APPROVED

JINR DIRECTOR

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PROJECT PROPOSAL FORM

Opening/renewal of a research project/subproject of the large research infrastructure project within the Topical plan of JINR

1. General information on the research project of the theme

1.1 Theme code (for extended projects) - *the theme code includes the opening date, the closing date is not given, as it is determined by the completion dates of the projects in the topic.*
04-9-1077-2009

1.2 Project code (for extended projects)

1.3 Laboratory

Laboratory of Radiation Biology

1.4 Scientific field

Radiation Research in Life Sciences

1.5 Title of the project

Molecular, genetic and organism effects of ionising radiations with different physical characteristics

1.6 Project leader(s)

Boreyko Alla Vladimirovna, Lobachevsky Pavel Nikolaevich

1.7 Project deputy leader(s) (scientific supervisor(s))

2 Scientific case and project organization

2.1 Annotation

The aim of the project is to study the regularities and mechanisms of molecular, genetic and organism effects of ionizing radiation with different physical characteristics. The use of ionizing radiation of a wide range of linear energy transfer in radiobiological experiments allows us to obtain unique information about the nature of damage of the DNA structure of cells during irradiation, the mechanisms of induction of gene and structural mutations in cells with different levels of genome organization, the regularities of the action of particle radiation on tumour during radiation therapy. Within the framework of the Project, fundamental and applied issues of modern radiation biology will be addressed: the formation and repair of cluster DNA damage in normal and tumour cells following exposure to accelerated charged particles; the study of the radiosensitizing effect of the DNA repair modifier (AraC) in combination with various molecular biological complexes during irradiation of tumour cells and tissues; the study of the regularities of induction of gene and structural mutations in

normal and tumour cells following exposure to charged particles; investigation of primary and long-term morphological and functional changes in the mammalian central nervous system following exposure to radiation with different physical characteristics.

2.2 Scientific case (aim, relevance and scientific novelty, methods and approaches, techniques, expected results, risks)

Introduction

Ionizing radiations of different physical properties represents an efficient tool for solving *fundamental and applied* problems in modern biology and medicine. Radiobiological studies with heavy charged particles proved fruitful and allowed to reveal and investigate a special type of radiation induced DNA damage – clustered lesions that play an important role in the emergence of various mutations initiating the development of cancer. The lesions of this type are very difficult to repair by cells and they largely determine the biological efficiency of various types of ionizing radiations on such endpoints as normal and tumour cells death, induction of gene and structural mutations and radiation effect at organism level.

Investigation of biological effects of such type of radiations is especially important for addressing challenges in *radiation medicine*. Radiation tumour therapy that exploits beams of accelerated protons and carbon ions is one of the most efficient treatments of hard-to-reach malignant neoplasms such as brain tumours. The investigation of various aspects of the effect of high-energy heavy ions on the whole organism is also important for solving problems of space radiobiology to ensure radiation safety of crews during deep space flights.

The presence of a wide range of radiation sources, including beams of heavy ions of various energies, at the JINR facilities provides a unique opportunity to solve these problems. The planned radiobiological experiments at the Institute's accelerators will be aimed at studying the mechanisms of action of heavy ions at the molecular, cellular, tissue and organism levels of biological organization. Special attention will be paid to the study of new approaches for increasing the biological effectiveness of radiation therapy with charged particle beams. The analysis of disorders in the central nervous system of experimental animals will be continued aiming to assess the risk of radiation exposure to the body of astronauts during interplanetary flights.

Scientific and methodological justification

In contrast to electromagnetic types of ionizing radiation, the energy of which is uniformly distributed over the volume of the nucleus of the irradiated cell, the energy of heavy ions passing through the condensed phase is distributed along the particle track, causing complex cluster DNA damage. Compared to electromagnetic radiation, charged particles have an inverse depth dose distribution: the minimum energy during the passage of particles through the tissue substance is released in the initial section, and the energy deposition increases sharply at the end of the range (Bragg peak).

Among a wide range of different DNA lesions induced by ionizing radiation, the most severe damage leading to cell death are simultaneous disruptions of the integrity of two DNA strands - double-strand breaks, which represent cluster-type of damage. Double–strand breaks (DSB) are formed either as a result of a direct break of two complementary sites - direct DSB (DDSB), due to the energy deposition at a local DNA site and resulting in disruption of its integrity, or are formed from other lesions as "repair costs" in the process of repair enzymes operation. This type of damage belongs

to the category of enzymatic DSB (EDSB). Following exposure to radiation with increasing values of LET, changes in the spectrum of induced cellular DNA damage are observed. At low LET values, base damage and single-strand breaks (SSB) of DNA are induced with high frequency. Following exposure to heavy charged particles with high LET values, predominantly DSB are induced, mainly of the DDSB type, and the number of SSB decreases. The yield of EDSB during irradiation depends on many factors, both physical and biological in nature. The peculiarities of the action of charged particles on the molecular genetic structures of cells must be taken into account when solving fundamental and applied problems of modern radiobiology. Most acutely, this concerns aspects of improving the approaches for radiation tumour therapy.

