltare Medico-Militară "Cantacuzino" (INC, CSII Dr. Crina Stavaru)
Institutul National de Cercetare-Dezvoltare pentru Tehnologii Izotopice si Moleculare (INCDTIM, CSI Dr. Rodica Turcu)
Universitatea din Bucuresti, Facultatea de Biologie (UB, Prof. Dr. Anca Dinsichiotu)

- Transfer tehnologic si de cunoastere
    - 2 experimente realizate in comun de echipa de cercetare din IFIN-HH si IVB in perioada 12-23.11.2019.

Vizite de lucru ale cercetatorilor de la INCDTIM la IFIN-HH

Tip de stagiu/vizita	**Nume**	**Perioada**	**Scop**
Stagiu de pregatire pentru tineri cercetatori	Ispas George	28.10.2019 – 31.10.2019	Metodele de preparare a nanoparticulelor magnetice
Vizite de lucru ale cercetatorilor cu experienta	Nan Alexandrina	19.11.2019	Protocoale de absorbtie a polimerului functionalizat cu peptide pe suprafata nanoparticulelor magnetice
	Patran Anca	19.11.2019 – 20.11.2019	
	Turcu Rodica	19.11.2019 – 21.11.2019	Discutarea rezultatelor obtinute pana in present privind proprietatile nanoparticulelor si polimerilor pentru atasarea radiofarmaceuticelor. S-au prezentat metodele de analiza a nanoparticulelor functionalizate.

In ultimele 2 etape 3 si 4 am realizat:

3.1-4.1 producerea si caracterizarea radio-chimica a unei prime transe de radiofarmaceutice selectate pentru nanoformulare si pentru experimentare in model animal. Rezultate

- 4 tipuri de neuropeptide marcate radioactiv cu ^{68}Ga pentru imagistica moleculara: ^{68}Ga-DOTA-NT (neurotensina), ^{68}Ga-DOTA-NMN (neuromedina N), ^{68}Ga-DOTA-PEG(4)-BBN(7-14) (bombezina), ^{68}Ga-DOTA-NMB (neuromedina M).

Metodologia de preparare a radiofarmaceuticelor este descrisa in articolul Chilug L et al. *Molecules* 2020, 25(15), 3363; https://doi.org/10.3390/molecules25153363, realizat in cadrul proiectului si prezentat in Anexa 1(incarcata in platforma proiectului Anexe1_6Proiect 3ONCORAD).

3.11 Producerea si caracterizarea unei prime transe de nanosisteme incarcate cu radiofarmaceutice tintite, selectate pentru experimentare in model animal.

4.2 Realizarea modelelor animale tumorale si transferul acestora catre IFIN-HH pentru tratament cu radiofarmaceutice. 2 modele de tumori umane (carcinom de colon din linia HCT116 sau HT29 si carcinom de prostata din linia DU-145) realizate in soareci imunosupresati din linia NU/J (The Jackson Laboratory, USA, https://www.jax.org/strain/002019).Soarecii NU/J au genotip Foxn1^{nu} (absenta genei care codifica proteina Forkhead box N1). Soarecii homozigori NU/J nu au celule T adulte si nu dezvolta imunitate mediata celular, ceea ce ii face adecvati pentru implantare de tumori umane, cu probabilitate redusa de rejectie a celulelor umane. Pe scurt, liniile celulare tumorale au fost achizitionate de la colectia ATCC (HT29: ATCC® HTB-38™, HCT116: ATCC® CCL-247™, DU145: ATCC® HTB-81™) si au fost mentinute in cultura conform indicatiilor producatorului. Cu 48h inainte de experimentare celulele au fost pasate, reinsamantate la densitatea de 20.000 celule / cm^2. Dupa 48h celulele au fost recoltate din cultura (cand celulele nu au atins confluenta, evitatand-se inhibitia de contact care ar fi scazut capacitatea proliferativa a celulelor tumorale). 1 x 10^6 celule tumorale in 200µL tampon fosfat salin (PBS) au fost inoculate sub-cutan in flancul drept al fiecarui soarece NU/J. Periodic s-a determinat diametru mediu al tumorilor. Celulele tumorale si radiofarmaceuticele au fost investigate ulterior in model animal. Datele experimentale sunt prezentate in Anexa 3 si in articolul Chilug L et al. *Molecules* 2020, 25(15), 3363, realizat in cadrul proiectului. S-a demonstrat faptul ca radiofarmaceuticele investigate se incorporeaza rapid in celulele tumorale, indiferent daca au fost sau nu nanoformulate.

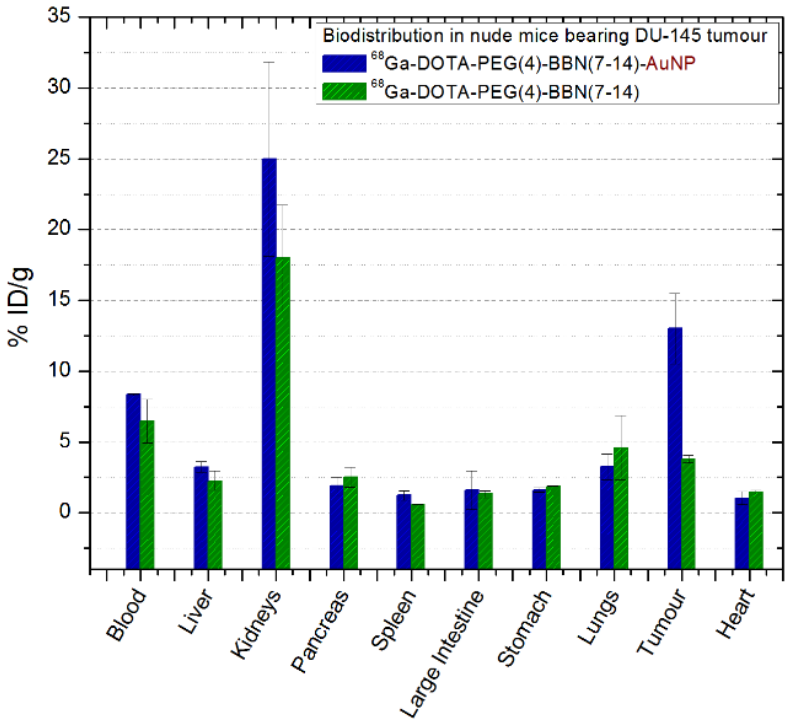


Figura. Biodistributia radiofarmaceuticului ^{68}Ga-DOTA-PEG(4)-BBN(7-14)-AuNP in soareci NU/J purtatori de carcinom uman de prostata din linia DU145. Comparatie cu biodistributia ^{68}Ga-DOTA-PEG(4)-BBN(7-14).

S-a demonstrat de asemena ca nanoformularea creste semnificativ retentia radiofarmaceuticelor in celulele tumorale, cele mai notabile rezultate obtinandu-se cu radiofarmaceuticul nanoformulat **^{68}Ga-DOTA-PEG(4)-BBN(7-14)-AuNP** in cazul celulelor de carcinom de prostata DU145, caz in care se ating

valori ale retentiei in celule de aproximativ 75% comparativ cu radiofarmaceuticul care nu a fost nanoformulat si care inregistreaza o retentie de 38% .Cea mai slaba internalizare ca si o retentie fluctuanta a fost inregistrata in cazul radiofarmaceuticelor tintite de neuromedina B in celule de carcinom de colon HT29, ceea ce sugereaza ca acest tip de radiofarmaceutic ar trebui sa se adreseze in principal carcinomului de prostata.

3.14 Demonstratie preliminara in model animal a posibilitatii de eficientizare a tehnicilor de tratament cu radioizotopi prin inhibarea sistemului antioxidant endogen cu ajutorul modulatorilor farmacologici ai factorului de transcriptie NRF2. Rezultate: 3 studii biologice .

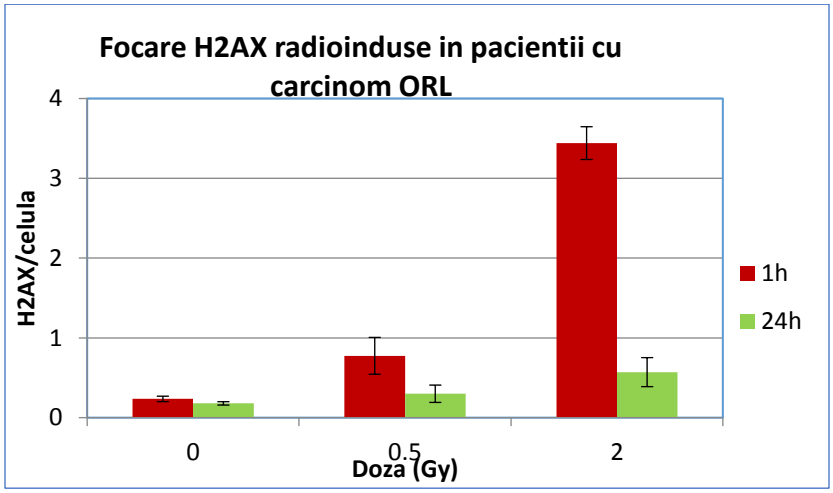
3-15 Elaborarea documentatiei preliminare pentru brevetarea unui nanosistem radiofarmaceutic

3.16 Diseminarea rezultatelor(Vezi la final lista conferintelor si articolelor consortiului).

Proiect 4/

S-au realizat cu succes obiectivele planificate privind testarea preliminara a radiosensibilitatii probelor biologice de la pacienti inaintea si respective dupa radioterapie. Efectele secundare induse de radioterapie afecteaza un numar important de pacienti bolnavi de cancer. Astfel, calitatea vietii pacientului post-tratament este amenintata si implica eforturi din partea sistemului de sanatate pentru a ajuta pacientul. De aceea este imperios necesara dezvoltarea unor biomarkeri care pot detecta pacientii care prezinta riscul sa sufere efecte adverse in urma tratamentului radioterapeutic. Prezentul proiect este focalizat pe dezvoltarea unei tehnologii de identificare pre-terapeutica a pacientilor radiosensibili bolnavi de cancer cervical.Puterea de predictie a biomarkerilor propusi in acest proiect va fi testata pe probe de sange folosind metode standardizate, prin corelarea radiosensibilitatii celulare in vitro cu efectele adverse toxice aparute in urma radioterapiei pacientilor.

Rezultate preliminare:



Numarul focarelor H2AX radioinduse de iradierea cu raze X in limfocitele de la pacienti cu neoplazii din sfera ORL

Toxicitatea indusa de radiatie in pacientii tratati cu radioterapie. In Figura 1 si Tabelul1 este ilustrata distributia pacientilor care sufera de reactii toxice adverse la nivelul pielii, mucoaselor, faringelui/esofagului, glandei salivare si laringelui.

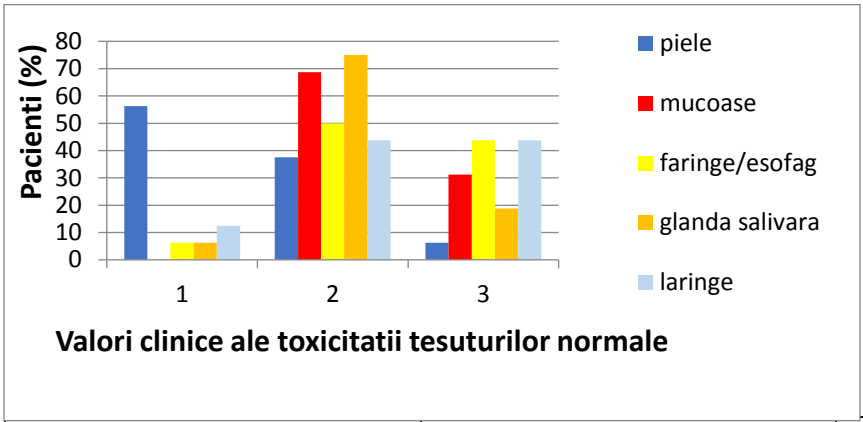


Figura 1 Valorile clinice ale toxicitatii tesuturilor normale induse de radioterapia pacientilor

Tabel 1. Efectele toxice inregistrate conform sistemului de clasificare RTOG in pacientii cu neoplasme in sfera ORL supusi radioterapiei

Reactii adverse	Clasificare RTOG	Pacienti cu neoplasme in sfera ORL (%)
Piele	G1	56,25
	G2	37.5
	G3	6.25
Mucoase	G1	0
	G2	68.75
	G3	31.25
Faringe/esofag	G1	6.25
	G2	50
	G3	43.73
Glanda salivara	G1	6.25
	G2	75
	G3	18.75
Laringe	G1	12.5
	G2	43.75
	G3	43.75

La nivelul pielii, cei mai multi pacienti (56.25%) au suferit reactii de gradul 1, in timp ce la nivelul mucoaselor, glandei salivare si faringelui/esofagului majoritatea (68.75%, respectiv 75% si 50%) a suferit reactii de gradul 2. La nivelul laringelui, 43.75% au suferit reactii de gradul 2, iar alti 43.75% au suferit reactii de gradul 3.

