РЕЗЮМЕТА НА НАУЧНИ ПУБЛИКАЦИИ НА АНГЛИЙСКИ ЕЗИК

НА **ГЛ. АС. Д-Р СЕВЕРИНА ЙОРДАНОВА СЕМКОВА**

КАНДИДАТ ЗА УЧАСТИЕ В КОНКУРС ЗА ЗАЕМАНЕ НА АКАДЕМИЧНА ДЛЪЖНОСТ „ДОЦЕНТ“ В ОБЛАСТ НА ВИСШЕ ОБРАЗОВАНИЕ 4. ПРИРОДНИ НАУКИ, МАТЕМАТИКА И ИНФОРМАТИКА, ПРОФЕСИОНАЛНО НАПРАВЛЕНИЕ 4.3. БИОЛОГИЧЕСКИ НАУКИ, ПО НАУЧНА СПЕЦИАЛНОСТ „БИОФИЗИКА“, КЪМ СЕКЦИЯ „ЕЛЕКТРОИНДУЦИРАНИ И АДХЕЗИВНИ СВОЙСТВА“, ИНСТИТУТ ПО БИОФИЗИКА И БИОМЕДИЦИНСКО ИНЖЕНЕРСТВО, БЪЛГАРСКА АКАДЕМИЯ НА НАУКИТЕ, ОБЯВЕН В ДВ БР. 27/24.03.2023 Г.

❖ Резюмета на научни публикации на английски език в издания реферирани и индексирани в световноизвестни бази данни с научна информация (Web of Science и Scopus), отнасящи се към **група от показатели В (Хабилитационен труд)**:

(публикации извън дисертационен труд за придобиване на ОНС “доктор”)

[B1] Nikolova, B., **Semkova, S**., Tsoneva, I., Antov, G., Ivanova, J., Vasileva, I., Kardaleva, P., Stoineva, I., Christova, N., Nacheva, L. - Characterization and potential antitumor effect of a heteropolysaccharide produced by the red alga Porphyridium sordidum. Engineering in Life Sciences, 2019, 19(12), 978-985. (IF_{2019}= 1.934, Q2), https://onlinelibrary.wiley.com/doi/epdf/10.1002/elsc.201900019

Abstract: Taking into account the rising trend of the incidence of cancers of various organs, effective therapies are urgently needed to control human malignancies. However, almost all chemotherapy drugs currently on the market cause serious side effects. Fortunately, several studies have shown that some non-toxic biological macromolecules, including algal polysaccharides, possess anti-cancer activities or can increase the efficacy of conventional chemotherapy drugs. Polysaccharides are characteristic secondary metabolites of many algae. The efficacy of polysaccharides on the normal and cancer cells is not well investigated, but our investigations proved a cell specific effect of a newly isolated extracellular polysaccharide from the red microalga Porphyridium sordidum. The investigated substance was composed of xylose:glucose and galactose: manose:rhamnose in a molar ratio of 1:0.52:0.44:0.31. Reversible electroporation has been exploited to increase the transport through the plasma membrane into the tested breast cancer tumor cells MCF-7 and MDA-MB231. Application of 75 μg/mL polysaccharide in combination with 200 V/cm electroporation induced 40% decrease in viability of MDA-MB231 cells and changes in cell morphology while control cells (MCF10A) remained with normal morphology and kept vitality.

[B2] Nikolova, B., Antov, G., **Semkova, S.,** Tsoneva, I., Christova, N., Nacheva, L., Kardaleva, P., Angelova, S., Stoineva, I., Ivanova, J. - Bacterial Natural Disaccharide (Trehalose Tetraester): Molecular Modeling and in Vitro Study of Anticancer Activity on Breast Cancer Cells, Polymers, 2020, 12(2), 499. (IF_{2020}= 4.329, Q1), https://www.mdpi.com/2073-4360/12/2/499