It is well known that the ***strategy of radiation therapy*** is based on establishing conditions that provide implementation of the following basic principles. The ***first*** one is based on conformal irradiation of the target (tumour) - deposition the maximum necessary amount of energy of the radiation to the tumour tissues while sparing the adjacent healthy tissues as much as possible. Compared to radiation of electromagnetic nature, proton beam irradiation of deep tumours in the Bragg peak achieves the maximum level of tumour irradiation with a lower level of irradiation of normal tissues adjacent to the tumour, as well as critical organs. Even greater differences in the level of absorbed doses at different parts of the Bragg curve are inherent to accelerated carbon ions. The ***second*** principle related to the first one is based on the requirement to deliver maximum damage to tumour tissue cells during irradiation. This is achieved, as mentioned above, by physical features of charged particle energy deposition. A sharp increase in LET value with decreasing energy of charged particles in the area of Bragg peak results in an increase in the yield of radiation damage in irradiated cells, causing their death. The higher biological effectiveness of accelerated carbon ions compared to protons is due to the high values of LET of heavy ions. Taking this into account, a number of countries have built specialized centres for carbon therapy. However, it should be noted that the cost of such accelerators is extremely high compared to proton machines, therefore there are few such carbon therapy centres so far and the cost of patient treatment is also extremely high.

Biological effectiveness of particle beams is determined by factors of different nature: a physical factor related to the character of energy deposition in sensitive cell targets (particle LET, dose rate, etc.) and a biological factor affecting the yield of damage causing cell death (cell repair status, oxygenation, cell cycle phase, tumour microenvironment, etc.). The yield of DNA DSB of enzymatic nature following irradiation can be influenced by a certain modification of DNA repair processes. It was demonstrated in *in vitro* experiments that following exposure to γ-irradiation and protons in the presence of DNA synthesis inhibitors 1-β-D-arabinosylcytosine (AraC) and hydroxyurea (HU) the yield of DNA DSB was significantly increased during post radiation incubation of cells due to transformation of non-lethal DNA damage into EDSB. In preliminary *in vivo* studies with grafted mouse melanoma tumour, a threefold reduction in tumour growth rate during post radiation period was demonstrated following proton irradiation in the presence of AraC as compared to proton irradiation only (Fig.1). Further development of the proposed approach will probably allow a substantial convergence of the areas of application of proton and expensive carbon accelerators for therapeutic purposes, as well as to increase the therapeutic efficiency of photon radiation sources.

Plans for deep space exploration present new challenges for space radiobiologists. The spectrum of galactic cosmic rays (GCR) comprises high-energy protons and ions with high charge and energy. The latter, despite their low fluences, significantly contribute to the radiation risk for astronauts given their high biological effectiveness. Most of the knowledge related to the effects of GCR is based on

ground-based experiments conducted on charged particle accelerators. From this point of view, the basic facilities of JINR provide wide opportunities for modelling of biological action of cosmic radiation. In the modern concept of radiation risk for manned interplanetary flights the ergonomic risk throughout the duration of whole mission, associated with the malfunction of the central nervous system (CNS), is recognized as paramount. The main objectives here are to investigate in detail the character and mechanisms of heavy charged particles effect, firstly, on cell cultures of nervous system, especially on cells with high proliferative activity, and secondly, on CNS structures of laboratory animals and their behaviour.

In view of the above, the solution of the considered fundamental and practical problems urgently requires further detailed study of the regularities and mechanisms of the action of heavy charged particles at the molecular, cellular, tissue and organismal levels of biological organization. Studies of molecular changes in genetic structures are primarily important in the context of analysing the incidence of the most severe DNA damage - DSB. An efficient approach for three-dimensional analysis of clustered DNA DSB (DNA repair foci method) developed and implemented in the LRB will allow to study the formation of the most severe damage of genetic machinery following exposure to multiply charged ions and provide an opportunity to investigate the induction and repair of genetic lesions both in the proliferating tissues and in the highly differentiated elements of the nervous system. Identification of the mechanisms of response to the exposure to charged particles of various energies will provide the basis for understanding the tissue reactions of highly differentiated cell systems - structures of various parts of the central nervous system to radiation exposure. In turn, these studies will allow assessing the disruption of integrity of the system – changes in cognitive functions, behavioural reactions. The applied focus of such comprehensive investigations for different fields of practical activity and, first of all, for solving the problems of human space radiobiology is quite obvious.

Special attention during the proposed research will be paid to elucidating the mechanisms underlying the increase in the efficiency of the biological effect of proton and photon beams on radioresistant tumour cells, modelling the radiation effect on tumours grafted into experimental animals. Taking into consideration the previously obtained results related to modifying effect of agents such as arabinoside cytosine in combination with other drugs on the yield of DNA DSB following exposure to ionizing radiation of different quality, as well as possible prospects for the application of DNA synthesis inhibitors of this type and ionizing radiation in clinic, further studies on the proposed topic are necessary.

