

XXVI Gliwice Scientific Meetings



Gliwice, November 18-19, 2022

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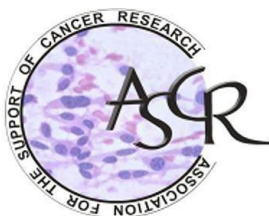
Organizers of Gliwice Scientific Meetings 2022:

Association for the Support of Cancer Research

Maria Skłodowska-Curie National Research Institute of Oncology, Gliwice Branch

Silesian University of Technology

Polish Academy of Sciences



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XXVI Gliwice Scientific Meetings, 2022

Scientific Program

Friday, 18th November 2022

9.00 – 9.15 **Opening of the conference**

Welcome addresses

Announcement of prof. Mieczysław Chorąży Fellowship laureate

9.15 – 9.50 **Professor Mieczysław Chorąży Lecture 2022:**

Philip Maini (*Wolfson Centre for Mathematical Biology, Oxford*): Can mathematical modelling help us understand cancer growth and progression?

9.50 – 10.00 Coffee Break

10.00 – 13.00 Session “**New and experimental cancer therapies and approaches**”

Chairpersons – Tomasz Cichoń and Ryszard Smolarczyk

- **Tatsuya Morisaki** (*Colorado State University, Fort Collins*): Technology developments to visualize and quantify translation at single molecule resolution in living cells.
- **Jesus Prieto** (*University of Navarra, Pamplona*): An AAV-based platform for cancer immunotherapy.
- **Sebastian Giebel** (*NRIO, Gliwice*): Chimeric antigen receptor (CAR) T- cells in oncology. Current status and future perspectives.
- **Urszula Foryś** (*Warsaw University, Warszawa*): Can a CAR-T cell treatment be efficient against solid tumors? A mathematicalinsight.

11.30 – 11.45 Coffee Break

- **Ekaterina Pylaeva** (*University Hospital, Essen*): Therapeutic modulation of neutrophil tumorigenic activity in head-and-neck tumor.
- **Aleksander Sochanik** and **Joanna Jazowiecka-Rakus** (*NRIO, Gliwice*): A novel viral oncolytic and chemotherapeutic combination approach to glioma therapy.
- **Ryszard Smolarczyk** (*NRIO, Gliwice*): The tumor microenvironment polarization as a goal of cancer immunotherapy.
- **Dorota Ścieglińska** (*NRIO, Gliwice*): Reconstructed human epidermis for *in vitro* studies on the HSPA2 gene function.
- **Paweł Kania** (*NanoTemper Technologies*): Employing Spectral Shift Technology and Microscale Thermophoresis in the modern drug discovery. Monolith.

13.00 – 13.15 Coffee Break

13.15 – 14.45 Session “**Biology of Extracellular Vesicles**” (part 1)

Chairpersons – Monika Pietrowska and Piotr Widłak

- **Janusz Rak** (*McGill University, Montreal*): Oncogenes, extracellular vesicles and cancer-associated vascular pathology.
- **Fabrice Lucien-Matteoni** (*Mayo Clinic, Rochester*): Tumor-derived extracellular vesicles in antitumor immunity and response to radiotherapy.
- **Jan Kranich** (*Ludwig-Maximilians-University, Munich*): Extracellular vesicles from antigen-presenting cells boost TCR-signaling and effector gene expression in CD8+ T cells during viral infections.

14.45 – 16.15 Lunch and **Poster Session**

16.15 – 18.00 Session “**Biology of Extracellular Vesicles**” (part 2)

- **Jadwiga Jabłońska** (*University Hospital, Essen*): Modulation of tumorigenic activity of neutrophils *via* tumor-derived EVs.
- **Piotr Widłak** (*Medical University of Gdańsk, Gdańsk*): Radiation-induced bystander effect mediated by exosomes involves the replication stress in recipient cells.
- **Anna Wojakowska** (*Institute of Bioorganic Chemistry, Poznań*): Metabolomic and proteomic profiling of serum exosomes from cancer patients treated by radiotherapy.
- **Aneta Żebrowska** (*NRIO, Gliwice*): Proteomic and metabolomic profiles of T cell-derived exosomes isolated from human plasma.
- **Daniel Fochtman**: *In vitro* and *in vivo* neutrophil models overview – an examination of advantages and disadvantages for neutrophil-derived small extracellular vesicles release.

19.00 Conference dinner

Saturday, 20th November 2022

9.00 – 11.30 Session “**Oncology Meets Physics, Mathematics and Numerical Methods**” (part 1)

Chairpersons – Zbigniew Grzywna and Krzysztof Fujarewicz

- **Khanh Ngoc Dinh** (*Rice University, Houston*): Modeling and simulation of cancer evolution in single cells.
- **Philip Maini** (*Oxford University, Oxford*): Mathematical challenges arising in cancer modeling.
- **Bartłomiej Waclaw** (*University of Edinburgh, Edinburgh*): Darwinian evolution in spatial models of cancer.
- **Ryszard Rudnicki** (*Institute of Mathematics, Warszawa*): Some aspects of mathematical modeling of cell cycle.
- **Krzysztof Fujarewicz** (*Silesian University of Technology, Gliwice*): Individualized and personalized models in biology and medicine.
- **Krzysztof Puszyński** (*Silesian University of Technology, Gliwice*): Mathematical modelling and analysis to search for molecular targets for therapy.

11.30 – 11.45 Coffee Break

11.45 – 12.30 Session “**Oncology Meets Physics, Mathematics and Numerical Methods**”
(part 2)

- **Roman Jaksik** (*Silesian University of Technology, Gliwice*): Whole genome sequencing - beyond driver mutation discovery.
- **Paulina Trybek** (*Silesian University, Katowice*) and **Agata Wawrzekiewicz-Jalowiecka** (*Silesian University of Technology, Gliwice*): On the use of nonlinear methods of patch-clamp data analysis to unravel the unical features of the BK channel gating in human glioblastoma cells.

12.30 – 14.10 Session “**Modifications of Nucleic Acids and Cancer**”

Chairpersons – Marek Foksiński

- **Edyta Reszka** (*Nofer Institute of Occupational Medicine, Lodz*): Epigenetics of a molecular clock in cancer.
- **Tomasz K. Wojdacz** (*Pomeranian Medical University, Szczecin*): Increasing significance of methylation biomarkers in clinical cancer management.
- **Marta Starczak** (*Collegium Medicum, Bydgoszcz*): N6-methyladenine metabolism – potential relationships with leukemia development.
- **Joanna Bogusiewicz** (*Collegium Medicum, Bydgoszcz*): New diagnostic opportunities in oncology based on microextraction methods.
- **Maciej Gawroński** (*Collegium Medicum, Bydgoszcz*): Vitamin C as a key regulator of the active DNA demethylation process.

14.10 – 14.40 **Poster Session** – Presentations of Awarded Posters

14.40 – 14.45 Closing Remarks

14.45 – Lunch

A great friend of Polish science, Professor Ronald Hancock, passed away on October 4th 2022

Ronald Hancock was born in 1933 in Epsom, England (now a district of London). During the Second World War, like many children of Londoners, he was sent by his parents to the estate of his uncle in the British countryside. After the war, he completed his college studies and obtained a PhD in Microbiology from the University of Cambridge. After graduation, he worked for several years as intern (post-doc), at the famous Harvard Medical School in Boston, United States, where he had the opportunity to work with top-class scientists, including Harold Amos. At the Harvard Medical School he became interested in research into genetic material and mechanisms regulating the formation of complexes and structures observed in cells and their participation in reading the genetic information. He remained faithful to these interests throughout his life. After the internship, he moved to the Cancer Research Institute at the University of Lausanne in Switzerland, where he took the position of an independent researcher. In 1986, he moved to Quebec, Canada, where he took up the position of professor and researcher at Laval University. In 2010, he came to Poland where he settled for good.

The choice of Poland and Gliwice as a place for further life was dictated by several reasons. Professor Ronald Hancock was associated with Gliwice from the 1970s, when he made friends with Professor Mieczysław Choraży,

who worked at the Institute of Oncology in Gliwice. Prof. Hancock was active in the organizing committee of the “Wilhelm Bernhard Workshop on the Cell Nucleus” [1] which was an



international conference initiated by Wilhelm Bernhard to make it easier for scientists from behind the “iron curtain” to learn about the achievements of the rapidly developing cell biology and molecular biology and to meet scientists from the institutions in the Western world (N.B., in 2019, during 26th Wilhelm Bernhard Workshop in Dijon, France, Prof. Hancock was awarded with the Wilhelm Bernard medal).

At that time, Polish science was in poor condition, in no small way due to the non-convertibility of the Polish currency into that of the “free world”, resulting in shortage of money to buy modern equipment and subscribe to foreign scientific journals. Professor Hancock actively helped to fill in the shortcomings of which he learned from Polish scientists whom he was meeting at various conferences. The organizing committees also financially supported the participation of “colleagues from the East” in these conferences. Professors Hancock and Choraży met and became friends at one of such conferences, and for many years

the Department of Tumor Biology was a source of scientific information published in Western journals such as Nature, Science, Biochemistry, Journal of Molecular Biology, Nucleic Acids Research which were all subscribed by Professor Hancock.

Professor Hancock was friends and supported also scientists from the University of Warsaw; he collaborated with prof. Shugar, the founder of Polish Biophysics, participated in many conferences organized by Polish centers in Gdańsk, Warsaw and Cracow. He supported the organization of international, specialized courses in the field of molecular biology organized in Poland, and also in other countries, e.g. in Tuzla, Bosnia, after the Balkan conflict and war, he was one of the organizers and lecturers of the molecular biology course and organized in Canada a collection of books and scientific literature for research centers in war-devastated Bosnia. In his laboratory in Lausanne and later in Canada, he accepted scholarship holders, PhD students, long-term and short-term postdoctoral fellows. Among the post-doc trainees in his laboratory were, among others, Prof. William Garrard, the discoverer of the mechanisms of cellular suicide death, or Jiri Fajkus, currently director of the Mendelian Plant Research Center at Masaryk University in Brno. Quite a large group of visitors to the Professor Hancock laboratory in Quebec were scientists from Gliwice from the Institute of Oncology and from the Silesian University of Technology visiting the laboratory to learn new methods. Professor Hancock not only made his laboratory available, but usually received guests, organized sightseeing trips and friendly meetings.

He settled in Poland because here he could still feel useful while lecturing at the University of Technology, supporting the English-language publishing work of his younger colleagues, but above all he had the opportunity to continue his research passion. Not without significance for his presence in Poland was also the fact that he married a Polish professor working in the same field and with whom he spent over 21 years of his life.

Unfortunately, he did not complete his research on chromosomes and his theory of “molecular crowding” forcing the formation of cellular structures. He was a perfectionist and still lacked the “last” experiment.

He was a true scientist who did not care about advertising himself, he loved nature, music, animals and people. We will miss his daily presence at the Biotechnology Center of the Silesian University of Technology, his wise advice on manuscripts, his quiet work and his example. There are few such people left among us in today’s world.

Prof. Joanna Rzeszowska, Ron’s wife

Translation: Dr. Aleksander Sochanik

[1] Pederson T. The Wilhelm Bernhard workshop: half a century of collegiality. Nucleus. 2019 Dec;10(1):218-220. doi: [10.1080/19491034.2019.1649844](https://doi.org/10.1080/19491034.2019.1649844)

History of the Gliwice Scientific Meetings

(by Prof. Katarzyna Lisowska, translated by Dr. Aleksander Sochanik)

Between 1966 and 1978, twelve Scientific Meetings, sometimes referred to as "Autumn Scientific Meetings" were held at the Institute of Oncology in Gliwice. The main Animator and Organizer of these Meetings was the late Professor Mieczysław Choraży, then a doctor at the Department of Tumor Biology.

In this early era, starting fifty years ago, various committees of the Polish Academy of Sciences (PAN) were invited to participate in the co-organization of these research conferences in Gliwice; alternatively, the latter were held jointly with various PAN Reporting Sessions or some other events. For example, the VIIth Scientific Meetings were organized together with the celebration of the Institute of Oncology 25th anniversary.

It is worth stressing that the scientific content of these early Meetings, despite "iron curtain" and relative isolation from the scientific community at large, has always been up-to-date with the most current issues of cancer research worldwide. For example, the invitation to the XIth Scientific Meetings (1977) read: "We would like to devote the meeting to the issue of genome research using 'genetic engineering' methods. In particular, we would like to discuss the current state of research on isolating and cloning specific DNA sequences of eukaryotic cells, summarizing information about restriction enzymes and plasmids used in prokaryote and eukaryotic DNA cloning experiments".

Table 1. Summary of early Scientific Meetings (based on surviving documents)

No.	Date (no of lectures)	Accompanying events	Foreign lecturers
III	29-30.11.1968 (8)		
IV	28-29.11.1969 (15)	Workshop "Nucleic acids and protein biosynthesis"; moderator: Prof. P. Szafranski	G.P. Georgiev (Moscow): Some features of the early stages of gene expression in animal cells P.M.B. Walker (Edinburgh): Satellite DNA in animal cells
VI	20.11.1971 (51)	6 th Conference of the Commission on Ultrastructure of Cells and Tissues (Committee of Cytopathophysiology of the Polish Academy of Sciences) on November 18-19, 1971	W. Bernhard (Villejuif): The contribution of electron microscopy to cancer research K. Smetana (Praha): Notes on the fine structure of the nucleoli during the cell maturation and differentiation P. Petrov (Sophia): Visualisation and functional variations of extranucleolar RNA I.B. Zbarsky (Moscow): Biochemical properties of the nucleolar envelope and its relation to nucleo-cytoplasmic

			information exchange
VII	17-18.11.1972 (21)	25 th anniversary of the Institute of Oncology in Gliwice; conference organized in cooperation with the Cancer Biology Committee and the Cancer Immunology Committee of the VI th Faculty, Polish Academy of Sciences	J. Bubenik (Praha): Immunology of human urinary tract cancer
VIII	15-16.11.1973 (18)	Reporting meeting of the problem group 09.3.1, Polish Academy of Sciences (Biological functions of nucleic acids)	
IX	16.11.1974 (9)		
X	26-27.11.1976 (12)		
XI	15.12.1977 (11)	Reporting session of the thematic group "Biology and biochemistry of cancer cells" of the Government Program PR-6	
XII	14-16.1978 (18)	Reporting session of the thematic groups "Biology and biochemistry of neoplastic cells" and "Biological foundations of radio- and cancer chemotherapy" of the Government Program PR-6	
XIII	4-5.05.1989 (21)		

After a 10-year break, an attempt was made to restore the tradition of holding the Scientific Meetings in Gliwice. Such event (XIIIth Scientific Meeting) was held on May 4-5, 1989. Its Promoters and main Organizers were Professors Joanna Rzeszowska and Zdzisław Krawczyk (at that time both doctors). The leading themes of that Conference were "DNA - research methods and organization in the cell nucleus" and "Gene expression in specific cell states".

No scientific meetings were held for several years later under this name, although the Department of Tumor Biology was involved in organizing other conferences. Between June 11-13th, 1992, a Workshop was held on "Assessment of Cancer Hazard in Silesia" (photo below). Among the sponsors of that conference were National Cancer Institute (Bethesda, USA) and Polish Ministry of Environmental Protection. The workshop aimed at summarizing



the results of research conducted at the Environmental Mutagenesis Laboratory by the team led by Mieczysław Chorąży and including Ewa Grzybowska, Grażyna Motykiewicz and Jan Szeliga. These studies were conducted in cooperation with the US and Swedish research teams headed by Federica Pereira (New York) and Kari Hemminki (Stockholm). Dr. Ansley Weston in her conference summary report written for NCI stated: "In conclusion, the dramatic pollution of air, water and soil as a result of industrial processes in Poland in Upper Silesia has been identified and well documented. This pollution seems to pose a serious threat to human health and a cancer risk in Silesia". The results were widely published, including a paper that appeared in *Nature* (F.P. Perera, K. Hemminki, E. Grzybowska, G. Motykiewicz, J. Michalska, R.M. Santella, T.-L. Young, C. Dickey, P. Brandt-Rauf, I. DeVivo, W. Blaner, W.-Y. Tsai, M. Chorąży (1992) *Molecular and genetic damage in humans caused by environmental pollution in Poland. Nature, 360, 256-258.*)

A memorable scientific conference organized by the Department of Cancer Biology was held in 1995 to commemorate the 70th birthday anniversary of Professor Mieczysław Chorąży. The Meeting was entitled "Progress in Tumor Biology" (October 13-14, 1995) and hosted many distinguished guests, friends and collaborators of Professor Chorąży (including Prof. Hilary Koprowski, inventor of the world's first effective live polio vaccine; Prof. Robert Gallo, HIV co-discoverer; Prof. Kari Hemminki, world-class mutagenesis researcher; Prof. Jorgen

Kieler, research collaborator and 2nd World War Danish resistance movement fighter; also Prof. Klaus Scherrer from the Jacques Monod Institute). Among the lecturers were Professor Choraży's former students working abroad: Jan Filipiński and Zenon Stęplewski. Laudation speeches were delivered by eminent Professors: Kazimierz Dux, Jan Steffen and Kornel Gibiński. The information brochure about this event features the Professor's bio and an interesting interview conducted with him by Dr. Piotr Widlak. The chief Organizer of this conference was the late Professor Stanisław Szala.

The last attempt to revive the tradition of Scientific Meetings in Gliwice was made in 1996 by Professor Joanna Rzeszowska (then Head of the Department of Experimental and Clinical Radiobiology at the Institute of Oncology) and her team. Following Professor Rzeszowska's initiative, an organizational platform to conduct the meetings was brought to life (the Association for the Support of Cancer Research). One of the main statutory goals of this Association has been the organization of an annual scientific event called "Gliwice Scientific Meetings". The Association has ensured successful sustainability of this initiative and in 2022 it is organizing the twenty-sixth Meetings in this new formula.

The first actual "Gliwice Scientific Meetings" took place in 1997. Its subject matter was devoted to the DNA-protein interactions. The only foreign guest then was Valentin Schick from Moscow who in his "Analysis of DNA and protein microchips" lecture spoke about the then brand-new microarray-based technology. The second "Gliwice Scientific Meetings" (1998) were held jointly with the celebration of the 50th anniversary of the Institute of Oncology

in Gliwice and enjoyed a handful of foreign lecturers (Wolfgang Henning, Wolf H. Strätling, Jan Filipiński and Regen Drouin) and participants. Four sessions (Mathematical models in biology; Chromatin structure and gene expression; Genetic polymorphism and cancer; DNA damage, cell repair and death) were held, featuring a total of 18 lectures, including four delivered by foreign guests. Since the 5th Gliwice Scientific Meetings the conference went international with English as the conference language. The subsequent years witnessed the Silesian University of Technology, a vibrant academic entity located in Gliwice, to join the organization of the Gliwice Scientific Meetings, and provide its modern Conference Center as the Meetings venue.

The IXth Scientific Meetings (2005) were an occasion to celebrate the 80th birthday of Professor Mieczysław Choraży. During the Covid-19 pandemic the Meetings went virtual (the XXIVth Meetings in 2020, with online lectures only), or adopted a hybrid formula (the Jubilee XXVth Meetings in 2021). Sadly, the latter event was also the first without Professor Mieczysław Choraży presence. Our long-time Mentor and eminent Scientist passed away on February 21, 2021 at the age of 95. In memoriam, the Conference organizers decided that the opening lecture would since become the "Mieczysław Choraży Lecture" (in 2021 it was given by Prof. Kari Hemminki). The same year the Association for the Support of Cancer Research established and co-funded the Professor Mieczysław Choraży memorial scholarship for young scientists (the first scholarship was awarded to Dr. Alexander Cortez from the Institute of Oncology).

Calendar

1966 – 1st Scientific Meetings at the Institute of Oncology in Gliwice; initiated and organized in the fall by Professor Mieczysław Chorąży and the staff of the Department of Tumor Biology.

1966 - 1978 – Scientific Meetings (12 held in total, except for 1970 and 1975).

1989 – 13th Scientific Meetings; reactivation after a decade-long break; re-initiated by Professors Joanna Rzeszowska and Zdzisław Krawczyk.

1992 – Workshop "Assessment of Cancer Hazard in Silesia" in part sponsored by National Cancer Institute, NIH, USA.

1995 – Scientific Conference "Progress in Tumor Biology" jointly with celebration of the 70th birthday anniversary of Professor Mieczysław Chorąży.

1996 – Association for the Support of Cancer Research (ASCR) is established on the initiative of Professor Joanna Rzeszowska.

1997 – 1st Gliwice Scientific Meetings organized by ASCR.

1998 – 2nd Gliwice Scientific Meetings organized by SWBR along with commemoration of the 50th anniversary of the Institute of Oncology in Gliwice.

2005 – 9th Gliwice Scientific Meetings and the 80th birthday jubilee of Professor Mieczysław Chorąży.

2021 – 25th Jubilee Gliwice Scientific Meetings; honorary "Mieczysław Chorąży Lecture" given for the first time; Mieczysław Chorąży Scholarship for the young scientists awarded for the first time.

Professor Mieczysław Choraży Lecture 2022

Can Mathematical Modelling Help Us Understand Cancer Growth and Progression?

Philip Maini

Wolfson Centre for Mathematical Biology, Oxford, Great Britain

In this talk I will review how mathematical models, combined with experimental observations, can be used to help us understand certain aspects of solid tumour growth. Specifically, I will discuss models for cancer cell invasion that incorporate the acid-mediated invasion hypothesis, phenotypic co-operation and somatic evolution. I will then discuss how mathematical models can be used to address therapeutic strategies, specifically in the context of adaptive therapy.

LECTURE ABSTRACTS

Session

**“New and experimental cancer therapies
and approaches”**

[L-1] Technology developments to visualize and quantify translation at single molecule resolution in living cells

Tatsuya Morisaki

Colorado State University, Fort Collins

Translation is one of the essential processes in gene expression regulation. It is known that misregulation of translation could cause many diseases, including cancer. Until recently, it was not possible to study translation in detail in living cells. I will talk about the technology development to visualize and quantify translation at single mole resolution in living cells and some of the applications of this technique. I will mainly discuss the translation shutoff process during stress responses and briefly mention translational frameshifting, IRES-mediated translation, and translational repression by miRNA.

[L-2] A new AAV-based platform for cancer radioimmunogene (RIG) therapy

Jesus Prieto

CIMA. University of Navarra. Pamplona. Spain

Tumor microenvironment (TME) constitutes a highly immunosuppressive, growth-promoting and pro-angiogenic milieu which is required for tumor progression. Modifying TME to create a pro-immunogenic, antiangiogenic and growth-inhibiting atmosphere is a desired therapeutic goal as this might lead to tumor elimination. IL-12 is a potent immunostimulatory and antiangiogenic molecule that is produced by activated antigen presenting cells (APCs) including dendritic cells (DCs). IL-12 stimulates CD8 T cells and NK cells to produce IFN γ which inhibits tumor vascularization, displays cytostatic/cytotoxic effects on malignant cells and induces the production of chemokines that attract effector T cells.

We decided to treat solid tumors using a strategy based on gene transfer of IL-12 to transform the TME and promote tumor rejection. To this aim, we used vectors based on adenoassociated virus (AAV), since they are well tolerated and have been used in multiplicity of clinical trials. AAV are non-cytopathic single-stranded DNA parvoviruses composed of a capsid made of 3 proteins named VP1, VP2 and VP3 and a genome of 4.7 kb possessing only two genes, rep and cap. The viral genome is flanked by two inverse terminal repeats (ITR) which are required for its encapsidation. Depending on the aa sequence of the VPs, there are diverse serotypes which differ in tissue tropism. AAV vectors are produced by replacing rep and cap by an expression cassette containing the therapeutic gene while maintaining the ITRs. Following cell entrance, the single DNA strand is copied to form double-stranded DNA which is the transcriptionally active form of the vector. Recombinant AAVs remain mostly episomal and enable long-term expression of the transgene. Many AAV serotypes, including AAV8, AAV9, AAV5 and AAV2, are hepatotropic and therefore transduce liver cells efficiently.

We used an AAV8 encoding single-chain IL-12 which was administered by intra-tumor injection. We found that AAVs were quite inefficient at transducing tumors. However, previous irradiation of the tumor significantly increased transduction efficiency of the tumor tissue. Hence, AAV could be applied to treat cancers that are subjected to radiation therapy. These represent about 50% of all solid tumors. To secure the production of IL-12 within the irradiated tumor we designed a synthetic radiation-responsive promoter to drive transgene expression.

Since IL-12 is toxic if given systemically, our aim was to raise intra-tumor IL-12 levels without significantly increasing its serum concentration. However, when the vector is injected into the tumor, a portion of the vector dose escapes to the circulation and will transduce the liver. This would entail the risk that IL-12 would be produced in this organ with resulting elevation of its levels in the circulation. Hence, it was critical to prohibit transgene expression in hepatocytes. We were able to achieve this objective by placing at the 3' end of the expression cassette binding sites for a microRNA highly expressed in hepatocytes. The resulting vector was named AAV.IL12T. This vector when administered locally in irradiated tumors enabled us to enrich the TME in IL-12 while maintaining very low, or undetectable, values in serum. AAV.IL12T was tested in different murine tumor models (MC38 colorectal cancer and B16 melanoma) produced by injecting syngenic tumor cell lines subcutaneously. Irradiation with 8 Gy followed by intratumor injection of AAV.IL12T resulted in potent and sustained inhibition of tumor growth (with complete tumor rejection in many cases) and development of antitumor immunity rendering the animal resistant to rechallenge with the same tumor cell line. The antitumor effect was accompanied by strong changes in TME with increased leucocyte infiltration, elevation of CD8 $^+$ T cells and marked reduction of Treg and of suppressive tumor associated macrophages.

Our data indicate that combination of radiotherapy with local injection of AAV encoding immunostimulatory cytokines represents a promising approach to treat tumors. This new therapeutic approach, named radioimmunogene (RIG) therapy, is ready to be tested in phase I/II clinical trials.

[L-3] Chimeric antigen receptor (CAR) T- cells in oncology. Current status and future perspectives

Sebastian Giebel

Head: Dept. of Bone Marrow Transplantation and Onco-Hematology, Maria Skłodowska-Curie National Research Institute of Oncology, Gliwice Branch, Gliwice, Poland

Chimeric antigen receptor (CAR) T-cells are T-cells that have been genetically engineered to produce an artificial T cell receptor for use in immunotherapy. T-cells are collected by leukapheresis from patient's peripheral blood and ex vivo transduced with gene construct encoding for receptor specific for tumor antigen. After stimulation and ex vivo expansion they are reinfused to patient's bloodstream. In vivo they recognize and destroy malignant cells. So far, four anti-CD19 CAR T-cell products have been approved for the treatment of relapsed/refractory B-cell lymphomas and B-cell acute lymphoblastic leukemia. Two products with specificity against B-cell maturation antigen have been approved for relapsed/refractory multiple myeloma. Hundreds of clinical trials are being conducted

all over the world on application of CAR T-cells in other hematological indications as well as in solid tumors and autoimmune diseases. Future perspectives include the use of allogeneic NK or NKT cells, as well as on targeting more than one antigen to increase the treatment efficacy.

[L-4] Can a CAR-T cell treatment be efficient against solid tumors? A mathematical insight

Urszula Forys¹, Marek Bodnar², Juan Belmonte-Beitia³

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In [1] mathematical models of a solid tumour response to a CAR-T cell treatment in the presence of immune suppression was proposed. The first model is two-dimensional and describes only the dynamics of necessary components of the system, that is the population of tumour cells and the population of CAR-T cells. Next, four dimensional system with additional components are included. The authors called this model “dual CART-T cells model” because they considered two localizations of CAR-T cells, at the tumour site and outside it. Moreover, except tumour cells, a-variable describing normal B cells is included. In both models the underlying tumour growth law is assumed to be exponential. CAR-T cells are administrated, they destroy tumour cells and their proliferation is stimulated by the tumour. On the other hand, tumour cells creates an immunosuppressive environment that suppress CAR-T cells.

In [1] impulsive treatment is analysed. It is shown that for biologically plausible model parameters, CAR-T cells can be efficient. Moreover, the authors performed a systematic study of the possibility of controlling tumour using “single” and “double” CAR-T therapies concluding that dual CAR-T improves the possibility of success.

In our study we assumed that the tumour growth is exponential or it can be limited (logistic or Gompertz). We mainly focused on the constant treatment and two dimensional case. For such a simple model we proved that for some range of parameters the tumour size can be controlled by medically achievable doses of treatment (the doses was taken from [2]) if the tumour size is sufficiently small. On the other hand, if it is large, it cannot be controlled by this type of treatment. We also checked that the dose of treatment in the case of constant inflow which is efficient corresponds to the mean dose of efficient treatment in the impulsive case. The advantage of the model with constant treatment lies in the fact that we are able to precisely describe the model dynamics with respect to the dosage.

Coming back to impulsive treatment, we showed that the analytical results are valid for a wide range of functions describing the tumour growth law. However, it occurs that for the Gompertz growth it is not the case. It does not mean that in this case numerical methods are not applicable, but we are not able to find efficient doses analytically.

We hope that our study will help in choosing doses in clinical trials to make the CAR-T therapy more widely applicable and efficient.

References

- [1] Odelayisy León-Triana, Antonio Pérez-Martínez, Manuel Ramírez-Orellana and Víctor M. Pérez-García, Dual-Target CAR-Ts with On- and O?-Tumour Activity May Override Immune Suppression in Solid Cancers: A Mathematical Proof of Concept *Cancers*, 13, 703 (2021), doi: 10.3390/cancers13040703
- [2] Christine E. Brown et al., Regression of Glioblastoma after Chimeric Antigen Receptor T-Cell Therapy, *The New England Journal of Medicine*, 375, 2561-9 (2016), doi: 10.1056/NEJMoa1610497

[L-5] Therapeutic modulation of neutrophil tumorigenic activity in head-and-neck cancer

Ekaterina Pylaeva

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Microenvironment of tumors and tumor-draining lymph nodes (LN) is a potent stimulus attracting neutrophils from blood stream and inducing phenotype that distinct from circulating cells. A continuum of anti- and pro-tumoral neutrophil activities arises there in a stage-dependent manner. In early non-metastatic tumor stage neutrophils possess anti-tumor properties (cytotoxicity, stimulation of adaptive immunity), while on late tumor stages and in presence of LN metastases, neutrophils significantly contribute to cancer progression via the stimulation of angiogenesis and metastatic spread, and suppression of adaptive immunity. Among other factors, lack of Type I IFNs signaling, and elevated amounts of G-CSF, GM-CSF via STAT3 activation drive neutrophils into tumor-supportive phenotype.

Here, we investigated therapeutical strategies, targeting migration and microenvironment-driven polarization of tumor-supportive neutrophils. Migration of myeloid cells is guided by solid-phase gradient of chemokines bound by glycosaminoglycans on endothelium and extracellular matrix. Peptide fragments of chemokines can block chemokine binding to matrix, disturb the guiding chemokine gradient and prevent their migration and tumor-mediated polarization (PD-L1/PD-1 axis) at the tumor site and LNs, leading to significantly suppressed tumor growth. IFNAR1-independent rescue of interferon signaling using type III IFNs (IFN-lambda) improved the immunostimulatory capacity of neutrophils in LNs and contributed

to the suppression of tumor growth. Antagonists of growth factors and STAT3 reverse tumor-supportive polarization of neutrophils in metastatic LNs. Moreover, blockade of G-CSF receptor downstream signaling pathways (NAMPT, SIRT1 inhibitors) in neutrophils suppresses their tumor-supportive polarization with the prominent inhibition of angiogenic activity, leading to repressed tumor growth.

In the light of still missing efficient anti-cancer approaches, modulation of neutrophil migration and activity may become a promising therapeutic target to suppress tumor growth and spread.

[L-6] A novel viral oncolytic and chemotherapeutic combination approach to glioma therapy

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Glioblastoma is rapidly proliferating and difficult to treat neoplasm affecting CNS structures. Brain tumor initiating cells (BTICs) are deemed responsible for recurrence and potentially fatal outcome in virtually all involved. A therapeutic strategy has been pondered that explores intraarterial delivery of a BTIC-targeting oncolytic agent *via* adipose-derived mesenchymal stem cells (ADSC). In quest for therapeutic synergy the viral oncolytic approach mediated by a oncoviral construct with deleted crucial antiapoptotic gene will be compounded by explore novel designer glioma chemotherapeutics also administered systemically using BBB-crossing agents. The talk will feature the basics of this experimental approach, its pros and cons, potential obstacles as well as ways to overcome it using immunocompetent mice with orthotopically induced (*via* stereotactic surgery) glioma foci and undergoing intralesional treatment, as well as mice with residual disease. We believe the proposed therapeutic platform is a potentially interesting weapon against glioblastoma.

This study has been supported by grant No. 2016/22/M/NZ6/00418 from National Science Centre, Poland, to JJ-R.

[L-7] The tumor microenvironment polarization as a goal of cancer immunotherapy

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The tumor microenvironment is a composition of different cells surrounded by heterogeneous matrix. In addition to cancer cells, it includes cancer associated fibroblast, endothelial cells and numerous immune cells such as macrophages, neutrophils, lymphocytes and NK cells. Many works indicate that immune cells present in tumor microenvironment have low cytotoxic activity. Furthermore, anti-cancer activity of such cells present in the tumors is inhibited by a tumor microenvironment immunosuppressive properties. Therefore, efficient polarization of tumor associated immune cells should effectively inhibits the growth of tumors.

The aim of the research was to design an effective therapy polarizing the tumor microenvironment from immunosuppressive to immunostimulatory.

Experiments were performed in two mouse models: triple negative breast cancer (4T1) and murine melanoma (B16-F10). In our experiments, we focused on the use of an anti-cancer STING agonist – cGAMP. Binding of cGAMP by STING protein activates a number of transcription factors like NF- κ B, IRF3, IRF7, STAT3 and STAT6 that stimulate the immune system to respond against cancer cells. The analyzes of immune cells infiltration and their state of activation (M1/M2 macrophages, NK cells, CD8 T cells, N1/N2 neutrophils) were performed using flow cytometry. Density of blood vessels was analyzed using confocal microscopy.

We have shown that the use of a STING agonist polarizes the tumor microenvironment from pro-tumorigenic to anti-cancer and inhibits the growth of tumors. Infiltration and activation of neutrophils after STING agonist administration were observed. cGAMP effectively stimulated the neutrophils which changed their phenotype from the pro-tumorigenic N2 to cytotoxic N1.

To increase the therapeutic efficacy of cGAMP combination with anti-vascular drug - combretastatin A4 phosphate (CA4P) was designed. Combination therapy significantly inhibited tumor growth of both models. The main therapeutic effect was associated with the NK cells infiltration and their activation in tumors. Decrease of tumor blood vessel density after CA4P monotherapy and combination therapy have been noticed. Additionally, the combination therapy promote switch of macrophages phenotype from pro-tumor (M2) toward anti-tumor (M1). Increase of CD8 cytotoxic T lymphocytes number was observed in tumors after cGAMP monotherapy and in combination therapy.

STING agonist administration efficiently polarize tumor microenvironment into immunostimulatory one by polarization of neutrophils, macrophages and NK cells into anti-tumor phenotypes. Driven by cGAMP switch of the tumor microenvironment phenotype acts as a basis of the effective combination therapy with anti-vascular compound providing more efficient tumor growth inhibition than the administration of each of the factors separately.