Abstract Isolation and characterization of new biologically active substances a_ecting cancer cells is an important issue of fundamental research in biomedicine. Trehalose lipid was isolated from Rhodococcus wratislaviensis strain and purified by liquid chromatography. The e_ect of trehalose lipid on cell viability and migration, together with colony forming assays, were performed on two breast cancer (MCF7—low metastatic; MDA-MB231—high metastatic) and one —normal‖ (MCF10A) cell lines. Molecular modeling that details the structure of the neutral and anionic form (more stable at physiological pH) of the tetraester was carried out. The tentative sizes of the hydrophilic (7.5 Å) and hydrophobic (12.5 Å) portions of the molecule were also determined. Thus, the used trehalose lipid is supposed to interact as a single molecule. The changes in morphology, adhesion, viability, migration, and the possibility of forming colonies in cancer cell lines induced after treatment with trehalose lipid were found to be dose and time dependent. Based on the theoretical calculations, a possible mechanism of action and membrane asymmetry between outer and inner monolayers of the bilayer resulting in endosome formation were suggested. Initial data suggest a mechanism of antitumor activity of the purified trehalose lipid and its potential for biomedical application.

[B3] Kadinov, B., Nikolova, B., Tsoneva, I., **Semkova, S**., Kabaivanova, L., Dimitrova, D. - Trehalose lipid biosurfactant reduced cancer cell viability but did not affect the isometric contraction of rat mesenteric arteries in vitro. International Journal Bioautomation, 2020, 24(1), 79-86. (SJR_{2020}=0.178, Q4), https://biomed.bas.bg/bioautomation/2020/vol_24.1/files/24.1_07.pdf

Abstract: Trehalose lipid biosurfactant from Nocardia farcinica strain is a naturally derived substance with potent anticancer activity. The increasing interest in naturally derived substances-based modality of cancer treatment requires investigations of the possible adverse effects of these substances, including the effects on vasculature. Therefore the present study was designed to investigate the effect of Trehalose lipid on isometric contraction of isolated rat mesenteric arteries. The contractile responses of

arteries under Trehalose lipid was studied using wire myography for small blood vessels. The isometric contractions of rat mesenteric artery rings with intact endothelium were examined. The effect of this biosurfactant was assessed in arteries precontracted with 42 mM KCl as a vascular smooth muscle depolarizing stimulus. The results showed that Trehalose lipid (75 μM) failed to change high K^+-induced contractions. The observed lack of effect of Trehalose lipid biosurfactant on the contractility of rat mesenteric arteries *in vitro* together with finding of reduced cancer cells viability makes it to be a suitable for potential medical application.

[B4] **Semkova, S.,** Antov, G., Iliev, I., Tsoneva, I., Lefterov, P., Christova, N., Nacheva, L., Stoineva, I., Kabaivanova, L., Staneva, G. - Rhamnolipid Biosurfactants - Possible Natural Anticancer Agents and Autophagy Inhibitors. Separations, 2021, 8(7), 92. (IF_{2021}= 2.777, Q3), https://www.mdpi.com/2297-8739/8/7/92

Abstract: Background/Aim: A number of biologically active substanceswere proved as an alternative to conventional anticancer medicines. The aim of the study is in vitro investigation of the anticancer activity of mono- and di-Rhamnolipids (RL-1 and RL-2) against human breast cancer. Additionally, the combination with Cisplatin was analyzed. Materials and Methods: Breast cell lines (MCF-10A, MCF-7 and MDA-MB-231) were treated with RLs and in combination with Cisplatin. The viability was analyzed using MTT assay, and investigation of autophagy was performed via acridine orange staining. Results: In contrast to the healthy cells, both tested cancer lines exhibited sensitivity to RLs treatment. This effect was accompanied by an influence on the autophagy-related acidic formation process. Only for the triple-negative breast cancer cell line (MDA-MB-231) the synergistic effect of the combined treatment (10 μM Cisplatin and 1 μg/mL RL-2) was observed. Conclusion: Based on studies on the reorganization of membrane models in the presence of RL and the data about a higher amount of lipid rafts in cancer cell membranes than in non-tumorigenic, we suggest a possible mechanism of membrane remodelling by formation of endosomes. Shortly, in order to havea synergistic effect, it is necessary to have Cisplatin andRL-2 as RL2 is a molecule inducingpositive membrane curvature.

