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RESEARCH REPORT 2022

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DEPARTMENT OF EXPERIMENTAL ONCOLOGY
Head: Professor Leon Strządała, Ph.D.

Laboratory of Experimental Anticancer Therapy
Head: Professor Joanna Wietrzyk, Ph.D.

Effect of increased expression of osteopontin (OPN) under the influence of vitamin D on the phenotype of cancer-associated fibroblasts (CAFs) from breast cancer patients

Research was conducted as an introduction to further work in this field, namely the analysis of the effect of CAFs isolated from breast cancer patients treated with calcitriol on cancer cells. Conditioned medium (CM) was harvested from 18 cultures of CAFs starved for 24 hours in serum-free medium. CM was applied to breast cancer cells of the MCF-7 and MDA-MB-231 lines to check the effect on migration and on the expression of selected proteins. CAFs CM significantly accelerated the migration of both tumor cell lines. In addition, a decrease in MCF-7 migration was observed after stimulation with CM collected from CAFs treated with 10nM calcitriol compared to CM from untreated CAFs. In addition, attenuation of the pro-migration activity (for MCF-7) of CAFs after calcitriol stimulation is observed only for CAFs from postmenopausal and metastatic cancer patients. In MCF7 and MDA-MB-231 cells stimulated for 72 hours with CAFs CM levels of E-cadherin, OPN precursor (pre-OPN), OPN and ZEB1 were assessed. In the MCF7 and MDA-MB-231 cell lines, E-cadherin levels were decreased after stimulation with CAFs CM treated *ex vivo* with calcitriol.

Research on the use of new biologically active compounds in anticancer therapy

The research was aimed at assessing the anticancer activity of thiosemicarbazones, which are obtained in cooperation with the Wrocław University of Technology. Forty new thiosemicarbazones were obtained. According to the literature data, in terms of antitumor activity, the substitution of N(4) nitrogen with one or two aliphatic substituents is the most preferred. Therefore, the synthesized derivatives contained linear aliphatic chains from C1 to C8 and unsaturated substituents, as well as modifications such as in the structures of DpC and COTI-2, one of the most active thiosemicarbazones described in the literature and in clinical trials. The antiproliferative activity of the obtained compounds against normal and neoplastic cells was determined. The most active compounds turned out to be 122, 124 and 127. The most interesting compounds turned out to be 107, 108 and 109, which were characterized by high antiproliferative activity against human breast cancer cells and at the same time lack of toxicity against cells of normal breast epithelium.

In vitro studies on the influence of proinflammatory cytokines on transendothelial migration of phagocytic cells

The ability of RAW 264.7 cells to migrate in the presence of M-CSF; 72-hour *in vitro* culture of MC38 cells; 72-hour *in vitro* culture medium of MC38 cells; and 72-hour *ex vivo* culture medium of cells isolated from MC38 tumor tissue was analyzed. A medium with 5% FBS was used as a control. Increased ability of RAW264.7 phagocytic cells to migrate in the presence of M-CSF or factors secreted by MC38 tumor cells cultured *in vitro* and *ex vivo* was demonstrated in relation to unstimulated cells. The highest migratory capacity of RAW 264.7 cells was demonstrated in the presence of a 72-day medium containing factors produced by MC38 cells grown *in vitro*. The obtained data will allow us to start the next stage of research,

which will be the creation of a model enabling the determination and regulation of changes in the infiltration of neoplastic tissue by phagocytic cells in the presence of extracellular matrix.

The role of the miR-125b molecule in the anticancer activity of calcitriol and paclitaxel

The MDA-MB-231 breast cancer cell line, which is characterized by a high level of miR-125b compared to the MCF-7 and MCF-10A lines, was used for the study. The cells were transfected with the miR-125b inhibitor and the negative control, and then the cytotoxic activity tests of calcitriol, tacalcitol and cytostatics: paclitaxel, doxorubicin, 5-fluorouracil and cisplatin were performed. Description of the most important achievements: The use of the miR-125b inhibitor did not affect the sensitivity of MDA-MB-231 cells to calcitriol, tacalcitol and the tested cytostatics. The obtained results will affect the modification of the research hypothesis of the grant project being prepared.

Laboratory of Tumor Molecular Immunobiology
Head: Professor Wojciech Kałas, Ph.D.

Cellular senescence is a natural process that gradually intensifies with age. Anti-cancer therapy is a potent trigger of the sudden senescence induction that affects normal and cancer cells. It is unclear if the induction of senescence of cancer cells is a positive or negative treatment outcome. Significantly, there is insufficient direct data on the impact of senescence induced by chemotherapeutics on immunosurveillance. In our current studies, we asked about the relation of cellular senescence, cancer therapeutics, and immune surveillance with natural killer cell activity. There are a few experimental systems suitable for measuring NK cell activity. All experimental set-ups for measuring NK cell activity require vast quantities of NK cells, which limits accuracy and practicality. Thus, we decided to design and work out an improved system for measuring the anti-cancer activity of NK cells based on the direct interaction of cells. Last year, we used that system to evaluate the cytotoxic activity of NK92 cells against cancer and cancer senescent cells. A three-day-long treatment with low-dose etoposide-induced the senescence. We found that the activity of NK92 cells against senescent cells was impaired. The other feature of senescence is its unique secretory phenotype (Senescence-associated secretory phenotype; SASP). We collected SASP of etoposide-induced cancer senescent cells and found that pretreatment of NK92 cells with SASP impacts the activity of NK92 cells against cancer cells.

Our results strongly suggest that drug-induced senescence harms NK cells' ability to recognize and kill cancer cells.

Laboratory of Biomedical Chemistry
Head: Professor Tomasz Goszczyński, Ph.D.

Boron cluster-based strategies to overcome antibiotic resistance of pathogens

The rapid spread of antibiotic-resistant pathogens is a global problem forcing governments, health organizations, and the scientific community to increase efforts to develop novel classes of antibiotics. One of the main threats are infections caused by *Candida* species, which developed resistance to existing drugs and are among the leading causes of morbidity and mortality worldwide. Currently, only four main classes of antifungal drugs are available: polyenes, azoles, echinocandins, and 5-flucytosine. Development of new classes of antifungal drugs requires using all chemical space to search for novel chemical leads. Boron clusters are inorganic compounds with versatility and ease of modification similar to organic compounds,

providing new opportunities to develop unprecedented antimicrobial agents. Boron clusters are abiotic structures, resistant to metabolism; nevertheless, they interact with components of biological systems, such as proteins, lipid membranes, and nucleic acids, but through different mechanisms. Boron cluster-containing compounds showed antimicrobial and anti-biofilm activity against Gram-positive and Gram-negative bacteria and fungi. Importantly, their activity remains unchanged when used against multi-drug resistant (MDR) pathogens.

In our studies, we synthesized organic-inorganic hybrid molecules containing one of the boron cluster family members – a metallacarborane cobalt bis(dicarbollide) (COSAN). The COSAN derivatives were tested against a panel of bacterial and fungal strains: Gram-positive *S. aureus*, Gram-negative *P. aeruginosa*, *E. coli*, and *K. pneumoniae* and a pathogenic yeast *C. albicans*. The compounds showed high to moderate antimicrobial activity. Next, the derivatives were tested against other representatives of the *Candida* genus and a panel of 100 clinical *C. albicans* isolates. All tested strains, even those resistant to antifungal drugs amphotericin B (AmpB) and fluconazole (Flu), were susceptible to an iodine derivative of COSAN (I-COSAN). Additionally, several other COSAN derivatives showed good activity against selected clinical isolates. Additionally, I-COSAN showed synergistic activity in combination with AmpB.

To evaluate the toxicity of the compounds, they were tested against the MCF 10A human breast epithelial cell line and mouse BALB/3T3 fibroblasts. Depending on the structure, the derivatives had high to low toxicity against the cell lines with I-COSAN being nontoxic. The anionic O-linked derivatives had low toxicity, whereas zwitterionic N-linked derivatives showed high antiproliferative activity. The derivatives were also tested against fish embryos to determine their embryotoxic properties. The modification of I-COSAN decreased its toxicity and spectrum of developmental malformations.

In conclusion, we showed that metallacarborane derivatives can have a broad antimicrobial activity while remaining low toxicity against eukaryotic cells and fish embryos. These results highlight the potential of COSAN as a lead structure for development of potent antimicrobials.

DEPARTMENT OF CLINICAL IMMUNOLOGY

Laboratory of Clinical Immunogenetics and Pharmacogenetics
Head: Professor Katarzyna Bogunia-Kubik, Ph.D.

The Laboratory of Clinical Immunogenetics and Pharmacogenetics is engaged in a multicentre, interlaboratory and international cooperation based on scientific projects financed by NCBiR (National Centre for Research and Development) and NCN (National Science Centre – Opus and Preludium projects). The focus of these projects is to identify diagnostic and prognostic biomarkers. The studies were performed on samples from patients with haematological disorders, breast cancer, as well as in an *in vitro* model using cell lines and organoids.