The work is a result of the research projects no. UMO-2018/31/B/NZ5/01825 and UMO-2019/35/N/NZ5/02506 financed by National Science Center, Poland.

[L-8] Reconstructed human epidermis for *in vitro* studies on the HSPA2 gene function

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A tissue-engineered model of reconstructed human epidermis (RHE) when combined with genetic manipulation methods, is a powerful tool for studying the effects of a particular protein on the integrity and function of the human skin. HSPA2, a member of the multigene and cytoprotective HSPA (HSP70) family, was originally regarded as a testis-specific chaperone essential for male fertility. Previously, we found that this protein is expressed in certain human somatic tissues including multilayered epithelia. In the epidermis HSPA2 is present in keratinocytes of the basal layer, but its functional significance is poorly understood. Surprisingly, this protein has minimal contribution to protection of keratinocytes against apoptosis-inducing stimuli.

To search for biological processes that rely on HSPA2 in epidermis we generated RHE model from HSPA2-null or HSPA2-overexpressing immortalized human epidermal keratinocytes.

We found that the loss of HSPA2, but not its overproduction resulted in aberrant stratification of RHE. The ones that were formed by HSPA2-null cells had undeveloped granular layer when compared to control. Transcriptome profiling of RHE based on RNAseq method provided another evidence that the terminal differentiation of keratinocytes is dependent on HSPA2. Pathway enrichment analyses revealed that the over-represented signaling pathways in HSPA2-null RHE are related to the late stages of epidermal stratification. Interestingly, among signaling pathways that have been sensitive to HSPA2 loss are those related to immunomodulatory processes.

We confirmed that HSPA2-null RHE produced higher levels of pro-inflammatory chemokines. The presence of HSPA2 in keratinocyte-derived small extracellular vesicles further suggests its potential immunomodulatory role in the human epidermis.

In summary, we found that in the human epidermis HSPA2 regulates late stages of keratinocyte differentiation and may impact on immunomodulatory processes. The last aspect highlights the need for further research on immunomodulatory role of HSPA2 in the epidermis and other stratified epithelia.

This work was supported by the National Science Centre, Poland grant 2017/25/B/NZ4/01550.

[L-9] Employing Spectral Shift Technology and Microscale Thermophoresis in the modern drug discovery. Monolith

Pawel Kania

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The road to the successful characterization of challenging molecular interactions is filled with roadblocks often imposed by the limitations of the widely established biophysical methods — like dependence on a surface immobilization, high sample consumption, buffers constraints, or interacting partners molecular masses differences. There is a clear need for a biophysical technology that gives scientists the solution they need to finally study challenging interactions that involve molecules like cell surface receptors, small molecular ligands, PROTACs, AAVs, liposomes, RNAs, DNA, or other complex molecules.

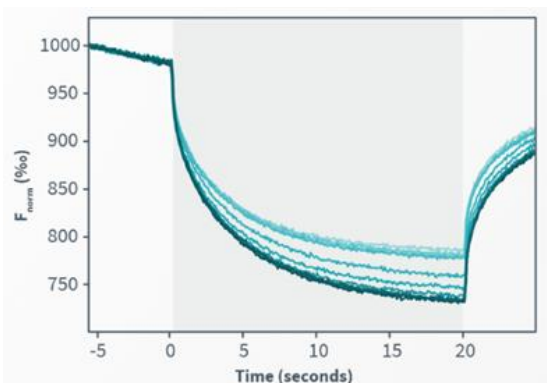
The new Dianthus — a plate-based and microfluidics-free platform that performs measurements in solution with a very low sample consumption, and yet unmatched sensitivity — now with breakthrough Spectral Shift technology combined with our well-established TRIC technology excels precisely at the measurement of these demanding interactions, independently of buffer composition.

TRIC (temperature-related intensity change) technology allows for quantification of molecular interactions between a target and ligand by detecting changes in fluorescence intensity while a temperature gradient is applied over time. The fluorescent signal comes from the target that is either fluorescently labeled or has intrinsic fluorescence and becomes an extremely sensitive reporter for the interaction.

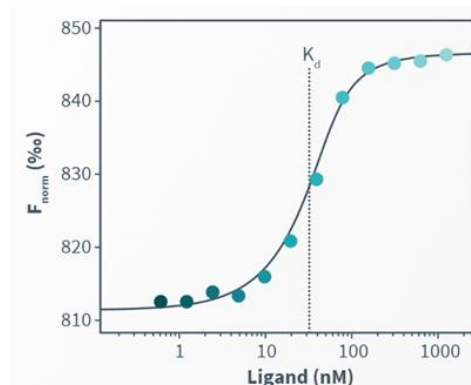
The second and yet our newest method in Dianthus; spectral shift in order to quantify a molecular interactions utilizes an experimental procedure during which a fluorescently labelled target generates a particular emission spectrum, and if a ligand binds to this target, the fluorophore's local chemical environment is changed, causing a shift in its fluorescence spectrum. This particular Monolith detector exploits this phenomenon by performing ratiometric measurements at two emission wavelengths of a labelled target in the presence of various concentrations of a ligand. In both of the detectors types which can be combined in the Dianthus instrument- the binding affinity is automatically determined at the end of each run without additional and lengthy data analysis. (Figure 1.).

Figure 1. The affinity constant (K_d) is calculated from a fitted curve (B) that plots normalized fluorescence (A) against concentration of ligand.

A)



B)



In this talk we will share with you the biophysical principles of both our methods harnessed in the new Dianthus, as well as a set of examples of successful characterization of challenging interactions with high-quality data, and actionable results from real-life samples some of which really challenging; containing aggregates, impurities, or precipitates, that reduce the time in assay development, towards obtaining highly reproducible data.

LECTURE ABSTRACTS

Session

“Biology of Extracellular Vesicles”

(part 1)

[L-10] Oncogenes, extracellular vesicles and cancer-associated vascular pathology

Janusz Rak

McGill University, Montreal, Canada

Oncogenic mutations play a defining role in progression of human cancers including aggressive brain tumours, such as glioblastoma (GBM). While the role of oncogenes has long been thought to be confined to intracellular processes of growth regulation and signalling, their non-cell autonomous influences have also emerged over the years as epitomised by vascular responses to changes in cancer cell secretome, such as angiogenesis, and non-angiogenic forms neovascularization. These extracellular changes include not only release of soluble growth factors, but also oncogene-driven shift the composition of the particulate secretome, including profiles of extracellular vesicles (EVs) and particles (EPs). EVs are membranous cellular fragments containing unique repertoires of lipids, proteins and nucleic acids that are reflective of the identity, state and function of their parental cells. Notably, we have observed that EV release from cancer cells enables transfer of oncogenic signalling activities from transformed cells to recipient cellular populations, such as endothelial cells, a process resulting in non-canonical vascular responses. In particular, mesenchymal subtype of glioma stem cells (GSCs) is associated with an EV-mediated release of oncogenic epidermal growth factor receptor (EGFR). These oncogenic EVs (oncosomes) interact with endothelial cells triggering a non-angiogenic vascular growth process that is resistant to classical anti-angiogenic agents. Conversely, EVs emitted by endothelial cells contain molecular effectors (including proteases) that modulate the phenotype of proneural GSCs, in which they trigger more mesenchymal and pro-invasive properties. Thus, oncogenic pathways and epigenetic regulatory circuits modify the reciprocal interaction between glioma stem cells and endothelium resulting in a modulation of vital biological processes involved in disease progression. Targeting these unique responses may open new avenues in treating GBM with agents that modulate tumour vasculature and stroma.

[L11] Tumor-derived extracellular vesicles in antitumor immunity and response to radiotherapy

Fabrice Lucien-Matteoni

Mayo Clinic, Rochester

Immune checkpoint blockade has revolutionized the therapeutic landscape of patients with metastatic cancer. However, despite remarkable outcomes in some patients, only a minority achieves complete and durable clinical response. To overcome this, combination of radiotherapy and immune checkpoint blockade has gained popularity to turn up the heat on cold tumors. While tumor irradiation can elicit an immunogenic cell death triggering tumor antigen presentation and T-cell priming, immune checkpoint blockade can enhance the expansion of tumor-reactive T cells which culminates with regression of distant non-irradiated metastases, referred as “abscopal effect”. The synergy of radiotherapy and immune checkpoint blockade has shown to improve oncological outcomes in some patients but rates of abscopal effect remain scarce which points toward uncharacterized immunosuppressive tumor-intrinsic mechanisms promoting local and systemic inhibition of antitumor immunity. Uncovering the molecular features that prevents a durable antitumor immune response is critically needed to accelerate the discovery of new therapeutic targets. Furthermore, there is an unmet need to develop non-invasive tools that can help predict which patients will benefit from radiotherapy alone from those who need combination therapies.

We investigated the clinical utility of tumor-derived extracellular vesicles (tdEVs) in two clinical trials evaluating the efficacy of radiotherapy in metastatic prostate cancer. Using high-resolution flow cytometry, we found that pre-treatment tdEV levels can predict risk of disease recurrence following radiotherapy. Furthermore, we found a negative correlation of blood concentrations of tdEVs and peripheral levels of tumor-reactive CD8 T cells suggesting that EVs can be a negative regulator of radiotherapy-associated antitumor immunity. Using a murine model of prostate cancer, inhibition of tdEVs combined with radiotherapy led to abscopal response. In vitro co-culture of tumor cells and CD8 T cells showed that tdEVs released upon irradiation dampen CD8 T cell cytotoxic function. Proteomic profiling of tumor cells identifies several immunomodulatory molecules dysregulated in response to radiotherapy. Altogether, our data demonstrate the immunosuppressive role of tdEVs on CD8 T cells which impairs with radiotherapy-induced antitumor immunity. Targeting immunosuppressive tdEVs is a valuable therapeutic strategy to boost antitumor immunity and increase rates of abscopal response in patients with advanced cancers.

[L-12] Extracellular vesicles from antigen-presenting cells boost TCR-signaling and effector gene expression in CD8+ T cells during viral infections

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CD8+ T cells are crucially essential to clear pathogens during the acute phase of viral infections. The pro-inflammatory conditions during infection concomitantly increase circulating extracellular vesicles (EVs) and we have found that especially CD8+ T cells interact with these EVs. However, if this EV – T cell interaction is only a epiphenomenon or can modulate CD8+ T cell immune responses is a matter of controversy. To address this chicken or egg dilemma, we used a new method to visualize cell-bound EVs and analyze EV-binding T cells in vivo. The present study shows that EVs increase transiently during viral infections and bind to activated but not naive CD8+ T cells. This EV-T cell-binding directly induces TCR signaling in antigen-specific T cells, causing increased nuclear translocation of nuclear factor of activated T cells cytoplasmic 1 (NFATc1) in vivo. Consequently, these EV-binding, but not EV-free CD8+ T cells, showed enriched gene expression signatures of proliferating effector T cells and reduced signatures of memory T cells. Our data show that EVs have particular adjuvanting effects on activated antigen-specific CD8+ T effector cells.

LECTURE ABSTRACTS

Session

“Biology of Extracellular Vesicles”
(part 2)

[L-13] Modulation of tumorigenic activity of neutrophils via tumor-derived EVs

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Neutrophils are newly recognized key players regulating immune responses and angiogenesis in cancer. Previously, we observe significant activation of tumor-associated neutrophils in HNC patients, such as elevated survival/ decreased apoptosis, elevated capacity to produce NETs, elevated pro-angiogenic capacity and significant immunoregulatory role. Moreover, we observe an increase of pro-tumoral neutrophil numbers, which is associated with an adverse prognosis of patients. However, the mechanisms regulating neutrophil function and activation in tumor are still not well understood.

Recently, exosomes (also known as small extracellular vesicles, sEVs) have come to the center of attention as potential signal transmitters between cells, also in tumor situation. Exosomes

are nano-sized particles ranging from 30-120 nm in size and are characterized by their vesicular shape in electron microscopy. We were interested if and how mechanistically sEVs influence neutrophil tumorigenic activity and which cargo plays an essential role in this process.

To address this, we first analyzed plasma exosomes isolated from patients at different stages of cancer disease and compared their composition to healthy EVs. We could identify several members of serpin family, which were significantly upregulated along tumor progression. Interestingly, serpins are known to inhibit neutrophil serine proteases that trigger neutrophil cell death pathways, resulting in the suppression of neutrophil apoptosis and NETosis, thus prolonging their survival. Importantly, we could also detect the presence of serpins in EVs isolated from HNC cell lines – once again demonstrating that the phenomenon that we observe in cancer patients (neutrophil pro-tumoral activation, prolonged survival, elevated NETosis) could be mediated by cancer-derived EVs.

To confirm the direct impact of these proteins on neutrophils, we educated neutrophils from HD (healthy donors) with tumor-derived EVs and assessed their survival and activation. In agreement with the content of EVs and corresponding to the situation observed *in vivo*, we observed similar changes in neutrophils: cell activation (downregulation of CD62L, increased expression of CD11b and CD66b), increased migratory capacity (upregulated CXCR2) and elevated ROS release. Moreover, we observed downregulated apoptosis and significantly prolonged survival of such neutrophils. It demonstrates that serpins that are contained in tumor-derived EVs efficiently modulate the activity and survival of neutrophils.

Summarizing, we suggest that knock-out of serpins in EVs or block of EVs/ neutrophil interaction could provide a therapeutic approach in cancer.

[L-14] Radiation-induced bystander effect mediated by exosomes involves the replication stress in recipient cells

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Exosomes released by irradiated cells mediate the radiation-induced bystander effect, which is manifested by DNA breaks detected in recipient cells; yet, the specific mechanism responsible for the generation of chromosome lesions remains unclear. In this study, naive FaDu head and neck cancer cells were stimulated with exosomes released by irradiated (a single 2 Gy dose) or mock-irradiated cells. Maximum accumulation of gamma H2A.X foci, a marker of DNA breaks, was detected after one hour of stimulation with exosomes from irradiated donors, the level of which was comparable to the one observed in directly irradiated cells (a weaker wave of the gamma H2A.X foci accumulation was also noted after 23 h of stimulation). Exosomes from irradiated cells, but not from control ones, activated two stress-induced protein kinases: ATM and ATR. Noteworthy is that while direct irradiation activated only ATM, both ATM and ATR were activated by two factors known to induce the replication stress: hydroxyurea and camptothecin (with subsequent phosphorylation of gamma H2A.X). One hour of stimulation with exosomes from irradiated cells suppressed DNA synthesis

in recipient cells and resulted in the subsequent nuclear accumulation of RNA:DNA hybrids, which is an indicator of impaired replication. Interestingly, the abovementioned effects were observed before a substantial internalization of exosomes, which may suggest a receptor-mediated mechanism. It was observed that after one hour of stimulation with exosomes from irradiated donors, phosphorylation of several nuclear proteins, including replication factors and regulators of heterochromatin remodeling as well as components of multiple intracellular signaling pathways increased. Hence, we concluded that the bystander effect mediated by exosomes released from irradiated cells involves the replication stress in recipient cells.

[L-15] Metabolomic and proteomic profiling of serum exosomes from cancer patients treated by radiotherapy

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Institute of Bioorganic Chemistry Polish Academy of Sciences, Poznań, Poland

The search for new potential cancer biomarkers, including proteins and metabolites, is increasingly carried out with the use of modern multi-omics tools. In recent years, there has also been increased interest in the possibility of using exosomes as a source of cancer biomarkers. Here we were looking for molecular components related to the progression of cancer and the response to treatment, with particular emphasis on the participation of exosomes modulating these processes. The developed methodology for conducting comprehensive proteomic and metabolomic analyzes of exosomes isolated from serum, using mass spectrometry techniques, made it possible to conduct appropriate experiments on serum samples of patients diagnosed with head and neck cancer and locally advanced rectal cancer treated with ionizing radiation. We have shown that the metabolomic profile of exosomes isolated from serum in relation to whole serum is different in head and neck cancer after radiotherapy. However, cancer-specific features of energy metabolism could be detected in both types of samples. Moreover, we have shown that the molecular composition of exosomes isolated from serum can be used to predict the response to neoadjuvant radiotherapy in rectal cancer. Integration of metabolomic and proteomic data, which enables the identification of disrupted metabolic pathways and signaling processes connected with neoplastic disease and response to radiation therapy, presents a modern look at the analysis of the global response to cancer treatment from the level of systems biology.

Funding: This study was supported by the National Science Centre, Poland, Grant 2017/26/D/NZ2/00964

[L-16] Proteomic and metabolomic profiles of T cell-derived exosomes isolated from human plasma

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Exosomes (small extracellular vesicles - sEV) sized between 30-150 nm are produced by all types of cells, including cancer cells. They are present in all body fluids, including plasma, urine, breast milk, saliva, bile, amniotic fluid etc. The molecular content of sEV reflects the nature of parental cells. However, sEV of different cellular origins may carry molecules that are cell-type specific and health-condition specific. That is why molecular profile of exosomes may serve as liquid biopsy. sEV are essential in intercellular signaling and communication, which impact the physiological balance and homeostasis of human body, e.g. tumor-derived exosomes play a key role in tumor-induced suppression of immune effector cells and in the promotion of tumor growth; exosomes released by T cells are key messengers between normal tissue cells, malignant tumor cells and the immune system cells. Understanding of sEV different subsets biology and functions is difficult as extracellular vesicles circulating in patients' plasma are a heterogeneous mix of sEV originating from multiple tissues, including immune cells. Isolation of T cell-derived exosomes may bring substantial knowledge about the crosstalk between immune cells and tissue-resident normal or pathologically altered cells.

Blood samples were obtained from 10 consented healthy donors (HDs) (IRB approval #04-001). Total exosomes were isolated by the mini-SEC method. Fraction #4 containing the majority of exosomes was separated into T cell-derived exosomes (CD3(+) exo) and not T cell-derived exosomes (CD3(-) exo) fractions using the immunoaffinity capture method with anti-CD3 mAbs, which recognizes an epitope selectively expressed only on T cell receptor-positive (TCR+) T cells. LC-MS/MS analysis of tryptic peptides was performed using the Dionex UltiMate 3000 RSLC nanoLC System coupled with the Q Exactive Plus Orbitrap mass spectrometer (Thermo Fisher Scientific). Using a shotgun proteomics approach, 418 proteins were identified. We found 36 sEV proteins upregulated in CD3(+)exo and 56 sEV proteins upregulated in CD3(-)exo. As the next step, we identified biological functions/processes associated with differentially expressed proteins upregulated in either CD3(+)exo or CD3(-)exo fractions. We found that among the most abundant subsets of proteins upregulated in either the CD3(+)exo or CD3(-)exo fractions were proteins associated with immune-related processes and response to stress. It is noteworthy, however, that immunity-related proteins upregulated in CD3(+)exo and CD3(-)exo fractions of plasma sEV were associated with different types of immune cells (with leukocytes and neutrophils respectively). Hence, functions associated with proteins upregulated in the two analyzed fractions of plasma exosomes confirmed their origin from T lymphocytes carrying CD3 antigen (CD3(+)exo fraction) and other types of cells (CD3(-)exo fraction) including platelets and neutrophils. Importantly, this discrimination of exosome subsets in plasma of HDs provides a basis for future investigations of CD3(+) exosomes in plasma of patients with pathological conditions, including autoimmune diseases or cancer.

This research was funded by the National Science Centre, Poland, grant number 2016/22/M/NZ5/00667 and in part by NIH grants number R01-CA168628 and U01-DE029759.

[L-17] In vitro and in vivo neutrophil models overview – an examination of advantages and disadvantages for neutrophil-derived small extracellular vesicles production

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Introduction: Neutrophils are the part of the innate immune responses, as well as play an important role in the regulation of tumor progression and metastasis. These functions may involve small extracellular vesicles (sEVs) which facilitate cell-cell communication. Studying neutrophil-derived sEVs necessitates a proper model selection. This work provides an overview of the available neutrophil models along with their advantages and disadvantages in the context of sEV production.

Methods: Human HI60, murine 32D and HoxB8 cell lines were differentiated following published protocols[1–3]. Human polymorphonuclear neutrophils (PMNs) were isolated from peripheral blood of healthy donors by density gradient centrifugation. Obtained PMNs were cultured in standard medium (N0) for 24h or in medium with N1/N2 polarization cocktail containing pan-caspase inhibitor for 48h. Granularity of all neutrophil populations was assessed by May-Grünwald-Giemsa (MGG) staining. Cells with segmented nuclei were quantified, neutrophil-specific marker expression and viability were determined by flow cytometry. sEVs were isolated by size exclusion chromatography and their protein content was determined by BCA assay.

Results: Published protocols for HI60 and 32D cell lines resulted with poor quantity of cells with segmented or lobed nuclei, as assessed by MGG staining. After 5 days of culture in differentiation medium, 60% of HoxB8 cells contained ring-shaped nuclei indicative of murine neutrophils. Compared to undifferentiated HoxB8 culture, expression of CD11b and Ly6G was respectively 1.9 and 4.4 times higher ($p < 0.05$), but no change in monocyte-specific Ly6C expression was observed. Isolation of human PMNs yielded 96% of non-eosinophilic cells with segmented nuclei. Presence of pan-caspase inhibitor allowed their 48h culture where 89% of population was viable. Expression of FasR and CD54 receptors, for neutrophils polarized to the anti-tumor N1 phenotype, was upregulated 3.5- and 6.8-fold compared to the N0 group ($p < 0.05$) and 1.8- and 2.1-fold compared to the N2 group ($p < 0.05$), however no difference in expression was detected for the CXCR2 protein.

Conclusions: In this study, selected neutrophil models for sEV production were compared. In vitro models allowed scalability and thus controllable sEV output, but depending on the cell line, part of the population was not differentiated. On the contrary, in vivo models allowed high population purity, although obtaining acceptable sEV yield proved difficult and necessitated model alterations. Since the use of primary cells is often technically challenging, it should be noted that the in vitro HoxB8 model produced best results when differentiation efficiency and sEV yield were compared to other cell lines.

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LECTURE ABSTRACTS

Session

**“Oncology Meets Physics, Mathematics
and Numerical Methods”**

(part 1)

[L-18] Modeling and simulation of cancer evolution in single cells

Khanh Ngoc Dinh

Rice University, Houston, Texas, USA

Recent advances in single-cell whole genome sequencing enable profiling of copy number aberrations at high resolution in thousands of cells. Single-cell genomics data from these technologies has enabled quantitative measurements of tumor dynamics, and measurements of the rate of chromosomal aneuploidy, whole-genome duplications and replication errors in tumors. We have developed a simulation algorithm for studying single-cell dynamics in a population of cells, incorporating somatic copy number changes, clonal selection of driver mutations and accumulation of neutral passenger mutations. The simulator follows population dynamics as input by the user, generates the clonal evolution forward in time, where clones are defined by their copy number and driver mutation profiles. The phylogeny of a sample is then computed backward in time. The algorithm is designed to be efficient for large cell populations while maintaining statistical accuracy.

We present several examples from the simulator package. The first is an application in testing the performance of copy number callers in single cells. The second investigates the evolution of high-grade serous ovarian cancer (HGSOC) driven by genomic instability. The simulator may also be used to calibrate clonal reconstruction algorithms used on single-cell DNA sequencing data.

[L-19] Mathematical Challenges Arising In Cancer Modelling

Philip Maini

Wolfson Centre for Mathematical Biology, Oxford, Great Britain

In this talk, I will ask the question: How can we develop a macroscopic model from microscopic properties? The motivation here will be tumour-induced angiogenesis where, starting from a simple hybrid cellular automaton model for individual cell behaviour, we systematically derive a coupled nonlinear partial differential equation model for cell density. We find that this model is different to the classical phenomenologically-derived snail-trail model and we compare and contrast both models. I will then briefly discuss the mathematical challenges arising from cell-invasion models with a degenerate density-dependent cross-diffusion term.

[L-20] Darwinian evolution in spatial models of cancer

Bartłomiej Waclaw

University of Edinburgh, Edinburgh, Great Britain

Mathematical modelling of cancer has a long history but it traditionally focused on replicating growth laws observed for different tumour types, the role of angiogenesis, or predicting the outcome of chemotherapy. Recently, advances in genomics have made it possible to investigate Darwinian evolution in populations of cancer cells. This has opened up many interesting questions. In particular, as the cancerous tumour grows, cells accumulate further mutations. Are these mutations neutral “passengers”(i.e. they do not change the net growth rate) or are some of them “driver mutations” that increase the growth rate? Is there evidence of selection acting on certain traits of cancer cells? How genetically diverse a typical tumour is? How is evolution affected by the spatial structure of the tumour? In this talk I will discuss how computer models can be used to shed light on these questions. I will show how different processes: replication, death, migration, and mechanical interactions between cells in a tumour affect its structure and genetic composition. I will also discuss how different model compare to experimental data, and their implications for cancer therapy.

[L-21] Some aspects of mathematical modelling of cell cycle

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Modeling of cell cycle is one of the fundamental subject of mathematical biology because it could help to solve such problems as synchronization of cell division in cancer therapy and allows to understand dynamics of growth of cellular populations (e.g. tissues). There are many different models of cell cycles - some of them are based on application of semigroups of operators. In my talk I am going to give a short introduction to this subject, in particular, I will briefly discuss the models that I have studied: a model given by partial differential equations of the first order with time delay and space variable retardation [1], and a model described by a piecewise deterministic process [2]. The main subject of my talk will be a new age-size structured model based on the cell cycle length [3]. The model is described by a first order partial differential equation with initial-boundary conditions, which leads to a positive semigroup on some L^1 space. We establish new criteria for an asynchronous exponential growth of solutions to such equations. A discussion on the generalizations of the model will be a good excuse to present some new challenges in cell cycle modelling. We also compare some popular hypotheses regarding the cell cycle with theoretical results and with existing data.

References

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[L-22] Individualized and personalized models in biology and medicine

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The problem of mathematical modeling for a set of objects, for example patients undergoing medical treatment, will be presented. On the one hand, in such a case it is possible to build one common model. This approach is most often used in statistics, where a model is built based on a sample taken from the population and applied to any element of the population. On the other hand, it is possible to build separate models for each of the objects. Both opposite approaches have advantages and disadvantages. For example, in the case of a small data set, creating separate models may lead to numerically ill-conditioned problems with parameter estimation.

An individualized compromise approach, free from the main drawbacks of the above-mentioned approaches, will be presented. In this approach, some parameters are estimated separately for each object, and the remaining common parameters are estimated on the basis of the aggregated data set for the entire cohort.

The advantages of the proposed approach will be illustrated by modeling the development of the Covid-19 pandemic for a set of European countries. Thanks to the individualized approach, it is possible to answer the question which government interventions are effective in fighting the development of the pandemic.

Literature

Wilk, A. M., Łakomic, K., Psiuk-Maksymowicz, K. et al. Impact of government policies on the COVID-19 pandemic unraveled by mathematical modelling. *Sci Rep* 12, 16987 (2022). <https://doi.org/10.1038/s41598-022-21126-2>

[L-23] Mathematical modelling and analysis to search for molecular targets for therapy

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As experimental techniques improve and the number of experiments carried out increases each year, our knowledge of how cells, tissues and whole organisms work is growing. Unfortunately, with it comes an increasing number of factors that need to be taken into account when considering observed disturbances in the normal functioning of biological systems that lead to disease states. This is where mathematical modelling and model analysis begin to play their part, which can help to structure biological knowledge and understand observed phenomena.

One of the key issues in analysing disease states and considering potential therapies, is the search for potential molecular targets for therapy. Here, too, biomedical engineering with its methods can assist experimental researchers.

Here, one possible method for the sensitivity analysis of mathematical models will be presented, which, in contrast to the commonly used methods aimed at acquiring the overall picture,

is directed at the search for molecular targets for therapy. It will be shown that the method leads to biologically relevant conclusions, identifying processes suitable for targeted pharmacological inhibition, represented by the reduction of kinetic parameter values. That, in turn, facilitates subsequent search for active drug components.

LECTURE ABSTRACTS

Session

**“Oncology Meets Physics, Mathematics
and Numerical Methods”**

(part 2)

[L-24] Whole genome sequencing - beyond driver mutation discovery

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Whole genome sequencing (WGS) is becoming increasingly important component of oncological care, providing invaluable information used to develop personalized therapies, through machine learning methods. Studies focusing on individual cancer driving variants are severely limited by the detection sensitivity of currently used methods, and by our understanding of the processes involved in carcinogenesis, which can lead to similar outcomes through seemingly unrelated genomic alterations. For this reason methods that focus not only on the driver mutations but also their impact on genomic stability, manifested by specific variant signatures, are becoming increasingly important predictors used to develop personalized treatment solutions.

In this talk I will focus on challenges associated with the development of genomic classifiers and describe successful solutions, which are based on the study of mutation patterns. I will also show that through the study of somatic mutation patterns in cancer cells it is possible to gain invaluable insights into the mechanisms utilized in normally functioning human cells.

This work was partially supported by the Polish National Science Centre grant 2018/29/B/ST7/02550 and Silesian University of Technology statutory research funds.

[L-25] On the use of nonlinear methods of patch-clamp data analysis to unravel the unical features of the BK channel gating in human glioblastoma cells

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Introduction: Glioblastoma multiforme (GBM) is a malignant type of cancer arising from glial cells. It turns out that the available therapies (surgery, chemotherapy, radiotherapy, and even immunotherapy) have still low efficiency in case of GBM. In the search of a novel drug target, we investigate the activity of the large-conductance voltage- and Ca²⁺-activated K⁺ channels in glioblastoma cells (gBK). The gBK channels regulate the cancer cells' growth and extensive migration, which makes them a promising candidate for future therapies.

Methods: The single- and multichannel patch-clamp recordings on U-87 MG cells and controls (rat hippocampal cells, human dermal fibroblasts and endothelium) were obtained using operational amplifier (Axopatch 200B, Axon Instruments) and converter (Clampex 11, Axon Instruments). The experiments were performed in mitoplast-attached (to study mitochondrial channels) and inside-out modes (analysis of channels from cell membrane). The signals were sampled at 10 kHz and low-pass at 1 kHz. Symmetrical solution was used in our experiments, which included 130 mM potassium gluconate, 5 mM KCl, 10 mM HEPES, 2 mM MgCl₂, 20 μM EGTA, and CaCl₂ at different concentrations ranging from 20 μM to 200 μM. The membrane potential was fixed during each recording (-60 mV to 60 mV; 20 mV step). Each experimental trace was analyzed by standard non-linear methods and the nonlinear ones.

Results: The kinetic analysis revealed increased sensitivity of gBK channels to Ca²⁺ as well as the Ca²⁺-and voltage-dependent cooperative open-reinforcing behavior within the clusters of gBK channels, which was not observed in control data. The nonlinear analysis including: the sample entropy, multiscale entropy, Hurst exponent, detrended fluctuation analysis, empirical mode decomposition and cross-correlation dwell-times' analysis indicated that the gBK channels gating exhibits unique features, which allow us to discern its conformational dynamics from the other BK channel isoforms.

Conclusions/Novel aspect: Our results show that the gating dynamics of the gBK channels exhibit specific characteristics, which suggests that this channel isoform should differ from the other ones by the number of stable conformations as well the switching kinetics between them. Moreover, the gBK channels can strongly cooperate and stabilize their conducting states, if only they are located sufficiently close to each other within the membrane. These features can deeply affect the physiological meaning of gBK channels. Possibility of the regulation of the gBK channel gating and cooperation can indicate a new direction in GBM-related research.

Keywords: patch-clamp; BK channels; glioblastoma; cooperative channels; calcium sensitivity

* presenting author,

LECTURE ABSTRACTS

Session

“Modifications of Nucleic Acids and Cancer”

[L-26] Epigenetics of a molecular clock in cancer

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Circadian clocks are under genetic and epigenetic regulation. Recent findings have greatly increased our understanding of epigenetic plasticity governed by circadian clock. Significant findings interlocking circadian clock, epigenetics and cancer have been revealed, particularly in breast, colorectal cancer and blood cancer. We observed that methylation of clock gene genes may affect the circadian gene expression level in tissues derived from the mammary gland of breast cancer patients, especially observed in more aggressive breast cancer tumors (G3) and those lacking ER such as Basal-like or HER2-enriched. Aberrant methylation of circadian genes promoter regions may play an important role (individually or as a result of interactions) in the breast cancer ethiology and disease process, including recurrence and progression.

[L-27] Increasing significance of methylation biomarkers in clinical cancer management

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In general terms epigenetic mechanisms of gene expression regulation alter gene expression without changing primary gene sequence. There is a number of epigenetic mechanisms that orchestrate gene expression and render cell phenotype and malfunctioning of those mechanisms leads to disease such as neoplastic transformation. In principle each of those mechanisms can be targeted by treatment or a become source of biomarkers. However, currently the most compiling research evidence indicates that changes of DNA methylation can be effectively utilized as biomarkers at all stages of the clinical disease management: from risk assessment through early diagnosis and treatment personalization to post treatment surveillance. Specifically, one of the largest clinical trials have recently shown that methylation biomarker-based cancer detection in liquid biopsy allows to detect cancer in patients without clinical symptoms with remarkable specificity and sensitivity. Moreover, detectable in blood methylation of *BRCA1* gene has been proposed as biomarker of predisposition to breast and ovarian cancers. And methylation of *MGMT* gene has long been used to guide treatment of glioblastoma multiforme patients.

With those developments methylation biomarkers can significantly contribute and change cancer management as well as play a significant part in personalized medicine. Thus, in my talk I will review current applications of the methylation biomarkers in clinical disease management.

[L-28] N6-methyladenine metabolism – potential relationships with leukemia development

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Epigenetic modifications, such as DNA methylation and histone modification, alter DNA accessibility and chromatin structure, thereby regulating patterns of gene expression. The best characterized DNA epigenetic modification in mammals is methylation of the cytosine, which forms the 5-methylcytosine (5-mCyt). Methylation can also occur on the other nucleotides. Methylation of deoxyadenosines has been identified and is a well-described epigenetic feature in prokaryotes. Formation of N6-methyl-2'-deoxyadenosine (N6-mdA) plays role in various biological pathways in bacteria: host-pathogen interactions, DNA replication, nucleotide segregation, mismatch repair, transcription and translation. DNA N6-mdA modification used to be considered as absent in eukaryotes including humans since it was not detectable in earlier generations of studies. Recently N6-mdA was found to be present in lower eukaryotes, vertebrates and mammals. Few recent studies reported presence of this modification in human tissue. Aberrant epigenetic landscapes promote tumor initiation and progression. The focus of cancer research has been on the global and local aberrations of 5-mCyt levels. Genome wide DNA 5-mCyt hypomethylation and promoter specific 5-mCyt hypermethylation may contribute to cancer formation and these alterations coexist in most, if not all, human cancers. The role of other methylated nucleobases in cancer remain obscure. Only few studies analyzed N6-mdA levels in human cancer. The N6-mdA level was significantly decreased in gastric, liver, lung and triple negative breast cancers tissues compared to normal tissues. Interestingly, the opposite tendencies were observed in case of esophageal squamous cell carcinoma, hepatocellular carcinoma, and glioblastoma where cancer tissues were characterized by higher levels of N6-mdA. However latest study showed that the content of this modification significantly decreases in glioma compared with normal brain tissues. Another characteristic feature of malignant cells is a profound decrease in level of 5-hydroxymethylcytosine (5-hmCyt), product of 5-mCyt demethylation. Interestingly, the recently published study reports the presence of N6-(hydroxymethyl)-2'-deoxyadenosine (N6-hmdA) in mammalian DNA. The level of this modification was significantly increased in lung carcinoma tissues compared to tumor-adjacent normal tissues. We hypothesize that this modifications in cancer may undergo similar changes to those observed with 5-mCyt and its oxidation products. For this reason, N6-methyladenine and its derivatives may play a role in leukemia development. In order to determine the abovementioned modifications in nucleic acids we will use a highly advanced technique, namely automatic online two-dimensional ultra-high performance liquid chromatography with tandem mass spectrometry (2D-UPLC-MS/MS). This method allows us reliable quantification of N6-methyladenine and its derivatives in material from patients with leukemias. We tested the content of N6-mdA in leukocytes from the patient with CLL and healthy controls (Fig. 4). The level of this modification was significantly lower in CLL patients than in controls. Moreover, we were able to determine the level of N6-hmdA in DNA from leukocytes from patients with CLL and AML. The results of our study may contribute to better understanding of adenine methylation/demethylation processes and the role of N6-methyladenine and its derivatives in cancer development.