Analiza transcriptomica prin microarray

Selectarea genelor de interes (prin utilizarea bioinformaticii) si validarea lor prin RT-PCR si Western-blotting. In urma prelucrarii bioinformatice a datelor de expresie genica in software GeneSpring 12.0 au fost obtinute seturi de gene exprimate diferential conform conditiilor de tratament.

Conditia I – 2Gy & pre-iradiere vs proba control (STDA). In setul de date Microarray au fost obtinute 3 gene radiosensibile implicate in repararea leziunilor genetice (ATM; XRCC1; XRCC3).

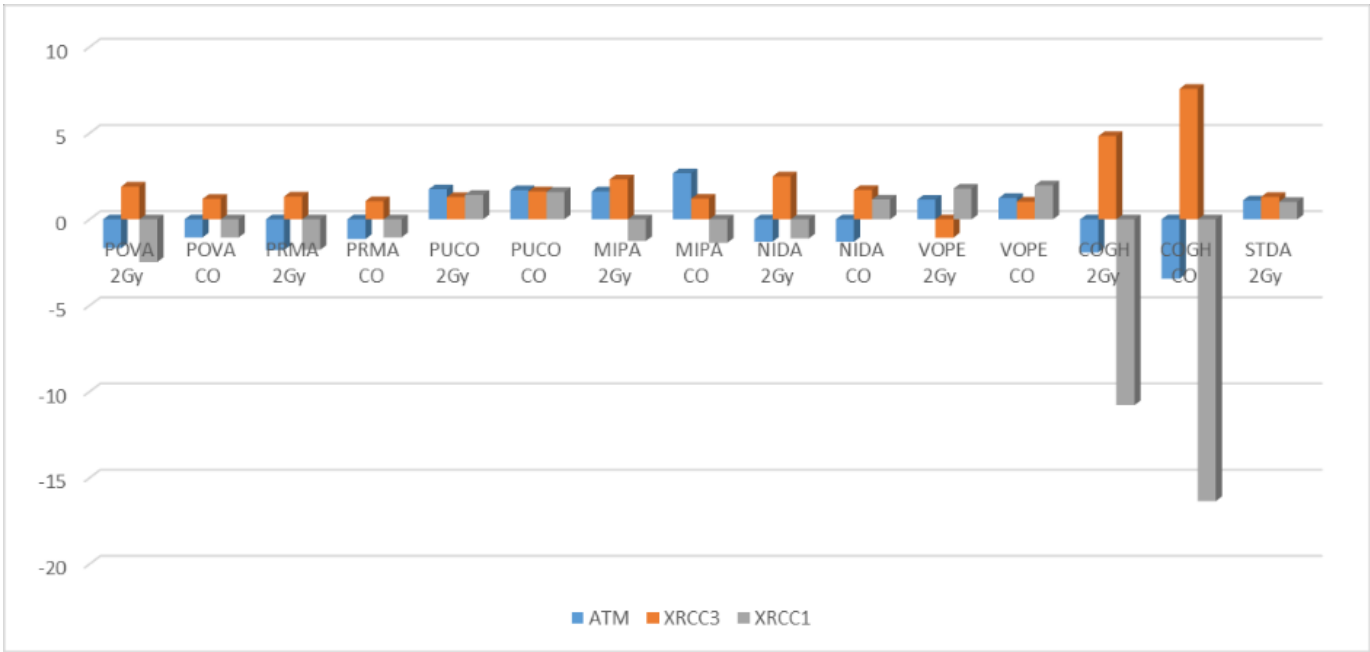
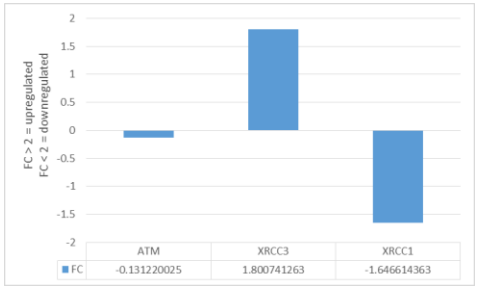


Figura 2 - Expresia genica diferentiala prin comparatie a probelor tratate cu 2Gy si pre-iradiere vs proba control (STDA)

Datele evidentiaza o supra-expresie a genei XRCC3 invers proportionala cu expresia relativa a genelor XRCC1 si ATM la conditiile pacientilor radiotratati cu 2Gy comparativ cu proba control provenita de la un pacient sanatos. In schimb, in contextul expresiei genice diferentiale a probelor pre-iradiere vs proba control, datele evidentiaza o supraexpresie statistic semnificativa a genei XRCC3 (FC > 2) invers proportionala cu subexpresia genei XRCC1 (FC < -2).



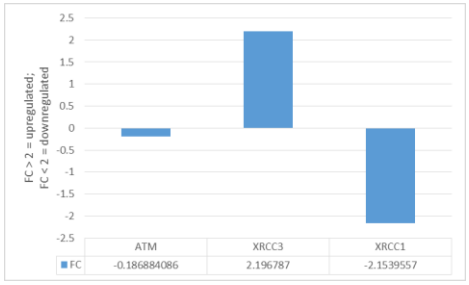
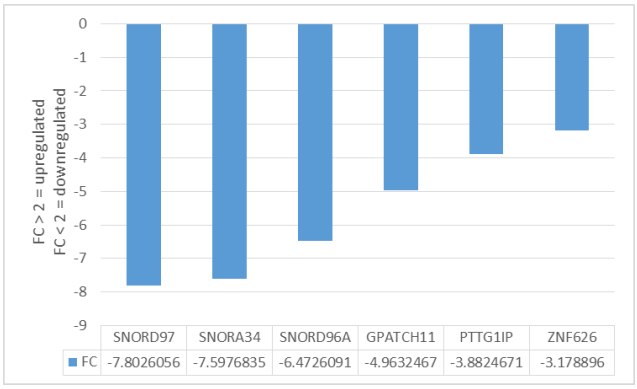


Figura 3 stanga - Media expresiei genice diferen-tiale a probelor radio-tratate 2Gy vs proba control (STDA)

Figura 4 dreapta – Media expresiei genice diferentiale a probelor pre-iradiere vs proba control (STDA).

In concluzie, au fost obtinute semnaturi specifice de expresie genica pe loturile de pacienti in corelatie cu calitatea probelor de ARN. Rezultatele indica ca gena XRCC1 prezinta un nivel de expresie scazut pentru conditia I de comparatie (paccienti tratati 2Gy vs pacienti control), insa XRCC3 prezinta un nivel de expresie crescut. In schimb se observa ca gena XRCC3 este subexprimata pentru conditia 2 (pacienti radiorezistenti vs pacienti radiosensibili in urma radioterapiei cu 2 Gy), iar gena XRCC1 este supraexprimata. Acest patern de expresie in tandem intre cele doua conditii poate indica gradul de toxicitate la pacientii radiosensibili comparativ cu cei radiorezistenti si poate fi utilizati ca markeri de prognostic al radioterapiei la pacienti cu cancer mamar sau din sfera ORL



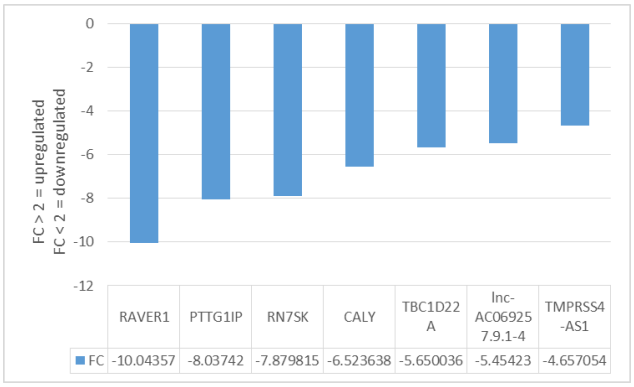


Fig 5stanga–Media subexpresiei genelor comune ale fractiei leucocitare 20 vs probe radiotratate cu 2Gy; Figura6– Media subexpresiei genelor comune ale fractiei leucocitare 20 vs probe pre-iradiere

Toti partenerii proiectului *IFIN-HH*, *ICF*, *IBAR*, *IC*, au participat la implinirea obiectivelor de faza.

1) Articole stiintifice scrise publicate in consortiu

1a -publicate deja fie in print fie electronic, sau manuscrise deja acceptate spre publicatie

1-Preclinical Evaluation of NHS-Activated Gold Nanoparticles Functionalized with Bombesin or Neurotensin-Like Peptides for Targeting Colon and Prostate Tumours, (2020) Chilug, L. E., Niculae, D., Leonte, R. A., Nan A., Turcu R., Mustaciosu C., Serban, R. M., Lavric, V., Manda G., *Molecules* 25 (15) 2020, pg 3363, DOI: 10.3390/molecules25153363

2- Poly(1-vinylimidazole) grafted on magnetic nanoparticles - attainment of novel nanostructures(2020) Nan A. and Turcu R., *Rev. Roum. Chim*., 2020, *65*(6), 611-616 DOI: 10.33224/rrch.2020.65.6.12

3- Characterization of the Nuclear Magnetic Resonance Relaxivity of Gadolinium Functionalized Magnetic Nanoparticles**(2020)** Nan A. , Suciu M., Ardelean I. , Şenilă M. and Turcu R. (2020), *Analytical Letters*, DOI: 10.1080/00032719.2020.1731522

4- Engaging health professionals and patients in the medical field: role of radiological protection culture and informed consent practices (2020) , C. Schieber, C. Pölzl-Viol, M.-C. Cantone, N. Železnik, S. Economides, R. Gschwind, B. Abelshausen, D. Savu, S. Lafage, L. Liutsko, S. Charron, C. Turcanu and R. Geysmans, *Radioprotection*, epub https://doi.org/10.1051/radiopro/2020039

5- Efficient uptake and retention of iron oxide-based nanoparticles in HeLa cells leads to an effective intracellular delivery of doxorubicin (2020) R. C. Popescu, D. Savu, I. Dorobantu, B. S. Vasile, H. Hosser, A. Boldeiu, M. Temelie, M. Straticiuc, D. A. Iancu, E. Andronescu, F. Wenz, F. A. Giordano, C. Herskind and M. R. Veldwijk, *Sci Rep 10 10530* https://doi.org/10.1038/s41598-020-67207-y /

6- Magnetic Nanoparticle Systems for Nanomedicine—A Materials Science Perspective(2020) Magnetochemistry/ Vlad Socoliuc , Davide Peddis, Viktor I. Petrenko, Mikhail V. Avdeev, Daniela Susan-

Resiga, Tamas Szabó, Rodica Turcu, Etelka Tombácz and Ladislau Vékás
doi:10.3390/magnetochemistry6010002

7- The transcription factor NRF2 shapes the identity of radio-resistant tumor cells (2020) *Journal of Cell Identity*, G. Manda, A. Cuadrado , (2020) 1, pg 49-81, DOI: 10.47570/joci.2020.004

8- Radiofrequency EMF irradiation effects on pre‐B lymphocytes undergoing somatic recombinationE. Ionita, A. Marcu, M. Temelie, D. Savu, M. Serbanescu and M. Ciubotaru ***Sci Rep* 11,** 12651 (2021) https://doi.org/10.1038/s41598-021-91790-3

9-Dobre M, Boscencu R, Neagoe IV, Surcel M, Milanesi E, Manda G. Insight into the Web of Stress Responses Triggered at Gene Expression Level by Porphyrin-PDT in HT29 Human Colon Carcinoma Cells. Pharmaceutics. 2021 Jul 7;13(7):1032. doi:10.3390/pharmaceutics13071032. PMID: 34371724; PMCID: PMC8309054

10- Responsiveness assessment of cell cultures exposed to poly(tartaric acid) and its corresponding magnetic nanostructures, I-V. Ganea , A. Nan, A. Ciorîţa , R. Turcu, C. Baciu, Journal of Molecular Structure 1248, (2021), 131459, https://doi.org/10.1016/j.molstruc.2021.131459

2)Prezentari la conferinte nationale si Internationale sub forma de 2a) prezentari orale, 2b) sub forma de poster, 2c) workshop sau prezentari via web

1. Automated production and purification of copper medical radioisotopes in a variable energy cyclotron using solid targets, Dana Niculae, Simona Ilie, Radu Leonte, Livia Chilug, Liviu Craciun The 23rd International Symposium on Radiopharmaceutical Sciences (ISRS 2019), Beijing, China, 26-31 May 2019.

