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Abstracts

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Pharmacogenetics

Pharmacogenetics: Do You Have Your DNA Passport?

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Adverse drug reactions are responsible for 5–7% of hospitalizations each year. Effectiveness of drugs is reported to be only 25–60%. Interindividual variation in drug metabolism is a key factor underlying part of these unwanted effects. Genetics causes an important part of this variation: DNA variants in metabolizing enzymes make that not each individual has the same metabolizing capacity in the liver. Since it is genetic, it can be determined before starting therapy using DNA from blood or saliva. This enables prescribing based on personal information, resulting in less Adverse Drug Reactions and an increase in drug efficacy. There is a huge demand from patients, physicians, pharmacists, insurers and regulators for this approach. With over 5,000 articles per year being published on potential genomic markers to guide drug therapy, choosing the most promising markers for routine use is a challenge. Genetic polymorphisms in the Cytochrome P450 system, involved in the metabolism of 80% of all drugs, is an important contributor. In the Netherlands, routine testing for pharmacogenetic markers is being done for over 10 years, and is now rapidly increasing, with main drivers being CYP2D6 testing for psychiatry, oncology and cardiology, DPYD testing for capacetabine and HLA-B*5701 testing for abacavir. The DNA information can be used in any pharmacy in The Netherlands to obtain personalized dosing for more than 80 drugs. This successful approach is the basis for the European Pharmacogenetics Implementation Consortium (www.eu-pic.net) in which the experiences in The Netherlands are combined with those of 18 European countries by means of a laboratory network, organized in collaboration with the IFCC and ESPT.

Endocrine Disorders and Psychiatry: Drug Discovery and Pharmacogenomics

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Drug response depends on: *i.* condition of drug's target; *ii.* effectiveness of drug transport and *iii.* effectiveness of drug metabolism. Impairment at any step of drug action often is a result of genetic polymorphism or rare mutation. The main distinctive feature of our approach is accounting variations at all those steps of drug action. We have attempted to use pharmacogenetic testing in psychiatric practice - area of medicine with the highest percentage of non-responders to drug therapy and high frequency of disabling/life-threatening adverse drug reactions. Considering genetic status of MDR1 and CYP450s we have developed an interpretive algorithm that allows psychiatrist

to choose individual strategy of patient treatment according to his inherited peculiarities. It is noteworthy that often in the absence of any common polymorphism patient still does not respond to the drug therapy. It may occur due presence of individual rare mutations, that alter structure or functioning of drug target, drug transporters or drug metabolizing enzymes. In that case, it is expedient to investigate altered component individually what is of very importance in drug discovery. We are working on enzymes – cytochrome P450s relevant to human health. Breast cancer, prostate cancer, resistant hypertension, Cushing's syndrome – are just few examples of the human P450s dysfunction. By applying a protein family-based approach in structure-functional studies of these proteins new selective drugs can be designed and developed to treat life-threatening diseases.

Pharmacogenetics in Immunosuppressive Management (Transplantation and Rheumatoid Arthritis)

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Allogenic solid organ transplantation and rheumatoid arthritis management require administration of drugs interfering with function of the immune system, i.e. graft maintenance and disease control in rheumatoid arthritis relay on successful administration of immunosuppressants. Drug concentrations in target cells are determined, among others, by activity of drug metabolizing enzymes and transporters. Several enzymes and transporters implicated in immunosuppressant drug pharmacokinetics as well as pharmacodynamics (drug targets) are encoded by highly polymorphic genes. Therefore, a large part of interindividual variability in drug dosage and response could be determined by genetic factors. There is growing evidence that polymorphisms in genes coding for thiopurine S-methyltransferase (TPMT) determines toxicity of azathioprine, CYP3A4/CYP3A5 genetic variants are associated with pharmacokinetic variability of calcineurin inhibitors (especially tacrolimus), UGT1A9 polymorphism affects the course of treatment with mycophenolic acid, genes of enzymes from reduced folate pathway (e.g. MTHFR) modify response to methotrexate, and activity of drug transporters (e.g. ABCB1, SCL19A1) influence drug availability in target cells, and thus treatment efficacy. The presentation summarizes the current state of knowledge in pharmacogenetics of immunosuppressive drugs used in the treatment of rheumatoid arthritis and management of solid organ transplantation focusing on possibilities of clinical application of genetic testing. Pharmacogenetic approach, as evidenced in available data, may serve as a tool to individualize pharmacotherapy, making it more effective and safe.

Genetic and Molecular Factors and Their Role in Response to Therapy in Selected Haematological Malignancies

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There has recently been a considerable progress in the therapy of haematological malignancies with completely new drugs being introduced. However, we are still looking for new prognostic factors that could not only classify patients into risk groups, but also predict response to new therapies. micro-RNAs are small novel molecules involved in post-transcriptional gene expression, and many of them were shown to influence risk for acute myeloblastic leukaemia (AML). While micro-RNA expression alone is an important parameter, the polymorphism of micro-RNA genes can also have an important role in the course of AML and response to new therapies. In the case of multiple myeloma (MM), introduction of a new class of immunomodulating drugs (IMiDs), such as thalidomide or lenalidomide, has changed favourably clinical outcome in many patients. We know that activity of IMiDs depends on cereblon expression, but it may also be influenced by other signalling pathways. We analysed expression and polymorphism of selected micro-RNAs in AML patients in relation to clinical outcome and response to therapy. We also examined the effect of polymorphism in cereblon and beta-catenin genes. We showed that aberrant micro-RNAs expression has a significant role in the course of disease in AML patients. Polymorphism of the genes coding for cereblon and beta-catenin was found to have a role as a risk factor in MM and response to IMiDs. Our results show that

various discrete genetic variants may significantly affect course of disease and therapy. This knowledge can potentially lead to more efficient and personalised medicine.

High Rate of In-Stent Restenosis Appearing Shortly after Endovascular Treatment of Peripheral Arterial Occlusive Disease Can Result of Reduced Pharmacological Platelet Blockage Associated with Cytochrome P450 2C19*2 Variant

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The potential and mechanisms of restenosis are: a) stent implantation (recoil, poor apposition, bad deployment, injury of the stent, vascular injury; all leading to thrombosis), b) pathological hyperactivity to stent material resulting also in thrombosis, c) late response with proliferation of the neo-intimal tissue and acute/subacute thrombosis as a result of platelet activation and aggregation on the stent surface in patient without antiplatelet treatment or resistance to antiplatelet treatment. Patients with so called "high on-treatment platelet reactivity" (HPR) have been classified as being nonresponsive or resistant to anti-platelet treatment. The term "resistance" means on-treatment reactivity to ADP during clopidogrel therapy (it indicates persistent response of the P2Y12 receptor which is the clopidogrel target). Several observational studies have demonstrated a strong link between HPR and recurrent ischemic events in endovascularly treated patients. But so far prospective evaluating personalized antiplatelet therapy based on platelet function testing has not demonstrated clinical benefit. In patients with resistance to clopidogrel the relationship with cytochrome P450 (CYP) C19*2 was described. This genotype occurs in about 30% of population but among patients with coronary artery disease only the half of this number are clopidogrel resistant patients (in laboratory tests). Our study evaluated the association between cytochrome CYP 2C19*2 genotype and antiplatelet effect of clopidogrel therapy in patients with restenosis/reclusion after endovascular treatment of peripheral arterial occlusive disease.

Pharmacogenetic Testing for Statin-Induced Myopathy: Method Implementation and Risk Variant Distribution in a Czech Population

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Genetic factors are involved in the pathogenesis of statin-induced myopathy (SIM). In this regard, variants (polymorphisms) of genes coding for transport proteins and enzymes affecting absorption, distribution, metabolisation and excretion of statins have been described. The aim of our work was to develop and implement a method for determination of single nucleotide polymorphisms in *SLCO1B1* and *GATM* genes, considered to be risk variants for SIM. In a cohort of 183 probands of Czech ethnicity (127 males, 56 females), MassArray technology was used to determine genotypes of *SLCO1B1* (rs4149056, T>C) and *GATM* (rs9806669, G>A) and obtained genotype and allele frequencies were compared with their distribution in other European populations. The frequency of the C allele (polymorphism rs4149056) was 18.9%, which corresponded to the occurrence of this *SLCO1B1* variant around Europe. The frequency of the A allele (polymorphism rs9806669) was 31.1%; similar to the reported distribution of this *GATM* variant. The utilised methodology was reliable and relatively fast; results were available in 36–48 h after sampling 1–2 ml of peripheral blood. In conclusion, we have at our disposal a technique for determination of two genetic markers which may contribute to early identification of patients predisposed to myopathy as a complication of statin therapy.

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Characterisation of CYP2C19 Gene Variants by Massarray and Point-of-Care Techniques and Its Application for Pharmacogenetics of Antithrombotic Therapy

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Cytochrome P450 2C19 isoenzyme (CYP2C19) gene was implicated in altered Clopidogrel metabolisation thus emphasizing need for individual drug dosing according to extent of genetic risk. We wished to characterise distribution of risk genotypes in patients on Clopidogrel treatment. Further, we compared the efficacy of a point-of-care (POC) technique for fast determination of basic pharmacogenetic profile. A multiplex molecular analysis combined with mass-spectrometry (MassArray, AgenaBioscience, USA) was utilised to determine CYP2C19 genotypes in 520 patients undergoing coronary stenting. Genotyping was controlled using reference DNA (Corriell, USA) and confirmed by EPT (Instand, Germany). In a subset of 50 patients, POC technique (Spartan RX, Canada) was employed to test in parallel selected variants (CYP2C19 *2,*3,*17) from a buccal swab specimen. Distribution of CYP2C19 variants did not differ from Europeans/populations of European descent. Extent of genetic risk was characterised as: high risk genotype (+++, poor Clopidogrel metabolisers) – presence of homozygotes of CYP2C19*2AA; Medium risk genotype (++, slow metabolisers) – presence of CYP2C19*2AG and CYP2C19*17TT. CYP2C19*17CT represented a low risk [+] genotype, with possible alterations of Clopidogrel metabolisation. We report the distribution of genotype subgroups in our patients. Summarising, MassArray technology represents a robust and reliable method for assessing CYP2C19 polymorphisms in our clinical laboratory. In approx. 1/3 of patients, medium to high risk of altered Clopidogrel metabolisation was observed suggesting the need to correct dosage and/or to switch to another antithrombotic drug. Utilised POC test was non-invasive and fast (1 h) but was not able to determine all selected CYP2C19 variants in every tested subject.