[L29] New diagnostic opportunities in oncology based on microextraction methods

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Detection of alterations at the genetic level is widely used in cancer diagnosis and therapy. Although, it does not give full information about cancer biology so there is a need to enrich diagnostic and therapeutic protocols with new approaches. Thus, many developments were related to the methods based on the detection of small molecules characterizing current biochemical phenotypes - lipidomics and metabolomics. They were used to profile tumors to find their biomarkers, explain their origin and growth, and identify the associated mechanisms of drug resistance. Traditional analytical methods, however, involve sample-, time- and labor-consuming procedures, which makes them unsuitable for intraoperative testing. Thus, solid-phase microextraction (SPME), also called a chemical biopsy, was introduced as an alternative sampling and sample preparation methodology. SPME probes enable low invasive and simple sampling without time- and labor-consuming protocols.

SPME probe consists of the solid support (mesh, blade, fiber) coated with sorbent particles. Before its utilization, SPME probe requires activation of coating and sterilization if *in vivo* animal or human studies are considered. For sampling, SPME device is inserted directly into the sample to extract analytes. In the next step, it is quickly rinsed in water to remove potential cell debris, blood constituents, etc. which can be loosely attached to the surface. Following that, the probe is ready for transport, storage, or analysis. The analytes extracted by the sorbent can be desorbed with a mixture of solvents and the obtained extract can be introduced to the analytical platform.

The presented methodology was applied in a wide range of oncological studies. Brain tumors with various histological types (meningiomas and gliomas), grades, and genetic changes (such as IDH, 1p/19q co-deletion, or NF) were characterized based on their metabolomic and lipidomic profile. It was also reported that the level of selected drugs used in the treatment of neoplasms can be assessed along the metabolomic profiling of sampled tissue. For instance, the spatial and temporal distribution of doxorubicin was studied during *in-vivo* lung chemo-perfusion.

To sum up, a chemical biopsy can be applied in oncological studies in several ways; one of them is profiling of chemical composition of the studied tissue and searching for potential biomarkers. Another approach is related to the rapid determination of the level of selected substances with intra-operative diagnosis as the final goal.

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[L-30] Vitamin C as a key regulator of the active DNA demethylation process

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Introduction: Vitamin C is a potent antioxidant and a crucial co-factor of Fe²⁺ and alpha-ketoglutarate dependent dioxygenases such as family of Jumonji histone demethylases and TET (ten-eleven-translocation) family proteins involved in active DNA demethylation. TET enzymes (TET1, TET2 and TET3) are able to oxidize 5-methylcytosine to 5-hydroxymethylcytosine (5-hmCyt) and subsequently to 5-formylcytosine (5-fCyt) and 5-carboxycytosine (5-caCyt) - modifications involved in epigenetic regulation of gene expression. After each step of oxidation, catalytic Fe ion in TET's active center must be restored to 2+ state by vitamin C, thus its optimal cellular concentration is crucial for proper functioning of epigenetic mechanisms. Therefore, in many studies involving epigenetic make-up, gene expression, cell maturation and differentiation supplementation of the vitamin C in physiological concentrations seems to be indispensable for obtaining most accurate and reliable data. Despite that fact, vitamin C is not routinely added to the culture medium, mostly because ascorbic acid in aqueous solutions is unstable, quickly degrades and may change pH of culture medium. Therefore, observations from experiments without vitamin C supplementation describe cells in a state of profound malnutrition, and not in their physiological state. Moreover, in many cells the effect of vitamin C supplementation may be affected by abnormalities in the functioning of vitamin C transporters. Here we show results of experiments providing the rationale behind necessity of vitamin C supplementation in epigenetic research.

Methodology: Cancer cell lines A549, CA46, HCT116, HAP1, MDA231 K562, RAJI, PC-3 were cultured in standard conditions and exposed to vitamin C in different concentrations (10-1000 µM) for 24 hours. DNA from harvested cells was isolated by phenol extraction and subsequently enzymatically hydrolyzed to obtain nucleosides. The modifications in cellular DNA - were measured by two dimensional ultraperformance liquid chromatography with tandem mass spectrometry (2D-UPLC MS/MS).

Results: In all cell lines significant and dose dependent increase in the levels of 5-hmCyt, 5-fCyt and 5-caCyt was observed after incubation with vitamin C. In HAP1 cells a gradual increase in the level of 5-hmCyt, 5-fCyt and 5-caCyt (peak between 18 and 24 hours) was observed, strongly and statistically significantly correlating with the intracellular concentration of vitamin C.

Conclusions: The activity of TET proteins, and thus also of other intracellular dioxygenases, is strongly dependent on the intracellular concentration of vitamin C. Supplementation of cells with physiological concentrations of vitamin C affects not only the activity of these enzymes, but also enables the proper functioning of the cell epigenome and therefore should be routinely used in epigenetic research. Liposomal form of vitamin C stimulate activity of TET proteins in the same manner as a free ascorbic acid, but is more stable in cell culture conditions. Taking this into consideration, liposomal vitamin C seems to be a better alternative for vitamin C supplementation, as it is as efficient as ascorbic acid in stimulating enzymatic activity of TET proteins (and potentially other dioxygenases), is well soluble in aqueous solutions and does not change the pH of the culture medium. Moreover, due to passive transport of liposomes to the cells, effect of liposomal vitamin C supplementation is potentially unaffected by the expression patterns or mutational status of vitamin C transporters.

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POSTER ABSTRACTS
(alphabetically, by first author name)

[P1] Conducting polymer-based antibacterial surfaces decorated with silver and gold particles

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Progress of contemporary research on electrotherapy implies the development of materials, which not only have a healing and biocompatible effect, but also antibacterial properties. The most serious complications after implantation are implant-associated infections, which can be fatal for the patients. The aim of this study was to engineer a surface modification, using conducting polymer (PEDOT, poly(3,4-ethylenedioxythiophene)) decorated with gold and silver particles. The resulting PEDOT-Au/Ag and PEDOT-Ag surfaces exhibited the strongest toxicity against *Escherichia coli* as confirmed by LIVE/DEAD® studies. The antibacterial properties were associated with surface roughness and the presence of silver, which is widely known as an antibacterial agent. Besides, developed surfaces are promising materials to prevent implant-associated infections, particularly those occurring on the surface of bioelectronic devices.

[P2] Design of transdermal polymeric drug carriers - analysis of diffusion through the membrane and initial biological properties

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Introduction The aim of the study was to obtain nanoparticles based on polymer-drug conjugates of methotrexate (MTX), examine their diffusion through the membrane that imitate the human skin and determine their toxicity. The drug carriers were based on star-shaped copolymer of *N, N*-dimethylaminoethyl methacrylate and 2-hydroxyethyl methacrylate (G-MTX) and its miktoarm analogue with an additional biodegradable poly(D, L-lactide-*co*-glycolide) arm (M-MTX).

Methods Drug conjugates were prepared by nanoprecipitation. Hydrodynamic dimeters (Dh) of nanoparticles were determined by DLS measurements using Microtracs Nanotracs Flex. SEM analysis was made with Volumescape 2 microscope. Transdermal diffusion test were carried out in vertical diffusion cells Phoenix DB-6 at 32.5 °C using PBS pH 7.4 or pH 5.4 as a medium. Measurement of the light absorption of the samples were made on UV-VIS Thermo Scientific Evolution 300 spectrometer. Determination of mitochondrial activity of colon cancer cells (HCT-116) treated with polymeric nanoprodugs was performed using the MTT assay.

Results The mean Dh values of the obtained nanoparticles ranged from 258 to 418 nm. SEM images showed that mainly nanospheres of nanoprodugs were obtained but some nanodiscs and aggregates were also formed. The optimal conditions of MTX and M-MTX diffusion through membrane were in PBS solution pH 7.4. However, for G-MTX conjugate no passage through the membrane was observed. Interestingly, MTX without the carrier did not pass through the membrane until 24 hours after the start of the experiment, whereas MTX released from M-MTX started to pass through the membrane after 48 hours. The maximum amount of pure MTX which passed through the membrane after 168 h was 1,2%, whereas maximum amount of MTX released from M-MTX at the same time was equal 2,8%. The calculated IC₅₀ value for G-MTX conjugate nanoparticles was equal 156 µg/ml in comparison to the pure methotrexate (IC₅₀ = 169 µg/ml).

Conclusions The efficiency of the drug and the nanoprodug transport strongly depended on the environment in which the given compounds were dissolved. In the case of polymer-drug conjugates, it was noticed that the presence of the polyester arm had a significant impact on the transport of nanocarriers across the membrane under the same conditions. Cytotoxicity studies have shown that nanoprodugs had lower IC₅₀ value compared to MTX without a carrier.

Keywords: polymeric DDS, psoriasis, vertical diffusion cells

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[P3] How is SLAMF7 regulated by p53?

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Introduction p53 protein is best known as the „ guardian of the genome”. Our earlier research has shown that p53 protein regulated some genes involved in immunity. An interesting protein engaged in innate immunity is SLAMF7. This protein is present on natural killer cells (NK cell), but also on cancer cells. The interaction SLAMF7-SLAMF7 on cancer cells and NK cells, can facilitate the destruction of neoplastic cells. SLAMF7 protein is used as a target in the immunotherapy because it is highly expressed in multiply myeloma cells, and the monoclonal antibody against SLAMF7, Elotuzumab, can mark them for destruction. In our project we are focusing on how SLAMF7 is regulated by p53.

Methods We employed four human cancer cell lines: A549 (non-small cell lung cancer cell line) U-2OS (osteosarcoma cancer cell line), NCI-H1299 (non-small cell lung carcinoma cell line) and NCI-H292 (human lung mucoepidermoid carcinoma cell line). In each cell line p53 was activated by combination of actinomycin D and nutlin-3a (A+N). The camptothecin (CPT) was a positive control and a negative control was dimethyl sulfoxide (DMSO), which is the solvent for the above-mentioned substance. Moreover, we used p53-deficient cells (and controls) generated by CRISPR/Cas9 technology. The expression of SLAMF7 was determined by Western blotting or qRT-PCR. Moreover, we cloned regulatory fragment of *SLAMF7* gene (putative enhancer) in reporter vector and tested its sensitivity to p53 by luciferase reporter tests.

Results Activation of the *SLAMF7* gene is mediated by p53. We observed the weakening of *SLAMF7* activation in p53-deficient cells. In addition, we observed that one of the form of the SLAMF7 protein (50 kDa), is secreted into the extracellular space. The reporter tests demonstrated that cloned regulatory region of SLAMF7 is activated by p53. Thus, *SLAMF7* is the gene directly regulated by p53. Unexpectedly, we found that paclitaxel can also stimulate the expression of *SLAMF7*, however this activation is not governed by p53.

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[P4] eDAVE - extension of GDC Data Analysis, Visualization, and Exploration Tools – a new platform for integrated methylomics and transcriptomics data analysis

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Rationale Increasing amounts of methylomics and transcriptomics data are currently being generated and deposited in publicly available repositories. These datasets are a valuable source of information that can be utilized to perform exploratory data analysis as well as to validate the results of independent studies. However, deposited datasets are frequently unstructured and poorly integrated. That makes mining of those datasets challenging for researchers without bioinformatics expertise and appropriate IT infrastructure. To address these challenges, we developed eDAVE (available from: <https://edave.pum.edu.pl/>), which allows for analysis of over 11000 whole methylome and transcriptome profiles for around 180 tissues and cancer types.

Methods Python environment was used to develop web app as well as scripts integrating datasets from Genomic Data Commons repository. The platform uses univariate analysis (an appropriate statistical test is selected on the basis of the data distribution) to identify differentially methylated positions (DMPs) or differentially expressed genes (DEGs) between datasets of interest. Multivariate analysis (designed for simultaneous analysis of plurality of CpGs or genes) for selected groups of compared samples is performed using decompositions algorithms (such as: PCA - principal component analysis or t-SNE - t-distributed stochastic neighbour embedding). Optimal number of clusters in specific comparison is defined using Calinski-Harabasz clustering efficiency metric. Analysis of interaction between CpG methylation and gene expression is performed using linear or non-linear regression models.

Results The eDAVE functionalities allows for:

- identification of DMPs/DEGs between groups of samples;
- one-dimensional statistical analysis of methylation or expression levels across groups of samples;
- multi-dimensional cluster analysis based on set of genes or CpGs;
- analysis of association between methylation of specific CpG site and gene expression.

Conclusions In summary, we have developed a user-friendly interface that allows a user without prior IT expertise to perform exploratory research as well as validation of the results of independent studies using large number of independent transcriptomics and methylomics datasets. Moreover, this tool is useful for fast assessment of specificity of methylation and expression biomarker candidates.

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[P5] Molecular abnormalities in unresectable soft tissue sarcomas

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Introduction: Soft tissue sarcomas (STS) are rare, mesenchymal neoplasms. The biology and molecular profile of STS remain poorly understood. The study aimed to assess molecular abnormalities in sarcomas that may be a potential diagnostic or predictive biomarkers in STS.

Methods: We analysed preoperative core biopsy samples of 19 patients who participated in the phase II clinical trial (NCT03651375). Enrolled patients included 10 patients with undifferentiated pleomorphic sarcoma (UPS), 1 patient with leiomyosarcoma (LMS), 5 patients with myxofibrosarcoma (MFS), 1 patient with pleomorphic liposarcoma (PLPS), 1 patient with dedifferentiated liposarcoma (DDLPS), 1 patient with malignant peripheral nerve sheath tumor (MPNST). Patients were treated 5×5 Gy radiotherapy combined with 3 cycles of doxorubicin-ifosfamide chemotherapy. For Next-Generation Sequencing (NGS) Illumina NGS TruSight Oncology 500 kit was used. The response to neoadjuvant therapy was assessed using the European Organization for Research and Treatment of Cancer-Soft Tissue and Bone Sarcoma Group (EORTC-STBSG) recommendations. For mutation pathological prediction the VarSome genome browser was used.

Results: In the analysed cohort an average of 8 **mutations** per patient were reported. Genes encoding proteins involved in cell cycle, apoptosis, and DNA repair were mutated. Interestingly promoter of *TERT* gene was mutated in UPS.

High-level **amplification** with ≥ 6 copy number of *MDM2*, *CDK4*, *CCNE1* genes were identified in 8 cases. At the same time 1 to 5 amplified copies of *MYC*, *EGFR*, *FGFR1*, *FGF10*, *PDGFRA*, *PIK3CA*, *PIK3CB*, *RICTOR*, *JAK2*, *BRAF*, *KRAS*, *NRAS*, *CDK6*, and *MET* were also found in selected cases. Among these amplifications of the *MYC*, *FGFR1*, *RICTOR*, *JAK2*, *FGF10*, and *NRAS* genes were identified only in UPS patients

Loss of the *BRCA2*, *TFRC*, *EGFR*, *NRG1*, *FGF6*, and *CCND1* genes were found in four UPS and MFS cases. In next three cases **fusions** were identified: *JAK2* gene fusion in a patient with MFS, *EML4* gene and the *AR/ELL* genes fusion in a patient with MPNST, and *TMPO/PIK3CA* and *TP53/ATP1B2* genes fusions in a patient with UPS. There was no correlation between the number of mutations and response to the treatment (Spearman's rho = -0,351, *p-value* = 0,141), while all patients with fusions were poor responders.

Conclusions: Cell cycle, apoptosis, DNA repair and proliferation pathways are deregulated in STS. Selected mutations detected only in patients with UPS may be potential diagnostic markers. Identified gene fusions could be candidates for predictive biomarkers. More research is needed on a larger group of patients.

[P6] Chemopreventive activities of spent hops (*Humulus lupulus* L.) extract against colorectal cancer

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Introduction: Colorectal cancer is one of the most frequent cancers occurring in the modern world with high annual mortality rates. The anti-cancer drugs used in the cancer treatment are expensive and their use is known to cause a range of side effects and complications. Therefore, chemopreventive agents inhibiting the development of colorectal cancer are subjects of many studies. The spent hops, prepared by the hops (*Humulus lupulus* L.) extraction by supercritical CO₂, are the source of polyphenols with high biological activity, including anti-cancer. The aim of the present study was to evaluate the effect of spent hops extract (SHE) on the invasion and migration of colorectal adenocarcinoma cells, as well as on the expression and activity of type IV collagenases, matrix metalloproteinase-2 (MMP-2) and -9 (MMP-9).

Methods: Two colorectal adenocarcinoma cell lines (SW-480 and HT-29) were cultured at 37°C in an atmosphere of 5% CO₂ plus air in medium supplemented with SHE (100-600 µg/mL). Epigallocatechin gallate (EGCG), one of polyphenol with proved anti-cancer activity, was used as a positive control. The Matrigel BM matrix assays were used to determine the impact of SHE on the cell invasion and migration. In turn, quantitative real time polymerase chain reaction (Q-PCR), enzyme-linked immunosorbent assay (ELISA), and zymography were performed to evaluate mRNA expression, protein expression and MMPs activity, respectively.

Results: SHE significantly inhibited both cell invasion and migration. However, stronger effect was observed for SW-480 cell line than HT-29 cells. After incubation with 200 µg/mL SHE, SW-480 and HT-29 cell invasion fell by 98.5% and 89% vs. controls, and migration was inhibited by 99% and 88% vs. controls. These changes were accompanied by a decline of MMP-2 and MMP-9 expression and activity.

Conclusions: Our data shows that SHE has anti-metastatic properties against colorectal cancer. The tested extract may be an effective chemopreventive agent acting via the inhibition of invasion and migration of colorectal cancer cells. In addition, we revealed a potential mechanism of the SHE activity.

[P7] Spent hops (*Humulus lupulus* L.) extract attenuates inflammation and angiogenesis of the retina via NF- κ B and Akt/ERK pathways

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Introduction Retinal diseases, mainly age-related macular degeneration and diabetic retinopathy, are associated with visual impairment, reduced quality of life, and may even lead to vision loss. The efficacy of available treatment targeting at a limitation of inflammation and angiogenesis, important processes of the diseases, is not satisfactory. Recent studies suggest that polyphenols have a lot of pro-health properties. Spent hops extract (SHE) is characterized by high content of polyphenolic compounds. The aim of our study was assessment of the anti-inflammatory and anti-angiogenic activities of SHE in *in vitro* model of the retinal inflammation. We wanted to prove that the SHE was able to inhibit the retinal angiogenesis and inflammation by restriction of expression and activity mediators engaged in this pathological process.

Methods Human retinal pigment epithelial cell line (ARPE-19) was cultured at 37 °C in an atmosphere of 5% CO₂ plus air in medium supplemented with SHE. Tumor necrosis factor alpha (TNF- α) was used to stimulate the inflammatory and pro-angiogenic response. The determination of the impact of SHE on the cell viability was performed using the MTT assay. The influence of SHE on gene expression was assessed by quantitative real time polymerase chain reaction (Q-PCR) analysis. The effect of SHE on protein expression was estimated by Western blot and by enzyme-linked immunosorbent assay (ELISA). The activities of matrix metalloproteinase-2 (MMP-2) and -9 (MMP-9) were evaluated by zymography assay.

Results: SHE modulated the TNF- α -induced expression of vascular endothelial growth factor (VEGF) and VEGF receptor 2 (VEGFR2), as well as reduced the activity of MMP-2 and MMP-9. Also, the inhibitory effect of SHE against the retinal inflammation and angiogenesis resulted from suppressing the nuclear factor kappa B (NF- κ B), protein kinase B (Akt) and extracellular signal-regulated kinase (ERK) pathways.

Conclusions Our results indicate that the SHE is able to inhibit inflammation and angiogenesis of the retina *via* reduction of the expression of mediators of these pathological processes, which results from the downregulation of NF- κ B, Akt, ERK signalling pathways. It shows that the SHE may occur as a support for the therapeutic management of diseases of the retina associated with angiogenesis, such as age-related macular degeneration or diabetic retinopathy. Nevertheless, further studies, including animal and clinical researches, should be conducted to confirm pro-health effects of the spent hops extract revealed in *in vitro* study by our researchers group.

[P8] Liquid target production of zirconium-89 for antibody labeling

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Introduction Positron emission tomography using zirconium-89-labelled antibodies (immuno-PET) is an important tool for personalised medicine allowing better patient selection for targeted therapies and improving drug development. Zirconium-89 (⁸⁹Zr, T_{1/2} = 78 h, E_{β⁺} = 396 keV, 23% positron yield) is a radiometal with near ideal physical and chemical properties for immuno-PET. The most common route to produce ⁸⁹Zr is via the ⁸⁹Y(p, n)⁸⁹Zr reaction on commercially available ⁸⁹Y solid target foils (ST). While the ST approach is advantageous for obtaining high production yields (94-95%) and high radionuclidic purities (>99%) of the isotope, it has high operational costs and requires a dedicated solid target system. The production of ⁸⁹Zr via liquid target (LT) systems can expand the availability of this isotope for cyclotron facilities by leveraging the existing infrastructure in place for clinical production of fluorine-18. Therefore, we have recently demonstrated the feasibility of producing ⁸⁹Zr using LT system on our IBA Cyclone18/9 cyclotron.

Methods As the target material the aqueous solution of yttrium (III) nitrate (99,8%) in 0,8 M nitric acid (69%) was used. The material was loaded into the niobium insert of LT on a IBA Cyclone18/9 and irradiated using two different energies of proton beam: 17,1 MeV and 13,4 MeV. For degradation of beam 250 μm thick disc was used. Extraction and purification of ⁸⁹Zr was carried out in 3 steps on Reform-Plus APS-3300 synthesis module. The final radionuclide purity was measured by gamma spectrometer with HPGe detector.

Results Initially using the beam energy of 17,1 MeV we managed to produce 39,27 MBq of ⁸⁹Zr. The purification yield was 20,3% with radionuclide purity of 98,54%. Subsequently, the same target material was degraded by beam energy of 13,4 MeV. This led to obtaining lower purification yield (13,16 ± 8,21%) but higher radionuclide purity (99,84±0,23%). Finally, when the preparation of target material was changed while using the same beam energy of 13,4 MeV, the radionuclide purity stayed the same 99,32% but the purification yield dropped even further (9,15%).

Conclusions We demonstrated the feasibility of producing and purifying ⁸⁹Zr in-house using IBA cyclotron and liquid target. We confirmed that lower beam energies (<14 MeV) decrease the production yield of ⁸⁹Zr as well impurities as ⁸⁸Y and ⁸⁸Zr. Further improvement of the process is needed to make this production approach more attractive for larger studies and clinical applications.

[P9] ILC1, ILC3 and ILC3 NKP46+ (NCR+) dysregulation in immunological response in Diffuse Large B Cell Lymphoma (DLBCL)

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The understanding of the cancer development and tumor microenvironment have been recently expanded with the recognition of the important role of innate lymphoid cells (ILCs). In the initial phase of the anti-tumor immune response, tissue-resident innate immune cells, such as macrophages, are stimulated to produce pro-inflammatory cytokines and chemokines. ILCs may play an crucial role in the tumor-associated host response and may represent interesting target cell populations for future therapeutic interventions. The potent antitumor properties of NK cells, a well-known type of cytotoxic cell that is currently ascribed to the ILC1 family, are well understood. In contrast, the role of non-cytotoxic ILC helper populations remains poorly understood. Based on functional criteria, cytokine production characteristics, and transcription factor expression profiles adult ILC can be generally divided into four lineages: conventional natural killer (cNK) and noncytotoxic group 1, 2 and 3 ILC. Group 1 innate lymphoid cells have a wide range of functions, including immunity to viruses and cancer cells, macrophage activation, cytotoxicity, and chronic inflammation. ILC3s might promote anti-tumor responses by enhancing leukocyte invasion, promoting tertiary lymphoid structure induction and trough the anti-tumor effect of IL-17 and IL-22. ILC3 (Lin⁻CD127⁺CD117⁺) comprise two subsets of cells defined on the basis of their cell surface expression of natural cytotoxicity receptors (NCR).

In this study, we analyzed the incidence of ILC and related factors in PBMC in patients with DLBCL. Finding a link between ILC and disease progression could help gather new information for further research into immune status in lymphoma patients.

Peripheral blood collected from adult patients positively verified for DLBCLs at the Clinic of Bone Marrow Transplantation and Oncohematology of the National Institute of Oncology in Gliwice was used. The control group consisted of healthy donors of bone marrow. Percentage of total ILC (Lin⁻ CD127⁺) was assessed by flow cytometry, as well as three ILC subsets, defined as ILC1 (Lin⁻ CD127⁺ cKit⁻ CRTH2⁻), ILC2 (Lin⁻ CD127⁺ CRTH2⁺), total ILC3 (Lin⁻ CD127⁺ cKit⁺ CRTH2-NKp46⁻) and NCR + ILC3 (Lin⁻ CD127⁺ cKit⁺ NKp46⁺).

In the studied group of patients (n = 54), a significantly lower level of circulating ILC (p = 0.004) as well as ILC1 (p = 0.01) and ILC3 (p = 0.009) was observed compared to the control group (n = 42). Similarly, in lymphoma patients there was a statistically significant (p < 0.0003) decrease in the median frequency of NKp46 + ILC3 cells from 0.087% in healthy subjects to 0.043% in the group of patients. Analysis of the ILC2 subpopulation showed no significant differences.

Our results will help to better define ILC interactions with the adaptive immune system to understand the initiation, maintenance and suppression of immune responses in lymphoproliferative diseases.

Obtained results may have priceless significance for revision and understanding the questions about contribution these cells in malignant transformation, tumor growth and their role in the microenvironment of lymphoproliferative diseases.

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[P10] Proliferation analysis of HeLa cells after pulsed treatment of 50Hz irradiation

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All living species, including humans, are exposed to the electromagnetic field (EMF). This research is focused on the investigation of biological effects of extremely low-frequency electromagnetic field (ELF-EMF) at the cellular level. EMF is defined by its parameters such as electric field intensity, magnetic flux density, and frequency. Because of today's fast-paced and technologically advanced society connected with an increasing number of electrical devices, it is important to effectively examine the effect of ELF-EMF on living organisms.

Non-ionizing electromagnetic field interactions with biological objects at various levels have lately become a fast-growing research area. Questions regarding its possible health risks are even more frequent and this particular area remains unclear, especially in a long-term radiation. The main purpose of this work was to verify the effects of ELF-EMF on the neoplastic cells' necrosis pathway.

Funding within the framework of the 8th call for project-oriented learning funding - PBL (Programme of Excellence - Research University Initiative) winter semester academic year 2022/2023; 02/040/BK_22/1022.

[P11] Verification of anticancer properties of polymersomes loaded with doxorubicin

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Cancer nanomedicine is a fast developing field which employs nanoparticles to target and destroy cancer cells even in hard to reach areas. Many nanoscale drug delivery systems have been developed to improve anticancer efficiency with simultaneous reduction of off-target toxicity. Among numerous vehicles, polymersomes have attracted a lot of attention thanks to their low toxicity, high stability, and high drug loading capacity. Polymersomes are synthetic amphiphilic block copolymers. Analogously to liposomes, they carry hydrophilic solution in the core, surrounded by a bi-layer membrane, which can carry hydrophobic drugs. Nonetheless polymersomes exhibit longer circulation and increased chemical versatility. Encapsulation of chemotherapeutic doxorubicin inside of polymeric nanocarrier allows for effective treatment while decreasing cardiotoxicity.

Polymersomes were obtained by conjugating poly(lactide) (PLA) with poly(ethylene glycol) (PEG) and loaded with doxorubicin or fluorescein. The experiments were conducted on cancer cells (4T1 – murine breast cancer, B16-F10 – murine melanoma), and normal cells (NIH 3T3 – murine fibroblasts, H5V – murine endothelial cells). Firstly we tested if synthesized polymersomes with fluorescein are able to enter cells. Secondly we assessed cytotoxicity of polymersomes, empty and loaded with doxorubicin using MTS assay. Cells were cultured in medium containing different concentrations of synthesized polymersomes. Next we observed cells after incubation with polymersomes in different time points. Medium above cells was discarded, cells were washed with DPBS and observed under confocal microscope and semi-confocal time laps microscope.

We confirmed that empty polymersomes have no influence on the cells growth. No cytotoxic effect was observed. Using polymersomes with fluorescein we observed that after 1h polymersomes would penetrate cells in dose dependent manner. Longer incubation (48h) showed that the polymersomes were still in the cytoplasm of the cells and have the potential to further drug release. Doxorubicin-loaded polymersomes were cytotoxic to all tested cell lines. Cytotoxicity was dose dependent and increased with time in each cell line. Cells 4T1, B16-F10 and NIH 3T3 were considerably more sensitive comparing to H5V cells that showed reduced infiltration of polymersomes both with fluorescein and doxorubicin.

In further studies we plan to functionalize polymersomes, by adding an azobenzene linker connecting polymers, so delivered drugs should be released only in hypoxic condition. Regions of hypoxia appear in 90% of solid tumors as formed vasculature fails to supply sufficient amount of oxygen to quickly proliferating cancer cells. Thanks to specificity of hypoxic microenvironment it is possible to design polymersomes reactive only in these regions.

The work is a result of the research project no. UMO-2020/39/B/NZ5/00745 financed by National Science Center.

[P12] Cardiac MRI in mice at 9.4T – towards protocol optimization

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Introduction/Rationale Application of magnetic resonance techniques in cardiac imaging permits non-invasive evaluation of heart contractile function, perfusion, oedema and viability. This method is useful in the studies of mouse models of heart disorders to test efficacy of novel drugs. However, cardiac imaging in these animals is challenging due to the small size of the organ and the fast heart rate (~600 bpm). The purpose of this work was to develop the imaging protocol permitting multiparametric evaluation of the mouse heart.

Methods Experiments were performed on a 9.4 T vertical 89 mm bore system (Bruker BioSpin, Germany) equipped with a Bruker Micro 2.5 gradient system and a transmit/receive birdcage radiofrequency coil with an inner diameter of 30 mm. During data acquisition, the animals were anaesthetized with 2-3% sevoflurane. Body temperature, ECG signal and respiration rate were monitored using ECG/respiratory unit (SA Instruments, Inc). The protocol starts from acquisition of several fast scout scans in order to plan geometry of self-gated CINE images (acquired using Intradate FLASH sequence) in the short and long axis orientation of the heart. End diastolic volume, end systolic volume, stroke volume and ejection fraction are functional parameters that can be determined by segmentation of ventricles in the short axis images. Prospectively gated multislice multiecho sequence was exploited for transverse relaxation time (marker of myocardial oedema) quantitation. Highly T1 - weighted self-gated CINE images were also measured to evaluate early gadolinium enhancement (indicator of tissue hyperemia) and late gadolinium enhancement for assessment of myocardial tissue variability. The kinetics of the signal change in T1 – weighted images of the heart after intravenous injection of Gadovist was compared to the kinetics obtained after intraperitoneal administration. The developed protocol was evaluated in 4 healthy Balb/c mice.

Results The end diastolic volume of left ventricle was equal to $36.7 \pm 1.4 \mu\text{l}$, end systolic volume of this ventricle - $14.5 \pm 2.3 \mu\text{l}$ and ejection fraction – $60.5 \pm 5 \%$. The transverse relaxation time determined from mono-exponential fitting was found to be $22.5 \pm 1.5 \text{ ms}$ in the healthy myocardium. After intravenous injection, the signal intensity in T1-weighted CINE images peaked earlier and was higher than after intraperitoneal administration. The contrast agent wash-out from the myocardium was more rapid after intravenous injection.

Conclusions/Novel aspect The developed protocol permits to obtain high quality MRI images of the heart in healthy animals. Further studies are required to optimize imaging parameters in mouse models of cardiovascular disorders.

[P13] STING agonist cGAMP and vascular disrupting agent induce anti-tumor response of poorly immunogenic tumor by inflaming tumor microenvironment

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Immunotherapy has emerged as one of the most promising cancer treatments. However, despite tremendous progress over the recent years, most patients will not respond or develop resistance mechanisms to applied treatment. Therefore, adequate combination of anticancer agents with immunotherapy is required to powerfully activate antitumor response.

The aim of the study was to examine whether the vascular disrupting agent improves effectiveness of immunotherapy in poorly immunogenic tumor.

Experiments were performed in a poorly immunogenic mouse model of triple negative breast cancer (4T1 tumors). BALB/c mice were inoculated subcutaneously with 2×10^5 4T1 cells. Combretastatin A4 phosphate (CA4P) was used as vascular disrupting agent (50mg/kg body weight intraperitoneally), cGAMP (STING agonist) as immunotherapeutic agent (5 μ g/mouse intratumorally). The analyzes of immune cells infiltration and their state of activation (M1, M2 macrophages, NK cells, CD8 T cells) were performed using flow cytometry. Density of blood vessels was analyzed using confocal microscopy.

We have shown that combination of anti-vascular agent CA4P with immunostimulatory agent cGAMP synergistically enhanced antitumor effect against 4T1 breast cancer. The combination therapy effectively reduced tumor blood vessels density. We have seen synergistic anti-vascular effect of cGAMP in combination with CA4P. We have also demonstrated that combination immunotherapy of the STING agonist cGAMP and vascular disrupting agent CA4P boost innate immunity. We showed that macrophages were polarized into pro-inflammatory, anti-tumorigenic M1 phenotype, NK cells massively infiltrated tumor and exhibited activated phenotype. We have also shown that combination therapy resulted in a reduction of exhausted tumor-infiltrating CD8+ T cells (CD8+PD-1+ T cells). However, triple combination of anti-PD-1 antibody with STING agonist and CA4P failed to improved overall benefit over cGAMP and CA4P double combination.

In conclusion, we have shown that anti-vascular therapy can effectively improve the antitumor response of immunotherapy by shifting the tumor microenvironment of non-immunogenic tumors into more immunogenic.

The work is a result of the research projects no. UMO-2018/31/B/NZ5/01825 financed by National Science Center, Poland.

[P14] Transdermal ferroptosis propagation from cancer to co-incubated normal HaCaT skin cells

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Introduction Signal transduction from extra- and intra-cellular cascades, in response to stress inducers, could lead to regulated cell death (RCD). Excessive or insufficient RCD can cause disorders in physiological and pathological conditions. RCD can take many forms (apoptosis, necroptosis), such as ferroptosis, a type of non-apoptotic, programmed iron-dependent cell death.