2. Comparative preclinical evaluation of 68Ga-labelled Neuromedin N and B for targeting glioblastoma malignant tissues Livia E. Chilug, R. A. Leonte, Gina Manda, C. Mustaciosu, V. Lavric and Dana Niculae International Symposium on Trends in Radiopharmaceuticals (ISTR-2019), IAEA Vienna, Austria, 28 October - 1 November, 2019

3. Comparative Radiobiological Evaluation of Intracellular Effects Induced by $^{64}CuCl_2$ in Different Tumor Cells, Dana Niculae, Livia E. Chilug, R. M. Serban, C. Mustaciosu, Mihaela Temelie, Ramona Dusman, R. A. Leonte, Doina Draganescu, D. A. Niculae, Diana Savu, Anca Dinischiotu, International Symposium on Trends in Radiopharmaceuticals (ISTR-2019), IAEA Vienna, Austria, 28 October - 1 November, 2019.

4. Radiobiological Evaluation of Copper-64 on Tumor Cells, D. Niculae, R. Leonte, R. Serban, L. Chilug, D. Draganescu, M. Temelie, D. Savu, 32nd Annual Congress of the European Association of Nuclear Medicine, Barcelona, Spain, October 12-16, 2019

5. Activated Gold Nanoparticles Conjugated with 68Ga-DOTA-PEG(4)-BBN(7-14) for Targeting Tumours Expressing GRP Receptors, D. Niculae, L. E. Chilug, R. M. Serban, A. J. Abrunhosa, R. A. Leonte, R. Turcu, A. Nan, V. Lavric;32nd Annual Congress of the European Association of Nuclear Medicine, Barcelona, Spain, October 12-16, 2019

6. Evaluation of the Radiotoxicity of Gallium-68 Labelled Pharmacological Compound Serban, R.M., Temelie, M., Niculae, D., Draganescu, D., Dinischiotu, A. PROCEEDINGS OF THE ROMANIAN NATIONAL CONGRESS OF PHARMACY, 17TH EDITION: 21ST CENTURY PHARMACY - BETWEEN INTELLIGENT SPECIALIZATION AND SOCIAL RESPONSIBILITY, 2018

7. Synthesis and Biological Evaluation of Gallium Radiopharmaceuticals Compounds Raicu, A., Min, E.A, Chilug, L.E., Leonte, R.A., Serban, R.M., Tuta, C.S., Draganescu, D., Niculae, D. PROCEEDINGS OF THE ROMANIAN NATIONAL CONGRESS OF PHARMACY, 17TH EDITION: 21ST CENTURY PHARMACY - BETWEEN INTELLIGENT SPECIALIZATION AND SOCIAL RESPONSIBILITY, 2018

8. An emerging medical radioisotope: Copper-64 from production to clinical application Dana Niculae Nuclear Medicine Days, Sinaia, Romania, June 7-8, 2019.

9. Activated gold nanoparticles conjugated with 68Ga-DOTA-PEG(4)-BBN(7-14) for targeting tumours expressing GRP receptors Livia Chilug, Radu Leonte, Dana Niculae, Doina Draganescu, Rodica Turcu, Alexandrina Nan, Gina Manda, Vasile Lavric Nuclear Medicine Days, Iasi, Romania, 01-04 November 2018.

10. 19th International Balkan Workshop on Applied Physics and Material Science, Constanta, Romania, July 16-19 / lectie invitata / Advances in Bio-medical Application of Ionizing Radiation at IFIN-HH & ELI-NP, M. Radu, M. Temelie, M. Bacalum, R. Popescu, D. Savu, L. Craciun, D. Niculae, M. Straticiuc, I. Burducea, P. Vasos, D. Stutman / 2019

11. H2020 ENGAGE workshop, Bratislava, Slovak Republic, September 11-13 / comunicare poster / MEDICAL Informed consent in medical use of radiation for cancer treatment in Romania Diana Iulia Savu and Daniela Stroe / 2019

12. 35th Congress of Turkish Physics Society, Bodrum, Turcia, September 4-8 / lectie invitata / New developments in radiobiology with biomedical applications for cancer therapy, D. Savu, M. Temelie, R. Popescu, M. Bacalum, M. Straticiuc, L. Craciun, M. Radu / 2019

13. Eighth International Conference On Radiation In Various Fields Of Research July 20-24, Herceg Novi VIRTUAL CONFERENCE | www.rad-conference.org / comunicarea poster Evaluation of individual in vitro radiation response in PBMC samples of H&N cancer patients correlated with severity of side effects following radiotherapy. Mihaela TEMELIE, Patricia MEDESAN, Mihaela TUDOR, Daniela STROE, Diana SAVU / 2020

14. Eighth International Conference On Radiation In Various Fields Of Research July 20-24, Herceg Novi VIRTUAL CONFERENCE | www.rad-conference.org/ comunicare poster Bystander effects induced by proton-irradiated human chondrosarcoma cells. Mihaela Tudor, Mihaela Temelie, Roxana Cristina Popescu, Anna Rzjanina, Gennady Mytsin, Francois Chevalier, Diana Iulia Savu / 2020

15. Eighth International Conference On Radiation In Various Fields Of Research July 20-24, Herceg Novi VIRTUAL CONFERENCE | www.rad-conference.org/ comunicare poster Radiosensitization effects of doxorubicin-iron oxide nanosystem in charged particle radiation therapy for human

chondrosarcoma. Mihaela Tudor, Roxana Cristina Popescu, Anna Rzjanina, Gennady Mytsin, Mihai Radu, François Chevalier, Diana Iulia Savu / 2020

16. Eighth International Conference On Radiation In Various Fields Of Research July 20-24, Herceg Novi VIRTUAL CONFERENCE | www.rad-conference.org/ comunicare orala Drug delivery nanosystems for intracellular release of doxorubicin improve the clonogenic inactivation of X-Rays in human cervical adenocarcinoma cells. Roxana Cristina Popescu, Verena Kopatz, Hiltraud Hosser, Frank Schneider, Ecaterina Andronescu, Frederik Wenz, Frank Giordano, Carsten Herskind, Wolfgang Doerr, Marlon, R. Veldwijk, Diana Iulia Savu / 2020

17. Eighth International Conference On Radiation In Various Fields Of Research July 20-24, Herceg Novi VIRTUAL CONFERENCE | www.rad-conference.org/ comunicare poster In vitro magnetic targeted delivery of doxorubicin using iron oxide nanoparticles leads to enhanced cell death in glioblastoma. Roxana Cristina Popescu, Alexandru Daniel Olarescu, Oana Gherasim, Stefan Banita, Mihai Straticiuc, Dragos Alexandru Mirea, Radu Florin Andrei, Bogdan Stefan Vasile, Gabriel Socol, Ecaterina Andronescu, Diana Iulia Savu /2020

18. 45th Annual Meeting of the European Radiation Research Society September 13 - 17, Lund, Sweden / comunicare poster / Bi-modal treatment using radiation therapy and drug delivery nanosystems for enhanced cytotoxicity in radio-resistant tumor models. Roxana-Cristina Popescu, Mihai Straticiuc, Cosmin Catalin Mustaciosu, Roxana Trusca, Hiltraud Hosser, Carsten Herskind, Mihai Radu, Marlon R. Veldwijk, Diana Iulia Savu / 2020

19. National Online Conference of Biophysics -CNB 2020- Braşov, 14th – 16th June / comunicare poster / In vitro Radiosensitivity Evaluation of Peripheral Blood Lymphocytes Isolated From H&N and Breast Cancer Patients. Patricia Medesan, Mihaela Tudor, Daniela Stroe, MihaelaTemelie and Diana Savu / 2020

20. " Noi aficorpi marcati radioactiv pentru imagistica si terapie directionata " 2019, SZEDLACSEK Stefan Eugen, Academia de Stiinte Ungare / Institutul de Cercetari Nucleare (ATOMKI) Debrecen

21. Hypoxia and antioxidant signaling in human colon carcinoma HCT116 exposed in vitro to 64-Cu, Gina Manda, Dana Niculae, Ionela Neagoe, Radu Serban, Dragos-Serban Niculae, Elena Milanesi, Maria Dobre. Conferința ”Ninth International Conference on Radiation in Various Fields of Research, 14-17.06.2021, Muntenegru. Prezentarea a fost realizată ca ePoster.

22. Biological evaluation of innovative theranostic agents based on peptides-functionalized iron oxide nanoparticles for colon cancer, Livia E. Chilug, Radu A. Leonte, Ramona Dusman, Gina Manda, Rodica Turcu, Alexandrina Nan, Dana Niculae. Workshop ”Adaptation of the tumour and its ecosystem to radiotherapies - Mechanisms, imaging and therapeutic approaches, 22-25 septembrie 2021, Le Bono, Franta. Prezentare orală.

3) elaborare de cereri de brevet -Depunerea unei cereri de brevet la OSIM pentru o metoda imagistica alternativa in oncologie: derivati de 4-(4-(DIALCHILAMINO)BENZILIDEN)OXAZOL-5(4H)-ONĂ fluorescenti

necitotoxici cu aplicatii biomedicale, care contin un radical arilsulfonil in pozitia 2 (cerere nr. A2020/00490) avand ca prim-autor pe doctoranda Elena-Valentina Rosca angajata in proiectul 3;

-Nanostructuri magnetice pe bază de polimer funcționalizat cu peptida neurotensină, marcate cu radioizotopul ^{64}Cu pentru diagnostic în patologia oncologică/OSIM/16.09.2021 (nr. inregistrare la OSIM A100558/16 sept2021), Proiect 3.

- Cerere Brevet submisa prin posta catre evaluare OSIM cu titlul " Procedeu rapid pentru obținerea selectivă de anticorpi din plasmă imunizată cu idiotip ce interacționează eficient cu antigenul" Nr de insotire posta S89/22.09.2021, Nr adresa de insotire 261/22.09.2021. IFIN-HH, Proiect1.

