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RESEARCH REPORT 2015

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LABORATORY OF BACTERIOPHAGES
Head: Professor Andrzej Górski, M.D.

The presence of bacteriophages in the alimentary tract of patients with inflammatory bowel diseases

The aim of our investigation was to evaluate frequencies and titers of bacteriophages for the *E. coli* strains from examined individuals and the laboratory strains *E. coli B, E. coli 1962* and *E. coli DSM 13127* in the stools of 90 patients with inflammatory bowel diseases (IBD) and 28 healthy volunteers. The dependence between the number of *E. coli* bacteria and frequencies and titers of coliphages in stools of patients and volunteers was analyzed. The material from adult patients was obtained from the Clinic of Gastroenterology and Hepatology of the Academic Clinical Hospital in Wrocław.

Lower frequencies of coliphages and isolation of *E. coli* bacteria in stools of patients compared with volunteers were observed. The titers of coliphages and *E. coli* bacteria in stools of patients were higher than in volunteers. A positive correlation between the number of *E. coli* bacteria and frequencies and titers of coliphages in stools of patients and volunteers was observed. Statistically significant differences were found for frequencies of coliphages for subjects' own strains of *E. coli* and *E. coli* DSM 13127 between patients and volunteers.

Phage neutralization by sera of patients receiving phage therapy

The aim of our study was to verify whether phage therapy (PT) can induce antiphage activity of sera (AAS). The AAS was evaluated for sera from 203 patients of the Phage Therapy Unit in Wrocław before and during PT using a neutralization test. The control was sera from 30 healthy volunteers. The rate of phage inactivation (K) estimated the level of phages' neutralization by sera.

Low AAS was observed in sera of volunteers and in patients before PT. High AAS was observed in 10.8% of patients during local PT (n=20) or local/oral PT (n=2) between days 15 and 66 of PT. High AAS was detected in patients treated with some *S. aureus*, *P. aeruginosa*, *E. faecalis* and *S. marcescens* phages. Low K rates were observed during oral PT (n=49). The results suggest that the level of neutralization of phages depends on the route of phage administration and phage type. The high AAS appearing during PT does not exclude clinical improvement.

The effect of bacteriophage preparations on intracellular killing of bacteria by phagocytes

Intracellular killing of bacteria is one of the fundamental mechanisms against invading pathogens. Impaired intracellular killing of bacteria by phagocytes may be the reason for chronic infections and may be caused by antibiotics or substances that can be produced by some bacteria. Therefore, it was of great practical importance to examine whether phage preparations may influence the process of phagocyte intracellular killing of bacteria. It may be important especially in the case of patients qualified for experimental phage therapy (approximately half of the patients with chronic bacterial infections have their immunity impaired).

Our analysis included 51 patients with chronic Gram-negative and Gram-positive bacterial infections treated with phage preparations at the Phage Therapy Unit in Wroclaw. The aim of the study was to investigate the effect of experimental phage therapy on intracellular killing of bacteria by patients' peripheral blood monocytes and polymorphonuclear neutrophils.

We observed that phage therapy does not reduce patients' phagocytes' ability to kill bacteria, and it does not affect the activity of phagocytes in patients with initially reduced ability to kill bacteria intracellularly. Our results suggest that experimental phage therapy has no significant adverse effects on the bactericidal properties of phagocytes, which confirms the safety of the therapy.

Research projects

Mammalian host-versus-phage immune response determines phage fate in vivo

Awareness of the emerging drug resistance in bacteria draws attention to bacteriophages as a therapeutic alternative. Examples of phages combating bacteria abound. However, negative examples also occur despite careful phage selection by testing of their antibacterial activity *in vitro*. We have identified how the immunological response determines antibacterial potency of phage *in vivo*. Anti-phage activity of phagocytes, antibodies and serum complement was identified by direct testing and by high-resolution fluorescent microscopy. We accommodated the experimental data into the mathematical model. As a result of numerical simulations, we propose a universal schema of the impact of innate and adaptive immunity on phage pharmacokinetics. Contrary to the general understanding of interactions between phages and bacteria (phages inhibit bacteria, bacteria stimulate phages), we have demonstrated an indirect pathway of phage inhibition by bacteria. This counterintuitive pathway proceeds through innate immunity, which is stimulated by bacteria to protect against them but has the consequence that the response also inhibits phage. We conclude that bacteria hijacked the innate immunity of hosts to inhibit phage. This state of affairs may determine the results of experimental phage applications in animal models, especially those based on acute septicemia and overt animal morbidity. This situation further implies that the immune status of a patient may have a crucial effect on the outcomes of the therapy. We propose that the complexity of mammalian immunity and the mammalian host-versus-phage (MHvP) immune response should be taken into account in considering the medical use of phage to combat bacteria.

Oral application of T4 phage induces weak antibody production in the gut and in the blood

A specific humoral response to bacteriophages may follow phage application for medical purposes, and it may further determine the success or failure of the approach itself. We conducted a long-term study of antibody induction in mice by T4 phage applied *per os*: 100 days of phage treatment followed by 112 days without the phage and a subsequent second application of phage up to day 240. Serum and gut antibodies (IgM, IgG, secretory IgA) were analyzed in relation to microbiological status of the animals.

T4 phage applied orally induced anti-phage antibodies when the exposure was long enough (IgG day 36, IgA day 79); the effect was related to high dosage. Termination of phage treatment resulted in a decrease of IgA again to insignificant levels. The second administration of phage induced secretory IgA sooner than that induced by the first administration. An increased IgA level antagonized gut transit of active phage. Phage resistant *E. coli* dominated the gut flora very late: on day 92. Thus the immunological response

emerges as a major factor determining phage survival in the gut. Phage proteins Hoc and gp12 were identified as highly immunogenic. A low response to exemplary foreign antigens (from Ebola virus) presented on Hoc was observed, which suggests that phage platforms can be used in oral vaccine design.

LABORATORY OF GLYCOBIOLOGY AND CELLULAR INTERACTIONS
Head: Professor Danuta Duś, Ph.D.

Mechanisms of tumor progression. Intercellular adhesive interactions during metastatic spread of cancer cells

Biology of endothelial progenitor cells

In 2015 in our laboratory the investigations on the biology of human endothelial (HEPC-CB.1 and HEPC-CB.2 cell lines) and mesenchymal progenitor cells (MSC25SVT cell line) were continued. The studies aimed to determine the participation of these cells in regenerative processes.

The secretion profile of mesenchymal progenitor MSC25SVT cells was determined using a commercial protein membrane assay. The influence of factors secreted by MSC25SVT cells on mature endothelial cell, fibroblast, keratinocyte and human breast cancer cell (SKBR3) *in vitro* proliferation was measured, and we found a significant (about 50%) increase in proliferation of all above-mentioned cells except SKBR3 cancer cells. Moreover, the chemotaxis of endothelial HUVEC cells and breast cancer SKBR3 cells was determined. It was found that factors secreted by MSC25SVT cells significantly increased the chemotaxis of SKBR3 cells, whereas HUVEC cells responded weaker to these factors. Additionally, a functional assay on the reconstituted extracellular matrix (Matrigel) was performed. Formation of tubular structures by HUVEC cells on Matrigel was augmented in the presence of MSC25SVT supernatant and slightly augmented where HEPC-CB.1 and HEPC-CB.2 supernatant was used.

Both supernatants from HEPC-CB.1 and HEPC-CB.2 and from MSC25SVT cells inhibited migration of mature endothelial cells (HUVEC and HSkMEC.2) as measured in the standard wound healing test, whereas HEPC-CB.1 supernatant increased the migration rate of fibroblast cell lines (FS4 and WI38). The supernatants from HEPC-CB.1 and HEPC-CB.2 did not change HSkMEC.2, HUVEC or fibroblast cell line proliferation modes.

Influence of multidrug resistance proteins on endothelial precursor cell biology

Investigations on the influence of multidrug resistance proteins on endothelial precursor cell differentiation and their functionality were performed in 2015. HEPC-CB.1 and HEPC-CB.2 cell lines with downregulated MRP1 and MRP4 protein expression as well as with overexpression of these proteins were prepared. It was found that downregulation of these multidrug resistance proteins only slightly changed proliferation, migration and angiogenic properties of HEPC-CB.1 and HEPC-CB.2 cells after the differentiation process, whereas the overexpression resulted in increased tube formation in the angiogenic assay.

IL-10 influence on the suppression mechanisms of tumor-derived myeloid suppressor cells (MDSCs)

Investigations on tumor-derived myeloid suppressor cells (MDSCs) were also started in 2015. The goal of our research project is to define the involvement of IL-10 in the activation of the suppression mechanisms utilized by MDSCs. Trials with third generation lentiviral vectors encoding shRNA sequences that silence the expression of IL-10 or IL-10R will allow us to decide whether and how IL-10 regulates the phenotype profile of MDSCs, as well as to unveil the role of IL-10 in modulation of the expression of transcription factors as well as molecules responsible for the suppressor activity of MDSCs.

Metallothionein-3 increases triple-negative breast cancer cell invasiveness via induction of metalloproteinase expression

To clarify the role of metallothionein-3 (MT3) in breast cancer progression, we analyzed the effect of MT3 overexpression on proliferation, invasiveness, migration, and tumorigenesis of breast cancer MDA-MB-231/BO2 cells. It was found that MDA-MB-231/BO2 cells overexpressing MT3 were characterized by increased invasiveness *in vitro*, compared to the control cells. Interestingly, this increased invasiveness correlated with a highly increased concentration of matrix metalloproteinase-3 (MMP3) in the culture supernatants. Our data suggest that MT3 may regulate breast cancer cell invasiveness by modulating the expression of MMP3. These experimental results, obtained using triple-negative MDA-MB-231/BO2 cells, were further supported by clinical data. It was found that in triple-negative breast cancer (TNBC), nuclear MT3 immunoreactivity in cancer cells tended to be associated with patients' shorter disease-specific survival, suggesting that nuclear MT3 expression may be a potential marker of poor prognosis of triple-negative TNBC cases. The project was conducted in collaboration with the Department of Histology and Embryology, Medical University of Wroclaw.

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

Laboratory of Tumor Molecular Immunobiology
Head: Professor Leon Strządała, Ph.D.

Cytotoxicity of newly developed lanthanide nanomaterials suited for use as fluorescent probes

The possibility to exploit lanthanide-doped fluoride nanocrystals either as contrasting agents for magnetic resonance imaging or luminescent probes has been proposed already some years ago. It has been proved that due to specific properties of trivalent lanthanide ions, they can both convert near-infrared light to visible light and exhibit magnetic properties. Although they are considered very promising materials for bio-imaging and bio-sensing, deep understanding of the toxicity of these materials, and particularly the toxicity of nanomaterials, is still insufficient or lacking. This knowledge is of great importance in the light of growing use of biofunctionalized nanoparticles (NPs). Some questions about the safety of these highly promising nanomaterials are raised since the very same properties of NPs that are desirable

and potentially useful from a technological or biomedical perspective may also give rise to unexpected and hazardous toxicities.
The chemical cytotoxicity of nanomaterials may be reduced by using different coating agents, whose role is at least threefold. Firstly, such coating makes the NPs prone to bio-functionalization and ready for further bio-applications. Secondly, the coating may also help to engineer the circulation time and clearance of NPs from out of the cells and bodies. Finally, the coating may prevent NC dissolution and possible leakage of toxic ions into the biological environment

We investigated the cytotoxicity of bare Yb^{3+}:Er^{3+} codoped $NaGdF_4$ nanocrystals and compared it with the cytotoxicity of NPs coated with PEG2000, SiO_2 or SiO_2-NH_2 shells. The studies were performed using two model cell lines, i.e. RAW264.7 mouse macrophages and NIH3T3 fibroblasts. The former cells are able to phagocyte NPs, while the latter are commonly used in biocompatibility studies and widely present in body tissues as structural cells, which are unlikely to actively take up the nanocompounds. Viability of cell cultures was determined using the MTS assay, and for selected samples, the propidium iodide apoptosis detection assay and direct proliferation assay were performed. Additionally, we took advantage of fluorescent up-conversion capabilities of the tested nanocompounds and observed their direct interactions with the cells.

Although lanthanide-doped $NaGdF_4$ nanocrystals are considered as non-toxic, here we present data showing the fatal effect of newly synthesized $NaGdF_4$:Yb^{3+}:Er^{3+} on chosen types of cells. Bare $NaGdF_4$:Yb^{3+}:Er^{3+} nanocrystals were cytotoxic and induced apoptosis of both NIH3T3 and RAW264.7 cells. Their cytotoxicity was reduced by PEGylation, at the expense of minimizing direct interactions between the compound and the cell. On the other hand, coating with silica reduced cell death induced by Yb^{3+}:Er^{3+} co-doped $NaGdF_4$ nanocrystals (but proliferation was still inhibited). The NH_2-modified silica-coated nanoparticles were clearly less cytotoxic than pristine nanoparticles, which suggests that both silica and PEG coatings are reasonable approaches to reduce cytotoxicity of the nanocrystal labels. Interestingly, the intracellular deposition of NH_2-modified silica does not lead to increased cytotoxicity, but may raise the question of the long-term safety of using such compounds. The silica and PEG shell should also enable and simplify further bio-functionalization of these luminescent labels.

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

Studies on the mechanisms of tumor progression and metastasis and on the effects of experimental antitumor therapy

Studies on the potential application of bisphosphonates in anti-cancer treatment
The aim of the studies was:

1. To evaluate the anti-proliferative activity of new bisphosphonates towards murine and human macrophages and tumor cells; to evaluate the mechanism of action of these new compounds in murine and human macrophages and tumor cells.
2. To examine the potential usefulness of new and commercially available bisphosphonates in anti-tumor therapy in the models of breast cancers of different molecular types.

In 2015, the studies on anti-proliferative activity of new bisphosphonates towards normal and cancer cells were continued. Based on the obtained results, we focused on determining the activity of two aminomethylidenebisphosphonic acids, WG9001B and WG8185B2, in comparison to zoledronate – commercially available and the most active bisphosphonate. In previous studies, we proved that the new bisphosphonates show particular and often selective antiproliferative activity towards J774E macrophages, which are a model of osteoclasts for *in vitro* studies. Thus, we evaluated their influence on the proliferation rate of other macrophage-like cells – RAW246.7. Compared to zoledronate, the compound WG9001B shows nearly four times higher anti-proliferative activity against RAW246.7 cells. The IC_{50} values calculated for WG9001 and zoledronate were respectively 4.9 and 19.2 μg/ml. The anti-proliferative activity of the new bisphosphonates seems not to result from the direct cytotoxic effect against normal cells. The IC_{50} values of WG8185B2 and WG9001 calculated for murine Balb/3T3 fibroblasts were respectively 38.9±17.9 and 35.0±13.0 μg/ml. Their anti-proliferative activity is related to the arrest of cell cycling in the S phase. The cellular effect of compound WG8185B2 is manifested after 24 hours of incubation. For the compound WG9001B changes in the course of the cell cycle include transient arrest at the G2/M phase and, after 72 hours of incubation, in the S phase. We have also observed that breast cancer cells show different susceptibility to bisphosphonates. In the case of MCF-7 cells (luminal A type) compound WG8185B2 showed moderate and compound WG9001B poor anti-proliferative activity (IC_{50} 44.0 and 156.8 μg/ml, respectively). In contrast, 4T1 and MDA-MB-231 cells (basal type) appeared to be much more susceptible to the new bisphosphonates, with IC_{50} values of 31.5 and 9.4 μg/ml for the compound WG8185B2.
In 2015, stable sodium salts of new bisphosphonic acids were synthesized: the salt WG12399C for WG8185B2, and the salt WG12592A for WG9001B. These salts are much more soluble, while retaining their antiproliferative activity *in vitro*. The anti-proliferative activity of WG12399C and WG12592 salts against Eph 1424 murine breast cancer cells was examined. The compound WG12399C significantly inhibited the proliferation of normal mammary epithelial cells, as well as cancer cells, both non-metastatic and forming lung and kidney metastasis. The compound WG12592A showed high anti-proliferative activity towards normal and non-metastatic cells but rather poor activity against cells with high metastatic potential. This may indicate a different molecular mechanism of action of these two new bisphosphonates.