The studies involved an analysis of expression and polymorphism of two genes, basigin (BSG, CD147) and SLC16A1 (MCT1), in patients with acute myeloid leukaemia (AML) and multiple myeloma (MM). It was found that BSG together with SLC16A1 (MCT1), a gene closely associated with BSG in energy metabolism, are overexpressed in AML cell lines compared to normal cells. Additionally, an analysis of serum was performed and showed that soluble BSG (sBSG) was higher in AML patients than in healthy individuals. Furthermore, high sBSG was associated with worse overall survival and higher blast percentage (Łacina et al., J Clin Med. 2022). Serum sBSG concentration was also increased in patients with MM, especially in patients with more advanced disease, and high sBSG was associated with shorter progression-free survival. Soluble BSG concentration decreased after remission in MM patients

(Łacina et al., Curr Issues Mol Biol. 2022). BSG and MCT1 gene variants rs4919859 C, rs4682 C and genotype rs1049434 AA were associated with shorter overall survival in patients with AML (Łacina et al., J Clin Med. 2022). These results were of major importance in the context of previous studies on the subject of BSG, and showed that serum sBSG may be a potential prognostic biomarker in haematological disorders. Moreover, rs4444903 AA homozygosity within the gene coding for a proangiogenic factor EGF was found to associated with shorter overall survival and hypercalcaemia in MM.

A study on patients undergoing allogeneic stem cell transplantation showed that those who developed chronic Graft-versus-Host-Disease (cGvHD) had a different microRNA expression profile in microvesicles isolated from plasma than patients who had developed no cGvHD symptoms. A panel of 3 microRNAs (miR-630, miR-374b-5p, miR-29c-3p) is expressed differently in cGvHD patients and can be a potential cGvHD diagnostic marker. This study was the first to use the novel NanoString technology in a study of microRNA in cGvHD (Łacina et al., Adv Clin Exp Med. 2022).

A study on women with breast cancer and on organoids (3D *in vitro* cell cultures) derived from them was conducted and showed an association between the expression of specificity protein 1 (SP1) and myelocytomatosis oncogene (MYC). Furthermore, a correlation between expression of the telomerase catalytic subunit TERT (telomerase reverse transcriptase) and tumour protein 53 (TP53) was observed. In breast cancer patients, TERT genetic variation was studied in the context single nucleotide substitutions, telomere length and TP53/SP1 gene expression and related to clinical data. The results showed that analysis of TERT gene polymorphism, TERT expression, and telomere length may be potential biomarkers of breast cancer (Dratwa et al. Int J Mol Sci. 2022).

In conclusion, our multifunctional, multicentre studies on patients with blood and breast cancers allowed us to identify biomarkers associated with risk and course of disease, as well as with treatment success.

Laboratory of Immunogenetics and Tissue Immunology
Head: Professor Izabela Nowak, Ph.D.

The association of *HLA-G* gene polymorphism and its soluble form with male infertility

Successful reproduction depends on many factors. Male factors contribute to infertility in approximately 50% of couples who fail to conceive. Seminal plasma consists of secretions from different accessory glands containing a mixture of various cytokines, chemokines, and growth factors, which together can induce a local immune response that might impact on a male's as well as a female's fertility. Human leukocyte antigen (HLA)-G expression has been suggested as an immunomodulatory molecule that influences pregnancy outcome. The *HLA-G* gene encodes either membrane-bound or/and soluble proteins. The aim of our study was the evaluation of *HLA-G* polymorphisms and their impact on soluble HLA-G (sHLA-G) production. We tested the *HLA-G* polymorphism in three positions: rs1632947: c.-964G>A; rs1233334: c.-725G>C/T in the promoter region; rs371194629: c.∗65_∗66insATTTGTTCATGCCT in the 3' untranslated region. We tested two cohorts of men: 663 who participated in *in vitro* fertilization (test material was blood or sperm), and 320 fertile controls who possessed children born after natural conception (test material was blood). Since 50% of men visiting assisted reproductive clinics have abnormal semen parameters, we wondered if men with normal sperm parameters differ from those with abnormal parameters in terms of **HLA-G** polymorphism and secretion of sHLA-G into semen. We found that certain rs1632947-rs1233334-rs371194629 *HLA-G* haplotypes and diplotypes were associated with male infertility, while others were protective. Normozoospermic men with the A-C-del

haplotype and A-C-del/A-C-del diplotype secreted the most sHLA-G into semen (574.1 IU/mL and 1047.0 IU/mL, respectively), while those with the G-C-ins haplotype and G-C-ins/G-C-ins diplotype - the least (80.8 IU/mL and 75.7 IU/mL, respectively). Men with the remaining haplotypes/diplotypes secreted sHLA-G at an intermediate level. However, only in one haplotype, namely G-C-ins, did we observe strong significant differences in the concentration of sHLA-G in the semen of men with teratozoospermia compared to men with normal sperm parameters ($p = 0.009$). In conclusion, fertile men differ in the profile of *HLA-G* polymorphism from men participating in IVF. Among all *HLA-G* haplotypes, the most unfavorable for male fertility is the G-C-ins haplotype, which determines the secretion of the lowest concentration of the soluble HLA-G molecule. This haplotype may reduce sperm parameters.

The impact of soluble HLA-G in IVF/ICSI embryo culture medium on implantation success

The HLA-G molecule is widely accepted as an important factor for pregnancy success. Its expression has been detected in the extravillous trophoblasts. Soluble HLA-G (sHLA-G) was found in the genital tract, pre-implanted embryos as well as in seminal fluid. In our study, we investigated the concentration of sHLA-G (sHLA-G1 and sHLA-G5) in media from 344 single cultured embryos following *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI). The level of sHLA-G (U/ml) was tested with a sandwich enzyme-linked immunosorbent assay (ELISA) kit. We correlated sHLA-G secretion with ovarian stimulation protocols, the type of embryo transfer (fresh or frozen cycle) and the quality of the embryos. The ovarian stimulation protocol affects the secretion of sHLA-G by the embryo. Embryos obtained from the long agonist protocol secreted more sHLA-G than those originating from the short antagonist protocol ($p = 0.0001$). Embryos whose transfer resulted in a clinical pregnancy and/or live birth secreted more sHLA-G compared to those whose transfer ended without pregnancy. This was particularly observable in embryos following the long ovarian stimulation protocol and from a frozen embryo cycle. In conclusion, sHLA-G secreted by the embryo has an impact on implantation and live birth and could be a developmental potential marker of the embryo. Its concentration depends on the ovarian stimulation protocol used.

Laboratory of Clinical Immunology
Head: Professor Andrzej Lange, M.D., FRCP (London), Dr med Sci

The main topic of this report: Study on a profile of the immune response against SARS-CoV 2 raised by the vaccination and/or the disease.

The Laboratory undertook this issue in response to society's call to help in fighting SARS-CoV 2 infection. We examined social welfare workers for the presence of SARS-CoV 2 antibodies with the use of the immunoblot technique which enables the detection of antibodies against epitopes of SARS-CoV2, MERS, and SARS CoV1 as well as the presence of antibodies against the epitopes of seasonal coronaviruses and against Papain-like Protease (PlPro) and ACE. All had anti-RBD antibodies (vaccination) some the anti-S2 antibodies but five individuals lacked anti-Nucleocapsid(N) Abs (reflecting the infection with SARS-CoV2). In the 2022 study, those negative for N antibodies in 2021 became positive. This suggests that they got the infection.

The conclusion was that infection with SARS-CoV 2 is common among social workers, which means that the vaccination did not necessarily protect against the infection of the SARS-CoV 2 variants surging in 2022.

To elucidate this issue, we examined social home residents for the presence of SARS-CoV 2 antibodies by employing the same microblot system (designed at the delta variant era). The residents live in two buildings where they are in contact with each other daily and have open contact with Wrocław residents employed in the Home or during frequent visits to the neighbouring institutions for shopping. All were vaccinated and had anti-RBD (except 2) and anti-N antibodies (except 3). During the 2022 SARS-CoV 2 epidemic surge, in spite of that 56 individuals had febrile disease, which was fatal in 0 cases. The Omicron variant was validly suspected, thus in 2022, in addition to the microblot, an ELISA suited for the detection of the S1 Omicron epitopes antibodies was used. The study was successful with the results as follows:
1. RBD Abs were present in all except two; the negative cases were or not vaccinated;
2. Positive readings in the ELISA suited for the Omicron S1 was positive in all except 2 (1 worker and 1 resident) and were found irrespective of the level of the microblot nucleocapsyd Abs;
3. Of note, 11 residents (9 positive, 2 borderline – it is not negative?) were positive for NL – 63 coronavirus NP epitope and two of them were also positive of HCoV 229E antibodies;
4. In 7 cases antibodies against papain – like protease (PlPro) was detected (the same 5 positive and 2 borderline);
5. It was a good correlation between the level of anti NL 63 antibodies and anti-PlPro antibodies, and nucleocapsid antibodies but not with RBD antibodies.