ENGLISH

Results obtained throughout the project

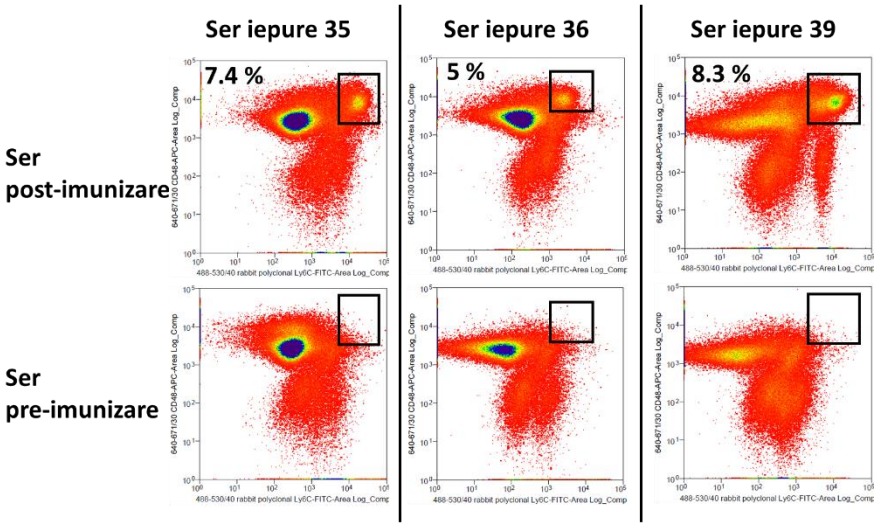
From the 1st of May 2018 until 30th Sept. 2021, finalized project.

Project PN-III-P1-1.2-PCCDI-2017-0769, Contract 64/2018

The development in oncology of novel radiopharmaceuticals and nuclear techniques for diagnostic imaging and personalized treatment at molecular level **(ONCORAD)**

Project 1

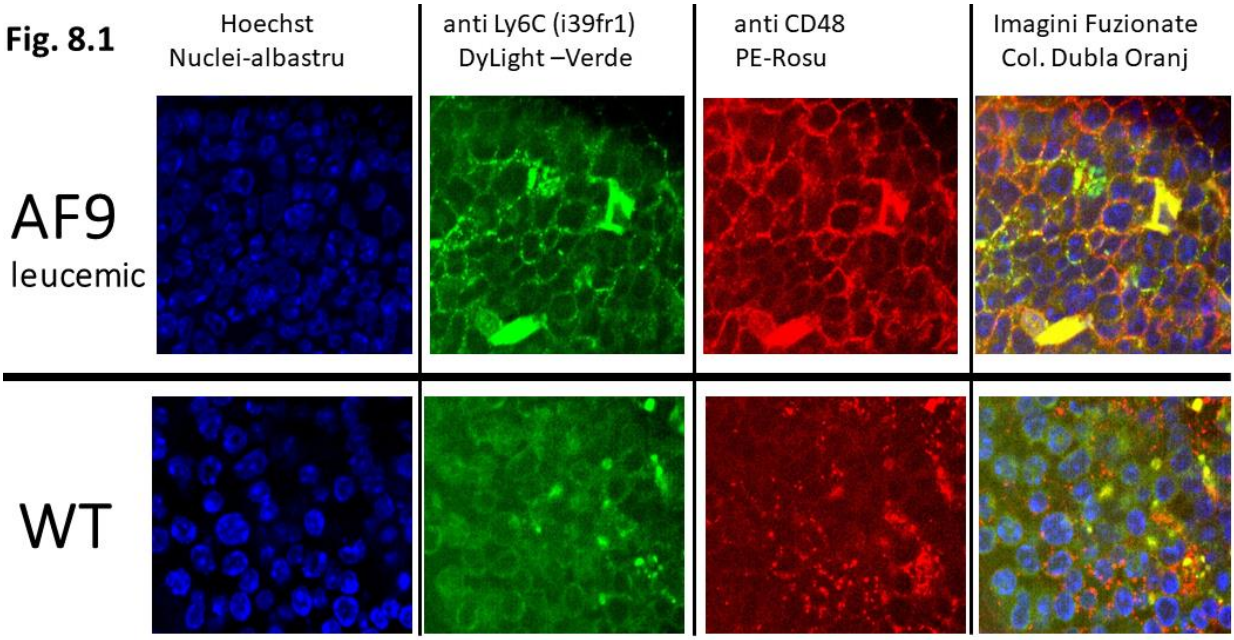
We successfully fulfilled all planned objectives, namely, expressing and purifying sufficient antigen required to conclude last animal immunization boosts before their sacrifice. The sera and B lymphocytes obtained from these animals were futher characterized and their reactivity was confirmed individually for each of the two antigen markers of cellular myeloid differentiation Ly6C and ScaI. During immunizations the sera from animals were tested by ELISA and their reactivity titers proved significant affinity enhancement. We obtained a double positive CD48/Ly6C+ FACS profile discriminative for pre-immune versus immune sera for all immunized animals (35, 36 and 39), using pre-sorted adult mouse CD48+ cells (shown Figure).



We also have sorted mouse bone marrow cells to investigate both surface and intracellular markers characteristic for the specific myeloid differentiation stage yielding the pathological clone in the manifest leukemia mice(MLL-AF9). Remarkably, for the MAC(CD11b) sorted mouse cells we detected by RT-PCR in transcripts from cell lysates the presence of all three cMYC exons which suggests the occurrence of MYC-201(containing all 3 exons) which is associated with early differentiation myeloid stages involved in the leukemic oncogenesis.

The proposed objectives of the IIIrd stage 2020 were all successfully accomplished; 1-the final stages of boost immunizations for ScaI antigens in rabbits, followed by their euthanization and collection of immune sera and biological material for our project biobank(spleen organs and bone marrow samples) followed by, 2- isolation of 4 specific immune reactive anti Ly6C fractions further tested for their binding affinity for soluble affixed Ag(BLI method) and for tissue reactivity by differential comparative immunofluorescence staining of spleen sections from same age

Fig. 8.1



normal(WT) and leukemic MLL-AF9 mice of similar background. Using cells from bone marrow and spleen tissue from these mice we also have identified a set of molecular biomarkers to characterize in evolution the leukemic disease in the murine model MLL-AF9.Especially important for our project is the result obtained for immunofluorescence colocalization of Ly6C and CD48+ markers on spleen sections(shown in the adjacent figure). Using thin cryotome slices Splenic tissue either from normal (WT) or from leukemic(AF9) 25 weeks old mice were coloured (from left to right)with : Hoechst for nuclear profiles (blue), with our specific immune fractions anti-Ly6C coloured with DyLight-green, with anti CD48 for monocytes/macrophage lineage marker coloured with PE(red), and in the ast right figures all previous figures merged to emphasize CD48 &Ly6C colocalization.Images were taken at 100x objective.

Remarkably one can see the perinuclear round orange circular contours in leukemic splenic tissue(AF9 upper right, large myeloid leukemic cells with 85% of their volume nuclei)whereas in the images from normal splenic tissue(right bottomWT) one can detect only punctiform orange local colouring.This set of immunohistological staining was checked for all our 4 immune anti Ly6C reactive fractions but the results shown are for the most reactive i39fr1 of them. This result proves the reactivity of our immune fragment not only for the soluble Ag but also for the cellular one in the splenic tissue, and shows unequivocally that anti Ly6C immune fragments may be used as biomarkers to characterize the evolution of myeloid monocytic acute leukemia in the murine MLL-AF9.

Using lysates from bone marrow or splenic cells from normal or leukemic MLL-AF9 mice we have also shown comparatively by RT-PCR their c-*myc*201(leukemic marker) transcription

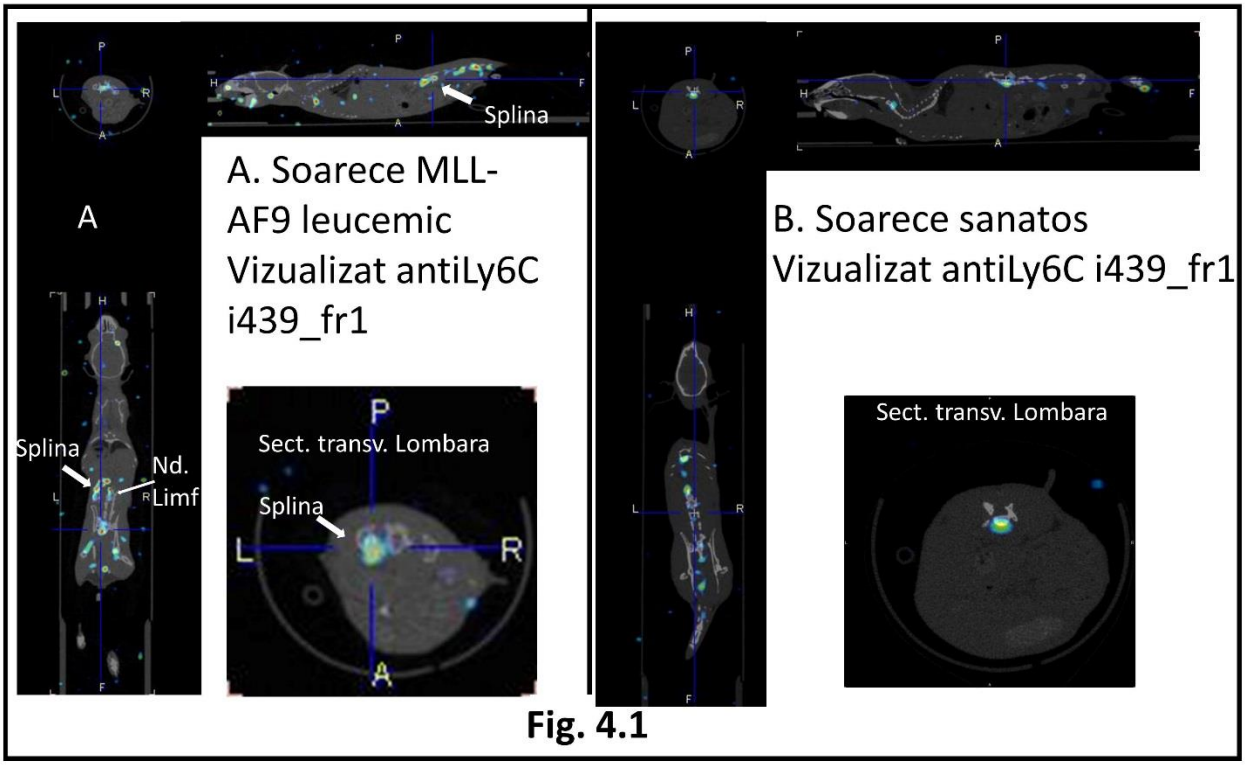


Fig. 4.1

profiles(on all its 3 exons) in FACS MAC(CD11b+) or CD48+ sorted populations. This cancer evolution analysis must be correlated with the cell division dynamics in early stages(CD11b) versus adult differentiation stages prevalent in 25weeks old sick mice(CD48+). The transcription profile was complemented in the same cell sorted populations by 6 evolution base biomarkers(c-

MYC associated transduction partners) using imunobloting detection: cMYC, Ly6C, SOX2, CDK4, CyclinD2, p53 si TIP60.