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Impact of the NKG2D Genetic Variants on Clinical Response to Anti-TNF Therapy

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The NKG2D acts as a powerful activating and co-stimulatory receptor on immune effector cells including NK and T cells. The NKG2D-ligand interaction is involved in immune surveillance against transformed and infected cells. Disruptions within this signaling pathway may trigger exacerbated immune response and promote autoimmune reactions. The aim of this study was to evaluate a plausible role of polymorphisms within the NKG2D gene as predictors of clinical outcome of anti-tumor necrosis factor (anti-TNF) therapy in rheumatoid arthritis (RA) patients. A total of 280 RA patients receiving anti-TNF therapy were enrolled to the study. Genotypings for NKG2D rs2255336 (A>G) and rs1154831 (C>A) were performed using a polymerase chain reaction (PCR) amplification employing LightSNiP assays. Clinical response was evaluated according to the European League Against Rheumatism (EULAR) criteria at 12th and 24th week after initiation of the therapy. The NKG2D rs225336 was significantly associated with efficacy of anti-TNF treatment among patients. Inefficiency of anti-TNF therapy was more frequently observed in patients with GG genotype than in patients bearing the A allele (p=0.005). Also the presence of the G allele in patients correlated with worse EULAR response after 12 weeks of anti-TNF treatment (p=0.005). Moreover, patients carrying the heterozygous genotype achieved significantly better EULAR responses to anti-TNF agents than patients with other genotypes (p=0.010). With regard to the NKG2D rs1154831 no association between this polymorphisms and response to

anti-TNF treatment was detected. Data from the present study provide evidence that NKG2D rs225336 polymorphism may affect clinical response to anti-TNF inhibitors in RA patients.

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Organ Transplantation

Transplantology: Challenges for Today

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Clinical transplantology in Poland had its 50th anniversary this year. With the early and long results comparable to the best achieved in the world leading centers, we face old and completely new challenges for this medical specialty. Main and growing challenge is insufficient number of available organs. With less than 15 donors/mln population/year Poland stay in the lower row of European countries in this measurement of transplant activity. Donation system is not efficient enough and we lose a big number of potential donors still. Living donation (with the exception for the fragments of the liver) remain low despite of different initiatives made so far on the national and local levels. DCD (donation after cardiac death) is possible from the point of Polish juridical regulations, but since last three years had not showed real impact on country donation rates (only two procedures done). Methods of tissue typing remain slow and cause relatively long times of cold ischemia for kidney programs. Second main challenge is chronic rejection causing loss of organs in the long term follow-up and no efficient treatment employed. The emerging possibility of tolerance induction, despite of plenty of new protocols proposition in the publications does not show up a clinical everyday practice in work. The same is with xenotransplantation promises, even we were informed recently that till 2030 such genetically modified porcine organs will be available. The next challenge is production of organs and tissues from own recipients cells installed on the different scaffolds or 3D printed. Other challenge is the personnel working in this field. We observe like in the other European countries lack of new candidates for work in this field together with serious problems of nursing staff, being a catastrophic perspective in country medical service in general, not only in transplant centers. The last but not least challenge is financial side of transplant programs and growing bureaucracy.

Antigen Match, Ischemia and Death and Transplant Immunogenicity

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Considering organ retrieval and transplantation, death is a dissociated process, leading to gradual functional and physical decay of tissues. Thus, an organ harvested from the dying corpse has to survive hypothermia and ischemia outside human body as long as it takes to transport the graft and match the recipient. Then, when blood flow is restored, an organ is subjected to oxygen challenge. During process of dying, inflammatory reaction is initiated. An apex of this reaction can be seen after reperfusion, when increase in serum pro-inflammatory cytokines, activation of endothelia, overexpression of histocompatibility antigens on surface of the cells, resulting in increased graft immunogenicity. Hypothermia is known to induce endothelial injury, increase adhesion molecules expression, and show denuded basal membrane proteins. Prolonged ischemia seems to potentiate tissue inflammation and immunogenicity additionally, as severe leukocyte infiltrates and humoral rejection proportional to ischemia time were noted in experimental studies. Hence, a conflict between an urge to minimize ischemia time and need for tissue typing to reduce mismatching antigens exists – the results of transplantation are proportional to the number of matching antigens in locus A, B and DR. Correct strategy probably lays in between, and appropriate logistical solutions, aimed at pre-retrieval tissue typing, avoiding the need for elongation of preservation time are sought for. Interventions to inhibit systemic activation of inflammatory process in deceased organ donor body can additionally increase organ tolerance to ischemia, decreasing its immunogenicity.

Role of BAFF Cytokine in Kidney Transplant Rejection

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Current immunogenetic tests before transplantation do not provide information about B cells which participate in the antibody response against the transplanted organ. BAFF (B cell activating factor) plays an important role in proliferation, maturation and differentiation of B cells. Several recent studies have suggested that BAFF could serve as a predictor/or marker of antibody-mediated rejection (AMR) in kidney transplant patients. Our aim was to correlate levels of soluble BAFF cytokine pre- and post-transplantation with the clinical course and incidence of acute rejection after transplantation. The study included 77 kidney recipients in the age range 19–76 years. BAFF cytokine levels were determined using the Luminex technique before transplant, three months, six months and one year after transplantation. In the first year, eight patients had cellular rejection (CR), four patients had AMR and five patients had simultaneously CR and AMR. Before transplantation the levels of BAFF were virtually the same in patients with rejection and without rejection (AMR: 3.0 ± 1.6, CR 2.4 ± 1.5 and without rejection: 3.4 ± 1.4 ng/ml). During the first three months post-transplant patients without rejection had higher levels of soluble BAFF than patients with AMR (3.4 ± 1.5 vs. 1.4 ± 1.2). During episodes of AMR, patients had significantly lower levels of the BAFF cytokine than patients without rejection. The concentrations of BAFF cytokine before transplantation were virtually the same in patients with rejection and without rejection. Patients with acute antibody-mediated rejection had lower levels of BAFF than patients free of rejection.

Impact of HLA Allele Polymorphisms in Liver Transplantation

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Liver transplantation is the only successful method for treatment of end-stage liver diseases. The aim of the present study was to examine association of HLA genes and development of liver disease and effect of HLA mismatches on liver transplant survival in liver transplantation. Study was conducted on a group of 175 patients with end stage of liver disease (autoimmune liver disease, ALD, n=18; alcoholic cirrhosis, AC, N=70 and hepatocellular carcinoma, HC, n=33). Two hundred unrelated healthy individuals were used as controls. All subjects were tested for HLA-A, -B and -DRB1 genes by PCR-SSO or PCR-SSP methods. Distribution of HLA genes between tested groups and controls showed the following significant differences: HLA-DRB1*13 was less present among AC patients in comparison to controls (7.9% vs. 16.5, p=0.007); HLA-B*13 (11.1% vs. 3.5%, p=0.05) and DRB1*04 (19.4% vs. 9.0%, p=0.05) demonstrated a significantly higher frequency in the group of ALD patients in comparison to controls; HLA-B*39 was more present in HC patients in comparison with AC patients (0.7% vs. 2.5%, P=0.03). The number of HLA mismatches (MMs) were observed to have an effect on several clinical outcomes. Firstly, a higher number of MMs was associated with a higher rate of disease recurrences in cases of ALD and HC. Second, HLA-DRB1 locus MMs contributed to a better survival of patients with AC, while there was a negative effect of HLA-DRB1 MMs on survival of patients with autoimmune etiology (shorter survival for patients with one or two mismatches compared with zero mismatches). This study demonstrated the possible role of HLA allele polymorphisms in liver transplantation. However, a more specific investigation of HLA typing, especially for locus C and DPB1 may be necessary.

Outcomes of Basiliximab and Thymoglobulin Induction an Recipients under 50 Years Old after Heart Transplantation

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To compare outcomes of induction therapy by Basiliximab vs. Thymoglobulin in recipients after heart transplantation (HT). From 2010 to 2015 we heart transplanted 34 recipients under 50 years old (mean age: 33±11 years; 20 male, 14 female). The induction therapy was: 1st group – basiliximab (50%, *n*=17; mean age: 37 ± 11 years), 2nd group – thymoglobulin (50%, *n* = 17; mean age: 30±10 years). Also they managed with triple-drug therapy – calcineurin inhibitors, mycophenolic acid and steroids. Before HT the presence of anti-HLA antibodies was diagnosed by ELISA test in 15% cases (*n*=8), 60% (*n*=3) of them were in 2nd group. Patients with the level of panel reactive antibodies (PRA) >30 % underwent desensitization treatment (DT). We estimated results of endomyocardial biopsies (EMB), frequency of rejection and graft failure during one month, six months and one year follow-up. According to EMB results R2 took place more often in 1st group (<one month –24.3%; (*n* = 9) vs. 16.6% (*n*=6); six months –15.1% (*n* = 16) vs. 10.9 % (*n*=11); over one year –12.3% (*n*=16) vs. 10.9% (*n* = 14)). However, during one month after HT the frequency of AMR1 was higher in 2nd group (5.6% (*n*=2) vs. 2.7 % (*n* = 1)), while in both groups anti-HLA antibodies were negative. As a result of positive anti-HLA antibodies (PRA = 12.5%) in six months after HT one recipient with previous DT took under control. Graft failure was diagnosed in 18% (*n*=3) patients from 1st group. Recipients with high immunological risk need to be HLA-screening more often. To treat by thymoglobulin is more effective as a prevention of rejection, graft failure.