These results reveal the bisphosphonate WG9001B to be a potentially interesting compound showing anti-osteoporotic and anti-tumor activity in the case of basal type breast cancers. These results also suggest a different mechanism of action of the two studied aminomethylidenebisphosphonates, despite the fact that they belong to the same group of N-bisphosphonates.

The miRNA influence on vitamin D receptor (VDR) expression in human leukemia and lymphoma cells

The aim of these experiments is to clarify the role of microRNAs (miRNAs) in the human leukemia and lymphoma cell differentiation process induced by calcitriol ($1,25(OH)_2D_3$) and its analog tacalcitol (PRI-2191).

We conducted preliminary studies, using different types of human leukemia (HL-60, K562, KG-1, Thp-1, MV-4-11) and lymphoma (U2932, Jurkat, Daudi, Raji) cell lines. The MTT assay was performed after 120 h exposure to calcitriol or PRI-2191 to calculate the antiproliferative activity. Calcitriol and its analog PRI-2191 revealed strong antiproliferative activity against three leukemia cell lines, HL-60, Thp-1 and MV-4-11, and the inhibitory concentration 50% values were calculated. For the less sensitive cells the percentage growth inhibition was calculated for the highest concentration (1000 nM) used in the experiment. All those human leukemia and lymphoma cells express VDR protein. However, the expression of VDR protein does not correlate with the sensitivity of tested cells to calcitriol or its analog. Therefore we studied the expression of selected miRNA molecules (miR-32, miR-125b, miR-181a and miR-181b) in these cell lines. Untreated leukemia and lymphoma cells have a different microRNA expression profile. Our study showed that the lowest level of these molecules is observed in the MV-4-11 cells most sensitive to calcitriol. Interestingly, lymphoma cells expressed miR-32 at a higher level as compared to other microRNA molecules. We observed a low level of VDR mRNA in all human lymphoma cells, with the exception of the Raji cell line (the most sensitive to calcitriol) and its analog lymphoma cell.

The differences observed in the expression of miRNA studied between cell lines sensitive and insensitive to calcitriol, as well as different expression profiles of these molecules in regard of leukemia or lymphoma cells, are the most interesting achievements of this study.

Evaluation of the effect of vaccines based on the cells transduced with IL-2 and IL-12 lentiviral carriers on the tumor environment in mouse colon carcinoma models

The aim of our study was to complete the scheme of application of genetically modified cells, which were planned as an independent vaccine, an adjuvant of conventional therapeutics or modulators of the immune response.

We used the lentiviral vectors of the third generation bearing gene sequences of IL-2 and IL-12 (and initial attempts with the IL-15 gene) to modify the murine tumor cell lines of TC1 (lung cancer), MC38 (colon carcinoma), B16 (melanoma), X63-Ag 8.653 (multiple myeloma) and the myeloid origin dendritic cells of the JAWS II line. The purpose of this stage of the study was to obtain stable tumor cell lines, and the functional and phenotypic characterization of transduced cells. An evaluation of the effects of the construction of the IL-2 and IL-12 expression vector was performed by electrophoresis analysis, kinetics of cell proliferation, and examination of the level of cytokine production. In addition, the effect of transduced cells on priming of splenic cells was analyzed. Introduction of vectors containing cytokine genes took place under the control of the puromycin resistance gene. This process did not affect the change in the characteristics of tumor cells, but modified the expression of MHC antigens as well as CD86 on dendritic cells. Transductants applied for several days to stimulate splenocytes did not induce significant changes in the size of the subpopulations of these cells. IL-12 gene-transduced MC38 cells or JAWS II cells were able to stimulate the splenocytes to high production of IFN-γ and low production of IL-10. This suggests the possibility that these transductants can be used as effective inducers of an anti-tumor response. The most important achievement of the study was to implement new techniques, extending the scope of the research to enrich the study in thematic projects.

Laboratory of Biomedical Chemistry
Head: Professor Janusz Boratyński, Ph.D., Eng.

Protein-boron conjugates

1. Boron clusters represent a vast family of boron-rich compounds with extraordinary properties that provide the opportunity of exploitation in different areas of chemistry and biology. In addition, boron clusters are clinically used in the boron neutron capture therapy (BNCT) of tumors. In this paper, a novel, solid-state (solvent-free), thermal method for protein modification with boron clusters is proposed. The method is based on a cyclic ether ring opening in an oxonium adduct of cyclic ether and a boron cluster with nucleophilic centers of the protein. Lysozyme was used as the model protein, and the physicochemical and biological properties of the obtained conjugates were characterized

The main residues of modification were identified as arginine-128 and threonine-51. No significant changes in the secondary or tertiary structures of the protein after tethering of the boron cluster were found by mass spectrometry and circular dichroism measurements. However, some changes in the intermolecular interactions and hydrodynamic and catalytic properties were observed.

To the best of our knowledge, we have described the first example of an application of cyclic ether ring opening in the oxonium adducts of a boron cluster for protein modification. In addition, a distinctive feature of the proposed approach is performing the reaction in a solid state and at an elevated temperature.

The proposed methodology provides a new route to protein modification with boron clusters and extends the range of innovative molecules available for biological and medical testing.

2. Two complementary methods, “in solution” and “in solid state”, for the synthesis of lysozyme modified with metallacarborane (cobalt bis(dicarbollide), Co(C2 B9 H11)2 (2-)) were developed. As metallacarborane donors, oxonium adducts of cobalt bis(dicarbollide) and 1,4-dioxane or tetrahydropyran were used. The physicochemical and biochemical properties of the obtained lysozyme-metallacarborane conjugates were studied for changes in secondary and tertiary structure, aggregation behavior, and biological activity. Only minor changes in primary, secondary, and tertiary protein structure were observed, caused by the single substitution of metallacarborane on lysozyme. However, the modification produced significant changes in lysozyme enzymatic activity and a tendency toward time- and temperature-dependent aggregation.

Removal of endotoxins from bacteriophage preparations

The method of removing pyrogen contaminating bacteriophage preparations by water immiscible solvent extraction was developed. During this process most of the phage lytic activity is retained in the aqueous phase, while endotoxin accumulates in the organic solvent. The levels of endotoxin in the aqueous bacteriophage-containing fraction determined by limulus amebocyte lysate or EndoLISA assay were exceptionally low. While the initial endotoxin levels in the crude phage lysates ranged between 10^3 and 10^5 EU/ml, the average level after organic extraction remaining in the aqueous fraction was 5.3 EU/ml. The purification procedure is scalable, efficient and applicable to all the bacteriophages tested: T4, HAP1 (*E. coli*) and F8 (*P. aeruginosa*).

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

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

Studies on the pathogenesis of some diseases of bacterial etiology and the role of bacterial surface glycoconjugates and protein antigens in the immune response

The main topics of studies are mechanisms of pathogenicity of diseases with bacterial etiology, the role of bacterial glycoconjugates and proteins in the immune processes, and the structure and functions of bacterial exopolysaccharides and endotoxins (lipopolysaccharides, LPS). Studies on the O-polysaccharides of serotypes O24 and O56 of *Escherichia coli* containing sialic acid in their lipopolysaccharide (LPS) structures revealed that various human tissues are recognized by anti-O24 and anti-O56 antibodies. The epitope recognized by anti-O56 antibodies is a new marker specific for glandular epithelium and nervous tissue. Further studies should be performed to determine the structure of the tissue epitope recognized. Regarding the studies on identification of clinically important strains of actinobacteria, the use of an upgraded MALDI-TOF Biotyper Database containing representatives of the suborder *Corynebacterineae* deposited in the Polish Collection of Microorganisms has been described. This newly created database was used for identification of the strain isolated from a nocardial brain abscess mimicking brain tumor in an immunocompetent patient with no underlying risk factors. Other studies presented a new type of highly sensitive label-free microwave sensor in the form of an interdigital capacitor coated with T4 bacteriophage gp37 adhesin recognizing *Escherichia coli* LPS. The C-terminal part of the adhesin consists of receptor-binding amino acid residues which are involved in a specific interaction with two terminal glucose residues of the bacterial *E. coli* B LPS. The change of the sensors' capacitance and conductance due to LPS presence is an indicator of the detection. The measurements in the frequency range of 0-3 GHz utilizing the vector network analyzer have been carried out at different concentrations to verify experimentally the proposed method. The measured capacitance change between the reference and the biofunctionalized sensor equals 15% in the entire frequency range and the measured conductance change exceeds 19%. The changes of both parameters can be used as good indicators of LPS detection. The selectivity has been confirmed by ELISA experiments and tested by sensor measurements of LPS from *E. coli* B, *E. coli* O56, *E. coli* O111, *Pseudomonas aeruginosa* NBRC 13743 and *Hafnia alvei* 1185.

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

Study on nonspecific immunity in viral infections

The hypothesis was that preadipocytes would have an intrinsically elevated propensity to differentiate into mature adipocytes due to AdV9 infection. To test this hypothesis, the metabolic and molecular mechanisms responsible for AdV9-induced adipogenesis were examined. An association between anti-AdV9 antibodies and human obesity was also

identified. 3T3L1 cells were used as a surrogate model to analyze the preadipocyte proliferation, differentiation, and maturation. Expression of E4orf1, C/EBP-b, PPAR-g, GAPDH, aP2, LEP and fatty acid synthase genes, intracellular lipid accumulation and cytokine release were assessed. The presence of anti-AdV antibodies, serum lipids, plasma leptin, and CRP was evaluated in 204 obese and non-obese patients. AdV9-infected cells accumulated more intracellular lipids in comparison to uninfected controls. AdV9 enhanced the expression of C/EBP-b and PPAR-g, leading to increased differentiation of preadipocytes. Overexpression of aP2 and fatty acid synthase and decreased expression of leptin confirmed increased accumulation of intracellular lipids due to AdV infection. Secretion of TNF-α and IL-6 from AdV9-inoculated cells was decreased strongly. About 24.5% prevalence of anti-AdV9 antibodies was reported in the study group. AdV9-infected subjects presented higher body weights, body mass index (BMI), waist-hip ratio (WHR), and central obesity. The presence of anti-AdV9 antibodies was associated with changes in serum lipid level but neither elevated CRP nor decreased leptin levels were related to obesity due to AdV infection. Data obtained from this study provide evidence that AdV9 is a second adenovirus which has an influence on differentiation and lipid accumulation of 3T3L1 cells. The results were published in *Journal of Medical Virology, 2015; 87: 230-239.*

Natural killer (NK) cells are an important element of innate immunity against viruses, although their numbers decrease in the liver during chronic HCV infection. NK cells express a large panel of inhibitory and activating receptors. The most polymorphic of these are killer cell immunoglobulin-like receptors (KIRs), which are encoded by multiple genes that may be present or absent in given individuals depending on their genotype. This variability results in differential susceptibility to viral infections and diseases, including HCV infection and its consequences. The aim of this study was to test whether chronic infection with HCV and the viremia levels are associated with any *KIR* gene in the Polish population. We typed 301 chronically HCV-infected patients and 425 non-infected healthy individuals for the presence or absence of *KIR* genes and their ligands, *HLA-C C1* and *C2* groups as well as *HLA-B* and *HLA-A* Bw4-positive alleles. We found that males, but not females, possessing *KIR2DS2* and *KIR2DL2* genes had a 1.7-fold higher probability to become chronically HCV-infected than males negative for these genes ($p = 0.0213$). In accord with this, the centromeric B region, containing *KIR2DS2* and *KIR2DL2* genes, was also associated with chronic HCV infection in males. In addition, patients of both genders possessing the *KIR2DS3* but not the *KIR2DS5* gene exhibited, on average, a 2.6 times lower level of viremia than HCV-infected individuals with other genotypes ($p = 0.00282$). This was evident in those infected at a young age. *KIR2DS3*-positive patients also had lower mean levels of bilirubin than *KIR2DS3*-negative ones ($p = 0.02862$). Our results suggest a contribution of the *KIR2DS2* and *KIR2DL2* genes (*cenB* haplotype) to the susceptibility to chronic HCV infection, and an association of the *KIR2DS3* gene in the absence of *KIR2DS5* with low viremia levels. The results were published in *Human Immunology, 2015; 76: 102-108.*

The aim of this study was to develop a minimal medium for the cultivation of *Escherichia coli* B, which could be especially suitable for the industrial propagation of bacteriophage T4. The newly defined, minimal SM-1 culture medium contains free amino acids as the only nitrogen source and enables the bacteria generation time to be prolonged and satisfactory

phage titers to be achieved. The presence of organic ingredients, such as meat extracts, yeast hydrolysates, and enzymatic protein hydrolysates, in a culture medium may cause problems in the case of bacteria or phage cultures for therapeutic purposes. In the present study, we introduce a new medium, together with some procedures and applications for its usage. We also present new kinetics of *E. coli* B growth. Some traits such as the lack of high molecular proteins, bacterial growth comparable to that in a rich medium, and the cost effectiveness of the medium, make it highly competitive with currently used microbiological media. The surprisingly high titers of bacteriophage T4 obtained in our experiments suggest that SM-1 medium has the potential to find a broad application in medicine, especially in infectious disease therapy, pharmacy and biotechnology. The results were published in *Journal of General and Applied Microbiology, 2015; 61: 75-81.*

Nanoemulsions (NEs) are adjuvants that enhance antigen penetration of the nasal mucosa, increase cellular uptake of antigens by both epithelial and dendritic cells, and promote the migration of antigen-loaded dendritic cells to regional lymph nodes within 24 h of vaccine administration. The objective of this study was to elucidate cell death caused by W805EC NE and identify caspases and genes associated with death pathways. Consistent with this aim, we show that exposure of human epithelial cells (EC), both RPMI2650 and FaDu, to NE results in the activation of caspases (1, 3/7, 6, 8, and 9) and the expression of genes involved in apoptotic as well as autophagy and necrosis pathways. Interestingly, the NE activates caspase 8, which promotes "immunogenic apoptosis". The rescue assay was employed to investigate the fate of RPMI 2650 cells treated with W805EC NE. After four-hour treatment with as little as 0.03% NE, no cells were rescued at 72 h. Remarkably, immediately after four-hour treatment, the cells morphologically resembled untreated cells and most of the cells were alive. Altogether, these results suggest that NE induces death of human EC through multiple pathways. Epithelial cell death caused by W805EC may have further implications for antigen uptake, processing, and presentation by dendritic cells. The results were published in *Vaccine, 2015; 33: 2289-2296.*

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

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

Effects of lactoferrins on susceptibility to viral infection of B cells from young versus old mice and B cell lines of immature and mature phenotype

B-cell-enriched splenocytes from 3-month-old and 13-month-old CBA mice were infected in culture with: encephalomyocarditis (EMCV), vesicular stomatitis (VSV) and human herpesvirus 1 (HHV-1) viruses. Bovine native, recombinant human lactoferrins and recombinant mouse lactoferrins (LFs) were used prior to or after infection. No significant differences were observed in the susceptibility of cells from young versus old mice. The suppressive effects of LF on replication of the viruses were stronger than those resulting from

competition of LFs and the viruses for the common receptor. In addition, LFs were equally protective in both age categories.

The studies with B-cell cell lines revealed that immature WEHI-231 cells appeared to be less susceptible to viral infection (in particular in the case of VSV) in comparison with A-20 cells. In WEHI-231 cells significant protective activities of bovine and mouse LFs against VSV and EMCV were observed and of bovine LF against HHV. For A-20 cells the protective actions of lactoferrins were stronger than for the WEHI-231 cell line (for HHV-1 the virus titer decreased by 3-5 log values). The protective effects of both LFs were, however, weak for EMCV and moderate for VSV infection.

In summary, B cells from aged mice preserve the resistance to viral infection and can still be protected by lactoferrins. The studies on B-cell lines will be continued and show a higher susceptibility to viral infection of more mature B cells.

