Hirszfeld Institute of Immunology and Experimental Therapy
Polish Academy of Sciences
Rudolfa Weigla 12, 53-114 Wrocław

RESEARCH REPORT 2023

Contents Page

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

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

Development of new orthotopic cancer models (with particular emphasis on bladder and colon cancer) using modern *in vivo* intravital imaging techniques

The presence of normal cells of a given organ, naturally occurring blood and lymphatic vessels and interactions with the immune system differentiate the orthotopic model completely from the subcutaneous model. It allows for a comprehensive, multi-factorial assessment of the impact of a developing tumor on the mouse body, providing a more complete and reliable picture of the effectiveness of the new therapeutic regimens being tested. Based on previously developed orthotopic models of bladder cancer (induced under ultrasound control), a number of therapeutic experiments were performed, while at the same time refining procedures on animals (accelerating procedures and thus reducing distress for animals). Refined research models (based on both tumor cells with induced resistance to cytostatics and wild-type) allow for 99% injection effectiveness and very small differences in the rate of tumor tissue growth in individual mice.

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

A group of 14 compounds was selected from the N-substituted thiosemicarbazone derivatives obtained in 2021-2022. They were characterized by high anticancer activity against MCF-7 cells (human breast cancer) and SK-N-MC (human neuroepithelioma), with simultaneous low toxicity against normal MCF10A cells (human mammary epithelium) and BALB/3T3 (mouse fibroblasts). The antiproliferative activity of these compounds was determined against a panel of cancer and normal human cells. Very interesting results were obtained indicating that these compounds have selective activity against cancer lines originating from the breast (MDA-MB-468, MCF-7, MDA-MB-231), ovaries (A2780) and cervix (C33A). In the case of sensitive lines, the selectivity index (SI) for 14 thiosemicarbazones ranged from 3 to over 500. In turn, the lines of human pancreatic cancer (MiaPaCa-2) and lung cancer (NCI-H1975) turned out to be similar to the action of thiosemicarbazones towards normal cells. These results may indicate the presence of a specific molecular target for this group of thiosemicarbazamines in selected groups of cancers.

In vitro studies on the influence of proinflammatory cytokines on the transendothelial migration of cells capable of absorbing antigen

In the conducted research, cells of the RAW 264.7 mouse macrophage cell line were stimulated for 24 hours with B4C preparations of various particle sizes, which were then applied to the plates based on previously optimized test conditions. To induce the migration effect, the 72-hour supernatant from the *in vitro* culture of MC38 colorectal cancer cells was used, and culture medium with 5% FBS was used as a control. After 16 hours of incubation, the ability of RAW 264.7 cells to move was assessed. Untreated and B4C 1-loaded cells migrated more efficiently through the insert surface into the supernatant from the tumor cells than into the control medium. The opposite effect was observed in the case of cells loaded with B4C 2, where the number of migrating cells was lower compared to control

macrophages. The obtained data contribute to the complete development of a model enabling the determination and regulation of changes in the infiltration of cancer tissue by phagocytic cells in the presence of the extracellular matrix.

The role of lncRNA and miRNA regulatory molecules in the anticancer activity of calcitriol in breast cancer

The research used cells of the breast cancer lines MCF-7, T47D, MDA-MB-231, MDA-MB-468, CAL-51 and the normal mammary epithelium cell line MCF-10A. The expression level of MALAT1, which belongs to the lncRNA group, was assessed in these cells. Lysates were collected from cells treated with calcitriol and tacalcitol for 72 h and appropriate controls (solvent calcitriol and tacalcitol) to determine MALAT1 levels. Lower levels of MALAT1 have been demonstrated in three triple-negative breast cancer lines (TNBC): MDA-MB-231, MDA-MB-468 and CAL-51.

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

Acute myeloid leukemia (AML) is an aggressive cancer of hematopoietic stem cells. Treatment for AML patients relies on the use of Ara-C alongside daunorubicin and/or darubicin. However, up to 40% of high-risk cytogenetic AML cases exhibit primary resistance to chemotherapy. Despite the complex, time-consuming, and costly molecular diagnosis of AML, the efficacy of these markers is questionable. Furthermore, despite the extensive classification of AML leukemias, therapeutic options remain limited to the "3+7" regimen (or very similar chemotherapy) and bone marrow transplantation.

It has been repeatedly shown that *ex vivo* tests can be useful for predicting therapy effectiveness. It is proposed that early detection of chemoresistance or sensitivity of cancer cells could assist doctors in deciding to exclude an ineffective drug. The aim of the research task is to develop and validate flow cytometry parameters that could help predict chemoresistance.

For the preliminary studies, we selected two leukemia cell lines that significantly differed in sensitivity to daunorubicin, reflected by approximately a 10-fold difference in IC50 parameter. Utilizing the fluorescent properties of daunorubicin, we measured the increase in drug fluorescence within individual cells over time. We observed significant differences in drug uptake by sensitive and resistant cells during short-term exposure to the drug. To describe these differences, we proposed the use of empirical parameters, considering the observed intracellular drug fluorescence and cell size, which can be determined in a short functional test.

Further studies on nine additional cell lines allowed for the analysis of the correlation between the proposed parameters and IC50 for daunorubicin. One of the proposed parameters (Susceptibility Index) shows a strong statistically significant correlation with the IC50 of the tested cell lines.

Additional bioinformatic analysis of two selected cell lines with extreme Susceptibility Index parameters suggests the involvement of overexpression of proteins related to the NAD(P)H redox system in daunorubicin resistance. We are convinced that our results provide a good foundation for more extensive research on the development of a rapid sensitivity test for topoisomerase inhibitors, which could assist in therapeutic decision-making.

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

Interactions of a metallacarborane cobalt bis(dicarbollide) with DNA

Cobalt bis(dicarbollide) (COSAN) is a metallacarborane used as a versatile pharmacophore to prepare biologically active hybrid organic–inorganic compounds or to improve the pharmacological properties of nucleosides, antisense oligonucleotides, and DNA intercalators. Notwithstanding these uses, it was unclear how COSAN interacts with nucleic acids, which hindered the creation of new metallacarborane-based drugs.

While some studies have demonstrated that COSAN intercalates into DNA, COSAN-containing intercalators do not, and while intercalators are frequently highly toxic, COSAN exhibits modest cytotoxicity. Because of its extreme flexibility, DNA can accommodate molecules of all sizes and forms. From a purely spatial perspective, COSAN might intercalate or bind to a groove in DNA to form complexes; however, this would require significant structural alterations in DNA that COSAN is unable to offset by creating new bonds. Furthermore, an anionic COSAN cannot provide the electrostatic component that is often included in the interactions between the cationic ligand and anionic DNA.

Through the use of a variety of methods, such as UV-Vis absorption, circular (CD) and linear (LD) dichroism, nuclear magnetic resonance (NMR) spectroscopy, thermal denaturation, viscosity, differential scanning calorimetry (DSC), isothermal titration calorimetry (ITC), and equilibrium dialysis measurements, we thoroughly characterized interactions between COSAN and DNA. The structure, length, stability, and hybridization of DNA were unaffected by COSAN. DNA, in turn, had no effect on COSAN's absorption spectrum or diffusion coefficient, nor did it induce CD or LD signals. COSAN titration into DNA had no heat effects. Additionally, we showed COSAN was as toxic to nuclear cells as to red blood cells (RBCs) without a cell nucleus or organelles. The membranolytic effect of COSAN on RBCs suggests that COSAN cytotoxicity is independent of DNA and more likely explained by its interactions with cell membranes. Theoretically and in light of our experimental findings, COSAN cannot form complexes with DNA and does not require DNA to cause toxicity, which is most likely protein- or cell membrane-dependent.