Project 1 IV th stage 4/2021 (Et4). The genes encoding the variable domains of IgG from Sca I immunized rabbits were isolated and amplified and two purified anti ScaI antibodies were obtained. One of them used as reference for our studies anti ScaI i40_2.3 has been characterized using bilayer interferometry BLI with a dissociation equilibrium saturation constant Kd=216,3± 55nM(i40_2.3). With this antibody and with the one developed last year the anti Ly6C(i39fr1) we had comparatively characterized the flow cytometry monocytic profile of bone marrow cells from syngeneic mice graft recipients of MLL-AF9 leukemic

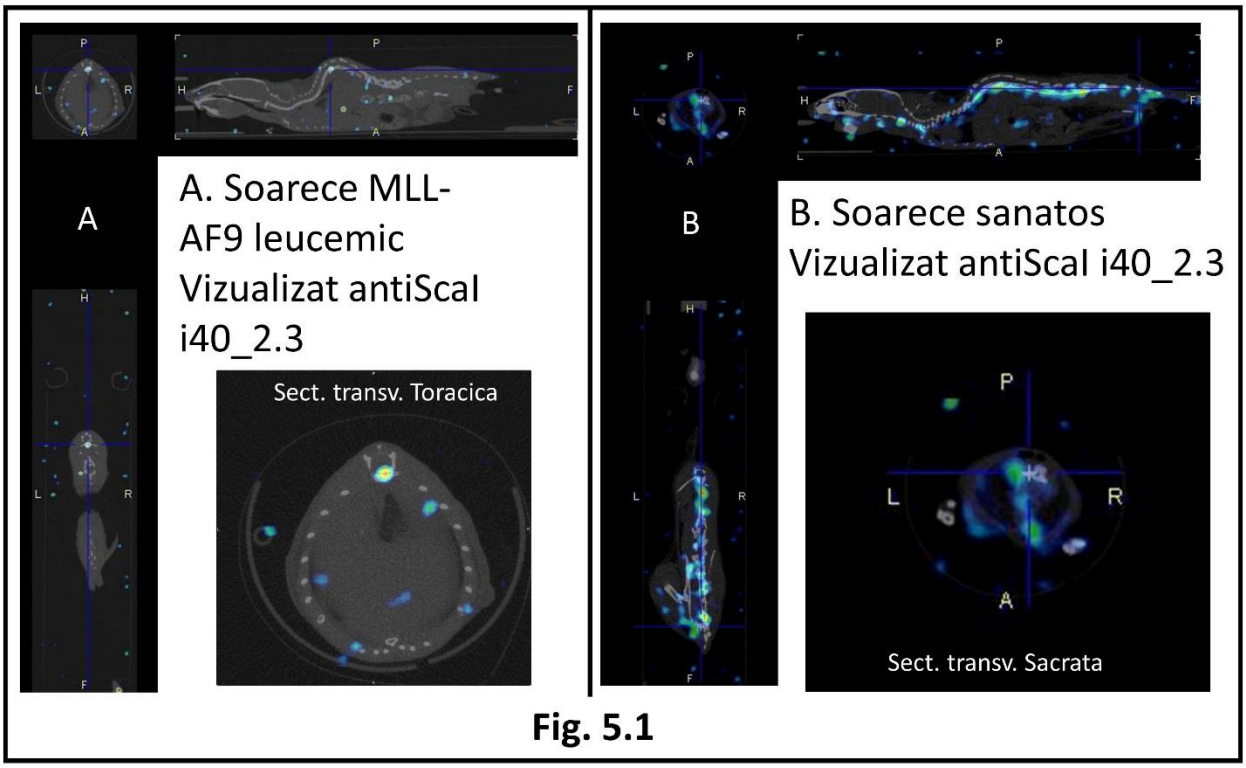


Fig. 5.1

marrow versus bone marrow cells of mice receiving healthy bone marrow transplants. Besides their flow cytometry profile we also characterized the immunohistology of bone marrow cells from both types of graft recipients. (Described in detail in Act4.1.2 of RST_Et4_2021 pg 1-3). The same two purified antibodies were radiolabeled with ^{89}Zr and used for PET(positron emission tomography) on graft recipient mice transplanted with normal versus MLL-AF9 leukemic bone marrow (Act4.3). The combined PET/CT images define the enlarged oncologic splenic profile with anti Ly6C (Fig.4.1A), by contrast with the same organ profile in normal mice(Fig. 4.1B) which thus highlight the potential biomarker abilities of anti Ly6C antibody i39fr1 when diagnosing monocytic acute leukemia in murine disease model. Moreover, the two antibodies (anti Ly6C i39fr1 and anti ScaI i40_2.3) can be used in conjunction, the second pointing in its PET/CT images the dramatic reduction of bone marrow stem cell distribution in leukemic mice, versus

strong radio imaging of spinal cord profile of vertebral bodies or cranial and hip wide bones(Fig. 5.1B in an anticorrelation effect) (Act4.1. fully accomplished RST_Et4_2021 pg 3-5).

All four partners on Project 1 *IFIN-HH, IBAR, IC, INCD Victor Babes* have contributed to the presented results.

Project 2

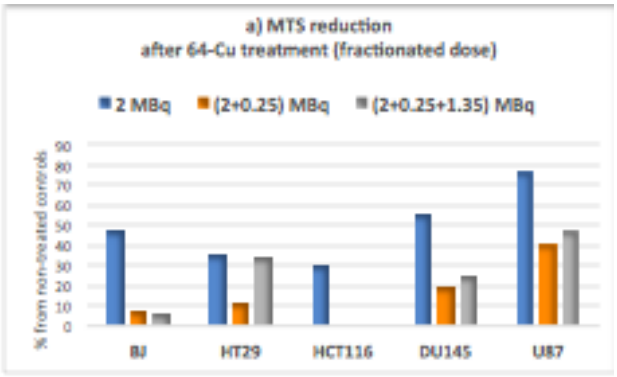
Our teams have focused on how to prepare and formulate the pharmaceutical agents used for molecular PET imagistics, their preclinical testing on tissue cultured tumoral cells in vitro and on standardized animal models bearing characterized tumors. One has radio-labeled biological compounds with medical radio-isotopes (Cu-64 si Ga-68) generated by us . We have also provided a thorough physico-chemical analysis of the obtained radiopharmaceuticals and their comparative biological side by side evaluation in parallel with unlabeled similar compounds, here including: (^{64}Cu-DOTA-NT, ^{68}Ga-DOTA-PEG(4)-BBN(7-14), ^{68}Ga-DOTA-NeuromedinB). To validate them we have initiated in vitro cultures of tumoral cells on whose receptors we tested the binding effects of radiolabeled peptides. We also have tested the mentioned radiopharmaceuticals on tumoral animal models for PET-CT imagistics and pharmaco-kinetical studies. We have achieved cathegory D3 type support activities-working visits, training the young employed researchers in training sessions on sample preparations for XRF electronic microscopy, XPS and IR. We have also participated in working visits designed to complement the expertise of more senior members of the labs in microscopy techniques for XRF/XPS/IR. A particular attention was given in training our staff in the latest trends in good manufacturing practices(GMP) in pharmaceutical industries

Irradiation optimization , radiochemical synthesis , setting standard pharmaceutical formulas and study of endotherapy control factors (systemic therapy using radioisotopes), biological preclinic testing of tumoral cell lines and of cells from animal tumor models, PET-CT animal scanning.

1-Synthesizing the medical radioisotope Cu-64, its radiochemical purification, physico-chemical purity analyses and radiochemical labeling of biological functional agents. Cu-64 medical radioisotope was generated at TR-19 cyclotron using the optimized validated technology developed in the previous 2nd stage(2019) . Thus ^{64}Cu was further used to label new compounds and radiopharmaceuticals all taking place in the " Centrul de Cercetare Radiofarmaceutică" using irradiation at TR19 cyclotron TR-19 (ACSI Canada-technology),

with accelerated protons of the ^{64}Ni solid targets, through nuclear reaction ^{64}Ni(p,n)^{64}Cu. The following peptides were labeled with ^{64}Cu: gastrin secreting class (GRP) and from glucagon class (GLP-like) as well as bombesin analogue (BBN 1-14 si BBN 7-14) and NeuromedinB.

2-In vitro pre clinical tests. Tumoral cellular lines(human and murine) were maintained and characterized for our in vitro experiments in the presence of the α/β emitting radiolabeled peptides and their radiotherapeutic effects were quantified and investigated (see the adjoining Figure). The effects of $^{64}CuCl_2$ exposure can be seen quantified for the dynamics of the number of metabolically active cells present in culture monitored by MTS levels human tumoral cell lines (HT29 colon carcinoma, HCT116 prostate carcinoma , DU145 glioblastoma U87 MG) and healthy normal cells (fibroblasts from BJ line).



Our data show that ^{64}Cu cell exposure induces an abrupt decrease in MTS levels at 24 hours post treatment, remarkably the most treatment reactive cell lines can be seen to be HCT116 colon carcinoma .

3- ***^{64}Cu exerted effect on stress genes expression profiles in normal or tumoral cell lines*** Using qRT-PCR one has analyzed for 84 critical genes their transcription levels induced by 24 h ^{64}Cu exposure (initial dose 2 MBq/mL) during cell stress response. The list of analyzed genes (Human Stress & Toxicity PathwayFinder, Qiagen) See adjacent table:

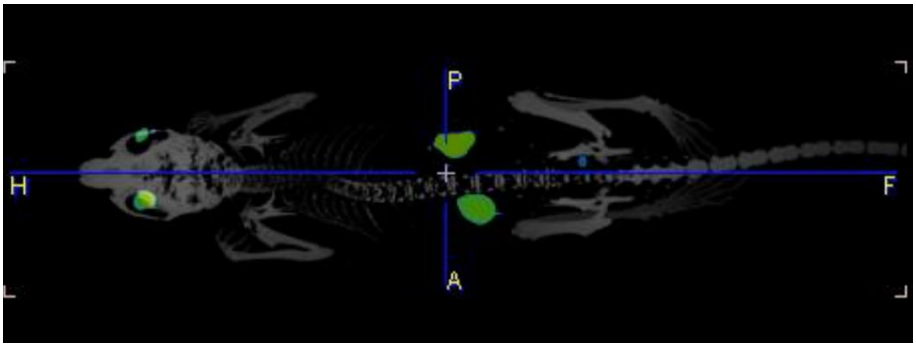
Stress	**Genes**
oxidative Stress	FTH1, GCLC, GCLM, GSR, GSTP1, HMOX1, NQO1, PRDX1, SQSTM1, TXN, TXNRD1, AKR1B1
Hypoxic Stress	ADM, ARNT, BNIP3L, CA9, EPO, HMOX1, LDHA, MMP9, SERPINE1 (PAI-1), SLC2A1, VEGFA
Osmotic Stress	AQP1, AQP2, AQP4, CFTR, EDN1, HSPA4L (OSP94), NFAT5, SLC5A3
Genotoxic Stress	
- Cell cycle modulators	CDKN1A (p21CIP1, WAF1), CHEK1, CHEK2 (RAD53), DDIT3 (GADD153, CHOP), HUS1, MRE11, NBN, RAD17, RAD9A, GADD45A
- DNA damage response	ATM, ATR, DDB2, GADD45A, GADD45G, RAD51, TP53 (p53), XPC

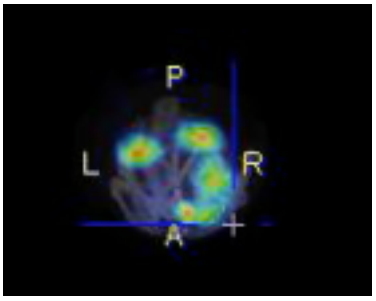
Protein Toxic Stress	ATF4, ATF6, ATF6B, BBC3 (PUMA), BID, CALR, DDIT3 (GADD153, CHOP), DNAJC3, HSP90AA1, HSP90B1, HSPA4 (HSP70), HSPA5 (GRP78)
Inflammatory stress	CCL2 (MCP-1), CD40LG, CRP, CXCL8 (IL8), IFNG, IL1A, IL1B, IL6, TLR4, TNF
Cell Death	
- apoptosis	CASP1 (ICE), FAS, MCL1, TNFRSF10A (TRAIL-R), TNFRSF10B (DR5), TNFRSF1A (TNFR1)
- necrosis	FAS, GRB2, PARP1 (ADPRT1), PVR, RIPK1, TNFRSF10A (TRAIL-R), TNFRSF1A (TNFR1), TXNL4B
- autophagy	ATG12, ATG5, ATG7, BECN1, FAS, ULK1

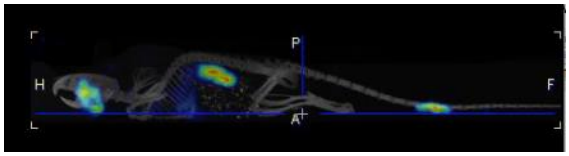
The experimental results show the comparative differential (^{64}Cu irradiation exposed/non-exposed)stress genes expression profiles in HCT116 human colon carcinoma and DU145 human prostate carcinoma supporting the therapeutic efficacy of irradiation based upon reduction in genes expression of those genes involved in cell viability and cell division. The HCT116 are more sensitive to cytotoxic ^{64}Cu exposure than DU145 cells based upon their stress gene induction profiles.