The results make the following conclusions possible:

Omicron antibodies do not correlate with the nucleocapsid antibodies, if the latter had contact with the past disease, the Omicron Abs positivity does not correlate with the level of nucleocapsid (reflecting the immunity against SARS CoV2 due to the infection), which may suggest that both diseases do not overlap significantly on the way of the immunity neither in serology nor in the immunity against the viruses of different variants specific immune response. The residents were exposed to the seasonal viruses – mainly to NL 63 – and this exposure was quite recent as suggested by the good correlation with PlPro antibodies (the presence is associated with rather a recent infection). Therefore, both viruses – the Omicron variant and seasonal coronavirus – circulate at a similar time in a cohort composed of social home residents. This creates a threat of the possible exchange of genetic material, allowing for the appearance of the virus chimera, which may harbor the features of the both coronaviruses circulating at the same time.

DEPARTMENT OF PHAGE THERAPY
Head: Professor Andrzej Górski, Ph.D.

Bacteriophage Laboratory
Head: Professor Andrzej Górski, M.D.

The main outcome of our studies is the optimalization of our program of experimental phage therapy and a decisive step forward confirmed by a grant for its clinical trial of safety and efficacy awarded by ABM (Agency for Medical Research): „Non-commercial clinical trial to confirm safety and efficacy of phage therapy in the treatment of chronic sinusitis – RHINOPHAGE"). Those achievements have recently been emphasized by Ministry of Science and Education's decision to award our Institute the highest ranking in medical sciences (category A Plus, the only such rank among all scientific and academic institutions in Poland). This decision has also been highlighted by the recent publication revealing the top publishing output worldwide of our Institute and our scientists (4 of them listed among the first 7 top scientitsts) during the past 21 years (Maimaiti Z.et al.; Global trends and hotspots of phage

therapy for bacterial infection: a bibliometric visualized analysis from 2001 to 2021. *Front. Microbiol.* 2023;13:1067803).

In addition, we have noted progress in the following studies: 1) an antiseptic agent containg silver does not inactivate phage (Raniseptol) in contrast to other often used preparations (Octanisept, Pronosan). Therefore, a combined antibacterial treatment with phage and silver could be possible; 2)the procurement of Acinobacter phage has been improved thus enabling isolation of more phage with their higher titer; 3)the procurement of new Enterobacter phage and their genomic and functional characteristics applicable in phage therapy; 4)while patients treated with phage produce serum anti-phage antibodies their level drops with time after termination of the therapy but the kinetics of that decrease varies from patient to patient.

Furthermore, we have made further progress in confirming the veracity of our hypothesis on the perspectives of „phage repurposing" in terms of their immunomodulating properties by showing that phage against Gram+ and Gram- bacteria may enance Il-10 production by human monocytes *in vitro* (Międzybrodzki R. Grant from NCN for studies on immunomodulating activity of phage). Il-10 has been considered as playing an important role of mediating immune tolerance and anti-inflammatory effects.

Conclusions: our studies carried out in 2022 have led to further progress in determination of the place of phage therapy in combatting antimicrobial resistance and extending its potential applicability also to non-bacterial pathologies.

Laboratory of Phage Molecular Biology
Head: Professor Krystyna Dąbrowska, Ph.D.

Studies of immunogencity in modified bacteriolytic proteins derived from bacteriophages; investigation of these proteins effects on physiological processes in mouse models and in tissue cultures

Bacteriolytic enzymes (endolysins) are promising antibacterial agents; however, as prokaryotic proteins, they can induce a normal immune response *in vivo*. In this study, we applied a modified approach of epitope scanning (EndoScan) for the identification of antigenic epitopes within two antibacterial enzymes, the endolysins Pal and Cpl-1. Antigenic epitopes were identified up to the key reactive amino acids. New variants of the enzymes were designed *in silico* by amino acid substitutions or deletions. Variants were expressed in a bacterial expression system and those demonstrating good antibacterial activity were tested for their intrinsic immunogenicity (ability to induce specific IgG) and for cross-reactivity to specific IgG induced by the wild-type (WT) enzymes. One new variant of Pal (257-259 MKS → TFG) demonstrated decreased immunogenicity while a similar one (257-259 MKS → TFK) demonstrated increased immunogenicity. One variant (280-282 DKP → GGA) demonstrated similar immunogenicity to WT Pal; however, it possessed significantly higher antibacterial activity than the WT enzyme, and furthermore, it was not cross-neutralized by antibodies induced by the WT enzyme. We propose this variant as a newly engineered endolysin with increased antibacterial activity capable of escaping cross-neutralization by antibodies induced by WT Pal. This variant has good potential as an antibacterial agent in repeated treatments, when previous rounds of treatment with WT Pal induced an efficient antibody response. Our observation demonstrates that antigenic epitopes identified by epitope scanning can be deimmunized by substitutions of identified key amino acids. Additionally, efficient antibacterial enzymes that escape cross-reactions and cross-neutralization by IgG can be developed. Importantly, this approach is universal and can be applied to many active proteins, so its applicability reaches far beyond endolysins and has the potential to be used in designing numerous biological drugs.

Identification of immunoreactive antigenic epitopes of SARS-CoV-2 virus by phage display method

The major factor shaping the global perspective for the increase or diminution of successive pandemic waves of COVID-19 is immunological protection. The SARS-CoV-2 virus is constantly developing new variants, and the capability of immune evasion is a major factor promoting variant spreading in the human population. After two years of the pandemic and virus evolution, it is almost impossible to explain all the possible combinations of previous infections with different variants, a few types of vaccinations, and new variants that may infect an individual patient; population levels generate myriads of combinations. Instead of variant-to-variant comparisons, identification of key protein regions that are linked to immune evasion could be a more efficient method. Here we report an approach with a phage display library of oligopeptides (96 623 oligopeptides) derived from multiple variants of SARS-CoV-2 for experimental identification of SARS-CoV-2 protein regions that (i) have characteristics of cross-reacting IgG hot-spots, and (ii) are highly immunogenic. Cross-reacting IgG hot spots are regions of protein frequently recognized in many variants by cross-reacting antibodies. Immunogenic regions efficiently induce specific IgG production in SARS-CoV-2 infected patients. Immunoprecipitation of the library with sera from patients hospitalized due to COVID-19 followed by next generation sequencing (NGS) revealed four regions that demonstrate both significant immunogenicity and the activity of a cross-reacting IgG hot-spot in protein S, and two such regions in protein N. Their distribution within the proteins suggests that they may be useful in vaccine design and in serological diagnostics of COVID 19.

LABORATORY OF BIOLOGY OF STEM AND NEOPLASTIC CELLS
Head: Professor Aleksandra Klimczak, Ph.D., D.Sc.

Isolation of mesenchymal stem cells from peripheral nerves and analysis of their biological properties

The aim of the study was to develop an immortalized human peripheral nerve mesenchymal stem cell line (PN-MSC) and its phenotypic characteristics as compared to primary cells of peripheral nerve-origin. Immortalization of primary MSCs isolated from a peripheral nerve was carried out using the hTERT and pSV40 plasmids according to the procedure established in the laboratory. Cells treated with plasmids were then selected using puromycin and geneticin. The analysis of the phenotype of immortalized PN-MSC cells showed that the immortalized cells had a similar expression level of surface markers as compared to the primary cells. Expression of mesenchymal stem cell markers CD73, CD90, CD105 (91%-99% of positive cells), HLA ABC marker (86% of positive cells) and CD44 (98% of positive cells) has been confirmed. A small percentage of cells (13%) also expressed the CD146 adhesion molecule characteristic of vascular endothelial cells. The examined cells were negative for the presence of surface markers specific of hematopoietic cells CD34, CD45, HLA-DR and for the CD56 marker. An immortalized cell line of human peripheral nerve mesenchymal stem cells (PN-MSC SVT) origin with expression of a primary MSC phenotype has been established.

The stable PN-MSC SVT cell line will allow for further research on the assessment of the secretory profile of the obtained cells and their potential use in the regeneration the peripheral nervous system and neuromuscular disorders.

Assessment of the effect of microvesicles derived from mesenchymal stem cells in the inhibition of the proliferative activity of ovarian cancer cells

The primary cancer cells were isolated from human postoperative ovarian cancer tissues and from the ascitic fluid found in the peritoneal cavity of the patients. After isolation, the cells were magnetically sorted with magnetic beads coupled with the CD133 antibody - one of the markers characteristic for cancer stem cells (CSCs). CD133+ cells constitute a small population within the tumor cells (0.1% - 18.0% in the examined cancer cells); therefore, to perform the planned analyses, studies were carried out on the entire population of ovarian cancer cells. Phenotype analysis using flow cytometry revealed that cancer cells constitute a heterogeneous population, positive for MSC markers (CD73, CD90, CD105) and CSCs markers (CD24, CD44, CD133), at various levels of expression. Within the examined cells, CD45+ cells were detected, which proves the presence of the tumor infiltrating lymphocytes in the population of isolated cells. Phenotype analysis using immunofluorescence staining and microscopic imaging confirmed the expression of CD44 and the presence of markers characteristic for tumor-associated fibroblasts (FAP, PDGFRa). Tumor cells were also positive for pluripotency markers (Oct4, Sox2, Nanog) and CSCs markers including ALDH1 and c-kit. F-actin expression showed a varied cytoskeletal organization and the presence of filopodia (F-actin-rich). In addition, cancer cells expressed epithelial-mesenchymal transition markers Snail and vimentin. Real-time RT-PCR analysis showed the expression of mRNAs related to pluripotency (Oct4, Sox2) and mRNAs with proto-oncogenic properties (p53, p21, c-myc). Microvesicles derived from the human adipose tissue mesenchymal stem cell line (HATMSC2) internalized into primary ovarian cancer cells inhibited metabolic activity (MTT assay) of cancer cells and decreased cell survival as confirmed by live/dead assay.