Aim In co-cultivated healthy HaCaT cells, ferroptosis death could be induced *via* biological response based on different modalities: chemical; specific recognition by cell-surface receptors; direct relations between cells developed on special junctions or nexus. In this research, the membrane TFRC receptors and enzyme ACLS4 – specific markers of ferroptosis in HaCaT were studied, preceded after cancer skin 1205_Lu and 451_Lu cells stimulation. **Methodology** The neoplastic skin cells (1205_Lu and 451_Lu) were used for ferroptosis induction by Erastin [5 and 10 μ M] and used for 96h long-term bystander co-cultivation with HaCaT. With qRT-PCR reaction the ferroptosis markers expression, for genes of TRFC receptor and ACLS4 enzyme, was evaluated. For both, normal and cancer cell lines, the viability was evaluated by 24h MTT assay.

Results The ferroptosis induction by Erastin in normal skin HaCaT cells is not visible by direct 24 h exposure, opposite to the sensitive cancer 1205_Lu cells. Resistance to death stimuli of cancer 451_Lu cells come from reduction of genes expression of receptor TFRC and ferroptosis execute enzyme ACLS4. Ferroptosis induction in HaCaT cells is visible on the expression level of those ferroptosis markers (TFRC and ACLS4) after signals release from co-incubation with cancer skin cells.

Conclusions Cancer cells propagated death stimuli for ferroptosis into bystander normal skin cells.

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[P15] Targetome profiling *in vitro* reveals *KSR2* as a target for tumor suppressor miR-143-3p in T-cell acute lymphoblastic leukemia

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Introduction T-cell acute lymphoblastic leukemia (T-ALL) is a heterogeneous and aggressive malignancy arising from T-cell precursors. miRNAs are implicated in negative regulation of gene expression and, when aberrantly expressed, contribute to various cancer types, including T-ALL. Previously we demonstrated the decreased level of miR-143-3p in pediatric and adolescent/young adult T-ALL patients compared to healthy controls. Here, we present the tumor suppressor activity of this miRNA in T-ALL cell lines and unravel its potential involvement in RAS pathway via targeting *KSR2* gene.

Methods 2 T-ALL cell lines with low endogenous miR-143-3p expression, JURKAT and ALL-SIL, were transduced for constitutive overexpression of this miRNA. Flow cytometry-based GFP competition assay was used to assess the effect of miR-143-3p on cell growth. Ago2 RNA immunoprecipitation (RIP) followed by RNA-seq was used for miR-143-3p targetome profiling. RT-qPCR and Western Blot were used to evaluate the mRNA and protein level of *KSR2*. CRISPR inhibition (CRISPRi) with the use of dead Cas9-KRAB construct was used to evaluate the effect of *KSR2* repression in T-ALL cell lines.

Results We showed the loss of growth advantage in T-ALL cell lines upon overexpression of miR-143-3p, pointing to its tumor suppressor activity. Next, using combined RIP-seq and RNA-seq approach, we unraveled the spectrum of direct miR-143-3p target genes as well as its global effect on the transcriptome of T-ALL cells. We identified *KSR2* and *EGR1* as miR-143-3p targets of potential importance for T-ALL biology. *KSR2* is a positive regulator of RAS pathway, which is overactivated in many cancers and related to oncogenic transformation. *EGR1* is a transcription factor involved in activation of interleukin-6 expression in T-ALL cells. We confirmed the decreased *KSR2* protein level in both T-ALL cell lines upon miR-143-3p overexpression. Since the oncogenic role of *KSR2* has not been previously studied in T-ALL, we evaluated the effect of its CRISPRi-mediated inhibition on the survival of T-ALL cells *in vitro*. We observed that loss of *KSR2* expression leads to the loss of growth advantage of T-ALL cell lines and thus phenocopies the effect of miR-143-3p overexpression.

Conclusions miR-143-3p is downregulated in T-ALL patients and exhibits tumor suppressor activity by decreasing the growth of T-ALL cells, upon forced overexpression *in vitro*. Its effect is at least partially mediated by negative regulation of *KSR2*. *KSR2* is a novel potential oncogene in T-ALL and its loss leads to decreased growth of T-ALL cells *in vitro*.

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[P16] The effect of STING activation on tumor associated neutrophils phenotype in 4T1 (breast cancer) and B16-F10 (melanoma) model

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Tumor Associated Neutrophils (TANs) constitute gradually increasing population of tumor-associated myeloid cells during tumor progression. Their abundance is a predictor of poor outcome, therefore new approaches are designed to overcome their unfavorable impact. Stimulator of interferon genes (STING) is crucial protein of innate immune response pathway. Currently, it appears as promise target for anti-cancer therapies which through stimulation of type I interferons could polarize tumor-associated myeloid cells into anti-tumor phenotype.

The aim of the work was to assess neutrophils polarization state in the tumor microenvironment after intratumoral STING agonist administration in 4T1 and B16-F10 cancer models.

The experiments were conducted on 4T1 murine breast cancer model (BALB/c mouse strain) and B16-F10 melanoma model (C57BL/6Crl mouse strain). 14 days after cancer cells inoculation, STING agonist (2'3'-cGAMP) was administered intratumorally in a dose 5 µg/mice. The phenotype of neutrophils (CD11b+Ly6G+) was determined after 12h by flow cytometry analyses of mononuclear cells. Neutrophils activation state was determined by CD69 molecule staining. Additionally, the percentages of Fas+, PD-1+, and PD-L1+ TANs were determined. The level of MMP9 molecule was determined by flow cytometry and immunohistochemistry staining.

In both tumor models there was observed increased percentage of activated (CD11b+Ly6G+CD69+) TANs after STING agonist administration. There was also observed increased percentage of Fas+ and PD-L1+ TANs after 2'3'-cGAMP administration in both analyzed models. There were observed differences between models regarding percentages of TANs presenting PD-1 and MMP-9 molecules. In 4T1 model significant decrease in the percentage of PD-1 molecule and slight increase of MMP-9 molecule were observed on TANs after 2'3'-cGAMP administration. In B16-F10 model the results were the opposite - there was observed increase in the percentage of PD-1 molecule and not significant - slight decrease of MMP-9 molecule on TANs after 2'3'-cGAMP administration.

The obtained results indicate that STING protein stimulation leads to neutrophils activation in both tumor models. STING stimulation increase percentage of Fas+ and PD-L1+ TANs in both tumor models and provide different TANs phenotype regarding MMP-9 and PD-1 molecule according to tumor model. Summarizing, STING activation-induced phenotype switch of TANs depends on tumor model.

The work is a result of the research project no. UMO-2019/35/N/NZ5/02506 financed by National Science Center, Poland.

[P17] Proteomic profile of primary colon tumors could discriminate patients with different status of metachronous distant metastasis: retrospective study

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Introduction Colon cancer patients' mortality is primarily caused by aggressive metastatic disease progression, rather than the primary tumor. Despite the progress in surgical techniques and oncological diagnosis, in some cases, it is not possible to predict the risk of progressive disease and consequently undertake proper treatment strategies. Therefore there is an urgent need to search for new molecular prognostic markers of colon cancer and to expand the knowledge of cellular processes responsible for the spread of tumor. In order to look for molecular signatures of colon cancer distant metastasis (DM) we applied proteomic profiling of FFPE primary tumors from patients with different status of metachronous DM.

Methods Primary right-sided colon cancer tissue was collected during surgery from 31 patients without synchronous distant metastasis and secured for routine histopathologic analysis as formalin-fixed paraffin-embedded (FFPE) specimens. Colon cancer patients were classified into two groups, depending on the occurrence of metachronous DM during the 5-year follow-up period: group A – with DM (11 tumors) and group B – without DM (20 tumors). Specimens were processed using MED-FASP method after deparaffinization of FFPE tissue with n-heptane and lysis in TLB buffer. Proteomic profiling was performed using LC-MS/MS approach with an additional ion filtering stage using FAIMS interface.

Results Label-free LC-MS/MS approach allowed the identification of about 7500 proteins in FFPE specimens. An untargeted proteomic analysis revealed about 500 proteins, whose levels discriminated between patients with different statuses of colorectal cancer distant metastases. These molecules were associated with a few pathways relevant to metastasis, including mitochondrial translation, post-translational protein phosphorylation, neutrophil degranulation, RHO GTPase cycle, WNT ligand biogenesis and trafficking, glycolysis and glucose metabolism. Among the proteins that most properly discriminated patients with different status of metachronous distant metastasis were CD300A, TBC1D15, LAD1, EPS8L1 and DENND2D with AUC > 0.90 and accuracy > 73%.

Conclusions This study revealed a specific proteomic pattern of FFPE primary colon tumors, which could discriminate patients with different status of metachronous distant metastasis. The proposed panel of proteins, associated with molecular processes relevant to metastasis, could be promising candidates for signatures of colon cancer DM.

Keywords: colorectal cancer; metastasis; FFPE; proteomics; mass spectrometry.

Funding: This study was supported by the National Science Centre, Poland, Grant 2017/26/D/NZ2/00964 (for KD, LM, MP, AW).

[P18] Research on the cytotoxicity of a conducting polymer, PEDOT, decorated with Ag and Au particles

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The main advantage of the PEDOT polymer selected for the study is its high biocompatibility and electrical conductivity. To provide it with antibacterial properties, it was decorated with gold and silver particles in a process of electrochemical deposition. In order to check how this modification influence the biocompatibility, the HNDF (Normal Human Dermal Fibroblasts, Adult) skin cell survival test was performed. Tested materials were compared with platinum, which is a standard electrode material to be used in bioelectronics.

Our results indicated that while PEDOT increased cell viability, in combination with silver, the biocompatibility was significantly decreased. This effect can be remedied by adding gold to the mixture of silver and PEDOT as it helps to reduce cytotoxicity.

Therefore, PEDOT/Au/Ag materials were shown as promising materials combining electroactivity, biocompatibility, and antibacterial properties.

[P19] Cyclic oligosaccharides derivatives as biocompatible initiators of star-shaped polymers via green ATRP

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Cyclodextrins (CD) are cyclic oligosaccharides composed of α -D-glucose units linked by α -1,4-acetal bonds. The most widely studied variants of CDs are α -, β -, and γ -cyclodextrins, which contain 6, 7 or 8 α -D-glucose units, respectively. CDs are toroidal in shape, with all primary hydroxyl groups located on the narrow side and the secondary hydroxyl groups on the wider side. The toroidal shape makes CDs hydrophilic on the outside, though the cavity stays hydrophobic. This structure enables the formation of guest-host interactions between CDs and hydrophobic compounds. Such guest-host complexes can be used in drug delivery systems, due to the fact that CDs are known to be non-toxic, biocompatible and non-immunogenic.

The aim of the research was to obtain bromoisobutyryl-terminated CDs derivatives as biocompatible atom transfer radical polymerization (ATRP) initiators. The esterification reaction of α -cyclodextrin (α -CD), β -cyclodextrin (β -CD) or (2-hydroxypropyl)- β -cyclodextrin (β -H-CD) was carried out in *N*-methylpyrrolidone (NMP), in the presence of triethylamine (TEA) and 4-(*N,N*-dimethylamino)-pyridine (DMAP) as deacid reagent and catalyst, respectively. The structures of the obtained initiators were confirmed by ¹H NMR and ATR-IR analysis. Finally, initiators based on β -CD and β -H-CD were used in an enzymatically assisted ATRP (glucose oxidase), also known as breathing ATRP, to obtain 8-arm and 15-arm star-shaped poly(*N,N*-dimethylaminoethyl methacrylate) (PDMAEMA).

The esterification yields depended on the CD variant, stayed in the range between 17% to 77%, and increased with the increasing amount of α -bromoisobutyryl bromide used as acylation reagent. The results showed that the obtained initiators are good candidates for the synthesis of star-shaped polymers *via* breathing ATRP mechanism, although the DMAEMA conversion was two and a half times lower compared with the utilization of commercially available 2-hydroxyethyl 2-bromoisobutyrate as bifunctional initiator. Despite the fact that the reaction conditions are still to be refined, the advantages of obtaining well-defined stimuli-responsive polymers according to the open-air green ATRP mechanism, are invaluable.

Keywords: cyclodextrins, initiators, breathing ATRP, DMAEMA

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[P20] Optimization of a tissue preparation procedure for MALDI-TOF mass spectrometry imaging of formalin-fixed paraffin-embedded specimens of colorectal cancer

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Introduction MALDI mass spectrometry imaging (MALDI-MSI) has gained considerable interest in molecular analysis of animal tissues as it enables to combine molecular data obtained from mass spectrometric analysis with localization of an analyzed molecule within a tissue section. However, the success of MALDI-MSI measurements is highly dependent of the quality of tissue section preparation which in turn is a product of the quality of the successive stages of the section preparation procedure. In the presented work we have shown results of optimization of a protocol of tissue preparation for MALDI-MSI-based analysis of colorectal cancer (CRC) specimens.

Methods Formalin-fixed paraffin-embedded tissue specimens were used in the study. A general protocol of tissue preparation for MALDI MSI measurements was composed of the following steps: tissue dewaxing, heat-induced reversal of protein crosslinks (*), enzyme deposition onto a tissue, protein on-tissue digestion in a humidified chamber (*), matrix deposition onto the tissue. Steps marked with an asterisk were optimized, since they are tissue dependent. Quality of results obtained on each step of the procedure were assessed taking into account: quality of spectra (intensity of spectra, number of peaks on the average spectrum), as well as results of unsupervised segmentation analysis of spectra performed in SCLS Lab software.

Results Heat-induced reversal of protein crosslinks is a crucial step in the case of formalin-fixed tissues, as the fixation process results in formation of methylene bridges between proteins, thus deteriorating their availability during subsequent on-tissue enzymatic digestion. Three temperature programs of Decloaking Chamber automatic pressure cooker were tested: 110°C, 20 min; 95°C, 40 min, and 80°C, 60 min. Although, generally, higher temperatures allow for more efficient crosslinking reversal in a shorter time, the performed tests highlighted the problem of tissue damage in elevated temperatures. Only boiling at 80°C left the tissue intact. The subsequent tested step: the so called on-tissue enzymatic digestion, was initially performed in a dedicated SunDigest incubation chamber following the conditions recommended by the manufacturer, i.e. 50°C, 2h, 95% humidity). However, the attainable digestion efficiency was not satisfactory, especially for cancer regions. Higher digestion yield was obtained using a home-made digestion chamber (chamber volume: 0.2 L, 37°C, 18 h, moisturizing mixture: 100 mM NH₄HCO₃ with 5% MeOH, mixture volume: 0.2 mL). Finally, performing crosslinking reversal at 80°C and enzymatic digestion in a home-made chamber were selected as optimal conditions.

Conclusions The obtained CRC tissue preparation protocol will be implemented in subsequent analysis of immune system cells in the tumor microenvironment (TME) using MALDI-MSI combined with immunohistochemistry (IHC) analysis.

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[P21] Radiomics signature for time-to metastasis prediction in non-small cell lung cancer patients

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Early and accurate diagnosis as well as tumor stage assessment is extremely important in the context of planning an effective treatment strategies in every neoplastic disease. Lung cancer is the leading cause of cancer-related deaths in Poland and in the world, and due to non-specific symptoms, patients are most often diagnosed in the advanced clinical stage. The main problem in locally advanced non-small cell lung cancer (NSCLC) is treatment resistance, and a high potential for progression and metastasis, resulting in poor prognosis. Especially in NSCLC, which is asymptomatic in its initial stages, positron emission tomography and computed tomography (PET/CT) turns out to be an effective diagnostic and monitoring tool, characterized by high sensitivity, specificity and accuracy. PET/CT is the most accurate method of solid tumor imaging in clinical oncology, and plays an important role in detecting metastases. Taking into account such unfavorable survival rates of NSCLC patients, it's necessary to identify new methods and factors that will allow better prediction of the disease course and prolong the patient's survival by selecting an appropriate therapeutic strategy.

In this study, the prognostic value of radiomic features in predicting the time to distant metastases was investigated in 97 NSCLC patients, treated with chemoradiotherapy (CHRT). Based on the radiomic features, extracted from PET/CT images, we built a Cox regression model, that predicts metastases and the time of their occurrence in the study group. The proportional hazard assumption was verified with Schoenfeld residuals, and the c-index was used to evaluate the model performance. High correlations between the radiomic features were observed. In the Cox regression model, the highest values of the median c-index across folds were observed when fewer radiomic features were analyzed.

The obtained results suggest that certain radiomic features have a potential as a predictors of metastatic relapse and therefore can be considered as a prognostic factor in NSCLC. Analysis of the oncological patient's radiomics signature may help in undertaking individualized therapeutic strategies.

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[P22] Global DNA methylation profiles in OSCC tumour and margin samples

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Introduction DNA methylation is one of epigenetic modifications. DNA methylation pattern changes during development and is also connected with many pathological processes, such as carcinogenesis. Oral squamous cell carcinoma (OSCC) is a common cancer worldwide and occurs due to many risk factors such as smoking, alcohol consumption and viral infections. This study aimed to evaluate global DNA methylation levels in OSCC tumour and margin samples. The potential influence of clinicopathological and demographic parameters, smoking, drinking and human papilloma virus presence was also analysed.

Methods The study population comprised 39 patients with OSCC (including 25 men and 14 women) and 50 age- and sex-matched healthy individuals (27 men and 23 women). Patients were recruited at the Department of Otorhinolaryngology and Oncological Laryngology in Zabrze, Medical University of Silesia in Katowice, Poland. The study was approved by the Bioethics Committee of the Medical University of Silesia (no: KNW/0022/KB1/49/16 and KNW/0022/KB1/49/II/16/17) and was supported by the Ministry of Science and Higher Education ((KNW-1-011/N/9/0). Global DNA methylation was analysed in tumour and surgical margin samples collected from OSCC patients and in epithelial cells of the oral mucosa collected from healthy individuals. After DNA isolation, the percentage of 5-mC was examined by a 5-mC DNA ELISA Kit (Zymo Research, Germany). The presence of human papilloma virus was detected using the GenoFlow HPV (Human Papillomavirus) Array Test Kits (DiagCor Bioscience Inc., Hong Kong).

Results We observed a significantly higher global DNA methylation level in OSCC tumour and surgical margin samples in comparison to the control group ($p < 0.001$ and $p < 0.001$, respectively). However, no significant difference was found between the tumour and margin samples ($p = 0.451$). Interestingly, a significantly higher global DNA methylation level in tumour samples was found in patients with advanced tumour stage (T3) ($p = 0.042$). On the other hand, significantly lower global DNA methylation was found in the tumour with histological grade G3 compared to patients with lower grades ($p = 0.016$). Furthermore, we reported a significant negative correlation between global DNA methylation and N stage ($r_s = -0.33$, $p = 0.039$) in margin samples, while no similar correlation was observed in the OSCC tumour group. In HPV(+) tumour samples, significantly lower global methylation levels were found than in HPV(-) patients ($p = 0.013$). In the control group, we found higher global methylation levels in HPV16(+) cases than in HPV16(-) cases ($p = 0.047$).

Conclusion Our study shows that changes in the global DNA methylation level were associated with the early stage of carcinogenesis and progression of OSCC. On the other hand, alternations in global DNA methylation could be the result of ongoing carcinogenesis. Moreover, environmental factors such as viruses have an influence on changes in the global DNA methylation level.

[P23] Effect of hypoxia on the adhesion properties of acute myeloid leukemia cells (AML) to the stromal cells of the bonemarrow (HS-5)

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Introduction The aim of the study was to evaluate the effect of sonidegib (LDE-225) on the adhesive properties of acute myeloid leukemia (AML) cells to the mesenchymal stromal cells using optical tweezers (OT) under hypoxic conditions. AML therapy fails due to, among other things, the protective effect of bone marrow cells. Reducing adhesion will weaken the protective effect of bone marrow cells. For this purpose, LDE-225 was used. In science, hypoxia is usually defined as an oxygen concentration below 21%, but it should be remembered that the O₂ concentration in a living organism is usually much lower. Traditional cell cultures are carried out in 21% oxygen, but bone marrow cells are physiologically distributed in an oxygen gradient from 0.1 to 5%. Clinical trials on the effectiveness of sonidegib in hematological cancers, including AML, are currently underway. However, there are no studies linking the effects of sonidegib treatment on AML adhesion under hypoxic conditions, which are believed to be closely related to drug resistance and treatment failure. The adhesion of a single leukemia cell to the HS-5 spheroid was assessed using optical tweezers designed and constructed at the Wrocław University of Science and Technology on the Olympus IX71 inverted biological microscope (Olympus, DE).

Methods Mesenchymal stromal cells (HS-5) were obtained from ATCC (USA) and OCI-AML3 from DSMZ (DE). HS-5 spheroids were prepared on an agarose gel according to the manufacturer's instructions. In brief, the 3.2×10^4 of HS-5 cells were seeded in 190 μ l of medium per gel using mold 12-256 of the commercially available PetriDish® 3D system (Microtissues Inc., USA). OCI-AML3 cells were seeded with 5.0×10^5 cells per well in 6 well plates. Cultures were grown in 21% oxygen and 1% oxygen at 37 °C in 5% CO₂ for 24 hours, and then 10 μ M sonidegib (Selleck Chemicals, USA) was added to the culture medium. Controls were cells grown in a medium without a compound. After 48 hours of incubation, cells were used to measure the adhesion time in OT.

Results The study showed an inhibitory effect of sonidegib on the adhesion of leukemia cells to the HS-5 spheroid. Under hypoxic conditions, a significant reduction in adhesion of the treated cells (mean $26.0 \text{ s} \pm 11.94$) compared to the control group ($15 \text{ s} \pm 5.06$) was observed. In 21% of O₂, there was also a decrease in drug adhesion ($17.75 \text{ s} \pm 10.97$) compared to control ($11.75 \text{ s} \pm 3.85$). As can be seen, the contact time necessary for AML to attach to the stromal cells was also lengthened due to the hypoxia itself.

Conclusions Optical tweezers enable accurate measurement of the time required for the adhesion of leukemia cells to the HS-5 spheroid. In our studies, we proved the effect of sonidegib on reducing the adhesion of acute myeloid leukemia cells to stromal cells in 21% and 1% of O₂. However, the increase in the time required for cells to adhere was even more evident under hypoxic conditions. Using an innovative tool in the form of optical tweezers allowed us to accurately study the adhesion time of leukemia cells impossible to achieve with traditional methods.

[P24] T cell regeneration after allogeneic haematopoietic stem cell transplantation from fully matched vs haploidentical donors

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Introduction/Rationale: Allogeneic haematopoietic stem cell transplantation (alloHSCT) is an effective and often the only therapeutic option in haematopoietic malignancies. The alloHSCT procedure is preceded by the high-dose radio- or chemotherapy, which is aimed to destroy the patient's defective hematopoiesis. During the first months after alloHSCT immune recovery must be rebuilt. That process relies on the peripheral expansion of donor T cells present in the transplanted cell material (memory T cells). The most affected and the last to restore after alloHSCT are CD4+ T cells. Especially valuable, but regenerated very late are so-called 'recent thymic emigrants' (RTE) which are produced in the thymus and are the earliest T lymphocytes detected in the blood. While detailed T-cell reconstitution has been fairly well described for alloHSCT from a donors fully matched in HLA, it is still poorly understood for alloHSCT from donor matched in only half of the HLA (haploidentical HSCT). Although rarely performed, haploHSCT is becoming increasingly important for patients who do not find HLA-matched donor.

Methods: The study was performed on 96 patients who underwent alloHSCT (49 haploHSCT patients and 47 MUD-HSCT patients). Immunophenotypic detection was carried out before and 100 days after HSCT including the following T cell subpopulations: Th (CD4+), Tc (CD8+), naive/memory T lymphocytes (CD5RA+ phenotype) , double negative T cells (CD3+ CD4- CD8-), Treg regulatory lymphocytes (CD4+ CD25 high CD127-), RTE (recent thymic emigrants, CD4+ CD45RA+ CD62L+ CD31+).

Results: Our results show that early post transplant naïve CD4+ T cell compartment, RTE cells and naïve Treg cells were restored more efficiently after haploidentical haematopoietic stem cell transplantation (haplo-HSCT) when compared to matched unrelated haematopoietic stem cell transplantation (MUD-HSCT). The most significant differences were observed in percentage of circulating Th CD4+ cells ($p = 0,01$), naive CD4+ T cells ($p = 0,0001$), recent thymic emigrants (RTE) ($p = 0,006$) and naive Treg cells ($p = 0,00005$). However, we found no substantial differences in regeneration of general population of lymphocytes ($p=0,37$), CD3+ lymphocytes ($p=0,33$), Tc lymphocytes CD8+ ($p=0,06$) and total Treg cells ($p=0,8$) between patients after haploHSCT vs matched unrelated HSCT. On the contrary, post-transplant CD3+ CD4- CD8- (DN) T cells percentage was lower for patients after haploHSCT compared to patients after MUD-HSCT ($p = 0,000001$).

Conclusions/Novel aspect: Although haploHSC is regarded as the last chance for patients, our results show that this therapeutic procedure is less harmful for naïve T cell recovery than matched HSCT. It can be also concluded that naïve T cell, particularly RTE cells are restored post haploHSCT regardless of other lymphocyte populations. We hope that our results will allow a deeper understanding of immune restoration after alternative forms of alloHSCT.

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[P25] N-glycome of CD4-negative peripheral blood mononuclear cells (CD4- PBMC) changes in Graves' disease

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Introduction Graves' disease is the most common type of hyperthyroidism, with annual incidence rate of approximately 14 per 100.000, affecting mainly women. The presence of autoantibodies, which bind to the thyroid hormone receptor (TSHR), triggers the most characteristic manifestations of the disease – goiter and Graves' ophthalmopathy. Methimazole is a drug normalizing TSH levels, with local immunosuppressive effects, commonly used to treat hyperthyroidism. Peripheral blood mononuclear cells (PBMC), implicated in thyroid autoimmunity, express a wide range of *N*-glycosylated proteins, such as: TCR, CD8, MHC II. Our study focuses on this most common post-translational modification of the proteome, which can alter many crucial protein qualities. The main goal was to assess the differences in *N*-glycome of PBMC after Th CD4+ depletion in GD development and the influence of the methimazole treatment on *N*-glycosylation of these immune cells.

Methods Blood samples were obtained from healthy volunteers (CTR), patients with Graves' disease before (GD) and after TSH normalization (GD/L) as a result of methimazole treatment. Blood was centrifuged in Histopaque-1077 gradient and PBMCs were collected. After the depletion of CD4+ cells (analysed separately), remaining cells were lysed in RIPA buffer. Total proteins were precipitated from the extracts, denatured and then de-*N*-glycosylated using *N*-glycosidase F (PNGase F). Released *N*-glycans were labelled with anthranilic acid (AA) and subjected to MALDI-ToF mass spectrometry. The selected *N*-glycan peaks (GPs) were further analysed quantitatively, and statistical significance of differences between studied groups was checked using the non-parametric Kruskal-Wallis test ($p < 0.05$).

Results Among *N*-oligosaccharide structures assigned to the most abundant GPs in the mass spectra we found the significant changes in *N*-glycan content between both Graves' disease groups and healthy donors. The amount of monosialylated biantennary complex-type *N*-glycans (H5N4S1, H5N4F1S1, H5N4F2S1) was increased, while the content of oligomannose *N*-glycans (H5N2, H9N2, H6N2, H8N2, H7N2) was lower in both GD groups comparing to control. The statistically significant differences between the GD and GD/L groups were not observed. It may indicate that methimazole does not affect PBMC CD4- *N*-glycosylation or it can result from not enough time after TSH stabilisation to observe changes.

Conclusion The identified differences in *N*-glycosylation of PBMC CD4- between healthy donors and study groups may have implications for the development of GD thyroid autoimmunity. However, further studies are needed to confirm this hypothesis.

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[P26] The human Heat Shock Protein A2 (HSPA2) chaperone controls the organization of the epidermis *in vitro* and modulates signalling pathways involved in cornified envelope development

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Background HSPA2, a member of the HSPA (HSP70) chaperone family, was originally described as a testis-specific protein involved in spermatogenesis and regulation of the fertilization process. We showed that HSPA2 is also synthesized in selected human somatic tissues, among others in stratified and pseudostratified epithelia including skin epithelium. However, the role of HSPA2 in extra-testicular cells is unknown. Our preliminary observations suggested that HSPA2 can modulate keratinocyte differentiation. This study was aimed at examining the influence of HSPA2 on epidermal development and morphology using 3D reconstructed human epidermis equivalents. Also, we searched for molecular mechanisms that rely on HSPA2 in terms of epidermis functions.

Methods We used CRISPR/Cas9-mediated genome editing to establish the human epidermal keratinocyte with the *HSPA2* gene knockout (KO). Control and HSPA2-KO HaCaT cells were used to construct epidermal equivalents *in vitro*. Next, epidermal structure and distribution of differentiation-specific markers in 3D epidermal substitutes were evaluated. In parallel, we analyzed HSPA2-dependent changes in the global transcriptome using RNA profiling based on next-generation sequencing (RNA-seq). Validation of selected genes was performed.

Results The deficit in HSPA2 caused spatial alterations in the upper layer of reconstructed epidermis equivalents (RHE). Histological and immunophenotypic analysis of epidermal differentiation markers revealed that the knockout of the *HSPA2* gene promoted a more mature phenotype of the 3D epidermal constructs. RHE formed from HSPA2-null cells had an undeveloped granular layer.

In the study, we attempted to search for molecular mechanisms that rely on HSPA2 and are related to keratinocytes' ability to form a fully-developed epidermis. For this purpose, using RNA-seq technique we analyzed HSPA2-dependent changes in the global transcriptome in epidermal equivalents derived from control and HSPA2-KO HaCaT cells. Transcriptome profiling of RHE revealed that HSPA2 deficit impairs the late stages of keratinocyte differentiation. Gene set enrichment analysis (GSEA) showed that among differentially expressed genes and over-represented signalling pathways were those associated with keratinocyte differentiation and formation of the cornified envelope.

Conclusions: We showed that HSPA2 plays an important role in controlling the late stages of keratinocyte differentiation including the formation of the epidermal barrier. Given that disturbances in cornified layer development underlie different pathological skin conditions it would be interesting to find whether, and to what extent, HSPA2 can be a novel marker and/or a drug target in human skin diseases.

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[P27] Novel cytidine derivatives with non-steroidal anti-inflammatory drugs for pancreatic cancer treatment

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Modifications of the chemical structure of pyrimidine antimetabolites, such as gemcitabine, are commonly used to improve the metabolic stability and cytotoxic activity profile in anti-cancer therapy. Due to the close similarity of gemcitabine to cytidine, it was decided to use this molecule in a preliminary study on synthesizing new derivatives with non-steroidal anti-inflammatory drugs (NSAIDs) with proven biological activity. In addition to their anti-inflammatory properties, NSAIDs may play a role in anti-cancer activity through their ability to induce apoptosis, inhibit angiogenesis, and enhance the cellular immune response. Therefore combining NSAID derivatives with chemotherapeutics seems to be justified for finding a drug that will target the inflammatory environment of cancer.

A three-step synthesis route has been designed. Modifications of cytidine were made by using the previously synthesized acid chlorides of NSAIDs (aspirin, ibuprofen, ketoprofen, naproxen, and flurbiprofen). The introduction of NSAIDs acid chlorides at the N4 amino group and the C5 ester group lead to the preparation of a series of (pro)drugs. Selective substitution at the amino group has been achieved by simultaneously blocking the C3 and C5 hydroxyl groups of the cytidine molecules with the 1,3-dichloro-1,1,3,3-tetraisopropylidisiloxane silyl group (TIPDSiCl₂). The purification of compounds was performed by liquid-liquid extraction and column chromatography, respectively. The proposed synthetic procedure has produced the (pro)drugs with good yield efficiency. The structural identity of the purified compounds underwent analysis and verification by ¹H and ¹³C NMR spectroscopy.

New cytidine derivatives with NSAID derivatives raise hopes of finding a drug that will be effective against cancer cells and will have an affinity for inflammatory sites such as tumor beds. The anticancer effect of the synthesized derivatives will be verified by testing their cytotoxicity impact on pancreatic cancer cells cultured *in vitro*.

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[P28] Effect of plasma-derived exosomes from HNSCC patients on NF- κ B signaling in macrophages

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Introduction: As one of the most immunosuppressive cancers, head and neck squamous cell carcinomas (HNSCC) show an increased NF- κ B activation with downstream production of regulatory T cell (Treg)-attracting immunosuppressive factors. Plasma-derived exosomes from HNSCC patients contain immune modulatory molecules, which can contribute to the immunosuppressive tumor microenvironment (TME). Here, we investigate the influence of plasma-derived exosomes of HNSCC patients on NF- κ B signaling and changes in the immunosuppressive properties of macrophages.

Material & Methods: Exosomes were isolated from plasma of HNSCC patients and healthy donors by size-exclusion chromatography. Monocytes from buffy coats were used to generate primary macrophage cultures, which were incubated with plasma-derived exosomes to investigate their effects. Exosome-uptake by macrophages was investigated by incubation of PKH-stained exosomes with macrophages and investigation by microscopy and Flow cytometry. NF- κ B nuclear translocation was determined and downstream signaling was evaluated by CCL5, CXCL12 and CCL22 ELISA. T cell attraction was investigated by migration assays. Polarization of macrophages was determined by measuring M1/M2 specific markers *via* Flow Cytometry.

Results: Macrophages internalized exosomes in a time-dependent manner resulting in accumulation of exosomes around the nucleus. Exosomes increased NF- κ B activation in macrophages, which was reversible by addition of NF- κ B inhibitors Bay and CAPE. The strongest inhibition, however, was achieved by Curcumin, a natural NF- κ B inhibitor. Interestingly, activated NF- κ B signaling resulted in production of higher levels of CCL22 in HPV positive, but not HPV negative patients. Regarding macrophage polarization, exosomes prevented differentiation into M1 type macrophages, compared to negative control.

Conclusion: Plasma-derived exosomes from HNSCC patients can alter immunosuppressive properties of macrophages. The reversion of NF- κ B activation by several inhibitors may be useful for future clinical therapeutic strategies on modulation of macrophages in the TME. However, HPV-status of the patients has to be considered.

[P29] Differences in miRNA impact on transcript and protein level in two human cancer cell lines

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Introduction MicroRNAs (miRNAs) are small 19-22 nucleotide long RNAs responsible for translation modulation by mRNA degradation or inhibition of protein synthesis. MiRNAs work through RNA-induced silencing complex (RISC) where with Argonaute (AGO) proteins they form the core that targets transcripts by sequence complementarity. In human cells, the degrading properties have only one of four AGO proteins while the others work through translation inhibition or even p-bodies or stress granules forming.

The aim of our study was to examine how different microRNAs influence the expression and translation process of reporter genes in different cell lines.

Materials and methods Two cancer cell lines – HCT116 and Me45 – were transfected with a psiCHECK-2 plasmid with two reporter genes – *Renilla* luciferase and firefly luciferase. *Renilla* luciferase had 8 miRNA target motifs in its 3'UTR end. Targeted sequences were designed for Let-7, miR-24, or miR-21 miRNA. Transient transfection was done with polyethylenimine and after 24 hours levels of mRNAs and proteins were assessed with RT-qPCR or protein activity measurement respectively. Polysome profile was conducted with the sucrose gradient method 48 hours after transient transfection. The presence of transcripts in the fractions was assessed by RT-qPCR.