[B5] Todinova, S., Nikolova, B., Iliev, Ivan; **Semkova, S.,** Krumova, S., Taneva, S. - Thermodynamic behavior of breast cancer cell lines after miltefosine and cisplatin treatment. Journal of Thermal Analysis and Calorimetry, 2022, 147, 7819–7828. (IF_{2021}= 4.755, Q2), https://link.springer.com/article/10.1007/s10973-021-11094-6

Abstract: Breast cancers exhibit different response to drug treatment. In this work, we analyze and compare the effect of two anticancer drugs differing in their primary action, miltefosine and cisplatin (cis-Pt), on two different breast cancer (the low—(MCF-7) and high—(MDA-MB-231) metastatic) cell lines, and one normal epithelial (MCF-10A) breast cell lines. The effect of cip-Pt and miltefosine on the thermodynamic behavior of the cancer cell lines was analyzed by differential scanning calorimetry, the cell morphology and viability were determined by optical microscopy and MTT test. We revealed distinct effects of miltefosine and cis-Pt on the thermodynamic behavior and viability of the cancer and normal cells. Importantly, the normal MCF-10A cells were drastically affected by miltefosine, while not by cis-Pt. MDA-MB-231 cell line, on the other hand, is more susceptible to cis-Pt than MCF-7 cells, while both cancer cell lines are equally affected by miltefosine. The drug-associated alteration of the thermal unfolding of the cells constituents correlated with the changes in the cell viability. The altered thermodynamic behavior of the cancer cells upon the drug treatment strongly indicates altered conformations of the proteins in cancer cell membrane and cellular matrix, and the DNA-containing structures.

[B6] Tsoneva, I., **Semkova, S.**, Bakalova, R., Zhelev, Zh., Nuss, Ph., Staneva, G., Nikolova, B. - Electroporation, electrochemotherapy and electro-assisted drug delivery in cancer. A state-of-the-art review. Biophysical Chemistry, 2022, 286(2022), 106819. (IF_{2021}=2.352,expected IF_{2021}=3.628, Q2)
https://www.sciencedirect.com/science/article/abs/pii/S0301462222000618?via%3Dihub

Abstract: This review focuses on electrochemotherapy that consists in the delivery of anti-cancer drugs using high-voltage electrical pulses. Technical issues, choice of drugs, and protocol of drug delivery are still under investigation and no consensus has been achieved yet. The different aspects of electrochemotherapy are discussed in the present paper. It includes interrogations about the choice of the preferred anti-cancer drug and dose to be delivered on the solid tumors. Another promising area is related to the electro-assisted release of nanoparticles (quantum dots) in xenografted solid tumors. Molecular mechanisms of enhanced drug delivery are discussed in terms of high cholesterol level and large fraction of lipid rafts in cancer cells. Electrochemotherapy is a paradigmatic example of cooperation between physicists, biophysicists, chemists, technicians, manufacturers, biologists, clinicians, and patients to improve a very promising treatment delivery in line with the conception of personalized medicine.

❖ Резюмета на научни публикации на английски език, в издания реферирани и индексирани в световноизвестни бази данни с научна информация (Web of Science и Scopus), отнасящи се към **група от показатели Г**:

*(публикации **извън** дисертационен труд за придобиване на ОНС "доктор")*

[Г1] Semkova S., Nikolova, B., Zhelev, Zh., Tsoneva, I., Zlateva, G., Aoki, I., Bakalova R. - Loading Efficiency of Polymersomes with Contrast Agents and their Intracellular Delivery: Quantum Dots Versus Organic Dyes. Anticancer research, 2018, 38(2), 825-831. (IF_{2018}= 1.935, Q2), https://ar.iiarjournals.org/content/38/2/825

Abstract: Background/Aim: Contrast nanocarriers as drugdelivery systems, capable of selective delivery to cancer cells and solid tumors, are essential for the development of new diagnostic and therapeutic (theranostic) strategies. The present study aimed to investigate the loading efficiency of chitosan-based polymersomes with fluorescent contrast substances [quantum dots (QDs) and conventional organic dyes] and the possibility to control their release from the polymer matrix into the cells by chemical modifications and electroporation. Materials and Methods: All investigated fluorophores were retained within the polymer globule via electrostatic and hydrophilic–hydrophobic interactions, without conjugation with the polymer. The fluorophoreloaded polymersomes were characterized by dynamic light scattering, zeta-potential titration, and fluorescence spectroscopy. The release of fluorophore from the polymersomes, passively or after electroporation, was detected by 5-step spin-ultrafiltration, combined with fluorescence spectroscopy of the upper phase (supernatant) of the filter unit. Passive intracellular delivery of the nanoparticles to HeLa cells was detected by fluorescence confocal microscopy. Results: The QDs were retained tightly and continuously in the polymer matrix, while the organic fluorophores [fluorescein isothiocyanate (FITC), FITCdextran10,000 and FITC-dextran70,000] were released rapidly from the polymersomes. The detergent Brij significantly increased the retention of FITC-dextran10,000 in the polymer globule. Electroporation up to 1000 V/cm did not induce release of QDs from the polymersomes, but accelerated the release of FITC-dextran10,000 Brij from the polymer matrix. High-voltage pulses (over 750 V/cm) induced also fragmentation or aggregation of the nanoparticles. QD_labeled polymersomes penetrated passively in cancer cells after 24-hour incubation. Conclusion: The results suggest that QD-labeled polymersomes are appropriate fluorescent probes and a nano-drug delivery system with high tracing opportunities for in vitro and in vivo applications. Furthermore, loading polymersomes with organic dyes with different molecular weights (such as FITCdextrans) is a simple model for visualizing and predicting the rate of release of small organic molecules (e.g.