In summary, we demonstrated that COSAN is a DNA-neutral pharmacophore and has no impact on the length, stability, or structure of DNA.

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 aimed to identify diagnostic and prognostic biomarkers of the outcome of allogeneic haematopoietic stem cell transplantation (HSCT), selected haematological diseases as well as those being associated with successful ageing. The analyses were performed on samples collected from HSCT recipients suffering from various disorders and their donors, patients with multiple myeloma, and healthy elderly and young people from four different populations. The latter study was conducted within the 18th International and Histocompatibility Workshop.

Our multicentre studies on adult patients undergoing HSCT allowed us to describe polymorphic variants of the genes coding for HLA-E and MICB molecules as well as their soluble protein serum levels as predictive factors of CMV infection and graft-versus-host disease (GvHD) development after transplantation.

Increased soluble HLA-E (sHLA-E) levels detected in recipients' serum 30 days after HSCT seemed to play a prognostic and protective role. Recipients with higher sHLA-E levels were less prone to chronic GvHD or more severe acute GvHD grades II-IV. Our results also confirmed the unfavorable role of HLA-E donor-recipient incompatibility in CMV infection development after transplantation. Moreover, patients with CMV infection characterized with elevated frequencies of NK cells (both CD56dim and CD56bright) expressing NKG2C, especially 30 and 90 days post-transplantation, and increased percentages of NKG2C+ NK cells lacking NKG2A expression. In addition, recipients carrying an *NKG2C* deletion characterized with decreased frequency of NKG2C+ NK cells. This study highlights the effect of HLA-E and NKG2C genetic variants, sHLA-E serum concentration, as well as NKG2C surface expression on transplant outcome (Siemaszko et al. Front Immunol. 2023).

Furthermore, our results also revealed a favorable role of *MICB* rs1065075 *G* allele. Recipients with donors carrying this genetic variant were less prone to develop chronic GvHD. Moreover, both donor and recipient *MICB* rs1065075 *G* allele was associated with lower incidence of CMV reactivation. The *MICB* rs1065075 *G* variant was also found to be associated with decreased soluble MICB (sMICB) 30 days after transplantation, while sMICB concentrations were significantly higher in recipients diagnosed with CMV infection or chronic GvHD when compared to recipients without those complications. A protective role of the *G* allele was also found for the rs3828903 polymorphism, as it was more frequently detected among donors whose recipients developed no chronic GvHD symptoms. This suggests that *MICB* genetic variants as well as serum levels of sMICB may serve as predictive factors for the risk of development of chronic GvHD and CMV infection after allogeneic HSCT (Siemaszko et al., Arch Immunol Ther Exp. submitted).

In patients with multiple myeloma (MM), telomere length and *hTERT* genetic variants were found to act as potential prognostic markers of the disease. Significantly shorter telomeres were detected in patients than in healthy controls, and in patients suffering from more advanced disease as compared to those presenting with lower stages of MM. Moreover, the presence of *hTERT* rs2736100 *T* allele characterized patients with significantly shorter progression-free survival and was less common in patients with disease progression in response to treatment (Dratwa et al., Sci Rep. 2023).

We also conducted a multicentre study involving elderly and young Bulgarians, Romanians, Turks and Poles, showing for the first time various relationships between HLA haplotypes, telomere length and longevity (Bogunia-Kubik et al., personal communication, HLA. 2023; Dratwa et al., in preparation).

In conclusion, our multifunctional, multicentre studies on patients with blood cancers and healthy individuals allowed us to identify biomarkers associated with risk and course of disease, treatment outcome, as well as successful ageing.

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

Pro- and anti-inflammatory cytokines and growth factors in patients undergoing *in vitro* fertilization procedure treated with prednisone

Embryo implantation is a key moment in pregnancy. Abnormal production of pro- and anti-inflammatory cytokines, their receptors and other immune factors may result in embryo implantation failure and pregnancy loss. The aim of this study was to determine the immunological profile of pro- and anti-inflammatory factors in the blood plasma of patients undergoing *in vitro* fertilization (IVF) and control women who achieved pregnancy after natural conception. The examined patients were administered steroid prednisone. We present

results concerning the plasma levels of IFN-ɣ, BDNF, LIF, VEGF-A, sTNFR1 and IL-10. We found that IVF patients receiving steroid treatment differed significantly from patients who were not administered such treatment in terms of IFN-γ and IL-10 levels. Moreover, IVF patients differed in secretion of all tested factors with the fertile controls. Our results indicated that women who secrete at least 1409 pg/ml sTNFR1 have a chance to become pregnant naturally and give birth to a child, while patients after IVF must achieve a concentration of 962.3 pg/ml sTNFR1 in blood plasma. In addition, IVF patients secreting VEGF-A above 43.28 pg/ml have a greater risk of miscarriage or a failed transfer in comparison to women secreting below this value. In conclusion, fertile women present a different profile of pro- and anti-inflammatory cytokines, and growth factors compared to patients with recurrent implantation failure (RIF). Moreover, the use of steroids by infertile patients should be carefully considered due to the changes they cause in the immune environment of the uterus and in the peripheral blood.

EULAR study group on 'MHC-I-opathy': Identifying disease-overarching mechanisms across disciplines and borders

The 'MHC-I (major histocompatibility complex class I)-opathy' concept describes a family of inflammatory conditions with overlapping clinical manifestations and a strong genetic link to the MHC-I antigen presentation pathway. Classical MHC-I-opathies, such as spondyloarthritis, Behçet's disease, psoriasis and birdshot uveitis, are widely recognised for their strong association with certain MHC-I alleles and gene variants of the antigen processing aminopeptidases ERAP1 and ERAP2, which implicates altered MHC-I peptide presentation to CD8+T cells in the pathogenesis. Progress in understanding the cause and treatment of these disorders is hampered by patient phenotypic heterogeneity and lack of systematic investigation of the MHC-I pathway. Here, we discuss new insights into the biology of MHC-I-opathies, which strongly advocate for disease-overarching and integrated molecular and clinical investigation to decipher the underlying disease mechanisms. Because this requires transformative multidisciplinary collaboration, we introduce the EULAR study group on MHC-I-opathies to unite clinical expertise in rheumatology, dermatology and ophthalmology, with fundamental and translational researchers from multiple disciplines such as immunology, genomics and proteomics, alongside patient partners. We prioritise standardisation of disease phenotypes and scientific nomenclature and propose interdisciplinary genetic and translational studies to exploit emerging therapeutic strategies to understand MHC-I-mediated disease mechanisms. These collaborative efforts are required to address outstanding questions in the etiopathogenesis of MHC-I-opathies towards improving patient treatment and prognostication.

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

Prof. Andrzej Lange is known for his studies on the role of the dysregulation of the immune system and that of HLA-associated factors in carcinogenesis. Studies on HLA benefited his clinical activity in the area of hematopoietic progenitor cell transplantation (more than 1300 procedures). He founded the Lower Silesia Centre of Cellular Transplantation with the National Bone Marrow Donors Registry (granted with the EBMT, EFI, NMDP, and WMDA accreditations). The close cooperation with the Laboratory of Clinical Immunology of the Institute made it all possible. The scientific activity is focused on the biology and genetics of alloreactivity after HSCT. Experience has geared his research and

clinical activity toward the use of marrow-derived cells in the regeneration of the vasculature bed and worn-out joints. It was a successful activity documented by 20 years of observation.