Results On the mRNA level there were differences in the Let-7 family influence as in HCT116 cells transcript level of *Renilla* luciferase didn't differ from the unregulated transcript (control) but in Me45 this level was lowered compared to the unregulated one. The non-targeted firefly luciferase behaved in similar way. On the protein level, there were differences in the action of the miR-24 and Let-7 family. The efficiency of translation was rising with miR-24 influence in Me45 cells – a higher level of *Renilla* luciferase as for HCT116 there was observed a slight decrease in level. Let-7 family influenced the cells in the opposite direction – in HCT116 was an increase in protein level and in Me45 cells was observed decrease in protein level. The non-targeted firefly luciferase mostly behaved in similar way. Only when the Let-7 regulation was introduced on plasmid firefly luciferase behaved in opposite direction in Me45 cells. Polysome profiles did not differ much between cells but there were more significant differences between miRNA-targeted transcripts and unregulated ones in Me45 cells.

Conclusions Different miRNAs show variety of action on mRNA levels and translation process. MiRNA-targeted mRNAs influence non-targeted transcripts.

[P30] DIFFERENCES IN SIGNALLING INDUCED BY TRANSFORMING GROWTH FACTOR BETA IN NORMAL AND MALIGNANT MAMMARY EPITHELIAL CELLS

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TGFβ1 inhibits epithelial cell cycle progression and promotes apoptosis, contributing significantly to tumor suppression. However, the ability of TGFβ1 to induce and promote epithelial-to-mesenchymal transition (EMT) is associated with increased tumor cell motility and invasion. Thus, TGFβ1 is also regarded as a metastasis inducer. These contrasting TGFβ1 behaviors are known as the “TGFβ1 paradox”. To investigate mechanisms induced by TGFβ1 in, and mammary epithelial cells, we treated MCF10A (normal) and MCF7 (malignant) cells with TGFβ1 and analyzed their morphology, signal transduction (by western blot), and global transcriptional changes (by RNA-seq).

Changes in cell morphology (towards mesenchymal phenotype) were seen starting from the third day of TGFβ1 treatment in both cell lines. In addition, changes suggesting apoptosis initiation were observed in MCF7 cells. TGFβ1 treatment resulted in the activation of canonical signaling (SMAD3 phosphorylation) already after one hour. It was connected with elevated levels of SNAI1 (that is known to repress epithelial E-cadherin) and extinction of the response only in MCF10A cells. On the contrary, SMAD3 phosphorylation was observed for several days in MCF7 cells after a single stimulation, which suggested that TGFβ1 may induce positive feedback, possibly via released cytokines. RNA-seq results revealed that indeed, the level of *TGFβ1* transcript was strongly induced (auto-stimulation) only in MCF7 cells, while *SNAI1* was only in MCF10A. Moreover, only in MCF7 cells, the expression of *NOG* (coding for an inhibitory protein of BMPs, which act also in SMAD signaling) was strongly induced. Thus, activation of genes from enriched terms: *Hallmark tgf beta signaling* and similar *Hallmark epithelial-mesenchymal transition*, was different in both cell lines. In addition, GSEA analysis indicates for inhibition of genes involved in DNA replication and cell cycle after TGFβ1 treatment. This effect was better visible in nontumorigenic MCF10A cells than in cancer MCF7 cells. Moreover, only in MCF10A cells, genes involved in RNA biogenesis and translation were downregulated, while those involved in DNA repair (e.g. base excision repair) were upregulated, which possibly facilitate surviving of these cells after TGFβ1 treatment. In MCF7 cells, which are estrogen-dependent, genes involved in cholesterol biosynthesis and estrogen signaling were downregulated, which may result in the inhibition of processes regulated by estrogen (e.g. proliferation). Genes involved in the regulation of inflammatory responses and p53 signaling (especially *TP63* and *CDKN1A*) were more strongly induced in MCF7 than MCF10A cells, which may be associated with the induction of apoptosis observed only in MCF7 cells.

Summarising, TGFβ1 induced a different set of genes in normal and cancer breast epithelial cells that may reflect the observed difference in their response.

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[P31] Antitumor effect of a different doses of TLR7 agonist in murine melanoma model

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Introduction Imiquimod (IMQ), a drug from aminoquinoline group, is a TLR7 agonist. It acts as an immunostimulant and radio-sensitizing agent. IMQ stimulates both non-specific and specific immune response. Despite studies conducted, there are no unambiguous data showing how IMQ affects the condition of tumor blood vessels. Tumor vasculature play the main role in tumor progression. Formation of new abnormal blood vessels which increase area of hypoxia leads to cancer relapse.

Methods We studied the therapeutic effect of different doses of imiquimod in murine melanoma tumors. We checked how various doses of IMQ (10µg, 50µg or 100µg/ in 100µL sterile endotoxin-free water) affect the blood vessels in tumors of treated mice compared to a group of control mice. We also evaluated the effect of imiquimod on B16-F10 melanoma tumor cells and H5V endothelial cells death *in vitro*.

Results We observed that imiquimod in a single dose of 10µg and 100µg, injected directly into tumors, similarly inhibits (around 50%) the growth of B16-F10 murine melanoma tumors compared to the control group. Increasing the dose to 100µg of IMQ did not improve the antitumor therapeutic effect. The 50µg of IMQ inhibits the growth of tumors more efficiently (around 75%) compared to other doses and the control group. It seems that a single dose of 50µg most effectively reduced tumor blood vessel density and increased the pericyte-covered blood vessels. Hypoxia regions in sections obtained from tumors of treated mice were decreased. Additionally, we observed that IMQ causes death of tumor and endothelial cells in a dose depending manner *in vitro*.

Conclusions Imiquimod may change the condition of tumor blood vessels in a selected dose. This knowledge may help with optimization of the combination IMQ with e.g. radiotherapy to elicit synergistic effect in controlling further tumor growth. The obtained data are preliminary and requires further research.

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[P32] Activation of the NF- κ B pathway as a hypothetical element of the “radiation-induced bystander effect” mediated by exosomes released by irradiated cells

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Introduction/Rationale The aim of the analyzes was to verify the hypothesis that exosomes released by irradiated cells have the ability to activate the NF- κ B pathway in non-irradiated target cells. Exosomes are nano-vesicles released by cells into the extracellular environment and play an important role in communication between cells. A number of studies indicate that exosomes derived from irradiated cells change the resistance of target cells to radiation and may be mediators of the so-called radiation-induced bystander effect (RIBE). The transcription factor NF- κ B is involved in the regulation of the cellular response to various types of stimuli, including the inflammatory response. One of the factors that activate the atypical NF- κ B pathway is ionizing radiation (IR). A series of data indicate that exosomes released by irradiated cells contain proteins and RNA molecules potentially involved in regulating the inflammatory response in target cells. However, there is no direct evidence that target cells receive such exosomes to activate the NF- κ B pathway, which would be key to understanding the mechanisms involved in the RIBE mechanism.

Methods Human FaDu and HCT116 cells were used as a research model. Exosomes were isolated from the culture medium by the method combining centrifugation and molecular filtration (SEC). Cells were irradiated with 2 Gy or 8 Gy. The isolated exosomes were administered to "naive" cells, and the effects induced by them were tested between 15 minutes and 4 hours after administration (exosomes released by non-irradiated cells will be used as controls). The ability to activate the RIBE mechanism by exosomes was confirmed by analyzing the clustering of phosphorylated histone gammaH2A.X (immunocytochemistry). The kinetics of NF- κ B activation in target cells was examined by Western-blot method using specific antibodies to proteins involved in the regulation of this pathway. Activation of specific genes regulated by NF- κ B was analyzed by quantitative PCR.

Results The activation of the NF κ B pathway at the transcriptional and protein levels was much stronger after incubation with TNF α than after direct irradiation of the cells. Changing the medium just before the time of the factor stimulation has a significant effect on the activation of the NF κ B pathway. The exosomes derived from irradiated FaDu as well as HCT116 cells caused the so-called Radiation Induced Bystander Effect (RIBE) in acceptor cells - increased number of γ H2AX foci in the nucleus compared to untreated control. NF- κ B pathway activation was not observed either at the protein level or at the level of genes activated upon induction of this pathway.

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[P33] PSMA theranostics – opportunities and challenges

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Rationale Prostate-specific membrane antigen (PSMA) is highly expressed in poorly differentiated, metastatic and castration-resistant prostate cancers. A number of diagnostic (e.g. 68Ga-PSMA-11, 18F-PSMA-1007) and therapeutic radiopharmaceuticals (177Lu-PSMA-617, 225Ac-PSMA-I&T) have been successfully developed and are currently in routine clinical use as well as in clinical trials.[1] We are producing on-site a 68Ga-PSMA-11, a well-studied diagnostic agent, and have recently also initiated the production of 18F-PSMA-1007. The adenoid cystic carcinoma (ACC), the major subtype of salivary gland cancer, often shows PSMA overexpression.[2] Therefore, our goal is to investigate whether 18F-PSMA-1007 uptake correlates with the antigen expression in ACC, and to give an outlook regarding the potential of 177Lu-PSMA-617 radionuclide therapy for patients with ACC.

Methods The nonradioactive reference compound PSMA-1007 has been purchased from ABX (Germany). The 18F-PSMA-1007 has been produced by a one-step procedure using quaternized ammonium salt of PSMA-1007 as a precursor (C₄₉H₅₅N₈O₁₆NH₄⁺). The quality control of the final product was performed according to the European Pharmacopeia and involved the assessment of radiochemical (HPLC, TLC), chemical (HPLC), radionuclide (MUCHA) and biological purity.[3] The binding affinity of the radioligand was evaluated *in vitro* using cancer cell lines. NEMA phantom studies with 68Ga and 18F ligands were performed to compare the PET image quality. Both radiopharmaceuticals were diluted to a concentration of 1.78 MBq/mL and injected into NEMA phantom with 6 spherical inserts ranging in size followed by 2-min PET acquisitions.

Results The overall synthesis time of 18F-PSMA-1007 was about 60 min, including dispensing of the product into sterile vial. The average radiochemical yield of 49,71% ± 9,78 (70-120 MBq) was obtained (not decay-corrected). The identity of the radiotracer and its radiochemical purity (92,01 ± 4,87%, n = 5) were confirmed by radio-TLC and met the requirements specified in the European Pharmacopoeia. Furthermore, the retention time of the radiotracer (γ detection) corresponded to that of the cold compound on HPLC (UV detection). Isotope identification was performed with MUCHA and in connection with half-life measurement radioisotopic purity was confirmed. The sterility of the product was evaluated by the external provider. The images of NEMA phantom have shown the superior resolution of the fluorinated ligand compared to the gallium-labelled analogue. The specificity binding studies *in vitro* are on-going.

Conclusion The preliminary studies with 18F-PSMA-1007 showed promising characteristics of the radioligand. Further experiments in our hospital are scheduled to translate the conjugate into the clinical trial for diagnosis and monitoring ACC progression.

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[P34] Studies on the well-defined choline ionic liquid linear copolymers carrying *p*-aminosalicylate: synthesis, physicochemical characterization and in vitro drug release

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Over the past few years the drug delivery systems (DDS) based on conjugates and self-assembled polymers, including linear, grafted, and star-shaped polymethacrylates have been extensively studied to achieve improvements in therapeutic index and bioavailability at the point of medication administration[1]. The developed technologies allow for precise targeting of the place in the body where medicine should be delivered to enhance drug effectiveness and decrease its side effects. One of the convenient strategies in the synthesis of bioactive ionic liquid (IL) polymers is the modification of a monomeric IL with a relevant pharmaceutical anion to create therapeutically activated monomers, which can be used further to obtain the well-defined copolymers by controlled atom transfer radical polymerization (ATRP).

In this research, the existence of chloride counterions in the quaternary ammonium groups in choline based monomeric IL, e.g. [2-(methacryloyloxy)ethyl]trimethylammonium chloride (ChMACl), was stimulated to undergo anion exchange with *p*-aminosalicylate sodium salt (NaPAS) as the source of the pharmaceutical anion. The resulted [2-(methacryloyloxy)ethyl]trimethylammonium *p*-aminosalicylate (ChMAPAS) was copolymerized with methyl methacrylate (MMA) by ATRP to attain the linear choline functionalized copolymers with various content of the pharmaceutical anions (41%– 52%), which was regulated by the initial ratio of ChMAPAS to MMA and monomer conversion degree. The physicochemical characterization was performed by a variety of techniques, which let to verify monomer structure and polymer composition. The length of polymeric chains was evaluated by the total monomer conversion (53%– 71%) and yielding degree of polymerization ($DP_n = 216– 312$). Apparent average molecular weights (M_n) and dispersity indices (\bar{D}) were obtained by SEC giving values in the range of 51600– 96700 g/mol and 1.29–1.36, respectively. The progress of drug release was monitored by the UV-vis method showing that depending on the polymer carrier structure 69–100% of *p*-aminosalicylate anions were exchanged within 26– 74 hours by phosphate anions in PBS imitating a physiological fluid.

In conclusion, the synthesized well-defined linear copolymers P(ChMAPAS-*co*-MMA)s with different ionic contents were investigated to demonstrate their potential as carriers in DDS. The results confirmed that the use of pharmaceutically active trimethylammonium-based IL monomer carrying PAS is excellent to design polymeric carriers containing the adjusted amount of therapeutically active anion. The application of studied DDS is focused on the treatment of bacterial infections and respiratory diseases, including tuberculosis.

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[P35] The effect of normalization techniques - comparison of methods on the example of Western Blot analysis for HeLa cells

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Data analysis is necessary for correct interpretation of the results. In experimental research, a very important stage in analysis is data normalization. Normalization is a stage of preliminary data processing that consists in comparing the samples of interest with reference or control samples. Skilful analysis and normalization are fundamental in the case of research dealing with the regulation of gene expression and the dynamics of changes in expression levels.

In the case of studies on gene expression, especially protein levels using Western blot, the most commonly used method for normalization is with compare protein levels to so-called housekeeping proteins (HKPs). These are proteins that are highly stable and do not change in expression in the presence of a stress factor (such as microRNA, IR, UV radiation, or chemical compounds). However, when lesions caused by stress factors affect signalling pathways in which such housekeeping genes are involved, it appears that HKPs may not be used as references in all cases as they exhibit strong interactions with other components of the signalling pathway. Besides the reference to the HKPs there are also methods of normalization to the total amount of protein that can be determined by e.g. staining the membrane with Ponceau S dye or a fluorescent dye.

In this work, three analyses were compared, depending on the standardization method. Normalization was performed for the GAPDH antibody, Ponceau S and Fluorescent method.

Keywords: *Western Blot, normalization*

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[P36] Evaluating loss of isozyme diversity in breast cancer on transcript level

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Introduction The phenomenon of loss of isoenzyme diversity (LID) contributes to metabolic reprogramming, which is a hallmark of malignant neoplastic transformation. The reprogramming concerns homologous proteins that are involved in catalyzing the same enzymatic reaction, but they can have different kinetic properties, cell localization, and tissue specificity. The LID phenomenon relates to changes in the expression of isozymes. The dominant tumor-losing enzymes that catalyze the response in the survival-critical metabolic process represent potential therapeutic targets.

Methods LID was evaluated for each of the XX human enzymatic reactions on a transcript level. Three selection steps were applied to identify the most promising therapeutic targets:

(i) selecting the reactions that correspond to 2-10 transcripts.

(ii) Identification of reactions showing LID.

(iii) data normalization and ordering of isozymes based on approximation of their expression rate to the ideal LID expression profile using target selection score, which was reflected by the five separate expression features from M1 to M5. Two different approaches were used for LID finding: (i) only one transcript is significantly increased or altered in cancer compared to the corresponding normal tissue, while all other transcripts catalyzing the same reaction show lower expression in cancer; (ii) at least one transcript of a given gene show increased expression compared to normal tissue, while the rest of the transcripts of genes involved in catalyzing the reaction show lower expression. Upregulated transcripts have been defined as therapeutic targets, while the rest with decreased expression have been defined as complementary transcripts.

Results Using the first method, we determined 31 chemical reactions with LID, and for the second method, 67. For the first method, the score that came closest to the ideal LID expression belonged to the 6-alpha sialyltransferase (EC 2.4.99.7) and the worst result was obtained for the dodecenfoyl-CoA isomerase (EC 5.3.3.8). Using the second method, we determined that the plasmalogen synthase (EC 2.3.1.25) has the best expression profile, while the PTEN isozyme (EC.3.1.3.67) is the least similar to the ideal LID profile.

Conclusions We found 41 chemical reactions that complement the list of LID-based targets, which was estimated on the gene level. We found that the 12 reactions were identical for both methods along with the reactions stated in the original study. New targets can be further tested as candidates for new treatments and to extend the current biological knowledge on metabolic genes involved in cancer development.

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[P37] The Oxygen consumption Assay as a new tool for measurement exogenous cell compounds triggering oxidative stress in mitochondria of mice platelets

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Introduction: Molecular oxygen is the key substrate of aerobic metabolism. Is a powerful tool for the monitoring of cell oxygenation, mitochondrial function and the metabolic implications of cell signalling; having been shown to facilitate the real-time assessment of transient changes in cell respiration, oxygen gradients and physiological responses across a range of cell models. Oxidative stress, in addition to studies related to neurodegenerative diseases, cancer or type 2 diabetes, also includes hematopoietic diseases. Oxygen consumption assay can be a useful for analysis of oxidative DNA damage in genome. In turn blood-borm markers are essential to detect different diseases pathologies, inducing proinflammatory states.

Aim of the work: We studied modulation of repair activity in blood in mice receiving antiplatelet, and/or endothelial environmental exogenous compounds (EECs) by monitoring the effects of platelet intracellular and extracellular mitochondrial reactivity. EECs is as a new group of compounds including Carbon MonOxide Releasing Molecule-A1 (CORM-A1), acetylsalicylic acid (ASA), sodium nitrite, 5-bromo Ploty's acid (PABr, HNO donor), protein disulfide isomerase (PDI) inhibitor LOC14, PDI-inhibitor CCS642 and NaHS (H₂S donor). When added to DNA bases, all these compounds might generate preneoplastic lesions as an oxidative DNA damage.

Materials and Methods: The eighty C57Bl6/cmdb mice (20-22 g), (8 or more animals per group) were treated i.p. with EEC at doses of: CORM-A1 30 µM/kg, ASA 75 mg/kg, sodium nitrite 0,17 mM/kg, PABr 100 mg/kg, LOC14 3mg/kg and 30 mg/kg, CCS642 3 mg/kg and 30 mg/kg and NaHS 3mg/kg of body weight, respectively. Control mice treated with vehiculum (Phosphate Buffered Saline, 2ml/kg) were used in these study. The highest dose was chosen to be approximately one-half of the 50% lethal dose for the compound. Mice were kept on their normal diet for all time of experiment duration. Animals were terminated in a one day and 2 ml of the fresh blood on 3.2% citrate or EDTA was collected for direct platelet and mitochondrial DNA isolation.

Results and conclusions: Based on the results we can notice that, after treatment with appropriate platelet mitochondrial compounds the oxygen consumption in the real time is the greatest for ASA, for CORM-A1, NaNO₂ and than for other used compounds. This is probably due to the selective passing of substances of different molecular weight from which the aspirin is the smallest. ASA inhibits the ability of platelets to aggregate and form clots. Salicylic acid and other analyzed substances relaxe the outer layer of the membrane that probably surrounds the blood platelets and penetrates directly into its mitochondria and might inhibit cyclooxygenase which it is responsible for the production of thromboxane. These processes can affect the differences of 8-oxoG repair rate observed in mitochondrial DNA within one day in all EECs compounds in descending order; CA1>LOC14>NAHS>CCS642>ASA>NaNO₂ >PABr. Probably the repair of 8-oxoG depends on the spatial conformation which fit into its active center of motif zing finger (herpin-helix-herpin) and availability of apendonucleases as APE1, which can increase OGG1 turnover on damaged DNA

[P38] A novel method of α -aminobisphosphonates synthesis and their potential application

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Bisphosphonates (BPs) are a group of compounds that are extensively studied around the world. The interest in these compounds results from their high and multidirectional biological activity (including antiresorptive, antibacterial, antiviral, antiprotozoal, and anticancer), as well as their high affinity to hydroxyapatite found in bones. An important subclass of bisphosphonates are α -aminobisphosphonates, for which a broad spectrum of biological activity has also been repeatedly proven.[1] Thanks to their high affinity for hydroxyapatite and the presence of the amino group which enables their further structural modification, they have also become of interest for new drug delivery systems to bone tissue.[2] Due to the important applications of α -aminobisphosphonates, the development of a general and efficient method for their synthesis is highly sought after.

Herein, we present the results of our studies on the development simple and efficient methodology for the preparation of *N*-protected bisphosphonic acids analogs. The key step of the proposed method involves a one-pot, three-component reaction of 1-(*N*-acylamino)-1-ethoxyphosphonates with triphenylphosphonium tetrafluoroborate and triethyl phosphite.[3] The starting diethyl 1-(*N*-acylamino)-1-ethoxyalkylphosphonates were synthesized according to a previously described two-step protocol which consists of acylation of the imidate hydrochloride with an acyl chloride and the Michaelis–Becker-like addition of diethyl phosphite to ethyl *N*-acylimidate.[4] The proposed strategy facilitates good to excellent yields of products and allowed us to obtain 14 structurally diverse bisphosphonic analogs of protein and non-protein α -amino acids under mild reaction conditions.[3]

The possibility of selective deprotection of the amino group of the obtained compounds and the fact that the proposed method leads to bisphosphonate esters, not acids, enables their further use in the synthesis of targeted therapeutics as bone-seeking agents. As numerous data suggest that BPs can enhance the effect of various anticancer drugs,[5] for now, it is planned to subject the obtained compounds to hydrolysis and explore their synergistic action with other well-known cytostatics.

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[P39] Stochastic modeling of the metastatic process in non-small cell lung cancer

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Introduction Lung cancer is the most common type of cancer in Poland. There are known two main types of lung cancer non-small cell lung (NSCLC) and small cell one (SCLC). The most common type of lung cancer is NSCLC (85% of all lung cancer cases). Lung cancer is usually diagnosed at an advanced stage due to unspecific symptoms.

Lung cancer is highly metastatic leading to the situation that patients die not because of the tumor present in the lung but a metastatic tumor. Thus, it is crucial to predict when and where a metastatic tumor will appear.

Methods To better understand the emergence of metastasis, we constructed a stochastic mathematical model describing the process of lung cancer dissemination. The model is in form of stochastic differential equations (SDEs).

The model describes the primary tumor dissemination via two routes lymphatic and blood vessels. Primary tumor, tumor in the local lymph nodes, and distant tumor forms separated compartments where dissemination is a process that can transport cancer cells from one to another compartment. As the model does not include cancer treatment, it only describes palliative patients.

The model was calibrated to clinical data from lung cancer patients treated at the National Research Institute of Oncology (NRIO), Gliwice Branch. The cohort includes

Results The mathematical model faithfully describes the lung cancer dissemination rate as the resulting Kaplan-Meier plot from the simulations fits the one from the available data.

Next, we performed the global sensitivity analysis of the model to find key variables affecting the time-to-metastasis (MFS). We have found that five variables are affecting the MFS. Among them, is the carrying capacity of the primary tumor.

Conclusions The developed mechanistic model was applied to find the key variables affecting so-called metastatic free survival that is the time elapsed from the diagnosis until metastatic relapse. Interestingly, a tumor carrying capacity that reflects tumor vascularization.

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[P40] Hexokinase 2 as a target for anticancer therapy

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In designing anti-cancer therapies, the drug selectivity is an important factor. Currently, therapies targeting cancer-specific isoforms of metabolic enzymes are among the most promising anti-cancer strategies. Hexokinase 2 (HK2) is a prominent enzyme which level is higher and its induced expression is observed in many tumours compared with normal cells. However, the metabolic HK2 inhibitors known so far are characterised by a low specificity of action and a high toxicity towards normal cells. Therefore, the researchers face the challenge of finding new HK2 inhibitors that will live up to their expectations.

The aim of this study was to evaluate the antitumour activity of potential hexokinase 2 inhibitors. The research hypothesis assumed *that the used compounds act through the inhibition of HK2 activity*. Human hepatocellular carcinoma cell lines: HepG2 and HUH7 and a normal cell line HMEC-1 were used and treated with the tested compounds for 72 h. The MTT assay was involved to cell survival assessment as well as the HK2 activity and its expression were verified by Western Blot technique.

Based on the results, it was shown that tested compounds inhibited HK2 activity and exhibited their cytotoxic effects. It was observed that HepG2 cells were significantly more sensitive to the tested compounds than HUH7 cells. Hepatocellular carcinoma (HCC) cells were also shown to be metabolically different from normal cells, as they exhibited higher expression of hexokinase 2.

Keeping in mind that HK2 expression is very low in most normal tissues but much higher in HCC, the targeting of HK2 may allow to selective elimination of HCC with a significant reduction in the risk of side effects. Thus, hexokinase 2 inhibitors seem very promising anticancer compounds, but still there is a need for further research to understand exactly how they work.

[P41] Analysis of changes in the proteome of human neuroblastoma cells with silenced *PHLDA1* gene

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Introduction Neuroblastoma (NB) is a common solid tumor of early childhood. Our previously performed studies demonstrated that *PHLDA1* protein (pleckstrin homology-like domain family A member 1) expression was strongly increased in IMR-32 NB cells treated with 14G2a anti-GD2 therapeutic antibodies, which was correlated with decreasing survival of the cells [1]. In this study we created *PHLDA1*-silenced IMR-32 cell line in order to better understand the role of this protein. Modified cells display numerous changes in the proteome, one of the most interesting is ABCB1 overexpression.

Methods *PHLDA1* gene in IMR-32 NB cells was silenced by transfection with shRNA plasmid. The cells with downregulated *PHLDA1* (sh*PHLDA1*) and control cells (shCtrl) were subjected to the global mass spectrometry analysis. *PHLDA1*-silenced and control cells were exposed to hEGF at concentration of 20 ng/ml for 15 min or treated with EGFR inhibitors, lapatinib or gefitinib, at concentration of 5 μ M for 72h. Relative ATP content, protein concentration in lysate and relative number of the cells (living, dead and total) were measured, in comparison to control cells treated with diluent (DMSO). Statistical significance was determined by two-way ANOVA with *post-hoc* Tukey test. Protein levels were detected via western blot.

Results Cells with silenced *PHLDA1* display changes in the global proteome pattern. Mass spectrometry analysis revealed that in sh*PHLDA1*-modified cells the level of ABCB1 efflux pump is strongly upregulated, as compared with shCtrl cells, which was confirmed by western blot. Interestingly, an analysis with the R2 programme showed that high level of ABCB1 in patients suffering from NB correlates with longer survival rate. Silencing of *PHLDA1* led also to changes in the phosphorylation pattern in NB cells, where the phosphorylations of the proteins involved in mRNA splicing were the most affected. There were no changes in oxidation and acetylation patterns in *PHLDA1*-downregulated cells. Additionally, inhibition of *PHLDA1* gene affects tyrosine kinases pathways. The level of EGFR and its phosphorylated form is significantly higher in sh*PHLDA1* cells. Moreover, the cells with silenced *PHLDA1* are more sensitive to EGFR inhibitors treatment.

Conclusion We showed that downregulation of *PHLDA1* in NB cells causes overexpression of ABCB1 transport protein, which is believed to be a major protein associated with drug resistance in many cancers [2]. We also found the correlation between *PHLDA1* inhibition and EGFR upregulation, which led to sensitization of sh*PHLDA1* cells to EGFR inhibitors used in therapies.

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[P42] Prognostic Value of Molecular Intratumor Heterogeneity in Primary Oral Cancer and Its Lymph Node Metastases Assessed by Mass Spectrometry Imaging

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Introduction/Rationale Different aspects of intra-tumor heterogeneity (ITH), which are associated with the development of cancer and its response to treatment, have postulated prognostic value. Mass spectrometry imaging (MSI) combines the analytical power of mass spectrometry with the ability to register spectra for individual positions (pixels) across the surface of tissues, which reveals molecular information annotated spatially with morphological pictures. Here we searched for potential association between phenotypic ITH analyzed by MSI and prognosis of head and neck cancer.

Methods The study involved tissue specimens resected from 77 patients with locally advanced oral squamous cell carcinoma, including 37 patients where matched samples of primary tumor and synchronous lymph node metastases were analyzed. A 3-year follow-up was available for all patients which enabled their separation into two groups: with no evidence of disease (NED, n = 41) and with progressive disease (PD, n = 36). After on-tissue trypsin digestion, peptide maps of all cancer regions were segmented using an unsupervised approach to reveal their intrinsic heterogeneity. Pairwise similarity index was calculated to assess similarities between a compared pair of spectra. The similarity index was computed in two manners: within particular ROI (intra-ROI similarity; e.g., intra-tumor [intra-T], intra-metastasis [intra-N]), and between different ROIs (inter-ROIs similarity; e.g., inter-T/N) creating all possible combinations of compared spectra pairs. Cohen's [d] effect size was calculated to indicate discriminatory components between pairwise compared subsets of spectra. Simpson's diversity index was calculated to assess T-ROIs heterogeneity. Testing of statistical hypotheses was applied to determine significance of differences in the number of clusters, calculated values of Simpson's diversity index, and cluster sizes between the NED and PD patient groups.

Results We found that intra-T similarity of spectra was higher in the PD group and diversity of clusters identified during image segmentation was higher in the NED group, which indicated a higher level of ITH in patients with more favorable outcomes. Furthermore, a positive correlation between ITH and histopathological lymphocytic host response was observed.

Conclusions/Novel aspects We found that phenotypic ITH revealed by MSI within tumor tissue resected from patients with locally advanced oral cancer was higher in patients with favorable outcomes. On the other hand, neither clinical cancer stage nor cancer localization was associated with the observed ITH, which suggested an independent prognostic value of this parameter. Moreover, the level of ITH was associated with histopathological pattern of infiltrating lymphocytes and the signature of molecular components that correlated with long-term outcomes could be associated with proteins involved in the immune functions. Hence, we hypothesized that higher ITH observed by MSI in cancers with a better prognosis could reflect the presence of heterotypic components such as infiltrating immune cells enhancing the response to the treatment.

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[P43] Sampling-oriented modelling of cancer evolution

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Introduction: The rapid development of Next Generation Sequencing (NGS) methods resulted in the development of many methods for the deconvolution of tumor subclonal structure from the NGS data. Whereas many of them figure out tumor subclonal structure by clustering the mutations with similar allelic frequencies, recently published MOBSTER applies the population genetics model not only to recognize the tumor clonal structure but also to infer the evolutionary parameters of identified subclones. Despite the criticism of the model's assumptions, it offers an interesting way to investigate the process of tumor evolution. An important limitation of this method is its requirement for deeply sequenced Whole Genome Sequencing (WGS) data. MOBSTER might be unable to recognize the low-frequency neutral mutations tail if many of them were removed by the variant calling algorithm due to data quality issues like sequencing depth. In our work, we reimplemented this model aware of this *sampling* event to fit the powerlaw neutral mutations tail into Whole Exome Sequencing data (WES), cheaper and more often available than WGS data.

Materials & Methods: Sequencing reads from our unpublished yet breast cancer study (WES, 10 patients, 2-3 tumor samples per patient) and the Acute Myeloid Leukaemia study of Shlush (2017) (WGS, 10 patients x 2 tumor samples, 50x sequencing depth) were aligned to GRCh38 genome using BWA aligner. Then the aligned reads were prepared for variant detection using the GATK toolkit and finally, short SNV variants and indels were detected using Mutect2. We fit our model in three steps. First, we use MJ Williams's formula (2016) for the cumulative number of mutations which should be linear for the $M(f) \sim \alpha * 1/f$ neutrally evolving mutations. The formula is modeled with many linear models in the moving window and the lowest fitted is selected to model power-law neutral tail. Finally, we fit the binomial distributions to the residuals of the neutral power-law model. The algorithm of model fitting was implemented in an R package *cevomod*. For users not familiar with R we also created a GUI application to facilitate browsing of the results.

Results: Sampling-aware implementation of the model successfully fitted models with neutral tails to the sequencing data in both datasets, where MOBSTER had previously failed. It also allowed us to estimate sampling rates - fractions of lowfrequency mutations filtered out due to quality reasons, and the total numbers of mutations in the neutral tails under the assumption of shape of the neutral tail.

Conclusions Although MOBSTER's model assumptions were recently challenged by some authors, model-based approaches in the analyses of intratumor heterogeneity can greatly improve our understanding of tumor evolution. Our implementation allows extending the usage of MOBSTER-like models on the wider range of lower-quality sequencing data.

[P44] MCPIP1 protects from the development of hepatocellular carcinoma

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The MCPIP1 protein is one of the regulators of inflammation. As a result of RNase activity regulates level of many transcripts, including pro-inflammatory cytokines for example IL-1B and IL-6. Additionally, inhibits the activation of NF-κB transcription factor which is involved in the immune response. Recent studies show that MCPIP1 level decrease during the progression of breast cancer and clear cell renal cell carcinoma (ccRCC). Our team's research indicates an important protective role for the MCPIP1 protein in the ccRCC development. Inhibition of the expression of the *Zc3h12a* gene, coding MCPIP1, resulted in faster tumor growth. Additionally, our results indicate the participation of MCPIP1 protein in the regulation of tumor migration and metastasis.

Currently we work on a mouse model lacking the expression of the *Zc3h12a* gene in the liver (Mcpip1 KO). In order to better study the differences between wild-type (wt) and Mcpip1 KO mice groups, hepatocytes were isolated by perfusion method, and then NGS analysis was performed. To induce hepatocellular carcinoma (HCC) we administered diethylnitrosamine (DEN) to two-weeks old mice. After 40 weeks we collected material and performed RT-PCR and Western Blot analysis.

It should be emphasized, that long-term exposure of mice to DEN leads to the development of HCC in almost 100% of males, while in females, the effectiveness ranges from 10-30% according to current literature. The effectiveness of HCC induction after DEN administration in our model is dependent on the MCPIP1 protein. In the group of control mice, no tumor development was noted, while in females lacking the MCPIP1 protein, tumors developed in almost 100% of cases. Knockout of the *Zc3h12a* gene in mouse livers leads to change in several signaling pathways for example steroid hormone biosynthesis or protein export. Moreover, in this group we observed immune cell infiltrates and an increase in the level of transcription factors responsible for the development of the mesenchymal phenotype.

The obtained results indicate protective role of MCPIP1 protein in HCC development and progression which has not yet been described in literature.

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[P45] Comparative analysis of human fibroblasts and tuberous sclerosis cells

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Introduction Tuberous sclerosis (TSC) is an uncommon but multi-system genetic disease caused by mutations in the *TSC1* or *TSC2* genes, which encode hamartin and tuberin proteins. They are responsible for regulating the mTOR protein, which controls various processes, including protein synthesis and cell growth control. The mTOR pathway is activated by the lack of hamartin and tuberin inhibitory complex (1-2). TSC symptoms vary from mild to life-threatening ones. As a result, the patient's quality of life may be worsened. For example, benign tumors may develop in numerous organs like in the brain, kidneys, lungs and on the skin. Despite many studies on the pathomechanisms of disease development, there is still a lack of optimal targeted therapy (3-4).

Methods Cells were isolated from the skin of a patient suffering from tuberous sclerosis: TSC fibroblast-like from the benign tumor on the skin and normal fibroblast (NHFib) from healthy skin. Cell growth was followed during the standard cell culture. Cell morpho-physiological properties were investigated on the optical and fluorescent microscopes, and also on the atomic force microscope (AFM) in the spectroscopy mode. The motility and adhesion of cells were quantified by the scratch and the cell-substrate adhesion assays, respectively. Finally, cell metabolism was analyzed during cell treatment with staurosporine by the dehydrogenase (MTT) and esterase (FDA) activity assays, as well as the reactive oxygen species (ROS) production assay.