conventional drugs, other contrasts, stabilizers, and supplements) from the polymer matrix

[Г2] Zhelev, Zh., Georgieva, E., Lazarova, D., **Semkova, S.,** Aoki, I., Gulubova, M., Higashi, T., Bakalova, R. - "Redox imaging" to distinguish cells with different proliferative indexes: Superoxide, hydroperoxides, and their ratio as potential biomarkers. Oxidative medicine and cellular longevity, 2019, vol. 2019, Article ID: 6373685, 18 pages. (IF_{2019} = 5.076, Q1), https://www.hindawi.com/journals/omcl/2019/6373685/

Abstract: The present study was directed to the development of EPR methodology for distinguishing cells with different proliferative activities, using "redox imaging". Three nitroxide radicals were used as redox sensors: (a) mito-TEMPO – cell-penetrating and localized mainly in the mitochondria; (b) methoxy-TEMPO – cell-penetrating and randomly distributed between the cytoplasm and the intracellular organelles; and (c) carboxy-PROXYL – nonpenetrating in living cells and evenly distributed in the extracellular environment. The experiments were conducted on eleven cell lines with different proliferative activities and oxidative capacities, confirmed by conventional analytical tests. The data suggest that cancer cells and noncancer cells are characterized by a completely different redox status. This can be analyzed by EPR spectroscopy using mito-TEMPO and methoxy-TEMPO, but not carboxy-PROXYL. The correlation analysis shows that the EPR signal intensity of mito-TEMPO in cell suspensions is closely related to the superoxide level. The described methodology allows the detection of overproduction of superoxide in living cells and their identification based on the intracellular redox status. The experimental data provide evidences about the role of superoxide and hydroperoxides in cell proliferation and malignancy.

[Г3] Ivanova, D., Zhelev, Zh., **Semkova, S.**, Aoki, I., Bakalova, R. - Resveratrol modulates the redox-status and cytotoxicity of anticancer drugs by sensitizing leukemic lymphocytes and protecting normal lymphocytes. Anticancer Research, 2019, 39(7), 3745-3755. (IF_{2019}= 1.994, Q2), https://ar.iiarjournals.org/content/39/7/3745

Abstract: Background/Aim: The study is directed to the effect of resveratrol on the redox-status and viability of leukemic and normal lymphocytes, as well as its ability to sensitize leukemic lymphocytes to anticancer drugs.

Materials and Methods: Cytotoxicity was analyzed by trypan blue staining,

apoptosis – by Annexin V test, and oxidative stress – by the intracellular levels of reactive oxygen species (ROS) and protein-carbonyl products.

Results: Incubation of resveratrol in combination with the majority of anticancer drugs resulted in higher toxicity than resveratrol or drug alone. In the case of leukemic lymphocytes treated with barasertib and everolimus in the presence of resveratrol, synergistic cytotoxicity was accompanied by strong induction of apoptosis, increased levels of hydroperoxides and insignificant changes in proteincarbonyl products. None of these parameters changed in normal lymphocytes.

Conclusion: Resveratrol is a promising supplementary compound for anticancer therapy, that may allow reduction of the therapeutic doses of barasertib and everolimus, minimizing their side-effects.