In this study, we confirmed the presence of CSCs in the population of primary ovarian cancer cells and proved the inhibition of proliferative activity and decreased rate of survival of ovarian cancer cells exerted by HATMSC-derived microvesicles.

DEPARTMENT OF ANTHROPOLOGY
Head: Professor Sławomir Kozieł, Ph.D.

Age at menarche in urban girls exposed to lead in the Copper Basin, Poland

Lead negatively affects human growth and development. This research aimed to assess the effect of elevated blood lead level on age at menarche (AM), controlling for body mass index (BMI) and estimated fatness. The sample included 490 girls aged 7-16 examined in Polkowice (Copper Basin, Poland) in 2008. Measurements included the following: height, weight, skinfold thicknesses and estimated percentage of body fat. AM was assessed using the status quo method. Blood samples were taken for lead level assessment. Two groups were defined based on the median blood lead level for the total sample of children (3.7 μg/dl). Logistic regression models were used to assess the association between AM and independent variables. The results indicated that menarche in the higher blood lead level group was occurred significantly later compared to the lower blood lead level group ($p<0.01$). This relationship remained only marginally significant when BMI ($p<0.10$), sum of skinfolds ($p<0.09$) or percentage fat ($p<0.08$) were controlled. The results revealed that a lower blood lead level (3.7 μg/dl) than the currently acceptable (5 μg/dl) is related to a later AM; however, this relationship is moderated by body fatness. Also, the favourable living conditions in the community may, to some extent, mitigate the potentially negative effect of lead on AM.

Prenatal and early postnatal exposure to a natural disaster and Attention-Deficit/Hyperactivity Disorder symptoms in Indian children

The aim of this study was to assess the relation between early exposure to stressful event and a level of Attention-Deficit/Hyperactivity Disorder (ADHD) symptoms in children, based on outcomes from a natural experiment. It was hypothesized that children pre- and postnatally exposed to cyclone Aila have a higher level of ADHD symptoms compared to the controls, and the effect depends on timing of exposure. Indian children (8-11y) prenatally (N=336) and early postnatally (N=216) exposed to Aila were compared to non-exposed control group of their peers (N=285). ADHD symptoms were assessed using the Conner's Teacher Rating Scale Revised. The main effect of exposure to the cyclone on total ADHD symptoms' score, ADHD index, Hyperactivity and Oppositional symptoms was significant and independent of covariates. Both prenatally and postnatally exposed girls, and only postnatally exposed boys, showed significantly higher level of Oppositional symptoms compared to the controls. Cognitive problems/Inattention symptoms were increased in both prenatally and postnatally exposed boys, but not girls, compared to non-exposed children. The timing of programming the later behavior characteristics by stressful experiences due to natural disaster is not limited to fetal life but extends at least into infancy. Sex is a significant modulator of the early stress-ADHD symptoms association.

DEPARTMENT OF MICROBIOLOGY

Laboratory of Molecular Biology of Microorganism (LMBM)
Head: Professor Anna Pawlik, Ph.D.

Secondary metabolism in *Streptomyces*

Bacteria from the genus *Streptomyces* are potent producers of bioactive compounds with highly diverse structures and functions. Our laboratory focuses on coelimycin synthesis regulation - the earliest coloured specialized metabolite synthesized in the life cycle of *Streptomyces coelicolor* A3. *cpkO* and *cpkN*, two SARP activators of the coelimycin synthesis, raised our particular interest. We recently characterized how CpkO and CpkN affected coelimycin synthesis and stationary-phase antibiotics production. Currently, we want to find out how *Streptomyces* control coelimycin synthesis in the context of a complex life cycle, intercellular communication and the production of subsequently synthesised secondary metabolites such as actinorhodin and undecylprodygiosin.

Antimicrobial therapy

Bacterial resistance to antibiotics becomes more and more problematic. Thus, new antimicrobial compounds and therapies are required to eradicate pathogenic bacteria efficiently. We are primarily interested in the photochemical eradication of bacteria in skin infections using the flexible organic light-emitting diode (OLED) and new photosensitizing chemicals with potential bactericidal activity. New photosensitizing compounds are investigated using *Staphylococcus aureus* as a model species and clinical strains - bacteria isolated from patients' diabetic foot.

Bacterial response to stress

We are interested in bacterial factors regulating stress response in bacteria belonging to selected species of Campylobacterota. We have recently focused on HP1021-like atypical response regulators that control genes' transcription and chromosome replication initiation. We aim to determine regulons controlled by these proteins in selected pathogenic Campylobacterota species to increase our understanding of their cellular processes and pathogenesis.

Laboratory of Microbiome Immunobiology
Head: Professor Sabina Górska, Ph.D

The ability of selected bacteria of the genus *Bifidobacterium* to regulate the mechanisms of innate immunity

Our research aimed to:

- decipher whether TLR2 and/or TLR4 are involved in the production of NO induced by selected *Bifidobacterium* species
- investigate whether JNK and p38/NF-κB/iNOS signaling pathways are responsible for the regulation of nitric oxide production.

Inductive nitric oxide synthase (iNOS) is a characteristic enzyme of M1 macrophages. The nitric oxide (NO) produced by it is a local mediator that regulates several physiological processes in the human body. It acts as an antimicrobial effector molecule and regulates several cellular processes, including cell differentiation and function by nitration of key molecules involved in transcriptional or signaling pathways. Moreover, the low concentration of NO protects the intestinal mucosa and influences mucus secretion regulation. However, it was observed that inhibition of iNOS activity or deletion of the iNOS gene, resulting in a significant decrease in nitric oxide levels, increased intestinal inflammation. In turn, the administration of exogenous NO precursors produced a therapeutic effect.

The MAPK mitogen-activated kinases (which include: ERK1/2, JNK, and p38) regulate the activation of the NF-κB transcription factor, leading to iNOS expression and NO production in macrophages under the influence of signals from TLRs (including TLR2 and TLR4). Previous studies have shown the ability of the tested *Bifidobacterium* strains to produce NO in the pathway dependent on ERK 1/2 kinases. It was, therefore, important to investigate whether TLRs 2 and 4, as well as JNK and p38 kinases, are also involved in the regulation of this process.

The studies were performed on murine macrophages of myeloid origin (BMDM) (WT) line. Macrophages were incubated with selected strains of *Bifidobacterium* for 24 h at 37 °C in the presence or absence of kinase inhibitors: JNK (SP600125) and p38 (SB203580). Changes in kinase phosphorylation levels were determined by Western blotting. The level of secreted NO was determined in the supernatants by the Griess method. The BMDM (WT), BMDM TLR4 (-), and BMDM TLR2 (-) cell lines were incubated with selected *Bifidobacterium* strains for 24 h at 37 °C. The level of secreted NO was determined in the supernatants by the Griess method.

Previously, we demonstrated that tested strains activated the ERK1/2 kinases, NF-κB factor as well as iNOS expression and NO production by macrophages of the BMDM line. The current study showed that the tested *Bifidobacterium* strains are capable of activating JNK kinase, but no changes in p38 kinase activation have been demonstrated. Pre-incubation of BMDM cells with a selective JNK inhibitor significantly inhibited NO production, confirming that iNOS expression and NO production in macrophages are also under the control of JNK kinases. It has

also been shown that NO production is dependent on the activation of TLR 2 (strains 219, 367, and 370) and TLR4 (strains 218, 368, 369, 371, 372, and 373).

DEPARTMENT OF TUMOR IMMUNOLOGY

Laboratory of Molecular and Cellular Immunology
Head: Professor Małgorzata Cebrat, Ph.D.

Analysis of the influence of mutated forms of renin identified in patients with genetic kidney diseases on the intracellular transport and activity of this protein and stress parameters of cells producing it

We have identified a rare mutation in the signal sequence of the renin gene (W17R) in a patient suffering from chronic kidney disease. This allowed him to be diagnosed with ADTKD-REN (autosomal dominant tubulointerstitial kidney disease) and to start treatment in order to delay the process of kidney degeneration. Due to insufficient data on the effect of this mutation on the intracellular transport and activity of the REN protein as well as the stress parameters of the cells producing it, we developed a research model based on the HEK293T cell line, derived from human kidney embryonic cells. The cells have been modified to provide stable expression of wild-type or mutated renin. The correct expression and processing of the wild-type protein and the lack of secretion of its mutated form have been demonstrated. The mutated renin is expressed in the form of preprorenin and is not subjected to further maturation. It is suggested that the molecular mechanism of the destructive effect of the mutated protein on the cells is the induction of chronic endoplasmic reticulum (ER) stress, leading eventually to apoptosis. Using Real-Time RT-PCR analysis and luciferase reporter vectors in order to monitor the expression of stress-related multigene markers, we did not detect any symptoms of activation of such a mechanism in cells producing the W17R mutated renin. On the other hand, we have confirmed the induction of ER stress by the expression of other form of mutate renin (L381P), which was previously described in the literature. This points to different molecular mechanisms underlying the different forms of ADTKD-REN. This observation is of particular importance in the light of the emergence of new therapies for diseases attributed to ER stress; pinpointing the actual mechanism of cell degeneration (whether it is ER stress-related or not) would be crucial for appropriate therapeutic choices.