Results Genetic instability of TSC cells was confirmed by the observance of DNA damage *via* the immunofluorescence staining and single cell gel electrophoresis. Although morphologically similar, TSC and NHFib cells presented differences in the growth rate, motility, and adhesion. However, no significant changes to the cell elasticity (AFM measurements) and cytoskeleton (immunofluorescent staining) were observed. Cell exposure to staurosporine caused significant changes in reactive oxygen species production. TSC cells were also more sensitive to staurosporine treatment than the NHFib cells.

Conclusions The results indicate that although the morphological and mechanical properties of cells do not change significantly, the other investigated properties of TSC cells show undeniable differences in comparison with NHFib cells.

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[P46] Mesenchymal prognostic signature in ovarian cancer

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Background In our previous microarray study we identified the 96-gene signature related to differential survival of patients with high-grade serous ovarian cancer (OC) [1]. Top differentially expressed genes were e.g. POSTN, COL11A1, SFRP2, MFAP5, ITGBL1, LOX. Similar mesenchymal signature has been observed also by others, but it has been ascribed to cancer associated fibroblasts, not epithelial cells. However, we postulate that these genes can be also expressed by cancer cells themselves.

Materials and methods For survival analysis we used Kaplan-Meier Plotter and Microarray Gene Expression Database of OC Subtype (CSIOVDB). Proteins expression was assessed by Heterogeneity_Analysis_Portal (Imdomics.org). Interaction networks were judged by STRING. Molecular cloning was performed using retroviral gene transfer; in vitro functional tests were done according to standard procedures; gene expression analyzed by PCR.

Results STRING algorithm applied to our prognostic signature showed interactions typical for proteins engaged in the function and structure of extracellular matrix. Our own qRT-PCR analysis, as well as Kaplan-Meier Plotter and CSIOVDB analysis confirmed that mRNA level of majority of genes from our negative prognostic signature is significantly related to survival of OC patients. Using Heterogeneity_Analysis_Portal we analyzed 24 out of these genes and found that they are strongly expressed by tumor stromal cells, while weakly by epithelial cells. We analyzed ten of these genes in several OC cell lines by semi-quantitative RT-PCR, and we found that they are expressed by epithelial cells as well. By functional in vitro assays we observed that overexpression of these genes (ITGBL1, MFAP5, SFRP2) may affect OC cells phenotype (migration, invasiveness, proliferation, chemosensitivity).

Conclusions Mesenchymal signature with negative prognostic significance in OC is expressed mostly by stromal, but also by epithelial cells, and may affect phenotype of the latter. Exact role of these genes in OC cells remains to be assessed.

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[P47] Analysis of early and late effects of doxorubicin on histomorphological and ultrastructural changes of mouse lungs

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Doxorubicin (Dox) is very often used in systemic treatment of variable cancers. The cytotoxic effect is based on the production of an excessive amount of free radicals and reactive oxygen species, consequently leading to oxidative stress. Unfortunately, it has been shown that intensive anticancer treatment of breast cancer with doxorubicin can have a negative effect on heart, lungs and breathing. Pulmonary complications can occur either during treatment, shortly after its completion or many years later. Anticancer therapy can damage the lungs by an inflammatory mechanism leading to infection and subsequent pulmonary fibrosis causing impaired oxygen delivery into the bloodstream.

The aim of this study was to evaluate the early and late effects of doxorubicin on the histomorphological profile of lung tissue and the ultrastructure of lung cells in healthy mice.

The experiment was carried out on female C57Bl/6J mice. The animals were kept at a constant temperature (21-22°C) on a daily cycle of 12 hours light, 12 hours dark, with constant access to water and food "*ad libitum*". Mice were divided into three control groups and six experimental groups, which were given doxorubicin at a dose of 4 mg/kg, b.w. (twice a week for two weeks, 1 mg/kg b.w.) and 8 mg/kg b.w. (twice a week for two weeks, 2 mg/kg b.w.). Lung fragments were collected 10, 20 and 40 weeks after injection of Dox, and then fixed in 3% glutaraldehyde in 0.1M cacodyl buffer (pH 7.3). After 2 hours, they were rinsed in 0.1M cacodyl buffer they were postfixed in 2% OsO₄ in 0.1M cacodyl buffer for 1 hour, dehydrated in a graded series of ethanol and propylene oxide series and embedded in EPON 812 resin. Semi-thin (80-150 nm) and ultra-thin (40-60 nm) sections were sliced on a Reichert-Jung ultramicrotome. The ultrastructure of the cells was observed on a Tesla BS-500 transmission electron microscope with a 1K-Frame Transfer-CCD digital camera.

Histomorphological results after doxorubicin administration showed swelling of endothelial capillary, alveolar wall discontinuity, decreased alveolar lumen, increased numbers of type II pneumocytes, alveolar wall discontinuity and thickened alveolar septa. In contrast, ultrastructural analysis showed mitochondrial damage, vacuolization of the cytoplasm, condensation of nuclear chromatin, swelling of the rough endoplasmic reticulum and lamellar bodies in mouse pneumocytes. Histomorphological and ultrastructural changes in mouse lungs correlated with drug dose and time point after treatment. Doxorubicin induced the greatest changes in the lungs at a dose of 8 mg/kg, 40 weeks after drug injection.

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[P48] Healthy women with detectable in blood methylation of BRCA1 display other significant genome-wide methylation changes

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Introduction There is currently a lack of reliable biomarkers of disease predisposition for women who do not carry pathogenic variants in high-penetrance susceptibility genes, such as BRCA1. However, recent research shows that detectable in blood cells epimutation of BRCA1 gene is significantly associated with risk of incidence of triple-negative breast cancers (TNBCs) and high-grade serous ovarian cancers (HGSOCs). It is, however, unknown whether the presence of BRCA1 methylation in the peripheral blood is an isolated event or a proxy of multiple genome-wide methylation changes.

Methods We obtained peripheral blood samples from 68 healthy women, 35 of whom were BRCA1 epimutation carriers and 33 non-carriers. The DNA was subjected to bisulfite modification using Zymo Research kit and BRCA1 methylation was confirmed with EpiMelt BRCA1 methylation Kit from MethylDetect ApS. The genome-wide methylation profiling was performed using EPIC 850k microarray, Illumina Inc. The data was processed in R using ChAMP pipeline, Qlucore by Qlucore Lund, and FUMA. The model was adjusted for WBC fractions and batch effect.

Results We identified 1046 significantly differentially methylated probes (DMPs) with methylation difference of ≥ 0.05 in patients with PCR confirmed BRCA1 methylation that were not present in patients negative for this epimutation. The identified DMPs annotated to 691 genes and FUMA-based GSEA (Gene Set Enrichment Analysis) showed that those genes were significantly enriched in 2 gene sets: MIKKELSEN-MEF-HCP-H3K27ME3 with enrichment of 52 genes (adjusted p-value = $1.37e-8$) and BENPORATH-ES-WITH-H3K27ME3 with enrichment of 73 genes (adjusted p-value = $8.38e-8$).

Conclusion Our results suggest that detectable in blood BRCA1 methylation is a surrogate of larger genome-wide methylation changes. Those changes are may be involved in differentiation of estrogen-negative breast cancer. Nevertheless, further studies are required to determine whether those changes are linked with increased risk of breast cancer. Interestingly, our data also confirmed that methylation level of BRCA1 detectable with PCR-based EpiMelt BRCA1 assay was less than 1% and that level of methylation was not detectable with EPIC 850k microarray.

Keywords: breast cancer; epigenetics; methylation; BRCA1

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[P49] S1 protein-induced viral infection from neoplastic cells to co-incubated HaCaT keratinocytes

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Aim SARS-CoV-2 is the virus that causes the COVID-19 disease characterized by, inter alia, acute respiratory failure. The pathogen enters the host cell using the S-surface protein, which plays a key role in the adsorption of the virion to the cell surface and its fusion with the cell membrane. The S protein consists of two subunits: S1 and S2. The S1 subunit recognizes the ACE2 receptor on the surface of the host cells, while the S2 subunit is involved in the fusion of the viral membrane with the host cell membrane. The activator of the reaction is the TMPRSS2 receptor. These receptors are found in many cells in the human body, incl. in skin cells. Inflammation may occur during viral infections and this may lead to an increase in the expression of interleukins.

The bystander effect is a biological response of a not directly traversed cell to an event in close proximity. These effects rely on intercellular communication based on different modalities: chemical; specific recognition by cell-surface receptors; direct relations between cells developed on special junctions or nexus. **We studied intracellular communication during co-incubation of S1-induced cells with no direct exposed one to the pathogen stimuli.**

Methodology The tests were carried out on lines of normal skin (HaCaT) and neoplastic skin (1205-Lu and 451-Lu). The cells were seeded on the plate (HaCaT) and inserts (melanomas), after 24h co-incubation was started. A fragment of the recombinant surface protein S1 of the SARS-CoV-2 virus was used as the factor simulating the course of the first phase of infection. The experiment simulated the first phase of infection in which virus adsorption takes place on the surface of the affected cell. Spike protein medium (S1) was added to the insert at three doses of 5, 10 and 20 ng / ml, followed by incubation for 72 hours. The mRNA level of activated ACE2 receptor and pro-inflammatory cytokines, infection marker genes (ACE2, IL6 and IL8) was checked by qRT-PCR reaction.

Results IL6 and 8 were expressed after ACE2 over-stimulation in bystander HaCaT cells during co-incubation with direct S1-exposed neoplastic cells. ACE2 becomes activated and causes rapid expression of interleukins, "cytokine storm". On the other hand, neoplastic cells influenced co-incubated HaCaT, depending on the degree of malignancy, with the production of interleukin IL6, preceded by 1205_Lu and IL8 by 405_Lu presence, respectively.

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[P50] Design, synthesis, and *in vitro* studies of new ciprofloxacin derivatives with potential antiproliferative activity

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Ciprofloxacin is a second-generation fluoroquinolone, characterized by high antimicrobial activity, a wide range of indications and rare adverse effects. Despite good antimicrobial activity, ciprofloxacin is also known from its anticancer properties such as cell cycle arrest and damages of nucleic acids, which trigger apoptosis. The anticancer and pro-apoptotic activity of ciprofloxacin was found in nonsmall cell human lung cancer melanotic melanoma, triple-negative breast cancer, bladder, colorectal and pancreatic cancer as well as in mouse melanoma and rat glioblastoma. Moreover, the current research indicates that blocking of MITF and Mcl-1 proteins by ciprofloxacin could be considered as a potential target in malignant melanoma treatment.

The purpose of this study was to design and synthesize new ciprofloxacin derivatives with antiproliferative properties. In the first stage of the study, 120 molecules containing the ciprofloxacin system were selected for virtual selection from an in-house library of compounds. The GOLD program was used in the docking procedure. The MITF and Mcl-1 proteins were selected as molecular targets. Based on the results of the docking procedure, 20 ciprofloxacin derivatives were selected for synthesis. New potential MITF and Mcl-1 inhibitors were synthesized using a copper (I) catalyzed azide-alkyne cycloaddition (CuAAC) protocol. The selected compounds were initially subjected to an evaluation of their antiproliferative activity.

Preliminary studies done on melanoma cells (COLO829, G361) indicate that the new compounds exhibit high cytotoxic activity against the analyzed melanoma cell lines. When assessing the structure-activity relationship, it was observed that ciprofloxacin derivative which has a cyano group in the para position of the phenyl substituent, had the strongest cytotoxic effect. In the case of the compound, which is a substrate for the remaining analyzed hybrid compounds, significant cytotoxic activity was observed only against melanoma cells of the COLO829 line.

[P51] Metabolomic status of BY2 cells adapted to (long-term) osmotic stress

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Over the coming decades, water shortages and osmotic stress will likely have growing adverse effects on plant cell functioning. Surprisingly, despite the response of plant cells to abiotic stress conditions being well studied, we know little about the molecular rearrangements that allow cells to adapt to chronic adverse conditions.

Here we present data on tobacco BY2 suspension cells adapted to osmotic stress in 2006: a perfect and unique model for investigating the molecular background of adaptation processes. The adapted BY2 cells are smaller than those living under normal conditions, similar to many plants living under chronic stress. It has been postulated that this decrease in mass and size is a 'cost' of the stress response; plant cells that cannot overcome this cost, die, whereas those that can, develop a state of 'new molecular homeostasis'. Despite this assumption being widely accepted, the molecular basis for the smaller size of plant cells adapted to long-term stress remains unclear.

We utilized an untargeted GC /MS analysis to study four BY2 lines adapted to various stress conditions (450 mM mannitol/sorbitol and 190 mM NaCl/KCl) and controls. The high-throughput metabolome study was supplemented with mitochondria structure analysis and NGS mRNA study focused on mitEC changes as well as with biochemical examination of MDA, radical levels, etc.

Indeed, the abundance of numerous core metabolites was unchanged in adapted cells compared to controls suggesting the 'stable state' of essential cellular molecular pathways. Nevertheless, the clear signals in cells' adaptation to osmotic stress were visible with significantly up-regulated proline or sorbitol. Moreover, some signs of the different molecular states of years-adapted cells compared to known data on shorter stress exposition were found. For example, the increased level of MDA was not correlated with radical up-regulation, which suggests the existence of some unknown defense mechanisms of cells exposed to years-long stress. The number of differently abundant metabolites was higher in BY2 lines adapted to salt (osmotic) stress compared to Mannitol/sorbitol exposed BY2.

The source of energy for the biosynthesis of stress-related compounds remained not fully recognized in adapted cell lines, as the energy-related genes/metabolites levels were generally unchanged in adapted all BY2 lines. However, signals of increased networking of mitochondria were found.

CONCLUSION The increased levels of compounds enabled cells to function in higher osmoticum found in adapted cells coexisting with symptoms of new molecular homeostasis, in the form of a relatively unchanged core metabolic pathway, together with signals of unknown protective mechanisms of plant cells.

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[P52] The role of MCPIP1 protein in hepatocellular carcinoma resistance to sorafenib

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Introduction Hepatocellular carcinoma (HCC) is highly malignant and most common type of liver cancer and the third leading cause of cancer related deaths worldwide. Currently used therapy is surgical resection followed by radiotherapy. For patients with advanced HCC, the first line treatment is sorafenib, a multikinase inhibitor. Sorafenib acts as anti-angiogenic agent, through the inhibition of tumor endothelium growth and cell survival impairment. However, after an initial period of stable improvement, most patients acquire a strong resistance to this drug followed by relapse. Our group has recently shown that MCPIP1 protein may play an important role in the tumor development and acquiring resistance to sunitinib and sorafenib in clear cell renal cell carcinoma.

The main aim was to **determine the role of MCPIP1 in HCC resistance to sorafenib.**

Methods HCC cell lines resistant to sorafenib were examined to check the level of MCPIP1. We stimulated two human and one murine HCC cell line Huh-7, HepG2 and Hepa 1-6 with DMSO (control) or sorafenib. Next, cells were analyzed for MCPIP1 level (mRNA by real-time PCR and protein by western blot). Cells were checked for the levels of CSCs markers. Tissues from HCC patients were examined for the expression of MCPIP1 and correlated with healthy tissue.

Results We found a decrease in the level of MCPIP1 in HCC patients with tumor progression and lower level of MCPIP1 in tumor tissues compared to healthy adjacent tissue. Moreover, HCC cell lines resistant to sorafenib also expressed lower level of MCPIP1 than sorafenib sensitive cells. We found that 7 days stimulation with sorafenib leads to increased expression of c-Met, STAT3 and epithelial to mesenchymal transition (EMT) markers ZEB2 and Vimentin. In addition we found a slight decrease in the level of MCPIP1 after sorafenib stimulation. MCPIP1 overexpression partially reverses the sorafenib effect.

Conclusions Our data indicates that MCPIP1 may affects the process of acquiring resistance to sorafenib treatment. The obtained results may contribute in enhancing the knowledge of hepatocellular carcinoma, and may help identifying new, more effective therapeutics in the future.

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[P53] Smart pH/Redox Dual-Responsive Nanogels as Doxorubicin Delivery Systems

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Introduction Doxorubicin hydrochloride (DOX) is one of the most potent chemotherapeutic agents being developed for treating a range of malignancies. Free DOX, on the other hand, has poor bioavailability, a tendency to aggregate into fibril-like structures under physiological conditions, which causes cardiotoxicity. Myriad nanocarriers have been developed for encapsulation of DOX, but the colloidal stability of nanocarriers containing DOX is commonly either not addressed or not properly explained.

Aims This research aimed to develop degradable nanogels (with pH/redox sensitivity) to encapsulate DOX, inhibit DOX aggregation in biological media, and enhance DOX release in particular conditions mimicking the cancer milieu.

Methods A photoinitiated free radical polymerization method was used to successfully create nanogels composed of *N,N*-dimethylacrylamide (DMAM) as the main monomer, 2-carboxyethyl acrylate (CEA) as an anionic monomer, and *N,N'*-bis(acryloyl)cystamine (CBA) as a degradable crosslinker. Utilizing the electrostatic attraction between the positively charged DOX and the negatively charged nanogels, the loading procedure was carried out. Three different compositions were prepared, differing in the molar ratios of anionic units of nanogel to DOX (NG-CBA/DOX-1,2) and the content of anionic units (NG-CBA/DOX-3). Non-degradable nanogel with *N,N'*-methylenebisacrylamide (MBA) crosslinker was also prepared (NG-MBA/DOX-1) as control. The colloidal stability of DOX-loaded nanogels was measured at different DOX concentrations up to 278.9 µg/mL.

Results Free DOX was aggregated in biological media at concentration higher than 20 µg/mL, while the use of nanogels could prevent DOX aggregation (in the presence of serum) at DOX concentrations up to ~160 µg/mL. In addition, in conditions of reducing environment (pH 5.0 and 5 mM GSH), DOX release was enhanced from nanogels compared to those in physiological conditions and from non-degradable nanogels. Cytotoxicity study showed that DOX-loaded nanogels had higher cytotoxic effects than free DOX on HCT 116 colon cancer cell line.

Conclusions Smart pH/redox-sensitive nanogels can be used to better control DOX release in reducing environments, resist DOX aggregation in biological media, and improve DOX cytotoxicity.

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[P54] The effect of radiotherapy on cell survival and cytokine secretion in the co-culture model of HNSCCs and normal cells

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Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide. The current treatment options for HNSCC mainly include surgery and radiotherapy. Interactions between cells in the tumor microenvironment have a significant impact on the effectiveness of therapy. The tumor microenvironment consists of cancer cells, fibroblasts, endothelial cells, mesenchymal stromal cells and immune cells.

The aim of the study is to investigate the effect of radiotherapy on cell survival and cytokine secretion in the co-culture model of HNSCCs with fibroblasts or endothelial cells.

We examined two HNSCC cell lines: FaDu, A253 and two normal cell lines: HUVEC (endothelial cells) and Wi-38 (fibroblasts). Cells were irradiated with single-dose: 2 Gy or 10 Gy in a phantom, at a depth of 10 cm. Photon radiation X 6 MV, with a beam power of 400 MU/min, was used. Studies were carried out on cancer cells monocultures and co-cultures with cancer cells and normal cells. Three days after irradiation, apoptosis was determined using Annexin V/7-AAD staining and phases of the cell cycle were analyzed using flow cytometry. After 72h cells were stained with anti-caspase-3 antibody to determine apoptotic cells using confocal microscope. The type and quantity of cytokines and growth factors secreted by the cells was examined using LEGENDplex™ Human Inflammation Panel and ELISA kits. Seven days after irradiation, the ability of cells to colony formation was assessed.

In co-cultures of FaDu with Wi-38 and A253 with HUVEC the percentages of cancer cells in the SubG1 and S phases of the cell cycle were increased and the percentages of cells in the G2/M phase were decreased after 2 or 10 Gy irradiation compared to monocultures. The ability of cells to colony formation decreased in co-cultures of cancer cells with Wi-38 and A253 with HUVEC compared to monocultures. Co-culture of cancer cells with HUVEC and A253 with Wi-38 increased the percentage of cells in the early phase of apoptosis after 10 Gy irradiation compared to monocultures. The area of caspase-3 positive cells decreased in co-cultures of cancer cells with Wi-38 and A253 with HUVEC compared to monocultures after 2 or 10 Gy irradiation. Cells in all co-cultures secreted increased amount of inflammatory cytokines/chemokines: IFN- α 2, IFN- γ , MCP-1, IL-6, IL-10, IL-12p70, IL-17, IL-23 and IL-33. The largest differences between monocultures and co-cultures were observed for IL-6 and MCP-1.

Interaction in co-culture between fibroblasts or endothelial cells with cancer cells change the secretome and ability to survive after irradiation. Presence of fibroblasts or endothelial cells arrest function of the G2/M checkpoint of the cell cycle and promote the proliferation of cancer cells. At the same time, activation of the early phase of apoptosis was observed, followed by the inhibition of the executive phase of apoptosis.

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[P55] Functionalization of monomeric ionic liquid with pharmaceutical anions for the preparation of ionic graft conjugates serving as drug delivery systems

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The concept of drug delivery utilizing polymers as their carriers is one of the most studied nowadays due to improved solubility of a drug and control during its *in vivo* release. Biocompatible polymers can be applied, in general to encapsulate or conjugate desired drug. Moreover, copolymers with specific topology like grafted ones offer high stability of drug carrier. One of the novel drug delivery systems are ionic conjugates where drug in the form of a salt is incorporated with polymer through ionic bonding between drug and ionic liquid (IL). Preparation of such systems might be done by functionalization of IL monomers with pharmaceutical anions and their forward polymerization. In this work, the well-defined graft copolymers with attached polymerized ionic liquids (PIL) containing pharmaceutical anions in the side chains are presented.

Monomeric ionic liquid, based on biocompatible choline, i.e. [2-(methacryloyloxy)ethyl]-trimethylammonium (TMAMA/Cl⁻), was functionalized via an ion exchange reaction with pharmaceutical anions, i.e. antibacterial cloxacillin (CLX⁻) and fusidate (FUS⁻). Subsequently, the obtained IL monomers (TMAMA/CLX⁻ and/or TMAMA/FUS⁻) were copolymerized with methyl methacrylate (MMA) to prepare the graft copolymers. Functionalized monomers as well as copolymers were analyzed using ¹H NMR spectroscopy. The synthesized single and dual drug carriers were investigated at *in vitro* drug release studies by dialysis method, where pharmaceutical anions were released during the exchange reaction with phosphate ones in PBS medium (37°C, pH = 7.4).

Efficiency of ion exchange for CLX⁻ and FUS⁻ was 71% and 75%, respectively. Synthesized ionic graft copolymers contained 19-50 mol% of TMAMA units. Amount of conjugated CLX⁻ was 44% and 41% in single and dual system, respectively, whereas regarding FUS⁻ it was approximately 53% in single and 33% in dual system. *In vitro* studies demonstrated that after 50 hours, 24% of CLX⁻ (2.7 µg/mL) and 27% of FUS⁻ (3.6 µg/mL) was released from the single delivery systems, while in case of co-delivery system, 21% of CLX⁻ (2.2 µg/mL) and 23% of FUS⁻ (2.0 µg/mL) was exchanged.

Polymerizable TMAMA/Cl⁻ was successfully biofunctionalized with antibacterial CLX⁻ and FUS⁻. The satisfactory drug contents and the amount of released pharmaceuticals were reached. Cytotoxicity tests indicated high cell viability i.e. above 60% for normal cells treated by conjugate samples. Preparation of ionic drug polymer conjugates from functionalized choline-based monomers allowed to obtain innovative drug delivery or co-delivery systems bearing CLX⁻ or/and FUS⁻ anions.

Key words: dual drug systems, ionic conjugates, graft copolymers, co-delivery systems.

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[P56] The impact of chemotherapy on DNA demethylation modifications and intracellular vitamin C levels in women with breast cancer

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Introduction Chemotherapy remains an important treatment modality for patients with breast cancer. Because epigenetic regulation, mainly DNA methylation, drives hematopoiesis, it has been widely speculated that chemotherapy would have a profound impact on DNA methylation of blood leukocytes.

The level of 5-methylcytosine (5-mC) may be altered by active demethylation proces. Active demethylation of 5-mC in DNA occurs by oxidation to 5-hydroxymethylcytosine (5-hmC) and further oxidation to 5 while further iterative oxidation reactions results in the formation of 5-formylcytosine and 5-carboxylcytosine, and is carried out by enzymes of the ten-eleven translocation family (TETs 1, 2, 3). L-ascorbic acid (vitamin C) is involved in many biological processes involving enzymatic reactions that are catalyzed by members of dioxygenases (e.g TET), which use Fe(II) and 2-oxoglutarate as a co-substrate. Several studies demonstrated that ascorbate may enhance generation of 5-hmC, acting as a cofactor of TET enzymes. 5-hmC level is substantially decreased in almost all investigated cancers models (including breast cancer), what may be linked with its progression. Level of vitamin C inside the cell may affect to the TETs activity, following changes in the level of 5-hmC and its derivatives.

The aim of the study was to find out whether chemotherapy in women with breast cancer affects the level of: 1) vitamin C, which is a cofactor of TET enzymes responsible for the formation of 5-hmC and its derivatives, 2) 5-mC, 5-hmC and its derivatives.

Methods The study was performed on leukocytes isolated from peripheral blood obtained from 16 women with breast cancer. The blood samples were taken: before the start of chemotherapy (sample A), after doxorubicin and cyclophosphamide treatment (sample B), after taxanes treatment (sample C). The level of epigenetic DNA modifications in DNA from leukocytes as well as intracellular vitamin C concentration were determined by two-dimensional ultraperformance liquid chromatography with tandem mass spectrometry (2D-UPLC-MS/MS).

Results We didn't observe statistically significant differences in the intracellular concentration of vitamin C and the level of 5-mC and 5-hmC and its derivatives between individual points.

Conclusions Considering the small sample size, it is difficult to draw a clear conclusion. Results indicate that chemotherapy may not affect on the level of DNA demethylation modifications and the intracellular concentration of vitamin C in women with breast cancer. It is recommended to include larger group of patients to confirm the validity of the obtained results.

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[P57] Comparison of serum metabolome profiles in women with different types of solid cancers and healthy controls

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The research on metabolites and metabolism supplements the existing knowledge regarding the physiological state of humans, including systemic changes related to the disease. Here, to better understand the influence of the neoplastic process on the human organism, we compared serum metabolome profiles in healthy women and women with diagnosed different types of solid tumors.

Metabolites were analyzed in sera collected from patients with breast (n = 121), head and neck (n = 35), lung (n = 37), and colorectal (n = 31) cancers as well as in a group of healthy controls (n = 95). Quantitative analysis was performed using the Absolute IDQ p400 HR kit, which hypothetically enabled the detection and quantification of 408 metabolites (amino acids, biogenic amines, and lipids). Measurements were made by combining assay: 1) direct flow injection for lipids, and 2) liquid chromatography high-resolution mass spectrometry for amino acid and biogenic amines.

We observed increased levels of phosphatidylcholines (mainly those with 30-40 carbon atoms in the structure) in cancer patients. On the other hand, decreased levels of lysophosphatidylcholines were observed in cancer patients compared to healthy controls. Moreover, decreased levels of amino acids (especially glutamate) were typical for cancer patients. Six differentiating metabolites were common to all tumor types and comprised pan-cancer signature. Moreover, we noticed that the metabolic profile of breast cancer patients was the most distinct from other solid cancers.

We concluded that the analysis of serum metabolome provides new information about pathophysiological changes generally related to cancer disease as well as to find the differences between different types of cancer.

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[P58] Blender: a free alternative for 3D modeling of protein localization in cell nuclei

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Visualization of protein activity is essential in proteomic science. The typical approach, though, involves using a professional software which may be very expensive. Moreover, not every scientist may have access to such software through their organization. Here we briefly show how to use Blender 3D (current release 3.3.1 LTS) to model and visualize protein activity and interaction with DNA. Blender 3D is a free, open-source based tool, popular among graphic designers.

Here, we describe how to use Blender 3D to prepare beautiful and precise protein and DNA visualization for free. For the purpose of the presentation we use the data obtained from EdU staining of DNA (analysis of replication) and Cdc6 detection in cell nucleus using immunocytochemistry.

We show two methods, most suited for publications. The first method generates the landscape-type model that uses the original protein activity image as a height map. It's the simplest model that can be generated in a matter of minutes. The second method allows us to visualize (in the presented case) the whole nucleus and show the localization of the analyzed factor within it. The second model uses the original images as a density map for a particle system and is thus more demanding, both in terms of learning and time needed to generate the image.

The output results are 3D models that are based directly on the original data acquired from the microscope. Models can be adjusted to show specific features or regions important for the topic of the study. The models can also consist of multiple layers (e.g. DNA, protein 1, protein 2) to show the interactions between the analyzed components. The approach shown here can be applied to visualize DNA, proteins, cell structures etc. simply depending on the data acquired. Presented solution for visualization is both free and highly customizable.

[P59] Iron octacarboxyphthalocyanine and myeloperoxidase as structural analogues in the degradation of diclofenac in the presence of hydrogen peroxide

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Phthalocyanines (Pc) are a group of chemical compounds which are structurally analogous to naturally occurring porphyrins. Phthalocyanines do not occur in nature – they are obtained by synthesis [1]. Due to their numerous and interesting photophysical and photochemical properties phthalocyanines are increasingly applied in electronics and nonlinear optics and they can also be used as catalysts [2] Metallophthalocyanines are potentially interesting research areas because of their structural similarity to naturally occurring hemoproteins. Naturally occurring hemoprotein that plays an antioxidant role is myeloperoxidase (MPO).

In our work, the degradation process of diclofenac (DCF) by hematoprotein myeloperoxidase (MPO) and iron octacarboxyphthalocyanine (FePcOC) in the presence of hydrogen peroxide was compared [3]. The oxidation of DCF in the presence of myeloperoxidase (or iron octacarboxyphthalocyanine) and hydrogen peroxide also provided yellow-coloured solutions with an absorption maximum at $\lambda_{\text{max}} = 451$ nm. However, the mechanism of the diclofenac degradation with hematoprotein myeloperoxidase is more complex than with iron octacarboxyphthalocyanine.

Moreover, the biological activity of diclofenac and DCF dimer (iron octacarboxyphthalocyanine and hydroxyl radicals degradation product) was investigated [3]. Promising applications of DCF and its derivatives could be used in anticancer therapies, specially for novel photodynamic therapy (PDT) protocols. The diclofenac degradation products could play a role of cellular dead inductors.

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[P60] The effectiveness of emodin and aloe-emodin in photodynamic therapy on skin cancer cells

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In the present study, the potential of emodin and aloe-emodin as photosensitizers in photodynamic therapy has been investigated. Emodin and aloe-emodin are natural anthraquinones derivative found in a variety of popular medicinal plants. They have variety of pharmacological activities. The conducted research present for the first time comparison of the phototoxic and anti-cancerous effects of emodin and aloe-emodin on skin cancer cell lines, including SCC-25 representing cutaneous squamous cell carcinoma, MUG-Mel2 representing a melanoma cell line, and normal human keratinocytes HaCaT representing control normal skin cells. The aim of the study was to compare the effectiveness of both substances in photokilling of skin cancer cells after low and high doses of the blue light irradiation, to measure their ability to accumulate in three cell lines, to generate apoptosis and impact cells' capacity to proliferate and migrate after proposed therapy.

Tests were carried out on the skin cancer cells SSC-25 and MUG-Mel2, and normal keratinocytes HaCaT cell line with the use of emodin and aloe-emodin and photodynamic therapy. We performed MTT assay measuring cytotoxicity of natural compounds, cellular uptake, apoptosis with flow cytometry, and a wound-healing assay.

The MTT experiment was conducted in a broad range of doses of the natural substance - from 2.5 μM to 50 μM for 24 h. The statistically significant difference in keratinocyte viability was observed in a concentration of 20 μM for both analyzed substances after irradiation, whereas, in malignant cell lines, the viability was decreased by almost 50%. The proposed therapy revealed lower phototoxicity against normal skin cells in comparison to malignant cells. To explore the number of apoptotic cells, which can arise in cell lines by incubation with emodin or aloe-emodin, TUNEL assay was performed. Both cancer cells demonstrate that PDT with aloe-emodin caused a stronger apoptotic effect than emodin. Furthermore, melanoma cells reveal twice as many cells damaged by programmed cell death in comparison to squamous cell carcinoma cell lines. Additionally, as shown in the wound healing experiments, migration and invasion of skin cancer cells decreased more obviously in the PDT-treated groups. Emerge that aloe-emodin is a more potent photosensitizer for the photodynamic treatment of melanoma cells.

Although emodin and aloe-emodin are isomers and differ only in the position of one hydroxyl group, our phototoxicity and apoptosis detection results show that both substances affect skin cancer cells, and normal keratinocytes in different ways. Based on our results, conclusions emerge that aloe-emodin is a more potent photosensitizer for the photodynamic treatment of melanoma cells. To sum up, more studies are necessary to confirm the potential anticancer activity of aloe-emodin-PDT and shed light on various cellular and molecular mechanisms which are behind our findings.

[P61] Aronia melanocarpa leaf extracts modulate MMP-2/-9 expression, migration, and invasion in colon cancer HT-29 and SW-480 cells

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Matrix metalloproteinases are zinc-dependent endopeptidases that play a role in several biological processes, such as tumorigenesis, angiogenesis, and metastasis. Their involvement in all stages of carcinogenesis makes them potential targets for reducing cancer promotion and progression. There are several ways modern medicine tries to improve the efficacy of cancer therapies, including drugs, surgery, and radiation therapy. Since prevention is better than treatment, naturally occurring compounds are in the spotlight. Polyphenols are dietary components that possess extraordinary health properties. We previously found that aronia leaf crude phenolic extract (ACE) and purified phenolic-rich extract (APE) had cytotoxic and antioxidant effects against colon cancer cells (1). Additionally, the UPLC-Q-TOF-MS analysis confirmed that chlorogenic acid (5-O-caffeoylquinic acid) and quercetin-3-rutinoside were the predominant polyphenols in both extracts.

In this study, we decided to examine the influence of the same aronia leaf extracts on the expression of *MMP-2* and *MMP-9* in colon cancer SW-480 and HT-29 cells using qPCR. The effect of these extracts on the inhibition of *MMP-2* and *MMP-9* activity was also evaluated by gelatin zymography. In addition, ELISA was used to determine the protein expression levels of both MMPs. Considering the importance of migration and invasion mechanisms in determining metastasis ability, both extracts were also evaluated for their impact on these parameters.

Our results showed the inhibition of *MMP-2* expression in SW-480 cells, while the expression of this gene was not detected in HT-29 cells. *MMP-9* mRNA expression was stimulated by a rising APE concentration in SW-480 cells but significantly reduced by ACE. Furthermore, both extracts reduced the expression of *MMP-9* in HT-29 cells, and the changes were considered statistically significant. The inhibition of *MMP-9* expression by ACE was also confirmed on the protein level in both cell lines. In SW-480 cells, 300 µg/mL APE slightly stimulated *MMP-9* protein expression while in HT-29 cells, it decreased. Gelatin zymography confirmed that tested aronia extracts inhibited the enzymatic activity of *MMP-9* and *MMP-2*. The observed effect was strong and concentration-dependent. Moreover, both extracts reduced colon cancer cell migration and invasion equally strongly and concentration-dependently.