[Г4] Semkova, S., Zhelev, Zh., Miller, T., Sugaya, K., Aoki, I., Higashi, T., Bakalova, R. - Menadione/ascorbate induces overproduction of mitochondrial superoxide and impairs mitochondrial function in cancer: comparative study on cancer and normal cells of the same origin. Anticancer Research, 2020, 40(4), 1963-1972. (IF_{2020}= 2.480, Q2), https://ar.iiarjournals.org/content/40/4/1963

Abstract: Background/Aim: The menadione/ascorbate (M/A) combination has attracted attention due to the unusual ability of pro-vitamin/vitamin combination to kill cancer cells without affecting the viability of normal cells. The aim of this study was to elucidate the role of M/A in targeting cancerous mitochondria. Materials and Methods: Several cancer and normal cell lines of the same origin were used. Cells were treated with different concentrations of M/A for 24 h. The cell viability, mitochondrial superoxide, mitochondrial membrane potential, and succinate were analyzed using conventional analytical tests. Results: M/A exhibited a highly specific suppression on cancer cell growth and viability, without adversely affecting the viability of normal cells at concentrations attainable by oral or parenteral administration in vivo. This effect was accompanied by: (i) an extremely high production of mitochondrial superoxide in cancer cells, but not in normal cells; (ii) a significant dose-dependent depolarization of mitochondrial membrane and depletion of oncometabolite succinate in cancer cells. Conclusion: The anticancer effect of M/A is related to the induction of severe mitochondrial oxidative stress in cancer cells only. Thus, M/A has a potential to increase the sensitivity and vulnerability of cancer cells to conventional anticancer therapy and immune system.

[Г5] Bakalova, R., **Semkova, S.,** Ivanova, D., Zhelev, Zh., Miller, T., Takeshima, Ts., Shibata, S., Lazarova, D., Aoki, I., Higashi, T. - Selective targeting of cancerous mitochondria and suppression of tumor growth using redox-active treatment adjuvant. Oxidative Medicine and Cellular Longevity, 2020, vol. 2020, Article ID: 6212935, 30 pages. (IF_{2020}= 6.543, Q1), https://www.hindawi.com/journals/omcl/2020/6212935/

Abstract: Redox-active substances and their combinations, such as of quinone/ascorbate and in particular menadione/ascorbate (M/A; also named Apatone®), attract attention with their unusual ability to kill cancer cells without affecting the viability of normal cells as well as with the synergistic anticancer effect of both molecules. So far, the primary mechanism of M/A-mediated anticancer effects has not been linked to the mitochondria. The aim of our study was to clarify whether this "combination drug" affects mitochondrial functionality specifically in cancer cells. Studies were conducted on cancer cells (Jurkat, Colon26, and MCF7) and normal cells (normal lymphocytes, FHC, and MCF10A), treated with different concentrations of menadione, ascorbate, and/or their combination (2/200, 3/300, 5/500, 10/1000, and 20/2000 μM/μM of M/A). M/A exhibited highly specific and synergistic suppression on cancer cell growth but without adversely affecting the viability of normal cells at pharmacologically attainable concentrations. In M/A-treated cancer cells, the cytostatic/cytotoxic effect is accompanied by (i) extremely high production of mitochondrial superoxide (up to 15-fold over the control level), (ii) a significant decrease of mitochondrial membrane potential, (iii) a decrease of the steady-state levels of ATP, succinate, NADH, and NAD+, and (iv) a decreased expression of programed cell death ligand 1 (PD-L1)—one of the major immune checkpoints. These effects were dose dependent. The inhibition of NQO1 by dicoumarol increased mitochondrial superoxide and sensitized cancer cells to M/A. In normal cells, M/A induced relatively low and dose-independent increase of mitochondrial superoxide and mild oxidative stress, which seems to be well tolerated. These data suggest that all anticancer effects of M/A result from a specific mechanism, tightly connected to the mitochondria of cancer cells. At low/tolerable doses of M/A (1/100-3/300 μM/μM) attainable in cancer by oral and parenteral administration, M/A sensitized cancer cells to conventional anticancer drugs, exhibiting synergistic or additive cytotoxicity accompanied by impressive induction of apoptosis. Combinations of M/A with 13 anticancer drugs were investigated (ABT-737, barasertib, bleomycin, BEZ-235, bortezomib, cisplatin, everolimus, lomustine, lonafarnib, MG-132, MLN-2238, palbociclib, and PI-103). Low/tolerable doses of M/A did not induce irreversible cytotoxicity in cancer cells but did cause irreversible metabolic changes, including: (i) a decrease of succinate and