Tissue Typing Center Zagreb - View From Outside and Inside Eurotransplant

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The solid organ transplantation program started at the University Hospital Zagreb in the early 1970s, when Tissue Typing Center (TTC) was founded and the first successful kidney transplantation was performed. In the next 35 years immunogenetic testing in TTC Zagreb supported 908 kidney transplantations, 119 heart transplantations, 257 liver transplantations and 51 pancreas transplantations in three transplantation centers. In 2007 Croatia became a full member of Eurotransplant, the biggest European non-profit organization for optimal use and cross-border sharing of cadaveric donor organs. Joining Eurotransplant requested fulfilling numerous organizational and legislative prerequisites having an European Federation for Immunogenetics (EFI) accredited tissue typing laboratory being the one of them. Croatia fulfilled these criteria when TTC in Zagreb obtained the EFI accreditation in 2007, becoming the first Croatian medical laboratory with European accreditation. Today, TTC Zagreb is responsible for the immunogenetic testing of patients from four transplantation centers (KBC Zagreb, KB Merkur, KB Dubrava and KBC Osijek) and for donor typing from 15 donor hospitals. In the 2007–2015 period immunogenetic testing was conducted for 1842 kidney recipients, 364 heart recipients, 1074 liver recipients and 92 pancreas recipients. In the same period, testing was done to support 1423 kidney transplantations, 230 heart transplantations, 774 liver transplantations and 75 pancreas transplantations. Such an intensive donor and transplantation program put Croatia among the world's most successful countries with TTC Zagreb being its integral part with an important role.

Solid Phase Assay Anti-HLA Antibodies And Their Impact on Pediatric Kidney Graft Function in One-Year Perspective

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Presence of complement-binding anti-HLA antibodies (abs.) is regarded as a risk factor in kidney transplantation, however the clinical relevance of abs. detected by sensitive solid phase assays at variable time pre- and post-transplant is still discussed. Previously, we reported no correlation between presence of anti-HLA abs. and complement-dependent crossmatch results as well as no impact on early graft survival. Here we analyzed the correlation between the presence of anti-HLA abs. and one-year graft survival and renal function. Anti-HLA abs. were present in 24/52 patients (46.2%). No differences in allograft loss rate were observed between antibody positive and negative groups (3 of 24 vs 0 of 28; *p*=0.09) or graft function after one-year follow-up, evaluated by estimated glomerular filtration rate (eGFR; modified Schwartz formula) (79.1 vs 81.5 ml/min/1.73 m^2). Donor specific anti-HLA abs. (DSA) were

present in 24 patients, among whom 12 received induction in immunosuppression with blocking ab. (anti-IL2R). No difference in rejection rate or allograft loss was observed (3/12 vs 3/12 and 1/12 vs 2/12, respectively). Despite limited number of patients, there was a non-significant trend towards lower eGFR in subgroup of patients receiving induction (79.5 vs 77.4 ml/min/1.73 m^2). In conclusion, the presence of solid-phase-assay detected anti-HLA abs. *per se* is not a risk factor for deteriorated renal function, graft rejection or graft loss at one-year post-transplant. The relevance of DSA in longer follow-up deserves further evaluation.

Analysis of the IL-17 Receptors Genetic Variants in Relation to Polymorphisms and Expression of the IL-17A and IL-17F Encoding Genes Patients Awaiting Kidney Transplantation

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IL-17A and IL-17F are proinflammatory cytokines produced by activated $CD4^+$ cells, which receptors are present on different cell types, with specific expression on cells of the spleen and kidney. These proteins are mediators of the inflammatory responses, following the activation of T cells and are associated with certain inflammatory diseases and transplant rejection reactions. The present study aimed to assess the relationships between polymorphisms within genes coding for IL-17A and IL-17F and their receptors in dialyzed patients and controls, and *IL17A* and *IL17F* genetic variants and serum levels of these cytokines in dialyzed patients. For this purpose patients (n=109) and controls (n=125) were genotyped for *IL17A* (rs2275913), *IL17F* (rs763780), *IL17RA* (rs4819554) and *IL17RC* (rs708567) alleles using Light SNiP assays. Levels of both cytokines in the patients' serum samples was assess by ELISA. It was observed that patients presented more frequently with the G allele of the *IL17F* (rs763780; A7488G) polymorphism as compared to controls (OR=22.44; CI 95% 10.65–47.30; p<0.001). Furthermore, the presence of the G allele was associated (p=0.016) with higher IL-17F serum levels (>6 pg/ml). No significant differences were observed while analyzing polymorphism and expression of the IL-17A encoding gene. Also similar distribution of the *IL17RA* and *IL17RC* genotypes was observed when dialyzed patients and controls were compared. These results showed that patients awaiting kidney transplantation and healthy controls differ with respect to the distribution of the *IL17F* genetic variants and that *IL17F* polymorphism correlates with IL-17F protein expression.

Occurrence of Autoantibodies in Highly Immunized Potential Kidney Recipients

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The aim of the study was the estimation of presence and the type of autoantibodies in a highly immunized kidney recipients which can affect the process of nephropathy after transplantation. Ninetieth-nine sera of highly immunized potential kidney recipients were tested. The identification of anti-HLA antibodies was performed (Luminex x-MAP method using LABScreen Single Antigen Class I and II tests – OneLambda) as well as identification of autoantibodies using the indirect immunofluorescence assay (IIF) and confirmatory tests (EUROLINE Profile 3 – EUROIMMUN). In 82 patients (83%) in the IIF assay revealed the presence of autoantibodies. In 21 patients (25%) within this group the presence of specific antibodies was confirmed by the EUROLINE assay. The presence and diversity of autoantibodies was more often found (11 patients/40 tested) in the group of recipients positive for both classes of anti-HLA antibodies (statistically insignificant, p=0.425. Patient No. 1 – PCNA, PM-100, Scl-70; No. 9 – Ro-52; No. 14 – Th/To; No. 17 – Jo; No. 21 – Ku, No. 26 – ACA; No. 31 – RP155; No. 32 – ASMA; No. 38 – SS-A, SS-B; No. 28, 37 – AMA. In the case of group of patients with only anti-HLA class I antibodies – autoantibodies were observed in 4 patients/30 tested (No. 70 – ASMA; No. 77 – PM-75; No. 78 – PM-100; No. 87 – dsDNA). In patients positive for anti-HLA class II antibodies, autoantibodies were obtained for 6 patients/29 tested (No. 43 – histones, nucleosomes, dsDNA, Ro-52; No. 47 – histones, nucleosomes, dsDNA, Ro52; No. 58 – RP11, RP155; No. 61, 62 – PCNA; No. 64 – Scl-70). There was no correlation between the presence of specific anti-HLA antibodies and type of autoantibodies. However, simultaneously determination of auto- and alloantibodies in kidney recipients can help in the selection of individual immunosuppressive treatment, what is associated with graft survival.

Analysis of Anti-HLA Class II Antibodies in Patients Classified to Kidney Transplant Including the Antibodies Against DP and DQ Antigens

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Assessment of anti-HLA class II antibodies against antigens DQ, DP and DR of patients classified to a kidney transplant. Sera of 104 patients classified to renal transplant were analyzed for the presence of anti-HLA class II antibodies using LAB Screen Mixed Class I and II test (One Lambda®) and then specificity of these antibodies was determined by the LAB Screen Single Antigen Class II test (One Lambda®). The presence of anti-HLA-DQ antibodies was demonstrated in 80% of patients (84/104), anti-HLA-DR in 76% (80/104) and the anti-HLA-DP in 53% (56/104). Coincidence of the three types of antibodies: anti-HLA-DP, DQ and DR were found in 35% of patients (37/104), while the presence of two antibodies types: anti-HLA-DQ and anti-HLA-DR were detected in 27% of cases (29/104), anti-HLA-DP and HLA-DR in 8% (9/104), and anti-HLA-DP, and anti-HLA-DQ in 4% (5/104). The presence of one type of antibodies: anti-HLA-DQ or anti-HLA-DP or anti-HLA-DR was found in respectively: 12% (13/104) 4% (5/104) 4% (5/104) of the cases. In conclusions: 1/ The most common antibodies of anti-HLA class II are antibodies against DQ antigens. 2/ Anti-HLA-DQ and DP antibodies have been detected in 20% (23/104) of the patients without presence of anti-HLA DR antibodies. Thus, it is justified to routine determination and consideration of compatibility between donor and recipient not only within the HLA DR antigen, but also within the HLA DQ, and DP during choosing of the most suitable kidney recipient.

Allogeneic Skin Graft Application in the Treatment of Burn Wounds

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Autologous skin grafting, performed after resection of necrotic tissue is the best way to close the burn wound. Unfortunately, each harvest of skin leads to another wound, which may cause further complications. Currently, a good solution is application of allogeneic skin graft. The objective of this study was to evaluate the effectiveness of the allogeneic and autologous skin grafts in wound closure and impact assessment of these two methods on the duration of hospitalization in two groups of patients. The experimental group consisted of 22 patients (7 females, 15 males) who were treated with allogeneic skin grafts, while the control group of the same size (6 females, 16 males) received autologous skin grafts. The average age of all patients was 51 years, the mean total burn surface area was 12% and the average burn degree was II/III. The study showed that the average length of hospital stay among patients from experimental group (16 days) was less than the mean duration of hospitalization of patients from control group (22 days). Furthermore, in 16 (73%) patients in experimental group, allogeneic graft resulted in fully healed wounds, while remaining 6 (27%) required autologous skin transplantation. In the control group wounds of 18 patients (82%) were healed and autologous regrafting was performed in remaining 4 (18%). The results of this study confirm that autologous skin grafting is the best method to burn wound closure, however allogeneic skin graft application seems to be an alternative solution taking lower traumatization and shorter hospitalization length into account.

The Impact of Hyperbaric Oxygen Therapy on Prolongation of Skin Allograft Survival Among Burn Patients

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Human cadaveric cutaneous allografts are used in burns surgery usually as a temporary dressing of partial thickness burns. In many cases concurrent application of mentioned grafts and hyperbaric oxygen therapy results in definitive burn wound closure increasing the time of graft adhesion to the wound. The aim of this study was to evaluate the influence of hyperbaric oxygen on allogeneic skin graft

ingrowth into wound bed and on lengthened the time to its rejection thereby reducing the need for autologous skin transplantation. The experimental group consisted of 20 patients who were treated with allogeneic skin grafts and hyperbaric oxygen, while the control group of the same size received only allogeneic skin grafts. In the experimental group the mean time of allogeneic skin adherence to the wound was 16 days. Among the 14 patients complete wound healing under allogeneic graft succeeded, while remaining 6 patients required autologous skin graft. The average hospital stay was 22.5 day. In the control group the average of allogeneic skin adherence to the wound was 12.4 day. The total epithelialization and complete wound closure under allogeneic skin occurred in 7 patients, while in the remaining 13 patients autologous skin transplantation was performed. Hospitalization time of each patient was equal to an average of 30.4 day. Hyperbaric oxygen can help maximize the viability of the wound bed while revascularization takes place, thereby extending skin allograft survival and reducing the need for regrafting procedures, which incur further operations and increased donor site morbidity.

