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RESEARCH REPORT 2025

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DEPARTMENT OF EXPERIMENTAL ONCOLOGY

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

Development of novel orthotopic cancer models using *in vivo* imaging

Objective: To develop and optimize modern orthotopic tumor models for preclinical studies of experimental anticancer therapies, with a focus on bladder and colorectal cancers.

Key achievements: Optimized colorectal cancer models (HT-29 and HCT-116) were histopathologically validated, confirming successful tumor engraftment and invasion into intestinal structures. The HCT-116 model showed aggressive metastasis within the peritoneal cavity, including distant sites such as the diaphragm, highlighting its value for studying advanced disease. In bladder cancer research, new tumor cell lines were introduced, some demonstrating muscle invasion, vascular infiltration, and distant metastases (e.g., liver), making them suitable models of invasive cancer. New collaborations (University of Georgia, 4th Military Clinical Hospital in Wrocław) will enable the use of patient-derived organoid models. Initial attempts at ultrasound-guided orthotopic lung implantation confirmed technical feasibility, though efficiency depends on physiological factors. Tumor growth in mouse lungs was achieved in some cases, and further evaluation is ongoing.

Studies on novel bioactive compounds in anticancer therapy

Objective: To evaluate the biological activity and mechanisms of novel compounds with potential anticancer effects.

Key achievements: New thiosemicarbazone derivatives were synthesized and tested on cancer (MCF-7, SK-N-MC) and normal (MCF10A, BALB/3T3) cell lines. Some compounds showed strong antiproliferative activity comparable or superior to cisplatin. Two compounds demonstrated high selectivity toward cancer cells (selectivity index 12-82). Apoptosis induction was not significant, and further studies are ongoing to assess effects on the cell cycle.

Optimization of isolation and differentiation of human dendritic cells

Objective: To develop methods for generating and evaluating human dendritic cells (DCs) from peripheral blood.

Key achievements: A protocol for differentiating monocytes into dendritic cells (moDC) using GM-CSF and IL-4 was developed with expert consultation. A detailed flow cytometry panel was designed to assess phenotype and maturation status. The system enables evaluation of DC activation, cytokine production, and their ability to induce anticancer immune responses.

Role of lncRNA and miRNA in the anticancer activity of calcitriol in breast cancer

Objective: To assess the role of MALAT1 lncRNA and miR-125b in modulating the sensitivity of breast cancer cells to treatment.

Key achievements: In MCF-7 cells, increased miR-125b reduced the sensitivity to calcitriol and tacalcitol. In MDA-MB-231 cells, inhibition of miR-125b increased sensitivity, suggesting its regulatory role in therapy response.

Overall conclusion: The research advances both experimental cancer models and therapeutic strategies, integrating molecular, cellular, and *in vivo* approaches to improve understanding and treatment of cancer.

Laboratory of Tumour Molecular Immunobiology
Acting head: Andrzej Rapak, Ph.D., D.Sc.

In 2025, the Laboratory of Tumour Molecular Immunobiology conducted research on the study of new surface markers in the context of targeted melanoma therapy.

The aim of the task was to identify and characterize unique melanoma surface markers that can be used in experimental targeted therapy. The selected receptors can be targets for drug-linked antibodies and peptides, which should increase the effectiveness and specificity of skin cancer therapy. The selected specific receptors can also be used in diagnostics.

Western Blotting was used to examine the expression of several receptors in lysates from Merkel cell carcinoma (MCC1, MCC13, MKL-1), squamous cell carcinoma (SCC) (A431, HSC-1, HSC-5), and melanoma (A375, SKMel3, HS294) cells. The effect of a proteasome inhibitor (Bortezomib) on the viability and apoptosis of skin cancer cells was also examined.

The procedure for obtaining a monoclonal antibody against N-cadherin has been initiated.

High levels of N-cadherin expression were demonstrated in Merkel cell carcinoma cells and melanomas. N-cadherin expression was slightly lower in squamous cell carcinoma cells. All cells tested express the CD44 receptor, but it can also be present in normal cells. Integrin alpha5beta6 is also present on melanoma cells.

All skin cancer cells were highly sensitive to bortezomib, which induced high levels of apoptosis at concentrations of 5-20 nM.

The use of new markers and therapeutic conjugates in clinical trials could significantly impact treatment strategies for melanoma patients. The most promising conjugate appears to be an anti-N-cadherin antibody combined with bortezomib. The developed diagnostic tests will enable early detection of patients at high risk of cancer progression, enabling individualized therapy.

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

Antibacterial activity of metallacarborane-peptide conjugates

The rapid emergence of multidrug-resistant bacterial infections poses a major challenge to public health and underscores the urgent need for new antimicrobial strategies. Conventional antibiotics typically act on a single molecular target, which facilitates the rapid development of resistance. In contrast, antimicrobial peptides (AMPs) employ multitarget mechanisms, often involving interactions with bacterial membranes. However, their clinical utility is limited by susceptibility to proteolytic degradation, toxicity, and high production costs. To address these limitations, we designed AMP mimics by conjugating ultrashort cationic peptides with cobalt bis(dicarbollide) (COSAN) and its iodinated analogue (I-COSAN).

The conjugates retained the amphiphilic character typical of AMPs and exhibited potent antibacterial activity. They were particularly effective against Gram-positive bacteria such as *Staphylococcus aureus*, including resistant strains, and displayed moderate activity against Gram-negative species. Notably, iodinated derivatives showed enhanced antibacterial activity without a corresponding increase in toxicity, indicating that subtle modifications of the metallacarborane scaffold can significantly improve biological performance. Importantly, neither the peptide component nor the metallacarborane alone showed meaningful

antibacterial activity, demonstrating that the observed effects arise from a synergistic interaction between the two components.

Mechanistic studies revealed that these hybrids operate through a nonlytic mode of action. Unlike classical membrane-disrupting peptides, they did not cause membrane damage and leakage of intracellular components. Instead, they rapidly depolarized the bacterial membrane, dissipating the electrochemical gradient essential for cellular function. This lead to depletion of ATP, disruption of metabolic processes, and ultimately bacterial cell death. Although reactive oxygen species were generated during treatment, they did not play a primary role in the bactericidal effect. The overall mechanism resembles that of certain nonmembranolytic antibiotics and highlights membrane potential as a viable therapeutic target.

In addition to their antimicrobial activity, the conjugates demonstrated favorable biocompatibility profiles, with low cytotoxicity toward mammalian cells and moderate hemolytic activity. A key advantage was the marked improvement in proteolytic stability. While unmodified peptides were rapidly degraded by trypsin, metallacarborane–peptide hybrids resisted proteolysis. This suggests that metallacarborane modification can serve as an effective strategy to stabilize peptide-based therapeutics without the need for more complex modifications such as cyclization or incorporation of D-amino acids.

Overall, this work demonstrates that integrating inorganic metallacarborane scaffolds with short cationic peptides yields a versatile and effective platform for antimicrobial design. The resulting hybrids combine potent, broad-spectrum activity with enhanced stability and a distinct, nonlytic mechanism of action. These features position metallacarborane–peptide conjugates as promising candidates for the development of next-generation antibiotics capable of addressing the growing challenge of antimicrobial resistance.

DEPARTMENT OF CLINICAL IMMUNOLOGY

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

Genetic and functional determinants of Natural Killer (NK) cell activity and telomere length (TL) in the course of allogeneic haematopoietic cell transplantation (HSCT)

The massive proliferation of donor cells in the recipient's body for engraftment after HSCT results in accelerated telomere shortening. Genetic variability within the telomerase reverse transcriptase (TERT) gene influences telomerase activity and was shown to affect HSCT outcome. NK cells are the first donor-derived lymphocytes to reappear in the host organism following transplantation. Their activity is shaped by activating and inhibitory receptors, including C-type lectin-like NKG2, IL-T, NCR families and their ligands. Therefore, genetic variability and expression of their genes may play a significant role at various time points after HSCT.