NADH, (ii) depolarization of the mitochondrial membrane, and (iii) overproduction of superoxide in the mitochondria of cancer cells only. In addition, M/A suppressed tumor growth in vivo after oral administration in mice with melanoma and the drug downregulated PD-L1 in melanoma cells. Experimental data suggest a great potential for beneficial anticancer effects of M/A through increasing the sensitivity of cancer cells to conventional anticancer therapy, as well as to the immune system, while sparing normal cells. We hypothesize that M/A-mediated anticancer effects are triggered by redox cycling of both substances, specifically within dysfunctional mitochondria. M/A may also have a beneficial effect on the immune system, making cancer cells "visible" and more vulnerable to the native immune response.

[Г6] Nikolova, B., **Semkova, S**., Tsoneva, I., Stoyanova, E., Lefterov, P., Lazarova, D., Zhelev, Zh., Aoki, I., Higashi, T., Bakalova, R. - Redox-related molecular mechanism of sensitizing colon cancer cells to camptothecin analog SN38. Anticancer Research, 2020, 40(9), 5159-5170. (IF_{2020}= 2.480, Q2), https://ar.iiarjournals.org/content/40/9/5159.long

Abstract: Background/Aim: The aim of this study was to elucidate the possibility of sensitizing colon cancer cells to the chemotherapeutic drug SN38 and investigate its mechanism of action after combined treatment with electroporation (EP).

Materials and Methods: Cells were treated with SN38, EP and their combination for 24/48 h. The cell viability, actin cytoskeleton integrity, mitochondrial superoxide, hydroperoxides, total glutathione, phosphatidyl serine expression, DNA damages and expression of membrane ABC transporters were analyzed using conventional analytical tests.

Results: The combination of EP and SN38 affected cell viability and cytoskeleton integrity. This effect was accompanied by: (i) high production of intracellular superoxide and hydroperoxides and depletion of glutathione; (ii) increased DNA damage and apoptotic/ ferroptotic cell death; (iii) changes in the expression of membrane ABC transporters – up-regulation of SLCO1B1 and retention of SN38 in the cells.

Conclusion: The anticancer effect of the combined treatment of SN38 and EP is related to changes in the redox-homeostasis of cancer cells, leading to cell death via apoptosis and/or ferroptosis. Thus, electroporation has a potential to increase the sensitivity of cancer cells to conventional anticancer therapy with SN38.

[Г7] **Semkova, S.,** Ivanova, D., Nikolova, B., Zlateva, G., Bakalova, R., Zhelev, Zh., Aoki, I. - Inhibition of ATP-synthase potentiates cytotoxicity of combination drug menadione/ascorbate in leukaemia lymphocytes. Biotechnology & Biotechnological Equipment, 2021, 35(1), 1738-1744. (IF_{2020}= 1.632, expected $IF_{2021/2022}$ = 1.762, Q3), https://www.tandfonline.com/doi/full/10.1080/13102818.2021.1996268

Abstract: The combination drug menadione/ascorbate (M/A) manifests synergistic dose-dependent antiproliferative and cytotoxic effects towards cancer cells, but not towards normal cells of the same origin especially at concentrations that can be achieved in vivo by its oral and parenteral administration. It is assumed that M/A alters selectively dysfunctional cancerous mitochondria. However, the exact molecular mechanism is not clear yet. The aim of the present study was to elucidate the role of adenosine triphosphate (ATP) synthase activity and its suppression by oligomycin-A on M/A-induced cytotoxicity, mitochondrial superoxide and ATP level in leukaemic lymphocytes. Cells were treated with different concentrations of M/A in the absence and presence of oligomycin-A (100 ng/mL) for 24 h and 48 h. The cell growth and viability, steady-state ATP level and mitochondrial superoxide were analysed using conventional analytical tests. The results showed that suppression of ATP synthase activity by oligomycin-A decreased the cell growth and viability and increased the production of mitochondrial superoxide and depletion of ATP in cells treated with low/tolerable doses of M/A (up to 5/500 μM/μM), compared to the cells treated with M/A only. Oligomycin-A did not affect these parameters in cells treated with high doses of M/A (10/1000 and 20/2000 μM/μM). The inhibition of ATP synthase potentiated the cytotoxicity of M/A, particularly in leukaemic lymphocytes treated with low/tolerable doses. We assume that the cytotoxicity of M/A is tightly connected to impairment of oxidative phosphorylation, and mitochondrial ATP depletion is a crucial factor for cell death.