Prof. Lange is a licensed physician in haematology, clinical immunology, and transplantation, published 260 scientific papers with more than 4,500 citations. He received numerous awards, scientific medals, and honours in Poland and internationally.

Laboratory of Clinical Immunology profile

Laboratory of Clinical Immunology is focused on transplantation immunology and studies of the immune system competence in cancer patients receiving immunotherapy and in those with chronic viral infections. Similar analysis is performed in Covid-19 vaccinated and/or infected individuals.

The ongoing research revealed the following:

1. The heightened response to the immune-check points inhibitors is facilitated by the excessive tumour mutation burden, but other mutations that may affect the immune responsiveness play a role, as thyroiditis and also hypophysitis are present.
2. Responsiveness to Covid-19 vaccination diminishes in seven month but lasts longer if there is a coincidence of the infection.
3. In the population of the social home residents, at the time of SARS-CoV2 pandemic, seasonal coronaviruses circulated as shown by the presence of antibodies specific for nucleocapsid of HCoV NL 63 and HCoV 229E in a proportion of the residents. This makes the recombination of genetic material between the viruses of different virulence (pandemic and seasonal) possible.
4. Observation of the patients with chronic Herpes viruses infections revealed in the blood the presence of the suppressor/regulatory cells as an effect of recurrent inflammatory events, but a primary defect making the hosts susceptible to chronic virus infections is rather associated with genetic predisposition. The study on that is currently under analysis.

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

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

We sought to determine how the existing already for almost 20 years at our Institute program of phage therapy (PT) affected the awareness of this method in the general population. The study was conducted in collaboration with the Institute of Philosophy and Sociology, PAS with the aid of CAWI (Computer Assisted Web Interview). The analysis included 1,098 responses provided by questionnaires related to phages and PT.

While the general knowledge on antibiotics resistance was high (>90%), only 12% responders were aware of the associated risks. PT was known to 9% of non-employed people and almost 40% of those with higher education. Interestingly, 85% of responders were ready to take advantage of PT and bear related costs.

This pioneering study will be published and form part of a doctoral thesis. Its results suggest the need for further research is needed to promote knowledge of phages and PT.

We also continued our work on the isolation and characterization of phages potentially useful in PT. We were able to isolate 8 new phages against *Acinetobacter* and characterized optimal conditions for their amplification Likewise, similar studies led to isolation of 8 new lytic phages against *Enterobacter.* We also assayed lytic activity of 12 *Enterococcus* phages and the effect of various factors on it (storage temperature, pH). Importantly, 6 of those

phages were able to destroy bacterial biofilms. Phages varied with regard to their ability to destroy biofilm from some 20% to 80%. Furthermore, we studied optimal storage conditions (-80°C) of *Klebsiella* phages and found that they differ markedly in their requirements for supporting media and stabilizers used. These results enrich our bank of therapeutic phages and shed more light on their biology and activity and, therefore, should allow us to expand our means to fight dangerous pathogens using PT.

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

New Phage-Derived Antibacterial Enzyme PolaR Targeting *Rothia* spp.

Rothia is an opportunistic pathogen, particularly life-threatening for the immunocompromised. It is associated with pneumonia, endocarditis, peritonitis and many other serious infections, including septicemia. Of note, *Rothia mucilaginousa* produces metabolites that support and increase overgrowth of *Pseudomonas aeruginosa*, one of the ESKAPE bacteria. Endolysins are considered to be antibacterial enzymes derived from bacteriophages that selectively and efficiently kill susceptible bacteria without harming human cells or the normal microbiome.

We applied a computational analysis of metagenomic sequencing data of the gastric mucosa phageome extracted from human patients' stomach biopsies. A selected candidate anti-*Rothia* sequence was produced in an expression system, purified and confirmed as a *Rothia mucilaginosa*- and *Rothia dentocariosa*-specific endolysin PolaR, able to destroy bacterial cells even when aggregated, as in a biofilm. PolaR had no cytotoxic or antiproliferative effects on mammalian cells. PolaR is the first described endolysin selectively targeting *Rothia* species, with a high potential to combat infections caused by *Rothia mucilaginosa* and *Rothia dentocariosa*, and possibly other bacterial groups. PolaR is the first antibacterial enzyme selected from the gastric mucosa phageome, which underlines the biological complexity and probably underestimated biological role of the phageome in the human gastric mucosa.

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

Characterization of primary and immortalized mesenchymal stem cells from peripheral nerves, analysis of the secretory profile and functional potential

The study is a continuation of the research on mesenchymal stem cells (MSC) of human peripheral nerve (PN-MSC) origin. The aim was to compare the biological properties of primary PN-MSC and immortalized PN-MSC (PN-MSC SVT) cell line. The profile of bioactive factors secreted by these cells was determined using protein membranes (Human Neuro Discovery C2 Array, Ray Biotech). Primary PN-MSC cells showed the presence of 20 out of 30 analyzed factors involved in the processes of growth and neurogenic differentiation, signal transduction, inflammation, apoptosis and angiogenesis, present both in cells (cell lysate) and in the form of secreted proteins. The pool of secreted bioactive factors include e.g.: neuronal growth factor BDNF, pro-apoptotic factor Fas, cytokines/chemokines IL-1 β, IL-6, IL-8, MCP-1, MIP-1 β, TARC, the extracellular matrix metalloprotease MMP3 and its inhibitor TIMP1, and pro-angiogenic factor VEGF. Immortalized PN-MSC SVT cells showed similar secretory profile to primary PN-MSC cells, and in addition, to the above listed bioactive factors, neurotrophic factors CNTF and GDNF and IFNγ were detected.

The study demonstrated that PN-MSCs, both primary and immortalized, are capable of producing and secreting trophic factors essential for the differentiation and growth of neural cells. The immortalization process did not affect the ability of PN-MSC SVT to secrete the tested bioactive factors. This knowledge will allow for the study of the regenerative potential of the PN-MSC secretome in the treatment of diseases related to peripheral nerve dysfunction.

Assessment of the impact of microvesicles derived from mesenchymal stem cells in inhibiting the proliferative activity of ovarian cancer cell lines

Research was continued on the influence of microvesicles, isolated from the of mesenchymal stem cells line obtained from adipose tissue (HATMSC2-MVs), on ovarian cancer cells.

Primary tumor cells were isolated from human postoperative ovarian cancer tissues (using commercial tumor cell isolation kit) and from ascitic fluid. The phenotype characteristic for mesenchymal stem cells (CD73, CD90, CD105), and cancer stem cells CSCs (CD24, CD44, CD133) has been confirmed. Also, the expression of proteins responsible for maintaining pluripotency (Oct4, Sox2 and Nanog) and markers responsible for epithelial-mesenchymal transition (EMT), i.e. Snail and vimentin, has been detected.

The activity of HATMSC2-MVs on cancer cells was assessed in 3D culture on spheroids created from primary ovarian cancer cells. Microvesicles derived from the HATMSC2 cell line were able to internalize into spheroids formed from primary ovarian cancer cells decreasing their survival, which was confirmed by confocal microscopy analysis by identifying viable cells with Syto 9 staining and dead cells identified with propidium iodide. The most important achievement was the creation of spheroids composed of heterogeneous population of primary ovarian cancer cells, containing subpopulation of cells with the CSCs phenotype expressing CD44, CD133 and CD24. We confirmed decrease of tumor cell survival in 3D culture of primary ovarian cancer by internalization of HATMSC2-MVs into tumor cells.

