NANOGENE Report Summary

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Final Report Summary - NANOGENE (EU-Belarus-Russia Network in Nanomaterials-Driven Anti-Cancer Gene Therapy)

Project objectives
The main objective of NANOGENE project is to create the partnership between six universities and research institutions from European Union (Poland, France, Spain), Russia and Belarus in the field of nanomaterial-driven delivery of anti-cancer siRNA into cancer cells to develop new approaches and carriers for non-viral delivery of anti-cancer nucleic acids.

Results obtained in 2013-2016 (Final Report)
During the reporting period (48M), all Project activities indicated in Work packages 1-5 were successfully performed. The secondments were organized accordingly to Gantt Chart and individual reports of completed trainings were prepared and collected by the Project Coordination Committee in real-time mode. The results were analyzed and discussed on Coordination Consortium meetings. During reporting period (48 months) 69.8% (person-months) or 75.5% (number of visits) of secondments were completed. The promotion of the international collaboration (Task I) was realized by Project Partners by means of 154 of training visits and 25 joint collaborative or coordination workshops/meetings. The preparation and submission to European Commission on 13 January 2015 of the collaborative project DENDRI-BIO within Maria Sklodowska-Curie Actions Innovative Training Networks (ITN) call in frames of ‘Horizon 2020’; preparation and submission to EU Commission (Horizon 2020 Research and Innovation Framework Program) of the collaborative project “EU-Ukraine-Georgia-Belarus platform for nanomaterials-driven targeting of Alzheimer’s diseases”- was completed on April 28, 2016. As regards the collaboration and transfer of knowledge (Task II), the Consortium has performed 25 collaborative workshops/trainings and 4 coordination meetings (September 2013; September 2014, September 2015 and September 2016, Lodz). The mentioned activities are presented in the project Deliverables annexed to the current report. Scientific results of the Project (Task III) were published in 24 scientific papers. Two manuscripts describing possibility of using of carbosilane dendrons and dendrimers as platform for new drug delivery systems were submitted for the publication in European scientific journals. The results were also presented on 21 international conferences and congresses in EU, Belarus or Russia. Totally during two years of reporting period 42 conference abstracts were presented as oral (13), poster presentations (25) or invited lectures (4). The patent claim was submitted to the Polish Patent Office. The claimed patent describes the synthesis of new phosphorus dendrimers and their use as efficient siRNA transfecting agents for the application in anticancer therapies. To promote the scientific collaboration relevant to H2020 (Task I) the Project results were presented on Belarusian International scientific conferences “Molecular, Membrane and Cellular Basics for Functioning of Biosystems”. The conferences were held in Minsk on 17-20.06.2014 and 28-30.06.2016. The 4+12 abstracts were published in conference abstract books. Participants of Consortium have received several awards which were partly based on activity of current NANOGENE Project: 1) The Team of the Department of General Biophysics, University of Lodz (Poland) was awarded by the Rector of the University of Lodz for the publication activity in 2014; 2) The results obtained from Project were added in Top 10 of the best 2014 scientific results of the National Academy of Sciences of Belarus; 3) Prof. Jean Pierre Majoral (The team leader of CNRS Partner) obtained the title ‘Doctor Honoris Causa’ from the University of Lodz, May, 2014; 4) In March/April 2015 the manuscript [Shcharbin D., Janaszewska A., Klajnert-Maculewicz B., Ziembta B., Dzmitruk V., Halets I., Loznikova S., Shcharbina N., Milowska K., Ionov M., Shakhbazau A., Bryszewska M. How to study dendrimers and dendriplexes III. Biodistribution, pharmacokinetics and toxicity in vivo. J. Control. Release 181 (2014) 40-52] received enough citations to place it in the top 1% of highly cited publications in the Web of
Science; 5) M. Bryszewska (Poland) was awarded by the Rector of the University of Lodz for the “Best publishing person in the field of natural sciences in 2015 and next in 2016” 6) D. Shcharbin (Belarus) was awarded by the medal "Universitatis Lodzienis Amico” May 23, 2016; 7) K. Milowska (Poland) was awarded by the Rector of the University of Lodz for the cycle of publications on biomedical properties and biophysical application of nano-carriers. October 14, 2016; 8) M. Ionov (Poland) was awarded by the Rector of the University of Lodz for the habilitation thesis “Dendrimers as potential peptide carriers in anti-HIV-1 vaccine”. October 14, 2016; 9) D. Shcharbin (Belarus) was awarded by the Rector of the University of Lodz for the cycle of publications on biomedical properties and biophysical application of nano-carriers. October 14, 2016; 10) D. Shcharbin (Belarus) was awarded by the National Academy of Science of Belarus for one of Top 10 best scientific results of NASB in 2016; 11) V. Dzmitruk (Belarus) was awarded by the Higher Attestation Committee of the Republic of Belarus for the best PhD thesis in the field of life sciences. 2017; 12) V. Dzmitruk (Belarus) was awarded two diplomas for the best scientific reports at the International conference “Molecular, membrane and cell basics of functioning of Biosystems, Minsk, 28-30 June 2016, Belarus and at the I European Biotechnological School, Minsk, 30 May - 4 June, 2016, Belarus. During the reporting period (2015-2016) the number of PhD and Habilitation theses were successfully completed by project participants: 1) Ph.D. thesis. Olga Nowacka (Poland) „Modification of the insulin aggregation and fibrillation process by dendrimers”. January 19, 2016; 2) Ph.D. thesis. Sylvia Moreno Pinilla (Spain), “Dendritic nanosystems of carbosilane structure of different topologies for their use in biomedical applications”. April 28, 2016; 3) Ph.D. thesis. Volha Dzmitruk (Belarus) “Formation and properties of dendriplexes and their interaction with blood cells”. June 24, 2016; 4) Habilitation. Katarzyna Milowska (Poland), Dendrimers as potential protective agents in Parkinson disease”. September 27, 2016; 5) Habilitation. Maksim Ionov (Poland) “Dendrimers as potential peptide carriers in anti-HIV-1 vaccine”. January 27, 2015. For daily implementation purposes, all Project teams maintained regular email contacts. For financial reporting and audit purposes, each Beneficiary kept records on researchers’ mobility. The Coordinator had regular contacts with the REA. Risk evaluation was carried out every 6 months according to the risk assessment plan and the major and minor modifications of the Project were made to correct it and to improve the Project implementation.