Disease Association

Immunogenetic Factors Involved in Cancer

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Immunogenetics integrates two exciting scientific fields of immunology and genetics. It is getting clear that genetic variations in immune response genes may impact on differences related to the type and strength of antitumor immune response. The question arises whether and to what extend the patient‘s immunogenetic characteristics may influence the susceptibility to or protection from malignancies. Most studies pointed out the undeniable role of HLA polymorphism in susceptibility, prognosis, natural history, and response to immunotherapy in different cancers. Less is known about two other highly polymorphic gene systems – cytokines and KIR genes, but it is assumed that they could affect immune mechanisms of cancerogenesis. Complex investigation of variation at these loci gives an opportunity of discovering advanced diagnostic/prognostic biomarkers and molecular targets for prevention, immunopharmacology and immunotherapy relevant to a wide variety of cancers. In addition, the researchers might translate these novel understandings in a post-genomic era in personalized medicine. The advances in understanding immunogenetic factors involved in cancer, together with our experience of the relevance of HLA, KIR and cytokines genetic profiles as biologic predictors for the risk of cancer development will be summarized. Finally, we will attempt to integrate in schematics KIR and HLA genetic associations with different malignant diseases, their potential impact on NK cell function and the promotion of or protection from solid tumors and blood cancers.

MicroRNAs in Diagnostics and Prognostics of Human Diseases

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The discovery of microRNAs (miRs), non-coding regulators of gene expression, brought new insight into the pathology of human diseases. Deregulation of miRs results in aberrant levels of numerous protein-coding genes, underlying initiation and progression of the disorder. MicroRNA expression profiles are highly tissue and disease specific, and can serve as potent molecular diagnostic markers. Moreover, cellular miRs are excreted to body fluids and show high stability in serum or urine, what brings new possibilities for non-invasive monitoring of many diseases. MiRs are best studied in human malignancies such as leukemia or cancers of the thyroid, kidney, adrenal cortex and prostate, for which specific and sensitive miR-based diagnostic panels have already been elaborated. The role of miRs in autoimmune disease has been a subject of extensive investigations. MiR-148a has been recently identified as a critical regulator of B cell tolerance, and the expression of this miR is significantly elevated in patients with systemic lupus erythematosus. Different sets of miRs are associated with systemic sclerosis, rheumatoid arthritis or arteritis, and the studies on miRs in autoimmunity are still on-going. The cellular “microcosm” is emerging as a very promising field in translational research, and provides excellent diagnostic and prognostic tools for the management of human diseases.

Relationships Between the miRNA-146a Polymorphism and Expression in Patients with Rheumatoid Arthritis

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MicroRNA-146a (miR-146a) has been shown to play an important role in the negative regulation of inflammatory innate immune responses, and found to be differentially expressed in rheumatoid arthritis (RA). These observations prompted us to analyze the potential associations between miR-146 rs2910164 polymorphisms and miR-146a expression in patients' sera in relation to clinical outcome of the treatment as well as predisposition to the disease. One hundred and eleven patients and 130 healthy individuals were genotyped for the miR-146a (rs2910164, G>C) alleles with the use of Light SNiP assay. For analysis of the miR-146a expression, RNA was isolated from sera of 13 patients (before and three months after anti-TNF treatment) and 16 controls (Nucleospin®miRNA Plasma; MACHEREY-NAGEL GmbH&Co. KG) followed by cDNA synthesis (TaqMan® MicroRNA Reverse Transcription Kit; Applied Biosystems™ by Life technologies) and Real-time PCR amplifications with hsa-miR-146a TagMan specific and U6 snRNA control primers for each probe. The results were analyzed using the (ΔΔCt) calculations. The highest miR-146a expression was detected in controls as compared to patients, especially before anti-TNF treatment (5.302±2.112 vs 0.422±0.712; p=0.048). Moreover, patients after 3 months of TNF inhibitors administration had higher miR-146a levels in serum than those before treatment (1.809±0.658 vs 0.422±0.171; p=0.033) and were more frequently carrying the C allele (p=0.032). No significant association was found between miR-146a polymorphism and disease susceptibility or progression. These results support the hypothesis that miR-146 might not be acting as a negative regulator of inflammation in RA and imply that miR-146a polymorphism may be associated with mi-RNA levels in serum after anti-TNF treatment.

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ERAP2 Variants Influence the Risk of Psoriatic Arthritis in HLA-B27-Negative Patients

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The group of spondyloarthropathies includes ankylosing spondylitis, psoriatic arthritis (PsA) and other rheumatic inflammatory disorders. A common genetic feature of spondyloarthropathies is the presence of HLA-B27 antigen, but in the case of PsA approximately 75% of the patients are HLA-B27-negatives. Endoplasmic reticulum aminopeptidase 2 (ERAP2) is a zinc-dependent enzyme involved in trimming the MHC class I-presented peptides. Recent studies have shown that *ERAP2* gene variants are associated with HLA-B27-negative ankylosing spondylitis. The aim of this study was to analyze two single nucleotide polymorphisms (SNPs) of *ERAP2* gene in HLA-B27-negative PsA patients and controls of Romanian origin. HLA-B27 typing was carried out for 100 PsA patients and 123 controls using the B27-SSP low resolution kit (Olerup, Sweden). *ERAP2* gene SNPs rs2248374 and rs2910686 were genotyped using TaqMan Allelic Discrimination Assays (Applied Biosystems, USA). Association tests were performed with the software package PLINK v 1.9. Controls and patients were in Hardy-Weinberg equilibrium for the two SNPs. The HLA-B27-negative patients (n=79) and controls (n=111) were investigated for disease association. Both SNPs were associated with the disease at allele and genotype level, but only for rs2248374 the association remained significant after correction (minor allele A 45% in controls, 56% in PsA, p=0.02, pcorr=0.04, OR 1.6, 95%CI 1.063–2.417). The haplotypes analysis revealed that the protective haplotype rs2248374G/rs2910686T had a significant lower frequency in patients (43%) versus controls (55%, p=0.01). These results suggest that *ERAP2* gene variants influence the risk of psoriatic arthritis in Romanian HLA-B27-negative individuals.

Comparison of Associations of Endoplasmic Reticulum Aminopeptidase 1 Polymorphisms with Non-Small Cell Lung Cancer in Poles and Chinese

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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. Aminopeptidase residing in endoplasmic reticulum 1 (ERAP1) trims peptides to the optimal length for HLA-I binding. Single nucleotide polymorphisms (SNPs) in the ERAP1 gene result in changes in aminopeptidase activity and specificity. This may affect susceptibility to cancer. However, non-small cell lung carcinoma (NSCLC) has not been studied in this respect. We compared genotype and haplotype frequencies of four coding, nonsynonymous ERAP1 SNPs, rs26653G>C, rs26618T>C, rs30187C>T, and rs27044C>G, in NSCLC in two genetically distant populations, Chinese and Poles. We found associations of all four SNPs with NSCLC in Chinese but not in Poles. The differences in ERAP1-NSCLC associations 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.

Expression Level of Bone Morphogenetic Proteins 4 in Human Non-Small Cell Lung Cancer

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Lung cancer is a serious oncological problem, both in Poland and all over the world. Non-small cell lung cancer (NSCLC) represents the vast majority of lung cancer cases (80%). Relatively low level of five-year survival term is the reason of the late detection of the disease. The standard for advanced NSCLC in the first line therapy is platinum-based chemotherapy that, unfortunately, often results in the development of chemoresistance. The bone morphogenetic proteins (BMP), members of the transforming growth factor superfamily are phytogenetically conserved proteins, which are essential for embryonic development. Studies have suggested that highly homologous BMP-4 may be associated with a poor treatment response. The present study aimed to explore the prognostic implications of the expression level of BMP-4 in serum of NSCLC patients. Serum samples were collected before chemotherapy from 58 hospitalized patients in the Chair and Department of Pulmonology and Lung Cancer Wroclaw Medical University, Poland from September 2015 to December 2015. Control sera were collected from 22 blood donors in Wroclaw Regional Center of Blood Donation. BMP-4 levels were evaluated using The Thermo Scientific™ Human BMP-4 ELISA Kit. BMP-4 serum levels were significantly lower in NSCLC patients ($p<0.001$). Median level of BMP-4 in healthy control equalled 292.58 (lower-upper quartile: 188.35–403.61), whereas in patients was lower: 64.90 (lower-upper quartile: 24.29–184.13). The BMP-4 levels if correlated with the results of platinum treatment may be used as a potential predictor for the chemotherapy response and prognosis of advanced NSCLC leading to the improvement of patients' survival.

HLA Typing as a Diagnostic Tool for Narcolepsy

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Narcolepsy is a chronic sleep disorder affecting 0.02–0.18% of the general population. Human narcolepsy is a multifactorial disease, involving genetic and environmental factors. Two independent diagnostic units were established in ICSD2 (The International Classification of Sleep Disorders): narcolepsy with cataplexy (type 1) and narcolepsy without cataplexy (type 2). The association of DQB1*06:02 with the narcolepsy is known from 1980s. The DQB1*06:02 allele is present in patients with the diagnosis of narcolepsy-cataplexy in 96–100 % of cases. Genotype of locus HLA-DQB1 on high/medium resolution level has been analysed in the cohort of patients with the suspected diagnosis of narcolepsy. The testing was performed by PCR-SSP method. The analysed cohort consists of 971 patients (496 male, 475 female) with the suspicion of narcolepsy-cataplexy (type 1). The presence of DQB1*06:02 was confirmed in 373 of them (38 %), of whom 53 were homozygotes (5.5% of the whole cohort). The homozygosity of DQB1*06:02 is reported as a higher risk of narcolepsy without any influence on the disease progression. On the contrary the protective allele DQB1*06:03 was detected in 106 individuals (11%), in nine of them in homozygous status. In the patients without DQB1*06:02 (62%) the diagnosis of narcolepsy-cataplexy was excluded and other neurological diagnosis were identified. The genotyping of HLA in patient with the suspected diagnosis of narcolepsy-cataplexy is a useful diagnostic tool for the establishment of the correct diagnosis.