Studies on the mechanism of action of immunosuppressive compounds

In the course of our studies on immunosuppressive compounds we demonstrated that various classes of compounds such as isoxazoles, cyclic peptides and azaphenothiazines have an ability to suppress mitogen-induced proliferation of peripheral blood lymphocytes. The molecular studies showed that the common feature of these compounds was suppression of expression of caspases 3, 8 and 9 in Jurkat cells. In the cases of a cyclic nonapeptide and azaphenothiazine the expression of caspase 3 was blocked. In addition the nonapeptide caused fragmentation of DNA in Jurkat cells.

Laboratory of Immunopathology

Head: Professor Irena Frydecka, M.D, Ph.D.

Studies on the mechanisms of immune deficiency in neoplastic and autoimmune diseases

Association of* BTLA *gene polymorphisms with the risk of B-cell chronic lymphocytic leukemia in a Polish population

The contribution of the immune system to the pathogenesis of B-cell chronic lymphocytic leukemia (B-CLL) has been receiving growing attention. The important role of coinhibitory receptors in these processes has been considered. One of these receptors is B and T lymphocyte attenuator (BTLA) – a member of the immunoglobulin superfamily which negatively regulates immune responses. High expression of BTLA and its ligand, herpes virus entry mediator (HVEM), was observed in hematological cancer cells.

As polymorphisms in the gene encoding *BTLA* might result in abnormal expression and function of BTLA, the aim of this study was to evaluate the association between polymorphisms in the *BTLA* gene and susceptibility to B-CLL in the Polish population. Eight tag single nucleotide polymorphisms (SNPs) in the *BTLA* gene were investigated: rs2705511, rs1982809, rs9288952, rs9288953, rs2705535, rs1844089, rs2705565, rs2633580. Genotyping was done using allelic discrimination methods with the TaqMan SNP Genotyping Assays in 321 B-CLL patients and in 481 healthy controls. We found that three polymorphisms in the *BTLA* gene – rs9288953 located in intron 1, rs1982809 in the 3′ near gene position, and rs2705511 in the intragenic region – are associated with susceptibility to B-CLL. For rs1982809 SNP the frequencies of G allele and G+ carriers (genotype GG + GA) were higher

in patients compared with controls (0.29 vs. 0.23, p=0.008; OR 1.36, 95%CI 1.08-1.71 and 0.51 vs. 0.40, p=0.002; OR 1.56, 95%CI 1.17-2.08, respectively). The presence of C alleles (genotype CC + CA) in rs2705511 increased the risk of B-CLL about 1.6-fold (p=0.0009, OR 1.62, 95%CI 1.22-2.16). Furthermore, in rs9288953 SNP the frequencies of T allele and TT genotype were significantly increased in patients compared with controls (0.45 vs. 0.37, p=0.0014, OR 1.39, 95%CI 1.14-1.71 and 0.22 vs. 0.14, p=0.0035 OR 1.74, 95%CI 1.20-2.53, respectively). Other investigated SNPs showed no differences in allele or genotype distributions in group of patients and controls. Haplotype estimation analysis indicated that the haplotype rs2705511A, rs1982809A, rs9288952A, rs9288953C, rs2705535C, rs1844089G, rs2705565C, rs2633580C was represented more frequently in controls and significantly reduced the risk of B-CLL ($p_{corrected}$=0.006, OR 0.70, 95%CI 0.57-0.87), while the haplotype rs2705511C, rs1982809G, rs9288952A, rs9288953T, rs2705535C, rs1844089G, rs2705565C, rs2633580C was represented more frequently in B-CLL patients and significantly increased the risk of B-CLL ($p_{corrected}$=0.005, OR 1.59, 95%CI 1.20-2.11). Our results indicate that polymorphisms in the *BTLA* gene might be considered as a potentially low-penetrating risk factor for B-CLL, but the results need to be verified in further studies.

Patients with chronic lymphocytic leukemia (CLL) differ in the pattern of CTLA-4 expression on CLL cells: possible implications for immunotherapy with CTLA-4 blocking antibody

Recently, systemic administration of a human monoclonal antibody directed against cytotoxic T-lymphocyte associated antigen 4 (CTLA-4) expressed on circulating T cells in patients with chronic lymphocytic leukemia (CLL) has been considered. Also, CLL cells have been shown to express CTLA-4, increased levels of which in the leukemic compartment are a predictor of good clinical outcome. Since both CLL and Treg microenvironment cells can be targeted by the CTLA-4 blocking antibody in this immunotherapy approach, the investigation of the functional effect of CTLA-4 blockade on CLL cells might be of potential clinical relevance.

Based on the fact that CLL cells exhibit high variability of CTLA-4 surface expression, we divided the patients into low and high expressors of the CTLA-4 molecule, taking as a cut-off value the median percentage of surface CTLA-4-positive CLL cells (32.8%). The main aim of this study was to examine the effect of CTLA-4 blockade on proliferation activity and apoptosis of CLL cells in patients with low and high CTLA-4 expression. We found that in the high CTLA-4-expressing CLL group CTLA-4 blockade on the CLL cell surface resulted in a significant increase in the median percentages of $Ki67^+$ cells (p=0.03) and a non-significant decrease in the proportion of apoptotic cells (p=0.07). In contrast, in the low CTLA-4 expressors, CTLA-4 blockade did not affect the proliferation activity or the frequency of apoptosis. This study reports for the first time the different effect of CTLA-4 blockade on CLL cells in CLL patients depending on the levels of CTLA-4 expression. CTLA-4 blockade seems to induce pro-survival signals in leukemic cells from CLL patients exhibiting high CTLA-4 expression, suggesting that an immunotherapy approach based on the systemic use of monoclonal anti-CTLA-4 antibodies could be an unfavorable strategy for some CLL patients.

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

Immunological mechanisms associated with reproductive processes in health and disease

Novel immune regulatory mechanisms in pregnancy – preliminary studies on Treg lymphocyte induction by peptides as their putative epitopes

Treg lymphocytes play a dominant role in maintenance of immunological tolerance towards paternal antigens during pregnancy. On the other hand, lower frequency or function impairment of these cells is associated with pregnancy failure in women and mice females. Methods of Treg abundance control may be used in future treatment strategies of preventing pregnancy losses. The objective of the study was to determine the proliferative response of Treg lymphocytes stimulated by peptides known as Tregitopes. Peptides were selected by a mathematical model for prediction of their binding to MHC class II developed by Okoniewska et al. (2016 submitted for publication). The experiments will verify the usefulness of the model for designing appropriate peptides as Treg stimulators.

Investigations were carried out in C57Bl6 ^{FOXP3GFP} transgenic mice, maintained in the Institute of Immunology and Experimental Therapy and kept in accordance with Polish legal requirements. Spleen antigen presenting cells (CD11c and Cd11b) were sorted and cultured with selected peptides and then co-incubated with sorted spleen CD4 lymphocytes. Frequency of Treg cells was determined by flow cytometry.

Preliminary data indicate that peptides of different characteristics determined by the model have differential stimulating potential for proliferation of Treg cells.

Peripheral pregnancy recognition in splenic T CD4(+) lymphocytes

Pregnancy has a profound impact on the female immune system. The first signs of pregnancy recognition by the immune system are observed even before implantation. The most visible effects are present in the local compartment, i.e. in uterine draining lymph nodes and the decidua, while peripheral changes are less obvious. We showed that in normal pregnancy the TCD4+ lymphocytes' proteome differs from that in non-pregnant animals (Chelmonska-Soyta et al. 2015). In this study we wondered if the presence of a non-surgically transferred embryo may influence protein expression in peripheral lymphocytes. For that reason, we decided to investigate global protein expression in splenic T CD4(+) lymphocytes in order to identify and validate the most important biomarkers characteristic for the preimplantation period of pregnancy at the periphery.

1.5 dpc embryos derived from female mice (CD-1 strain) donors were cultured for 24 h *in vitro* without or in the presence of TNF-α (50 ng/ml) and non-surgically (NSET Device, ParaTechs, USA) transferred to hormonally synchronized recipients. The control group of pseudopregnant mice received a transfer of fresh culture medium alone. Twenty-four hours later recipients were euthanized. Two-dimensional electrophoresis (2-DE) and mass spectrometry (MS) were used to analyze the protein expression pattern of magnetically sorted TCD4(+) lymphocytes from spleens of females recipients.

The proteomic map consisted of 135±20 protein spots. Eight proteins were significantly altered (between the control group and both experimental groups). Four of them represent cytoskeleton-regulating proteins (CAPZ1, TPM3, GDIR2, LSP1), similarly to previous results

obtained in mice in natural pregnancy. However, embryo transplantation enhanced expression of IL-24 and peroxiredoxin. The results confirmed lymphocytes' awareness of preimplantation of the embryo at the periphery not only in natural pregnancy but also after transplantation. The project was supported by Polish National Science Centre grant No. N N311 523940 and Wroclaw Centre of Biotechnology, The Leading National Research Centre (KNOW) program for 2015-1019, and was performed in cooperation with the Faculty of Biotechnology and Animal Husbandry, West Pomeranian University of Technology, Szczecin.

DEPARTMENT OF MICROBIOLOGY

Laboratory of Molecular Biology of Microorganisms
Acting Head: Professor Anna Pawlik, Ph.D.

Replication of bacterial chromosomes

DNA synthesis is tightly controlled and strictly dependent on cell cycle progression. It is primarily regulated at the first step – initiation. In the Laboratory of Molecular Biology of Microorganisms (LMBM) we are interested in mechanisms of the initiation and regulation of bacterial chromosome replication, with special emphasis on characterization of the key factors engaged in initiation complex (orisome) formation, namely DnaA (the initiator protein) and *oriC* (the origin of chromosome replication). We are especially interested in orisome formation in the Epsilonproteobacteria, which comprise one of the classes within the phylum Proteobacteria. Recent studies have revealed that Epsilonproteobacteria, which are mostly microaerophilic or anaerobe Gram-negative, are globally spread and inhabit a wide variety of ecological niches including water reservoirs, sewage, oil fields and deep-sea hydrothermal vents. Many of the known Epsilonproteobacteria are obligate or facultative human and/or animal pathogens, including *H. pylori* – one of the most studied human-associated bacteria causing gastric ulcer or gastric cancer. Few other Epsilonproteobacteria are assumed to be emerging human pathogens. So far, initiation of chromosome replication has been characterized only for *Helicobacter pylori*. Some unique features have been revealed, such as bipartite structure of its origin region and topology-sensitive DnaA binding to DnaA boxes. These features might be common for all more closely related bacteria and/or bacteria sharing similar genetic and lifestyle properties. We identified the origins of chromosome replication in 4 species of Epsilonproteobacteria. The DnaA boxes as well as DNA unwinding elements have been mapped. The obtained results allowed us to characterize features of orisome formation unique to the Epsilonproteobacteria as well as those common to the majority of Proteobacteria. Thus the results will be beneficial for general knowledge concerning initiation of bacterial chromosome replication.

Polyketide synthesis and its regulation in Streptomyces

Polyketides are a large class of bioactive compounds with extremely diverse structures and functions. They are synthesized as secondary metabolites by giant multienzyme complexes – polyketide synthases. Our work is focused on the polyketide synthase Cpk from

S. coelicolor A3(2), which is responsible for synthesis of the yellow pigment coelimycin. Expression of *cpk* genes is tightly controlled by regulatory proteins encoded by the genes within the *cpk* cluster and probably by several pleiotropic regulators connected with regulation of secondary metabolite production as well as *Streptomyces* morphological differentiation. We are interested in deciphering the regulatory circuits governing the synthesis of coelimycin as well as in the discovery of its biological activity.

Laboratory of Signaling Proteins
Acting Head: Professor Janusz Matuszyk, Ph.D.

Studies on proteins and signaling pathways involved in activation of proinflammatory transcription factors and the response to hypoxia

Elucidation of transcription factors involved in tyrosine hydroxylase gene activation in PC12 cells in response to treatment with adenosine

Tyrosine hydroxylase (TH) is the rate-limiting enzyme in the synthesis of catecholamines inside pheochromocytes within the adrenal medulla. The results of our recent experiments indicated the involvement of the cAMP response element (CRE) in the promoter region of the *TH* gene and the CRE-binding protein (CREB) in activation of transcription of the *TH* gene in PC12 cells (rat pheochromocytoma) in response to treatment with adenosine. The *TH* promoter region also contains the Nur77/Nurr1-binding response element (NBRE), and other authors suggested that the orphan nuclear receptor Nurr1 directly transactivates the promoter activity of the *TH* gene. The NBRE motif is also a potential target for cAMP-dependent intracellular signaling pathways. However, our latest result obtained using site-directed mutational analysis of the *TH* promoter region upstream of the luciferase reporter gene does not support the role of the Nur77/NURR1 response element in *TH* gene activation, at least in PC12 cells following treatment with adenosine.

DEPARTMENT OF CLINICAL IMMUNOLOGY

Laboratory of Clinical Immunology
Head: Professor Andrzej Lange, M.D.

Multikinase inhibitor sorafenib contributes to salvage chemotherapy and is effective given alone for maintenance in AML FLT3 ITD patients relapsing after allo-hematopoietic stem cell transplantation

Cancer is usually associated with genetic or epigenetic abnormalities which if detected may be targeted by appropriate compounds including tyrosine kinase inhibitors and demethylation agents. At present we have a variety of tyrosine kinase inhibitors already validated for clinical use. The internal tandem duplication (ITD) in the juxtamembrane domain of the FLT3 gene encoding the SCF receptor leads to activation of the constitutive receptor, resulting in 30% of acute myeloid leukemia (AML) cases, and is associated with poor prognosis. This receptor with ligand interaction activates the signaling pathways (RAF/MEK/ERK and PI3K/AKT/mTOR kinases) promoting hematopoietic progenitor cell

expansion (proliferation and better survival). It was found that the multikinase inhibitor sorafenib originally synthesized for treatment of kidney cancer cases also blocks the constitutive activation of FLT3 in leukemia cases. Taking advantage of that observation, we followed the fate of two patients relapsing after matched unrelated donor (MUD) allo-HSCT who received chemotherapy tailored for their biologic performance. The chemotherapy was supported by the administration of the multikinase inhibitor. The present study focused on the impact of the multikinase inhibitor on the immune system of the patients.
We found:

The multikinase inhibitor was effective in supporting the induction chemotherapy given for blast reduction and showed further potent anti-leukemic potential given to the patient as the only treatment modality (biologic performance ECOG 3) and as an adjuvant to the maintenance protocol of AML treatment in the other patient.

1. The course of the treatment was common in both patients as they responded well, not exhibiting a leukemia relapse during 2 years of observation. Also in both cases the presence of CD8+ lymphocytes in the marrow was documented. A high proportion of them were CD279 and CD69 positive. These cells were more prevalent in the marrow than the blood. In addition, lymphocyte morphology evaluated at the microscopic level was characteristic for activated lymphocytes, frequently having azurophilic granules. Indeed, the cells were in proportion perforin positive.
2. The two studied patients differed with respect to the unwanted effect of the multikinase inhibitor treatment. One of these two patients developed acute graft versus host disease (GvHD) progressing to subacute GvHD with sicca syndrome, and skin and liver lesions. There was no response to the rapamycin treatment. In addition, avascular necrosis (AVN) of the head of the femur in those patients were seen together with the hand and foot syndrome (both known to be side effects of multikinase inhibitor treatment). Finally, the patient died 15 months after the relapse diagnosis and at that time he was free from leukemia but suffered from progressive GvHD. The second patient's case course was undisturbed by any abnormal findings except for transient thrombocytopenia, seen usually during the second week after each maintenance therapy course.

In conclusion, the multikinase inhibitor used did not reduce the immune responsiveness which was seen as the accumulation of CD8+ lymphocytes in the marrow cavity (graft vs leukemia effect), and consequently no mitigation of acute graft vs host reaction was seen. Acute GvHD was progressing. The multikinase inhibitor affected in this particular patient VEGF receptor associated kinases, which resulted in side effects, namely AVN. The multikinase inhibitor can be used in relapsing AML patients to facilitate the salvage chemotherapy, and the effect can be further continued with immune surveillance of leukemia, which is apparently not affected by the multikinase inhibitor.