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

Fine and gross motor skills in 7-10 year-old Indian children exposed to a natural disaster during early development

Fetal life and infancy are extremely sensitive to adverse environmental conditions. This study aimed to assess the effect of exposure to a natural disaster (cyclone Aila) in utero or during infancy on fine and gross motor functions in preadolescent Indian children. The study was conducted in West Bengal, India, and included approx. 700 children (7-10 years old) prenatally or postnatally exposed to cyclone Aila and non-affected group. Anthropometric measures included height, weight and birthweight. Socioeconomic status was based on parental education, family size and income. Motor functions were assessed using the short form of Bruininks-Oseretsky Test of Motor Proficiency (BOT-2). Statistical analyses included e.g. generalized linear models. There were no differences in motor functions depending on the timing of the exposure (trimester) during pregnancy. Compared to the controls, prenatal Aila-exposure resulted in poorer performance in all BOT-2 subtests, except for fine motor precision, strength and balance (the last in boys), while postnatal Aila-exposure - in fine motor integration, bilateral coordination, speed and agility, and upper limb coordination. Early life exposure to a natural disaster has long-term adverse effect on motor proficiency.

Therefore, during an environmental cataclysm, pregnant women and infants should be of particular concern for emergency and health services.

Height and integration in proximity networks among Tanzanian Hadza men

In recent years there has been much interest in the extent to which social status or prestige are related to integration in social networks of an individual. It has been shown that among hunter gatherers, social characteristics of an individual based on social status or prestige, such foraging reputation, friendship popularity and pro-social reputation, can influence the extent to which an individual is embedded in a social network. However, little is known regarding the extent to which height, a physical trait that in Western societies is often associated with social status, is associated with integration in social networks among small-scale hunter gatherers. Here, we investigated the relationship between height and the position an individual occupies in proximity networks among the Hadza men, hunter-gatherers living in Northern Tanzania. The results of our study show that height is neither related to status nor the position an individual maintains in proximity networks. We argue that in a relatively egalitarian small-scale hunter-gather societies, such as the Hadza, social interactions driving proximity networks might be influenced by social, such as popularity and hunting reputation, rather than physical traits, such as height.

DEPARTMENT OF MICROBIOLOGY

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

The research activity of LMBM is dedicated to addressing three crucial scientific issues: i/ secondary metabolism in *Streptomyces*, a key area in understanding microbial behaviour; ii/ the development of new compounds for antimicrobial therapy, a pressing need in the face of increasing antibiotic resistance, and iii/ bacterial response to stress, a fundamental aspect of bacterial survival and adaptation.

i/ Our laboratory is at the forefront of studying the regulation of coelimycin synthesis in *Streptomyces coelicolor* A3(2). We are particularly intrigued by the autoregulation of coelimycin biosynthetic cluster and the roles of two SARP activators, CpkO and CpkN, an orphan kinase CpkM, gamma-butyrolactone receptors and the transporter CpkF in coelimycin production. We aim to unravel the regulatory connection between coelimycin synthesis, which occurs at the switch from primary to secondary metabolism, and the control of other secondary metabolite biosynthetic gene clusters. These findings could potentially lead to the development of improved industrial *Streptomyces* strains.

ii/ Bacterial resistance to antibiotics is becoming increasingly problematic. As a result, new antimicrobial compounds and therapies are needed to eradicate pathogenic bacteria effectively. Our primary focus is on using flexible organic light-emitting diodes (OLED) and new photosensitizing chemicals with potential bactericidal activity to eradicate bacteria in skin infections photochemically. We are investigating new photosensitizing compounds using *Staphylococcus aureus* as a model species and clinical strains of bacteria isolated from diabetic foot infections.

iii/ We are studying bacterial factors that regulate stress response in certain species of *Campylobacteria*. Specifically, we focus on CemR atypical response regulators, which control gene transcription and chromosome replication initiation. We aim to identify the regulons controlled by these proteins in specific pathogenic *Campylobacterial* species to enhance 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

Macrophages play an important role in maintaining tissue homeostasis, in clearing apoptotic bodies, and are the first line of defense in infections. In response to alarm signals, there is a classic TLR-dependent activation of macrophages, resulting in the release of several pro-inflammatory factors, including nitric oxide (NO), oxygen free radicals (ROS) and pro- and anti-inflammatory cytokines.

Our studies have shown that selected *Bifidobacterium* strains are activators of inducible nitric oxide synthase iNOS and stimulate macrophage cells of the BMDM lineage to secrete significant amounts of NO. In addition, it was shown that this effect may be dependent on the activation of the TLR-4 receptor. Therefore, the next objective was to study the effect of selected *Bifidobacterium* strains on modulating the production and secretion of pro-, and anti-inflammatory cytokines, and to determine their antioxidant activity in terms of their ability to neutralize ROS and inhibit oxidative processes in the body.

The studies were performed on a mouse model of BMDM myeloid-derived macrophages and human whole blood cells. The first goal was to test whether the tested *Bifidobacterium* strains could *ex vivo* increase the ability to induce cytokines in human whole blood cells. Cytokine levels were determined by ELISA. The tested *Bifidobacterium* strains (218, 219, 366, 367, 368, 369, 370, 371, 372 and 373) were shown to be capable of stimulating whole blood cells to secrete significant amounts of cytokines. For TNFα, strains 368 and 370 showed the highest activity (874.8 ± 153.90 and 863.8 ± 91.03, respectively). A similar trend was observed for anti-inflammatory IL-10: 120.33 ± 136.42 for strain 368 and 124.2 ± 112.93 for strain 370. The highest amounts of IL-6 and IL-8 were released in response to strain 219. In the next step, the effect of *Bifidobacterium* on cytokine release by macrophages of BMDM WT, BMDM TLR (4-) and BMDM (TLR2-) lines was examined.

Cytokine levels were determined by Luminex technology using the MILLIPLEX MAP Human TH17 kit (Bio-RAD). BMDM WT macrophages were shown to release significant amounts of TNFα in response to strains 218, 367, 370 and 373. Strains 218, 366, 269, and 370 induced IL-6 production, whereas strain 366 mainly stimulated IL- 2 and IL-1β production. In the case of BMDM (TLR4-) macrophages, a significant increase in the level of released IL-1β was observed in response to strains 366, 369 and 371. In addition, all strains tested, except 369 and 370, induced TNFα production. In the case of BMDM macrophages (TLR2-), an increase in IL-1β, Il-6 and IL-10 levels was observed in response to strains 366 and 373, while strains 218, 360, 368, 369. 370, 371, 372 and 373 induced TNFα production. An attempt was also made to study the modulatory effect of *Bifidobacterium* under conditions of LPS-induced inflammatory response. It was shown that strains 219 and 366 significantly reduced the level of IL-6, while no changes were observed in the level of released TNFα.

The antioxidant activity of selected *Bifidobactertium* strains was evaluated. The antioxidant potential of bacterial culture supernatants was determined by methods: ABST, DPPH and FRAP. It was observed that the tested strains: 219, 366, 367, 368 and 371 showed significant ability to reduce ROS levels, with strains 367 and 368 showing the highest activity.

We demonstrated the regulatory effect of *Bifidobacterium* probiotic bacteria on the regulation of the production of pro- and anti-inflammatory cytokines by human whole blood cells and macrophage cells, as well as on the neutralization of oxygen free radicals, thus inhibiting oxidative stress.