The Project arrangements
1) Synthesis and the modification of carbosilane dendrimers to improve their transfection efficiency and biocompatibility. Task leader – UAH. 2) Synthesis and the modification of phosphorus dendrimers. Task leader – CNRS. 3) Synthesis and the modification of anticancer (pro-apoptotic) siRNA. Task leader – IBCFM. 4) Analysis of complexation between siRNA and dendrimers; studying biocompatibility of newly synthesized dendrimers and their complexes with siRNA; the analysis of anticancer effect of dendriplexes based on different dendrimers and siRNA. Task leaders - UL, HGM and IBCE.

Scientific highlights and research achievements. Reporting period, 2013-2014:
The synthesis of 15 phosphorus-based dendrimers of different generations (amino-terminated, neutral, hybrid, non-fluorescent and fluorescent) was made. The synthesis of 9 carbosilane-based dendrimers (amino-terminated, “bow-tie” hybrids, PEGylated, gold nanoparticles covered by carbosilane-based and PEGylated dendrons) was performed. The multi-walled carbon nanotubes with carboxyl groups on their surface were synthesized. All synthesized structures were characterized by NMR, mass spectrometry, fluorescence and other analytical techniques. These structures were presented in articles and presentations at the conferences.
The mechanisms of complexation between 4 siRNAs (anti-cancer siBCL-2, siBCL-xL, siMCL-1, scramble siRNA) and 11 dendrimers mentioned above were analyzed by circular dichroism, fluorescence polarization, ethidium bromide intercalation assay, zeta-potential, gel-electrophoresis. The size of complexes was estimated by zeta-size technique and transmission electron microscopy. Their stability in time and against nucleases was studied by fluorescence assay and gel-electrophoresis. The differences in a complex formation depending on a dendrimer nature and generation were found. Their impact on a dendrimer transfection efficiency was observed. The cellular uptake of complexes into cancer cells (HeLa, human leukemia cell lines HL-60 and 1301) was estimated by confocal microscopy and cytofluorimetry. The differences in uptake depending on a dendrimer nature and generation were observed. The dendrimers’ transfection efficiency was analyzed using the plasmid encoding the green fluorescent protein (pGFP) gene.
The difference in pGFP transfection to cells for different kinds of dendrimers was found. The cytotoxicity of pure dendrimers towards cells was studied. It was found that dendrimers in low concentrations are non-toxic. Finally, the dendrimer-driven
transfection of single anti-cancer siRNAs and their mixtures (‘cocktails’) was performed to HeLa cells, human leukemia cell lines HL-60 and 1301. The anti-cancer effect of siRNA ‘cocktails’ at low doses of siRNAs and dendrimers was found. The ways to improve dendrimers’ transfection efficiency were found.