INNOMED/I/1/NCBR/2014 Cell Therapy NCBiR grant*: Fresh marrow cell propagation for MSC using the Quantum Cell Expansion System*

Bone marrow cells obtained from 8 individuals (7 healthy donors and 1 patient with limb ischemia, age (years): range 39-71 years, 4 female/4 male) were cultured in a closed and automated medical device (Quantum Cell Expansion System). Twenty-five ml of fresh bone marrow cells were cultured in Minimal Essential Medium alpha and human platelet lysate

(5%). The final product was described functionally and phenotypically and was subjected to measurements of quality control parameters.

The cultures were terminated and passaged when the level of lactates reached 4.0 mmol/l (on days 9-16 of culture). After that the cells were usually passaged to the next culture in the same culture conditions.

Harvested cells' (after the first passage) characteristics were as follows. Total count (median) 7.6E+07 cells (6.91E+06 to 1.0E+08 cells), CD45- CD34- cells constituted (mean) 96.88%±2.0%, in the latter population we found 98.83±0.78%, 98.58±1.16% and 96.43±2.68% of CD105+, CD90+ and CD73+ cells, respectively. Microbiologic surveillance proved that the products were free from *Mycoplasma* and *Chlamydia* as well as other bacteria and fungi, and in addition the cultures were LPS-free. The donors were HBV DNA, HCV RNA and HIV RNA negative.

Culture of MSC from fresh bone marrow cells using the Quantum Cell Expansion System is effective in propagation of cells having mesenchymal/stromal cell characteristics.

Analysis of the regeneration potential of the marrow-derived mononuclear cell population

Thirteen years' experience in the use of marrow-derived mononuclear cells in patients with critical limb ischemia (CLI) leads us to the following conclusions:

1. Bone marrow cells harvested from the posterior iliac crest when enriched in mononuclear cells using a COBE Spectra separator contained the cells characterized with the following phenotypic characteristics: CD34-CD45- median 11.23% (4.45-24.35), CD45-CD34-CD90+ median 0.095% (0.008-0.31) and CD45-CD34-CD73+ median 0.077% (0.02-0.23).
2. Bone marrow derived mononuclear cells were used for revascularization of critically ischemic legs. For that 0.5 mL portions of these cells were injected into the calf muscles.
3. Patients responded promptly to the treatment with pain relief, wound healing and some increase in the distance to claudication. The improvement was long lasting and 59% of patients enjoyed the positive effect of the treatment at the 6 years post-implantation check point. 31% of patients deteriorated after one year and 7 had to have the leg amputated.
4. The novel aspect of this presentation relies on the observation that the treatment failure was associated with a higher proportion of CD34-CD45-90+ (median 0.19% vs 0.06%; p=0.13) and CD34-CD45-CD73+ (median 0.15% vs 0.03%; p=0.037) cells in the inocula than was seen in patients responding well to the treatment.

In conclusion, a higher proportion of cells with MSC phenotype do not facilitate revascularization but on the contrary they prevent stable angiogenesis.

Laboratory of Immunogenetics and Tissue Immunology
Head: Professor Piotr Kuśnierczyk, Ph.D.

ERAP1 single nucleotide polymorphisms in non-small cell lung cancer patients and healthy controls in Poland and China

This work was done in collaboration with Professor Li Shi and coworkers from the Institute of Medical Biology in Kunming, Chinese Academy of Medical Sciences (CAMS) and Peking Union Medical College, China.

An effective cytotoxic immune response to neoplastic cells requires efficient presentation of antigenic peptides to T lymphocytes by HLA class I (HLA-I) molecules. The HLA-I-bound peptide repertoire depends on antigen-processing machinery molecules. One of these, aminopeptidase residing in endoplasmic reticulum 1 (ERAP1), trims peptides to optimal length for HLA-I binding. Single nucleotide polymorphisms (SNPs) in the *ERAP1* gene result in changes in aminopeptidase activity and specificity. This affects susceptibility to many diseases, including cancer. However, non-small cell lung carcinoma (NSCLC), the most frequent type of lung cancer, has not been studied in this respect yet. We tested 420 cases and 385 healthy control individuals in China as well as 317 cases and 506 controls in Poland for genotype and haplotype frequencies of four coding and nonsynonymous ERAP1 SNPs: rs26653G>C [R127P], rs26618T>C [I276M], rs30187C>T [K528R], and rs27044C>G [Q730E]. We found associations of all four SNPs with NSCLC in Chinese but not in Poles. No differences in SNP frequencies between squamous cell carcinoma and adenocarcinoma were found, although a weak trend in Poles for two SNPs (rs26653 and rs30187) was observed. The differences between Chinese and Poles might be explained by highly significant differences in SNP genotype frequencies between Chinese and Poles (except for rs26618). In accord with this, the most frequent ERAP1 haplotypes were distributed differently in cases versus controls in Chinese, but not in Poles. Our findings add to the differences between Orientals and Caucasians in genetics of disease susceptibility.

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

Polymorphism and expression of proinflammatory factors in patients with rheumatic diseases treated with TNF-alpha inhibitors

Our previous studies documented that polymorphisms within IL-17A, IL-17F, TNF-alpha and its receptor encoding genes may affect the predisposition to rheumatoid arthritis (RA) or the efficacy of therapy with TNF-alpha inhibitors (*Arch Immunol Ther Exp*. 2015, *Joint Bone Spine* 2015). We also found that non-classical HLA-E alleles may play an important role in patients with RA (*Clin Exp Immunol*, 2015) and psoriatic arthritis (PsA) (*Human Immunol*, 2014).

The objective of the present study was to determine the polymorphism of genes coding for IL-6, IL-6R, IL-33 and NF-kB in patients with RA, PsA and in a control group of healthy individuals in order to detect potential associations between the polymorphic variants and susceptibility, course of disease as well as response to treatment.

In total, 289 individuals, including 86 patients with RA, 77 with PsA and 126 healthy controls, were investigated. Capillary electrophoresis and PCR-RFLP (restriction fragment length polymorphism) with *Pfl*M1 restriction enzyme were used to determine the polymorphic variants in the promoter region of the *NF-kB1* gene (rs28362491, -94 ins/del ATTG). The single nucleotide polymorphism (SNP) in the *IL-6* promoter (rs1800795, -187 C>G) was determined by TaqMan probe. LightSNiP assays using the high resolution melting (PCR-HRM) real-time PCR technique were employed to determine SNPs within the IL-33 (rs7044343, C>T) and IL-6 receptor encoding genes (rs2228145, A>C, Asp358Ala).

The results were as follows:
(1) - *NF-kB1* gene polymorphism:
• the *del/del* genotype was more frequent in women with PsA than in healthy women (p=0.025); peripheral arthritis was more common in patients with *ins/del* genotype, whereas axial arthritis was more common in those with *ins/ins* genotype; methotrexate (MTX) therapy more commonly was unsuccessful in *ins/ins* homozygotes as compared to patients with any other genotypes, while *ins/del* heterozygotes were better responders to MTX treatment; remission was more frequent in PsA patients with *ins/ins* genotype;
• RA patients with *ins/ins* genotype were unresponsive to anti-TNF therapy (61.1%) more frequently than patients with *del/del* (22.2%) and *ins/del* genotype (16.7%) (p=0.023);
(2) - *IL33* gene polymorphism:
• *IL33* homozygosity was more frequently detected in female than in male PsA patients (p=0.0598); PsA patients harboring the *CT* genotype had higher IL-33 levels (CT: 40.8 pg/ml, CC: 18.1 pg/ml; TT: 16.96 pg/ml);
(3) – *IL-6* and *IL-6R* polymorphisms:
• *IL6 GG* homozygosity was more common among healthy men than in male patients with PsA (p=0.019) as well as in healthy men than in healthy women (p=0.024);
• the *G* allele was associated with higher IL-6 and CRP levels in serum of patients with PsA ($p<0.05$ in both cases);
• PsA patients carrying the *IL6* heterozygosity showed a worse response to MTX therapy than patients harboring the *CC* or *GG* genotypes (p=0.005);
• no correlations were observed for the *IL-6R* SNP rs2228145 (A>C, Asp358Ala).
(4) - other observed associations:
• higher levels of IL-6 and IL-33 were detected in PsA patients before the initiation of therapy as compared to healthy controls; also, a correlation between high IL-6 levels and levels of the acute-phase protein CRP was detected in the group of PsA patients (R2=0.4296).

Cereblon-coding gene polymorphism and its role in lenalidomide therapy in patients with multiple myeloma

Lenalidomide (Revlimid; a derivative of thalidomide) is one of the immunomodulatory drugs used in treatment for multiple myeloma; however, its use is ineffective in some patients. Recent research suggests than cereblon (CRBN) is an important factor in metabolism of immunomodulatory drugs and that β-catenin (coded by the *CTNNB1* gene) is partly responsible for the lenalidomide resistance (and resistance to other immunomodulatory drugs) developing in some patients.

The objective of this research was to determine the polymorphic variants of the *CRBN* and *CTNNB1* genes and to analyze their relation to the response to lenalidomide treatment in patients with multiple myeloma (MM) (*Leuk Res.* 2015).
The polymorphisms of the cereblon and β-catenin encoding genes (*CRBN* rs121918368 C>T, *CTNNB1* rs4135385 A>G and rs4533622 A>C) were analyzed in 142 MM patients and 123 healthy individuals employing the LightSNiP assays and melting curve analysis.

No significant differences in the distribution of either allelic or genotype frequencies were observed between patients and healthy controls. The *CTNNB1* (rs4533622) *A* allele was more frequently detected in patients in stages II-III of the disease (according to the Durie-Salmon

criteria and International Prognostic Index, $p<0.05$ in both cases). As for the rs4135385 *CTNNB1* SNP, the *G* variant was more frequent among patients who did not respond to the CTD treatment (cyclophosphamide, thalidomide, dexamethasone; $p=0.047$), while the rs4533622 *C* variant was more frequent in patients with a worse response to treatment with thalidomide-containing regimens. No significant association with lenalidomide treatment was observed. Nonetheless, the occurrence of neutropenia during lenalidomide therapy was observed more frequently in *CTNNB1* (rs4135385) *AA* homozygotes ($p=0.019$), and high grade (3-4) neutropenia was characteristic for patients with *CTNNB1* (rs4533622) *AA* ($p=0.044$). These results seem to be in accordance with the expected role of β-catenin in the development of resistance to immunomodulatory drugs.

DEPARTMENT OF TUMOR IMMUNOLOGY
Head: Professor Paweł Kisielow, Ph.D.

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

Ikaros and RAG-2-mediated antisense transcription are responsible for inactivation of the promoter of the NWC gene in lymphocytes

NWC is the third evolutionarily conserved gene identified within the recombination activating gene (RAG) locus. The function of NWC protein is unknown. The predicted structure of vertebrate NWC proteins includes three unique and highly conserved domains, out of which two are present also in NWC homologs present in numerous invertebrate species, including *Trichoplax adhaerens* (Placozoa), *Nematostella vectensis* and *Strongylocentrotus purpuratus.* Considering the evolutionary conservation of NWC protein and its unique structure, an effort to learn about the function of the NWC gene and protein seems to be well justified. For this purpose we generated *NWC*-deficient mice and attempted to find proteins binding to NWC protein. We generated NWC-deficient mice unable to produce functional NWC protein by taking advantage of heterozygous B230118H07Rik$^{tm1a(KOMP)Wtsi}$ (NWC-KOMP) mice. These mice contain a gene trap cassette inserted in *NWC* intron 4, and exon 5 flanked by loxP sequences preventing generation of the transcript encoding functional NWC protein with conserved domains. NWC-KOMPcre mice with deleted exon 5 were obtained by crossing NWC-KOMP mice with cre-deleter mice. The homozygous NWC-KOMPcre mice were observed for 6 months and showed no obvious morphological, anatomical, physiological or reproductive abnormalities.

To look for possible subtle effects of NWC deletion we decided to analyze tissue that in normal mice expresses the highest level of NWC protein. Using the NWC-YFP reporter mouse strain we found that the highest level of expression of NWC protein is present in testes. These findings were confirmed by Western blotting experiments. We then compared testes from normal and *NWC*-deficient mice and found no difference in tissue organization, cellularity or morphology.

The failure to observe any visible effects on the phenotype of NWC-KOMPcre mice prompted us to use an indirect approach to gain insight into the function of *NWC* by searching for the protein partners binding to the NWC protein. To identify proteins interacting with

NWC protein we performed co-immunoprecipitation experiments. The proteins co-precipitated with NWC-specific antibody from lysates of testes from normal mice were identified by mass spectrometry. Protein lysates from testes of NWC-KOMPcre mice served as a negative control. Eight proteins, specifically and repeatedly precipitated from tissue lysates of normal mice, represent the possible candidates of NWC binding proteins. Several of these proteins are known to be involved in the formation and functioning of the cilia. To support these findings we checked whether NWC protein is present in those cell structures. To do this we induced ciliogenesis in NIH3T3 cells and stained them with Ab285 antibody and anti-acetylated alpha tubulin (cilia marker). The results showed that NWC is localized in most of the primary cilia projecting from the cell surface

Further experiments are needed to investigate the exact role of NWC protein in the intra-flagellar transport and function of cilia and its possible role in spermatogenesis.

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

Linker for activation of T cells (LAT) inhibits development of aggressive thymic lymphomas by mediating pre-TCR independent down-regulation of Notch-1 expression

Linker for Activation of T Cells (LAT) is a raft-associated, transmembrane adaptor protein whose expression promotes expansion and selection of thymic T cell precursors. The positive role of LAT in thymocyte maturation is however counterbalanced by its poorly understood inhibitory role in development of thymic lymphoma. This latter role can be evidenced when LAT deficient mice (*LAT-/-*) are crossed with pLFG-C (line 3073) transgenic mice harboring an allele of chronically active LCK kinase (LCK^{Y505F}). The resulting *LAT-/-* LCK^{Y505F} progeny but not control CD3ε*-/-* LCK^{Y505F} mice develop fully penetrant T-cell acute lymphoblastic leukemia (T-ALL).

We aimed to identify gene expression changes underlying the malignant transformation of precancerous to cancerous T-ALL stage in *LAT-/-*LCK^{Y505F} mice. We focused on T-ALL-related gene transcripts representative of 15 different signaling pathways. Comparative, transcriptomic analysis of CD4+CD8+ (DP) thymocytes from 3-week-old CD3ε*-/-* LCK^{Y505F} and *LAT-/-* LCK^{Y505F} mice prior to overt signs of neoplasia led to identification of early, critical, LCK-driven oncogenic signals leading to the initiation of T-ALL. By profiling thymomas of *LAT-/-* LCK^{Y505F} mice, thymoma-derived cell lines and lymph node metastases we found gene expression changes accompanying T-ALL progression. Our data revealed that T-ALL in *LAT-/-* LCK^{Y505F} is a Notch-driven leukemia and implied a novel role for LAT in down-modulation of Notch-1 and pTα expression during β-selection of thymocytes.

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

Laboratory of Glycoconjugate Immunochemistry
Head: Professor Hubert Krotkiewski, Ph.D.

Immunochemical and genetic studies on human glycophorin and other proteins active in the immune system

Analysis of IgG-derived N-glycans using LC-MS technique

Human serum IgG has been previously recognized as a biomarker in several rheumatic diseases. Briefly, it was shown that its carbohydrate moiety is affected under these pathological conditions by lacking the terminal galactose residues in the conservative N-glycans, which is proportional to the severity of the disease. Therefore, it is interesting to determine the status of galactosylation of these IgG N-glycans, firstly to determine the current clinical stage of the disease, and secondly to know the efficacy of the clinical treatment applied. IgG N-glycans can be analyzed in a native immunoglobulin molecule (ELISA method or SPR technique), as glycopeptides (after protease treatment) or as free oligosaccharides, released by the enzymatic or chemical method. In this investigation we applied the chemical method (1 M NaOH, 1 M $NaBH_4$, 100°C, 6 h). IgG was isolated from serum using affinity chromatography on a Sepharose-protein A/G column. The IgG samples were subjected to strong alkaline degradation and the released N-glycans were isolated in the reduced form, then they were re-N-acetylated using Carbograph minicolumns (GL Sciences, Tokyo). The samples of N-glycans were desalted using a BioGel P-4 column, eluted with Milli-Q water and then were subjected to LC-MS analysis. Based on identified molecular *m/z* ions 13 different structures of the N-glycans could be established. They were biantennary chains, partially sialylated, partially galactosylated, partially fucosylated and partially GlcNAc-bisected. Performed experiments revealed that it is possible to determine the glycosylation profile of serum IgG using the LC-MS technique on free, reduced N-glycans.