DEPARTMENT OF TUMOR IMMUNOLOGY

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

The use complementary sex determining gene diversity for studying the genetic structure of honey bee colonies

Polyandry, a mating system where a female mates with multiple males, is a crucial aspect of honey bee biology. Queen bees typically mate with 10-20 drones during their early life mating flights, storing sperm in the spermatheca to fertilize eggs throughout their lifetime. This polyandrous behavior enhances genetic diversity within the colony, promoting adaptability, disease resistance, and overall fitness. Analyzing patrilines, or groups of individuals sharing the same drone parent, is essential for understanding genetic diversity, colony health, and reproductive success. These insights are invaluable for breeding programs aiming to enhance traits like disease resistance and productivity. Current methods of analysing patrilines employ genotyping of worker brood using polymorphic microsatellite loci to estimate patriline diversity and origin. However, microsatellite markers have limitations, including homoplasy, null alleles, and limited allelic diversity, which can reduce analysis resolution.

Our studies explored the use of the complementary sex determining gene (*csd*) as a potential replacement or supplement to microsatellite markers for patriline identification. The *csd* gene, crucial for sex determination in honey bees, exhibits high polymorphism due to balancing selection, making it a promising candidate for genetic studies. The research analyzed worker bees from five colonies, using both microsatellite markers and *csd* gene fragments. Results indicated that *csd* genotyping alone identified more patrilines than five microsatellites markers combined, though combining both methods yielded the highest resolution. However, challenges of using *csd* genotyping include inefficient amplification of the *csd* HVR fragment and potential allele misassignment due to restriction pattern similarities.

Despite these challenges, *csd* genotyping offers significant advantages, such as higher variability and simpler maternal allele identification. This method reduces the need for extensive microsatellite analysis, making genetic studies more efficient and comparable across different research teams. Recent advancements suggest that *csd* gene variants can also be determined from complex samples like honey, further expanding its applicability.

In conclusion, combining csd and microsatellite genotyping substantially enhances genetic study resolution in honey bee colonies. The *csd* gene stands out as a promising tool for advancing genetic research, allowing for better insights into genetic diversity, reproductive success, and social dynamics. This combined approach can streamline genetic studies, improve breeding programs, and enhance the overall understanding of honey bee colony genetics.

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

Overexpression of c-Myc promoter binding protein (MBP-1) in melanoma cells: Why does cellular compartment matter?

C-Myc promoter binding protein (MBP-1) is a product of alternatively translated mRNA encoding alpha-enolase (ENO1). In contrast to ENO1, MBP-1 possesses no enzymatic

activity, but instead represses transcription from *cMYC*. Ectopic overexpression of MBP-1 was shown to reduce cell proliferation and tumorigenicity of numerous tumor cell lines, constituting an attractive target for cancer therapy. Here, we overexpressed MBP-1 or its C-terminal truncated variant (MBP-1ΔC-HA), using lentiviral transduction in two human melanoma cell lines (A375, WM9). Unexpectedly, we found that overexpressed MBP-1 variants predominantly localized in the cell cytoplasm and consistently only minimally decreased c*MYC* expression. Moreover, the proliferation rate of MBP-1-transduced cells increased in comparison to empty vector controls, as did the rate of glucose metabolism in hypoxia. When assessing cell migration, we found that overexpression of MBP-1, but not MBP-1ΔC, led to a substantial decrease in the cell migration capacity of WM9 but not A375. Collectively, our data suggest an unexpected tumor-promoting activity of MBP-1 that can be largely attributed to its artificial cytoplasmic overexpression

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

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

In vitro studies on the mechanism of action of yolkin

Yolkin, an egg yolk derived protein, is postulated to promote the development of the immune system in embryos. The studies investigated the effects of yolkin on mitogen-induced mouse and human blood lymphocyte proliferation, cytokine production by mouse splenocytes, tumor cell line growth and cell signaling in mouse splenocytes and mouse macrophage RAW 264.7 cells. Concanavalin A-induced thymocyte proliferation was regulated and phytohemagglutinin A-induced blood lymphocyte proliferation inhibited. The viability of splenocytes was enhanced but inhibited in lipopolysaccharide (LPS) - treated cells. Yolkin inhibited the growth of mouse lymphocytic leukemia L 1210 cells and exhibited an additive suppressive effect on cell growth with cisplatin. The protein induced production of tumor necrosis factor alpha (TNF α), interferon gamma (IFN ɣ), interleukin 6 (IL-6), but interleukin-10 (IL-10) only at a high concentration. LPS-induced TNF α, IFN γ and IL-6 was inhibited but stimulated with regard to IL-10. The changes in expression of signaling molecules in splenocytes and cell lines indicated elicitation of cell activation and differentiation. Yolkin elicited significant levels of expression and production of cyclooxygenases COX-1 and COX-2 in splenocytes and cell lines. LPS-induced cyclooxygenase expression and production was regulated depending on concentration of yolkin. The results contribute to explaining the mechanism of yolkin action and facilitate the interpretation of the *in vivo* studies on yolkin.

The effect of yolkin on the development of cellular and humoral immune response in adolescent mice

The immune system in weanling and adolescent mice is not fully functional. We have previously established properties of yolkin in promotion of differentiation of immature cells. Therefore, the effect of yolkin, administered to 4 and 5-week-old mice in drinking water, was investigated on the development of the cellular and humoral immune response and phenotype of the immunocompetent cells. The contact sensitivity to oxazolone and antibody production to ovalbumin were measured. We showed that administration of yolkin to 4-week-old mice stimulated the contact sensitivity, but not the humoral immune response. The stimulation of

contact sensitivity was accompanied by a decrease of regulatory CD8+ i CD25+Foxp3+ cell content in the spleen. Regulatory actions of yolkin on the phenotype of T cell subpopulations in the thymus and mesenteric lymph nodes and B cells in the bone marrow were also registered, indicating its effects on T and B cell maturation and homing.

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: Time to progression and response to therapeutic checkpoint inhibitors