Our study confirmed the adverse effect of mismatches in the MICA gene (Nihtilä et al. *Transplant Cell Ther*. 2025), which encodes the ligand for the activating NKG2D receptor. We also investigated the effect of NKG2E, NKG2F and KLRG1 receptors, which have not previously been analysed in the context of HSCT and transplant-related complications. Recipients whose donors carried the *NKG2F* rs2617170 *T* allele were characterized by a significantly worse one-year overall survival. A decreased risk of overall survival after HSCT and a higher risk of acute GvHD were also related to donor *ILT4* rs1128646 *T* allele, while the presence of the *ILT2* rs1061681 *T* variant in donors was associated with an increased risk of chronic GvHD (Siemaszko et al. *Clin Immunol.* 2025). In addition, recipients who developed chronic GvHD exhibited reduced serum CD107a concentrations compared to

patients without complications. Serum levels of soluble HLA-F and HLA-G were higher at day +30 than at day +90 after HSCT. Moreover, acute GvHD manifestation and a significantly shorter aGvHD-free survival were associated with the presence of *TERT*p rs2853669 *T* allele in the recipients, whilst a shorter TL characterised donors of patients with late complete chimerism at 180 day after HSCT (Dratwa-Kuzmin et al. *J Cancer Res Clin Oncol.* 2025).

NK and γδ T cells in patients with rheumatoid arthritis (RA) treated with anti-TNF drugs

RA is a heterogeneous autoimmune disorder with chronic synovial inflammation, in which NK cells play an important pathogenic role. Their cytotoxic activity depends on NCR receptors, perforin and granzyme B, which serve as markers of NK cell cytotoxicity. On the other hand, unconventional Tγδ lymphocytes may provide new insights into disease pathogenesis and help to understand RA mechanisms (Biały & Bogunia-Kubik, *Front Immunol.* 2025).

We previously studied the genetic variability of three common NK and γδ T cell receptor genes (FCγ3R, NCR3, and DNAM-1) and their role in rheumatic diseases (Biały et al. *Immunol Res.* 2024). Preliminary findings concerned the *NCR3* rs1052248 variants, which appeared to influence the clinical course of RA and the response to anti-TNF therapy, as well as the *FCγR3A* rs396991 polymorphism, associated with CRP levels and VAS scores. More recently, we showed that the presence of the *GZMB* rs8192917 *T* allele, as well as the *NCR3* rs1052248 *AA* genotype, in RA patients may constitute markers of a less favourable course of RA and patients' response to anti-TNF drugs. Compared with healthy subjects, patients with RA exhibited increased cytotoxic activity of NK cells (as reflected by higher granzyme serum levels and frequency of CD3-CD56+NCR3+ cells). Moreover, a significantly lower frequency of γδ T cells was observed in the peripheral blood of RA patients compared with the healthy control group. γδ T cells from RA patients also exhibited increased expression of inhibitory receptors, such as TIM-3, TIGIT, and PD-1, and decreased expression of activating receptors, including CD27, CD28 and DNAM-1. Furthermore, an increased proportion of terminally differentiated cells was detected, suggesting chronic antigenic stimulation and prolonged immune activation (Biały et al. in press).

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

Association of ERAP1 and ERAP2 gene polymorphisms and ERAP2 protein with the susceptibility and severity of rheumatoid arthritis in the Ukrainian population

Rheumatoid arthritis (RA) is a long-term autoimmune disorder that primarily affects joints. Although RA is chiefly associated with HLA class II, some HLA class I associations have also been observed. These molecules present antigenic peptides to CD8+ T lymphocytes and natural killer cells. HLA-I molecules bind their peptide cargo (8-10 amino acids long) in the endoplasmic reticulum. Peptides longer than 10 amino acids are trimmed by the endoplasmic reticulum aminopeptidases ERAP1 and ERAP2 to fit the peptide binding groove of the HLA-I molecule. We investigated the possible association of ERAP1 and ERAP2 polymorphisms with RA, and also any possible correlation between serum levels of the ERAP2 protein with disease severity. We used Real-Time PCR to genotype ERAP1 and ERAP2 and an ELISA test to detect ERAP2 protein. We found significant associations of ERAP1 rs30187, rs27044, and rs26618, as well as ERAP2 rs2248374, with susceptibility to RA. ERAP1 rs26653 and

ERAP2 rs2248374 were also associated with the Disease Activity Score (DAS28), and some polymorphisms were also associated with anti-citrullinated protein or anti-mutated citrullinated vimentin antibodies. RA patients secreted higher concentrations of ERAP2 than controls. Patients with mild disease activity (DAS28 < 3.2) released a concentration of ERAP2 four times lower than that of patients with severe disease activity (DAS28 > 5.1). We detected a higher level of ERAP2 in rheumatoid factor (RF)-positive patients than in RF-negative patients. ERAP2 concentration above 5.85 ng/mL indicated a severe phase of RA. In conclusion, some ERAP1 and ERAP2 polymorphisms seem to be related to susceptibility to RA or the severity of the disease. The ERAP2 protein tested in serum could be a valuable biomarker of RA severity.

Association of HLA-DRB1 allele group but not HLA-B or ERAP1-rs7063 gene polymorphisms with atopic dermatitis

Atopic dermatitis (AD) is one of the most common dermatoses. According to current data, 2.6% of the world's population suffers from AD. Atopic dermatitis is a multifactorial disease. The polymorphism of immune genes is also associated with an increased risk of AD. The human leukocyte antigen (HLA) system is a group of genes that play a crucial role in the immune response. The endoplasmic reticulum aminopeptidases ERAP (1 and 2) trim longer peptides to an optimal length for binding to HLA-I molecules. HLA class I (HLA-B) and class II (HLA-DRB1) typing and ERAP1-rs7063 genotyping were performed in 152 patients with AD and 187 control subjects. Frequencies of the HLA-B allele group and the majority of the HLA-DRB1 allele group did not differ between patients and controls. The exception was HLA-DRB1*07 allele group (OR = 0.34; 95% CI = 0.20; 0.61, p = 0.0039), whose frequency was lower in patients than in controls. ERAP1 19-exon to 20-exon ratio, governed by rs7063, did not seem to affect the susceptibility to AD.

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

Chronic CMV and EBV infections: the immune system training effect

Laboratory research has long focused on the impact of chronic herpes virus infection on the immune status of sufferers. The problem is complex, as immune deficiency *per se* may be a risk factor for persistent chronic infection, and the ongoing chronic infection may generate and/or exacerbate immune dysfunction. The argument used to stratify patients with herpes infection was based on IgG antibody levels specific for CMV, EBV, HSV, and VZV measured during routine diagnostic procedures. Levels of IgG antibodies exceeding at least 20 times the positive result boundary value were considered indicative of chronic infection. This approach was valid for CMV and EBV.

We found: 1. Analyzing EBV and CMV positive cases, two subgroups were identified, including the patients positive for both CMV and EBV, and those with EBV IgG antibodies. 2. Proportions of lymphocytes were negatively correlated with the CMV antibody levels, and tended to correlate positively with the number of monocytes, which was not seen when EBV antibody correlations with blood parameters were analyzed. 3. In patients with chronic CMV infection, independent of EBV seropositivity, the presence of myeloid-derived suppressor cells was documented. 4. The presence of myeloid suppressor cells (CD14+DR low -) and CD33+11b+DR- in the blood is elevated, supporting the notion of stressed myelopoiesis. The latter was associated with mutually elevated blood levels of the proinflammatory cytokines IL-1α, TNF-α, and IL-1β. IFN-α2 levels were correlated with lower lymphocyte counts.

Evaluating IFN-γ generation under *ex vivo* stimulation, CD56+ cells, but not CD4+ cells, were the main responders. The above findings were interpreted together with the presence of EBV DNA and activated lymphocytes, as well as monocytes, in the blood. The latter shows the biological significance of chronic EBV or CMV infection (diagnosed based on high levels of virus-specific IgG Abs).

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

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

Characterization of new therapeutic phages specific to *Enterobacter*

A comprehensive characterization of newly isolated Enterobacter-specific bacteriophages was performed. Phages Entb_43 and Entb_45 were assessed for their ability to inhibit biofilm formation by clinical *Enterobacter hormaechei* strains carrying biofilm-associated genes (*fimA*, *csgA*, *csgD*, *sdiA*). The results demonstrated that phage application was the most effective at the early stages of biofilm development and could also reduce established biofilms on the surfaces of urological catheters. Additionally, three newly isolated lytic phages, Entb_46–48, underwent comprehensive biological, morphological, and genomic characterization, confirming their therapeutic potential.

Optimization of the isolation process of phages specific to *Acinetobacter baumannii* and development of a method ensuring longer stability of these phages in the preparation

The isolation protocol and preservation strategies for phages targeting *A. baumannii,* a critical ESKAPE pathogen, were also evaluated. A novel Bait-Phage Method (BPM) was developed, enabling more efficient screening of environmental samples. Extensive stability studies of selected phages under different temperatures and storage conditions revealed that limiting light exposure significantly improves long-term phage activity.