Research areas

Molecular radiobiology

For a number of years, various molecular biological methods have been used at JINR LRB to investigate alterations in the genetic structures of normal and tumor cells exposed to ionizing radiation of a wide range of LET. In recent years, immunocytochemical and immunohistochemical research metods have been actively exploited for these purposes. Such methods provide an opportunity not only to quantify the formation of molecular alterations, but also to take into account their spatial distribution in genetic structures. The most severe lesions are DNA double-strand breaks (DSB). DNA DSB indution in certain regions of the genome leads to specific phosphorylation of the H2AX histone in the chromatin surrounding the lesion, which manifests itself in the formation of the so-called γH2AX foci. The internal structure of these radiation-induced foci (RIF) is a reflection of the interrelationship of the

biochemical processes triggered in response to the occurrence of damage and aimed at restoring DNA integrity. Of special interest is comparative analysis of the patterns and mechanisms of the induction and repair of DNA damage in normal and radioresistant tumor cells irradiated following exposure to γ-rays and accelerated protons and heavy ions of different energies.

We have previously investigated dose dependences of the formation frequency of RIF in human cells after exposure to radiation of different LET. A higher effectiveness of their formation following exposure to accelerated heavy ions as compared to γ-rays has been revealed. After cell exposure to accelerated heavy ions, compared to γ-rays, a higher rate of RIF induction and a slower kinetics of their elimination were observed. A detailed analysis of the structure of complex clustered damage in accelerated ion tracks was carried out based on a differentiated analysis of individual RIF in three-dimensional images reconstructing the entire volume of the cell nucleus. It has been established that following exposure to accelerated heavy ions of low and intermediate energies, in contrast to γ-rays, complex clusters are formed, including up to six or more individual foci. Changes have been observed in the structure, size, and shape of clustered damage, depending on particles' LET. It has been found that the kinetics of radiation-induced foci elimination in cells is slower after heavy ion exposure in comparison with γ-rays. Considering the possibilities of differentiated analysis of individual DNA foci in three-dimensional images, it is planned to study the nature of single-strand cluster-type damage in cellular DNA induced by protons in the energy range used for therapeutic purposes.

In view of the earlier results on the modifying effect of cytosine arabinoside (AraC) on DNA DSB formation in normal and tumor cells, it is planned to further study the effect of AraC on cell radiosensitivity in combination with other molecular biological complexes. Using the immunocytochemical method for analyzing DNA DSB formation and the quantitative cell survival method, it is planned to develop approaches to increase the sensitivity of various cell lines to radiation of different LET. It is planned to study changes in molecular biochemical mechanisms in the presence of modifiers following exposure to radiation in a wide LET range in normal and radioresistant cells of malignant tumors. Fibroblast cultures and human U87 glioblastoma cells, as well as mouse B16 melanoma cells will be used as objects of the study.

The research will involve not only normal and tumor cell cultures, but also neuronal cell cultures and histological sections of tissues of different parts of the central nervous system of irradiated animals.

Expected results:

- to study clustered DNA DSB formation after exposure to accelerated charged particles of different energies in the nuclei of human skin fibroblasts, tumor cells (U87, B16), and neurons of different parts of the central nervous system of irradiated animals;
- to study the repair kinetics of clustered DNA DSB in the post-irradiation period in the nuclei of human skin fibroblasts and radioresistant tumor cells;
- to study the patterns and mechanisms of the radiosensitizing effect of cytosine arabinoside in combination with various molecular biological complexes on normal and tumor cells after exposure to radiation with different LET;
- to study quantitative regularities of the survival of normal and tumor cells after radiation exposure in the presence of a combination of DNA repair modifiers.

Radiation genetics

The subject of mutagenic effect of ionizing radiation of different quality, especially the accelerated heavy ions on mammalian and human cells has not been sufficiently studied so far. It is proposed to continue the studies of regularities and mechanisms of induction of various types of gene and structural mutations and their dependence on the dose and LET of radiation, repair status as well as mechanisms of genetic stability, which are in progress in LRB at JINR.

Obviously, the study of regularities and mechanisms of mutation induction in human cells following exposure to various types of ionizing radiation is an extremely difficult task. Mammalian and lower eukaryote cell cultures offer convenient model objects for these purposes. Yeast *Saccharomyces cerevisiae* is a unicellular eukaryotic organism in which genetic control and molecular mechanisms of such fundamental cellular processes as replication, repair and transcription have been comprehensively studied. We use haploid tester strains to detect various molecular events (base pair substitutions, single and multiple nucleotide deletions, recombination rearrangements). The direct gene mutation testing system used in the laboratory allows detection of any gene mutations at a long DNA section, namely arising within arginine permease 1.8 kb gene. In addition to nuclear DNA, cells contain mitochondrial DNA (mtDNA), whose mutagenesis and functional significance are currently poorly understood. The ease of manipulation of mtDNA in yeast makes it an ideal subject for studying the role of mitochondria and mtDNA in the mutagenic effects of radiation, particularly those related to respiration and iron metabolism.