[Г8] Ivanova, D., Yaneva, Z., Bakalova, R., **Semkova, S.,** Zhelev, Zh. - The antimalaria drug artemisinin displays strong cytotoxic effect on leukaemia lymphocytes in combination with vitamin c and pro-vitamin K3. Bulgarian Journal of Veterinary Medicine, 2021, 24(4), 533-543. (SJR_{2021}=0.157, Q4), http://www.uni-sz.bg/bjvm/BJVM%20December%202021%20p.533-543.pdf

Abstract: This study investigated the anticancer effect of the anti-parasitic drug artemisinin in combination with two redox modulators: vitamin C and pro-vitamin K3 (C/K3) The experiments were conducted on leukaemia cells Jurkat. Cells were treated

with either artemisinin or C/K3 alone and with all three compounds. Cell proliferation and viability were analysed using trypan blue stating and automated cell counting. The results showed that artemisinin (>10 μM) suppressed cell proliferation activity, but did not induce cell death up to 500 μM. The drug demonstrated a clear cytostatic effect at concentrations 250 μM-500 μM – Jurkat cells did not proliferate, but were alive. The combination C/K3 (200:2, 300:3 μM / μM) applied alone did not affect cell proliferation and viability. Vitamins C/K3 in concentration ratio 500:5 (μM/ μM) decreased cell proliferation activity by ~10%. The triple combination artemisinin/C/K3 manifested synergistic anti-proliferative effects at all concentration ratios analysed. This synergistic effect increased with increasing C/K3 concentration. Based on literature data, it was assumed that the anti-proliferative effect of the triple combination was mediated by changes in the redox-homeostasis of cancer cells. The C/K3 redox system likely acted on cancer mitochondria and increased superoxide production and activation of pro-apoptotic signals, specific for cancer cells. On the other hand, artemisinin could generate hydroxyl radicals as a result of activation of Fenton reactions, depleting intracellular reducing equivalents. Both redox mechanisms lead to activation of signal pathways for induction of cancer cell death.

[Г9] Lazarova, D., **Semkova, S**., Zlateva, G., Tatsuya, H., Aoki, I., Bakalova, R. - Quantum sensors to track total redox-status and oxidative stress in cells and tissues using electron-paramagnetic resonance, magnetic resonance imaging, and optical imaging. Analytical Chemistry, 2021, 93(5), 2828-2837. (IF_{2020}= 6.986, expected IF_{2021}= 8.008, Q1), https://pubs.acs.org/doi/10.1021/acs.analchem.0c04116

Abstract: Total redox capacity (TRC) and oxidative stress (OxiStress) of biological objects (such as cells, tissues, body fluids) are one of the most frequently analyzed parameters in the life science. Development of highly sensitive molecular probes and analytical methods for detection of these parameters is a rapidly growing sector of BioTech R&D industry. The aim of the present study was to develop quantum sensors for tracking the TRC and/or OxiStress in living biological objects using EPR, MRI, and optical imaging. We describe a two-set sensor system: (i) TRC sensor – QD@CD-TEMPO; and (ii) OxiStress sensor – QD@CD-TEMPOH. Both redox-sensors are composed of small-size quantum dots (QDs), coated with multi-nitroxide-functionalized cyclodextrin (paramagnetic CD-TEMPO or diamagnetic CD-TEMPOH) conjugated with Triphenylphosphonium (TPP) groups. The TPP-groups were added to achieve intracellular delivery and mitochondrial localization. Nitroxide residues

interact simultaneously with various oxidizers and reducers, and the sensors are transformed from paramagnetic radical form (QD@CD-TEMPO) into diamagnetic hydroxylamine form (QD@CD-TEMPOH) and vice-versa, due to nitroxide redox-cycling. These chemical transformations are accompanied by a characteristic dynamics of their contrast features due to quenching of QD fluorescence by nitroxide radical. TRC sensor was applied for EPR analysis of cellular redox-status in vitro on isolated cells with different proliferative index, as well as for non-invasive magnetic resonance imaging of redox imbalance and severe oxidative stress in vivo on mice with renal dysfunction.