Study of the ability of phages to eradicate intracellular infection of gastrointestinal epithelial cells

The ability of bacteriophages to combat intracellular infections was investigated using an *in vitro* Caco-2 epithelial cell model. The study demonstrated that preincubation with phages can reduce intracellular bacterial load, indicating their potential role in preventing or limiting intracellular infections.

Effect of *Enterococcus* phages against biofilm in urinary tract infections (UTI)

Two *Enterococcus* phage cocktails were selected. Each of those cocktails contained three phages with the broadest lytic activity, causing destruction of biofilm produced by *Enterococcus* spp. (including HLGR isolated from patients with UTI). Selected phage cocktails caused marked destruction of biofilm with cocktail I (phages EF15, EF49, EF 62) reaching 75% biofilm elimination, while cocktail II (phages EF1, EF12, EF57) as well as monovalent phages were less efficient. These results suggest the usefulness of selected phages in the treatment of UTI.

Stability studies on *Enterococcus* phages active against bacterial strains isolated from patients with UTI (*E. faecalis* and *E. faecium*)

Studies included 11 phages with broad activity against *Enterococcus* spp. isolated from patients with UTI and stored in peptone water or commercially available media (BHI, LB, TSB) for up to 4 months. At -80°C, some phages were best preserved with TSB medium, while for the others, LB medium or peptone water was optimal. At 4^{o}C peptone water was optimal for all phages, while light had no effect on their lytic activity.

Those data may be helpful for ensuring the optimal conditions for storage of phages considered for phage therapy.

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

Antibodies targeting *Lactococcus* phage endolysin in humans

Phage-derived epitopes specifically recognized by the human immune system were identified using high-throughput serological profiling of healthy volunteers with a synthetic phageome (referred to here as PhageScan), adapted from the VirScan method developed by Xu et al. (Xu et al., Science, 2015). The analysis covered the full phage proteome available in the GenBank database (National Center for Biotechnology Information). The identified epitopes were derived from: (i) structural phage proteins, and (ii) non-structural proteins with bacteriolytic functions (endolysins, holins, and depolymerases).

To evaluate how the immunogenicity of phage antigens detected by PhageScan translates into physiological effects, we focused on a phage antigen targeted by specific antibodies in a large portion of the studied population. This antigen was a *Lactococcus* phage endolysin (NCBI: YP_004306215.1) (PMID: 20802084), in this work: ImmE (Immunogenic Endolysin), with ImmE-specific antibodies found in 59.9% of investigated serum samples.

We further selected three representative sera with high (187), medium (77), and low (10) numbers of normalized NGS reads for the ImmE epitope and tested whether they affected the antibacterial activity of ImmE in the SYTOX assay (PMID: 34759903). ImmE demonstrated significant bactericidal activity alone, while its action was inhibited by the tested sera. Efficacy of this inhibition was concordant with the normalized number of NGS reads mapping to the ImmE epitope in each sample.

Conclusions:

- Antibody reactivity detected in the human population using PhageScan translates into biological effects, particularly the inhibition of phage protein activity.
- Since phage enzymes can induce lysis from without, our findings indicate that humans produce antibodies capable of protecting bacteria targeted by phage endolysins.
- To our knowledge, this is the first demonstration that humans may produce antibodies that protect beneficial or probiotic bacteria from the activity of phage-derived enzymes.

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

Influence of adipose tissue-derived MSCs (HATMSC2) and their derivatives on the biological activity of human fibroblasts (continuation of the task)

The aim of the study was to investigate the effect of various derivatives obtained from adipose-derived mesenchymal stem cells (HATMSC2) on the biological activity of reference human fibroblasts (MSU-1.1 cell line).

The research included the assessment of the biological activity of MSU-1.1 fibroblasts in the presence of different HATMSC2-derived products, i.e. conditioned medium (CM) and/or microvesicles (MVs). The proliferation rate of target cells (fibroblasts) was assessed by the MTT assay at three time points (24 h, 48 h and 72 h) in the presence of: (1) unselected CM obtained from HATMSC2 cell cultures, (2) CM collected from HATMSC2 cell cultures and depleted of MVs, (3) only MVs isolated from CM of HATMSC2 cell cultures, (4) concentrated CM containing MVs, (5) concentrated CM depleted of MVs. Controls included: C(-) medium without serum, C(+) medium with serum, C RIPA - control for RIPA buffer, and LMVs - lysed MVs isolated from CM collected from HATMSC2 cell cultures. A significant increase in the proliferation rate of MSU-1.1 cells was achieved when treated with concentrated CM containing MVs collected from the supernatant of HATMSC2 cells.

The obtained results confirm the biological activity of CM collected from HATMSC2 cells as carriers of bioactive factors stimulating cell proliferation and provide a basis for further studies on the mechanisms underlying their action.

Phenotypic characterization and determination of the expression level of osteopontin (OPN) in cancer cells isolated from ascites fluid (A) and postoperative tissue (T) obtained from patients with ovarian cancer (a new task)

The aim of the study was to perform phenotypic characterization of primary isolates from ascitic fluid (ascites - A) and postoperative tissue (tumor - T) obtained from ovarian cancer patients. Subsequently, the level of osteopontin (OPN) expression was determined in the collected biological material.

A comprehensive analysis of the expression of selected molecules on the cell surface was performed for: CD73, CD90, CD105, CD133, CD44, CD24, CD34, and CD45 in material obtained from patients with high-grade serous ovarian cancer (N=11) and other histological types of ovarian cancer (N=4). Osteopontin expression at the protein level was assessed in primary ovarian cancer cell lysates using Western blot and by immunofluorescence staining of cytospin slides. Among cells obtained from patients with high-grade serous ovarian cancer, the percentage of CD73-positive cells was lower in the population of cells isolated from ascites (A) compared to cells isolated from postoperative tissue (T). However, a higher percentage of cells positive for CD44 and CD45 was found in ascites. No significant differences were found between the two groups for the markers CD90, CD105, and CD24. The percentage of CD133-positive cells was higher among cells obtained from postoperative tumor tissue than among cells obtained from ascites fluid.

In most analyzed cases, the relative level of OPN expression was higher in cells obtained from ascitic fluid (A) than in cells isolated from the corresponding postoperative tissue (T) from the same patient. Furthermore, of the 27 analyzed samples, only three showed lower levels of OPN expression after normalization to the reference protein β-actin. In the remaining cases, elevated OPN expression levels were observed. The results suggest that OPN may play a role in the progression of serous ovarian cancer, and its increased expression in ascitic fluid cells may potentially serve as a prognostic biomarker, which needs to be confirmed in a larger study group.

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

Variation in digit ratio (2D:4D) across different types of military services in Poland

The relationship between the second (2D) and fourth finger (4D) of the hand (2D:4D) is considered to be a proxy indicator of prenatal testosterone (PT) and oestrogen (PE) exposure in the first trimester of pregnancy. A lower 2D:4D indicates relatively higher PT exposure and *vice versa*. The 2D:4D is generally higher in women than in men. Lower 2D:4D is associated with greater physical ability, strength, better athletic performance, and a propensity for jobs that require more physical fitness and are more risky. The aim of the present study was to examine the differences in 2D:4D, if any, between three groups of men in Poland: military students (N = 250), soldiers of the Land Forces (N = 106) and volunteers of the Territorial Defense Force (N = 202). This cross-sectional study was carried out in the Military University of Land Forces (MULF) in Wroclaw, Poland. The measurements included body height, body weight and the lengths of the second and fourth fingers in both hands of each participant. The results showed significantly lower 2D:4D in land forces soldiers and military students than those belonging to the Territorial Defense Force. The results indicated the possible impact of foetal androgens on specific human abilities as well as choices for challenging occupations.