Distribution of Genes HLA-A, B Loci on Russian Patients with Ulcerative Colitis Living in Chelyabinsk Region

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Ulcerative colitis (UC) is a disease that causes inflammation and sores (ulcers) in the lining of the large intestine. Some studies indicated an association between genes of HLA II class and predisposition to UC. The data of assessment of the distribution of HLA I genes of patients with UC are contradictory. To determine the frequencies of genes HLA-A, -B in patients with UC Russian population of the Chelyabinsk region. DNA typing of HLA-A, -B genes was performed in 72 UC patients and 591 healthy controls Russian nationality of Chelyabinsk Regional Blood Transfusion Station. Determination of genes HLA-A, -B was performed by molecular typing – PCR SSP using sets of Protrans (Protrans, Germany). Data were analyzed using genes frequencies, the χ^2 test (Pearson's test) and the criterion OR with 95% confidence interval. It was established the distribution of genes HLA-A, -B in patients with UC. There were found the 13 specificities in HLA-A locus and 20 in HLA-B locus. It was determined that the frequency of genes HLA-A*02 (0.42 vs 0.31; p=0.018, OR=1.85), HLA-A*29 (0.035 vs 0.02; p=0.013 OR=3.6), and HLA-B*07 (0.24 vs 0.13; p=0.002, OR=2.18) in the group of patients was higher in comparison with the donors group. According to preliminary data, the alleles HLA-A*02, -A*29, -B*07 may be markers of ulcerative colitis in Russian Chelyabinsk region.

Altered mRNA Expression of *TMEM39A* on Multiple Sclerosis Patients

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Genome-wide association studies (GWAS) have identified hundreds of new potential genetic risk *loci* associated with numerous complex diseases such as multiple sclerosis (MS). Genes which have been discovered by GWAS are now the focus of numerous ongoing studies which goal is to confirm and understand their potential role in MS susceptibility. The present study, explore the *TMEM39A* (mRNA-transmembrane protein 39A) gene, which was found to be associated with MS susceptibility in GWAS performed by IMSGC (International Multiple Sclerosis Genetics 2010). First, we performed *TMEM39A* mRNA expression analysis in the group of 39 relapsing–remitting (RR) MS patients and 40 controls and then we examined the effect of two *TMEM39A* polymorphisms (rs17281647

and rs1132200) on its mRNA level. Moreover, we investigated *TMEM39A* methylation status. Additionally, we conducted a case-control study (on 336 patients and 322 controls) to find possible association of the investigated polymorphisms with susceptibility and/or progression of MS in well-defined Polish population. Although we were not able to confirm the association of *TMEM39A* polymorphisms (rs1132200 and rs17281647) with predisposition to multiple sclerosis, we showed, for the first time, the difference in *TMEM39A* mRNA expression between MS patients and controls ($T^2_{2;74}$ =5.429; p=0.0063). In our study the lower mRNA expression of *TMEM39A* gene in patients was associated neither with rs1132200 nor with rs17281647. It did not correlate also with higher methylation level of *TMEM39A* promoter. In conclusion, we showed that *TMEM39A* mRNA expression may have influence on susceptibility to MS.

Anti-HLA Antibody in Patients Group with SLE

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Patient's sera after blood transfusion or after transplantation as well as sera from pregnant woman are good specimen to investigate presence of anti-HLA antibodies. Sensization to polymorphic proteins mostly HLA class I and HLA class II molecules occur when patient is exposed to cells from other individuals. In present study we investigate presence of anti-HLA antibodies in patient group with systemic lupus erythematosus (SLE). In this specific autoimmune disease with presence of variety of antibodies pathogenetic importance of lymphocytotoxins was suggested. The study comprised sera collected from 40 female SLE patients. Blood donors were used as controls. We used LABScreen Luminex xMAP technology simplifies the process of identifying anti-HLA antibodies. HLA antibody screening were expanded by Fc gamma RIIa, Fc gamma R IIIb and bFGF genotyping with use of PCR-SSP. Anti-HLA antibodies were found in 18 of 37 of serum (45%) with SLE in comparison to 1 of 36 of serum (2.7%) in control group. We observed mostly anti-HLA class II. Fc gamma RIIa H/H genotype was not found in patient group with SLE. There was no difference for R/H and R/R genotypes regard patients with SLE and control group. We observed a higher frequency of Fc gamma R IIIb NA1/NA1 genotype in patient group (30%) than in control group (16%). There are many mechanism of HLA antibody activity. They have multitude of biological aspects it seems to be very interesting to consider autoimmunity in aspect of Fc receptors properties.

Polymorphisms in Co-Stimulatory *CD28/CTLA-4* Pathway Genes and the Risk of Prostate Cancer

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Polymorphisms in co-inhibitory genes are associated with susceptibility to malignancies. The aim of this study was to evaluate the possible associations between polymorphisms in *CD28* and *CTLA-4* gene and prostate cancer (PCa). A total of 301 PCa patients and 301 controls were genotyped for single nucleotide polymorphisms (SNPs) in the *CTLA-4* and *CD28* genes: *CTLA-4*c.49A>G, *CTLA-4*g.319C>T, *CTLA-4*g.*6230G>A(CT60), *CTLA-4*g.*10223G>T(Jo31), *CD28*c.17+3T>C and *CD28*c.-1042G>A. The distributions of the alleles, genotypes and haplotypes in the *CTLA-4* and *CD28* SNPs were similar in PCa patients and controls. However, the overrepresentation of *CTLA-4*c.49A>G[A] allele carriers in PCa patients as compared to healthy men was observed (0.86 vs 0.80, p=0.082, OR 1.46, 95%CI: 0.95–2.25). There was also insignificant prevalence of carriers of T alleles at *CTLA-4*g.319C>T SNP in PCa patients (0.26 vs 0.20, p=0.13 OR 1.35, 95%CI: 0.92–1.97). Moreover the risk of disease was significantly higher for carriers of susceptibility alleles for both SNPs: *CTLA-4*c.49A>G[A] and *CTLA-4*g.319C>T[T] as compared to carriers of protective genotypes (*CTLA-4*c.49A>G[GG] and *CTLA-4*g.319C>T[CC]) (OR 1.78 95%CI: 1.06–3.01; p=0.03). For other investigated polymorphisms similar distribution of alleles and genotypes was observed in PCa patients and healthy men. Our results indicate that polymorphisms in CTLA-4 gene CTLA-4c.49A>G and CTLA-4g.319C>T might be considered as low risk susceptibility locus for PCa patients, but keeping in view the correction for multiple hypothesis testing our results should be treated with caution and require further studies on larger group of patients.

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Gene-Gene Association in Cervical Squamous Cell Carcinoma Polish Population: Preliminary Studies

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Abnormalities in DNA repair system and regulation of tumorigenesis, inflammation, adipocyte differentiation and glucose homeostasis are one of the crucial factors implicated in carcinogenesis. Studies on the polymorphic variability within genes encoding molecule critical in those pathways may indicate whether a specific genetic variants are linked to cancer susceptibility and/or clinical outcome by affecting the function of their protein products. The main aim of our research was to assessed the gene-gene interaction between selected 13 SNPs located within two DNA repair genes: *ERCC4* and *XRCC3*, and a member of nuclear hormone receptor superfamily – peroxisome proliferator-activated receptor-γ (*PPARγ*) in cervical squamous cell carcinoma (CSCC). In population-based/-case-control association study in 280 Polish Caucasian women, including 139 CSCC patients, two *ERCC*4tagSNPs (rs3136176, rs1799798), seven *XRCC3*tagSNPs (rs3212079, rs3112102, rs861534, rs861537, rs709399, rs12432907, rs1799796) and four functional *PPARγ*SNPs:Val290Met (rs72551362), His449His (rs3856806), Phe380Leu (rs72551363) and Pro12Ala (rs1801282) were studied. Combined effects of all studied genetic marker on the development of CSCC as well as on remission achievement were analyzed by multivariate logistic regression analysis employing a quasi-Newton estimation method (Epi Info^{TM}7.1.1.14). The combined gene-gene association analysis pointed that four of studies polymorphism were independent CSCC risk factors. The most pronounced association was observed for *XRCC3*rs3212079 marker, which 4.13-fold increased risk of CSCC (p=0.0009). Weaker association were observed for *PPARγ*Pro12Ala (rs1801282) and *ERCC4*rs3136176 markers (p=0.03, OR=2.15, and p=0.04, OR=2.89, respectively). Contrary, *PPARγ*His449His (rs3856806) polymorphism was the independent CSCC-protection factor (p=0.04, OR=0.51). Altogether, this indicate a significant role of polymorphic variation of selected genes in the pathogenesis CSCC.

Interleukin 1 Beta Gene Variability Impact on Etiology of Recurrent Aphthous Stomatitis: Data From a Genetic Association Analysis in a Polish Cohort of Patients

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Recurrent aphthous stomatitis (RAS) belongs to the group of chronic, inflammatory, ulcerative diseases of the oral mucosa, usually leading to patient's suffering and pain. Its prevalence in a general population ranges between 5% and 20%. The potential trigger factors which can modify the immunologic response in patients with RAS include, e.g., viral and bacterial infections, systemic diseases, mechanical injuries and increased oxidative stress. The observations suggest that there are significant ethnic differences in a genetic basis of the disease. The results of the *Interleukin-I-β (IL-1β)* gene variability analysis in several European and non-European populations of patients are inconsistent. Therefore, we decided to perform IL-1β gene analysis in a Polish cohort of patients. The aim of our study was to estimate a distribution of the two following DNA polymorphisms: IL-1β T[-511]C and C[+3954]T among 104 patients with RAS and 273 control individuals (without RAS). The genotyping was carried out with the use of genomic DNA isolated from blood samples and PCR-RFLP approach. Differences of genotypes and alleles frequencies in the two groups did not reach statistical significance. Our preliminary results did not confirm the earlier suggestion about the association between IL-1β [-511]*C/IL-1β [+3954]*T alleles and RAS. As the immunogenetic background of RAS remains still not clearly defined, the treatment is mainly symptomatic and not very effective. Thus, the disease remains a challenge for clinicians of various specialities and discovering genetic defects of RAS may be helpful in both a risk prediction of the disease and an effective, causative therapy.