[Г10] Ivanova, D., **Semkova, S.,** Yaneva, Z., Nikolova, B., Zhelev, Zh., Bakalova, R., Aoki. I. - Docosahexaenoic Acid Potentiates the Anticancer Effect of the Menadione/Ascorbate Redox Couple by Increasing Mitochondrial Superoxide and Accelerating ATP Depletion. Anticancer Research, 2023, 43(3), 1213-1220. (IF_{2020}= 2.480, expected IF_{2021}= 2.435, Q2), https://ar.iiarjournals.org/content/43/3/1213

Abstract: Background/Aim: Mitochondria-targeted anticancer drugs (“mitocans”) of natural origin are attractive candidates as adjuvants in cancer therapy. The redox couple menadione/ascorbate (M/A), which belongs to the “mitocans” family, induces selective oxidative stress in cancerous mitochondria and cells, respectively. DHA has also been found to regulate the mevalonate pathway, which is closely related to the prenylation of the cytotoxic menadione to the non-cytotoxic menaquinone. The aim of this study was to elucidate the ability of docosahexaenoic acid (DHA) to potentiate the anticancer effect of M/A by increasing ROS production, as well as affecting steady-state ATP levels in cancer cells.

Materials and Methods: The experiments were performed on leukemic lymphocyte Jurkat. Cells were treated with DHA, M/A, and their combination (M/A/DHA) and four parameters were examined using the following assays: cell viability and proliferation, steady-state ATP, mitochondrial superoxide, intracellular hydroperoxides. Three independent experiments with two or six parallel measurements were performed for each parameter.

Results: The triple combination M/A/DHA was characterized by much higher antiproliferative activity and cytotoxicity than M/A and DHA administered alone. DHA significantly accelerated M/A-induced ATP depletion in cells, which was accompanied by an additional increase in mitochondrial superoxide compared to cells treated with

M/A or DHA alone.

Conclusion: DHA significantly enhanced M/A-induced cytotoxicity in leukemic lymphocytes by inducing severe mitochondrial oxidative stress and accelerated ATP depletion. Selective DHA-mediated suppression of cholesterol synthesis in cancer cells (involved in the prenylation of cytotoxic menadione to the less cytotoxic phylloquinone), as well as DHA-mediated inhibition of superoxide dismutase are suggested to underlie the potentiation of the anticancer effect of M/A.

[Г11] Semkova, S., Nikolova, B., Tsoneva, I., Antov, G., Ivanova, D., Angelov, A., Zhelev, Zh., Bakalova, R. - Redox-mediated anticancer activity of anti-parasitic drug Fenbendazole in triple-negative breast cancer cells. Anticancer Research, 2023, 43(3), 1207-1212. (IF_{2020}= 2.480, expected IF_{2021}= 2.435, Q2), https://ar.iiarjournals.org/content/43/3/1207

Abstract: Background/Aim: An increasing number of studies are reporting anticancer activity of widely used antiparasitic drugs and particularly benzimidazoles. Fenbendazole is considered safe and tolerable in most animal species at the effective doses as an anthelmintic. Little is known about the redox-modulating properties of fenbendazole and the molecular mechanisms of its antiproliferative effects. Our study aimed to investigate the possibility of selective redox-mediated treatment of triple-negative breast cancer cells by fenbendazole without affecting the viability and redox status of normal breast epithelial cells. Materials and Methods: The experiments were performed on three cell lines: normal breast epithelial cells (MCF-10A) and cancer breast epithelial cells (MCF7 – luminal adenocarcinoma, low metastatic; MDA-MB-231 – triple-negative adenocarcinoma, highly metastatic). Cells were treated with fenbendazole for 48-h and three parameters were analyzed using conventional assays: cell viability and proliferation, level of intracellular superoxide, and level of hydroperoxides. Results: The data demonstrated that MDA-MB-231 cells were more vulnerable to fenbendazole-induced oxidative stress than MCF-7 cells. In normal breast epithelial cells MCF-10A, fenbendazole significantly suppressed oxidative stress compared to untreated controls. These data correlate with the effect of fenbendazole on cell viability and the IC50 values, which is indirect evidence of the potential targeting anticancer effect of the drug, especially in MDA-MB-231 cells. Conclusion: The difference in the levels of oxidative stress induced by fenbendazole in MDA-MB-231 and MCF-7 indicates that the two types of breast cancer respond to the drug through different redox-related mechanisms.