Differential microRNAs and metabolites in the breast milk of mothers with adverse childhood experiences

Adverse childhood experiences (ACE) can strongly impact the physical and mental health of individuals. Recent evidence further suggests that children born to mothers with a history of ACE are at an increased risk for behavioral and metabolic perturbations. In this study, we investigated the impact of maternal ACE on small RNAs and fatty acids (FAs) in the breast milk from a cohort of Polish mothers (n = 103) and ascertained their association with the early temperament of their children. Small RNA sequencing followed by qPCR assays were performed to compare small RNAs in the milk from lactating mothers with high vs. low ACE. Additionally, milk from mothers with high vs. low ACE were compared on the short-, middle-, and long-chain FAs content. Our study revealed distinct microRNA and FA signatures of ACE in human breast milk, with increased expression of miR-142-3p, miR-142-5p, and miR-223-3p and reduced levels of middle chain FAs (MCFAs) in the breast milk of mothers with high ACE. Furthermore, a positive correlation was observed between the milk expression of miR-142-3p, miR-142-5p, and miR-223-3p and the ACE score in the mothers. Finally, milk expression of miR-142-5p and MCFAs correlated with infant temperament at the age of 5 and 12 months. The observed associations were not confounded by symptoms indicative of postpartum depression in the mothers. In conclusion, this study newly reveals changes in milk miRNAs and FAs as signatures of ACE in humans and highlights their potential as predictors of intergenerational transmission of the effects of ACE.

DEPARTMENT OF MICROBIOLOGY

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

Bacterial response to stress
Replication of bacterial chromosomes

The research activity of LMBM is dedicated to addressing two scientific issues: i/ bacterial response to stress; ii/ replication of bacterial chromosomes. We focuse primarily on species

from the *Campylobacteria* class, including the pathogenic species *Helicobacter pylori* and *Campylobacter jejuni*, as well as the emerging pathogen *Arcobacter butzleri*. Both processes, stress adaptation and chromosome replication, are fundamental to bacterial survival and adaptation.

i. Our laboratory studies the regulation of stress response in *Campylobacteria*. Specifically, we focus on the atypical response regulators CemR, which control gene transcription and chromosome replication initiation. We have shown that the CemR proteins are pleiotropic regulators, controlling the expression of many, often species-specific genes of multiple categories. However, central carbon metabolism, particularly glycolysis/gluconeogenesis, the tricarboxylic acid cycle, and oxidative phosphorylation, is controlled by CemRs in response to oxygen availability in all species analyzed. We have therefore named these regulators *Campylobacteria* energy and metabolism regulators, CemR. Regarding the molecular mechanism regulating the activity of CemR proteins, despite their general sequence similarity and, in particular, their protein structures, there are certain fundamental differences in the control mechanisms of CemR binding to DNA. In some species, CemR proteins are regulated via redox and/or Zn^{2+}, while in others they are insensitive to redox or divalent metal ions. If proteins respond to redox and divalent metal ions, this depends on the specific cysteine residues in the CemR protein. Using the two *H. pylori* strains 26695 and N6 as examples, we identified a set of genes that are conservatively regulated by CemR (i.e., under its control in both strains) and a set whose regulation is likely strain-dependent. We have also identified the non-coding RNA which are expressed under CemR direct or indirect response. We are currently expanding our research to include the analysis of how stress signals received by cells are integrated in the cell and how this integration triggers the cell's responses mediated by various transcriptional regulators.

ii. The aim of our research is to identify unknown factors that control the initiation of chromosome replication in the *Campylobacterial* species. At the present stage of research, only a few proteins controlling the initiation of chromosome replication in these species are known, such as HobA and CemR. In other species, such as model *Escherichia coli* or *Bacillus subtilis*, a significantly larger number of proteins controlling chromosome replication have been identified, and many of these proteins are species-specific. We conduct our research using a bacterial two-hybrid system to identify interactions with known conserved replication proteins, such as the initiator DnaA and the helicase DnaB. We also characterize the effect of post-translational modifications on the activity of known regulators, particularly CemR.

Laboratory of Biotechnology
Head: Krzysztof Pawlik, Ph.D., D.Sc.

Secondary metabolism in *Streptomyces*
Antimicrobial therapy

The research activity of LB is dedicated to addressing two crucial scientific issues: i/ secondary metabolism in *Streptomyces*, a key area in understanding microbial behaviour; ii/ the development of new methods for antimicrobial therapy, a pressing need in the face of increasing antibiotic resistance.

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 the coelimycin biosynthesis gene cluster (CPK BGC) 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. In particular, we have shown that intracellular CPK precursor(s) (preCPK) is/are involved in a negative feedback loop repressing the CPK BGC. We characterized the cluster-encoded efflux pump CpkF, showing that CpkF is essential for extracellular CPK production. We have also clearly shown that the coelimycin precursor is an inhibitor of the actinorhodin synthesis process. During the growth of *Streptomyces coelicolor* colonies, there are important decision points. The initiation of coelimycin synthesis under specific conditions causes changes in the levels of other secondary metabolites. These molecular regulatory interactions are the subject of our ongoing research. 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. A biomimetic model of cell membranes has been developed to study the molecular phenomena occurring during photodynamic therapy within bacterial biological structures. We have also identified species infecting wounds of diabetic patients and characterised their antimicrobial resistance. Based on the data obtained, we are developing concepts for the effect of APDT on bacteria. In collaboration with the angiology clinic at Wrocław University Hospital, we conducted a pilot study on the use of APDT therapy in patients with diabetic foot ulcers. The preliminary results are very promising.

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

The ability of extracellular vesicles (OMVs) produced by E. coli strain 083 to regulate innate immune mechanisms (*In collaboration with the group of Irma Schabussova, Ph.D.*)

The research aimed to investigate the molecular mechanisms regulating iNOS expression by extracellular vesicles (EVs) produced by the probiotic *E. coli* 083 strain.

The studies were conducted using a model of murine bone marrow-derived macrophages (BMDMs). Based on our previous findings that NO induction by EcO83-EVs is TLR4-dependent, we performed further analysis of signaling pathways. Specifically, we examined the effects of EcO83-EVs and EcO83 on MAP kinases ERK1/2 and JNK, as well as on NF-κB activation.

The results indicate that both EcO83-EVs and EcO83 significantly enhance the phosphorylation of ERK1/2 and JNK; however, the dynamics of these responses differ markedly. EcO83-EVs induced an early and sustained phosphorylation of ERK1/2, whereas EcO83 triggered an early but transient response. In the case of JNK, EcO83-EVs caused rapid and sustained activation, in contrast to the delayed response observed for the bacteria. Consistently, NF-κB phosphorylation occurred rapidly in response to EcO83-EVs, while stimulation with EcO83 led to a delayed but prolonged response.

We confirmed the involvement of ERK1/2 and JNK in NO production induced by EcO83 and its extracellular vesicles.

Semi-synthetic strategies for generating structurally defined curdlan sulfate polysaccharides (*In collaboration with the group of Prof. Emiliano Bedini*)

The research aimed to develop and characterize curdlan sulfate derivatives with controlled sulfation patterns and evaluate their impact on immune signaling pathways. We applied three semi-synthetic approaches to generate structurally diverse curdlan sulfate polysaccharides. Specifically, we employed direct sulfation, selective desulfation, and multistep protection strategies to precisely control the positioning of sulfate groups along the glucose backbone. Using NMR spectroscopy, we characterized the resulting compounds to determine how variations in sulfation patterns influence their structural and functional properties. We subsequently evaluated these derivatives in immunological assays using murine macrophage cells to assess their effects on inflammatory signaling pathways. Our analyses focused on how structural differences affect interactions with immune receptors and downstream signaling responses.

Our results demonstrated that specific sulfation patterns, rather than overall sulfate content, play a critical role in modulating interactions with immune receptors such as TLR4. These findings indicate that structurally tailored curdlan derivatives may serve as promising immunomodulatory agents for future therapeutic applications.

DEPARTMENT OF TUMOR IMMUNOLOGY

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

Quantitative Analysis of Sex-Specific *fem* Transcripts in *Apis mellifera*

In 2025, our research focused on elucidating the developmental dynamics of sex-specific *feminizer* (*fem*) transcripts in the honey bee (*Apis mellifera*), a key model for complementary sex determination. While the role of the upstream *csd* gene is relatively well established, quantitative data on downstream fem transcript expression across developmental stages remained limited and partly contradictory.

The primary objective of this study was to establish a robust and quantitative framework for analyzing female-specific (*femF*) and male-specific (*femM*) splice variants. We optimized Real-Time PCR conditions to overcome technical challenges associated with AT-rich sequences, repeated regions, and cDNA instability. This included the development of a custom reaction mix, identification of efficient primer pairs, and adjustment of elongation temperatures, enabling reliable detection of both transcript variants.