Expression and purification of the recombinant binding region of **Plasmodium reichenowi** *eba-140 ligand*

Plasmodium reichenowi, which infects chimpanzees, is the closest relative of *P. falciparum* – the causative agent of malignant malaria. They are morphologically identical and genetically similar parasites. Each of these parasites has co-evolved with its host, but that does not rule out chimpanzee to human transmission, or vice versa. Research on homologous *Plasmodium* species such as *P. reichenowi* and *P. falciparum* is important for understanding the restrictive host specificity of the *Plasmodium* genus.

We used synthetic cDNA coding for EBA-140 Region II of *P. reichenowi.* Expression of the recombinant form of EBA-140 RII with 6xHis and c-myc tags at its C-terminus was carried out in High Five cells using a baculovirus expression vector (BD Biosciences). The recombinant EBA-140 RII was purified from medium by affinity chromatography on NiNTa-agarose. The purity of the obtained recombinant protein was examined using Western blotting and SDS-PAGE methods. Binding of the recombinant EBA-140 Region II to chimpanzee erythrocytes was evaluated by flow cytometry using native, neuraminidase, trypsin-treated or chymotrypsin-treated red blood cells. It was found that EBA-140 Region II binds to normal and trypsin-treated chimpanzee erythrocytes, but its binding to erythrocytes treated with

neuraminidase or chymotrypsin was significantly decreased. These results indicated that baculovirus-expressed Region II is a functional protein and its interaction with chimpanzee erythrocytes is specific and sialic acid-dependent.

Laboratory of General Immunochemistry
Head: Professor Maria Janusz, Ph.D.

Studies on the mechanism of action of proline-rich polypeptide complex (PRP)

Comparative studies on a proline-rich polypeptide complex isolated from ovine colostrum and a complex isolated from hen egg yolk – Yolkin: effect on BDNF expression and secretion.

The development and function of the nerve tissue is under control of the nerve growth factor (NGF) and brain-derived nerve growth factor (BDNF). In the central nervous system (CNS) the hippocampus, neocortex and neuronal function as well as synaptic plasticity are controlled by BDNF. In neurodegenerative processes, lowered secretion of trophic factors is observed. In consequence of a reduced brain BDNF level, deterioration of cognitive and memory function takes place.

It was previously shown by us that a colostral derived polypeptide complex (PRP) with immunoregulatory and antioxidant effects, in the form of orally administered Colostrinin tablets, showed a positive therapeutic effect in the case of Alzheimer's disease. Hen eggs play a similar role for newborn chicken as colostrum plays for newborn mammals. In hen eggs the polypeptide complex with immunostimulatory properties was shown by us to be a fraction, similarly as in the mammal system, accompanying the main immunoglobulin fraction – IgY. Because of some similarities in both polypeptides' activities such as the effect on cytokine and nitric oxide release and taking into account that hen eggs are a convenient, effective and readily attainable source of Yolkin, it was interesting to compare the neuroprotective effects of PRP and Yolkin.

Purity and activity of both isolated preparations were evaluated by electrophoresis and ability to induce IL-6 secretion. Using rat PC12 cells the effect of PRP and Yolkin on BDNF expression and secretion was studied. It was found that both complexes were active cytokine inducers, and no cytotoxic effect was observed even in a dose of 150 μg/ml of cell culture. In the presence of Yolkin the mature form of BDNF was secreted in a dose-dependent manner (control = 0; $Y_{1\mu g/ml} = 0$; $Y_{10\mu g/ml} = 6$ pg; $Y_{100\mu g/ml} = 269$ pg/ml; $Y_{150\mu g/ml} = 525$ pg/ml). No effect on BDNF expression was observed.

It is suggested that Yolkin influences the release of BDNF stored inside the cell. This effect may be advantageous in therapy of neurodegenerative disorders.

Laboratory of Microbial Immunochemistry and Vaccines
Head: Professor Czesław Ługowski, Ph.D.

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

Lipopolysaccharide (LPS, endotoxin) is the main surface antigen of Gram-negative bacteria, called O antigen. LPS is built of lipid A substituted with a core oligosaccharide (OS) that is further substituted with the O-specific polysaccharide (O-PS) built of oligosaccharide repeating units (RU). In most species core oligosaccharides contain an outer core (hexose region) and inner core (heptose region). As the main virulence factor LPS constitutes a pathogen-associated molecular pattern and is the target for the rapid response of the innate immunity system. Lipid A is the most conservative region of LPS recognized by receptors present on the surface of target cells for LPS, such as the CD14/TLR 4/MD2 receptor complex. The core OS and O-PS modulate the activity of lipid A, influence persistence of LPS aggregates in the bloodstream and mask the lipid A region. Among substituents modifying LPS structure and activity are various amino acids, O-acetyl groups, phosphate groups (P), pyrophosphate groups (PP) and ethanolamine (Etn). They were reported to occur in all regions of lipopolysaccharides and, besides O-acetyl groups and amino acids, are common substituents of the core OS. It is known that modifications of core OS and lipid A with amine-containing substituents (Etn, ester-linked amino acids, glycine) or the lack of P and PP represent an adaptive mechanism of reduction of the net negative charge of the bacterial surface promoting cationic resistance against cationic antimicrobial peptides such as polymyxin B. Thus such structural modifications affect the virulence of pathogenic bacteria. For years glycine (Gly) was identified among nonsugar substituents in a variety of LPS preparations. The first reports described the presence of Gly in LPSs of *Escherichia coli* and *Salmonella enterica* serovar typhimurium. Afterwards Gly was found as an integral constituent of core OS fractions isolated from LPS of *Escherichia coli*, *Salmonella* spp., *Hafnia alvei*, *Citrobacter* spp., *Shigella sonnei*, *Vibrio cholerae*, *Thiobacillus* spp. and *Proteus* spp. The first indication of the covalent linkage between core OS and Gly was based on GC-MS analyses. Together with the development of modern analytical techniques, such as multiple stage electrospray ionization mass spectrometry (ESI-MSn), capillary electrophoresis (CE)-ESI-MS, and matrix-assisted laser desorption ionization time-of flight (MALDI-TOF) mass spectrometry (MS), occurrence of Gly was further supported by detailed MSn analyses. The described analytical techniques allowed for wide range screening of Gly substitution in LPSs of typeable and nontypeable *Haemophilus influenzae* strains, where the amino acid was predominantly identified in the inner core region of the core OS. Among other glycinylated examples are core OSs of lipopolysaccharides isolated from several strains of *Neisseria meningitidis*, *Proteus mirabilis*, *Shigella flexneri*, *Campylobacter jejuni* and *Plesiomonas shigelloides*. Since glycine residue substitutes mainly the core oligosaccharide of the LPS, especially the inner core region, it was also considered as a part of the common epitope for broad-reactive antimicrobial antibodies. The presented results supplemented described data with information about occurrence of Gly in core OSs of *H. alvei* lipopolysaccharides isolated from strains 32, PCM (Polish Collection of Microorganisms) 1190, PCM 1192, PCM 1200 and PCM 1209. This bacterium is an opportunistic human pathogen which contributes to bacteremia and septicemia in humans and animals, but also to acute cholecystitis, mixed hospital infections, appendicitis, community-acquired cholangitis and respiratory diseases. We used multiple-stage electrospray ionization mass spectrometry to identify glycine substitution in the core oligosaccharide type characteristic for *Hafnia alvei* LPS, and isolated from five strains of different O-serotypes: 32, PCM 1190, PCM 1192, PCM 1200, and PCM

1209. The location of glycine in the core oligosaccharide was determined in detail for LPS 1190 using ESI-MS n. Three glycoforms were identified, including two monoglycinylated and one diglycinylated core oligosaccharides.

Publication - 2015

Articles published in the journals from Thomson Reuters Master Journal List:

1. Antoszczak M, Sobusiak M, Maj E, Wietrzyk J, Huczyński A.: Synthesis and antiproliferative activity of new bioconjugates of Salinomycin with amino acid esters. *Bioorg Med Chem Lett*. 2015, 25, 3511-3514, DOI: 10.1016/j.bmcl.2015.06.086. **IF – 2,420 (25 pkt.)**
2. Ashline D.J, Reinhold V.N, Duk M, Lukasiewicz J, Lisowska E, Jaskiewicz E.: The structures of glycophorin C N-glycans, a putative component of the GPC receptor site for *Plasmodium falciparum* EBA-140 ligand. *Glycobiology*, 2015, 25(5):570-81. doi: 10.1093/glycob/cwu188. **IF – 3,147 (30 pkt.)**
3. Atarod S., Turner B., Pearce K.F., Ahmed S.S., Norden J., Bogunia-Kubik K., Wang X.N., Collin M., Dickinson A.M.: Elevated level of HSPA1L mRNA correlates with graft-versus-host disease. *Transpl Immunol*, 2015, 32(3), 188-94 S0966-3274(15)00006-4. **IF - 1,457 (20 pkt.)**
4. Awad S. M., Fathalla O. A., Wietrzyk J., Milczarek M, Soliman A. M, Mohamed Mosaad S.: Synthesis of new pyrimidine derivatives and their antiproliferative activity against selected human cancer cell lines. *Res Chem Intermed* 2015, 41:1789–1801, DOI 10.1007/s11164-013-1312-z **IF - 1,221 (20 pkt.)**
5. Bączkowicz D, Majorczyk E, Kręcisz K.: Age-related impairment of quality of joint motion in vibroarthrographic signal analysis. *Biomed Research International*, 2015, 2015, 591707 doi: 10.1155/2015/591707 (online) **IF – 1,579 (20 pkt.)**
6. Bil-Lula I, Sochocka M, Zatońska K, Szuba A, Sawicki G, Woźniak M.: Adenovirus type 9 enhances differentiation and decreases cytokine release from preadipocytes. *J. Med. Virol*, 2015; 87: 230-239 **IF - 2,347 (25 pkt.)**
7. Blach-Olszewska Z, Zaczynska E, Gustaw-Rothenberg K, Avila-Rodrigues M, Barreto GE, Leszek J., Aliev G.: The innate immunity in Alzheimer disease- relevance to pathogenesis and therapy. *Curr Pharm Des*, 2015 Jul 10, 21(25), 3582-8 **IF - 3,452 (35 pkt.)**
8. Blazejczyk A, Papiernik D, Porshneva K, Sadowska J, Wietrzyk J.: Endothelium and cancer metastasis: Perspectives for antimetastatic therapy. *Pharm Rep*, 2015, 67(4):711-8 **IF – 1,928 (25 pkt.)**
9. Bogunia-Kubik K, Mizia S, Gronkowska A, Nowak J, Kyrcz-Krzemień S, Markiewicz M, Dzierżak-Mietła M, Koclęga A, Sędzimirska M, Suchnicki K, Duda D, Lange J, Mordak-Domagała M, Kościńska K, Węzik S, Jędrzejczak WW, Kaczmarek B, Hellmann A, Kucharska A, Kowalczyk J, Drabko K, Warzocha K, Mika-Witkowska R, Goździk J, Lange A. CCR5 gene polymorphism affects the risk of GvHD after haematopoietic stem cell transplantation from an unrelated donor. *Br J Haematol*, 2015 Mar 30. doi: 10.1111/bjh.13387. **IF – 4,971 (40 pkt.)**

10. Bogunia-Kubik K., Mizia S., Polak M., Gronkowska A., Nowak J., Kyrcz-Krzemień S., Markiewicz M., Dzierżak-Mietła M., Koclęga A., Sędzimirska M., Suchnicki K., Duda D., Lange J., Mordak-Domagała M., Kościńska K., Jędrzejczak W.W., Kaczmarek B., Hellmann A., Kucharska A., Kowalczyk J., Drabko K., Warzocha K., Hałaburda K., Tomaszewska A., Mika-Witkowska R., Witkowska A., Goździk J., Mordel A., Wysoczańska B., Jaskula E., Lange A.: Beneficial effect of the CXCL12-3'A variant for patients undergoing hematopoietic stem cell transplantation from unrelated donors. *Cytokine*, 2015 May 14. pii: S1043-4666(15)00171-4 **IF - 2,664 (25 pkt.)**
11. Bogunia-Kubik K**.**, Świerkot J., Malak A., Wysoczańska B., Nowak B., Białowąs K., Gębura K., Korman L., Wiland P.: IL-17A, IL-17F and IL-23R Gene Polymorphisms in Polish Patients with Rheumatoid Arthritis. *Arch Immunol Ther Exp*, 2015; 63(3), 215-21 **IF - 3,176 (25 pkt.)**
12. Brzozowska E, Śmietana M, Koba M, Górska S, Pawlik K, Gamian A, Bock WJ.: Recognition of bacterial lipopolysaccharide using bacteriophage-adhesin-coated long-period gratings. *Biosens Bioelectron*. 2015 May 15;67:93-9. doi: 10.1016/j. bios.2014.07.027 **IF – 6,409 (40 pkt.)**
13. Butrym A., Rybka J., Łacina P., Gębura K., Frątkiewicz D., Bogunia-Kubik K., Mazur G.: Polymorphisms within beta-catenin encoding gene is of prognostic value for patients with multiple myeloma. *Leukemia Res*, 2015, pii: S0145-2126(15)30532-4. **IF - 2,351 (25 pkt.)**
14. Całkosiński I, Płoneczka-Janeczko K, Ostapska M, Dudek K, Gamian A, Rypuła K.: Microbiological analysis of necrosols collected from Urban Cemeteries in Poland. *Biomed Res Int,* 215;2015:169573. doi: 10.1155/2015/169573. **IF- 1,579 (20 pkt.)**
15. Całkosiński I, Rosińczuk-Tonderys J, Bronowicka-Szydełko A, Dzierzba K, Bazan J, Dobrzyński M, Majda J, Gamian A.: Effect of tocopherol on biochemical blond parameters in pleuritis-induced rats treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicol Ind Health*, 2015 Jun;31(6):510-22. doi: 10.1177/0748233713475497 **IF – 1,859 (25 pkt.)**
16. Chmielewska M, Symonowicz K, Pula B, Owczarek T, Podhorska-Okolow M, Ugorski M.: Dziegiel P.:Expression of metallothioneins I and II in kidney of doxorubicin-treated rats. *Exp Toxicol Pathol*, 2015, 67: 297 – 303 **IF – 2,005 (25 pkt.)**
17. Ciechomska M., van Laar J., O'Reilly S.: Current frontiers in systemic sclerosis pathogenesis. *Exp Dermatol*, 2015, 24(6), 401-6 **IF-3,76 (40 pkt.)**
18. Cyganek A, Nowaczyk M, Sańko-Resmer J, Pietrzak B, Grzechocińska B, Pączek L, Międzybrodzki R, Wielgoś M.: The effect of pregnancy on humoral rejection in patients after vascularized organ transplantation. *J Reprod Immunol*, 2015;112:115-119. doi: 10.1016/ j.jri.2015.08.005. **IF – 2,815 (30 pkt.)**
19. Cytarska J, Skowerski K, Jaworski S, Misiura K, Filip-Psurska B, Wietrzyk J.: The Disulfide Analogues of Isophosphoramide Mustard for Anticancer Therapy. *Lett Drug Design Discov*, 2015, 12(3):172-179 **IF – 0,770 (15 pkt.)**
20. Czerwiński M.: Grupy krwi: minusy i plusy. Czy antygeny grupowe krwi chronią nas przed chorobami zakaźnymi? *Post Hig Med Dośw,* 2015, 69, 703-722 **IF – 0,573 (15 pkt.)**
21. Delplanque A, Wawrzyńczyk D, Jaworski P, Matczyszyn K, Pawlik K, Buckle M, Nyk M, Nogues C, Samoć M.: DNA base pair resolution measurements using resonance energy transfer efficiency in lanthanide doped nanoparticles, *PLOS One* DOI:10.1371/ journal.pone.0117277 March 6,10(3) 2015 **IF – 3,234 (40 pkt.)**