Laboratory of Tumor Immunology
Head: Professor Arkadiusz Miążek, Ph.D.

In human cutaneous melanoma cell lines

c-*MYC* promoter binding protein (MBP-1) is a product of alternatively translated mRNA encoding alpha-enolase (ENO-1). In contrast to ENO-1, MBP-1 possesses no enzymatic activity but instead, it is able to bind the c-Myc promoter leading to the downmodulation of its transcription. C-*MYC* transcription is commonly observed in malignancies of numerous cell types and it is positively correlated with cell proliferation. Reducing c-*MYC* transcription through the overexpression of MBP-1 was correlated with a decrease in cell proliferation and tumorigenicity, hence constituting an attractive anticancer strategy.

We overexpressed the MBP-1 protein or its C-terminal truncated variant (MBP-1ΔC) by means of lentiviral transduction of two human melanoma cell lines (A375, WM9) and made three unexpected observations: (1) we found that overexpressed MBP-1 and MBP-1ΔC predominantly localized in the cell cytoplasm and consistently only minimally decreased c-

MYC mRNA expression, (2) the proliferation rate of MBP-1- transduced cells increased in comparison to empty vector transductants, and (3) the rate of glucose metabolism in hypoxia increased in MBP-1 and MBP-1ΔC transduced cells. When assessing *in vitro* cell migration, we also found that overexpression of MBP-1, but not MBP-1ΔC, led to a substantial decrease in the cell migration capacity of the metastatic WM9 cell line but not the A375 cell line isolated from the primary tumor lesion.

Collectively, our data provide evidence suggesting an unexpected tumor-promoting activity of MBP-1 that can be largely dissociated from its nuclear localization.

DEPARTMENT OF EXPERIMENTAL THERAPY
Head: Professor Michał Zimecki, Ph.D.

Laboratory of Immunobiology
Head: Professor Michał Zimecki, Ph.D.

Antiviral activity of a cyclic tetrapeptide

The core of Cyclolinopeptide A (CLA, cyclo(LIILVPPFF)), responsible for its high immunosuppressive activity, contains a Pro-Pro-Phe-Phe sequence. A recently synthesized cyclic tetrapeptide, cyclo(Pro-Pro-β^3-HoPhe-Phe), bearing the active sequence of CLA, was shown to exhibit a wide array of anti-inflammatory properties in mouse models. We also demonstrated that the peptide had a property to significantly inhibit replication of human adenovirus C serotype 5 (HAdV-5) and herpes simplex virus type 1 (HSV-1) in epithelial lung cell line A-549. Based on a previously established mechanism of its action, we propose that the peptide may inhibit virus replication by induction of PGE2 acting via EP2/EP4 receptors in epithelial cells. We also postulate that the antiviral actions of the peptide may have a better therapeutic benefit if combined with its anti-inflammatory properties.

Three dimensional cell culture

RANKL-RANK-osteoprotegerin (OPG) axis is crucial in the process of osteoclastogenesis, with tartrate-resistant acid phosphatase (TRAP) as a marker. Mouse fibroblasts (NIH 3T3 cell line) encapsulated in agar gel with accompanying cells (mouse peripheral blood –PBMC-or spleen cells) increased RANKL expression. The experiments showed that conduct of mixed culture of fibroblasts with PBMC resulted in an increase of TRAP expression, being a differentiation and activity marker of osteoclast-like hemopoietic cells.

Preliminary *in vitro* studies on activity of yolkin

Preliminary experiments were performed to evaluate some immunological activities of yolkin, a protein isolated from egg's yolk. We demonstrated immunoregulatory effects of yolkin in mitogen-induced proliferation of mouse thymocytes and human peripheral mononuclear cells. Yolkin alone, in mouse splenocyte cultures, induced marked levels of TNF α, IL-6 and IFN ɣ. On the other hand, yolkin inhibited lipopolysaccharide-induced production of TNF α and IFN ɣ, but not of IL-10 and IL-6. Such actions indicate anti-inflammatory character of yolkin in LPS-elicited production of proinflammatory cytokins. In a macrophage RAW 264.7 cell line yolkin significantly elevated expression of caspase 7, 9 and NFκB. In natural killer cell line NK-92 yolkin stimulated expression of NFκB and p53. These effects suggest induction of apoptotic pathways in the investigated tumor cell lines.

The *in vitro* immunotropic actions of a calf thymus extract – thymus factor X (TFX®) preparation

TFX® exhibited a co-stimulatory action of concanavalin A-induced mouse thymocyte proliferation and partially restored the mitogen-induced proliferation capability of mouse thymocytes exposed to hydrocortisone. The preparation also inhibited Herpes virus-1 replication in A549 cells when preincubated with the virus and when added to the infected cells. In addition, it weakly inhibited lipopolysaccharide-induced TNF α, IL-1β and IL-6 by the THP-1 monocyte cell line. The determination of mitogen activated protein kinase (MAPK) expression in Jurkat T cells revealed strong increases in ERK-2 kinase and p38α subunits. In WEHI 231 immature B cells, TFX® elevated p38α, and had a particularly strong elevating effect on p38ɣ. In HL-60 myeloblastic cells, the expression of p38α, β and ɣ was not detectable, almost blocked for p38δ and JNK, but accompanied by an increase in ERK-1. In turn, the effects of TFX® in J744E macrophages resulted in a strong increase in p38ɣ expression, moderate elevations of ERK and a drop in p38δ. Significant increases in MAPK expression were also found in cells from the lymphoid organs. In the bone marrow cell population, p38α, β and ɣ, in thymocytes p38α, ɣ and δ, and in splenocytes p38β and ɣ, subunit expression was elevated. We conclude that the changes in MAPK expression may be attributed to cell maturation and differentiation, and explain the beneficial therapeutic effects of TFX®.

Laboratory of Immunopathology
Head: Professor Edyta Pawlak, M.D., Ph.D.

Inappropriate expression of PD-1 and CTLA-4 checkpoints in myeloma patients is more pronounced at diagnosis: Implications for time to progression and response to therapeutic checkpoint inhibitors

Multiple myeloma (MM) is a hematologic malignancy characterized by severely profound immune dysfunction. Therefore, the efficacy of drugs targeting the immune environments, such as immune checkpoint inhibitors (ICIs), is of high clinical importance. Yet, several clinical trials evaluating ICIs in MM in different therapeutic combinations revealed underwhelming results, showing a lack of clinical efficacy and excessive side effects. The underlying mechanisms of resistance to ICIs observed in the majority of MM patients are still under investigation. The aim of the current study was to determine the usefulness of immune checkpoint expression assessment as a predictive biomarker of the response to therapeutic inhibitors. For this purpose, along with checkpoint expression estimated by flow cytometry, we evaluated the time to progression (TTP) of MM patients at different clinical stages (disease diagnosis and relapse) depending on the checkpoint expression level; the cut-off point (dividing patients into low- and high-expressors) was selected based on the median value. We confirmed the defective levels of regulatory PD-1, CTLA-4 receptors, and the CD69 marker activation in newly diagnosed (ND) patients, whereas relapsed/refractory patients (RR) exhibited their recovered values and reactivity. Also, substantially higher populations of senescent CD4+CD28- T cells were found in MM, primarily in NDMM subjects. These observations suggest the existence of two dysfunctional states in MM CD4 T cells with the predominance of immunosenescence at disease diagnosis and exhaustion at relapse, thus implying different responsiveness to the external receptor blockade depending on the disease stage. Furthermore, we found that lower CTLA-4 levels in NDMM patients or higher PD-1 expression in RRMM patients may predict early relapse. In conclusion, our study clearly showed that the checkpoint level for CD4 T cells may significantly affect the time to MM progression concerning the treatment status. Therefore, when considering novel therapies and potent combinations, it

should be taken into account that blocking PD-1 rather than CTLA-4 might be a beneficial form of immunotherapy for only a proportion of RRMM patients.

The role of dopaminergic genes in probabilistic reinforcement learning in schizophrenia spectrum sisorders and confirmation bias in the course of instructed reinforcement learning in schizophrenia-spectrum disorders

Schizophrenia spectrum disorders (SZ) are characterized by impaired probabilistic reinforcement learning (RL), which is associated with dopaminergic circuits involving the prefrontal cortex and basal ganglia. However, there are no studies examining dopaminergic genes in relation to probabilistic RL in SZ. Using Probabilistic Selection Tasks (PST), we examined the impact of polymorphic variation in dopaminergic genes: *COMT*rs4680, *DARP-32*rs907094, rs2734839, rs936461, rs1800497 and rs6277 in *DRD2*, rs747302 and rs1800955 in *DRD4*, rs28363170 and rs2975226 in *DAT1* on outcomes assessed in SZ patients compared to healthy controls (HC). A probabilistic RL task was performed by 59 SZ patients and 95 HC patients. SZ patients performed significantly worse in acquiring reinforcement conditionality during the task compared with HC.