Multiple myeloma (MM) is a hematologic neoplasm characterized by clonal proliferation in the bone marrow of B lymphocytes producing monoclonal proteins and progressive immune dysfunction. Drugs that interact with immune surveillance elements (including PD-1 and CTLA-4 checkpoints on T lymphocytes, or immune checkpoints, or ICs) are particularly important for controlling the dynamics of tumor growth. To date, clinical trials on the use of therapeutic PD-1/PD-1L and/or CTLA-4 axis inhibitors (IC inhibitors, ICIs) in patients with MM have been highly disappointing, revealing little clinical efficacy with a high number of serious complications mainly of an autoimmune nature. The mechanisms of resistance to ICIs in MM are still of interest. The aim of this study was to investigate in different phases of MM: 1) the expression of PD-1, CTLA-4, CD28 and CD69 in peripheral blood CD4 T cells in order to determine their role in the development of systemic immunosuppression and autoimmunity (cognitive goal) and the clinical utility of ICs as potential target proteins for therapeutic ICIs (practical goal); 2) the association of PD 1 and CTLA-4 expression with the clinical course of MM (predictive value for assessing the risk of MM progression). Material and Methods. The study was conducted in a group of 40 patients with active MM before treatment (26 NDMM and 14 RRMM) and a control group of 20 healthy subjects. The quantitative (fluorescence intensity) and qualitative (% of cells) expression of ICs (PD-1 and CTLA-4) in the population of peripheral effector T cells (CD4+CD127+) and regulatory T cells (CD4+CD127), as well as the size of the population of aging autoreactive CD4+CD28- T cells were determined by flow cytometry. We also correlated PD-1 and CTLA-4 expression with the length of time to progression (TTP) of MM. Results and conclusions. It was shown that the lymphocytes of patients with NDMM showed a defect in the expression levels of ICs, CD69 and CD28, while in the RRMM group a reversal of the abnormalities of ICs expression and reactivity to stimulation was observed, as well as a partial restoration of CD28 expression. The observed phenotypic differences in different phases of MM suggest the predominance of immunostained CD4 T-cell dysfunction in the developmental stage of the disease (NDMM) and cellular exhaustion in the progressive stage of MM (RRMM), indicating differential reactivity and susceptibility to blockade of extracellular signals (PD-1 and CTLA-4) dependent on the stage of the disease. Suboptimal expression levels of ICs (insufficient to achieve a clinical response to ICIs) accompanied by peripheral expression of autoreactive CD4+CD28- cells found in all MM patients (most pronounced in the NDMM group) may be indicative of impaired immune tolerance and increased potential for autoimmune processes both in the course of MM and during therapy with ICIs. Our work also showed that low levels of CTLA-4 expression in NDMM and high PD-1 expression in RRMM may promote markedly shortened TTP, suggesting a potential clinical benefit of blocking ICs in only a subset of RRMM patients; for NDMM patients, the use of ICIs may prove to be a disadvantageous therapeutic strategy by accelerating disease progression.

Cytokine alterations and gut permeability in the deficit subtype of schizophrenia

There is evidence that subclinical inflammation and increased gut permeability might be involved in the pathophysiology of schizophrenia. Less is known about these phenomena in patients with the deficit subtype of schizophrenia (D-SCZ) characterized by primary and enduring negative symptoms. Therefore, in the present study we aimed to compare the levels of zonulin (the marker of gut permeability) and immune-inflammatory markers in patients with D-SCZ, those with non-deficit schizophrenia (ND-SCZ) and healthy controls (HCs). A total of 119 outpatients with schizophrenia and 120 HCs were enrolled. The levels of 26 immune-inflammatory markers and zonulin were determined in serum samples. The following between-group differences were significant after adjustment for multiple testing and the effects of potential confounding factors: 1) higher levels of interleukin(IL)− 1β and C-reactive protein (CRP) in patients with D-SCZ compared to those with ND-SCZ and HCs; 2) higher levels of tumor necrosis factor-α and RANTES in both groups of patients with schizophrenia compared to HCs and 3) higher levels of IL-17 in patients with D-SCZ compared to HCs. No significant differences between the groups in zonulin levels were found. Higher levels of IL-1β and CRP were associated with worse performance of attention after adjustment for age, education and chlorpromazine equivalents. Also, higher levels of IL-1β were correlated with greater severity of negative symptoms after adjustment for potential confounding factors. In conclusion, individuals with D-SCZ are more likely to show subclinical inflammation. However, findings from the present study do not support the hypothesis that this phenomenon is secondary to increased gut permeability.

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

Immunological mechanisms associated with reproductive processes in health and disease

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

The aim of the planned study was to understand the effect of interleukin 33 on the expression of transcription factors in peripheral blood B lymphocytes.

CD19+ cells sorted from peripheral blood were stimulated with human recombinant IL-33 (100 ng) for 6 hours. RNA was isolated and reverse transcribed to generate cDNA strands for RT-qPCR analysis during which B cell-specific transcription factors (PRDM1, cMAF, HIF1A, STIM1, SLAMF5, IRF4, IRF8) selected from the literature were analysed. In addition, the expression of the receptor for IL-33 (ST2) was examined in CD19+ cells after IL-33 stimulation and in control cells.

Higher relative IL1RL1 (ST2) gene expression was observed in IL-33-stimulated cells compared to unstimulated cells. In contrast, there were no differences in the expression levels of selected transcription factors after a six-hour stimulation of B lymphocytes with interleukin 33. The study indicates for the first time the presence of the ST2 receptor in human B lymphocytes.

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

Differential effects of common germline variants of *PDCD1, CD274,* and *HAVCR2* genes on risk and prognosis of major subtypes of NSCLC: Adenocarcinoma and Squamous Cell Carcinoma

Our study aimed to investigate the association between single nucleotide polymorphisms (SNPs) of the *PDCD1*, *CD274,* and *HAVCR2* genes and the risk and outcomes of non-small cell lung cancer (NSCLC), in particular its subtypes: squamous cell lung cancer (LUSC) and lung adenocarcinoma (LUAD).

For this purpose, we genotyped the following SNPs: *PDCD1*: rs36084323, rs7421861, rs11568821, rs2227981, rs10204525; *CD274*: rs822335, rs10815225, rs17718883, rs2297136, rs4742098, rs4143815; *HAVCR2*: rs10057302, rs1036199 in 383 NSCLC patients (112 LUAD and 116 LUSC) as well as 433 unrelated, cancer-free subjects as controls, using TaqMan SNP genotyping assays or polymerase chain reaction-restriction fragment length polymorphism method.

We showed that CC genotype of rs4143815 and GG genotype of rs4742098 were associated with two times higher risk of developing LUSC (CC vs. GG + GC, OR = 2.31; 95% CI = 1.32, 4.06; P = 0.003; GG vs. AA + AG, OR = 2.26; 95% CI = 1.17, 4.36; P = 0.016, respectively). Moreover, rs4143815 was an independent predictor of the age at diagnosis of LUAD. The carriers of the C allele were diagnosed 4.81 years later (95% CI = 1.47, 8.15; P = 0.006) than patients with the GG genotype. The rs10057302 CA genotype was an independent predictor of overall survival in LUSC (adjusted HR = 0.13; 95% CI = 0.02, 0.93; P = 0.043). NSCLC carriers of rs11568821 T allele had almost double the risk of death (adjusted HR = 2.05; 95% CI = 1.28, 3.29; P = 0.003) compared to carriers of CC genotype.

Our results provided additional evidence that SNPs of genes for PD-1, PD-L1 and TIM-3 differentially modulate the risk and prognosis of LUSC and LUAD.

The association of BTLA gene polymorphisms with non-small lung cancer risk

Continuing our interest in inherited risk factors and prognostic factors within genes encoding immune checkpoints in NSCLC, we studied polymorphisms of the *BTLA* gene in that context.

Using TaqMan probes, we genotyped seven *BTLA* SNPs: rs2705511, rs1982809, rs9288952, rs9288953, rs1844089, rs11921669 and rs2633582 in similar group of patients and controls.

We found that rs1982809 within *BTLA* is associated with NSCLC risk, where carriers of rs1982809G allele (AG+GG genotypes) were more frequent in patients compared to controls. Since cigarette smoking is the prevailing risk factor for NSCLC, we also analyzed the influence of *BTLA* polymorphisms in relation to smoking status. We noticed that rs1982809G carriers are significantly overrepresented in never-smokers, but not in smokers compared to controls. Additionally, the global distribution of the haplotypes differed between the never-smokers and smokers. Furthermore, in additional analysis of subgroups we showed that the presence rs1982809G allele (AG+GG genotypes) as well as the presence of rs9288953T allele (CT+TT genotypes) increased NSCLC risk in female patients. After stratification by histological type, we noticed that rs1982809G and rs2705511C carriers were more frequent among LUAD patients. Moreover, rs1982809G and rs2705511C correlated with the more advanced stages of NSCLC (stage II and III), but not with stage IV. Furthermore, we showed that rs2705511 and rs1982809 significantly modified OS, while rs9288952 tend to be associated with patients' survival.