4- ***In vivo Pre-clinical tests***– We comparatively have evaluated the efficacy of β emitting /electrons Auger (Cu-64) compounds. We have performed microPET-CT studies to visualize the radiotherapy effect and monitor in real time monitoring distribution and efficacy of ^{64}Cu-DOTA-BBN(7-14); ^{64}Cu-DOTA-NeuromedinB, ^{64}Cu-DOTA-NT.

a-We wrote and elaborated a report for tumor animal model validation intended to support later translational oncological studies







b-We wrote and elaborated a biological evaluation report for the whole consortium for future interdisciplinary studies, one radio-pharmaceutical product was selected for its efficacy to be later developed for endo-therapy in clinical studiesi): ^{64}Cu-DOTA-BBN(7-14). *Parteneri: IFIN-HH, IC, UB, INCD Victor Babes, UMFCD Tumoral cells were commercially acquired from* ATCC bank and maintained in cultures at IFIN-HH and IVB (HT29, DU145, both lines expressing GRPR receptors) which then were implanted in nude mice strain NU/J. The shown adjacent images depict the location and size of the developed grafted tumors.

All partners in the project *IFIN-HH*, *UB*, *IC*, *INCD Victor Babes*, *UMFCD* were involved in the accomplishment of the phase objectives.

Component project 3

Development of innovative solutions for increasing the efficacy of radioendotherapy

Scientific objective: to develop innovative solutions for increasing the efficacy of imagistic diagnostic and radioisotope treatment techniques in solid tumors, through the following approaches:

1) nanoformulation of the newly designed targeted radiopharmaceuticals;

2) pharmacological modulation of the endogenous antioxidant response in tumor cells by modulation of the activity of the NRF2 transcription factor.

Scientific and technical novelty

a) investigation of molecular changes in the redox signaling network of normal and tumor cells for monitoring radioendotherapy;

b) the use of the NRF2 transcription factor as adjuvant therapeutic target in radioendotherapy for solid tumors;

c) using the concepts of “system biology” along with "omic" technologies for advanced investigations on the radioendotherapy of tumors (functional transcriptomics, MALDI protein profile).

Consortium

Coordinator: "Victor Babes" National Institute for Research and Development in the Field of Pathology and Biomedical Sciences (IVB, CSI Dr. Gina Manda)

Partners

"Horia Hulubei" National Research and Development Institute for Nuclear Physics and Engineering (IFIN-HH, CSII Dr. Dana Niculae)

"Cantacuzino" National Institute for Medical-Military Development Research

(INC, CSII Dr. Crina Stavaru)

National Institute for Research and Development for Isotopic and Molecular Technologies

(INCDTIM, CSI Dr. Rodica Turcu)

University of Bucharest, Faculty of Biology

(UB, Prof. Dr. Anca Dinsichiotu)

• Technological and knowledge transfer: 2 joint experiments performed by the research team from IFIN-HH and IVB between 12-23.11.2019.

Working training visits between researchers from INCDTIM to IFIN-HH

Tip de stagiu/vizita	**Nume**	**Perioada**	**Scop**
Training session for young researchers	Ispas George	28.10.2019 – 31.10.2019	Methods on how to prepare magnetic nanoparticles
Training sessions for experienced researchers	Nan Alexandrina	19.11.2019	Protocols on how to absorb on the surface of magnetic nanoparticles chemically functionalized polypeptides
	Patran Anca	19.11.2019 – 20.11.2019	

	Turcu Rodica	19.11.2019 – 21.11.2019	Analysis of the last obtained data with magnetic nanoparticles carriers for surface absorbed functionalized polypeptides

In the IIIrd 2020 stage we have obtained:

3.10 Production and radio-chemical characterization of radiopharmaceuticals intended as nano-scale formulas for animal models Results.

- 4 types of neuropeptides radiolabeled with ^{68}Ga for molecular level imaging studies: ^{68}Ga-DOTA-NT (neurotensin), ^{68}Ga-DOTA-NMN (neuromedine N), ^{68}Ga-DOTA-PEG(4)-BBN(7-14) (bombesine), ^{68}Ga-DOTA-NMB (neuromedine M).

We elaborated and optimized the preparation and labeling methodology of these radiopharmaceuticals described in the published study Chilug L et al. *Molecules* 2020, 25(15), 3363; https://doi.org/10.3390/molecules25153363, supported by the work developed in this project.

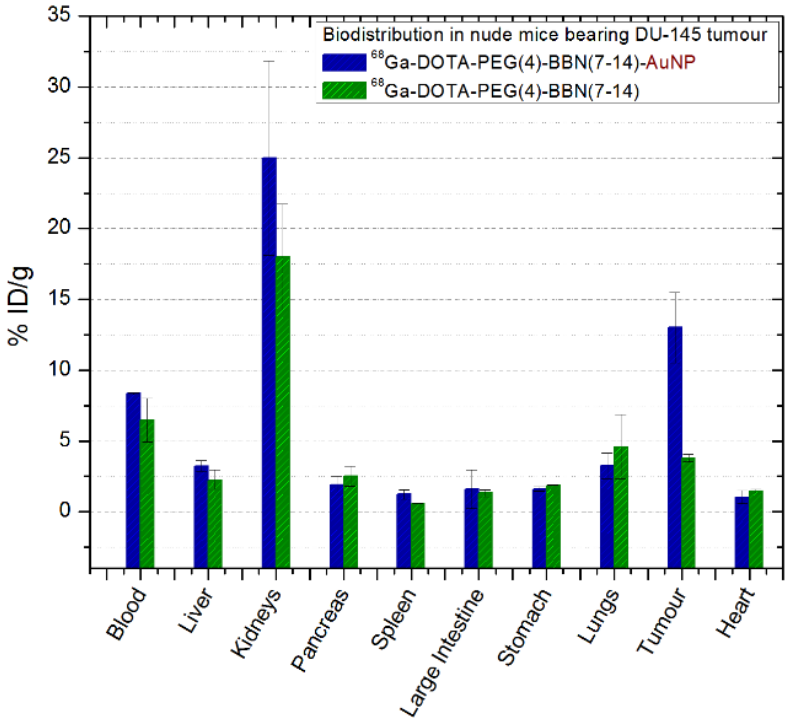
3.11 Production and characterization of radiopharmaceuticals intended as nano-scale system formulas filled with radiopharmaceuticals for animal models

4.2 We developed tumor animal models ready to be transferred to IFIN-HH for radiopharmaceutical treatment. Two human carcinoma models (colon carcinoma lines HCT116, HT29 or prostate carcinoma line DU-145) developed in immunosuppressed nude NU/J strain mice (The Jackson Laboratory, USA, https://www.jax.org/strain/002019).

NU/J mice have Foxn1^{nu} genotype (absence of the gene encoding Forkhead box N1 protein). Homozygous mice NU/J do not have T lymphocytes or T cell mediated immunity, which makes them suitable for grafting (they do not reject human tumor grafts), accepting the human tumor cell line implants. All cell lines were bought and maintained in culture according to ATCC producer reccommendations (HT29: ATCC® HTB-

38™, HCT116: ATCC® CCL-247™, DU145: ATCC® HTB-81™). Forty eight hours prior to the experiments the cells were diluted and suspended in fresh medium at an appropriate density of 20000 cells / cm^2. After 48h the cells were harvested from cultures at 1 x 10^6 in 200µL phosphate saline buffer (PBS) and subcutaneously injected on the right side of each NU/J mouse used. The tumor progression in diameter was checked regularly. The growing dynamic of tumoral cells and the efficacy of radiopharmaceutical treatment were assessed on the animal models and the experimental detailed data shown in Anexa 3(uploaded in the project platform Anexe1_6Proiect 3ONCORAD) and in our published study Chilug L et al. *Molecules* 2020, 25(15), 3363, supported entirely by our projects in this consortium. We have shown that the uptake of all investigated radiopharmaceuticals by tumor cells was quick and efficient regardless of their nano-scale or microscale formula.

Figure. Biodistribution of the radiopharmaceutical compound ^{68}Ga-DOTA-PEG(4)-BBN(7-14)-AuNP in NU/J mice grafted with human prostate carcinoma implants DU145. Comparative study versus ^{68}Ga-DOTA-PEG(4)-BBN(7-14) radiopharmaceutical biodistribution.



We also have shown that nano-scale formulas significantly increase the radiopharmaceutical tumoral tissue retention with the most significant results obtained for nano system formula of radiopharmaceutical **^{68}Ga-DOTA-PEG(4)-BBN(7-14)-AuNP** for DU145prostate cancer implants. In this particular case , nano-scale formula reach 75% tumor retention whereas the retention for conventional uptake was only of 38% .The lowest internalization uptake and oscillatory levels of tumor retention were obtained for neuromedine B radiopharmaceuticals in colon carcinoma cells HT29, studies which suggested us to attempt its use in the future for prostate carcinoma models.

3.14-4.1 Preliminary proof in animal model to effectively increase the tumor treatment with radioisotopes by inhibiting the endogenous antioxidant effects mediated by transcription factor NRF2. Results: include 3 biological studies.

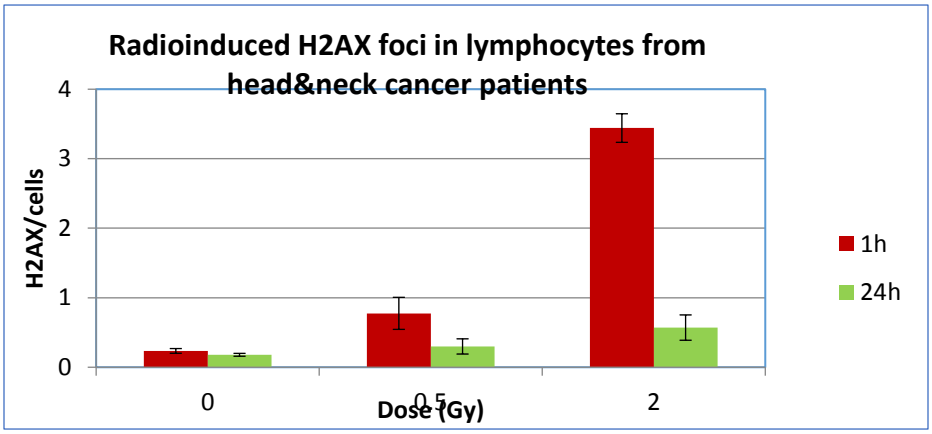
3-15 We elaborated and wrote the documentation needed in support for a patent application for a radiopharmaceutical nanosystem

Project 4

We studied the effects of radiosensitivity on patient's bioptic/blood samples to differentially establish by our methods the effects of radiotherapy. A significant part of the cancer patients treated by radiotherapy are affected by secondary effects of the treatment. This has consequences on the patient quality of life and management of the disease from the health system side. There is an imperious need to develop predictive biomarkers to personalize radiotherapy of cancer patients and reduce the risk of radiotherapy related clinical adverse effects. The present project will focus on the development of a new improved tehnology for pre-therapeutic identification of radiosensitive cervical cancer patients. The predictive power of the proposed biomarkers will be evaluated on relevant blood samples using a set of methods for assessment of the association of the in vitro individual radiosensitivity and normal tissue acute adverse reactions to radiotherapy.

Preliminary results:

H2AX foci induced by X-ray irradiation in lymphocytes of head & neck cancer patients



In the IIIrd stage 2020 we have studied extensively the genotoxic effects using microarray profiles.

Irradiation induced toxicity in patients following In the adjacent Figure 1 and Table1 it is shown the distribution of patients suffering adverse toxic reactions monitored at skin, mucosa, pharynx/esophagus larynx, and salivary glands level.