These findings for the first time revealed the anti-metastatic and anti-invasive potential of aronia leaf extracts against colon cancer cells. The results indicate that tested extracts might be a promising alternative source of bioactive substances in the future, that can be used as replacements for synthetic agents in the prevention of colon cancer.

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[P62] Surface modification via diazonium salts and poly-L-lysine to enhance neural cell adhesion

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Biosensor and bioelectronic systems play a crucial role in the diagnosis and monitoring of various diseases, and healthcare applications [1]. Significance of studying cell adhesion *in vitro* and *in vivo* has become increasingly important, particularly in the field of tissue engineering and regenerative medicine. In recent years, conducting polymers have sparked a lot of attention considering their biocompatibility and electroactivity, cells or tissue cultivated on them may be stimulated; as a result, they are becoming essential materials for biosensors, implants, drug delivery devices, and tissue engineering scaffolds [2]. To fulfil its potential, a number of obstacles must be overcome, notably those pertaining to maintaining the integrity of communication pathways between tissue and electrode surfaces of bioelectronics, which leads to prolonged inflammatory and scarring responses [3]. Biologically active compounds that function as cell adhesion promoters are electrografted onto organic monolayers, which is a potential approach to surface modification.

Here, we show how diazonium salts can be used to modify platinum (Pt) electrodes while poly-L-lysine further biofunctionalized them to improve cell adhesion sites. Infrared spectroscopy, Raman spectroscopy, profilometry, and other methods were used to characterize the functionalized electrodes. Cell adhesion was monitored by culturing human neuroblastoma SH-SY5Y cells on modified Pt electrodes.

The results of our study revealed that surface modification with diazonium salts and PLL is noteworthy in facilitating cell adhesion to the electrode, thus enhancing the functionality of neurological devices to either stimulate or record neural functions.

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[P63] Fractal electrodes and their biomedical applications

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The aim of this presentation is to provide a review on fractal electrodes and their application in biomedical engineering, particularly bioelectronics. The starting point is to present the topic of fractal, their equivalents in nature and the possibilities related to this unique geometry. The most promising fractal electrodes and their applications will be presented, particularly sensing and biosensing. Fractal electrodes are characterized by sensitivity and durability due to the increased contact surface with the analyte. This gives the possibility to expand the limits of detection, and thus the design of new biosensors with a reduced size and the same efficient operation. Particular attention will be given to fractal electrodes made of palladium, gold and silver, which allow the detection of many analytes in a wide range of concentrations. Finally, different strategies of fabrication of fractal electrodes will be briefly discussed.

[P64] DGCR8 inhibition enhanced levels of a fraction of primary miRNAs and reduced viability of Hodgkin lymphoma cells

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MicroRNAs are a class of small, non-coding RNAs that inhibit gene expression at the post-transcriptional level. Differential miRNA profiles are often observed in many types of cancer including B-cell lymphoma. miRNA biogenesis consists of several regulated steps including transcription of miRNA genes to long primary miRNA transcripts (pri-miRNAs) that are processed by Drosha-DGCR8 complex to precursor miRNAs (pre-miRNAs) and exported to the cytoplasm to be processed by Dicer to mature miRNAs.

In this study we aim to determine the effect of DGCR8 inhibition on miRNA biogenesis. We used two short hairpin RNAs, shDGCR8-1 and shDGCR8-2, to inhibit DGCR8 expression in Hodgkin lymphoma (HL) L1236 cells. We showed that the levels of both DGCR8 transcript and DGCR8 protein were significantly decreased upon shDGCR8-1 and shDGCR8-2 compared to scrambled negative control (SCR). Interestingly, DGCR8 inhibition caused death of L1236 cells as the percentage of living cells decreased 3 folds for shDGCR8-1 and 1.5 fold for shDGCR8-2 within 10 days as determined by flow cytometry. It is confirmed that cells had targeted the apoptotic pathway. The DGCR8 inhibition caused also increased percentage of cells in G1/G0 phase up to 4 folds for shDGCR8-1 upon 8Gy of irradiation compared to control in L1236 cells. Moreover, results obtained from RNA-seq showed that DGCR8 inhibition caused increased expression of only 15 pri-miRNAs. We confirmed some of these results using qRT-PCR method. DGCR8 inhibition prevented biogenesis of several miRNAs, including miR-155 and miR-19b. The levels of mature miR-155 and miR-19b decreased, whereas the levels of the pri-miR-155 and pri-miR-17~92 (pri-miR-19b) increased upon shDGCR8 compared to SCR introduction to L1236 cells. Interestingly, not all miRNAs were equally affected by DGCR8 inhibition, since the levels most of pri-miRNAs were not altered compared to SCR control.

In conclusion, we showed induced cell death and enhanced levels of a fraction of pri-miRNAs upon DGCR8 inhibition in L1236 HL cells. Further studies that involve analysis of additional miRNAs and pri-miRNAs using PCR are ongoing.

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[P65] Role of lncMYC15 in DNA damage response

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DNA damage response (DDR) is a complex process, essential for cell survival. Especially deleterious types of DNA damage are DNA double-strand breaks (DSB), which can lead to genomic instability and malignant transformation if not repaired correctly. The central player in DSB detection and repair is the ATM kinase which orchestrates the action of several downstream factors. Despite substantial knowledge of DNA repair processes, still several aspects of DNA damage detection and signaling are not fully understood. Recent studies have suggested that long non-coding RNAs (lncRNAs) are involved in DDR.

Here, we aimed to verify the hypothesis that **ATM-dependent lncRNAs are essential players involved in the DDR.**

DNA damage was induced by ionizing radiation (IR) in immortalized lymphoblastoid cell lines (LCLs) derived from 4 patients with ataxia-telangiectasia (AT) and 4 healthy donors. Cells were collected 1h and 8h after IR to allow identification of lncRNAs involved in the early and late response to DNA damage. A strand-specific RNA sequencing approach was applied to identify IR-induced lncRNAs and mRNAs. 7 mRNAs and 10 lncRNAs were significantly induced 1h after IR in healthy donors, whereas none in AT patients. 447 mRNAs and 149 lncRNAs were induced 8h after IR in the control group, while only 100 mRNAs and 3 lncRNAs in AT patients. The overlap between the control and AT patient groups was limited (19% and 6% for mRNAs and lncRNAs, respectively). Among IR-induced mRNAs, we found several genes with well-known functions in DDR. Gene Set Enrichment Analysis revealed delayed induction of key DDR pathways in AT patients compared to controls. Based on Transcription Factor ChIP-seq ENCODE data, we found 71 TFs with binding sites within 1kb from differentially expressed lncRNAs. The majority of TFs are involved in pathways connected with DNA repair, p53, and cell cycle regulation, which supports the involvement of lncRNAs in DNA damage response. One of detected lncRNA was lncMYC15. The induction and dynamics of lncMYC15 was confirmed by RT-qPCR at several time-points after IR on a larger cohort. Moreover, inhibition of ATM with a specific inhibitor (KU-60019) proved that lncMYC15 is dependent on ATM. lncMYC15 is localized next to protein-coding gene (MYC) and other lncRNA (PVT1). We observed that induction of lncMYC15 after IR is correlated with changes in expression of adjacent genes. Subcellular fractionation into chromatin, nuclear, and cytoplasmic fractions revealed that lncMYC15 is localized in chromatin, which further suggests its role in regulation of gene expression. The localization of lncRNAs remains unchanged upon IR- and IR+ conditions. Functional study showed that lncMYC15 knockdown affects expression of nearby gene - PVT1. Cell cycle analysis revealed accumulation of cells in S phase after lncMYC15 silencing.

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[P66] Effect of BTIC-targeting oncolytic myxoma virus construct on cultured glioma cells.

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Aim of research: Glioblastoma is a highly malignant rapidly proliferating and recurring neoplasm, particularly difficult to treat. Responsible for recurrence and fatal outcome are brain tumor initiating cells (BTICs) present in CNS tumors. We used a myxoma virus-based oncolytic construct vMyx-M011-KO with deletion (knock-out) of the M011L gene. The construct is capable of targeting BTIC cells and increases their apoptosis. The proposed therapeutic strategy has been aimed at systemically delivering the oncolytic virus (OV) with a cellular carrier (adipose derived mesenchymal stem cells, ADSC). The ultimate purpose is to bypass the adverse response of the immune system, whereby virus particles are degraded before they can reach tumor site and, at the same time, explore the immune response-stimulating properties of the OV construct.

Methods: the effect of both the knockout construct (vMyx-M011L-KO) and wild-type myxoma virus (vMyx-WT) on murine (GL-261 and GL-261luc) as well as human (LN18, T98G and U-251MG) glioma and mesenchymal stem cell line (ADSC). Infectiveness of both viral agents was evaluated by generating single-step and multiple-step growth curves. Cytotoxicity was compared using MTS viability test. Western blot analysis was performed to assess protein expression in the tested lines.

Results: the tested human glioma cell lines and carrier ADSCs are permissive to myxoma virus productive infection. The oncolytic agent kills to a large extent murine glioblastoma cells, which are highly sensitive for OV used. Unfortunately, human glioma cell lines appear so far less susceptible for MYXV. The vMyx-M011L construct achieved a stronger cytopathic effect compared to the wild-type virus. Use of vMyx-M011L-KO resulted in lower survival in GL-261 and T98G glioma lines. As a result of infection with the vMyx-M011-KO construct, the expression of apoptotic proteins was demonstrated in the human glioma cell lines (LN18, T98G) and ADSC line. The therapeutic time window in the case of *in vivo* systemic administration should be sufficient to employ ADSC as a cellular carrier allowing crossing of the blood brain barrier (BBB).

Conclusion: The oncolytic construct tested confirmed its potential usefulness for eliminating BTIC cells; satisfactory viability of infected ADSC cells makes the proposed therapeutic platform a potential weapon in the planned systemic therapy of glioblastoma using an immunocompetent mouse model.

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[P67] Mitoregulin is overexpressed in hodgkin lymphoma and involved in cell growth

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Mitoregulin was first identified as long non-coding RNA, LINC00116 and was shown to be upregulated in B-cell lymphoma compared to normal B-cells. Recently, LINC00116 was shown to encode a 56-amino acid peptide called Mitoregulin (MTLN) that was localized in the inner mitochondrial membrane. However, the functions of both LINC00116 and MTLN in B-cell lymphoma are unknown.

In this study, we focus on the role of MTLN in B-cell lymphoma. We showed that MTLN is significantly higher expressed in Hodgkin lymphoma (HL) cells compared to normal B-cells. Next, we inhibited the expression of MTLN with two short hairpin RNAs (shRNAs) in lentiviral vectors and used two negative control vectors (NT and SCR). We confirmed that the levels of MTLN and LINC00116 were downregulated upon inhibition with both shRNAs compared to NT and SCR. The vectors contained GFP, thus we analyzed the number of GFP+ transduced HL cells compared to wild-type (wt) cells in the GFP competition assay. The percentage of HL cells with inhibited MTLN decreased significantly, whereas the percentage of HL cells transduced with a negative control vectors did not change compared to wild-type cells within 21 days. Thus, inhibition of MTLN expression resulted in inhibition of HL cell growth. Additionally, we showed that this inhibition was not caused by apoptosis, since no significant difference in the apoptosis rate was observed for L540 HL cells with inhibited MTLN compared to negative controls.

In conclusion, we showed that MTLN is overexpressed in Hodgkin lymphoma and is important for HL cell growth. In further studies we aim to demonstrate possible differences in the function of LINC00116 and MTLN.

Keywords: long noncoding RNA, B-cell lymphoma, Mitoregulin, Hodgkin lymphoma

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[P68] Robust, sensitive and reproducible MGMT methylation assessment in glioblastoma

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Introduction Glioblastoma, previously known as glioblastoma multiforme (GBM), is the most common and aggressive primary cancer of the central nervous system. GBM has still a poor prognosis, with median survival rate in adults estimated at 14.6 months. The methylation of the *MGMT* gene promoter is associated with a favorable response of patient to temozolomide treatment. Recent studies show that even low level of methylation within *MGMT* gene promoter may result in a certain sensitivity to treatment with temozolomide and therefore *MGMT* methylation testing should be considered as a stratifying factor in decision-making for temozolomide treatment. Nevertheless, in Poland the assessment of methylation status of this gene is still rarely considered in treatment planning partly due to lack of standardized diagnostic methylation testing technology. We have assessed limitations of MethylDetect's ApS *MGMT* EpiMelt assay for standardized *MGMT* methylation testing.

Methodology DNA was extracted from 12 freshly frozen GBM samples using the salting-out method, in which 0,1 g of tissue was used. The quantity and quality of extracted DNA was assessed using NanoDrop (Thermo Fisher Scientific, USA) and QUBIT (Thermo Fisher Scientific, USA). DNA was modified with sodium bisulfite (EZ-96 DNA Methylation-Gold™ Kit, Zymo Research, USA) The methylation status of *MGMT* gene promoter was assessed using EpiMelt Assay (MethylDetect ApS, Denmark) and LightCycler® 480 High Resolution Melting Master (Roche, Switzerland) on mic qPCR version 2. 12. 6 platform (Bio molecular systems, Australia). Each sample was run in triplicates, in two technical repeats at two annealing temperatures to control for the sensitivity of the methylation detection.

Results Five of the samples in our study displayed signs of methylation of the *MGMT* promoter and were classified as heterogenous methylation. The remaining seven samples were non-methylated. The reproducibility and sensitivity of the results was 100% between both replicates and technical repeats at different testing temperatures.

Conclusions The EpiMelt *MGMT* testing assay along with the DNA sample preparation method that we optimized allows robust, sensitive and reproducible *MGMT* gene methylation assessment. Moreover, the assay allows detection of heterogenous methylation which is not possible with any other methylation assessment technologies. Importantly, as in our study all of the *MGMT* methylation positive samples displayed heterogeneous pattern of methylation the clinical significance of this phenomenon needs to be assessed in future studies.

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[P69] ²²⁵Ac labeled anti-HER2 2Rs15d sdAb as a potential therapeutic for targeted alpha therapy – an *in vitro* and *in vivo* evaluation

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Objectives Human Epidermal Growth Factor Receptor type 2 (HER2) overexpression is often associated with more aggressive form of cancer, metastatic activity and chemo-resistance. Intact mAbs are not always ideal vectors for radioimmunotherapy due to their slow pharmacokinetic. Single-domain antibody fragments (sdAbs) are the smallest (~15kDa) antibody-derived fragments with beneficial pharmacokinetic properties for molecular imaging and targeted radionuclide therapy (TRT), especially when labeled with α -particle emitters, which provide highly lethal and localized radiation to targeted cells. The goal of our studies was to evaluate the therapeutic potential of the ²²⁵Ac-labeled anti-HER2 sdAb 2Rs15d.

Methods 2Rs15d was coupled with the bifunctional chelator *p*-SCN-Bn-DOTA and labeled with ²²⁵Ac. The binding affinity, specificity for HER2 and immunoreactive fraction (IF), were evaluated on SKOV-3 (HER2+) cells. The *in vitro* cytotoxicity was assessed using clonogenic assay, while cell damage evaluated using γ H2AX phosphorylation assay. *In vivo*, [²²⁵Ac]Ac-DOTA-2Rs15d and a non-targeting control [²²⁵Ac]Ac-DOTA-R3B23 were evaluated in female athymic nude mice bearing subcutaneous SKOV-3 xenografts, both alone and with molar excess of unlabeled 2Rs15d. Therapeutic potential of one and three consecutive doses of the radiobioconjugate was compared to the trastuzumab and combination of both therapeutics in HER2+ metastatic mouse model.

Results [²²⁵Ac]Ac-DOTA-2Rs15d labeling yield was high (>90%), with radiochemical purity above 95%. *In vitro* bound specifically to HER2 receptors with ~75% IF, a KD of 3.50±0.17nM and lack of competition with trastuzumab. Cytotoxicity studies showed that [²²⁵Ac]Ac-DOTA-2Rs15d significantly reduced SKOV-3 cell viability in a dose-dependent and HER2-mediated manner. Tumor uptake in SKOV-3 xenografts was high and specific (~8%). The accumulation in kidneys was reduced almost 3-fold by co-injection of [²²⁵Ac]Ac-DOTA-2Rs15d with 150 mg/kg Gelofusine. Therapy studies indicated that [²²⁵Ac]Ac-DOTA-2Rs15d increased median survival significantly, which measured 101 days for one dose and 143 for three consecutive doses compared to about 60 days for animals treated with PBS or [²²⁵Ac]Ac-DOTA-R3B23, and to 99 days in case of trastuzumab regimen. The most extensive therapeutic effect (160 days) was observed for the combination of both three consecutive doses of [²²⁵Ac]Ac-DOTA-2Rs15d and trastuzumab as an add-on therapy.

Conclusions The strong therapeutic potential of ²²⁵Ac-labeled 2Rs15d was observed *in vitro*, which was confirmed *in vivo* in mice bearing SKOV-3 xenografts especially when applied in fractions. This study shows that the [²²⁵Ac]Ac-DOTA-2Rs15d can be a promising new radiobioconjugate for TRT and supports its further development towards the clinic.

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[P70] Multiple myeloma methylation signatures at the diagnosis appear to be associated with bortezomib-induced peripheral neuropathy

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Introduction In Poland, the incidence of Multiple myeloma (MM) is rising and leads to approximately 1100 deaths yearly. Bortezomib (BTZ) is a compound widely used as the first-line of MM treatment. However, peripheral neuropathy, one of the most common side effects of BTZ, is amongst the most significant challenges in the clinical management of MM, with 40% of patients suffering from this complication. The molecular bases of the development of BTZ-induced peripheral neuropathy (BiPN) are unknown, and there is no strong evidence for the genetic-based predisposition in patients. Our previous studies on MM cell line models showed that BTZ induces methylation changes within transcription factor (TF) binding sites of EBF, PAX, DLX, LHX, and HNF family and those TF take part in neurotransmission pathways. Thus, in this study, we investigated the association of MM methylation signatures at the diagnosis with the development of BiPN.

Materials and methods Bone marrow samples from 52 MM treatment-naïve patients were obtained at the diagnosis. On average, patients were followed up for 35 months and monitored for signs of BiPN. CD-138+ cells were isolated from bone marrow samples. Methylation profiling of 850,000 CpG was performed using Infinium MethylationEPIC Beadchip array. Data were analyzed using .R ChAMP package, Qlucore, and GENE2FUNC function of FUMA GWAS.

Results We identified 1343 statistically significant differentially methylated probes (DMPs) with methylation differences of ≥ 0.3 between patients with and without BiPN. The identified DMPs were annotated to 768 genes. The identified gene set has been shown to be specifically expressed in 8 types of brain tissue. Moreover, those genes were statistically significantly enriched in REACTOME curated gene sets participating in transmission across electrical (adjusted p-value = 6.27E-07) and chemical synapses (adjusted p-value = 3.79E-07), with enrichment of 29 and 24 genes, respectively. Our results suggest that identified methylation changes might interfere with neurotransmission and lead to BiPN development.

Conclusions Epigenetic alterations appear to play a role in the development of BiPN. The results are in line with our previous studies. Nevertheless, our analysis needs to be performed on larger patient cohorts.

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[P71] Melatonin as preconditioning agent for the improvement of extracellular vesicles secretion by mesenchymal stem cells

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Melatonin is a broadly investigated biological compound which role in majority is associated with regulation of circadian rhythm. However, it is known that melatonin possesses antioxidative and anti-inflammatory properties and its role is also investigated for example in anti-cancer studies. Studies showed that melatonin also changes the activity of mesenchymal stem cells such ability of differentiation into another types of cells. Mesenchymal stem cells secrete several factors that improves tissue regeneration. Among these factors there are extracellular vesicles (EVs) which transport different compounds such proteins, lipids or even nucleic acids like miRNA. This study focuses on the investigation of melatonin activity in EVs secretion by mesenchymal stem cells. Here we present the preliminary findings. The aim of the study was to evaluate the putative role of melatonin in secretion of extracellular vesicles by mesenchymal stem cells.

Mesenchymal stem cells were purchased from cell bank as the primary cells harvested from human adipose tissue. Adipose-derived stem cells (ADSC) were cultured in standard cell culture media (DMEM) with 10% of exosome-depleted FBS and 1% of antibiotics. The cell phenotype was investigated. Cells were identified by CD90, CD73, CD105 molecular markers and their differentiating potential was assessed by adipogenesis, chondrogenesis and osteogenesis. The differentiation process was assessed with histochemical staining such oil-red, alcian blue, alizarin accordingly. Furthermore, we assessed the expression of characteristic molecular markers of differentiation by immunofluorescence and confirmed ADSC phenotype accordingly to the ISCT (International Society for Cellular Therapy) recommendations.

For the EVs isolation, cells were passaged at 70% of confluence and seeded in T75 bottles. After the attachment of cells, the medium was changed for DMEM with 5% of exosome-depleted FBS and 1% of antibiotics with the addition of melatonin, melatonin with luzindole (inhibitor of melatonin receptors). No addition of active compounds served as control group. The medium was changed every 24 h through 4 days and conditioned medium was collected, centrifuged to pellet down dead cells and cell debris, then supernatants were collected in fresh tubes and stored at +4°C until further proceedings. Total fraction of heterogenic extracellular vesicles was isolated with the use of chemical precipitation method. EVs were visualized with transmission electron microscopy and the level of CD9 antigen was assessed with western blot method.

The studies on preconditioning of mesenchymal stem cells can be applied for targeted therapy. Successful preconditioning in this case would boost the secretion of extracellular vesicles which can be used for optimization of EVs production for therapeutic purposes.

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[P72] Regulation of adipose derived stem cells activity through melatonin receptors in aspect of viability and osteogenic differentiation

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Melatonin is a hormone produced by mammalian cells, involved in regulating circadian rhythms, secreted mainly by the pineal gland and acting through receptor and non-receptor pathways. There are many studies demonstrating that melatonin significantly increases the effectiveness of physiological osteogenesis but there is still not enough research proving the effect of melatonin on healthy, normal stem cells such as adipose derived stem cells (hADSCs). Mesenchymal stem cells from adipose tissue have potential to differentiate, among others, into mesenchymal osteoblast-like cells. However, the main problem in using hADSCs is time-related loss of properties and activity in cell culture, inefficient culture and cellular senescence. The aim of the study was to assess the influence of melatonin on hADSCs apoptosis and ability to differentiate into osteoblast-like cells.

The effect of melatonin on hADSCs has been analysed in several aspects. First, the viability of hADSCs was evaluated at different concentrations of melatonin and compared to control cells. Non-toxic concentration of melatonin was used for further proceedings. Next, cells were incubated for 48h with/without melatonin receptor inhibitors: luzindole or 4-P-PDOT. Cells were evaluated on the basis of ATP activity, and changes in phenotype, apoptosis and cell cycle measurable with FACS and RTqPCR, including cleaved Cas3 immunofluorescence staining and expression of *CASP3* and *CASP7* genes after 48h incubation with melatonin and inhibitors. Moreover, *MEL1A* and *MEL1B* receptor expression was analysed. Finally, the ability of hADSCs to differentiate into osteoblast-like cells after the treatment with/without melatonin and its inhibitors, was evaluated.

We proved that cells treated with melatonin at a concentration of 100µM had the highest viability, whereas concentration 1000µM was toxic for cells. We detected also that hADSCs treated with melatonin showed the highest activity of ATP, it was related to the number of viable cells in culture. Morphology of cells and phenotype after incubation with melatonin and its inhibitors has also remained unchanged. Moreover, apoptosis has not been induced. It suggested that promotion of hADSC proliferation do not affect its properties but it might be connected with melatonin receptors. The results demonstrated that melatonin regulates also hADSC differentiation into osteoblast-like cells through melatonin receptors and it generates overexpression of *SPPI* and *RUNX2*. However, we did not observe differences in mineralization of cells in culture containing supplements.

It was concluded that melatonin could be an important compound for cell culture, especially for hADSCs with poor viability or low proliferation potential. Melatonin might be used also as an extra osteogenesis differentiation factor in *in vitro* conditions. Treatment of MSCs with melatonin should efficiently enhance its function and regenerative capacities.

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[P73] Metabolic content of epithelial and stromal compartments in breast tumors revealed by 1H HR MAS NMR spectroscopy

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Introduction/Rationale There is substantial evidence that interaction between cancer and stromal cells within tumor microenvironment drives cancer initiation, progression, metastasis and response to treatment. The purpose of this work was to determine metabolic composition of epithelial and stromal compartments in breast tumors of different grades (I vs II/III) using NMR spectroscopy of intact tissues.

Methods The studied group consisted of 39 patients who underwent breast conserving surgery in National Institute of Oncology in Gliwice for the treatment of breast cancer. The patients were diagnosed with invasive ductal carcinoma (32 cases) and invasive lobular carcinoma (7 cases). Along with routine intraoperative histological assessment of the resected specimens, tissue samples from tumor and peritumoral tissue from each patient were collected. 1H HR MAS NMR spectra of these specimens were acquired on Bruker Avance III 400 MHz spectrometer. Linear regression analysis was used to examine the relationship between the metabolic levels and cancer cell fraction in the samples containing mainly cancer cells and intratumoral fibrotic stroma. Extrapolation of the linear relationships to 0% cancer content was used for determination of the metabolite levels characteristic of pure fibrotic stroma while extrapolation to 100% – for determination of those levels in a pure cancer tissue. The metabolic levels obtained for these tissue compartments were compared to the values found for the extratumoral fibrous connective tissue.

Results Positive correlations between cancer cells fraction and the levels of several metabolites (phosphocholine, glycerophosphocholine, ascorbate, phosphoethanolamine, taurine, scyllo-inositol, creatine, glutamine, succinate, lactate, glycine and glutamate) were observed in grade II/III breast tumors. Decrease of glucose in a function of tumor purity was detected in the lower grade (I) ones. The higher levels of lactate, succinate, glutamate, phosphoethanolamine, myo-inositol, scyllo-inositol, choline, ascorbate and creatine in the intratumoral fibrotic compartment than in extra-tumoral connective tissue were revealed in the analysis of tumors of all grades. While stromal accumulation of lactate, succinate and glutamate were visible in the separate analyses of both low grade (I) and higher grade (II/III) tumors subgroups, ascorbate, choline, myo-inositol, scyllo-inositol and creatine were found to be significantly increased in the fibrotic tissue in grade I tumors and trended towards increase in the higher grade (II/III) ones. Additionally, glycine, phosphoethanolamine and taurine accumulation was observed in the stroma of grade I tumors only.

Conclusions/Novel aspect Joint analysis of metabolic content of stromal and epithelial metabolic profiles in breast tumors of different grades contributes to the increased understanding of breast cancer biology.

[P74] Influence of surface modification by “onium” salts on PEDOT:PSS adhesion

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PEDOT:PSS is a conductive polymer, which is widely used as a neurostimulator coating [1] due to its well-proven biocompatibility. Unfortunately, its poor adhesion to metals precludes long-term applications [2]. In my investigation, I am working on the enhancement of PEDOT:PSS adhesion through an electrochemical reduction of iodonium and diazonium salts on the surface of Pt electrodes, what leads to the creation of an organic layer, which is able to increase the adhesion of polymers. The organic layer was created by cyclic voltammetry in a range of potentials matched to each salt, the electrodes were then coated with PEDOT:PSS by a drop coating technique and placed in a phosphate buffer solution for 30 days. After this time, UV-Vis spectra of the solutions were collected to investigate the presence of detached PEDOT:PSS. The results confirmed that electrografting of “onium” salts can be used to enhance the adhesion of PEDOT:PSS, e.g. in brain-stimulators production.

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[P75] Heat Shock Protein A2 is a novel extracellular vesicle-associated protein

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Introduction Heat shock proteins (HSPs) are a large family of evolutionarily conserved molecular chaperones. HSPs control all aspects of cellular proteostasis, including the assembly of multi-protein complexes and protection of proteins from aggregation both under physiological and stressful conditions. The heat shock proteins family A (HSPA/HSP70) members are well-known cancer-related proteins. HSAs have anti-apoptotic properties, and their high expression levels are frequently associated with resistance to chemotherapy and poor patient prognosis. Extracellular vesicles (EVs) are nanosized particles that are released by cells and play a key role in cell-to-cell signalling. HSPs are commonly considered as the constitutive EV proteins, however it is believed that it is mainly the HSPA1 one. In this study we investigate other HSPA family member – HSPA2 as another significant component of small extracellular vesicles. Although HSPA2 is well known as highly overexpressed in various types of cancers, as far its function is a mystery. Here we indicate its potential role in intercellular communication.

Methods We used HSPA2-CRISPR/Cas9 edited and HSPA2-overexpressing cell lines. Analysis of the intra- and extracellular proteins were performed using Western Blot procedure. EVs derived from cells cultured in vitro and human urine were isolated using size exclusion chromatography. Particle concentration and size distribution in EV samples were analyzed using NanoSight NS300 analyzer.

Results In the present study we demonstrated that autophagy is not involved in the HSPA2 degradation upon proteasome inhibition, while it is easily detectable in EVs derived from various sources such as established in vitro cell lines of different origins and urine from male and female donors. Our research for the first time provides evidence that during proteasome inhibition, loading of HSPA2 into EVs is executed at the expense of lowering the intracellular levels thereof. Moreover we were able to prove that the levels of HSPA2 in urinary EVs from healthy donors as well as patients were correlated with EVs markers.

Conclusions Our results extend the basic knowledge about HSPA2 protein and may have also clinical implications. We assume that HSPA2 can be proteotoxicity-associated DAMP (damage-associated molecular pattern). It suggests that HSPA2 may be signalling-associated protein and have effect on the tumour microenvironment especially under the proteasome inhibitors treatment conditions. Although we tested HSPA2 in terms of stress conditions, we believe that HSPA2 is constitutively present in extracellular vesicles, however its exact role in EVs needs further investigation.

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[P76] Genome-wide methylation changes in healthy individuals with low and high arsenic levels

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Introduction The arsenic exposure has long been associated with adverse health effects including neurotoxicity, cardiovascular disease and cancer. There is a consensus in the field that environmental exposures such as arsenic are not genotoxic. Therefore, it is likely that epigenetic changes induced by arsenic exposure are involved in development of arsenic related adverse health effects. In this study, we compared whole blood methylomes of 23 and 37 healthy women with low (average level = 0.45 ± 0.05) and high (average level = 2.89 ± 0.33) arsenic (As) levels to identify methylation changes associated with the As exposure.

Methods We measured total As concentration in blood samples with ICP-MS technique using Elan DRC-e (PerkinElmer, USA) instrument and extract DNA from those samples with salting out method. Then performed genome-wide DNA methylation profiling of those samples with the Infinium MethylationEPIC array (Illumina). The raw data were processed with ChAMP package and normalized with BMIQ method. We corrected the analysis for batch effect and adjusted methylation levels for cell fraction differences between groups. The methylation changes that we considered in analysis were $\geq 5\%$ with adjusted p-value ≤ 0.05 . To identify genes, which expression potentially could be affected by identified methylation changes, we used Genomic Regions Enrichment of Annotations using GREAT, an algorithm that predicts the functions of cis-regulatory regions by analyzing the annotation of nearby genes.

Results Our analysis identified 8111 differentially methylated CpG sites (1119 DMPs were hypo- and 6992 hypermethylated) in patients with high arsenic levels. The identified CpGs were significantly depleted in gene promoter regions (1stExon, TSS1500, TSS200), and enriched in regions which are localized outside of the promoters, such as 3'UTR and ExonBnd. Moreover, the top hit in the GREAT analysis based on identified DMPs was *PRDM8* gene harboring 6 DMPs in the promoter. *PRDM8* has been shown to suppress the PI3K/AKT/mTOR signaling cascade through the regulation of NAP1L1 and PI3K/AKT/mTOR signaling cascade is crucial to many aspects of cell growth and survival, in physiological as well as in pathological conditions (e.g., cancer).

Conclusions Our analyses indicate that globally, arsenic exposure affects methylation of the CpGs which are not localized in regions directly involved in gene expression regulation such as promoters but regions that, considering recent reports from other studies, may be involved in indirect gene expression regulation. Moreover, our results suggest that one of the critical genes for cancer development may be affected by As related methylation changes.

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[P77] The hydrogen potential of microorganisms

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Hydrogen is often said to be the fuel of the future, especially in the current climate crisis caused mainly by coal and petroleum. In discussions about lowering our emissions hydrogen is often mentioned in the context of heating, vehicles, power stations as their biggest advantage is how they produce no harmful emissions, especially carbon dioxide. However, our main source of hydrogen has been, so far, fossil fuels that take away the environmental aspect of the hydrogen economy. Thus, constantly growing demand for environmentally friendly sources of energy gives the opportunity for development of many interesting solutions. The most popular option - biohydrogen produced by bacteria is one of the most promising solutions that still needs to be refined.

Two hydrogen producing strains of bacteria were selected: *Pseudomonas fluorescens* and *Rhodospirillum rubrum*. For the first strain, maximization of production has been achieved by maintaining bacterial cultures in stress conditions. Levels of production were measured by solar pyrolysis and showed that nearly half of pyrolytic gas was molecular hydrogen (H₂). For the other strain, bacteria were grown in falcons filled with different media to find the one that provides the fastest growth and; furthermore, biohydrogen production.

The results show how the best chosen media were the lysogeny broth and a medium containing malic acid as a carbon source. Collected information should allow for easier development of the hydrogen economy.

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[P78] Women that developed breast cancer carry specific methylation changes in blood cells years before diagnosis

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Introduction Breast cancer continues to be the most prevalent cancer worldwide. Several pathogenic genetic variants are known to increase an individual's risk of breast cancer but these variants have been linked to only a small fraction of cases. Recently, detectable *BRCA1* epimutation in blood cells was shown to be strongly associated with the risk of incidence of triple-negative breast cancer and high-grade serous ovarian cancer. With these findings, there is an urgent need to study epigenetic changes with a similar effect as these changes can improve current cancer prediction biomarker indexes.

Methods We analyzed methylation profiles (EPIC array, Illumina) of peripheral blood cells collected from 21 female patients three to seven years before breast cancer diagnosis and 34 age-matched cancer-free controls obtained from the Gene Expression Omnibus database (GSE123914). We pre-processed the data using the default pipeline implemented in the R ChAMP package. Differentially methylated probes (DMPs) were defined as 1% of statistically significant ($FDR \leq 0.05$) CpGs with the largest difference in mean methylation levels between cases and controls. Annotation of identified DMPs to specific regions of the genome, including gene regions, relation to CpG islands, and specific genes, was performed using the Illumina EPIC manifest (v1.0 B5). Gene set enrichment analysis was performed using GREAT.

Results Our analysis identified 3921 CpG sites (61% hypomethylated and 39% hypermethylated) in the blood cells of women that eventually developed breast cancer with a mean methylation difference of more than 5%. The identified methylation changes are significantly depleted in the exon boundary ($FC = 0.69$) of a gene and enriched in CpG islands ($FC = 1.2$). The GREAT-based gene set enrichment analysis showed that differentially methylated CpGs are enriched in seven genes. Several of these genes have been previously associated with breast cancer either through indication of better breast cancer patient prognoses (*LHX6* and *HLA-DPA1*) or increased breast cancer cell proliferation (*SGCE* and *PEG10*).