Using these optimized methods, we performed a comprehensive analysis of *fem* transcript expression across embryonic, larval, and pupal stages in both sexes. Our results demonstrate that *femF* expression is strongly female-biased (~100-fold higher in females), confirming its high specificity. In contrast, *femM* exhibited only moderate male bias (~10-fold higher in males) and was increasingly expressed in females during later pupal stages. Notably, both transcripts were detectable in both sexes, challenging the assumption of strict sex specificity.

Importantly, quantitative comparisons revealed that femM expression in males is comparable to *femF* expression in females, suggesting that the male transcript is not efficiently degraded despite containing a premature stop codon. These findings provide new insights into the regulation of the sex-determination pathway and highlight the importance of quantitative approaches in interpreting gene expression data.

Overall, this work establishes a methodological and conceptual framework for future studies on sex determination in honey bees and contributes to resolving inconsistencies in the current literature.

DEPARTMENT OF EXPERIMENTAL THERAPY

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

The protective properties of yolkin in endotoxemic mice

Yolkin is an egg yolk-derived protein with immunoregulatory properties. The protein was evaluated as a protective agent in endotoxemic BALB/c mice. The mice were pretreated with yolkin either orally in drinking water or intraperitoneally (i.p.) before i.p. injection of *E. coli* lipopolysaccharide (LPS). Circulating blood leukocyte number, blood cell composition, serum levels of tumor necrosis factor alpha (TNF α), interleukin 6 (IL-6), and haptoglobin, as well as histological changes in the spleen and the liver, were examined. Yolkin differentially regulated the values of these parameters, depending on the administration protocol; however, the serum levels of TNF α and IL-6 were generally decreased, and the level of haptoglobin, the acute phase protein, was elevated. The pretreatment of mice with yolkin led to improved histological architecture in the investigated organs of endotoxemic mice, particularly in the liver, where yolkin diminished an increased level of vascular vessel permeability and reversed a decreased number of Kupffer cells. These changes were independent of the route of yolkin administration. In conclusion, yolkin proved effective in the amelioration of pathogenic consequences of LPS administration.

The stimulatory effect of yolkin on the pinocytic and phagocytic function of the immature RAW 264.7 macrophage cell line

We demonstrated that RAW 264 cells, incubated with yolkin for 48 h, significantly increase their capability to pinocytise natural red and phagocytise zymosan particles. Yolkin, at an optimal concentration of 10 μg/mL, elicited production of TNF α, IL-6 and IL-10, with a prevailing amount of TNF α, thus exhibiting a proinflammatory phenotype. The anti TLR4 and TLR2 antibodies partially inhibited the process of pinocytosis potentiated by yolkin. In conclusion, RAW 264.7 cells differentiate to a M1 phenotype following incubation with yolkin. As a result, the cells produce cytokines of the proinflammatory profile and demonstrate the property of pino- and phagocytosis. The results add new information on the immunotropic properties of yolkin and its potential antibacterial property in a developing embryo.

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

In 2025, the Laboratory of Immunopathology continued its research programme focused on immune dysregulation in cancer, chronic inflammatory diseases and stress-related molecular mechanisms affecting clinical outcomes. The major scientific achievements comprised studies on chronic lymphocytic leukaemia progression, myeloid-cell-mediated immune regulation in uveitis, population-level determinants of cancer stage among Ukrainian war refugees, and the statutory project investigating the *FKBP5*–miR-511 stress-regulatory axis in colorectal cancer patients.

A principal achievement concerned the identification of T-cell apoptosis and G1-phase cell-cycle deregulation as clinically relevant mechanisms in chronic lymphocytic leukaemia (CLL). The study demonstrated significantly increased spontaneous apoptosis of T lymphocytes in CLL patients compared with healthy controls, accompanied by impaired apoptosis of leukaemic B cells. Expression of the G1-phase regulators p27^Kip1^ and cyclin D2 was significantly elevated in both B and T lymphocytes. Importantly, enhanced T-cell apoptosis was characteristic of progressive disease, while high p27^Kip1^ expression and reduced apoptotic susceptibility of B cells, together with altered cyclin D2 expression and increased T-cell apoptosis, were identified as independent predictors of earlier progression. These findings indicate that deregulation of the p27^Kip1^–cyclin D2 axis contributes to the persistence of malignant B cells and supports the concept that T lymphocytes actively shape CLL biology rather than merely accompany tumour expansion.

The Laboratory also provided novel evidence on systemic immune dysregulation in uveitis. Circulating monocyte subsets and indoleamine-2,3-dioxygenase-expressing monocytic myeloid-derived suppressor cells were characterised in patients with different clinical phenotypes. Patients with cystoid macular oedema exhibited selective expansion of CD16+ IDO-expressing myeloid-derived suppressor cells and intermediate CD14^high^CD16+ monocytes, accompanied by relative Th1/Th17 overactivation and suppression of regulatory T cells. These results identify a CD16+ myeloid-cell phenotype and impaired IDO-dependent immunoregulation as potential biomarkers of clinical severity and mechanisms contributing to ocular inflammatory progression.

A further achievement was a retrospective population-based cohort study including 3,259 patients, demonstrating that Ukrainian war refugee status was associated with more aggressive malignancies and more advanced disease at diagnosis. Adjusted analyses showed an increased risk of stage III–IV cancer and G3 tumour grade among refugees. These findings have direct public-health relevance and support the implementation of dedicated screening, early diagnostic pathways and equitable oncology access for populations affected by war-related displacement.

Within the statutory task planned for 2024–2026, the Laboratory investigated the relationship between *FKBP5* expression, miR-511 expression, selected *FKBP5* polymorphisms and stress burden in colorectal cancer patients with and without intestinal stoma. FKBP5 is a key regulator of glucocorticoid receptor signalling and hypothalamic–pituitary–adrenal axis responsiveness, making it a biologically justified candidate in studies of stress vulnerability and psycho-oncological outcomes. Biological material was collected from colorectal cancer patients treated at the Lower Silesian Centre of Oncology, Pulmonology and Haematology in Wrocław. *FKBP5*mRNA and miR-511 expression were assessed using RealTime-qPCR, polymorphisms rs3800373, rs1360780 and rs9296158 were analysed by allelic discrimination, and psychological status was evaluated using PSS-10 and PHQ-9 questionnaires.

The study confirmed increased perceived stress in oncological patients. No significant differences in *FKBP5* or miR-511 expression were observed between patients with and without a stoma, and no significant correlation between *FKBP5* and miR-511 expression was found. However, logistic regression indicated that perceived stress and miR-511 expression were associated with increased risk of depressive symptoms, suggesting that the *FKBP5*–miR-511 regulatory axis may contribute to psychological vulnerability in colorectal cancer. Polymorphism analysis did not demonstrate significant associations with *FKBP5* or miR-511 expression, indicating that additional regulatory mechanisms, including epigenetic, inflammatory or treatment-related factors, should be investigated.

Collectively, the 2025 achievements of the Laboratory of Immunopathology demonstrate an integrated translational research profile combining cellular immunology, molecular

regulation, clinical phenotyping and public-health relevance. The obtained results strengthen the Laboratory's position as a unit addressing complex biomedical problems at the interface of immune regulation, cancer progression, inflammation, stress biology and patient-centred outcomes.

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

Immunological mechanisms associated with reproductive processes in health and disease

Research on the Role of the ST2 Receptor in a Murine Model of Pregnancy Threatened by Miscarriage

The aim of the planned study was to investigate the expression of the ST2 receptor on B lymphocytes in a murine model of pregnancy threatened by miscarriage and in normal pregnancy. The application of this experimental model was intended to evaluate the involvement of this receptor in the peripheral immune system, in uterine-draining lymph nodes, and within the reproductive system — particularly in the decidua — as well as to experimentally confirm the increased expression of the ST2 receptor on peripheral blood B lymphocytes observed in women after miscarriage.

Description of the work performed: A series of multicolor stainings and flow cytometric analyses were conducted to determine the frequency of Breg cell subpopulations — including B1a, B10, marginal zone (MZ) B cells, follicular (FO) B cells, T2-MZP B cells, and memory B cells — as well as the level of ST2 receptor expression on these cells. The analyses were performed using splenocytes, uterine-draining lymph nodes, peritoneal cells, and decidual tissue obtained from a murine model of immunologically mediated miscarriage (CBA/J♀ × DBA/2J♂ mating), a control model of normal pregnancy (CBA/J♀ × Balb/c♂ mating), and virgin mice.