Genetic Factors of Diabetes: Molecular Diagnosis of Selected Cases

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The course covers the latest news in the field of etiopathogenesis of diabetes in her broad terms, identifying both the substrate disease and therapeutic possibilities. Problems presented in the lecture relate to the genetic basis of disease, monogenic forms of diabetes identified in the pediatric population. The presentation will be presented previously known genetic basis of diabetes and possibilities of interpretation of the result, or "predictive" (providing consultative) affecting the behavior of the patient from the diagnostic and therapeutic e.g. the impact on the selection of therapy or verify (changes) have already implemented therapies patient and sick family members. At the same time the results of genetic identification in subsequent stages of the clinical – diagnostic in relation to the patient and his family plays a key role in genetic counseling and prognostic, not once drastically affecting the important decisions of family. The lecture also aims to approximate the problem of modern genetics and its use in specialized laboratories from both the diagnostic and scientific and visibility of the problem of selection of appropriate tools of molecular biology to identify the problem from the side of "molecular genetics". Interpretation of his confirmation and documentation is at the present moment is also a big problem and presenting it in a readable form for the clinician, mistakes such a process are also discussed in the above lecture. They will also be presented exemplary results and interpretation indicating problems differences in clinical practice.

One-Carbon Metabolism in Schizophrenia: Lessons From Genetic and Epigenetic Studies

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It is increasingly being recognized that schizophrenia patients are characterized by one-carbon metabolism alterations including elevated homocysteine and reduced folate levels. Several studies have also demonstrated positive association between homocysteine levels and the severity of negative symptoms. Importantly, these alterations have been reported in drug-naïve first-episode psychosis patients, implying that one-carbon metabolism alterations might originate from genetic underpinnings. Indeed, a number of meta-analyses have provided that the C677T polymorphism in the methylenetetrahydrofolate gene (*MTHFR*), which encodes one of key enzymes involved in one-carbon metabolism, serves as risk factor for schizophrenia. In addition, this polymorphism has been found to predict the development of metabolic alterations in the course of antipsychotic treatment. On the other hand, several studies have provided evidence for epigenetic dysregulation in schizophrenia patients. It has been observed that schizophrenia patients present impaired DNA methylation both in the brain and peripheral tissues. In addition, some studies have revealed concordant changes in DNA methylation in the brain and peripheral tissues. Differentially methylated genes in schizophrenia include those encoding proteins involved in neurotransmission and immune-inflammatory processes. Overall, these findings suggest that one-carbon metabolism disturbances and impaired epigenetic mechanisms play a role in the pathophysiology of schizophrenia. Future studies should disentangle clinical relevance of these alterations and their potential application as drug targets.

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Association Study of Functional Polymorphisms in Interleukins and Interleukin Receptors Genes: IL1A, IL1B, IL1RA, IL6, IL6R, IL10, IL10RA and TGFB1 in Schizophrenia

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Schizophrenia has been associated with a large range of autoimmune diseases, with a history of any autoimmune disease being associated with a 45% increase in risk for the illness. The inflammatory system may trigger or modulate the course of schizophrenia through complex mechanisms influencing neurodevelopment, neuroplasticity and neurotransmission. In particular, increases or imbalance in cytokine before birth or during the early stages of life may affect neurodevelopment and produce vulnerability to the disease. A total of 27 polymorphisms of IL1N gene: rs1800587, rs17561; IL1B gene: rs1143634, rs1143643, rs16944, rs4848306, rs1143623, rs1143633, rs1143627; IL1RN gene: rs419598, rs315952, rs9005, rs4251961; IL6 gene: rs1800795, rs1800797; IL6R gene: rs4537545, rs4845617, rs2228145, IL10 gene: rs1800896, rs1800871, rs1800872, rs1800890, rs6676671; IL10RA gene: rs2229113, rs3135932; TGF1B gene: rs1800469, rs1800470; each selected on the basis of molecular evidence for functionality, were investigated in this study. Analysis was performed on a group of 621 patients with diagnosis of schizophrenia and 531 healthy controls in Polish population. An association of rs4848306 in IL1B gene, rs4251961 in IL1RN gene, rs2228145 and rs4537545 in IL6R with schizophrenia have been observed. rs6676671 in IL10 was associated with early age of onset. Strong linkage disequilibrium was observed between analyzed polymorphisms in each gene, except of IL10RA. We observed that haplotypes composed of rs4537545 and rs2228145 in IL6R gene were associated with schizophrenia. Analyses with family history of schizophrenia, other psychiatric disorders and alcohol abuse/dependence did not show any positive findings. Further studies on larger groups along with correlation with circulating protein levels are needed.

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Aldosterone Synthase Gene Polymorphism in Hypertension And Obesity

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Hypertension has a multifactorial background based on genetic and environmental interactive factors. Elevated levels of steroid hormone aldosterone are associated with arterial hypertension, congestive heart failure, chronic kidney disease, and obesity. We aimed to test for the association of the aldosterone synthase (CYP11B2 C-344T (rs1799998)) gene polymorphism with hypertension and obesity. For this purpose, 48 patients with hypertension and 135 healthy individuals (71 with the BMI $\leq$25, 64 with the BMI >26, age: 18–65 years) were genotyped to identify SNP using the Light SNiP assay. Hypertensive cases presented with a higher frequency of the C allele (RR=2.282, p=0.051). Similarly, the C allele was more frequently detected among man with class I obesity (BMI 30–35; p=0.035). The CYP11B2 C-344T polymorphism (rs1799998) may contribute to a hypertension phenotype and obesity, especially in men.

Hematological Diseases and Stem Cell Transplantation

Single Centre Experience in Bioethical Problems in Children Stem Cell Transplantation

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Between 1994 up to 1050 patients aged underwent stem cell transplantation in Poland "Cape of Hope". We observed ethical problems in every phase of the complex transplantation procedure: pre-transplantation care, donor search, harvest, transplantation phase (which includes the preparative regimen, cell infusion and hospitalization until the patient has recovered from neutropenia), short-term follow-up care (1–3 months) and long-term follow-up care. However, bone marrow transplantation (BMT) is associated with a substantial risk of potentially life-threatening acute complications, as well as significant chronic organ toxicity. In some patients, BMT-related complications are superimposed on pre-existing chronic organ insufficiency, such as renal insufficiency related to previous nephrotoxic treatment. In addition, almost all

patients entering BMT treat show significant immunosuppression, either due to cytostatic or immunosuppressive pre-treatment or as result of the their underlying disease. These pre-existing factors, as well as BMT-related parameters such as the toxicity of the conditioning regiment, infection or graft-versus-host disease, may all contribute to the clinical course after BMT and ultimately determine outcome. The high cost per patient of hematopoietic stem cell transplantation causes this therapy to be the focus of much controversy, given the competing societal demands to provide all possible therapy to preserve life while simultaneously limiting global health care expenditures. Last but not least bioethical problem is that approaches to gene transfer and therapy can utilize transplantation methodologies and augment their effects. Selection of candidates for hematopoietic transplantation is another idea of tension between transplant centers and the maternal center of child and should be done according to international guidelines.

High Resolution HLA-Matching in Hematopoietic Stem Cell Transplantation

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Blood stem cell transplantation (SCT) has become an established treatment method for a variety of congenital or acquired disorders of the hematopoietic system. A key factor for the success of allo-SCT is HLA compatibility between patient and donor. According to the current German consensus for the selection of unrelated donors, compatibility is sought on allele level for the loci HLA-A, -B, -C, and -DRB1 -DQB1 (10/10). HLA incompatibilities lead to increased non-relapse mortality, particularly as a result of graft-versus-host disease associated complications. The influence of individual incompatibilities on the success of stem cell transplantation depends on the individual allele combination and the respective gene locus. There is common agreement, that the risk for early death after hematopoietic stem cell transplantation cumulatively increases with the number of HLA incompatibilities. Therefore, unrelated donors with more than two HLA incompatibilities are usually not accepted. In a single mismatched situation (9/10), allele and antigen incompatibilities for the loci HLA-A, -B, and possibly also -DRB1, are equally unfavorable. For HLA-C, antigen-incompatibilities are associated with increased risk. This has not been shown for HLA-C allelic incompatibilities, and may be caused by the presence of permissible combinations (for example, HLA-C*03:03 and HLA-C*03:04). For HLA-DQB1 incompatibilities, no clearly significant effect on survival has been shown, but there is evidence from our cohort, that HLA-DQB1 antigen-incompatibility, in contrast to HLA-DQB1 allelic incompatibilities, is possibly associated with an increased risk. Furthermore, additional loci in the extended HLA region (for example, HLA-DPB1, HLA-DRB*345, MICA) and donor KIR genes may gain importance for histocompatibility testing in future.

Prediction of NK Cell Licensing Level in Selection of Hematopoietic Stem Cell Donor

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NK cell licensing status depends on clonal expression of inhibitory killer cell immunoglobulin-like receptors (iKIR) and short term HLA environment. Licensed NK cells are more efficient in tumor killing than unlicensed NK cells. Cognate KIR-HLA pairs in stem cell transplant (SCT) donor and recipient are decisive for the possible change in the NK cell licensing status after SCT. We assessed clinical outcomes in 297 patients (malignant or MDS) in a model with upward licensing, downward resetting and unchanged licensing status after SCT. We found better disease free survival (DFS) and lower relapse incidence (RI) in recipients with upward licensing status and reduced DFS and highly increased RI among patients with the downward resetting status of repopulated donor NK cells after SCT, as compared with unchanged NK cell

licensing (for DFS: HR=2.32, p=0.0031, 95%CI: 1.33–4.17 and for RI: HR=4.76, p=0.0026, 95%CI: 1.72–14.29). The incidences of grade I–IV acute graft-versus-host disease (aGvHD; 80%, 62% and 51%, p=0.27±0.37) and grade III–IV aGvHD (21%, 15% and 11%, p=0.60±0.39) were not significantly different in three strata. We conclude that, better donors for malignant patients can be selected considering increased number of cognate HLA-iKIR pairs in recipient after SCT as compared with the pre-transplant donor status. Conversely, donors with increased risk of inferior DFS and RI can be ruled out based on prediction of decreased number of cognate HLA-iKIR pairs post-SCT.