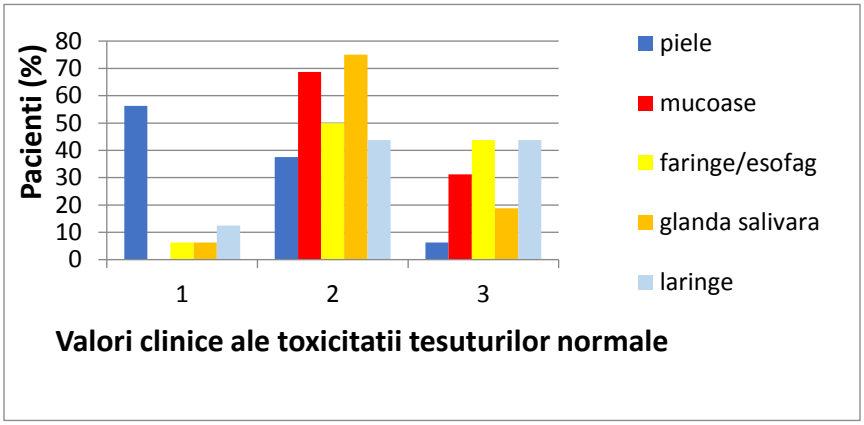


Figura 1 Toxicity clinical values in patients following radiotherapy

Tabel 1. Toxic recorded effects according to RTOG system observed in head & neck cancer patients following radiotherapy

Adverse Reactions	**RTOG Classification**	head & neck cancer patients **ORL (%)**
Skin	G1	56,25

	G2	37.5
	G3	6.25
Mucosa	G1	0
	G2	68.75
	G3	31.25
Pharynx/esophagus	G1	6.25
	G2	50
	G3	43.73
Salivary Glands	G1	6.25
	G2	75
	G3	18.75
Larynx	G1	12.5
	G2	43.75
	G3	43.75

The most frequent radiotherapy toxic effects were observed at skin level (56.25%) suffering 1 degree reactions, whereas at mucoasal level or , at salivary glands level or pharynx/esophagus, (68.75%, respectiv 75% si 50%) they were suffering 2nd degree reactions. At larynx level 43.75% were suffering 2nd degree reactions, while 43.75% only 3^{d} degree reactions.

Microarrays transcriptomic analysis

Selection of the pool of genes of interest (using a bioinformatics approach) and their validation by complementary RT-PCR and immunoblotting analysis. We used bioinformatic gene expression data associated with a data base of the GeneSpring 12.0 software, using target tissue levels according to treatment protocols. A set of 6 target conditions were used in correlative studies. We will exemplify with the first condition I

Ist Condition – 2Gy dose & pre-irradiation vs control (STDA).In the Microarray data set 3 radiosensitive genes involved in DNA damage repair were followed (ATM; XRCC1; XRCC3).

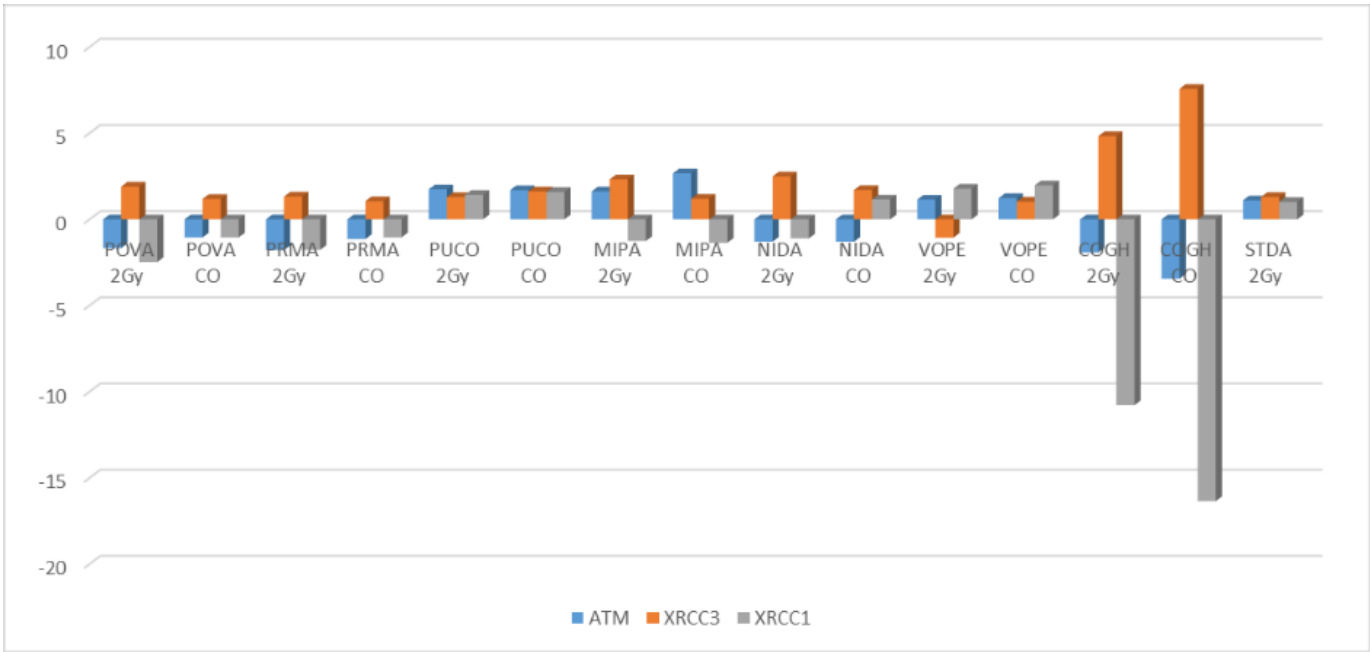
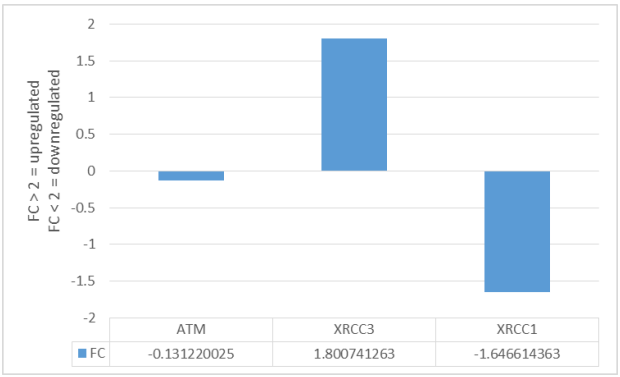


Figura 2 – Differential gene expression of irradiated at 2Gy versus non-exposed samples(STDA)

Our data clearly show an over expression of the *XRCC3* gene inversely proportional with that of XRCC1 and ATMgenes in radiotherapy treated patients (2Gy dose comparatively with the tissue sample from normal untreated healthy donor). In the context differential gene expression of pre-irradiated vs control sample, our data show statistically significant overexpression of gene XRCC3 (FC > 2) inversely proportional with underexpression of xRCC1 gene (FC < -2).



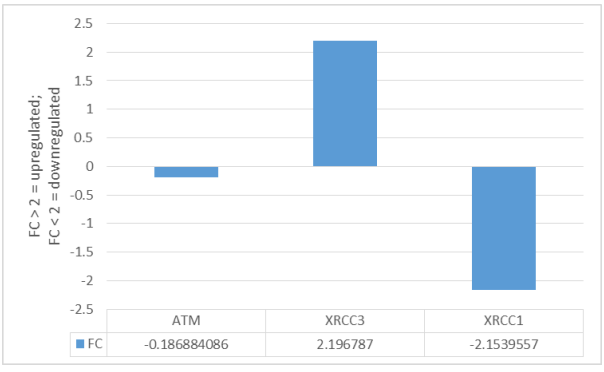
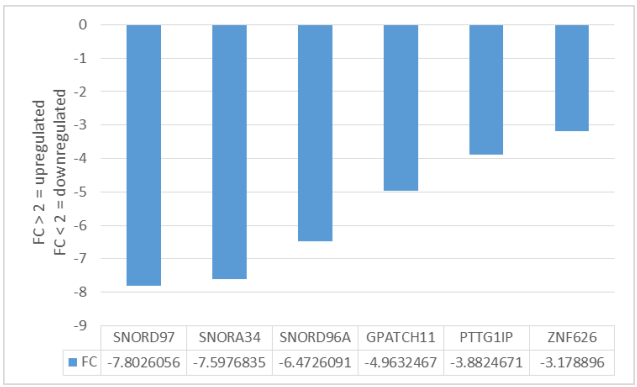


Figura3 Left– The differential average gene expression levels of 2Gy radio-treated samples versus the control sample (STDA) ; Figura 4 Right – The differential average gene expression levels of pre-irradiation vs control sample(STDA)

To summarize we have obtained specific gene expression signatures from radiotherapy subjected patient tissue samples according to their RNA quality. The results unequivocally show that XRCC1 gene is underexpressed for Condition 1 (patients 2Gy treated vs unirradiated control patients), but XRCC3 shows under same conditions overexpression. Interestingly, XRCC3 is underexpressed by our 2nd condition

(radioresistent patients vs radiosensitive patients following 2 Gy radiotherapy), but XRCC1 is overexpressed under 2nd condition. This in tandem pattern between the two sets of conditions may reveal the degree of toxicity in radiosensitive patients versus those radioresistant and may be used as radiotherapy prognostic markers for patients with breast cancer or head & neck cancer patients. A revealing study was to extend our differential gene expression profile analysis to white cell blood fractions 20 as shown in figures below(more accessible than tissue samples).



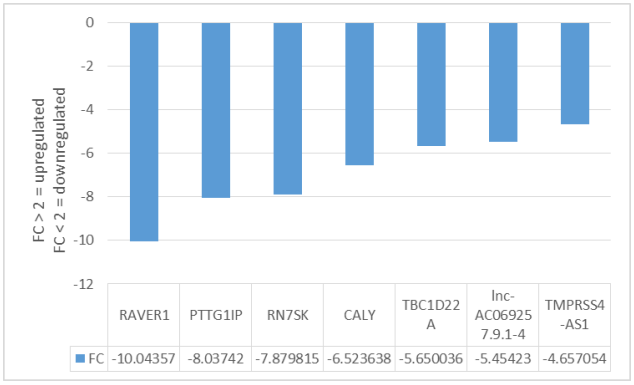


Fig 5Left– The differential average gene expression levels of 2Gy radio-treated white cell 20 blood fractions *samples; Fig6– The differential average gene expression levels of 2Gy pre-irradiated* white cell 20 blood fractions *samples*

All four partners on Project 1 *IFIN-HH*, *ICF*, *IBAR*, *IC*, have contributed to the results

2) <u>The list of published papers of the entire consortium</u>

1a -publicate deja fie in print fie electronic, sau manuscrise deja acceptate spre publicatie
1-Preclinical Evaluation of NHS-Activated Gold Nanoparticles Functionalized with Bombesin or Neurotensin-Like Peptides for Targeting Colon and Prostate Tumours, (2020) Chilug, L. E., Niculae, D., Leonte, R. A., Nan A., Turcu R., Mustaciosu C., Serban, R. M., Lavric, V., Manda G., *Molecules* 25 (15) 2020, pg 3363, DOI: 10.3390/molecules25153363

2- Poly(1-vinylimidazole) grafted on magnetic nanoparticles - attainment of novel nanostructures(2020) Nan A. and Turcu R., *Rev. Roum. Chim*., 2020, *65*(6), 611-616 DOI: 10.33224/rrch.2020.65.6.12

3- Characterization of the Nuclear Magnetic Resonance Relaxivity of Gadolinium Functionalized Magnetic Nanoparticles**(2020)** Nan A. , Suciu M., Ardelean I. , Şenilă M. and Turcu R. (2020), *Analytical Letters*, DOI: 10.1080/00032719.2020.1731522

4- Engaging health professionals and patients in the medical field: role of radiological protection culture and informed consent practices (2020) , C. Schieber, C. Pölzl-Viol, M.-C. Cantone, N. Železnik, S. Economides, R. Gschwind, B. Abelshausen, D. Savu, S. Lafage, L. Liutsko, S. Charron, C. Turcanu and R. Geysmans, *Radioprotection*, epub https://doi.org/10.1051/radiopro/2020039