No significant association was found between genetic polymorphisms and RL among SZ patients, while among HC participants with regard to the DAT1 rs28363170 polymorphism, those with the 10-allele repeat genotype performed better compared to carriers of the 9-allele repeat. The present study indicates the importance of the DAT1 rs28363170 polymorphism in RL in HC participants.

Next, using learning tasks designed to examine the function and interaction of the PFC and BG in patients with SZ spectrum disorders compared to healthy subjects (HC)Z, we assessed learning deficits in schizophrenia (SZ) involving systems involved in value representation (prefrontal cortex, PFC) and reinforcement learning (basal ganglia, BG), as well as impaired connectivity of these regions. In the Instructed Probabilistic Selection task (IPST), participants were falsely instructed on one of the stimuli used during probabilistic learning, leading to a confirmation phenomenon in which the instructed stimulus is overestimated compared to its actual experienced value. IPST was administered to 102 SZ patients and 120 HC subjects. We showed that SZ patients and HC subjects were equally influenced by false instructions in a probabilistic reinforcement learning (RL) task (p=0.441); however, HC subjects had significantly higher learning rates related to the process of overcoming cognitive biases compared to SZ patients (p=0.018).

SZ patients and healthy participants were equally influenced by false instructions in the RL probabilistic task (however, healthy participants had significantly higher learning rates related to the process of overcoming cognitive biases compared to SZ patients.

The results can be interpreted as a sign of impaired top down and bottom up connections between PFC and BG in SZ. However, the next step is to confirm our observations with neurofunctional imaging studies.

Laboratory of Reproductive Immunology
Head: Professor Anna Chełmońska-Soyta, Ph.D, V.D.

Immunological mechanisms associated with reproductive processes in health and disease

Effects of IL-33 on the expression of transcription factors in human B lymphocytes

IL-33 is a cytokine with a strongly pleiotropic effect. It is a cytokine known as an alarmin, whose regulatory role is predominantly directed toward the stimulation of type 2 responses.

Impaired IL-33 secretion is associated with reproductive failure including miscarriage in women. In mice, IL-33 stimulates the development of regulatory B lymphocytes. The aim of the study is to determine the transcriptional profile of human B lymphocytes under the influence of IL-33 in healthy women and in women after miscarriage.

The aim of the first stage of the planned study was to select an appropriate time of stimulation of isolated B lymphocytes with human recombinant IL-33 in order to recognize its effect on the expression of selected transcription factors in circulating B lymphocytes. This is the first time that direct stimulation of isolated human B lymphocytes with recombinant human IL-33 has been attempted.

The protocol for PBMC isolation and magnetic sorting of B cells was refined to obtain the highest possible concentration of cells needed for *in vitro* culture. B cells were stimulated with human recombinant IL-33 (100 ng/ml) for 3 and 6 hours, and the expression of selected proteins (phospho-STAT5, phospho-STAT3 and phospho-p38 MAPK) involved in IL-33-induced signal transduction pathways was analysed by flow cytometry. No statistically significant differences in the expression levels of these proteins were observed in both B ($CD3^{-}CD19^{+}$) and B1 ($CD19^{high}IgD^{low}$) cells, but an increasing trend was evident after 6 hours of cell culture with IL-33. The study is being continued.

Laboratory of Genetics and Epigenetics of Human Diseases
Head: Professor Lidia Karabon, Ph.D

Genetic and epigenetic control of immune response regulating molecules in disease development, progression and treatment

Analysis of miRNA-BTLA mRNA interactions in Chronic Lymphocytic Leukemia

Our previous study showed overexpression of BTLA mRNA in B cells of CLL patients while decreased BTLA protein level on the surface of CLL B cells compared to healthy controls (HC). This observation might indicate the posttranscriptional regulation of *BTLA* translation by micro RNAs (miRNAs). Therefore, the aim of our analysis was to identify potential miRNAs responsible for decreased BTLA protein levels in B cells of CLL patients. In fact, our previous results showed indirectly that blocking miR-155-5p expression partially restored BTLA protein level in CLL B cells. Currently, we are searching for other miRNAs regulating BTLA expression and performing experiments to confirm direct binding to BTLA mRNA of identified miRNAs including miR-155-5p.

Using three bioinformatic prediction tools TargetScan, mirDIP, and DIANA the analysis of possible miRNA-BTLA mRNA interaction was performed. The results common for 3 databases were selected for further analysis. Then, on the basis of literature data, we identified miRNAs that are dysregulated in CLL. Common in bioinformatic and literature survey miRNAs were further selected to predict miRNA recognition element (MRE) regions within BTLA 3'UTR sequence by TargetScan and DIANA tools. Next, Dual-Luciferase Assay was used to experimentally confirm miR-155-5p-BTLA mRNA interaction.

Data analysis showed that 79 miRNAs were predicted by all three prediction tools. Among them 19 have been found (based on literature search) to be underexpressed and 10 overexpressed in CLL patients. From overexpressed miRNAs, we selected six miRs: miR-155-5p, miR-150-5p, miR-28-5p, miR-136-5p, miR-660-5p, and miR-31-5p highly upregulated in CLL. Interaction analysis revealed that all six miRNAs bind to *BTLA* 3'UTR sequence by: 7mer-A1 (miR-155-5p), 7mer-m8 (miR-150-5p, miR-136-5p and miR-660-5p) or 8mer (miR-31-5p and miR-28-5p) canonical motifs.

Between identified miRNAs, miR-155-5p has been reported to be one of the most overexpressed miRNAs in CLL. To validate whether miR-155-5p indeed targets BTLA 3'UTR we performed Dual-Luciferase Assay. We noticed a significant decrease in luciferase activity in HEK 293 cells co-transfected with mimic miR-155-5p and reporter constructs containing BTLA 3'UTR fragments compared to the negative control. Additionally, luciferase activity remained invariable in cells co-transfected with mimic miR-155-5p and constructs containing mutations in the predicted miR-155-5p MRE region (1524-1539) of BTLA 3'UTR. Altogether those results showed that miR-155-5p directly targets BTLA and binds by 7mer-A1 canonical motif at the position 1524-1539 of BTLA 3'UTR.

In conclusion, BTLA 3'UTR sequence contains potential MRE regions for binding of miR-155-5p, miR-150-5p, miR-28-5p, miR-136-5p, miR-660-5p, and miR-31-5p, that are overexpressed in CLL. Therefore, our analysis suggests that miR-155-5p miR-150-5p, miR-28-5p, miR-136-5p, miR-660-5p, and miR-31-5p might be involved in the pathogenesis of CLL by blocking BTLA protein expression in CLL B cells.

Association between *TIM-3, LGALS9* gene variants and ccRCC risk

Numerous data showed associations between variants in genes encoding immune checkpoints (ICs) - crucial immune response modulators, with cancer risk and overall survival (OS). We aimed to study the influence of variations in genes encoding TIM-3, and its ligand GAL -9 (*LGALS9)* on the risk of clear cell renal cell carcinoma (ccRCC) and patient OS.
We found that the presence of rs10057302 A (*TIM-3*) and rs4794976 T (*LGALS9*) alleles decreased ccRCC risk, while the minor alleles in both SNPs (A and G, respectively) increased the risk of disease. Subgroup analysis showed an association of ICs gene variants with clinical features of the disease. Specifically, the presence of the rs4794976 G allele increased the risk of disease twice in females as well as in patients over 63 (median of age) years old. In rs10057302, we noticed overrepresentations of the A allele in patients with tumor bigger than 70 mm. In addition, ICs genes haplotype analysis revealed that particular haplotypes increased the risk of ccRCC. Moreover, rs1036199 (*TIM-3*) significantly influenced OS.

Our results indicate that *TIM-3*, and *LGALS9* variants might modulate ccRCC risk and OS.

DEPARTMENT OF IMMUNOLOGY OF INFECTIOUS DISEASES
Head: Professor Andrzej Gamian, Ph.D.

Laboratory of Medical Microbiology
Head: Professor Andrzej Gamian, Ph.D.