Our results indicate that *BTLA* polymorphic variants may be considered low penetrating risk factors for NSCLC, especially in never-smokers and in females, and are associated with OS of NSCLC patients.

Summing up, our study indicated that inherited variations in genes encoding immune checkpoint molecules influence NSCLC risk. However, additional clinical and environmental factors like NSCLC subtype, gender, smoking status, and tumor grade should also be considered.

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

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

Lipidomic analysis of propionic bacteria, characterization of extracellular vesicles secreted by *Cutibacterium* and studies on the structure and function relationship of microbial glycoconjugates, their participation in infection and immunity processes, as well as the studies of glycation products

The aim of the current stage of the study included lipidomic analysis of representatives of the genus *Cutibacterium* using the UHPLC-ESI-MS method. The dominant class of lipids identified in *Cutibacterium* spp. were acylglycerols with a predominance of triacylglycerols. Significant amounts of sphingomyelin and phosphatidylcholine were also found. In the course of lipidomics analysis of *Cutibacterium* cell extracts, the presence of fatty acid amides was found for the first time, in which the acyl group is attached to the nitrogen atom of ethanolamine (N-acylethanolamine), and the presence of phospholipids belonging to the cardiolipin subclass in these bacteria. Lipid markers characteristic of individual species of the genus *Cutibacterium* were also selected, which may have diagnostic potential. The MALDI-TOF MS analysis of the lipid extract obtained from the extracellular vesicles secreted by *C. acnes* serotype III showed a different lipid profile to the other serotypes of this species. Using an optimized method of vesicle isolation, these nanostructures can be obtained from other taxa of propionic bacteria. The understanding of the structure and biological function of bacterial extracellular vehicles will allow us to use them as drug carriers or in vaccine production. The next task was to develop a prototype of a conjugate vaccine based on the RYDERY peptide antigen, inducing a protective response of the immune system to infections caused by *Shigella* strains. Several protein carriers for the vaccine were tested and HSA was selected. An HSA-peptide conjugate was prepared with very good yields (>90 %) and peptide coating (28 peptide molecules per 1 HSA molecule). *In vitro* studies were performed to exclude the cytotoxic properties of the prepared preparation on a model of the K562 cell line. Obtaining an immunogenic preparation with protective properties will allow for the planning of preclinical studies.

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

Introduction. Periodontal disease (PeD) is the most common infectious inflammatory disease of the oral cavity. PeD is common in older adults and may lead to dysbiosis of the oral microbiome, being a major source of persistent, mild inflammatory activation caused by infection and toxic bacterial products. In recent years, evidence has emerged linking poor oral health and brain health. It is believed that PeD may also contribute to the

development/progression of Alzheimer's disease (AD). The aim of the study was to determine the number and composition of the oral microbiome of patients with AD and PeD. Currently, there is no clear evidence that the comorbidity of AD and PeD is associated with greater dysbiosis of the oral microbiome.

Methodology. The tested research hypothesis was that the microbiome composition of patients affected by AD and PeD differs from that of cognitively healthy people. Analyses:

Analysis of patients' oral microbiome. Profiling of the entire microbial community was performed in saliva samples by sequencing fragments of the 16S rRNA gene (high-throughput sequencing based on the NGS, MiSEQ, Illumina technique).

Bioinformatics analysis. The microbiome was analyzed using the QIIME2 program (microbiome analysis based on 16S rRNA sequencing data).

Key findings of this preliminary study include the following: determination of the detailed composition of the microbiome in saliva samples of 32 study participants – AD+/PeD+ patients and age-matched cognitively healthy people. Demonstration of significant differences between the studied groups in the number of bacteria, especially those belonging to the Type: *Actinobacteria*.

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 will improve 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.

During the period under review, the main focus was on developing an *in silico* model to describe breast cancer tumor cells. To initiate this process, data was collected from patients who had both transcriptomic and mutational profiles drawn up at LGiB. Material was also obtained from the Lower Silesian Centre for Oncology, Pulmonology, and Hematology. Additionally, similar material was taken from the Cancer Genome Atlas database, which had a comparable response to treatment.

In collaboration with the Institute of Computer Science at Wrocław University of Technology, work has begun to use deep learning algorithms to classify experimentally obtained data and build an *in silico* model. (Dr. Adrianna Kozierkiewicz, Prof. PWr)

Efficacy and safety profile of base editors delivered as mRNA and protein used to create an *in vitro* model of cutaneous squamous cell carcinoma associated with a dystrophic recessive form of bullous epidermal detachment (RDEB-SCC)

Bullous epidermal detachment (RDEB) is an inherited skin disease caused by mutations in the COL7A1 gene that encodes type VII collagen. This type of disease is dystrophic and

recessive, and it is rare. Patients affected by RDEB experience extensive blistering and non-healing skin wounds that cause them pain and intractable itching. These symptoms significantly reduce their quality of life. Also, excessive scarring and recurrent infections lead to reduced mobility and limb deformity. Patients with RDEB have an increased risk of developing aggressive and metastatic squamous cell carcinoma (SCC) of the skin. According to the US National EB Registry, the risk of SCC in this group increases with age, reaching more than 90% after 55 years of age.

The study of rare diseases like RDEB is challenging because of the limited availability of patient samples. There is a lack of universal cellular and animal models that can accurately reflect the phenotype caused by specific mutations in a patient. This makes it difficult to search for potential targeted therapies for RDEB-associated cancer (RDEB-SCC). To address this issue, an easily obtainable and patient-adaptable model of RDEB-SCC needs to be developed. Such a model could be useful for screening anti-cancer drugs and pre-testing gene therapies.

CRISPR/Cas9 is a highly accurate gene-editing tool that uses a Cas9 nuclease to cut double-stranded DNA. It also includes a 20-nucleotide guide RNA (gRNA) molecule that directs the Cas9 protein to the complementary DNA sequence in the genome. The cytosine base editor (BE3) is a modified version of CRISPR/Cas9 that has an attached cytosine deaminase subunit. This subunit catalyses the conversion of cytosine to uracil, which the cell reads as thymine during replication. According to data from the ClinVar database, there are 77 recorded pathogenic point non-sense mutations in the COL7A1 gene, of which 57 are C>T or G>A mutations that can be inserted using a cytosine base editor. Therefore, BE3 was selected as the precise tool to insert mutations causing the most severe, generalized form of RDEB into cells from a commercially available SCC.

Cas9 (or BE3) can be introduced into the cell in the form of: a plasmid (fully expressed in the cell), an mRNA (subject to translation in the cytoplasm) or a protein-RNA complex. It has been observed that the mode of Cas9 delivery affects the efficiency of editing, as well as its specificity - delivery in plasmid form results in the most unscheduled editing.

Main hypothesis:

By using a cytosine editor (BE3) to introduce RDEB-causing mutations into a commercially available SCC cell line (MET1), we can establish a clinically relevant model of RDEB-SCC.

Supporting hypothesis:

The delivery of BE3 in the form of mRNA or protein may affect the efficiency and safety of editing at the genome, epigenome and/or transcriptome level.