It is proposed to continue the studies of the patterns of induction of nuclear and mitochondrial mutations following exposure to sparsely and densely ionizing radiation using the aforementioned tester systems. The main focus of these studies will be to compare the contribution of point and structural mutations induced by radiations with different LET in order to identify the type of molecular DNA lesions leading to these mutations and the mechanisms of their formation, reflecting the relationship between the character of energy deposition in the charged particle track and the spectrum of induced mutations. To clarify the molecular nature of the mutations, sequencing of induced point mutations and electrophoretic and restriction analysis of deletion mutants will be used. Building on the results obtained earlier, it is also planned to investigate the effect of a number of factors that may both influence radiation-induced mutagenesis and interfere with methodological aspects of mutation detection. One of such factors is the inherent heterogeneity of the cell population in haploid yeast cells, so it is planned to evaluate mutagenesis in dividing (G1, S, G2), resting (G0, quiescent) and aging (senescent) cells.

To understand the mechanisms of spontaneous and induced mutations and rearrangements, it is planned to study the modifying effects of deficiency in genes of error-prone post-replication repair and in genes performing epigenetic modifications of histones, genetic control of accuracy and copy number of the mitochondrial genome, control of apoptosis and cell aging.

Mechanisms that increase radioresistance and reduce mutagenesis are of undoubted interest. Previously, we obtained and genetically characterized a nuclear mutation that has a wide range of actions, including increasing cellular radioresistance and affecting genetic stability. The plan is to investigate the mechanism of radioresistance, map this mutation, and analyse the effect of the mutation on genome expression. Preliminary protein electrophoresis data showed that the mutation is localized in the regulatory gene.

It is proposed to continue studies on the mutagenic effect of sparsely and densely ionizing radiation on mammalian cells and evaluate the mutagenic effect of radiation of different quality. Earlier it was shown that when the expression period was increased, there was an increase in mutagenesis to a maximum value, followed by a decrease to a spontaneous level. The position of this maximum depended on LET of the accelerated ions. With increasing LET, the value of the maximum shifts toward longer "expression times". It can be assumed that the increased level of radiation-induced mutagenesis is determined by the increased chromosomal instability of the irradiated cell population, and its manifestation at different "expression times" depends on the severity of the initial damage. The observed variability of cytogenetic parameters is apparently determined by the type of arising mutations following exposure to radiation with different LET. Perhaps, this is associated not only with point mutations, but also with structural changes in the gene. The consequences of this exposure can manifest themselves in a series of cell generations.

Expected results:

- to continue investigation of the patterns of point and structural mutation induction in *Saccharomyces cerevisiae* yeast cells by radiation with different LET;
- to study the influence of heterogeneity of cell population in haploid yeast on the radiation-induced mutagenesis; estimate mutagenesis in different phases of cell cycle;
- to study the influence of respiratory impairment as the result of mitochondrial DNA damage on the sensitivity to the mutagenic effect of radiation;
- to study the mechanism of radioresistance and its effect on radiation-induced mutagenesis in yeast mutant;
- to continue the study of radiation-induced mutagenesis and to compare yield of chromosome aberrations in Chinese hamster cells (line V-79) at maximal and minimal mutagenesis levels depending on time of expression and LET of accelerated ions;
- to analyse structural abnormalities in the *hprt* gene and their projection to abnormalities in the chromosome machinery of cells.

Radiation cytogenetics

The study of the induction of chromosome aberrations following exposure to ionizing radiation and other cytotoxic agents has a long history. The classical metaphase method of chromosome aberration analysis in peripheral blood lymphocytes has remained nearly the only method of human biodosimetry for more than half a century, allowing assessment of radiation dose received by an individual in radiation incidents, medical diagnostics, and radiotherapy of cancer. Combination of metaphase method and more informative modern method of 24-color chromosome staining mFISH (multi-color Fluorescence in situ Hybridization) gives invaluable advantage when studying cytogenetic effects of charged particles. This method, currently used in LRB, allows to identify each pair of human and animal chromosomes by hybridization of chromosome DNA with samples labelled with unique combinations of 5 fluorochromes and analyse all kinds of structural rearrangements between them.