5- Efficient uptake and retention of iron oxide-based nanoparticles in HeLa cells leads to an effective intracellular delivery of doxorubicin (2020) R. C. Popescu, D. Savu, I. Dorobantu, B. S. Vasile, H. Hosser, A. Boldeiu, M. Temelie, M. Straticiuc, D. A. Iancu, E. Andronescu, F. Wenz, F. A. Giordano, C. Herskind and M. R. Veldwijk, *Sci Rep 10 10530* https://doi.org/10.1038/s41598-020-67207-y /

6- Magnetic Nanoparticle Systems for Nanomedicine—A Materials Science Perspective(2020) Magnetochemistry/ Vlad Socoliuc , Davide Peddis, Viktor I. Petrenko, Mikhail V. Avdeev, Daniela Susan-Resiga, Tamas Szabó, Rodica Turcu, Etelka Tombácz and Ladislau Vékás doi:10.3390/magnetochemistry6010002

7- The transcription factor NRF2 shapes the identity of radio-resistant tumor cells (2020) *Journal of Cell Identity*, G. Manda, A. Cuadrado , (2020) 1, pg 49-81, DOI: 10.47570/joci.2020.004

8- Radiofrequency EMF irradiation effects on pre‐B lymphocytes undergoing somatic recombinationE. Ionita, A. Marcu, M. Temelie, D. Savu, M. Serbanescu and M. Ciubotaru *Sci Rep* **11,** 12651 (2021) https://doi.org/10.1038/s41598-021-91790-3

9-Dobre M, Boscencu R, Neagoe IV, Surcel M, Milanesi E, Manda G. Insight into the Web of Stress Responses Triggered at Gene Expression Level by Porphyrin-PDT in HT29 Human Colon Carcinoma Cells. Pharmaceutics. 2021 Jul 7;13(7):1032. doi:10.3390/pharmaceutics13071032. PMID: 34371724; PMCID: PMC8309054

10- Responsiveness assessment of cell cultures exposed to poly(tartaric acid) and its corresponding magnetic nanostructures, I-V. Ganea , A. Nan, A. Ciorîţa , R. Turcu, C. Baciu, Journal of Molecular Structure 1248, (2021), 131459, https://doi.org/10.1016/j.molstruc.2021.131459

2)List of national and international scientific conferences where we had 2a) invited talks, 2b) posters, 2c) workshops and web presentations

23. Automated production and purification of copper medical radioisotopes in a variable energy cyclotron using solid targets, Dana Niculae, Simona Ilie, Radu Leonte, Livia Chilug, Liviu Craciun The 23rd International Symposium on Radiopharmaceutical Sciences (ISRS 2019), Beijing, China, 26-31 May 2019.

24. Comparative preclinical evaluation of 68Ga-labelled Neuromedin N and B for targeting glioblastoma malignant tissues Livia E. Chilug, R. A. Leonte, Gina Manda, C. Mustaciosu, V. Lavric and Dana Niculae International Symposium on Trends in Radiopharmaceuticals (ISTR-2019), IAEA Vienna, Austria, 28 October - 1 November, 2019

25. Comparative Radiobiological Evaluation of Intracellular Effects Induced by $^{64}CuCl_2$ in Different Tumor Cells, Dana Niculae, Livia E. Chilug, R. M. Serban, C. Mustaciosu, Mihaela Temelie, Ramona Dusman, R. A. Leonte, Doina Draganescu, D. A. Niculae, Diana Savu, Anca Dinischiotu, International Symposium on Trends in Radiopharmaceuticals (ISTR-2019), IAEA Vienna, Austria, 28 October - 1 November, 2019.

26. Radiobiological Evaluation of Copper-64 on Tumor Cells, D. Niculae, R. Leonte, R. Serban, L. Chilug, D. Draganescu, M. Temelie, D. Savu, 32nd Annual Congress of the European Association of Nuclear Medicine, Barcelona, Spain, October 12-16, 2019

27. Activated Gold Nanoparticles Conjugated with 68Ga-DOTA-PEG(4)-BBN(7-14) for Targeting Tumours Expressing GRP Receptors, D. Niculae, L. E. Chilug, R. M. Serban, A. J. Abrunhosa, R. A. Leonte, R. Turcu, A. Nan, V. Lavric;32nd Annual Congress of the European Association of Nuclear Medicine, Barcelona, Spain, October 12-16, 2019

28. Evaluation of the Radiotoxicity of Gallium-68 Labelled Pharmacological Compound Serban, R.M., Temelie, M., Niculae, D., Draganescu, D., Dinischiotu, A. PROCEEDINGS OF THE ROMANIAN NATIONAL CONGRESS OF PHARMACY, 17TH EDITION: 21ST CENTURY PHARMACY - BETWEEN INTELLIGENT SPECIALIZATION AND SOCIAL RESPONSIBILITY, 2018

29. Synthesis and Biological Evaluation of Gallium Radiopharmaceuticals Compounds Raicu, A., Min, E.A, Chilug, L.E., Leonte, R.A., Serban, R.M., Tuta, C.S., Draganescu, D., Niculae, D. PROCEEDINGS OF THE ROMANIAN NATIONAL CONGRESS OF PHARMACY, 17TH EDITION: 21ST CENTURY PHARMACY - BETWEEN INTELLIGENT SPECIALIZATION AND SOCIAL RESPONSIBILITY, 2018

30. An emerging medical radioisotope: Copper-64 from production to clinical application Dana Niculae Nuclear Medicine Days, Sinaia, Romania, June 7-8, 2019.

31. Activated gold nanoparticles conjugated with 68Ga-DOTA-PEG(4)-BBN(7-14) for targeting tumours expressing GRP receptors Livia Chilug, Radu Leonte, Dana Niculae, Doina Draganescu, Rodica Turcu, Alexandrina Nan, Gina Manda, Vasile Lavric Nuclear Medicine Days, Iasi, Romania, 01-04 November 2018.

32. 19th International Balkan Workshop on Applied Physics and Material Science, Constanta, Romania, July 16-19 / lectie invitata / Advances in Bio-medical Application of Ionizing Radiation at IFIN-HH & ELI-NP, M. Radu, M. Temelie, M. Bacalum, R. Popescu, D. Savu, L. Craciun, D. Niculae, M. Straticiuc, I. Burducea, P. Vasos, D. Stutman / 2019

33. H2020 ENGAGE workshop, Bratislava, Slovak Republic, September 11-13 / comunicare poster / MEDICAL Informed consent in medical use of radiation for cancer treatment in Romania Diana Iulia Savu and Daniela Stroe / 2019

34. 35th Congress of Turkish Physics Society, Bodrum, Turcia, September 4-8 / lectie invitata / New developments in radiobiology with biomedical applications for cancer therapy, D. Savu, M. Temelie, R. Popescu, M. Bacalum, M. Straticiuc, L. Craciun, M. Radu / 2019

35. Eighth International Conference On Radiation In Various Fields Of Research July 20-24, Herceg Novi VIRTUAL CONFERENCE | www.rad-conference.org / comunicarea poster Evaluation of individual in vitro radiation response in PBMC samples of H&N cancer patients correlated with severity of side effects following radiotherapy. Mihaela TEMELIE, Patricia MEDESAN, Mihaela TUDOR, Daniela STROE, Diana SAVU / 2020

36. Eighth International Conference On Radiation In Various Fields Of Research July 20-24, Herceg Novi VIRTUAL CONFERENCE | www.rad-conference.org/ comunicare poster Bystander effects induced by proton-irradiated human chondrosarcoma cells. Mihaela Tudor, Mihaela Temelie, Roxana Cristina Popescu, Anna Rzjanina, Gennady Mytsin, Francois Chevalier, Diana Iulia Savu / 2020

37. Eighth International Conference On Radiation In Various Fields Of Research July 20-24, Herceg Novi VIRTUAL CONFERENCE | www.rad-conference.org/ comunicare poster Radiosensitization effects of doxorubicin-iron oxide nanosystem in charged particle radiation therapy for human chondrosarcoma. Mihaela Tudor, Roxana Cristina Popescu, Anna Rzjanina, Gennady Mytsin, Mihai Radu, François Chevalier, Diana Iulia Savu / 2020

38. Eighth International Conference On Radiation In Various Fields Of Research July 20-24, Herceg Novi VIRTUAL CONFERENCE | www.rad-conference.org/ comunicare orala Drug delivery nanosystems for intracellular release of doxorubicin improve the clonogenic inactivation of X-Rays in human cervical adenocarcinoma cells. Roxana Cristina Popescu, Verena Kopatz, Hiltraud Hosser, Frank Schneider, Ecaterina Andronescu, Frederik Wenz, Frank Giordano, Carsten Herskind, Wolfgang Doerr, Marlon, R. Veldwijk, Diana Iulia Savu / 2020

39. Eighth International Conference On Radiation In Various Fields Of Research July 20-24, Herceg Novi VIRTUAL CONFERENCE | www.rad-conference.org/ comunicare poster In vitro magnetic targeted delivery of doxorubicin using iron oxide nanoparticles leads to enhanced cell death in glioblastoma. Roxana Cristina Popescu, Alexandru Daniel Olarescu, Oana Gherasim, Stefan Banita, Mihai Straticiuc, Dragos Alexandru Mirea, Radu Florin Andrei, Bogdan Stefan Vasile, Gabriel Socol, Ecaterina Andronescu, Diana Iulia Savu /2020

40. 45th Annual Meeting of the European Radiation Research Society September 13 - 17, Lund, Sweden / comunicare poster / Bi-modal treatment using radiation therapy and drug delivery nanosystems for enhanced cytotoxicity in radio-resistant tumor models. Roxana-Cristina Popescu, Mihai Straticiuc, Cosmin Catalin Mustaciosu, Roxana Trusca, Hiltraud Hosser, Carsten Herskind, Mihai Radu, Marlon R. Veldwijk, Diana Iulia Savu / 2020

41. National Online Conference of Biophysics -CNB 2020- Braşov, 14th – 16th June / comunicare poster / In vitro Radiosensitivity Evaluation of Peripheral Blood Lymphocytes Isolated From H&N and Breast Cancer Patients. Patricia Medesan, Mihaela Tudor, Daniela Stroe, MihaelaTemelie and Diana Savu / 2020

42. " New affibodies radioactively labeled for imagistics and targeted therapy in oncology" 2019, SZEDLACSEK Stefan Eugen, Hungarian Academy of Sciences / National Institute of Nuclear Research (ATOMKI) Debrecen

43. Hypoxia and antioxidant signaling in human colon carcinoma HCT116 exposed in vitro to 64-Cu, Gina Manda, Dana Niculae, Ionela Neagoe, Radu Serban, Dragos-Serban Niculae, Elena Milanesi, Maria Dobre. Conferința ”Ninth International Conference on Radiation in Various Fields of Research, 14-17.06.2021, Muntenegru. Prezentarea a fost realizată ca ePoster.

44. Biological evaluation of innovative theranostic agents based on peptides-functionalized iron oxide nanoparticles for colon cancer, Livia E. Chilug, Radu A. Leonte, Ramona Dusman, Gina Manda, Rodica Turcu, Alexandrina Nan, Dana Niculae. Workshop ”Adaptation of the tumour and its ecosystem to radiotherapies - Mechanisms, imaging and therapeutic approaches, 22-25 septembrie 2021, Le Bono, Franta. Prezentare orală.

3) Patent applications submitted

-Patent application request to OSIM for a new method of imagistics and radiotherapy : ” 4-(4-(DIALKYLAMINO)BENZILIDEN)OXAZOL-5(4H)-ketone with an aril sulfonil radical in 2nd position” fluorescent derivatives with no citotoxicity for biomedical applications (application request nr. A2020/00490) first author graduate student Elena-Valentina Rosca in project 3;

-”^{64}Cu radiolabeled magnetic nanostructures based upon a polymer functionalized with Neurotensin peptide for diagnosis in oncology/OSIM/16.09.2021 (nr. Application request OSIM A100558/16 sept2021), Project 3

- Patent application request submitted by mail to OSIM cu title “ Rapid procedure for selective purification and preparation of antibodies with Antigen interacting idiotype, from sera of immunized animals” Postal accompanying Nr . S89/22.09.2021, Nr accompanying address 261/22.09.2021, IFIN-HH Project 1.