Studies of the structure and function relationship of microbial glycoconjugates, their participation in infection and immunity processes, studies of glycation products as well as lipidomic analysis of propionic bacteria and their extracellular vesicles

Propionic acid bacteria include probiotic, commensal, opportunistic and also human pathogenic strains. The aim of the current stage of the study was the creation of a database of MALDI-TOF mass spectra of lipid extracts of bacteria of the genus *Propionibacterium* for diagnostic purposes. A comparative analysis of extracellular vesicles (EVs) obtained from three phylotypes (subspecies) of *Cutibacterium acnes* (IA1, IB and II) was presented to show the existence of differences in their protein and lipid composition. We showed that bacterial EVs differ significantly in protein and lipid composition, depending on the phylotype from which they were isolated. These are important implications for future research on extracellular vesicles, which will require a case-by-case approach to the study of these nanostructures within

a given phylotype of the same species. Other studies concerned an epitope of the enterobacterial protein OmpC, recognized by umbilical cord blood antibodies, with a structure established in our laboratory. This protein immobilized in the affinity column was used to extract immunoglobulins from human serum, which are being investigated for use in the treatment of humoral innate immune deficiency. Next project was on the cross-reactivity of glycosylated sialic acid structure with glandular epithelial cells and nervous tissue. The monoclonal antibodies have been obtained in order to investigate the biosynthesis of these antigens by SEM and TEM electron microscopy. The practical significance of the results will be important for the differential diagnosis of cancer. The advanced glycation end-products (AGEs) described earlier in our laboratory have been subjected to quantitative studies in various diseases. The level of AGE was shown to be elevated in alcoholic liver disease and decreased in EBV infection, where these drop of antibodies level was associated with increased amounts of specific immune complexes. In addition, as part of the statutory activities, research was conducted on the activity of phage protein gp30 from bacteriophage F8 against *Pseudomonas aeruginosa*.

Laboratory of Virology
Head: Professor Egbert Piasecki, Ph.D.

Viral and bacterial diseases are among the greatest concerns of humankind since ancient times. Despite tremendous pharmacological progress, there is still a need to search for new drugs that could treat or support the healing processes. A rich source of bioactive compounds with antiviral potency include plants such as black chokeberry and elderberry. The aim of this study was to assess the *in vitro* antiviral ability of an originally designed double-standardized blend of extracts from *Aronia melanocarpa* (Michx.) Elliot and *Sambucus nigra* L. (EAM-ESN) or separated extracts of *A. melanocarpa* (EAM) or *S. nigra* (ESN) against four human respiratory tract viruses: influenza A virus (A/H1N1), betacoronavirus-1 (HCoV-OC43) belonging to the same β-coronaviruses as the current pandemic SARS-CoV-2, human herpesvirus type 1 (HHV-1), and human adenovirus type 5 (HAdV-5). Antiviral assays (AVAs) were used to evaluate the antiviral activity of the plant extracts in a cell-present environment with extracts tested before, simultaneously, or after viral infection. The virus replication was assessed using the CPE scale or luminescent assay. The EAM-ESN blend strongly inhibited A/H1N1 replication as well as HCoV-OC43, while having a limited effect against HHV-1 and HAdV-5. This activity likely depends mostly on the presence of the extract of *S. nigra*. However, the EAM-ESN blend possesses more effective inhibitory activity toward virus replication than its constituent extracts. A post-infection mechanism of action of the EAM-ESN make this blend the most relevant for potential drugs and supportive treatments; thus, the EAM-ESN blend might be considered as a natural remedy in mild, seasonal respiratory viral infections. The results were published in *Pharmaceuticals, 2022; 15: 619.*

Neurodegenerative disorders, including Alzheimer's disease (AD), are associated with a disruption of normal immune function that could potentially impact the brain. In AD sex and gender have been noted as relevant to disease prevalence or clinical manifestation. It is suggested that disease progression could vary as a result of the different inflammation state among males and females. The objective was to investigate sex-dependent difference in innate immunity of AD patients and healthy, age-matched controls. The level of innate immunity was measured with tests based on peripheral blood leukocytes (PBLs) resistance to viral infection (vesicular stomatitis virus, VSV) *ex vivo*. Cytokine: TNF-α, IFN-γ, IL-1β, IL-10 production by uninfected and VSV-infected PBLs *ex vivo* with enzyme-linked immunosorbent assay were examined. In contrast to controls, women with AD exhibit lower average level of innate immunity than AD men. The mean level of TNF-α, IL-10 and IL-1β was higher in AD men

than in AD women, whereas such changes were not observed among controls. The level of IFN-γ was higher in AD than in controls. PBLs from AD did not increase IFN-γ production after viral infection in contrast to controls. Leukocytes from women with AD exhibited a weaker response to viral infection and much less cytokine production compared to men with AD. It is important to consider sex as a biological variable in AD as it shows promises to advance our understanding of mechanisms of AD pathology and may be the basis for future treatment of AD. The results was published in *Archivum Immunologiae et Therapiae Experimentalis, 2022; 70: 16.*

Laboratory of Genomics & Bioinformatics
Head: Professor Łukasz Łaczmański, Ph.D.

Molecular characteristics of cancer cells - analysis of transcriptomes and epigenetic data

In the process of cancer transformation, three groups of genes play a key role: proto-oncogenes, suppressor genes and mutator genes. Identification of genetic changes, modifying individual response to drugs and potential prognostic and predictive molecular markers (response to treatment) is the key to improving the effectiveness of the treatment. This will allow the selection of groups of patients that require the introduction of a different therapeutic standard (individualization of treatment time, doses), thus improving not only the prognosis, but also the quality of life.

The aim of the project:

1. Finding transcriptome changes that could be markers to predict treatment responses.
2. Finding markers that could be predictors of the cancerogenesis based on small RNA.
3. Finding changes in the methylation profile associated with transcriptome tumor changes.
4. Developing a model by combining transcriptomic and epigenetic data.

An analysis of the transcriptomic profile of 51 samples from patients with breast cancer was performed. The patients were divided into two groups: responding well to treatment (Y) and responding poorly to treatment (Paclitaxel or Doxorubicin) - marked with N. First, a classic analysis of DE (differential expression) was performed between patients Y and N. The analysis showed no statistically significant differences. Subsequently, an analysis was performed on breast cancer cell lines. A group of approximately 220 genes strongly correlated (positively and / or negatively) with the response to Paclitaxel or Doxorubicin was selected. The machine-learning algorithm (sPLS-DA) was then used to select from these 220 genes that best separate responder and non-responder groups. A list of 36 genes was obtained, the expression of which may be correlated with the response to treatment with Paclitaxel or Doxorubicin.

A bioinformatic model of response to treatment with the given preparations will be made, based on the transcription profiles of 51 examined patients, containing the genes selected in the previous stage.

Figure 1. Typing of genes with high correlations with paclitaxel sensitivity of tumor lines by partial least squares (PLS) regression technique.

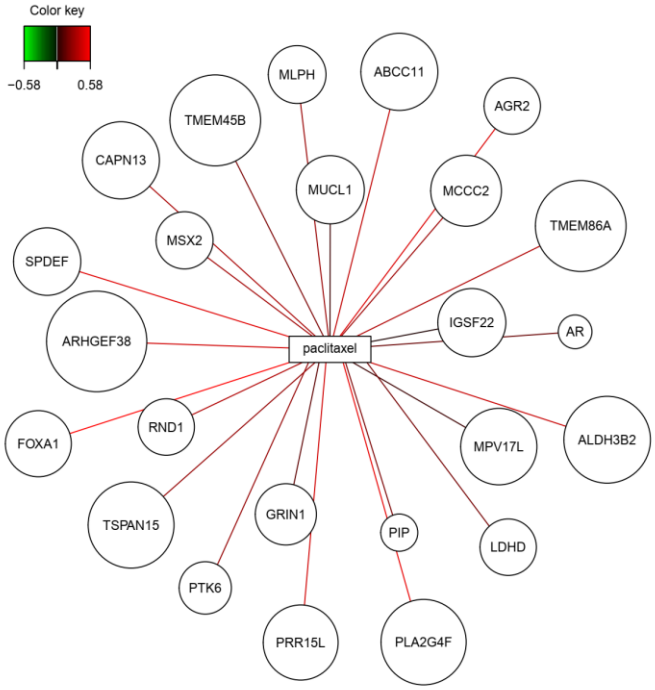


Figure 2. Analysis of the localization of expression of one of the selected markers (KCNE4) by single cell RNA-seq analysis. The gene showed weak expression on tumor pericytes and uneven expression on tumor cells.

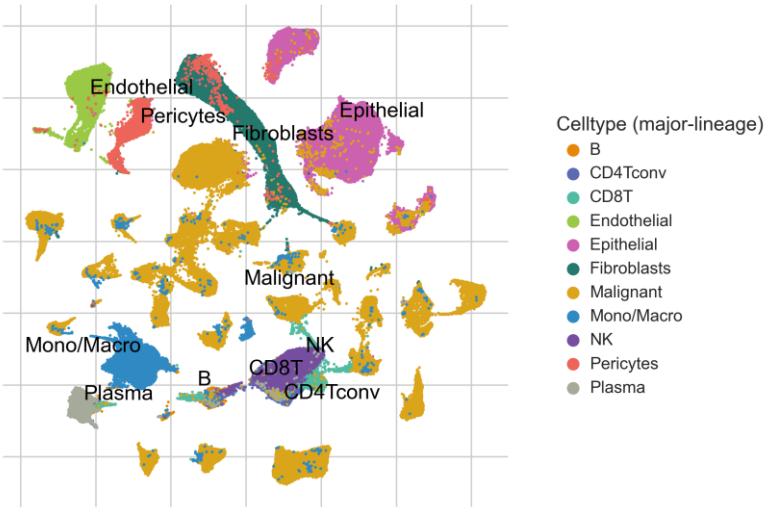
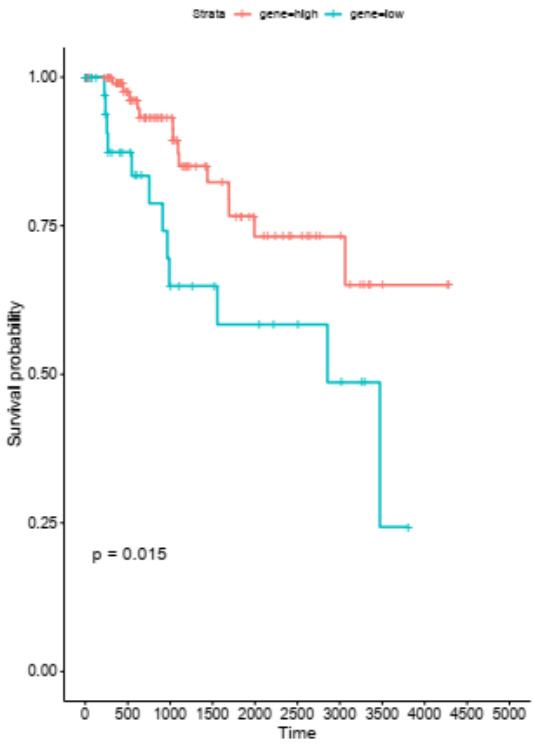