22. Donczew R, Makowski Ł, Jaworski P, Bezulska M, Nowaczyk M, Zakrzewska-Czerwińska J, Zawilak-Pawlik A.: The atypical response regulator HP1021 controls formation of the *Helicobacter pylori* replication initiation complex. *Mol. Microbiol*., 2015, Jan; 95(2):297-312. doi: 10.1111/mmi.12866 **IF – 4,419 (35 pkt.)**
23. Dylong A, Sowa M, Goldman W, Ślepokura K, Drożdżewski P**,** Szponar B, Matczak-Jon E.: Zinc(II) complexes derived from imidazo[1,2-a]pyridin-2-ylacetic acid (HIP-2-ac): [Zn(IP-2-ac)2(H2O)] and unexpectedly, [Zn3(IP-2-ac)6(H2O)]·11H2O. *J Coordin Chem*, 2015, 68: 2208-2224 **IF – 2,012 (25 pkt.)**
24. Felcenloben I, Piasecki T, Miller J, Rossowska J, Bańcyr E, Atamaniuk W, Nowak M, Świerkot J, Ratajczak K, Chełmońska-Soyta A.: Adoptively transferred Tregs accumulate in a site-specific manner and ameliorate signs of less advanced collagen-induced arthritis progress in rats. *Immunotherapy,* 2015;7(3):215-28. doi: 10.2217/imt.14.121. **IF – 2,070 (20 pkt.)**
25. Frydecka D, Beszłej JA, Pawlak-Adamska E, Misiak B, Karabon L, Tomkiewicz A, Partyka A, Jonkisz A, Szewczuk-Bogusławska M, Zawadzki M, Kiejna A.: CTLA4 and CD28 Gene Polymorphisms with Respect to Affective Symptom Domain in Schizophrenia. *Neuropsychobiology,* 2015, 71(3), 158-67. doi: 10.1159/000379751 **IF – 2,261 (25 pkt.)**
26. Frydecka D, Misiak B, Pawlak-Adamska E, Karabon L, Tomkiewicz A, Sedlaczek P, Kiejna A, Beszłej JA.: Sex differences in TGFB-β signaling with respect to age of onset and cognitive functioning in schizophrenia. *Neuropsychiatr Dis Treat*, 2015 Mar 5;11:575-84. doi: 10.2147/NDT.S74672 **IF – 2,154 (25 pkt.)**
27. Frydecka D, Misiak B, Pawlak-Adamska E, Karabon L, Tomkiewicz A, Sedlaczek P, Kiejna A, Beszłej JA.: Interleukin-6: the missing element of the neurocognitive deterioration in schizophrenia? The focus on genetic underpinnings, cognitive impairment and clinical manifestation. *Eur Arch Psychiatry Clin Neurosci*, 2015, 265(6), 449-59. doi: 10.1007/s00406-014-0533-5. **IF – 3,525 (30 pkt.)**
28. Futoma-Kołoch B, Godlewska U, Guz-Regner K, Dorotkiewicz-Jach A, Klausa E, Rybka J, Bugla-Płoskońska G. Presumable role of outer membrane proteins of Salmonella containing sialylated lipopolysaccharides serovar Ngozi, sv. Isaszeg and subspecies arizonae in determining susceptibility to human serum. *Gut Pathog*, 2015;7:18. doi: 10.1186/s13099-015-0066-0. eCollection 2015. **IF – 2, 281 (25 pkt.)**
29. Gnach A, Lipinski T, Bednarkiewicz A, Rybka J, Capobianco JA.: Upconverting nanoparticles: assessing the toxicity. *Chem Soc Rev*. 2015, 44(6):1561-84. doi: 10.1039/c4cs00177j. **IF – 33,383 (50 pkt.)**
30. Goldeman W, Nasulewicz-Goldeman A.: Synthesis and biological evaluation of aminomethylidenebisphosphonic derivatives of the β-arylethylamines. *Tetrahedron,* 2015, 71: 3282-3289. DOI: 10.1016/j.tet.2015.03.112 IF – **2,641 (30 pkt.)**
31. Goszczyński T, Kowalski K, Leśnikowski Z, Boratyński J.: Solid state, thermal synthesis of site-specific protein-boron cluster conjugates and their physicochemical and biochemical properties. *Biochim Biophys Acta (General Subjects),* 2015, 1850(2), 411-8. doi: 10.1016/j.bbagen.2014.11.015 **IF – 4,381 (35 pkt.)**
32. Gozdziewicz TK, Man-Kupisinska A, Lugowski C, Lukasiewicz J.: Occurrence of glycine in the core oligosaccharides of *Hafnia alvei* lipopolysaccharides--identification of disubstituted glycoform. *Carbohydr Res,* 2015; 408:119-26 **IF – 1,929 (25 pkt.)**

33. Goździewicz T.K, Łukasiewicz J, Ługowski C.: The structure and significance of enterobacterial common antigen (ECA). *Post Hig Med Dośw,* (online) 2015; 69, 1003-12 **IF – 0,573 (15 pkt.)**
34. Górski A, Dąbrowska K, Hodyra-Stefaniak K, Borysowski J, Międzybrodzki R, Weber-Dąbrowska B.: Phages targeting infected tissues: novel approach to phage therapy. *Future Microbiol*. 2015, 10, 199-204. **IF – 4,275 (35 pkt.)**
35. Grzywnowicz M, Karabon L, Karczmarczyk A, Zajac M, Skorka K, Zaleska J, Wlasiuk P, Chocholska S, Tomczak W, Bojarska-Junak A, Dmoszynska A, Frydecka I, Giannopoulos K.: The function of a novel immunophenotype candidate molecule PD-1 in chronic lymphocytic leukemia. *Leuk Lymphoma,* 2015, 56(10) 2908-13. doi: 10.3109/10428194.2015. 1017820 **IF – 2,891 (25 pkt.)**
36. Gutarowska B, Skóra J, Stępień Ł, Szponar B, Otlewska A, Pielech-Przybylska K. Assessment of microbial contamination within working environments of different types of composting plants. *J Air Waste Manag Assoc*. 2015, 65(4):466-78. doi: 10.1080/10962247.2014.960954 **IF – 1,342 (20 pkt.)**
37. Hodyra K., Dąbrowska K.: Molecular and chemical engineering of bacteriophages for potential medical applications. *Arch Immunol Ther Exp*, 2015, 63(2), 117-127 **IF – 3,176 (25 pkt.)**
38 Hodyra-Stefaniak K, Miernikiewicz P, Drapała J, Drab M, Jończyk-Matysiak E, Lecion D, Kaźmierczak Z, Beta W, Majewska J, Harhala M, Bubak B, Kłopot A, Górski A, Dąbrowska K.: Mammalian Host-Versus-Phage immune response determines phage fate *in vivo*. *Scientific Rep*, 2015, 5 14802 **IF - 5,578 (40 pkt.)**
39 Hoffmann K, Łakomska I, Wiśniewska J, Kaczmarek-Kędziera A, Wietrzyk J. Acetate platinum(II) compound with 5,7-ditertbutyl-1,2,4-triazolo[1,5-a]pyrimidine that overcomes cisplatin resistance: structural characterization, *in vitro* cytotoxicity, and kinetic studies. *J Coord Chem*, 2015, 68:17-18, 3193-3208, doi: 10.1080/00958972. 2015.1070954 **IF – 2,012 (25 pkt.)**
40. Huczyński A, Antoszczak M, Kleczewska N, Lewandowska M, Maj E, Stefańska J, Wietrzyk J, Janczak J, Celewicz L.: Synthesis and biological activity of salinomycin conjugates with floxuridine. *Eur J Med Chem*. 2015, 93C:33-41. doi: 10.1016/j .ejmech.2015.01.045 **IF – 3,447 (40 pkt.)**
41. Huczyński A, Klejborowska G, Antoszczak M, Maj E, Wietrzyk J.: Anti-proliferative activity of Monensin and its tertiary amide derivatives. *Bioorg Med Chem Lett*, 2015, 25(20):4539-43, doi: 10.1016/j.bmcl.2015.08.067. IF – **2,420 (25 pkt.)**
42. Huczyński A, Rutkowski J, Popiel K, Maj E, Wietrzyk J, Stefańska J, Majcher U, Bartl F.: Synthesis, antiproliferative and antibacterial evaluation of C-ring modified colchicine analogues. *Eur J Med Chem*, 2015**,** 90: 296-301 **IF** – **3,447 (40 pkt.)**
43. Iwaszko M., Świerkot J., Kolossa K., Jeka S., Wiland P., Bogunia-Kubik K.: Polymorphisms within the HLA-E gene and their associations with susceptibility to rheumatoid arthritis as well as clinical outcome of anti-TNF therapy. *Clin Exp Immunol*, 182(3), 270-7 **IF - 3,037 (30 pkt.)**
44. Iwaszko M., Świerkot J., Kolossa K., Jeka S., Wiland P., Bogunia-Kubik K.: Influence of CD94 and NKG2A variants on susceptibility to rheumatoid arthritis and efficacy of anti-TNF treatment. *Joint Bone Spine*, 2015 pii: S1297-319X(15)00162-1 **IF - 2,901 (25 pkt.)**

45. Jankowska E, Wojtas K, Kasztura M, Mazur G, Butrym A, Kalicinska E, Rybinska I, Skiba J, von Haehling S, Doehner W, Anker S, Banasiak W, Cleland J, Ponikowski P.: Bone marrow iron depletion is common in patients with coronary artery disease. *Int J Cardiol*. 2015, 182:517-22. doi: 10.1016/j.ijcard.2014.10.006 **IF – 4,036 (35 pkt.)**
46. Jasek M, Wagner M, Sobczynski M, Wolowiec D, Kuliczkowski K, Woszczyk D, Kielbinski M, Kusnierczyk P, Frydecka I, Karabon L.: Polymorphisms in genes of the BAFF/APRIL system may constitute risk factors of B-CLL - a preliminary study on a Polish population. *Tissue Antigens*, 2015,86, 279-84. doi: 10.1111/tan.12641 **IF – 2,137 (20 pkt.)**
47. Jaskula E, Dlubek D, Tarnowska A, Lange J, Mordak-Domagala M, Suchnicki K, Sedzimirska M, Borowik A, Mizia S, Lange A.: Anti-CMV-IgG positivity of donors is beneficial for alloHSCT recipients with respect to the better short-term immunological recovery and high level of CD4+CD25high lymphocytes. *Viruses*, 2015 Mar 23;7(3):1391-408. doi: 10.3390/v7031391 **IF – 3,353 (30 pkt.)**
48. Jeleń M, Pluta K, Zimecki M, Morak-Młodawska B, Artym J, Kocięba M. 6-Substituted 9-fluoroquino[3,2-b]benzo[1,4]thiazines display strong antiproliferative and antitumor properties. *Eur J Med Chem*, 2015, 89, 411-20 **IF – 3,447 (40 pkt.)**
49. Joachimiak Ł, Janczewski Ł, Ciekot J, Boratyński J, Błażewska K.: Applying the prodrug strategy to α-phosphonocarboxylate inhibitors of RabGGTase--synthesis and stability studies. *Org Biomol Chem*, 2015, 13(24), 6844-56. doi: 10.1039/c5ob00281h **IF – 3,562 (35 pkt.)**
50. Jurczyszyn A, Gdula-Argasińska J, Kosmaczewska A, Skotnicki AB. The role of the bone marrow microenvironment in the pathogenesis of multiple myeloma. *Post Hig Med Dosw, (online),* 2015 Apr 22;69:521-33. doi: 10.5604/17322693.1150216. **IF – 0,573 (15 pkt.)**
51. Karabon L, Markiewicz M, Kosmaczewska A, Partyka A, Pawlak-Adamska E, Tomkiewicz A, Ciszak L, Jagoda K, Dzierzak-Mietla M, Kyrcz-Krzemien S, Frydecka I.: Pretransplant donor and recipient CTLA-4 mRNA and protein levels as a prognostic marker for aGvHD in allogeneic hematopoietic stem cell transplantation. *Immunol Lett*, 2015, May;165(1), 52-9. doi: 10.1016/j.imlet.2015.03.011. **IF – 2,512 (20 pkt.)**
52. Karabon L, Markiewicz M, Partyka A, Pawlak-Adamska E, Tomkiewicz A, Dzierzak-Mietla M, Kyrcz-Krzemien S, Frydecka I.: A CT60G>A polymorphism in the CTLA-4 gene of the recipient may confer susceptibility to acute graft versus host disease after allogeneic hematopoietic stem cell transplantation. *Immunogenetics*, 2015 Jun;67 (5-6):295-304. doi: 10.1007/s00251-015-0840-7 **IF – 2,227 (20 pkt.)**
53. Kaszowska M, Niedziela T, Maciejewska A, Lukasiewicz J, Jachymek W, Lugowski C.: Core oligosaccharide of *Escherichia coli* B-the structure required for bacteriophage T4 recognition. Carbohydr Res, 2015; 413:51-4 **IF – 1,929 (25 pkt.)**
54. Klein D, Schmitz T, Verhelst V, Panic A, Schenck M, Reis H, Drab M, Sak A, Herskind C, Maier P, Jendrossek V: Endothelial Caveolin-1 regulates the radation response of epithelial prostate tumors. *Oncogenesis*. 2015 May 18;4:e148. doi: 10.1038/oncsis.2015.9. **IF – 3,952 (30 pkt.)**
55. Klekotko M, Matczyszyn K, Siednienko J, Olesiak-Bańska J, Pawlik K, Samoć M.: Bio-mediated synthesis, characterization and cytotoxicity of gold nanoparticles. *Phys. Chem. Chem. Phys*, 2015, Oct 28;17(43):29014-29019 DOI:10.1039/C5CP01619C. **IF – 4,493 (35 pkt.)**