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Impact of Killer Cell Immunoglobulin Like Receptors on Hematopoietic Stem Cell Transplantation

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In stem cell transplantation, donor natural killer cells contribute significantly to the eradication of residual leukemic cells in the recipient. Natural killer cells exert their cytotoxic effect through engagement of killer immunoglobulin-like receptors (KIRs) with their cognate HLA ligands on target cells. HLA and KIR segregate independently on different chromosomes; HLA-identical individuals have completely different KIR genotypes. Better engraftment and a significantly decreased incidence of relapse and acute graft-versus-host disease (aGVHD) was associated with activating KIR that is most prominent in patients suffering from acute myeloid leukemia. The aim of the study was to assess the impact of KIR/HLA genotype combination on transplant outcome including, GVHD, leukemic relapse, survival after hematopoietic stem cell transplantation (HSCT). The study included 65 patient/-donor pairs who received peripheral blood stem cell transplantation from HLA-matched identical siblings. KIR genotyping was done for all donors using reverse sequence specific oligonucleotide probes (rSSO) coupled with luminex technology and HLA-C genotyping was done for all patients using rSSO strip assay. Most frequent KIR haplotype was B/x 55/65 (84.6%) while A/A haplotype frequency was 10/65 (15.4%). The most frequent KIR ligand was HLA-C07; 28/130 (21.5%). KIR2DS4 was associated reduced risk of relapse (p=0.002). A trend towards reduced risk of relapse was observed with more than two CenB motifs (p=0.09), while homozygous HLA-C2 ligands was associated with increased relapse (p=0.04). Activating KIR and HLA-C alleles have an impact on HSCT outcomes and better selection of donors with favorable KIR genotype can improve HLA matched sibling HSCT outcome.

Methodology of Cell Chimerism Monitoring in Patients after Allogeneic Hematopoietic Stem Cell Transplantation

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The examination of cell chimaerism has been investigated in patients after allogeneic hematopoietic stem cell transplantation (allo-HSCT) indicated for malignant and nonmalignant diseases in the National Reference Laboratory for DNA Diagnostics in Prague since 1992. Early detection of donor/recipient ra tio of DNA and subsequent individual approach to patient is important as well as selection of method for monitoring. The examination comprises two parts. A) Informativity determination – the comparison of the recipient's and donor's DNA profiles using short tandem repeats (STR) polymorphisms and short insertion-deletion (indel) polymorphisms. Fragment analysis and real-time PCR are usually methods of choice. B) Quantitative assessment of cell chimaerism after allo-HSCT – evaluation of ratio of donor's/recipient's DNA. Chosen method (especially its sensitivity) and the most suitable polymorphisms are the most important for successful monitoring. The sensitivity of STR analysis is 1% and the sensitivity of real-time PCR is 0.035% (internal validation). Mixed chimaerism after allo-HSCT is regularly detected the 14th day after transplantation and therefore two STR polymorphisms are used for quantification. When the complete chimaerism (CC) or microchimaerism is detected, one indel analysis is added for quantification. In long-term monitored patients with CC or microchimaerism the combination of STR and indel analysis is used. If autologous haematopoiesis increases, two STR markers are examined additionally. Combination of methods according to their sensitivity is important for chimaerism testing and

selection of the method is strictly dependent on individual patient's chimaerism trend.

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Peripheral Blood Progenitor Cells Mobilization is Associated with G-CSF Pathway Genes Expression Changes on mRNA and Protein Levels

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The G-CSF/CSF3R pathway plays a key role in peripheral blood mobilization for transplantation purposes. In the present study, the CSF3R, STAT3, STAT5B and SOCS3 gene expression on mRNA level and *CSF3R* rs3917924 C>T polymorphism (with the *T* allele reported to reduce mobilization efficacy) was investigated in 32 (females/males: 16/16) patients. Additionally, changes in CSF3R protein expression on the surface of neutrophils (counted as $CD16^+$ granulocytes) and monocytes ($CD14^+$ blood mononuclear cells) were measured by flow cytometry in blood samples obtained from 7 (F/M=6/1) patients. Samples for both expression analyses were preserved on the fifth day and prior to the G-CSF-induced mobilization. A positive correlation was observed regarding changes in CSF3R expression in comparison with STAT3 (Pearson Correlation Coefficient R=0.673; $p<0.001$) or SOCS3 (R=0.629; $p<0.001$), while negative with STA5B (R=–0.436; $p=0.013$) levels. CSF3R mRNA level more often decreased due to mobilization in patients carrying the wild-type *C* allele than in *TT* homozygotes ($\Delta E<0.5$; 17/23 vs. 3/9; $p=0.049$). Number of neutrophils expressing CSF3R was reduced after the mobilization (from the mean of 93.6% to 51.1% of cells) in 6 of 7 patients and increased in 1 case only (34.8–84.3% cells). Changes in the percentage of monocytes expressing CSF3R did not seem to depend on the G-CSF administration and decreased/increased in three cases, while staying on the same level in the last patient. In conclusion, G-CSF pathway genes expression on mRNA and protein level changes during cytokine induced mobilization.

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Selection of Haploidentical Donors for Haematopoietic Stem Cell Transplantation, Revisions in Guidelines of Polish National Health Fund for Family Donor Selection

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Allogeneic hematopoietic stem cell transplants from haploidentical donors have been increasingly used in the last five-year period to treat severe hematological diseases, immune-based disorders and cancers. Haploidentical transplantations induce relatively limited immunological complications and haploidentical donors are more easily accessible than the fully matched family donors. The advent of haplotransplantation requires organizational and essential adaptation in donor selection algorithm, therapeutic approach and reimbursement. Basic difference in selection of haploidentical donor and fully HLA matched family donor depends on the recommended extended scope of HLA genes to be typed, methods used and the level of their resolution. The overall preference for haploidentical donors selection is third choice rather, after matched family donors (the first choice) and matched unrelated donors (the second choice). These and further diversities are considered in the revisions to the current guidelines of the National Health Fund for the family donor selection. The revisions proposed here involve introduction of two obligatory steps: i) the preliminary HLA testing at intermediate/higher resolution and ii) verifying HLA testing at high resolution. The scope of preliminary step is extended to five genes (A, B, C, DRB1 and DQB1) in order to analyze segregation of extended haplotypes in patients family. The verifying HLA testing at high resolution is performed in three HLA genes including DRB1 and two out of four genes (HLA-A, -B, -C, -DQB1), depending on clarity of preliminary haplotype segregation. The cost of testing was valued in accordance with the requirements of the Agency for Health Technology Assessment and Tariff System.

Evaluation of the Donor-Recipient Matching Process for the Haematopoietic Stem Cell Transplantation, the Efficacy, Duration and Donor and Patient-Related Causes of Failure

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The aim of the study was to analyze n=418 completed unrelated hematopoietic stem cell donor search procedures referred to the Department of Immunogenetics, Institute of Hematology and Transfusion Medicine in 2013–2015, including outcome and duration from the various stages of the matching of unrelated donor to hematopoietic stem cell (HSC) transplantation phase. 384 (92%) procedures were completed and the remaining were in progress (13/418) or has been terminated due to reasons related to the health condition of the patient (21/418). In 371 cases (96.6%) a fully matched or an acceptable HSC donor has been selected. The failure of the selection of acceptable donor occurred in only 13/384 (3.4%) cases, which proves the high effectiveness of used algorithm. Among 386 patients with reported transplantation or withdrawal, 208 (54%) received HSC. The median time of the search procedure was 51 (1–413) days and the median time from the start of the donor selection to the transplantation was 126 (24–408) days. The average time from the start of the donor selection to the transplantation was three days longer when donor was selected from abroad than from Polish registries but the difference was statistically insignificant (mean: 93 vs. 90 days; p=0.68). Segregation of extended MHC haplotypes has been performed using ML-based algorithm PHASE 2.0, and allowed to determine the disparity level of HLA alleles and extended MHC haplotypes between donors and recipients. Clinical data on the transplant outcome are collected for future analyses.

Profile of Inflammation-Associated Proteins in Early Post-Transplant Samples of Patients after Allogeneic Haematopoietic Stem Cell Transplantation

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Allogeneic hematopoietic stem cell transplantation (aHSCT) is used as a curative treatment in severe haematological malignancies, primary immunodeficiencies and hereditary metabolic disorders. Despite clear improvement of the aHSCT outcome, substantial proportion of patients still suffers from severe complications including graft-versus-host disease (GvHD). The aim of this study was, therefore, to investigate selected systemic inflammation-associated markers that may improve prediction of GvHD. Serum concentrations of 92 inflammation-associated proteins were measured in samples obtained from eighty aHSCT patients 14 days after transplantation and from 23 healthy control subjects by proximity extension assay technology (Olink Bioscience, Uppsala, Sweden) using Proseek Multiplex Inflammation kit. As expected, serum profiles of inflammatory proteins in patients after aHSCT were substantially different from those observed in control subjects. Particularly, the difference between aHSCT patients and controls reached significance level at $p<10^{-8}$ for seventeen markers (e.g. IL-6, IFN-γ, CXCL5, TRANCE, CASP-8). Among aHSCT patients, acute GvHD was associated with higher concentration of the chemokine CCL21 (p=0.021) and decreased levels of stem cell factor (SCF; p=0.06). Interestingly, we observed significant increase of the chemokine CX3CL1 (fractalkine) concentration in aHSCT patients who later developed chronic GvHD compared to those without chronic GVHD (p=0.002). In conclusion, systemic concentrations of the CCL21 and SCF proteins in early post-transplant period correlated with the development of acute GvHD after aHSCT. Observed upregulation of the CX3CL1 in patients with chronic GvHD is in compliance with the recent findings implicating this chemokine in the GvHD pathogenesis. Grant support: IGA MZCR NT12454-5 and IGA_LF_2016_011.