The main advantage of the fish method is the ability to study complex chromosome aberrations involving 3 or more breaks in two or more chromosomes with identification of each chromosome involved. The use of mFISH has shown that a large number of aberrations previously thought to be simple are part of a complex involving breaks in several chromosomes and their interaction with each other. Thus, the introduction of mFISH leads to the need to re-evaluate all postulates of classical

cytogenetics and changes our knowledge about chromosome aberrations, their spectrum and distribution in cells. Complex chromosome aberrations are characteristic of densely ionizing radiation and reflect the clustered nature of DNA DSB formation along the particle track. Thus, the use of mFISH method makes it possible to obtain new insights into the mechanisms of radiation-induced aberrations, reflecting the relationship between the character of energy deposition in the charged particle track, the level of complexity of DNA cluster damage and the formation of complex chromosome aberrations. On this basis, mFISH is supposed to be used primarily to assess complex chromosome aberrations induced by radiation of different quality.

Despite the continuing interest in proton radiotherapy of cancer and a huge number of works, including cytogenetic studies of proton irradiation, there are still no works using mFISH. Therefore, a detailed analysis of the spectrum of chromosome aberrations induced by proton beams is highly relevant.

Along with conducting experiments at the JINR accelerators, it is planned to conduct experiments at the new basic facilities of the LRB: SARRP X-ray irradiators (XStrahl, USA) 220 kVp and CellRad (Precision, USA) 130 kVp. The latter also has an operating mode of 30 kVp. Low-energy X-rays are widely used in medical diagnostics, although their biological efficiency is not sufficiently studied and has been the subject of ongoing discussions for the last 20 years. There are few known studies on the cytogenetic effects of this radiation, but there are no mFISH data that can give a complete picture of the number and spectrum of chromosome aberrations.

Studies using mFISH have been published for high-energy heavy ions with energies up to 1 GeV/n, such as iron, carbon, etc.; for α-particles and low-energy ions with energies up to 10 MeV/n, but no data are available for ions at intermediate energies (20-60 MeV/n). Such beams are available at the basic facilities of JINR. The effect of such accelerated ions on normal and tumour cells of humans and mammals is studied.

In combination with metaphase and mFISH methods for comparative assessment of induction and repair of chromatin breaks in normal and tumour cells it is planned to use the method of chemically induced premature chromatin condensation, which allows to measure the initial level of chromatin breaks induced by irradiation and the kinetics of their reparation.

Expected results:

- to finalise the study of the biological efficiency of proton beams by mFISH method;
- to study the biological efficiency of low-energy X-rays following *in vitro* irradiation of human blood lymphocytes using mFISH method;
- to evaluate the contribution of complex chromosome aberrations to the biological effectiveness of densely ionizing radiations following irradiation of human normal and tumour cells *in vitro*;
- to study the induction and kinetics of chromatin break repair by premature chromatin condensation in normal and tumour human cells exposed to sparsely and densely ionizing radiation.

Radiation Physiology

Radiation physiology studies in the forthcoming period will be aimed mainly at investigation of behavioural reactions of irradiated animals and of pathomorphological changes in different critical organs and systems of rodents. To solve this problem, behavioural reactions will be evaluated using a complete set of tools and methods of modern zoopsychology, including test systems for evaluation of long-term and short-term memory, learning ability, emotional reactivity, level of anxiety and motor reflexes, and social behaviour. Analysis of behavioural parameters will be carried out with the use of modern software and information systems of video tracking. The study of pathomorphological changes in tissues will be conducted exploiting modern histological and immunohistochemical methods using light and fluorescent microscopy equipment, with subsequent analysis using specialized software. The study of bioelectrical activity of the brain will be carried out using modern invasive methods of electrophysiology.

Normal neuron activity CNS is safeguarded by glial cells. This type of cells includes astrocytes, microglial and ependymal cells, oligodendrocytes and their precursors. They perform dynamic monitoring of the nervous system state, control and support integrative activity of the brain. A number of studies have established that the origin of radiation-induced neurodegenerative changes in CNS is a chronic neuroinflammatory process associated with microglia activation. This process leads to destruction of myelin sheath of axons, disruption of neuronal architecture, loss of dendrites, permeability of the blood-brain barrier, disruption of neurogenesis and other vital processes in CNS. A key component of this response is microglia, the resident immune cells of the CNS. Currently, microglia are being considered as a therapeutic target to prevent radiation-induced pathological changes in the brain leading to cognitive impairment. Therefore, it is planned to further investigate inflammatory processes and the role of microglial cells in the effect of ionizing radiation, as well as the possibility of modulating these processes using inhibitors to the receptors of signal cascades involved in the response of microglia to radiation exposure.

Expected results:

- to continue the study of primary and late morphological and functional changes in the central nervous system of rats following exposure to ionizing radiation with different physical characteristics;
- to conduct studies of pharmacological protection agents under the influence of ionizing radiation;
- to conduct a study of the effect of radiation with different LET on pathogenesis in organs and tissues of small laboratory animals;
- to continue the investigation of the activation of microglial cells in cell culture and inflammatory markers in the brain of mice following exposure to ionizing radiation of different quality;
- to investigate the possibility to modulate the activation of microglial cells in irradiated culture and neuroinflammation in the brains of irradiated mice by using inhibitors to the receptors of signalling pathways involved in these processes.