Figure 3. Analysis of the relationship of expression of one of the selected markers (KCNE4) on data from GDC aimed at examining its relationship with survival prognosis for patients. High gene expression was statistically significantly associated with prolonged survival of patients.



Analysis of the gut microbiome and bacterial transcriptome in relation to the diet and gastrointestinal symptoms in patients with autism

The aim of the project:
1. Determining the composition of the gut microbiota and the differences between the autistic group and the control group.
2. Establishing the transcriptional activity of bacteria colonizing intestines of children with autism.
3. Determination of the correlation between gastrointestinal symptoms, diet and intestinal dysbiosis in children with autism.

The preliminary results, based on the analysis of the V3V4 16S rRNA fragment (UniFrac index) show that statistically significant ($p < 0.05$) differences in the bacterial composition of fecal microbiota may be observed between the group of neurotypical siblings and children from the control group (PERMANOVA test, $p = 0.003$) and between neurotypical siblings and children with autism spectrum disorders (PERMANOVA test, $p = 0.001$). There was no statistically significant difference between the groups of children with autism spectrum disorders and the control group (PERMANOVA test, $p = 0.563$).

On the other hand, on the basis of the Bray-Curtis index we show that the diversity of the composition of the microbiota is statistically significant between all groups. Differences are observed between neurotypical siblings and children from the control group (PERMANOVA test, $p = 0.001$) and between neurotypical siblings and children with autism spectrum disorders (PERMANOVA test, $p = 0.001$), as well as between groups of children with autism spectrum disorders and children from the control group (PERMANOVA test, $p = 0.029$).

These results indicate that the trait (bacterial sequence) that differentiates ASD children from control children is close to the phylogenetic tree (because the UniFrac weighted index reduces the beta distance for similar traits in the phylogenetic tree).

In 2022, the size of the control group was increased and more people were recruited to the study groups (children on the autism spectrum and their neurotypical siblings). Questionnaires and biological material (faeces) were collected. DNA and RNA extraction was performed and

DNA libraries were prepared, 16S rRNA gene amplicons (V3V4 and V7V9) were sequenced on the MiSeq (Illumina) apparatus, obtaining data in the Fastq format, which were then analyzed using the bioinformatics data analysis platform - Qiime2, establishing a standard protocol microbiota data analysis. Another part of the project has been started, consisting in the analysis of data from dietary surveys, in the context of which further analyzes of the composition of the microbiota will be carried out.

DEPARTMENT OF IMMUNOCHEMISTRY
Head: Professor Czesław Ługowski, Ph.D.

Laboratory of Glicobiology
Head: Professor Marcin Czerwiński, Ph.D.

Basigin expression and characterization

Basigin (BSG) or EMMPRIN (CD147), is a glycosylated transmembrane protein that belongs to the immunoglobulin (Ig) superfamily. It is abundant on the surface of various types of tissues and cells, including erythrocytes. Recent studies have revealed its role as red blood cells (RBCS) receptor for *Plasmodium* merozoites. These parasites invade human RBCS, and an essential step in this process involves the ligand PfRh5, which forms protein-complex, and binds BSG on the host cell. Structurally, BSG consists of two extracellular Ig domains, Ig1 and Ig2; a single transmembrane domain; and a short cytoplasmic domain. The extracellular region of BSG contains three asparagine- linked oligosaccharide chains at positions: 44, 152 and 186.

In order to evaluate the role of basigin N-glycans in the interaction with Rh5 merozoite ligand, the recombinant BSG and its glycosylation deletion mutants and Rh5 ligand were expressed in HEK193 cells. We have obtained all recombinant proteins with good yield about 0.5mg/ml. The binding analysis was performed using surface plasmon resonance (SPR). We have revealed that the recombinant BSG and its triple glycosylation mutant, without any N-glycans, can bind Rh5 ligand, but with different affinity. Further studies will be performed to characterize the binding properties of all basigin glycosylation -mutants. These results may explain the molecular mechanism of BSG-Rh5 interaction crucial for human erythocyte invasion by malaria parasite *Plasmodium falciparum.*

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Biochemical characteristics of macromolecules involved in immunological processes: Immunochemical studies of bacterial endotoxins

The expertise of the Laboratory of Microbial Immunochemistry and Vaccines covers a variety of preparative, chromatographic, chemical, and instrumental analytical techniques (mass spectrometry, nuclear magnetic resonance spectroscopy, surface plasmon resonance) to elucidate structures of biologically relevant carbohydrates, especially lipopolysaccharides (LPS, endotoxins) of Gram-negative bacteria. In previous years, the current state of knowledge has been extended by an analysis of genes involved in LPS biosynthesis. Our research concerns Gram-negative species, such as *Klebsiella pneumoniae, Escherichia coli, Shigella sonnei*, *Bordetella* spp. and *Plesiomonas shigelloides*, and *Edvarsiella tarda*, which represent opportunistic human pathogens associated with extraintestinal and nosocomial infections in humans and animals. Moreover, *K. pneumoniae*, particularly ESBL- and KPC-strains, has been singled out in 2017 as *"priority 1. critical pathogen"* for health care by the WHO, CDC, and

the UK Department of Health. *B. pertussis, B. parapertussis, B. bronchiseptica,* and *B. holmesii*, are mammalian respiratory pathogens, having substantial economic impact on human health and agriculture. *B. pertussis* is responsible for whooping cough (pertussis) and *B. holm*esii is the second pertussis etiological factor, but the current anti-pertussis vaccines do not provide cross-protection. Major virulence factors and surface antigens of these species are: lipopolysaccharide (LPS, endotoxin, O antigen), capsules (antigen K, e.i. capsular polysaccharide - CPS and exopolysaccharide (EPS), and fimbriae. LPS is built up of lipid A, core oligosaccharide (OS), and O-specific polysaccharide (O-PS). LPS and CPS determine O serotype or K serotype, respectively. In particular, O serotype is defined by O-PS region built up of carbohydrate repeating units.

In 2022, we have reported the structure of exopolysaccharide of *Streptococcus bovis.* The species - *Streptococcus gallolyticus subspecies gallolyticus, known as Streptococcus bovis biotype I, is a facultative pathogen causing bacteraemia, infective endocarditis and sepsis. S. bovis* is also another example of bacterium linked with cancer, but contrary to *H. pylori* its connectivity with cancerogenesis is poorly characterised. The bacterium has been linked with colorectal cancer (CRC) since the 1950s, but this correlation is still unclear. Worldwide CRC is one of the major medical problems. It ranks as the third most common malignant neoplastic disease after breast and lung cancer in women and prostate and lung cancer in men. CRC is second cause, after lung cancer, of global cancer mortality, with an estimated 9.4% of cancer deaths in 2020.

Bacterial surface structures, such as the major sugar antigens exposed to the outside of the microorganism, are among potential virulence factors. One of the primary sugar antigens loosely attached to the cell surface is the biofilm component, exopolysaccharide (EPS). EPSs of *S. bovis* are poorly characterized molecules. Until now, only one *S. macedonicus* Sc136 EPS structure was known to the entire *S. bovis* group. The *S. gallolyticus* DSM 13808 EPS was investigated by chemical analysis, mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy. The hexasaccharide repeating unit of the EPS, containing four Glc, two Rha residues and one phosphate group, has been described " →6)-α-D-Glc*p*-(1→3)-β-L-Rha*p*-(1→4)-β-D-Glc*p*-(1→3)-[β-D-Glc*p*-(1→2)]-α-L-Rha*p*-(1→2)-α-D-Glc*p*-(1→P→"
*(*Maciejewska A et al. The first report on the *Streptococcus gallolyticus* (S. *bovis* Biotype I) DSM 13808 Exopolysaccharide structure was in the International Journal of Molecular Sciences, 2022. 23, no. 19: 11797; https://doi.org/10.3390/ijms231911797). The study was performed within the framework of the MINIATURA2 project (National Science Center, DEC-2018/02/X/NZ6/02532).