Description of the main findings: An increased frequency of B10 cells was observed in miscarriage-prone mice compared with the normal pregnancy model in the spleen and compared with control mice in the peritoneal fluid. However, no differences were observed in the frequency of these cells expressing the ST2 receptor on their surface in the spleen, lymph nodes, or peritoneal fluid. The median ST2 expression on ST2+ B1a cells was significantly higher in the peritoneal fluid of mice with normal pregnancy compared with both the control group and miscarriage-prone mice. No significant differences were observed for the remaining B-cell phenotypes (MZ, FO, T2-MZP).

Summary: In contrast to observations in women after miscarriage, mice in the model of pregnancy threatened by miscarriage did not exhibit differences in ST2 receptor expression. Increased expression of the ST2 receptor on B1a lymphocytes in mice with normal pregnancy, but not in miscarriage-prone or non-pregnant mice, suggests hormonal regulation of ST2 receptor expression.

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

Challenge in Optimization of Lymphocyte Transduction and Culture – Laboratory Experience with CAR-T Programming and Manufacturing

In 2024, the Medical Research Agency awarded funding for the project "Development of a therapeutic product based on genetically modified T cells for the treatment of relapsed and

refractory forms of plasmocytic myeloma: from CAR receptor DNA vector production to phase I/II studies, BECAME - B(e)CMA CAR-T in MM THERAPY" leaded by a consortium consisting of the Technology Reassurance Company, our Institute, and the Lower Silesian Oncology, Hematology and Pulmonology Center. The task of the Laboratory of Genetics and Epigenetics of Human Diseases is to determine the optimal culture and expansion conditions of CAR-T cells, which allow for achieving a high level of transduction and differentiation of cells toward the most desirable cellular subpopulations, i.e. naive-like and central memory T-cells, while minimizing effector memory and terminal effector T- cells, as well as obtaining the least exhausted cells possible.

During the first period of the realization of the project an extensive optimization of culture conditions was performed, including various activation systems, GMP-compliant media, and cytokine combinations. Systematic testing of four GMP-grade media, four activation methods, and multiple cytokine combinations (IL-2, IL-7, IL-15, IL-21) using cells from healthy donors showed that transduction efficiency and final product characteristics depend on both culture conditions and donor variability. Culture conditions influenced CD4/CD8 balance, generating products ranging from balanced (~50/50) to CD8-enriched (~80%) or CD4-dominant (~70%) populations, while naive T cells decreased and terminally differentiated cells remained low. Chosen cytokine combination favored a central memory phenotype (with $CD8^+CAR^+$ T_CM exceeding 60%) and reduced PD-1 and TIGIT expression, although LAG-3 increased, consistent with evidence linking low PD-1/TIGIT levels to enhanced antitumor activity. Based on these findings, GMP-compliant CAR-T culture protocols were developed and successfully scaled to 0.5 L and 1 L G-Rex bioreactors, yielding functional CAR-T products.

Next, CAR-T lymphocyte constructs targeting the BCMA and SLAMF7 antigens—key therapeutic targets in multiple myeloma—were developed and tested. Based on structural and functional analyses, two monospecific constructs (anti-BCMA and anti- SLAMF7) and eight bispecific CAR variants (anti-BCMA/SLAMF7) were designed, differing in binding domain configurations, linker sequences, and intracellular structures. Functional validation using multiple myeloma cell lines (MM.1S, U266, RPMI-8226) and additional lines (Reh, Rec-1), supported by holotomographic microscopy and cytotoxicity assays (LDH, luminescence, proliferation, and cytokine secretion), confirmed strong antitumor activity of anti-BCMA, anti-SLAMF7, and bispecific constructs, including S-HMA-B CAR-T, which were advanced to *in vivo* studies. Overall, the study shows that precise optimization of activation, cytokines, and culture media critically determines CAR-T differentiation, exhaustion, and cytotoxic efficacy, while donor-dependent variability remains a key factor influencing product heterogeneity. Functional assays demonstrated that optimized CAR-T cells for each construct achieved over 80% tumor cell lysis across all tested myeloma lines after 24 h of co-culture (E:T 5:1).

Lymphocyte-activation gene 3 and Galectin-3 in non-small cell lung cancer

Lymphocyte-activation gene 3 (LAG-3; CD223) is a transmembrane inhibitory receptor expressed, among others, on T cells, natural killer (NK) cells and regulatory T cells (Treg). LAG-3 is a next-generation immune checkpoint and has been linked to immunosuppression in the tumor microenvironment (TME). Galectin-3 (GAL-3) is considered one of the LAG-3 ligands. LAG-3 is frequently co-expressed with PD-1, providing a rationale for combinatorial blockade in cancer therapy. LAG-3 overexpression is observed in many cancers like CLL, AML and in multiple solid tumors, e.g. cervical cancer and non-small cell lung cancer (NSCLC). A growing number of reports indicate that in patients with NSCLC undergoing resection, high LAG-3 expression was associated with poorer overall survival and tumor aggressiveness.

We analysed LAG-3 and GAL-3 in 81 NSCLC tissues, including the two main histological subtypes, adenocarcinoma (LUAD) and squamous cell carcinoma (LUSC), at the mRNA (ddPCR) and protein (immunohistochemistry and/or multiparametric flow cytometry) levels.

Our results demonstrate a high correlation between LAG-3 and PD-1 expression at both the mRNA and protein levels. The expression of LAG-3 did not differ between LUSC and LUAD. We observed higher GAL-3 expression in LUAD than in LUSC. Neither LAG-3 nor GAL-3 expression was associated with lymph node metastasis or tumor clinical stage in our study. (This work was supported by the National Science Centre, Poland, Grant number 2019/33/B/NZ5/03029).

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

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

Lipidomic analysis of propionic bacteria, characterization of extracellular vesicles secreted by *Cutibacterium* and studies on a peptide vaccine against bacterial dysentery, as well as thermal stability of healthy and cancer cells

The study of four glycolipids (GL1–GL4) isolated from distinct *Cutibacterium acnes* phylotypes revealed that these compounds are glucoglycerolipids identified in cells and in extracellular vehicles (EVs) produced by these bacteria. Results are the basis for further investigation of EVs towards their interaction with human prostate epithelial and fibroblast cell lines to investigate their potential role in host-pathogen interactions. In the frame of the second task, the method has been optimized for the construction of a conjugate of the OmpC epitope peptide RYDERY with human serum albumin as a carrier protein. Results of experiments indicate that for OmpC and the received conjugate, IgA titers – unlike IgG – were significantly lower in patients with immunodeficiencies (PID) compared to healthy controls. Additionally, the specific anti-OmpC antibodies may be beneficial in the complementary therapy for patients with primary immunodeficiencies. The third task concerned the determination of the heat tolerance of different bacterial proteins. The resistance of proteins at temperatures in the range 37-42°C was assayed. Results will have therapeutic potential regarding the use of infection fever to facilitate cancer treatment. Moreover, in separate studies, we have found that advanced glycation end products, particularly the unique AGE10 epitope, may be a potential biomarker of immunopathology in rheumatic diseases. We analyzed serum AGE10 levels in patients with reactive arthritis (ReA)-caused by *Chlamydia trachomatis* and ReA with *C. trachomatis* during the reactivation of EBV infection. Additionally, ankylosing spondylitis (AS) patients were involved in the study, due to the potential evolution of ReA toward AS. A striking finding was the complete absence of detectable AGE10 antigen in the AS group, coinciding with notably elevated immune complex AGE10–anti-AGE10 levels. Diminished level of AGE10 could be caused, besides local accumulation, also by the formation of immune complexes, a pathogenic factor. Therefore, evaluating disease activity in ReA and AS is crucial to further our understanding of the pathophysiology of AGEs formation and predicting prognosis.

Laboratory of Virology
Acting head: Beata Orzechowska, Ph.D., D.Sc.

Development of a genetically modified T cell-based therapeutic product for the treatment of recurrent and refractory multiple myeloma: from CAR receptor DNA vector production to Phase I/II trials; BECAME-B(e)CMA CAR-T in THERAPY; 2022/ABM/06/00004

The aim of this study is to generate lentiviral vectors encoding CAR constructs for T cell transduction through optimization of transfection conditions, cell culture parameters of producer cell lines, purification processes, and cryopreservation conditions. Third-generation lentiviral vectors were generated in the HEK293-F producer cell line via co-transfection of a transfer plasmid encoding transgenes targeting TNFRSF17 (BCMA) or bispecific constructs (e.g., BCMA and additional targets), together with packaging plasmids (gag/pol and rev) and an envelope plasmid expressing VSV-G.