Molecular and radiobiological aspects of radiation therapy

Radiation-induced cell death is known to be predominantly caused by the formation of DNA DSB. Among a wide range of different radiation damage to DNA, such molecular damage is the most severe.

As mentioned above, they are formed either as a result of a direct rupture at two complementary sites - direct DSB (DDSB), due to energy deposition into a local DNA site, which leads to disruption of its integrity, or are formed from other damage as "repair costs" during the operation of repair enzymes. This type of damage belongs to the category of enzymatic DSB (EDSB). Following exposure to radiation with increasing LET, changes are observed in the spectrum of DNA damage induced in cells. At low LET values, base damage and DNA single-strand breaks (SSB) are formed most frequently. After irradiation with high-LET heavy charged particles, predominantly DDSB are formed, and the number of SSB decreases.

The yield of radiation-induced EDSB depends on many biological factors, and the frequency of their formation can be manipulated by exploiting certain approaches based on the modification of DNA repair processes. Given these circumstances, we can conclude that the biological effectiveness of radiation will be determined by factors of different nature: a physical factor related to the nature of energy deposition in sensitive cell targets, and a biological factor, which affects the yield of damage that causes cell death. The latter presents an opportunity to increase the efficiency of proton beams for therapeutic purpose.

Considering the important role of EDSB in cells' radiosensitivity, we have previously investigated the modifying effect of 1-β-D-arabinosidecytosine (AraC) on the sensitivity of normal cells to radiations in a wide LET range. It was demonstrated that the radiosensitivity of mammalian and human cells in culture exposed to γ-ray and accelerated proton, doubled in the presence of this agent, and the DNA DSB yield increased significantly during post-irradiation incubation. The mechanism of the enhancing effect of this agent on the radiosensitivity of cells is based on the blocking of DNA polymerases that are responsible for DNA reparative synthesis in the process of repair. As a result, there is a long-term fixation of emerging direct SSB or SSB formed during excision repair. Such lesions may become sites for EDSB formation as a result of type S endonucleases' attack on the strand opposite to the damaged site.

Considering the ability of AraC to significantly increase the DNA DSB yield after exposure to ionizing radiation of cell cultures *in vitro,* we studied the effect of this agent on the growth of B16 melanoma and a number of processes associated with the radiation response of the tumor to the combined action of this compound and accelerated proton exposure at a focal dose of 10 Gy, as compared with that after irradiation with a proton beam only *in vivo.* Significant inhibition of tumor growth was observed in both groups of irradiated animals compared with the control; the effect was most pronounced with combined exposure. Molecular indicators of cell death and proliferative activity changed approximately to the same extent after the studied exposures compared with the control. However, the fraction of cancer stem cells was reduced by 3.1-fold after combined exposure compared with irradiation only, which, to some extent, explains the strongest inhibition of tumor growth after irradiation in the presence of AraC. Given the importance of the obtained results on the modification of tumor growth inhibition by AraC in irradiated animals, further studies of the effect of AraC on tumor growth in combination with other molecular biological complexes are planned. To solve this problem, experiments will be done using animal tumor-bearing models with an assessment of tumor regression exploiting computed tomography methods at the SARRP facility, followed by histological examination.

Expected results:

- to study *in vivo* the radiosensitizing effect of cytosine arabinoside in combination with other molecular biological complexes on melanoma tumor growth in mice following the combined exposure to these agents and proton radiation;
- to evaluate the influence of the combined action of AraC and other molecular biological complexes on the survival of different normal and tumor cell lines based on clonogenic survival criterion upon X-ray and proton irradiation;
- to study the kinetics of the formation and elimination of DNA damage in U87 glioblastoma and other radioresistant cell cultures after proton and X-ray exposure in the presence of AraC and other molecular biological complexes;
- to study DNA DSB formation in different components of the central nervous system after *in vivo* irradiation with protons and X-rays in the presence of a combination of radiomodifiers.

Development of research infrastructure

As the project's international radiobiology research program progresses, equipment upgrades and new facilities need to be phased in. The development of research infrastructure includes the following components:

1) commissioning of equipment for molecular and cellular omics studies (mass spectrometers, high-performance sequencer, flow cytofluorimeter sorter, laser confocal scanning microscope, etc.);
2) modernization of the vivarium and commissioning of equipment for multimodal tomography of animals.

Risk assessment

Most critically, the project depends on providing the necessary time at JINR accelerators, as well as resources of the JINR Multifunctional Information and Computing Complex. Possible technical and organizational problems can be solved through interaction with coordinators from the collaborating JINR Laboratories.

The financial risks may be due to difficulties in the supply of unique high-tech equipment, materials, and reagents. In this case, it is possible to temporarily use equipment of organizations participating in the project.

The risks of personnel shortages are not foreseen. The participants include both world-renown scientists and a large number of young specialists.