Conclusions Women diagnosed with breast cancer appear to carry genome-wide methylation changes in peripheral blood cells years before cancer diagnosis. These changes are enriched in CpG islands suggesting that they may directly affect expression levels of annotated genes. Overall, these results indicate that apart from *BRCA1* epimutation, the methylome of these women appears to carry other methylation changes that may contribute to the risk of breast cancer.

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[P79] Aberrant glycosylation contribute to cancer-promoting effect of bladder cancer-derived ectosomes in terms of recipient cell viability and migratory properties

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Introduction/Rationale Bladder cancer is a malignancy that remains a therapeutic challenge and requires extensive research into mechanisms of its progression. Several studies showed that extracellular vesicles, such as ectosomes, promote angiogenesis, migration, and metastasis, and inhibit apoptosis in bladder cancer. This effect may depend on their glycosylation status; thus the aim of this study was to investigate whether glycosylation of ectosomes released by bladder cancer T-24 and normal urothelial epithelial HCV-29 cells is responsible for the effect exerted by them on the proliferation and migration of recipient cells.

Methods HCV-29 and T-24 cells grown under standard conditions and in the presence of 1-deoxymannojirimycin (DMJ) – a specific inhibitor of α 1,2-mannosidase I, a crucial enzyme involved in the maturation of N-glycans to the complex type. Ectosomes were isolated from conditioned media collected from subconfluent cultures. Collected media was cleared of cells and cellular debris, concentrated by low vacuum filtration, and then centrifuged at 18 000 x g to pellet ectosomes. The functional effect of HCV-29- and T-24-derived ectosomes as well as aberrantly glycosylated ectosomes (from DMJ-treated cells) on recipient HCV-29, T-24, Hs27 fibroblasts and human umbilical vein endothelial cells (HUVEC) was assessed in Alamar Blue cell viability assay and wound healing assay after 18h of incubation.

Results In Alamar Blue assay, the addition of T-24 ectosomes (60 μ g of protein) caused an over 4-fold increase in the measured fluorescence intensity of recipient T-24 cells, while its effect exerted on HCV-29 cells was significantly weaker. HCV-29 ectosomes increased viability of recipient T-24 cells (by approx. 2-fold) but did not induce any changes in viability of HCV-29 cells. Furthermore, stronger promigratory activity of T-24-derived ectosomes was observed in comparison to ectosomes from HCV-29 cells. Also, in wound healing assay HCV-29 cells were less responsive to ectosomes treatment than T-24 cells. When ectosomes were isolated from DMJ-treated cells aforementioned effects were diminished, suggesting that glycans carried by ectosomes were involved in modulation of recipient cell function. HCV-29- and T-24-derived ectosomes also increased viability and motility of endothelial HUVEC cells and Hs27 fibroblasts, to a greater degree in the case of T-24-derived ectosomes.

Conclusions/Novel aspect Glycoproteins carried by ectosomes are one but not the only factor involved in ectosomes-recipient cells interactions and subsequent biological processes which lead to increased cell viability and motility. Also the functional effect exerted by ectosomes may depend on the type of recipient cells since different responses were observed between recipient bladder cells, endothelial cells, and fibroblasts. Nevertheless, obtained results support the hypothesis that ectosome can modulate function of various cells present in the tumor microenvironment.

[P80] transcriptomic information captured in pathway activation scoring methods in single cell RNA-SEQ data analysis

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The scRNA-Seq is the newest high throughput technology method which allows us to understand the biological processes with higher precision than before. In contrast to microarrays and bulk RNA-Seq, in scRNA-Seq bioinformatical investigation of gene set enrichment is commonly concentrated on single-sample approaches. They led to representation of data in the form of pathway activation score (PAS) matrix (pathways x cells). This gives the ability of capturing each cell heterogeneity within investigated dataset based on well-defined pathways. In the presented study, we compared 11 different PAS algorithms regarding their information consistency and clustering effectiveness.

The scRNA-seq dataset of COVID patients' blood, consisting of 4,903 samples and 15,390 measured genes, was used. Cells were labels into 6 different cell types (B cell, erythroid lineage cell, monocyte, T cell, platelet and neutrophil). The log normalization in Seurat package was applied. Next, gene expressions were transformed into explainable sets of genes representing immunological signaling pathways or biological processes (in total 114 pathways). Here, PAS algorithms were used i.e.: AUCell, CERNO, PLAGe, sparsePCA, ssGSEA, GSVA, z-score, DropOut Ratio, JASMINE, Mean Value and Vision. For generated PASs, Silhouette coefficient (SI) was calculated within each cell type to estimate information consistency. This process was also performed on top 20% variable genes of original data. The ANOVA test with Tuckey Post-Hoc was used to check the significant differences between methods. Finally, hierarchical clustering was applied, and normalized mutual information (NMI) was calculated to assess clustering performance of PAS methods.

The statistical inference of SI shows significant difference of PAS matrix performance in terms of information consistency (ANOVA p-value=0.0284). The best results were obtained for PLAGe, sparsePCA and ssGSEA (median SI 0.65, 0.66 and 0.53 respectively). For the top 20% variable genes the SI has a poor outcome with median equal 0.15. Moreover, the PLAGe shows significant difference to the top gene level analysis (Tukey p-value=0.0465). This indicates the power of PAS transformation. The best clustering was observed for JASMINE and ssGSEA methods with NMI equal 0.8. PLAGe has NMI on 0.76 which is slightly worse compared to top genes (NMI=0.79).

The majority of PAS methods group cells more effectively than investigation on gene level. Methods which are derivatives of PCA together with ssGSEA have the best performance in terms of information consistency. Surprisingly the rank-based methods like AUCell, CERNO have worse results than simple average. Moreover, ssGSEA gives better clustering results compared to gene level analysis. The presented results need further investigation into a larger number of datasets.

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[P81] Analysis of selected aquaporins mRNA expression in HCT116 cells exposed to environmental water

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Introduction Aquaporins (AQPs) are a family of small transmembrane transport proteins distributed in various human tissues. AQPs have been shown to be involved in the regulation of oxidative stress, as well as in cell proliferation and migration. The change in the expression level of AQPs has been associated with the pathogenesis of many diseases, including civilization diseases such as cancer, diabetes and obesity. One of the factors that contributes to the development of civilization diseases is environmental pollution. Environmental pollutants can cause oxidative stress in cells, therefore our research focused on two aquaporins (AQP3, AQP5) belonging to porins, that is, those that transport hydrogen peroxide.

Aim The aim of the study was to investigate whether in selected environmental waters in Silesia there are factors influencing the expression level of AQP3 and AQP5 in cancer human cells (HCT116).

Methods Water samples were collected in May 2022 from two water reservoirs, one river and the effluent of one of the wastewater treatment plants. All sampling sites were located in Silesia. Basic parameters (chemical oxygen demand, nitrogen and phosphorus levels) were determined in the water samples using standard tests. To determine the expression level of AQP3 and AQP5, HCT116 cells were treated with MilliQ water (control) or a sample of environmental waters. After 24h incubation, the RNA was isolated from the cells and the expression level of AQP3 / AQP5 mRNA was determined by RT-qPCR.

Results The basic parameters of all environmental water tests were within the norms. However, the level of AQP5 expression in HCT116 cells treated with environmental water samples increased (2.7-5.4 times depending on the water sample) compared to the level of AQP5 expression in control cells. The impact of environmental water samples on the expression of AQP3 was weaker (0.9-2 times depending on the water sample).

Conclusions Research showed that in the investigated environmental waters investigated there is a compound that influence the expression level of AQP3 and AQP5 in HCT116 cells. Because the basic parameters of the environmental waters are within the norms, we assume that one of the micropollutants in the water is responsible for the change in the expression of the tested AQPs. The mechanism of regulation of aquaporin transcription is not yet fully understood, so it is not possible to clearly determine which compound present in the water induces changes in the level of the tested aquaporins. It is known, however, that in the AQP3 promoter region there is an estrogen response element [Yde *at al*, BBA - Biomembranes 1863 (2021) 183619], and at the same time estrogens have been shown to be present in the environmental waters of Silesia [Dudziak *at al*, Ochrona środowiska, R26(1) (2004) 21]. Perhaps, the expression of the tested AQPs is influenced by estrogens present in environmental waters. However, this hypothesis requires further research.

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[P82] Effect of environmental exposure to cadmium on hormone therapy of breast cancer

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Introduction Breast cancer is the most common female cancer in Poland. Endocrine therapy is widely used for treatment. Tamoxifen (TAM) is the most common drug for ER-positive tumors for both pre- and post-menopausal women. Unfortunately, some women acquire resistance to the drug during ongoing treatment, and some have intrinsic resistance to TAM. It is still unclear what contributes to resistance, but there is a lot of evidence that these mechanisms are complex and difficult to explore. One of the factors contributing to endocrine therapy resistance may be environmental exposure to heavy metals, which tend to accumulate in fatty tissues, including the mammary gland. Cadmium (Cd) is one of the heavy metals widely found in the environment, and the main route of exposure for humans is smoking and diet. There is evidence that Cd has estrogenic properties and specifically activates the estrogen receptor and downstream regulation pathways, suggesting that environmental exposure to Cd may also contribute to TAM resistance. To explore this hypothesis, we analyzed the joint effects of combined exposure to Cd and TAM in MCF-7 cells (ER-positive cells, sensitive to TAM).

Methods MCF-7 cells were exposed to cadmium (as CdCl₂) in a short and long-term manner, at three different concentrations: 1 µM and 10 µM (72h) and 0,01 µM (6 months). 24h before cytotoxicity assessment, cells were treated with active TAM metabolite – 4-OHT at 15 µM (IC₅₀). After the exposure, the MTT reduction test and the Sulforhodamine B assay were performed to assess cytotoxicity. During the long-term experiment, cells were collected after 2, 4 and 6 months to assess the concentration of Cd.

Results Exposure to Cd significantly decreased the sensitivity of MCF-7 cells to 4-OHT in a dose-dependent manner in a short-term experiment, and this effect was further confirmed in long-term exposure, upon treatment to a low, environmentally relevant concentration of metalloestrogen. Moreover, a significant increase in Cd concentration was observed over time after 2, 4 and 6 months ($p = 0.02$), which confirms the phenomenon of Cd accumulation in human breast cancer cells.

Conclusions This study indicated that Cd interferes with tamoxifen treatment in MCF-7 cells. This observation deserves further research, as it may suggest that chronic exposure to Cd, resulting in its accumulation in the mammary gland, may be involved in intrinsic resistance to hormone therapy in breast cancer.

[P83] Heat shock-induced inflammatory response could be stronger in hsf1-deficient cells

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Heat Shock Factor 1 (HSF1), a transcription factor frequently overexpressed in cancer, is activated by proteotoxic agents and participates in the regulation of cellular stress response. To investigate how cancer cells deal with cellular stress in the case of HSF1 deficiency, we analyzed the global gene expression profiles in breast adenocarcinoma MCF7 cells with decreased HSF1 levels and corresponding control cells with normal HSF1 levels. Although the general transcriptional response to heat shock was impaired due to HSF1 deficiency (mainly chaperone expression was inhibited), a set of genes was identified, including *ATF3* and certain *FOS* and *JUN* family members (subunits of AP1 transcription factor), whose stress-induced activation was stronger and persisted longer than in cells with normal HSF1 levels. Gene set enrichment analysis of RNA-seq results indicated that HSF1-proficient and HSF1-deficient MCF7 cells may differ in the heat shock-induced TNF α signaling via NF κ B. This prompted us to analyze the level of cytokines produced and secreted to culture media after heat shock by HSF1+ and HSF1- MCF7, RKO (colon carcinoma), and HAP1 (near-haploid cell line derived from the chronic myelogenous leukemia KBM-7) cells. IL10, IL1 β , IL2, and IL4 were not released under any condition by all analyzed cell variants while IL6 was not detected in RKO cells. The ELISA assay combined with the RT-qPCR analyses indicated that the heat shock-induced production of TNF α was higher in HSF1- cells. On the other hand, expression of IL6 after heat shock was delayed in HSF1- MCF7 and HAP1 cells and started to rise as the decline in HSF1+ began. These results suggest that the inflammatory response induced by heat shock could be stronger and extended in cells with low levels of HSF1. Thus, in addition to the fact that HSF1 drives a transcriptional program distinct from heat shock to support malignant phenotype, proteotoxic stress (e.g., induced by therapeutic hyperthermia) can elicit different effects depending on the level of HSF1 and the type of cancer. In addition to the different levels of chaperones that are dependent on HSF1, the inflammatory response mediated by AP1 and ATF3 can differ from tumor to tumor.

[P84] Apoptotic effect of *Hypericum perforatum* L. extracts on human melanoma cells cultured under normoxia and hypoxia

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Introduction Cutaneous melanoma (CM) is the most aggressive skin cancer. During CM progression, tumour cells reprogram their proliferation and survival pathways, and acquire resistance to treatment-induced apoptosis. Furthermore, the hypoxic microenvironment is one of the predominant causes of incomplete response to therapies in CM. Plant extracts of *Hypericum perforatum* L., containing numerous secondary metabolites. Among them, hyperforin, which shows the ability to inhibit the growth of cancer cells by inducing apoptosis, appears promising in the fight against cancer development. Therefore, **the aim of our study was** to evaluate the effect of *Hypericum perforatum* L. extracts and hyperforin salts on apoptosis of WM115 and WM266-4 human melanoma cell lines under normoxia and hypoxia conditions.

Methods Cell lines isolated from the same patient at two stages of melanoma progression, a primary cutaneous tumour (WM115) and a lymph node metastasis (WM266-4), were used for the study. *Hypericum perforatum* L. ethanolic extracts made from the herb collected in Krakow and commercially available from Herbapol were used to induce apoptosis. Cells were incubated under normoxia (21% O₂) and hypoxia (0.5% O₂) for 24 hours with different concentrations of extracts and hyperforin salts diluted in RPMI 1640 medium. The apoptotic effect was assessed by flow cytometry for cells labeled with annexin V and showing caspase 3 and 7 activity. A two-way ANOVA test (p<0.05) was performed to compare the effects of hyperforin salts and *H. perforatum* L. ethanolic extracts in normoxia and hypoxia.

Results Our study showed a dose- and oxygen level-dependent response of both melanoma cell lines to *H. perforatum* L. extracts. We observed an increase in the number of early apoptotic cells and those showing caspase 3/7 activity after incubation with the highest doses of ethanolic extracts. Both melanoma cell lines showed a greater susceptibility to apoptosis induced by extract components under hypoxia than under normoxia conditions. Hyperforin salt had a lower apoptotic effect than the *H. perforatum* L. extracts regardless of the oxygen level. In addition, WM266-4 metastatic melanoma cells showed greater resistance to secondary metabolites present in the analysed extracts than WM115 primary cell line.

Conclusions Both commercially available and self-made extracts exerted the apoptotic effect on melanoma cells, aggravated by hypoxia. We found that hyperforin is not the only component of the extracts that inhibits the growth of CM cells leading to cell death. Further studies are needed to identify other compounds involved in the induction of apoptotic pathways.

[P85] A high level of HSF1 is detected in triple-negative breast cancer cells with invasive phenotype

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Breast cancer is a heterogeneous disease with different pathological and molecular features. Among them, triple-negative cases (TNBC), negative for steroid hormone receptors (ER and PR) and HER2, are the most aggressive with a higher rate of distant recurrence and a worse prognosis. Because TNBC is still the least understood breast tumor with few therapeutic options, finding new biomarkers is important for the improvement of prognosis or development of novel therapeutic targets. Heat shock factor 1 (HSF1), a well-known regulator of the cell stress response, is frequently detected at high levels in invasive stages of cancer. Here, we investigated HSF1 levels in several cell lines originating from patients with triple-negative breast cancer (MDA-MB-468, MDA-MB-231, BT-549, CAL120, and HCC1395). Among them, MDA-MB-468 cells exhibited the lowest HSF1 level, MDA-MB-231 and CAL120, the moderate, while BT549 and HCC1395 cells, the highest. Interestingly, MDA-MB-468 cells were characterized with the expression of E-cadherin (CDH1, epithelial cell marker), which also correlated with epithelial CD44pos/CD24pos cell phenotype assayed by flow cytometry. Other cell lines, expressing a different level of HSF1 (but higher than MDA-MB-468 cells), expressed vimentin (VIM, a mesenchymal cell marker) and characterized predominantly mesenchymal CD44pos/CD24neg phenotype. By comparing the migratory ability of these cell lines in the trans-well assay, we noticed that MDA-MB-468 cells showed the lowest ability to migrate, while the highest was detected for BT549 cells. Other cells with the highest HSF1 level (HCC1395) did not have a high ability to migrate compared to BT-549 cells or cells with moderate HSF1 levels. It indicates that there is no correlation between the ability to migrate and the HSF1 level or CD44/CD24 phenotype of analyzed cell lines. Nonetheless, analyzing the cell growth in Matrigel, we noticed that BT549 and HCC1395 exhibit stellate invasive phenotype, while other breast cancer cells with low (MDA-MB-468) or moderate (MDA-MB-231 and CAL120) HSF1 levels exhibited less aggressive grape-like phenotype. Therefore, we hypothesize that the correlation between high HSF1 levels and the acquisition of invasive features by breast cancer cells may exist. However, the possible utility of HSF1 as a prognostic biomarker for TNBC patients or as a therapeutic target requires further research.

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[P86] HSF1 may be involved in heat shock- or estrogen-induced chromatin reorganization in MCF7 breast cancer cells through interactions with CTCF and ER α

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HSF1 (Heat Shock Factor 1) is activated by proteotoxic stress (e.g. heat shock) and is responsible for cytoprotection through the activation of genes coding for chaperones. It is also activated by estrogen in estrogen receptor α (ER α)-positive breast cancer cells. Our ChIP-seq analysis revealed a strong enrichment in HSF1 binding induced by heat shock in MCF7 cells: approximately 14 times more peaks were identified than in untreated cells. However, most of them were located not in promoters but in intergenic and intronic regions. Thus, we hypothesized that HSF1 may be involved in the reorganization of chromatin domains after heat shock through interactions with CTCF (CCCTC-Binding Factor), a known regulator of chromatin architecture. We found that HSF1 is likely to interact with CTCF in MCF7 cells (as assessed by the proximity ligation assay) and the number of interactions increased significantly after heat shock treatment. Thus, remodeling of the genome-wide binding of HSF1 after heat shock could be mediated by CTCF. Interestingly, HSF1 is also likely to regulate the CTCF expression as it may bind to CTCF regulatory sequences following heat shock. Western blot analysis indicated that the CTCF protein levels may slightly increase after heat shock, however, this seems to be HSF1-independent (a similar effect was observed in HSF1-proficient and HSF1-deficient cells). Interestingly, heat shock resulted in the strong, although transient, accumulation of the shorter (70 kDa) form of CTCF. It was previously shown that such CTCF form results from C-terminal truncation and is likely to be a product of premature termination of translation. It was suggested that it may enhance the transactivation capacity of the normal CTCF. We found that its levels were higher and kept longer during recovery from heat shock in HSF1-deficient than in HSF1-proficient cells indicating that HSF1-dependent mechanisms are responsible for the restoration of the full-length CTCF translation.

Our results indicate that HSF1, in addition to direct regulation of transcription (by binding to the promoters) may have an indirect impact on transcription by participating in chromatin organization via CTCF. Both HSF1 and CTCF are expressed ubiquitously, so this may be a universal mechanism not related to the cell type. We found that HSF1 was also able to participate in chromatin organization by interacting with ER α and the absence of HSF1 resulted in changes in the organization of chromatin loops that may affect transcription. Interactions of HSF1 with ER α are cell type-specific and could account for the observed differences between ER-positive and ER-negative tumors in the expression of HSF1-dependent genes.

This work was supported by Polish National Science Centre.

[P87] GPX4-modified CRISPR/Cas9 HCT116 cells avoided ferroptosis death under oxidative stress

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Introduction One of the greatest scientific achievements of recent years, the precise and fast CRISPR/Cas9 genome editing system has enabled groundbreaking results in the field of medicine and genetic engineering. This technique based on elements of immune system identified in *E. coli*, after modification is used to human genome editing *in vitro*. CRISPR/Cas9 renews the targeted genome editing approach in a more efficient and specific way, thus facilitating the smooth implementation of genome changes, e.g. by inducing DNA double strand breaks in any organism and cell type.

Aim A glutathione peroxidase 4 (GPX4) is an intracellular antioxidant enzyme that can directly reduce peroxidated phospholipids in the plasma membrane. GPX4 catalyzes the reduction of hydrogen peroxide, organic hydroperoxides and lipid peroxides at the expense of reduced glutathione, thereby protecting cells from oxidative stress. **The GPX4-knockout HCT116 cells were examined under the oxidative stress for ferroptosis induction.**

Methods Human colorectal cancer HCT116 cells were edited with CRISPR/Cas9 method for gene GPX4 knockout. Wild type and selected mutants (western blot confirmed) were exposed to Erastin (5, 10 μ M) for 24 h. Reactive oxygen species (ROS), mass and mitochondrial potential were measured using the Varioskan LUX multimode microplate reader, preceded by specific dyes addition. Using RT-qPCR, gene expression of ACSL4 and TFRC (propagators and ferroptosis markers) was evaluated. Cells viability was assessed by the MTT assay, and cell's morphology and damage by microscopic live observations (JuLI_FL™ apparatus).

Results In HCT116 WT and GPX4-modified CRISPR/Cas9 mutants increasing ROS level after ferroptosis induction, resulted from total glutathione, mass and mitochondrial potential elevation – for antioxidative protection of treated cells. Erastin addition overexpressed marker genes - ACSL4 and TFR1. GPX4-modified CRISPR/Cas9 mutants, lacking of ferroptosis-bloker, protein GPX4 still presented a good viability and morphology, because of compensation effects coming from a different than ferroptosis pathway.

Conclusions Inactivation of GPX4 leads to the accumulation of ROS and pro-oxidative enzymes production (ACSL4); however the HCT116-GPX4- mutants still avoid ferroptotic death.

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[P88] Developing a model to test the different functions of HSPA1 and HSPA2 chaperon proteins in human bronchial epithelium

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Environmental pollution and unhealthy lifestyle lead to an increased number of upper airway disorders. The development of new models for studying the respiratory tract would improve our knowledge of the mechanisms controlling homeostasis in the tissues.

Heat shock proteins (HSPs) are a large group of molecular chaperones. HSPs assist in the protein folding process and contribute to a protein quality control system. The HSPA (HSP70) family groups several cytoplasmic/nuclear chaperones of evolutionarily conserved proteins. Despite numerous studies, it is still unclear whether the functional significance of those proteins is fully redundant, or at least partially different. The Heat Shock Protein A1 (HSPA1) plays a key role in cytoprotection and contributes to adaptive stress responses including stress-induced immunomodulation. Heat Shock Protein A2 (HSPA2) is one of the most intriguing members of the human HSPA (HSP70) family. This protein over the years was regarded as a testis-specific chaperone essential for male fertility. Recently, we found that HSPA2 is also present in certain populations of somatic cells, including the basal cells in various stratified and pseudostratified epithelia (esophagus, epidermis, bronchus) [1]. We hypothesize that the HSPA2 protein, but not HSPA1 affects the basal cells in bronchial epithelium in terms of their proper differentiation and formation of functional epithelia. We have previously found that the differentiation of keratinocytes into multilayered epidermis depends on HSPA2 activity [2]. HSPA1 in turn could be implicated in protective mechanisms against environmental stresses.

This study has been designed to generate *in vitro* tissue-like model of genetically modified human bronchial epithelial cells BEAS-2B. We used CRISPR/Cas9 double nickase system with commercial plasmid and lipofection to obtain cells with the knockout of the *HSPA2* gene. The same system with sequences designed by the author (without the use of plasmid) and nucleofection was used to do the simultaneous knockout of two genes *HSPA1A* and *HSPA1B* (coding for HSPA1 protein). Cells after transfection have been selected to generate cell lines with a successful knockout of a particular gene. The derived *HSPA2*- and *HSPA1*-null cell lines are tested (cell viability, proliferation, adhesion test, clonogenic assay) to characterize the phenotypic changes caused by the knockout of one of those proteins. Afterward, we will use derived cell lines to create an ALI model to test the effect of protein knockout on their ability to form fully differentiated epithelium and their sensitivity to tobacco smoke components.

Keywords: heat shock proteins, CRISPR/Cas9, epithelial cells

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[P89] Tube formation assay shows the pro-angiogenic effect of melanoma-derived ectosomal integrins

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Background Integrins are heterodimeric transmembrane cell adhesion molecules known to play a crucial role in the initiation, progression, and metastasis of solid tumors. Investigation of whether the integrins delivered via melanoma-derived ectosomes have the ability to induce angiogenesis was the aim of this study.

Methods Ectosomes released by primary WM115 and metastatic WM266-4 cutaneous melanoma cells were isolated from conditioned media that had previously been concentrated by low-vacuum filtration by differential centrifugation. Then WM115- and WM266-4-derived ectosomes alone, or in combination with anti- $\alpha\text{v}\beta\text{3}$ or anti- $\alpha\text{v}\beta\text{5}$ integrin antibodies, as well as with cilengitide or echistatin, which are integrin antagonists, were added to human dermal microvascular endothelial cells (HDMEC) seeded on Matrigel. After 18 hours of incubation, images were taken and tube formation analysis was performed using Image J software with the addition of the angiogenesis plugin. Parameters such as the number of closed tubes, junctions, branching points, and total tube length were evaluated.

Results The addition of WM266-4-derived ectosomes increased the number of closed tubes and their total length but the effect was diminished when anti- $\alpha\text{v}\beta\text{3}$ or anti- $\alpha\text{v}\beta\text{5}$ antibodies were added. Ectosomes isolated from the metastatic cell line showed higher pro-angiogenic potential than primary ones. Echistatin showed a stronger anti-angiogenic effect compared to cilengitide. Moreover, the addition of ectosomes in combination with anti-integrin antibodies or integrin antagonists generally resulted in the reduction of junctions and total tube length.

Keywords: extracellular vesicles, integrins, angiogenesis, melanoma

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[P90] Chemopotentiating effects of low-dose fractionated radiation in normal fibroblasts from patients with head and neck cancer treated with LDFR combined with induction chemotherapy

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One possibility to benefit in the clinic from the low-dose hyper-radiosensitivity (HRS effect in which cells die from excessive sensitivity to low doses < 0.5 Gy of ionizing radiation) is using low-dose fractionated radiation (LDFR) as an enhancer of systemic chemotherapy. The fact that our National Research Institute of Oncology, as the first in Poland, has started a phase II clinical trial using LDFR (0,5 Gy fractions) combined with induction chemotherapy in patients with locally advanced squamous cell carcinoma of head and neck (SCCHN) gave us a unique opportunity to recognize potential predictors and still not well understood molecular mechanism underlying the clinical response to such treatment. Recently the Foray group proposed a reliable predictive assay based on the theory of nucleoshuttling of ATM kinase (RIANS). This theory is based on the ATM protein transit from cytoplasm to nucleus in irradiated cells and assumes that the rate of recognized DSB is proportional to the pATM forms that diffuse from the cytoplasm into the nucleus and phosphorylate H2AX at the DSB. Because the incidence of the HRS effect has never been studied in normal cells of SCCHN patients, the aim of the study is to determine: 1) whether the chemopotentiating effect of LDFR applies to normal cells and depends on the HRS status, and 2) whether the radiation-induced ATM nucleoshuttling (RIANS model) participates in the mechanism of chemopotentialization by LDFR and may be a predictive biomarker of such treatment.

The effect of low fractional doses (LDFR 4 x 0.5 Gy) compared to a single dose of 2 Gy on carboplatin and paclitaxel (cytostatics used in a clinical trial) in normal skin fibroblasts derived from 11 patients with SCCHN enrolled in the clinical study (LDFR+ChT) is assessed by clonogenic and immunofluorescence pATM and γ H2AX foci assays. Determination of the maximum number of recognized DSB (pATMmax and γ H2AXmax) in fibroblasts of each patient were quantified according to the number of pATM and γ H2AX foci scored after 10, 20, 30 minutes, 1 and 24 hours after irradiation with 0.2, 0.5 and 2 Gy.

Based on cell survival results, LDFR induced an inverse effect of dose fractionation and enhanced the effects of carboplatin and paclitaxel significantly greater or at least at the same level as a single dose of 2 Gy irrespective of the HRS effect (the HRS response was demonstrated for fibroblasts of 3 of the 11 patients). Preliminary results from immunofluorescence assay don't indicate the response of DSB recognition mechanisms on used cytostatics. Effects of LDFR were significantly greater (more DSB unrecognized or repaired until the last fraction) than those of 2 Gy single fraction in HRS-negative fibroblasts of one patient. The number of initial pATMmax and γ H2AXmax appears to be independent on the HRS status. Research is ongoing.

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[P91] Polymorphism or mutation in chondrosarcoma? – Tumor-only mutation detection pipeline with non-paired control, based on next-generation sequencing

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Introduction Chondrosarcoma (ChS) belongs to a rare and heterogeneous group of bone sarcomas and is the second most common malignant primary bone tumor. Genetics of ChS and the role of particular mutations in pathogenesis of these tumors is still poorly understood. Next-generation sequencing is a fast and efficient method of mutation profile analysis, however the subsequent analysis of the results, and selection of genetic variants are much more challenging. The best practice in tumor somatic variant detection is using matched normal samples from the same patient, which is often not possible to employ. The aim of this study was to identify mutations occurring in ChS and development of mutation detection methodology for cases with no matched normal bone tissue available.

Methods DNA was isolated from formalin-fixed paraffin-embedded (FFPE) tissues of 48 patients diagnosed with ChS of different histological subtypes (15 G1, 19 G2, 9 G3, 4 dedifferentiated, and 1 mesenchymal). Control DNA was isolated from 9 normal blood and FFPE tissues from local population. Mutational profile was analyzed with Next Generation Sequencing (NGS) on Ion Torrent platform with the use of a 409 genes panel. Sanger sequencing was used to confirm selected recurring mutations detected by NGS. Raw reads were processed by the Torrent Suite analysis pipeline and mapped to the hg19 human genome. Variant calling and selection were performed by GATK, Torrent SuiteTM and Ion ReporterTM software. Functional annotation of the variants was performed using Ensembl Variant Effect Predictor (VEP).

Results 650 potentially pathogenic genetics variants were detected in 48 ChS samples. 31% of these variants were also detected in normal samples. The most frequent, occurring both in tumor and normal samples, were variants in *THBS1*, *TAF1*, *HNFI1A*, *EPHB1*, *ESR1*, *GPR124*, and *KMT2D* genes. Pathogenic deletion (c.864del) in *HNFI1A* gene was detected in 50% of tumor samples and 55.5% of normal samples. However, Sanger sequencing, performed in both normal and tumor samples, did not confirm this deletion in *HNFI1A* gene. Instead, we identified a silent mutation (c.864G>C) in this gene. Finally, 356 variants were selected as potentially pathogenic in ChS, excluding variants identified in normal samples. *TAF1L*, *IDH1*, *LRP1B*, *ATRX*, *ARID1A*, *IDH2*, *TP53*, *BCL11B*, and *CSMD3* genes were found to be the most frequently mutated (in more than 10% of tumor samples).

Conclusions Analysis of normal tissues enables optimization of tumor mutation analysis pipeline. NGS analysis of normal samples may result in identification of polymorphisms and common mutations, not pathogenic in ChS development. We showed that tumor-only sequencing is not sufficient enough to accurately identify somatic mutations, without an additional control of normal tissue. Moreover, Sanger sequencing is essential in identifying NGS artefacts, as that observed in *HNFI1A* gene. Other mutations detected in the analysis need to be confirmed in the same manner.

[P92] Hyperthermia treatment as a potential anticancer strategy. Combination therapy with hyperthermia and cisplatin and its effect on ovarian cancer cell morphology

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Ovarian cancer is the second most common cancer of female reproductive system. In 2020, 4 669 new cases of this disease were detected in Poland, while 3 131 women died from it. Such a high mortality is a result of the lack of preventive examinations and ambiguous symptoms in the early stages of the disease. The standard first line treatment of patients with advanced-stage ovarian cancer is surgery, followed by chemotherapy with cisplatin and paclitaxel. The main disadvantage of this therapy are very frequent relapses of the disease, caused by the development of cisplatin resistance. For this reason, ways are sought to increase the effectiveness of cisplatin, and reduce the resistance of cells to its action. One of the possible solutions is the use of combination therapy consisting of simultaneous application of a cytotoxic drug and hyperthermia. The underlying concept of our study is the hypothesis that appropriate drug combinations, including cisplatin with hyperthermia, will exert maximal effects in cancer treatment while limiting the side effects of the cytostatic used. In this study, the efficacy of such anticancer therapy was tested, and the analysis of the cytotoxic effect of the two agents tested on ovarian cancer cells was verified by assessing cell morphology.

During conducted studies, SKOV-3 and TOV-21G tumor cell lines were treated with a cisplatin at a concentration of 50 nM (SKOV), and 5 nM (TOV-21), and with a temperature of 37°C, 39°C and 40°C. Then, the cells were stained with acridine orange, ethidium bromide and DAPI, and observed under fluorescence microscope. The obtained results indicate the occurrence of a number of morphological changes in the nucleus and cytoplasm of tested cells, indicating the activation of apoptosis and necrosis pathways in the tested cells. The conducted studies showed a significant improvement in the effectiveness of the cytotoxic effect of cisplatin in combination with moderate hyperthermia (temperature of 39°C), compared to standard conditions (temperature of 37°C). The increase in number of apoptotic cells was observed in both of the analyzed temperature points (39°C and 40 °C), however, the most effective temperature level was 39°C. The percentage of the apoptotic cells for SKOV-3 in those conditions was 20,25%, while in standard conditions it was 5,96%. For TOV-21G the similar situation was observed - percentage of the apoptotic cells in temperature of 39°C was 62,37%, while in temperature of 37°C was 7,8%.

Those observations confirm the results of other studies, indicating the influence of hyperthermia on the mechanisms of cisplatin resistance in ovarian cancer cells, leading to reduction of cisplatin resistance of these cells. Yet, there is still a need to continue research in this area, so that combination therapy based on the use of hyperthermia as a component of treatment can be introduced in the future.

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The band **AUTENTIKOS** was founded in August 2015 in Mikołów. It consists of five musicians with many years of stage experience:

- **Arkadiusz Kuś** - founder and drummer,
- **Marcin Tomaszewski** - bass guitar player,
- **Rafał Cieślak** and **Tomasz "Tiziano" Czerw** – guitar players,
- **Łukasz Kwapisiewicz** – pianist,
- **Sebastian Giebel** – a professor of medicine and hematologist – the vocalist and author of the lyrics.

In **2018**, the first AUTENTIKOS album entitled "Audioterapia" was released. In **April 2019**, the band performed in the concert studio of Radio Katowice, while recording material for the first live album entitled "Maria Curie". In **December 2022**, the second studio album will be released.

Songs with a variety of musical styles, from dance funky through hip-hop, poetic ballads, punk and progressive rock, were at the top of the Radio Katowice and the Warsaw "Radio dla Ciebie" hit lists. In the textual layer, they refer to the reality of the contemporary world, sometimes seen in a crooked, pastiche mirror through the eyes of the singer - the author of the lyrics.

The common denominator is the sincerity of the message, which became the basis for choosing the name of the band.

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