Scope of work: Optimization of cell culture in a bioreactor, transfection, lentiviral production, purification, and functional characterization of CAR-BCMA lentiviral vectors.

- Transfection and process optimization

Transfection was optimized using commercial, control (KON), and in-house (IIET PAS) plasmids across scales from shake flasks to ~1 L bioreactors. Bioreactor parameters (viability, pH, dissolved oxygen) and process conditions (harvest time, media, DNase treatment) were optimized based on physical (p24 ELISA, RT-qPCR) and functional (TU/mL, MOI, FACS) titers.

- Purification process optimization

Ultracentrifugation conditions were established, including spin parameters and buffer composition. In parallel, clarification via filtration (8–0.65 µm) is under evaluation, including impurity retention and vector loss.

- Vector validation

Batches were assessed for physical (p24 ELISA, RT-qPCR) and functional titers (TU/mL, FACS in HEK293-F/Jurkat). Selected studies included transduction of primary T cells.

- Plasmid comparison

In-house and control plasmids showed comparable transfection efficiency and yielded consistent physical and functional titers after ultracentrifugation.

- Downstream development

TFF and chromatographic purification (HPLC/ÄKTA) are under evaluation.

- Quality assessment

Quality control and stability studies, including purity evaluation, are ongoing.

Oral Health: A Window to Improve Systemic Immune Response in Alzheimer's Disease Patients (OASIS-AD). This international research project is funded by the Alzheimer's Association Research Grant (AARG)

The project is conducted by a consortium of multiple institutions, including the Institute of Immunology and Experimental Therapy of the Polish Academy of Sciences (IIET PAS), Wrocław Medical University, and the University of Connecticut (USA).

The primary objective of the study is to evaluate whether the treatment of periodontal disease (periodontitis, PeD)-a chronic bacterial disease- can enhance systemic immune function, beneficially modulate the oral microbiome, and improve both quality of life and cognitive performance in affected patients. Recent evidence indicates that untreated periodontitis is associated with a 70% increased risk of developing Alzheimer's disease. The relationship between periodontal disease and neuroinflammatory processes in the brain remains a relatively novel yet highly promising area of investigation. At the same time, the

initiative promotes awareness of Alzheimer's disease prevention, emphasizing the importance of periodontal health and daily oral hygiene practices, which are frequently underestimated. In the absence of effective curative treatments for Alzheimer's disease, all medical interventions that have the potential to extend life expectancy and improve its quality are essential and carry significant public health benefits.

First observations and results: In the first year of the project, 71 individuals diagnosed with MCI (37) or mild to moderate stages of AD (34) were recruited. During the course of the study, 4 participants withdrew, and 3 dropped out (due to serious illness or death). 64 patients continue the study. All included individuals (n=64) have either completed periodontal diagnostic and treatment (n=40) or are currently undergoing/awaiting them (n=24). Of the 64 participants included in the study, 28 underwent a follow-up assessment of all parameters (immunological, clinical) at the study start (T=0) and 3-month time point (T=3), while 17 participants completed the full study at T=0, the 3-month time point (T=3) and the 6-month time point (T=6). At the start of the study (T=0), the level of innate immune response was assessed in all patients using a VSV-based assay. Patients in the AD+/PeD+ and AD+/PeD- groups showed similar activation levels (3.2 vs. 3.5 log TCID50). Measurements were repeated at T=3 and T=6 in 17 participants, but correlation analyses have not yet been performed due to small sample sizes. A broad panel of cytokines and biomarkers was also measured in PBLs and plasma, with analyses prioritized for participants who completed the full study protocol. Ongoing analyses aim to evaluate the association of PeD with immune cell activation, systemic inflammation, and sex differences, and will include saliva microbiome assessment. Clinical monitoring continues to determine the effects of PeD and its treatment on immune response, biomarkers, quality of life, and cognitive function.

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

Efficacy and safety profile of base editors delivered in the form of mRNA and protein, applied to generate an *in vitro* model of cutaneous squamous cell carcinoma associated with the recessive dystrophic form of epidermolysis bullosa (RDEB-SCC) (*task continued*)

Recessive dystrophic epidermolysis bullosa (RDEB) is a rare, inherited skin disorder caused by mutations in the COL7A1 gene encoding type VII collagen. In patients affected by RDEB, extensive blisters and non-healing skin wounds develop. They are accompanied by pain and burdensome pruritus, which markedly reduce patients' quality of life, while excessive scarring and recurrent infections lead to restricted mobility and limb deformities. In addition, patients with RDEB have a substantially elevated risk of developing aggressive and metastatic cutaneous squamous cell carcinoma (SCC). According to the USA National EB Registry, the risk of SCC in this group increases with age, exceeding 90% after 55 years of age.

Research on rare diseases such as RDEB and the search for potential targeted therapies for the RDEB-associated malignancy (RDEB-SCC) is hampered by limited access to patient samples. Universal cellular and animal models are not always available and often do not faithfully reflect the phenotype produced by the specific mutations present in a given patient. The development of an easily obtainable RDEB-SCC model that can be tailored to the patient's needs could support anticancer drug screening and enable preliminary testing of genetic therapies.

CRISPR/Cas9 is an exceptionally precise gene-editing tool. It consists of the Cas9 nuclease, which cleaves double-stranded DNA, and a 20-nucleotide variable guide RNA

(gRNA) molecule that directs the Cas9 protein to a complementary DNA sequence in the genome. The cytosine base editor (BE3) is a modified version of CRISPR/Cas9 with an appended cytidine deaminase subunit that catalyzes the conversion of cytosine to uracil, which is read by the cell during replication as thymine. According to the data collected in the ClinVar database, among the 77 registered pathogenic point nonsense mutations in the COL7A1 gene, 57 are C>T or G>A mutations (which can likewise be introduced using a cytosine base editor); BE3 was therefore selected as the precise tool for introducing the mutations that cause the most severe, generalized form of RDEB into cells of a commercially available SCC line.

Cas9 (or BE3) can be introduced into the cell in the form of: a plasmid (undergoing full expression in the cell), mRNA (translated 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 the form of a plasmid produces the greatest number of unintended edits.

Principal hypothesis: By introducing the mutations that cause RDEB into a commercially available SCC cell line (MET1) using a cytosine base editor (BE3), we can obtain a readily available and clinically relevant RDEB-SCC model.

Auxiliary hypothesis: Delivery of BE3 in the form of mRNA or of protein may affect the efficiency and safety of editing at the level of the genome, the epigenome, and/or the transcriptome.

Progress in the implementation of the project:

- A workflow was established, spanning from *in silico* design of the construct to obtaining functional mRNA and its validation in cell lines. As a result, 4 different constructs for the cytosine base editor BE3 and a further 3 constructs for the newer-generation BE4max editors were obtained.
- A431 cells transfected with optimized mRNA and gRNA introducing the COL7A1 c.1732C>T and c.4894C>T mutations simultaneously, at two mass ratios, were separated into single cells by serial dilution in order to obtain a fully modified monoclonal line. A total of 227 lines were established, with an overall editing efficiency of 11–17% in this population. Screening genotyping of the established lines using ARMS PCR enabled selection of 53 lines for further evaluation by Sanger sequencing. Ultimately, from both tested transfection conditions, we obtained 7 lines with the heterozygous c.1732 C/T mutation, 9 lines with the heterozygous c.4894 C/T mutation, 3 lines carrying both homozygous mutations, and 5 lines with the target genotype c.1732 C/T and c.4894 T/T – corresponding to the mutation type most frequently observed in patients with the recessive dystrophic form of epidermolysis bullosa. Subsequently, studies will be conducted to compare the function of cells carrying the introduced mutations and their sensitivity to anticancer therapies.
- Transcriptome analysis of cells transfected with the base-editor mRNA revealed marked activation of antiviral response pathways, type I interferon (alpha/beta) production, the response to double-stranded RNA, and the DNA-damage response, as well as a decrease in the activity of pathways associated with cellular metabolism and the formation of axonal connections. The differences in expression observed at the RNA-seq level were confirmed by qPCR for the RELB and DDIT4 genes, which are involved, respectively, in the inflammatory response and the DNA-damage response. Confirmation of further genes by qPCR is in progress.
- The transcriptome analysis was complemented and deepened by an examination of alternative splicing and the formation of altered isoforms of the expressed genes. 33 genes showing differential splicing were identified in the samples treated with all three mRNA constructs. Among them, particular attention was drawn to the NEO1 gene, whose

alternative transcript encodes a truncated version of the neogenin protein, involved, among other things, in the process of forming axonal connections. Primers specific for the canonical and the alternative transcript forms were designed in order to confirm the observed phenomenon by qPCR. Detection of the truncated protein form in the cells by western blot is also planned.