2.3 Estimated completion date

2024-2028

2.4 Participating JINR laboratories

2.4.1 MICC resource requirements

Computing resources	Distribution by year				
	1st year	2nd year	3rd year	4th year	5th year
Data storage (TB) - EOS - Tapes	-	-	-	-	-
Tier 1 (CPU core hours)	-	-	-	-	-
Tier 2 (CPU core hours)	-	-	-	-	-
SC Govorun (CPU core hours) - CPU - GPU	-	-	-	-	-
Clouds (CPU cores)	-	-	-	-	-

2.5. Participating countries, scientific and educational organizations

Organization	Country	City	Participants	Type of agreement
YSU	Armenia	Yerevan	Harutyunyan R.M.	
RINPh BSU	Belarus	Minsk	Kulahova T.A.	coop. program
Inst. Physiology NASB	Belarus	Minsk	Kulchitsky V.A.	
IBCE NASB	Belarus	Minsk	Antonevich N.G.	
IE BAS	Bulgaria	Sophia	Avramov L.A.	
NCRRP	Bulgaria	Sophia	Hristova R.	protocol
Inst. Microbiology BAS	Bulgaria	Sophia	Danova S.	protocol
INPC VAST	Vietnam	Hanoi	Wu Thi Ha, Trinh Thi Thu Huong	
CENTIS	Cuba	Havana	Gonzalez I.	
PIBOC FAB RAS	Russia	Vladivostok	Kusaikin M.I., Ermakova S.P.	agreement

FRC KazSC RAS	Russia	Kazan	Samigullin D.V.	
FMBC FMBA	Russia	Moscow	Osipov A.N., Rozhdestvenskiy L.M.	
FCBN FMBA	Russia	Moscow	Belousov V.V.	
IBMC	Russia	Moscow	Lisitsa A.V., Trifonova O.P.	protocol
IHNA&NPh RAS	Russia	Moscow	Pavlova G.V.	
MSU	Russia	Moscow	Latanov A.V.	
NRC KI	Russia	Moscow	Moskaleva E.Yu.	
ICBFM SB RAS	Russia	Novosibirsk	Dymova M.A., Silnikov V.N.	
NMRRC	Russia	Obninsk	Selivanova E.I., Yakimova A.O., Mosina V.A., Koryakin S.N., Khvostunov I.K.	
Pavlov Institute of Physiology RAS	Russia	St.-Petersburg	Filaretova L.P.	
SRI MP	Russia	Sochi	Klots I.N.	
UMF	Romania	Buharest	Verga N.	
INS «Vinča»	Serbia	Belgrade	Stepić M., Adžić M., Vranješ S.	coop. program
IBISS	Serbia	Belgrade	Popov A.	coop. program
University of Kragujevac	Serbia	Kragujevac	Marković Z.	coop. program
JFMED CU	Slovakia	Bratislava	Balentova S.	protocol
iThemba LABS	SAR	Faure	Vandervoorde Ch.	coop. program
UWC	SAR	Bellville	Rahiman F.	coop. program

2.6. Key partners *(those collaborators whose financial, infrastructural participation is substantial for the implementation of the research program. An example is JINR's participation in the LHC experiments at CERN).*

3. Manpower

3.1. Manpower needs in the first year of implementation

№№ n/a	Category of personnel	JINR staff, amount of FTE	JINR Associated Personnel, amount of FTE
1.	research scientists	37	-
2.	engineers	10	-
3.	specialists	-	-
4.	technicians	-	-
	Total:	**47**	-

3.2. Available manpower

3.2.1. JINR staff

No.	Category of personnel	Full name	Division	Position	Amount of FTE
1.	research scientists	Bugay A.N. Krasavin E.A. Boreyko A.V. Lobachevsky P.N. Koshlan I.V. Koltovaya N.A. Zamulaeva I.A. Zadneprianets M.G. Koshlan N.A. Nasonova E.A. Chausov V.N. Komova O.V. Korogodina V.L. Matchuk O.N. Vinogradova O.O. Vinogradova Yu.V. Kutsalo P.V. Severiukhin Yu.S. Khramko T.S. Shvaneva N.V. Budionnaya N.N. Vinogradova V.C. Golikova K.N.	LRB	Director Scientific Director Deputy Director Department Head Scientific Secretary Lead Reseacher Lead Reseacher Group Leader Group Leader Group Leader Group Leader Senior Reseacher Senior Reseacher Senior Reseacher Reseacher Reseacher Reseacher Reseacher Reseacher Reseacher Reseacher Junior Researcher Junior Researcher Junior Researcher	37