Results:

We have successfully implemented a DNA cleavage detection assay using the USER enzyme mixture to confirm the *ex vivo* activity of the BE3 cytosine base editor protein we obtained and purified. Our findings showed a cleavage efficiency ranging from 6% to 37%, depending on the suspending buffer used. In order to improve the stability of the BE3 protein during storage, we tested two dedicated suspending buffers and different storage temperatures (-20°C and -80°C). We will continue to measure the protein's efficacy *ex vivo* to determine its maximum shelf life. Recent measurements taken three months after purification showed no significant decrease in activity.

To obtain the mRNA encoding the BE3 protein, we created a construct (plasmid) containing the T7 promoter, a 5' non-translational region derived from Tobacco etch virus (TEV), the sequence encoding the BE3 protein, and a 3' non-translational region from the mRNA for alpha-globin using Gibson cloning. We synthesized mRNA with a full uracil substitution for N1-methyl-pseudouridine, which prevents the development of an immune

response to long foreign mRNA constructs in cells. We obtained 42.5μg of purified full-length mRNA confirmed by capillary electrophoresis.

We acquired two cell lines: HEK293T - used to optimize transfection methods and confirm the efficacy of BE3 protein and mRNA in human cells, and A431 - derived from primary cutaneous squamous cell carcinoma from an immunocompetent patient with HPV-negative status. We will use line A431 to obtain a cell model of cutaneous squamous cell carcinoma associated with a recessive dystrophic form of bullous epidermal detachment. Both cell lines were tested for mycoplasma by nested-PCR, confirming their negative status.

We tested both the BE3 protein and mRNA construct on HEK293T cells, and confirmed their activity. The BE3 protein achieved an efficiency of 12% of edited sequences in the transfected cell population, while the mRNA achieved a maximum of 13%. To optimize transfection of A431 cells, we introduced mRNA encoding the fluorescent protein GFP into the cells using electroporation and lipofectamine, trying different conditions of both methods. Lipofectamine at an increased dose proved to be the most effective, and we will use it in the next step to introduce mRNA encoding BE3 and homing gRNAs into A431 cells.

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

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

In 2023, the Laboratory of Glycobiology has continued the projects that were started in the previous years. There were 3 main areas of study: structure and specificity of Gb3/CD77 synthase (alpha-4-galactosyltransferase), binding characteristics of *Plasmodium falciparum* (malaria parasite) to human red blood cells, and glycomics of protist animal cousins and early branching animals. A significant amount of work was also performed in connection to a collaborative project focused of the interaction of elements of the innate immune system with the SARS-CoV-2 Spike protein.

We have optimized the in-house method of N-glycome determination using MALDI-TOF mass spectrometry. The method involves a fast on-target labeling procedure, cutting the sample preparation time from 4-5 days to just 2 at a minimum. Using this method, we determined the glycomes of glycomically unstudied animals: a placozoan *Trichoplax* sp. H2, the sea anemone *Nematostella vectensis* and a ctenophore *Mnemiopsis leidyi*. The results indicate that the anemone and the placozoan both have an N-glycome similar to canonical vertebrate glycome, comprising both oligomannosidic structures and complex, tri-antennary structures. The sensitivity of this method is such that publication-ready spectra can be generated.

In addition, we used the computational infrastructure of the Hirszfeld Institute to study the evolution of a crucial Golgi glycosidase, the GH99 endomannosidase. We determined the importance of its structural motifs in the emergence of holozoans, as well as recognizing its indispensability for all vertebrates – all contain at least one putatively active form of the enzyme. We found that in a clade of eukaryotes that contains animals, the Filozoa, a rare switch of the enzyme specificity from mannosylated to glycosylated structures occurred. This might have optimized the GH99 endomannosidase for functioning as a nascent N-glycan trimming agent in the Golgi apparatus.

Laboratory of Microbial Immunochemistry and Vaccines
Head: Professor Jolanta Łukasiewicz, Ph.D.

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 the precious years, the expertise has been extended to include analysis of genes involved in LPS biosynthesis. Our research concerns Gram-negative species, such as *Klebsiella pneumoniae, Escherichia coli, Shigella sonnei, Bordetella* spp., *Plesiomonas shigelloides,* and *Edvarsiella* spp. that 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. Precisely, O serotype is defined by O-PS region built up of carbohydrate repeating units.

In 2023, for the first time, the chemical structure and genomics of core oligosaccharides of *E. piscicida*, *E. anguillarum*, *E. hoshinae* and *E. ictaluri* LPS were reported, including complete gene assignments for all core biosynthesis gene functions. *Edwardsiella* species cause infections mainly in fish, but they can also infect reptiles, birds or humans. Lipopolysaccharide (endotoxin) plays an important role in the pathogenesis of these bacteria. Research was carried out in cooperation with the Department of Genetic, Microbiology and Statistic at University of Barcelona (*Jordán M. et al. Structural Diversity among Edwardsiellaceae Core Oligosaccharides. Int J Mol Sci. 2023, 24, 4768*).

In collaboration with Swedish University of Agricultural Science, Department of Molecular Sciences, SLU in Sweden, we have provided a valid methodological approach that can be applied to the quantitative assessment of isotopically labeled glycans to improve detection capabilities and facilitate future structure-function relationship analysis of complex glycans. The metabolic labeling of hyaluronan (HA) using the bacterium *Streptococcus equi* subsp. *zooepidemicus* and the subsequent analysis by NMR and mass spectrometry was described. Hyaluronan has many diverse biological functions that vary a lot depending on the length of the HA chain and its concentration. A better understanding of the structure of different-sized HA at the atomic level is, therefore, crucial to decipher these biological functions. The level of ^{13}C and ^{15}N isotope enrichment at each position was determined quantitatively by NMR spectroscopy and was further confirmed by high-resolution mass spectrometry analysis. (*Xue Y et al. Metabolic labeling of hyaluronan: Biosynthesis and quantitative analysis of $^{13}C,^{15}N$-enriched hyaluronan by NMR and MS-based methods, Carbohydrate Research, 2023, 108888*).

In collaboration with the Bacteriophage Laboratory at IIET PAS we were involved in verification of clinical isolates of *K. pneumoniae* and *K. oxytoca* by MALDI-TOF Biotyper. Species identification was a prerequisite for the validation of biological properties, morphology, host specificity, lytic spectrum and sensitivity of phages against *Klebsiella* to

chemical agents along with their life cycle parameters such as adsorption, latent period, and burst size. Genomic sequences of selected phages were determined and analysed (W*eber-Dąbrowska B et al. Characteristics of environmental Klebsiella pneumoniae and Klebsiella oxytoca bacteriophages and their therapeutic applications. Pharmaceutics 2023,5(2), 434*).

Finally, the review was published to highlight the importance of a prior knowledge of the carbohydrate structural features and present current understanding of the in-cell processing of glycoconjugates. Microbial polysaccharides are depolymerized within antigen-presenting cells by reactive nitrogen and reactive oxygen species with no obvious structure alterations. The endosomal processing of glycoconjugates can modify the structure to a variable degree that correlates with ability to T-cell activation and immunogenicity of the antigen. During the in-cell processing of the glycoconjugate the immunodominant epitopes can be lost or altered and, in consequence, the protective efficacy of the vaccine antigens can be altered. Thus, the properties of both glycan and protein carrier should be considered in the design of optimal antigens, maximizing the glycopeptide specific T cell help and booster response (*Koj et al. In-cell depolymerization of polysaccharide antigens. Exploring the processing pathways of glycans and why some glycoconjugate vaccines are less effective than expected: A review. Carbohydrate Polymers, 2023, 315, 120969*).