- As part of the assessment of the precision and safety of the base editors, the Nano-OTS method was adapted for laboratory (*in vitro*) prediction of potential off-target mutation sites for a given gRNA. The method was applied to the gRNA introducing COL7A1 c.1732C>T and to genomic DNA of HEK293T cells, yielding more than 1,500 predicted potential off-target sites. Additionally, bioinformatic prediction of off-target sites was performed on the transcriptomic data using CRISPRroots, yielding more than 5,000 further potential off-target sites. As a third dataset, a combination of two *in silico* methods – CasOFFinder + BEdeepoff – will be used. A selected subset of 4,000–7,000 potential off-target sites will be validated in genomic DNA isolated from the cells after transfection. Long-read adaptive-sampling sequencing with Nanopore technology will enable the simultaneous assessment of mutation occurrence at several thousand selected positions in the genome.

DEPARTMENT OF IMMUNOCHEMISTRY

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

The role of glycans of recombinant human erythrocyte basigin in the interaction with the *Plasmodium falciparum* merozoite ligand Rh5

Basigin (BSG, extracellular matrix metalloproteinase inducer, CD147), encoded by the *BSG* gene, is a 269-amino-acid membrane glycoprotein that carries antigens of the Ok human blood group system. BSG is a critical receptor for *Plasmodium* invasion. Interaction of basigin with the Rh5 (Reticulocyte-binding protein homolog 5) ligand of merozoites enables parasite recognition and entry into erythrocytes. BSG contains three N-linked glycan chains, which can be of complex or high-mannose type.

Project aim: This project aims to determine the contribution of recombinant BSG oligosaccharide chains to Rh5 binding.

Project description: Recombinant human erythrocyte BSG and its four glycosylation mutants (lacking either all three or individual N-linked glycan chains) were obtained by transient expression in HEK293 cells. The proteins secreted into the culture medium were purified by nickel affinity chromatography. Binding of recombinant BSG variants to a recombinant biotinylated Rh5 ligand was analyzed using SPR.

Results: As Rh5 ligand binding to BSG is a key step in *Plasmodium* erythrocyte invasion, understanding the role of BSG glycans in this interaction is highly relevant. We determined that removal of all three N-glycosidic chains, particularly the N-glycan linked to Asn 44, increased the binding of BSG to the Rh5 ligand. This may suggest a protective role for BSG N-glycosidic chains in the invasion process.

Potential applications: These findings contribute to a better understanding of the molecular mechanisms underlying the specific invasion of human erythrocytes by malaria parasites, particularly the role of BSG glycosylation in this process.

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

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. The expertise has also been extended by 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 2024 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. holmesii* is the second pertussis etiological factor; however the current anti-pertussis vaccines do not provide cross-protection. Major virulence factors and surface antigens of the studied species are: LPS (O antigen), capsules (antigen K, 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. O serotypes are defined by O-PS regions built up of carbohydrate repeating units. O-antigens and other conservative surface antigens of bacteria are of interest as targets for antibodies protective against infections.

In 2025, structural studies of potential vaccine antigens against bovine respiratory disease (BRD) in dairy calves were completed. BRD in dairy calves causes significant economic losses due to treatment costs, reduced growth rates, and mortality. Immune serum was obtained from cows immunized with three conserved antigens of pathogens causing BRD (*Histophilus somni* rHsp60, *H. somni* rOMP40, and the LPS core oligosaccharide of *Actinobacillus pleuropneumoniae*) and characterized by broad cross-reactivity against various Gram-negative bacterial species. The sera were administered to calves subcutaneously as an adjunct to therapy with antibiotics and anti-inflammatory drugs, and IgG and IgM antibody titers were assessed. It was demonstrated that the additional use of immune serum (passive immunization) in combination with antibiotics and non-steroidal anti-inflammatory drugs (NSAIDs) may contribute to faster recovery and a reduction in the severity of clinical symptoms in dairy calves compared to treatment without serum. The study was conducted in collaboration between Wojciech Jachymek and the Department of Immunology and Veterinary Prevention at the University of Life Sciences in Wrocław (Sci Rep. 2025, 30;15(1):19088).

As part of a collaboration with the Faculty of Biology and Environmental Protection at the University of Łódź (J. Lukasiewicz and A. Maciejewska), the usefulness and complementarity of biochemical methods (the indole test and Api 20E), PCR, and MALDI Biotyper mass spectrometry for the species differentiation of 35 clinical strains belonging to the *Klebsiella pneumoniae* (KpSC) and *K. oxytoca* (KoSC) complexes were compared. A methodology suitable for conditions with limited whole-genome sequencing capabilities was employed. It was demonstrated that, among the biochemical methods, the indole test exhibited the highest discriminatory power, whereas growth at 10°C was the least effective. The *rpoB* gene was detected in all strains biochemically identified as *K. pneumoniae*, and only 12 *K. oxytoca*

isolates produced the *pehX* gene product. MALDI-TOF MS analysis proved to be the most reliable method for identifying *K. oxytoca* and *K. pneumoniae* in the studied group of isolates, although it required, in addition to the standard database comparison, a detailed analysis of mass spectra for *K. oxytoca* biomarkers (Front. Microbiol. 2025, 16:1514643). Using the resources of the laboratory and the NMR Facility, Tomasz Niedziela conducted research in collaboration with the Centre for Molecular Biophysics, UPR CNRS (France), aimed at assessing the microenvironment of erythrocytes in terms of hypoxia. The team's (NMR Facility) contribution involved the use of NMR spectroscopy to analyze the ^{31}P NMR spectra of 2,3-diphosphoglycerate (2,3-DPG), which provides information about the internal environment of erythrocytes. It has been demonstrated *in vitro* and *in vivo* that by modulating haemoglobin activity via the erythrocyte band 3 protein anion transporter, ITPP increases oxygen release and regulates the pH inside red blood cells. Its availability in the blood confirms the utility of ITPP-based strategies, which complement cancer treatment strategies with drugs that counteract hypoxia and normalize abnormal vascularization through more efficient oxygen release by haemoglobin in red blood cells (Journal of Cellular & Molecular Medicine, 29 (2), e70343). Jolanta Łukasiewicz, Ph.D., and Anna Maciejewska, in collaboration with Prof. Yin's group at Jiangnan University in Wuxi, China, continued their research on bacterial sugar antigens with a focus on identifying immunogenic epitopes. In collaboration with the group from China, using natural and synthetic variants of the natural O antigens of *Plesiomonas shigelloides* O51 as components of glycoconjugates, it was demonstrated *in vitro* and *in vivo* that the repeating trisaccharide unit is a key epitope, while the acetamidine group has no effect on immunogenicity (Carbohydrate Research, 550, 2025, 109388).

The results were disseminated in the form of four original publications: (i) Blicharski K., Bajzert J., Jawor P., Jachymek W., Kuczaj M., Stefaniak T. Hyperimmune serum containing Histophilus somni rHsp60, rOMP40, and *Actinobacillus pleuropneumoniae* LPS antibodies as supplementary treatment for calf respiratory diseases. Sci Rep. 2025, 30;15(1):19088. (ii) Palusiak A., Maciejewska A., Łukasiewicz J. The results of polymerase chain reaction and MALDI-TOF mass spectrometry versus phenotypic distinction between *Klebsiella pneumoniae* and *Klebsiella oxytoca*. Front. Microbiol. 2025,16. (iii) Koj S., Niedziela T., Rossowska T., Schmitt J.L., Lehn J.M., Nicolau C., Kieda C. Modulation of the oxygenation state and intracellular pH of erythrocytes by inositol trispyrophosphate investigated by the ^{31}P NMR study of 2,3-DPG (2025) Journal of Cellular & Molecular Medicine, 29 (2), e70343. (iv) Chunjun Qin, Wei Han, Guangzong Tian, Xiaopeng Zou, Anna Maciejewska, Jolanta Lukasiewicz, Peter H. Seeberger, Jing Hu, Jian Yin. Evaluation of the antigenicity of synthetic trisaccharides related to the *Plesiomonas shigelloides* serotype O51 O-antigen containing an acetamidino group. Carbohydrate Research, 550, 2025, 109388.