Scientific highlights and research achievements. Reporting period, 2013-2014:
The synthesis of new generation of cationic phosphorus dendrimers was performed in CNRS (France) with the participation of partners from ICBFM (Russia). Several synthesized dendrimers were selected to study their properties in the field of drug delivery. The synthesis of new carbosilane nanosystems containing ruthenium was performed in UAH (Spain). The synthesis and characterization of obtained compounds were described in published and submitted scientific papers. Dendrimers were synthesized in a controlled manner. They consist of a core and attached repetitive units (branches). At the ends of branches there are functional groups that can be connected to substituents and can interact with nucleic acids. In a case of carbosilane dendrimers the presence of ruthenium molecules in dendrimer structure can increase anticancer properties of dendrimer/siRNA complexes that allows to create a new, more effective tool, to treat the different types of tumors. The newly synthesized ruthenium terminated carbosilane dendrimers consisting of carbosilane dendrons functionalized with N-, NH2-donor monodentate and N,Nchelating ruthenium complexes were analyzed. The biophysical characterization, hemolytic activity and the cytotoxicity towards cancer (HL-60) and normal (B-14) cell lines of these dendrimers were determined. The data indicate that: 1) generation 0 metallodendrimers are the most effective drugs, being non-toxic to normal cells but inducing significant cytotoxicity (>90%) in cancer cells; 2) an increase of generation leads to increased cytotoxicity of the dendrimers; 3) coordination mode of ruthenium in a dendrimer scaffold did not correlate with their cytotoxicity towards normal and cancer cells. The mechanisms of complexes formation between anti-cancer siRNAs: siBCL-2, siBCL-xL, siMCL-1, and Ru-based carbosilane dendrimers were evaluated by transmission electron microscopy (TEM), circular dichroism (CD) and fluorescence. The zeta-potential and the size of formed dendriplexes were determined by dynamic light scattering (DLS). The internalization and cytotoxicity of dendriplexes were estimated using HL-60 cells. Results show that ruthenium dendrimers associated with anticancer siRNA have the ability to deliver siRNA as non-viral vectors into the cancer cells. Moreover, dendrimers can protect siRNA against nuclease degradation. At one of project workshops the decision was made to continue this research to examine the therapeutic potential of ruthenium dendrimers as well as dendrimers complexed with anti-cancer drugs in cancer cells. The results concerning new phosphorus AE dendrimers and their complexes with above mentioned anticancer siRNAs show that produced compounds can be applied for efficient delivery of gene material into cancer cells in presence of antibiotics, serum or serum proteins with high uptake (>80%) for both adherent and non-adherent cells. The new carrier obtained was shown to be 2-5 times more effective than commercially available standard transfection agent Lipofectamine. In addition, cytotoxicity of these dendrimers in used concentration was low (<20%). Obtained data suggest that new phosphorus dendrimers can be considered as alternative anticancer gene delivery agents.

Contact:
Prof., D.Sc., Maria Bryszewska,
NANOGENE Coordinator,
Head of Department of General Biophysics,
University of Lodz,
141/143 Pomorska str.,
90-236, Lodz, Poland
Email: marbrys@biol.uni.lodz.pl

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