56. Kmiecik A.M, Pula B, Suchanski J, Olbromski M, Gomulkiewicz A, Owczarek T, Kruczak A, Ambicka A, Erys J, Ugorski M, Podhorska-Okolow M, Dziegiel P. Metallothionein-3 increases triple-negative breast cancer cell invasiveness via induction of metalloproteinase expression. *PloS One*, 2015 10(5): e0124865. doi:10.1371 /journal.pone.0124865. **IF – 3,234 (40 pkt.)**
57. Koj S, Ługowski C, Niedziela T.: Bordetella pertussis lipooligosaccharide-derived neoglycoconjugates - new components of pertussis vaccine. *Post Hig Med Dośw* (online), 2015, 8; 69:1013-30 **IF – 0,573 (15 pkt.)**
58. Korzeniowska-Kowal A, Kochman A, Gamian E, Lis-Nawara A, Lipiński T, Seweryn E, Ziółkowski P, Gamian A.: Antibodies against Escherichia coli O24 and O56 O-Specific Polysaccharides Recognize Epitopes in Human Glandular Epithelium and Nervous Tissue. *PLoS One*. 2015 Jun 18;10(6):e0129492. doi: 10.1371/journal.pone.0129492. eCollection 2015. **IF – 3,234 (40 pkt.)**
59. Kosmaczewska A, Ciszak L, Swierkot J, Szteblich A, Kosciow K, Frydecka I.: Exogenous IL-2 controls the balance in Th1, Th17, and Treg cell distribution in patients with progressive rheumatoid arthritis treated with TNF-alpha inhibitors. *Inflammation,* 2015, 38(2), 765-74 doi: 10.1007/s10753-014-9987-x. **IF – 2,208 (20 pkt.)**
60. Kowal A, Wiśniewski A, Kuśnierczyk P, Jankowska R.: Human leukocyte antigen (HLA)-G gene polymorphism in patients with non-small cell lung cancer. *Thoracic Cancer,* 2015, 6, 61319 doi: 10.1111/1759-7714.12232 **IF – 0,743 (15 pkt.)**
61. Kowalski K, Goszczyński T, Leśnikowski Z, Boratyński J.: Synthesis of lysozyme-metalla-carborane conjugates and the effect of boron cluster modification on protein structure and function. *Chembiochem*, 2015, 16(3), 424-31. doi: 10.1002/cbic.201402611 **IF – 3,06 (30 pkt.)**
62. Krawczyk P, Twarog E, Kurowska E, Klopotowska D, Matuszyk J.: Establishment of a cellular model to study TrkC-dependent neuritogenesis. *In Vitro Cell Dev Biol Anim.* 2015 Mar;51(3):241-8. doi: 10.1007/s11626-014-9829-z. **IF - 1,145 (15 pkt.)**
63. Kuśnierczyk P, Mozer-Lisewska I, Zwolińska K, Kowala-Piaskowska AE, Bura M, Bereszyńska I, Pauli A, Żeromski J.: Contribution of genes for killer cell immunoglobulin-like receptors (KIR) to the susceptibility to chronic hepatitis C virus infection and to viremia. *Hum Immunol*, 2015, 76, 102-8. doi: 10.1016/j.humimm. 2015.01.020 **IF – 2,138 (20 pkt.)**
64. Lange A, Jaskula E.: Can determination of gene polymorphism be of practical value in tailoring the treatment of HSCT patients? *Bone Marrow Transplant*, 2015 Feb;50(2):161-2. doi: 10.1038/bmt.2014.295. **IF – 3,570 (30 pkt.)**
65. Litwin M, Radwańska A, Paprocka M, Kieda C, Dobosz T, Witkiewicz W, Baczyńska D.: The role of FGF2 in migration and tubulogenesis of endothelial progenitor cells in relation to pro-angiogenic growth factor production. *Mol Cell Biochem*, 2015 Dec;410(1-2):131-42 **IF – 2,393 (20 pkt.)**
66. Lundqvist LCE, Kaszowska M, Sandström C.: NMR study of the O-specific polysaccharide and the core oligosaccharide from the lipopolysaccharide produced by *Plesiomonas shigelloides* O24:H8 (strain CNCTC 92/89). *Molecules*, 2015, 20(4), 5729-39 **IF – 2,416 (30 pkt.)**
67. Maj E, Filip-Psurska B, Świtalska M, Kutner A, Wietrzyk J.: Vitamin D analogs potentiate the antitumor effect of imatinib mesylate in a human A549 lung tumor model. *Int. J. Mol. Sci.* 2015, 16(11), 27191-27207 **IF - 2,862 (30 pkt.)**

68. Majewska J, Beta W, Lecion D, Hodyra-Stefaniak K, Kłopot A, Kaźmierczak Z, Miernikiewicz P, Piotrowicz A, Ciekot J, Owczarek B, Kopciuch A, Wojtyna K, Harhala M, Mąkosa M, Dąbrowska K.: Oral application of T4 phage induces weak antibody production in the gut and in the blood. *Viruses*, 2015, 7(8): 4783-4799 **IF - 3,353 (30 pkt.)**
69. Matković K, Mitkiewicz M, Matuszyk J.: Ubiquitin as a regulator of IFN production in the antiviral response. *Post Hig Med Dośw,* 2015, 69, 864-873 **IF – 0,573 (15 pkt.)**
70. Mehlich A, Górska S**,** Gamian A, Myc A.: Selected aspects of Clostridium difficile infection. *Post Hig Med Dosw* (online). 2015 May 5;69:598-611. doi: 10.5604/17322693.1151340.**IF – 0,573 (15 pkt.)**
71. Michalski M, Swierzko A.St, Lukasiewicz J, Man-Kupisinska A, Karwaciak I, Przygodzka P, Cedzynski M.: Ficolin-3 activity towards the opportunistic pathogen, *Hafnia alvei*. *Immunobiology*, 2015, 220(1), 117-23 **IF – 3,044 (25 pkt.)**
72. Mieczkowski A, Makowska M, Sekula J, Tomczyk E, Zalewska E, Nasulewicz-Goldeman A, Wietrzyk J.: Bicyclic cytarabine analogues: synthesis and investigation of antitumor properties of novel, 6-aryl- and 6-alkyl-3H-pyrrolo[2,3-d]pyrimidin-2(7H)-one arabinosides. *Tetrahedron*, 2015, 71 (44): 8454-8461. **IF – 2,641 (30 pkt.)**
73. Mikulska JE.: *Analysis of response elements involved in the regulation of the human neonatal Fc receptor gene* (FCGRT). *Plos One*, 2015, 10 (8), 1-28; e0135141 **IF - 3,234 (40 pkt.)**
74. Mitkiewicz M.: Struktura, regulacja oraz funkcje błonowej cyklazyguanylanowej typu A. *Post Hig Med Dośw,* (online), 2015, 69, 457-468 **IF – 0,573 (15 pkt.)**
75. Morak-Młodawska B, Pluta K, Zimecki M, Jeleń M, Artym J, Kocięba M.: Synthesis and selected immunological properties of 10-substituted 1,8-diazaphenothiazines. *Med Chem Res.* 2015, 24, 1408-1418 **IF – 1,402 (20 pkt.)**
76. Niewiadomy A, Skrzypek A, Matysiak J, Głaszcz U, Wietrzyk J, Krajewska-Kułak E.: Synthesis and biological activity of novel N, N-cyclic-2,4-dihydroxythio-benzamide derivatives. *Acta Pol Pharm*, 2015, 72 No. 5 pp. 943-950, **IF – 0,737 (15 pkt.)**
77. Niezgoda N, Gliszczyńska A, Gładkowski W, Kempińska K, Wietrzyk J**,** Wawrzeńczyk C.: Phosphatidylcholine with *cis*-9,*trans*-11 and *trans*-10,*cis*-12 Conjugated Linoleic Acid Isomers: Synthesis and Cytotoxic Studies. *Australian J Chem*, 68(7) 1065-1075, **IF – 1,558 (25 pkt.)**
78. Nowak I, Barcz E, Majorczyk E, Malinowski A, Wilczyński JR, Banasik M, Motak-Pochrzęst H, Kuśnierczyk P.: Genetic polymorphism of KIR2DL4 in the Polish population. *Tissue Antigens*. 2015, 85, 450-7 doi: 10.1111/tan.12544. **IF – 2,137 (20 pkt.)**
79. Nowak I, Płoski R, Barcz E, Dziunycz P, Kamiński P, Kostrzewa G, Milewski Ł, Roszkowski PI, Senitzer D, Malejczyk J, Kuśnierczyk P.: KIR2DS5 in the presence of HLA-C C2 protects against endometriosis. *Immunogenetics*. 2015;67:203-9. doi: 10.1007/s00251-015-0828-3 (online) **IF – 2,227 (20 pkt.)**
80. Nowak J., Kościńska K., Mika-Witkowska R., Rogatko-Koroś M., Mizia S., Jaskuła E., Polak M., Mordak-Domagała M., Lange J., Gronkowska A., Jędrzejczak WW, Kyrcz-Krzemień S., Markiewicz M., Dzierżak-Mietła M., Tomaszewska A., Nasiłowska-Adamska B., Szczepiński A., Hałaburda K., Hellmann A., Czyż A., Gil L., Komarnicki M., Wachowiak J., Barańska M., Kowalczyk J., Drabko K., Goździk J., Wysoczańska B., Bogunia-Kubik K., Graczyk-Pol E., Witkowska A., Marosz-Rudnicka A., Nestorowicz K., Dziopa J., Szlendak U., Warzocha K., Lange A.: Polish Donor-Recipient Matching Study Group. Role of Donor Activating KIR-HLA Ligand-Mediated NK Cell Education Status

in Control of Malignancy in Hematopoietic Cell Transplant Recipients. *Biol Blood Marrow Transplant*, 2015; 21(5), 829-39 **IF - 3,404 (35 pkt.)**

81. Nowicka A, Liszkiewicz H, Nawrocka W.P, Wietrzyk J, Sadowska J.: Synthesis and *in vitro* antiproliferative activity of novel 2-arylideneaminobenzimidazole derivatives. *Acta Pol Pharm*, 2015, 72 No. 5 pp. 951-963. **IF – 0,737 (15 pkt.)**
82. Nowicka A, Liszkiewicz H, Nawrocka W.P, Wietrzyk J, Zubiak A, Kołodziejczyk W.: Synthesis and antiproliferative activity in vitro of new 2-thioxoimidazo[4,5-B]pyridine derivatives. *Acta Pol Pharm*, 2015, 72(1):101-11. **IF – 0,737 (15 pkt.)**
83. O'Keeffe J, Podbielska M, Hogan EL.: Invariant natural killer T cells and their ligands: focus on multiple sclerosis. *Immunology*, 2015, 145, 468-475 **IF – 3,795 (30 pkt.)**
84. Olejnik B, Jarząb A, Kratz EM, Zimmer M, Gamian A, Ferens-Sieczkowska M.: Terminal Mannose Residues in Seminal Plasma Glycoproteins of Infertile Men Compared to Fertile Donors. *Int J Mol Sci*. 2015 Jul 2;16(7):14933-50. doi: 10.3390/ijms160714933. **IF – 2,862 (30 pkt.)**
85. Orzechowska BU, Kukowska-Latallo JF, Coulter Alexa D, Szabo Z, Gamian A, Myc A.: Nanoemulsion-based mucosal adjuvant induces apoptosis in human epithelial cells. *Vaccine*, 2015; 33: 2289-2296 **IF - 3,624 (30 pkt.)**
86. Partyka A, Woszczyk D, Strzała T, Szczepańska A, Tomkiewicz A, Frydecka I, Karabon L.: Gene polymorphisms of novel immunotolerant molecule BTLA: distribution of alleles, genotypes and haplotypes in Polish Caucasian population. *Arch Immunol Ther Exp*, 2015 Feb;63(1):73-8. doi: 10.1007/s00005-014-0300-3 **IF – 3,176 (25 pkt.)**
87. Paściak M, Dacko W, Sikora J, Gurlaga D, Pawlik K, Miękisiak G, Gamian A.: Creation of an In-House Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry Corynebacterineae Database Overcomes Difficulties in Identification of Nocardia farcinica Clinical Isolates. *J Clin Microbiol*, 2015 Aug; 53(8):2611-21. doi: 10.1128/JCM.00268-15 **IF – 3,993 (35 pkt.)**
88. Pawlak A., Strządala L., Kalas W.: Non-genomic effects of the NR4A1/Nur77/TR3/ NGFIB orphan nuclear receptor. *Steroids*, 2015, 95, 1-6 **IF - 2.639 (25 pkt.)**
89. Pietrzyk AJ, Bujacz A, Mak P, Potempa B, Niedziela T.: Structural studies of Helix aspersa agglutinin complexed with GalNAc: A lectin that serves as a diagnostic tool. *Int J Biol Macromol*, 2015, 81, 1059-1068 **IF – 2,858 (25 pkt.)**
90. Polak-Berecka M, Choma A, Waśko A, Górska S, Gamian A, Cybulska J.: Physicochemical characterization of exopolysaccharides produced by Lactobacillus rhamnosus on various carbon sources. *Carbohydr Polym*. 2015, 117:501-9. doi: 10.1016/j.carbpol. 2014.10.006. **IF – 4,074 (40 pkt.)**
91. Poręba K, Pawlik K, Rembacz K, Kurowska E, Matuszyk J, Długosz A.: Synthesis and antibacterial activity of new sulfonamide isoxazolo[5,4-b]pyridine derivatives. *Acta Pol Pharm*, 2015, 72(4) 727-735 **IF – 0,737 (15 pkt.)**
92. Rossowska J, Anger N, Kisielińska J, Pajtasz-Piasecka E, Bielawska-Pohl A, Wojas-TurekJ, Duś. D.: Temporary elimination of IL-10 enhanced the effectiveness of cyclophosphamide and BMDC-based therapy by decrease of the suppressor activity of MDSCs and activation of antitumour immune response. *Immunobiology*, 2015, 220(3):389-98. **IF – 3,044 (25 pkt.)**

93. Rydzak J, Kaczmarek R, Czerwiński M, Lukasiewicz J, Taborowska J, Szewczyk B, Jaskiewicz E.:. The baculovirus-expressed binding region of Plasmodium falciparum EBA-140 ligand and its glycophorin C binding specificity. *PLoS One*, 2015, 10(1):e0115437 **IF – 3,234 (40 pkt.)**
94. Sava GP, Speedy HE, Di Bernardo MC, Dyer MJ, Holroyd A, Sunter NJ, Marr H, Mansouri L, Deaglio S, Karabon L, Frydecka I, Jamroziak K, Woszczyk D, Juliusson G, Smedby KE, Jayne S, Majid A, Wang Y, Dearden C, Hall AG, Mainou-Fowler T, Jackson GH, Summerfield G, Harris RJ, Pettitt AR, Allsup DJ, Bailey JR, Pratt G, Pepper C, Fegan C, Rosenquist R, Catovsky D, Allan JM, Houlston RS. Common variation at 12q24.13 (OAS3) influences chronic lymphocytic leukemia risk. *Leukemia*, 2015, 29(3):748-51. doi: 10.1038/leu.2014.311. **IF - 10.431 (45 pkt.)**
95. Sidoryk K, Świtalska M, Jaromin A, Cmoch P, Bujak I, Kaczmarska M, Wietrzyk J**,** Dominguez EG, Żarnowski R, Andes DR, Bańkowski K, Cybulski M, Kaczmarek Ł.: The synthesis of indolo[2,3-b]quinoline derivatives with a guanidine group: Highly selective cytotoxic agents. *Eur J Med Chem*. 2015, 105:208-219. doi: 10.1016/j.ejmech. 2015.10.022 **IF – 3,447 (40 pkt.)**
96. Skiera I, Antoszczak M, Trynda J, Wietrzyk J, Boratyński P, Kacprzak K, Huczyński A.: Antiproliferative activity of polyether antibiotic - Cinchona alkaloid conjugates obtained via click chemistry. *Chem Biol Drug Des*, 2015, 86(4): 911-7, doi: 10.1111/cbdd.12523. **IF – 2,485 (25 pkt.)**
97. Smietana M, Koba M, Brzozowska E, Krogulski K, Nakonieczny J, Wachnicki L, Mikulic P, Godlewski M, Bock WJ.: Label-free sensitivity of long-period gratings enhanced by atomic layer deposited TiO(2) nano-overlays. *Opt Express*, 2015 Apr 6; 23(7):8441-53. doi: 10.1364/OE.23.008441. **IF – 3,488 (40 pkt.)**
98. Smolarek D, Hattab C, Buczkowska A, Kaczmarek R, Jarząb A, Cochet S, de Brevern A.G, Lukasiewicz J, Jachymek W, Niedziela T, Grodecka M, Wasniowska K, Aronovicz YC, Bertrand O, Czerwiński M.: Studies of a murine monoclonal antibody directed against DARC: reappraisal of its specificity. *PLoS One*, 2015, 10(2):e0116472 **IF – 3,234 (40 pkt.)**
99. Sochocka M, Tomczyk T, Sobczyński M, Szermer-Olearnik B, Boratyński J.: The kinetics of *Escherichia coli* B growth and bacteriophage T4 multiplication in SM-1 novel minimal culture medium. *J Gen Appl Microbiol*, 2015, 61(3), 75-81 doi: 10.2323/jgam.61.75 **IF - 0,943 (15 pkt.)**
100. Swierkot J., Bogunia-Kubik K., Nowak B., Bialowas K., Korman L., Gebura K., Kolossa K., Jeka S., Wiland P.: Analysis of associations between polymorphisms within genes coding for tumour necrosis factor (TNF)-alpha and TNF receptors and responsiveness to TNF-alpha blockers in patients with rheumatoid arthritis. *Joint Bone Spine*, 2015, 82(2): 94-9. **IF - 2,901 (25 pkt.)**
101. Szermer-Olearnik B, Boratyński J.: Removal of endotoxins from bacteriophage preparations by extraction with organic solvents. *PLoS One*, 2015, 10(3) e0122672. doi: 10.1371/journal. pone. 0122672. eCollection 2015 **IF – 3,234 (40 pkt.)**
102. Szurek E, Cebula A, Wojciech L, Pietrzak M, Rempala G, Kisielow P, Ignatowicz L.: Differences in Expression Level of Helios and Neuropilin-1 Do Not Distinguish Thymus-Derived from Extrathymically-Induced CD4+Foxp3+ Regulatory T Cells. *PLoS One*, 2015 Oct 23;10(10):e0141161. doi: 10.1371/journal.pone.0141161 **IF – 3,234 (40 pkt.)**
103. Śmieszek A, Czyrek A, Basinska K, Trynda J, Skaradzińska A, Siudzińska A, Marędziak M, Marycz K.: Effect of metformin on viability, morphology, and ultrastructure of mouse

bone marrow-derived multipotent mesenchymal stromal cells and Balb/3T3 embryonic fibroblast cell lin. *BioMed Res. Int*, 2015, 2015, Article ID 769402, 14 pages. doi:10.1155/2015/769402 **IF – 1,579 (20 pkt.)**