		Zhuchkina N.I. Il'ina E.V. Kovalenko M.A. Kozhina P.A. Kokoreva A.N. Kolesnikova I.A. Krupnova M.E. Kuzmina E.A. Kulikova E.A. Melnikova Yu.V. Nurkasova A. Petrova D.V. Pronskih E.V. Tiunchik S.I. Utina D.M. Shamina D.V. Shipilova E.A.		Junior Researcher Junior Researcher Junior Researcher Junior Researcher Junior Researcher Junior Researcher Junior Researcher Junior Researcher Junior Researcher Junior Researcher Junior Researcher Junior Researcher Junior Researcher Junior Researcher Junior Researcher Junior Researcher Junior Researcher	
2.	engineers	Tiupikova T.V. Melnikova L.A. Isakova M.D. Smirnova I.V. Pakhomova N.V. Nguen Bao Ngok Bazlova T.N. Erzhan K. Lkhasuren P.O. Golubeva E.V. Tilavova G.T.		Leading Engineer Senior Engineer Engineer Engineer Engineer Engineer Sen. Tech. Assistant Sen. Lab. Assistant Sen. Lab. Assistant Lab. Assistant Lab. Assistant	10
3.	specialists	-	-	-	-
4.	technicians	-	-	-	-
	Total:	**51**			**47**

3.2.2. JINR associated personnel

No.	Category of personnel	Partner organization	Amount of FTE
1.	research scientists	-	-
2.	engineers	-	-
3.	specialists	-	-
4.	technicians	-	-
	Total:	**-**	**-**

4. Financing

4.1 Total estimated cost of the project

The total cost estimate of the project (for the whole period, excluding salary).

The details are given in a separate table below.

5 090 000 $

4.2 Extra funding sources

Expected funding from partners/customers – a total estimate.

–

Project Leader _____/_____/

Project Leader _____/_____/

Date of submission of the project to the Chief Scientific Secretary: _____

Date of decision of the laboratory's STC: _____ document number: _____

Year of the project start: _____

(for extended projects) – Project start year: _____

Proposed schedule and resource request for the Project

Expenditures, resources, funding sources			Cost (thousands of US dollars)/ Resource requirements	Cost/Resources, distribution by years				
				1st year	2nd year	3rd year	4th year	5th year
		International cooperation	590 k$	100	100	130	130	130
		Materials	500 k$	100	100	100	100	100
		Equipment, Third-party company services	3500 k$	700	700	700	700	700
		Commissioning	-	-	-	-	-	-
		R&D contracts with other research organizations	500 k$	100	100	100	100	100
		Software purchasing	-	-	-	-	-	-
		Design/construction	-	-	-	-	-	-
		Service costs (*planned in case of direct project affiliation)*	-	-	-	-	-	-
Resources required	**Standard hours**	Resources						
		- the amount of FTE,	235	47	47	47	47	47
		- accelerator/installation:						
		SARRP (LRB)	960	192	192	192	192	192
		U400M (FLNR)	1000	200	200	200	200	200
		Nuclotron (SIMBO station) (VBLHEP)	1000	200	200	200	200	200
		Linak-200 (DLNP)	150	30	30	30	30	30
		IREN (FLNP)	150	30	30	30	30	30
		- reactor,…	-	-	-	-	-	-
Sources of	**JINR Budget**	JINR budget *(budget items)*	4500 k$	900	900	900	900	900
		5, 6, 10	590 k$	100	100	130	130	130
		4						

funding	**Extra fudning (supplementary**	Contributions by partners Funds under contracts with customers Other sources of funding	-	-	-	-	-	-

Project Leader _____/_____/

Project Leader _____/_____/

Laboratory Economist _____/_____/

APPROVAL SHEET FOR PROJECT

TITLE OF THE PROJECT

Molecular, genetic and organism effects of ionising radiations with different physical characteristics

SHORT DESIGNATION OF THE PROJECT

PROJECT CODE

THEME CODE

04-9-1077-2009

NAME OF THE PROJECT LEADERS

Boreyko Alla Vladimirovna, Lobachevsky Pavel Nikolaevich

AGREED

JINR VICE-DIRECTOR	_____	_____	_____
	SIGNATURE	NAME	DATE
CHIEF SCIENTIFIC SECRETARY	_____	_____	_____
	SIGNATURE	NAME	DATE
CHIEF ENGINEER	_____	_____	_____
	SIGNATURE	NAME	DATE
LABORATORY DIRECTOR	_____	_____	_____
	SIGNATURE	NAME	DATE
CHIEF LABORATORY ENGINEER	_____	_____	_____
	SIGNATURE	NAME	DATE
LABORATORY SCIENTIFIC SECRETARY	_____	_____	_____
	SIGNATURE	NAME	DATE
THEME LEADERS	_____	_____	_____
	SIGNATURE	NAME	DATE
	_____	_____	_____
	SIGNATURE	NAME	DATE
PROJECT LEADERS	_____	_____	_____
	SIGNATURE	NAME	DATE
	_____	_____	_____
	SIGNATURE	NAME	DATE
APPROVED BY THE PAC	_____	_____	_____
	SIGNATURE	NAME	DATE