104. Świerkot J, Sokolik R, Czarny A, Zaczyńska E, Nowak B, Chlebicki A, Korman L, Madej M, Wojtala P, Lubiński Ł, Wiland P.: Activity of JAK/STAT and NF-kB in patients with axial spondyloarthritis**.** *Post Hig Med Dośw*, (online). 2015; 69,1291-1298 **IF - 0,573 (15 pkt.)**
105. Trojanowski D, Ginda K, Pióro M, Hołówka J, Skut P, Jakimowicz D, Zakrzewska-Czerwińska J: Choreography of the *Mycobacterium* replication machinery during the cell cycle. *MBio*, 2015 Feb 17; 6(1):e02125-14. doi: 10.1128/mBio.02125-14 **IF – 6,786 (40 pkt.)**
106. Trynda J, Turlej E, Milczarek M, Pietraszek A, Chodyński M, Kutner A, Wietrzyk J.. Antiproliferative activity and *in vivo* toxicity of double-point modified analogs of 1,25-dihydroxyergocalciferol. *Int J Mol. Sci*, 2015, 16(10), 24873-24894; doi: 10.3390/ ijms161024873 **IF - 2,862 (30 pkt.)**
107. Tupikowski K, Partyka A, Kolodziej A, Dembowski J, Debinski P, Halon A, Zdrojowy R, Frydecka I, Karabon L.: CTLA-4 and CD28 genes' polymorphisms and renal cell carcinoma susceptibility in the Polish population - a prospective study. *Tissue Antigens*, 2015, Nov; 86(5):353-61. doi: 10.1111/tan.12671 **IF – 2,137 (20 pkt.)**
108. Wagner M, Sobczyński M, Bilińska M, Pokryszko-Dragan A, Cyrul M, Kuśnierczyk P, Jasek M.: MS risk allele rs1883832T is associated with decreased mRNA expression of CD40. *J Mol Neurosci*, 2015, 56, 540-5 doi: 10.1007/s12031-015-0490-0 **IF – 2,343 (20 pkt.)**
109. Wagner M, Sobczyński M, Karabon L, Bilińska M, Pokryszko-Dragan A, Pawlak-Adamska E, Cyrul M, Kuśnierczyk Pr, Jasek M.: Polymorphisms in CD28, CTLA-4, CD80 and CD86 genes may influence the risk of multiple sclerosis and its age of on set. *J Neuroimmunol*. 2015, Nov 15; 288:79-86. doi: 10.1016/j.jneuroim.2015.09.004 **IF – 2,467 (25 pkt.)**
110. Wiśniewski A, Kowal A, Wyrodek E, Nowak I, Majorczyk E, Wagner M, Pawlak-Adamska E, Jankowska R, Ślesak B, Frydecka I, Kuśnierczyk P.: Genetic polymorphisms and expression of HLA-G and its receptors, KIR2DL4 and LILRB1, in non-small cell lung cancer. *Tissue Antigens*, 2015, 85, 466-75 **IF – 2,137 (20 pkt.)**
111. Wojciechowska A, Gągor A, Zierkiewicz W, Jarząb A, Dylong A, Zierkiewicz W.: Metal-organic framework in L-argininato coper(II) ions polymer - structure, spectroscopic properties, theoretical studies and microbiological activity. *RSC Adv*, 2015, 5**,** 36295-36306 **IF -3,840 (35 pkt.)**
112. Wojcieszak D, Kaczmarek D, Antosiak A, Mazur M, Rybak Z, Rusak A, Osekowska M, Poniedzialek A, Gamian A, Szponar B.: Influence of Cu-Ti thin film surfach properties on antimicrobial activity and viability of living cells. *Mater Sci Eng C Mater Biol Appl*, 2015 Nov 1; 56: 48-56. doi: 10.1016/j.msec.2015.06.013. **IF – 3,088 (25 pkt.)**
113. Wolański M, Donczew R, Zawilak-Pawlik A, Zakrzewska-Czerwińska J.: *oriC*-encoded instructions for the initiation of bacterial chromosome replication. *Front Microbiol*., 2015 Jan 6;5:735. doi: 10.3389/fmicb.2014.00735. **IF – 3,989 (35 pkt.)**
114. Wysoczanska B., Wrobel T., Dobrzynska O., Mazur G., Bogunia-Kubik K.: Role of the functional MNS16A VNTR-243 variant of the human telomerase reverse transcriptase gene in progression and response to therapy of patients with non-Hodgkin's B-cell lymphomas. *Int J Immunogenet*, 2015; 42(2): 100-5 **IF - 1,247 (15 pkt.)**

115. Wysokińska E., Cichos J., Zioło E., Bednarkiewicz A., Strządała l., Karbowiak M., Hreniak D., Kałas W.: Cytotoxic interactions of bare and coated $NaGdF_4$:Yb3+:Er3+ nanoparticles with macrophage and fibroblast cells. *Toxicol In vitro*, 2015, 27, 16-25 **IF – 2,903 (30 pkt.)**
116. Zabłocka A, Mitkiewicz M, Macała J, Janusz M.: Neurotrophic activity ofcultured cell line U87 is up-regulated by Proline-Rich Polypeptide complex andits constituent nonapeptide. *Cell Mol Neurobiol*, 2015, 35, 977-986 **IF – 2,506 (20 pkt.)**
117. Zerka A, Kaczmarek R, Jaśkiewicz E.: Z punktu widzenia zarodźca malarii – ewolucja *Plasmodium falciparum. Post Hig Med Dośw,* 2015, 69, 1519-1529 **IF – 0,573 (15 pkt.)**
118. Zielichowska A, Saczko J, Garbiec A, Dubińska-Magiera M, Rossowska J, Surowiak P, Choromańska A, Daczewska M, Kulbacka J, Lage H.: The photodynamic effect of far-red range phthalocyanines (AlPc and Pc green) supported by electropermeabilization in human gastric adenocarcinoma cells of sensitive and resistant type. *Biomed Pharmacother*, 2015 Feb;69:145-52. doi: 10.1016/j.biopha.2014.11.017 **IF – 2,023 (20 pkt.)**
119. Zimecki M, Artym J, Kocięba M, Obmińska-Mrukowicz B, Mączyński M, Ryng S.: Immune function in cyclophosphamide-treated mice is restored by the T-cell-tropic isoxazole derivative R-13. *J Immunotoxicol*, 2015, 12, 322-9 **IF – 2,054 (20 pkt.)**
120. Żaczek M, Weber-Dąbrowska B, Górski A.: Phages in the global fruit and vegetable industry. J. Appl. Microbiol. 2015, 118, 537-556 **IF – 2,479 (30 pkt.)**

Publications from the journals not included in JCR database:

1. Artym J: Laktoferyna – strażnik procesów przyswajania żelaza. *Post Biol Kom*, 2015, 42(2): 283-308
2. Brzozowska E, Pyra A, Miśków M, Górska S, Gamian A.: C-Terminal Sequence Determinants of T4 Bacteriophage Tail Fiber Adhesin for Specific Lipopolysaccharide Recognition. *SOJ Microbial Infect Dis*, 2015; 3:1-5.
3. Jaegermann Z, Ciołek L, Zaczyńska E, Czarny A.: *In vitro* biological evaluation of new dental biomaterials. Engineering of Biomaterials. 2015, 131, 32-39. ISSN 1429-7248. Publisher: Polish Society for Biomaterials in Krakow **(7 pkt.)**
4. Orzechowska B.U.: Patomechanizm wirusowych gorączek krwotocznych w świetle epidemii Ebola. *Post Nauk Med*, 2015; 28, 23-33 **(6 pkt.)**
5. Soliman Abdel M, Abdelhamid HF, Wietrzyk J, Pawlik A.: Evaluation of antiproliferative potency and induced biochemical parameters of novel pyridine derivatives against leukemia, lung, breast and colon cancer cell lines. *World J Pharm Res*, 2015, 8:100-115, ISSN 2277-7105.

Chapters of books:

1. Frydecka Irena: Czerwienica prawdziwa. str. 1722-1726. w: *„Interna”* Szczeklika, 2015, wydanie w oprawie miękkiej ISBN 978-837430459-7; wydanie w oprawie twardej ISBN 978-83-7430-461-0 Medycyna Praktyczna Kraków 2015
2. Frydecka Irena: Nadpłytkowość samoistna. str. 1726-1730. w: *„Interna”* Szczeklika, 2015 wydanie w oprawie miękkiej ISBN 978-837430459-7; wydanie w oprawie twardej ISBN 978-83-7430-461-0 Medycyna Praktyczna Kraków 2015

3. Frydecka Irena: Smoistne włóknienie szpiku. str. 1730-1733. w: *„Interna”* Szczeklika, 2015 wydanie w oprawie miękkiej ISBN 978-837430459-7; wydanie w oprawie twardej ISBN 978-83-7430-461-0 Medycyna Praktyczna Kraków 2015
4. Frydecka Irena: Czerwienica prawdziwa. str. 907. w: „*Interna*” Szczeklika, mały podręcznik 2015/2016 wydanie VII w oprawie miękkiej ISBN 978-837430460-3; Medycyna Praktyczna Kraków 2015
5. Frydecka Irena: Nadpłytkowość samoistna. str. 911. w: *„Interna*” Szczeklika mały podręcznik 2015/2016 wydanie VII w oprawie miękkiej ISBN 978-837430460-3; Medycyna Praktyczna Kraków 2015
6. Frydecka Irena: Smoistne włóknienie szpiku. str. 913. w: *„Interna*” Szczeklika mały podręcznik 2015/2016 wydanie VII w oprawie miękkiej ISBN 978-837430460-3; Medycyna Praktyczna Kraków 2015
7. Jundzill A, Klimczak A, Brzezicki G.: Chapter 48: Spleen Transplantation Model. In: *„Plastic and Reconstructive Surgery, Experimental Models and Research Designs”. (Edytor) M. Siemionow,* Springer London, Heidelberg, New York, Dordrecht, 2015; pp. 387-397 ISBN 978-1-4471-6334-3; ISBN 978-1-4471-6335-0 (eBook) DOI 10.1007/978-1-4471-6335-0-48
8. Janicka A, Zawiślak M, Zaczyńska E, Czarny A: „Innowacyjna metoda oceny toksyczności mieszanin gazowych Bat-Cell Bio-Ambient Tests”, rozdział w książce pt. *”Wybrane zagadnienia biologii i medycyny”.* Praca zbiorowa pod redakcją: E. Skopińskiej-Różewskiej, A. K. Siwickiego, R. Zdanowskiego, str. 345-359, Wydawnictwo EDYCJA s.c., Olsztyn 2015, ISBN: 978-83-94917-1-9.
9. Klimczak A, Siemionow MZ.: Chapter 70: Cellular Therapies in Vascularized Composite Allograft: Review. In: *„Plastic and Reconstructive Surgery, Experimental Models and Research Designs”. (Edtor) M. Siemionow,* Springer London, Heidelberg, New York, Dordrecht, 2015; pp. 569-579 ISBN 978-1-4471-6334-3; ISBN 978-1-4471-6335-0 (eBook) DOI 10.1007/978-1-4471-6335-0-70
10. Klimczak A.: Chapter 73: Cellular Therapies via Vascularized Bone Marrow Transplantation. In *„Plastic and Reconstructive Surgery, Experimental Models and Research Designs”. (Edytor) M. Siemionow,* Springer London, Heidelberg, New York, Dordrecht, 2015; pp. 605-616 ISBN 978-1-4471-6334-3; ISBN 978-1-4471-6335-0 (eBook) DOI 10.1007/978-1-4471-6335-0-73
11. Klimczak A, Jundzill A.: Chapter 74: Cellular Therapies in Vascularized Composite Allograft. In: *„Plastic and Reconstructive Surgery, Experimental Models and Research Designs”. (Edytor) M. Siemionow,* Springer London, Heidelberg, New York Dordrecht, 2015; pp. 617-627 ISBN 978-1-4471-6334-3; ISBN 978-1-4471-6335-0 (eBook) DOI 10.1007/978-1-4471-6335-0-74
12. Lange A. Zasady poszukiwania dawcy szpiku lub komórek krwiotwórczych z krwi obwodowej. Rozdział 33 w książce: „*Praktyka hematologiczna*”, wydawnictwo Termedia, red. W.W. Jędrzejczak, ISBN 9788379880102
13. Łukasik E, Waśniowska K, Grodecka M, Majorczyk E, Czerwiński M.: High-resolution melting analysis for genotyping Duffy blood group antigens. In: Methods in Molecular Biology, 2015;1310:83-95. doi: 10.1007/978-1-4939-2690-9_7. Print ISBN 978-1-4939-2689-3 (online) ISBN 978-1-4939-2690-9

Articles published without IITD affiliation:

1. Budziszewska BK, Pluta A, Sulek K, Wierzbowska A, Robak T, Giebel S, Holowiecka-Goral A, Sawicki W, Ejduk A, Patkowska E, Manko J, Gajkowska-Kulik J, Piszcz J, Mordak-Domagala M, Madry K, Holowiecki J, Kyrcz-Krzemien S, Nowakowska-Domagala M, Dmoszynska A, Calbecka M, Kloczko J, Wiktor Jędrzejczak W, **Lange A**, Razny M, Bilinski P, Warzocha K, Lech-Maranda E. Treatment of elderly patients with acute myeloid leukemia adjusted for performance status and presenceof comorbidities: a Polish Adult Leukemia Group study. *Leuk Lymphoma*, 2015, 56(8):2331-8. doi: 10.3109/10428194.2014.985672.**IF – 2,891 (25 pkt.)**
2. Cartmell J, Paszkiewicz E, Dziadek S, Tam PH, Luu T, Sarkar S, **Lipinski T**, Bundle DR.: Synthesis of antifungal vaccines by conjugation of β-1,2 trimannosides with T-cell peptides and covalent anchoring of neoglycopeptide to tetanus toxoid. *Carbohydr Res*. 2015 Feb 11;403:123-34. doi: 10.1016/j.carres.2014.06.024. **IF – 1,929 (25 pkt.)**
3. DiGiuseppe S, Keiffer TR., **Bienkowska-Haba M**, Luszczek W, Guion Lucile G.M., Müller M, Sapp M.: Topography of the human papillomavirus minor capsid protein L2 during vesicular trafficking of infectious entry. *J. Virol*, 2015; 89: 10442-10452 **IF - 4,439 (35 pkt.)**
4. **Lipinski T**, Bundle DR. Temporary Conversion of Protein Amino Groups to Azides: A Synthetic Strategy for Glycoconjugate Vaccines. *Methods Mol Biol*, 2015;1331:145-57. doi: 10.1007/978-1-4939-2874-3 9.
5. Neubauer K, Misa IB, Diakowska D, Kapturkiewicz B, **Gamian A**, Krzystek-Korpacka M.: Nampt/PBEF/visfatin upregulation in colorectal tumors, mirrored in normal tissue and whole blood of colorectal cancer patients, is associated with metastasis, hypoxia, IL1β, and anemia. *Biomed Res Int*. 2015; 2015:523930. doi: 10.1155/2015/523930. **IF – 1,579 (20 pkt.)**