TNF α A Allele (rs1800629 Polymorphism) as a Part Haplotype Consisting of HLA B*08 and DRB1*03 Associates with a Lower Risk of aGvHD in Patients Post Haematopoietic Stem Cells Transplantation

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Tumor necrosis factor (TNF)-α plays a role in the pathomechanism of acute graft-versus-host disease (aGvHD) post HSCT and its generation is influenced by the gene polymorphic features. We revised the association between the polymorphic variant of *TNFα* (rs1800629, –308G/A) and aGvHD in 286 10/10 HLA-allele MUD transplantations (MAC: 161, RIC: 107 (missing: 18), age range: 1–65, mean: 32). We found: 1) Distribution of *TNFα* alleles in donors were: A/G: 17/83%, AA/GG/GA: 4/70/26% (Hardy-Weinberg equilibrium: chi2=1.115). 2) Donors TNF-α allele A was associated with the presence of A*01, B*08 and DRB1*03 in 60%, 98% and 78%, respectively. 3) 60%, 46% and 41% (ns) patients had aGvHD (grade I–IV) in the patients being homozygotes, heterozygotes and lacking donors *TNFα* A allele, respectively. 4) Neither A*01, nor B*08 specificities associated with the higher frequency of aGvHD as compared to their counter partners lacking tested specificity. DRB1*03 patients had lower incidence of aGvHD as compared to these lacking this specificity (32 vs 50%; p=0.042) but this difference was not seen when *TNFα* A patients were excluded from the analysis. 5) aGvHD was less frequent in *TNFα* A allele patients if this polymorphic feature was present together with HLA A*01 (45 vs 51%, ns), B*08 (39 vs 61%; p=0.053), DRB1*03 (34 vs 62%; p=0.015), B*08DRB1*03 (26 vs 66%; p=0.001) or A*01B*08DRB1*03 (28 vs 59%; p=0.018) as compared to the patients transplanted from *TNFα* A donors lacking the above listed HLA specificities. *TNFα* polymorphic features may exert an impact on the incidence of aGvHD in the context of the other ancient haplotype HLA specificities.

The Associations Between *BTLA* Gene Polymorphisms and Susceptibility to Chronic Lymphocytic Leukaemia

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B and T lymphocyte attenuator (BTLA) negatively regulates immune responses. The aim of this study was to investigate the association between *BTLA* gene polymorphisms and susceptibility to chronic lymphocytic leukaemia (CLL). Moreover the *BTLA* mRNA expression level in T and B cells from CLL patients was analyzed in relation to *BTLA* single nucleotide polymorphisms (SNPs). The following SNPs rs2705511, rs1982809, rs9288952, rs9288953, rs2705535, rs1844089, rs2705565, rs2633580 were genotyped in 321 CLL patients and in 470 controls. The mRNA levels of human *BTLA* and *β2microglobulin* (β2m) were determined using Applied Biosystems assays. We found that three SNPs: rs1982809, rs2705511 and rs9288953 were associated with susceptibility to CLL. The frequency of rs1982809[G] allele and rs2705511[C] allele carriers were higher in patients compared to controls (0.51 vs. 0.41; p=0.004 and 0.56 vs. 0.44; p=0.0009, respectively). Moreover, rs9288953[TT] genotype was overrepresented in CLL patients compared to controls (0.22 vs. 0.14; p=0.004). The *BLTA* mRNA expression in $CD3^+$ cells in CLL patients was about seven times higher than in controls, while in B only 2.78 higher than in controls (p<0.000001 and p=0.0008, respectively). Additionally, we evaluated the influence of *BTLA* SNPs on *BTLA* mRNA expression in CLL patients.

The presence of rs1982809G allele was associated with lower median (±SD) *BTLA* mRNA expression in T cells as compared to AA homozygotes (0.009±0.013 vs. 0.026±0.012; p=0.03). Our results indicate that BTLA expression is altered in CLL patients and polymorphisms in the *BTLA* gene might be considered as potentially low-penetrating risk factor for CLL.

Expression of Toll-Like Receptors in Patients with Chronic Lymphocytic Leukemia

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B-cell chronic lymphocytic leukemia (B-CLL) presents with progressive accumulation of monoclonal B-cells in blood, bone marrow and lymphoid organs. B-CLL is characterized by heterogeneous clinical outcome. Toll-like receptors (TLRs) play an important role in B-cell activation and maturation. The expression of TLRs and their association with other prognostic factors in B-CLL patients remains unclear. The aim of our study was to evaluate the expression of TLRs and their significance as biological markers in patients with B-CLL. 60 patients with newly diagnosed B-CLL were evaluated. The median age of patients was 68 years. The healthy control group included 14 age-matched individuals. Using quantitative reverse transcriptase PCR, the mRNA expression of genes TLR2, TLR4 and TLR9 was measured. In comparison to control group TLR2 and TLR4 mRNA expression was higher in B-CLL patients than in healthy individuals (TLR2 6.46 vs 0.98 and TLR4 11.91 vs 2.21; p<0.05). TLR9 mRNA expression was higher in control group than in patients with B-CLL (TLR9 23.65 vs 3.35; p<0.05). We observed that patients with higher mRNA expression of TLR9 had significantly longer OS than patients with lower mRNA expression of TLR9 (p<0.05). TLR2 mRNA expression was higher in patients with advanced-stage CLL than in patients with early-stage disease (TLR2 19.10 vs 7.94; p<0.05). We found that expression of TLR2 in patients with anemia was significant higher than in patients without anemia (p<0.05). Our results suggest that TLRs could become new potential biological markers for the clinical outcome in patients with B-CLL.

Studies on Genetic Predisposition to Sporadic Chronic Lymphocytic Leukaemia

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Chronic lymphocytic leukaemia (CLL) is the most common type of adult leukaemia in Western countries. Currently, the co-inheritance of multiple low-risk susceptibility variants is proposed as a model of CLL inheritance. Despite the fact that the effect of single allele is small, the joint effect of many alleles, may have a significant influence on the risk of CLL. The *TNFSF13B* and *TNFRSF13C* genes which encode BAFF (B-cell activating factor), and BAFF-R (BAFF receptor), respectively, have been implicated as associated with non-Hodgkin lymphoma risk. BAFF shares two receptors: BCMA (B-cell maturation antigen) and TACI (transmembrane activator and calcium-modulator and cyclophilin ligand interactor) with APRIL (a proliferation-inducting ligand), which similarly to BAFF belongs to the tumor necrosis factor superfamily. The association between the haplotype consisted of four SNPs located in *TACI* (*TNFRSF13B*) gene and the risk of CLL has been also observed. Taking into consideration the above mentioned observations we conducted the case-control studies which aim was to investigate if the polymorphic variants of genes belonging to the BAFF/APRIL system may be associated with the risk and clinical course of sporadic CLL. We also examined the correlation between investigated SNPs and CLL clinical parameters as well as BAFF and APRIL plasma levels and BAFF and TACI protein expression. The obtained results indicate that genes of the BAFF/APRIL system may constitute potential risk factors of CLL and allowed for identification of new

variants potentially associated with the risk of CLL and the outcome of this disease.

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MNS16A Polymorphism in the Gene Encoding the Telomerase Catalytic Subunit in Patients with Chronic Lymphocytic Leukemia

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Telomerase reverse transcriptase (hTERT) is one of the two subunits of the human telomerase, which has a catalytic activity for DNA synthesis from the RNA template. The promoter activity of *hTERT* gene is influenced by polymorphic minisatellite tandem repeats (VNTR) called MNS16A. The aim of the study was to investigate the impact of the MNS16A polymorphism on susceptibility and clinical course of chronic lymphocytic leukemia (CLL). For this purpose 68 patients and 126 healthy individuals were studied using capillary electrophoresis. Five different VNTRs of MNS16A were detected including two short alleles S* (VNTR-243 and VNTR-274) and three long L* variants (VNTR-302, VNTR-333 and VNTR-364), occurring in nine different genotype patterns. The comparison of the MNS16A genotype and allele frequencies between CLL patients and healthy subjects did not show significant differences suggesting no association with predisposition to the disease. Patients above 60 years more frequently developed low and intermediate (0–II and A) stage of the disease at diagnosis (according to Raji and Binet criteria) as compared to younger patients (42/3 vs 15/8, p=0.005 and 38/7 vs 12/11 p=0.01), respectively. The MNS16A S* allel of *hTERT* gene was more frequently found in patients who developed low grade disease (Raji 0–II and Binet A) as compared to homozygous patients with LL genotype with more advanced stage (38/3 vs 19/8, p=0.02 and 34/7 vs 16/11, p=0.05), respectively. These results imply that the MNS16A S* may play a significant role in maintaining a low grade of disease in patients with CLL.

Interleukin-10 Gene Polymorphism in Patients with Multiple Myeloma

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Different cytokines play a significant role in the development of multiple myeloma (MM), for instance, interleukin (IL)-10 regulates the proliferation of myeloma cells, induces the production of oncostatin M and IL-11 receptor and leads to an increase of tumor cells quantity in plasma. It is known that single nucleotide polymorphisms (SNP) in the regulatory regions of cytokine genes can influence its production what in turn, can affect the development of disease. The aim of this study was to determine SNP in IL-10 (–889C/T) and (–592C/A) associated with the development of MM. A total of 52 MM patients (20 male and 32 female; mean age: 69.7±8.6 years) were studied. The diagnosis of symptomatic MM was verified based on the criteria of IMWG. The control group consisted of 40 healthy volunteers (16 male and 24 female; mean age: 51.2±6.9 years). All surveyed persons were the residents of St.-Petersburg. Genotyping was performed by PCR-SSP, p-values less than 0.05 were considered statistically significant. Based on the SNP analysis some differences were observed: IL-10 –889CC genotype frequency in control was lower than in the patient group (0.41 vs. 0.62), conversely, IL-10 –889CT genotype frequency in control was higher than in patients (0.49 vs. 0.29), $p \geq 0.05$. Furthermore, IL-10 –592CC genotype frequency in MM patients was significantly higher compare to the group of donors (0.62 vs. 0.42; $p \leq 0.05$). However, genotype IL-10 –592CA was detected with the lower frequency in the patient group (0.26) than in control (0.46; $p \leq 0.05$). Thus, our results allow to describe genotype IL-10 –592CC as a marker associated with the development of MM, while genotype IL-10 –592CA can probably be

considered as a marker of resistance of the disease development.

Modern Approaches in Therapy

Emerging Strategies of Targeting the Crosstalk Between Tumor Cell and The Microenvironment in Lymphoid Malignancies

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Classical Hodgkin lymphoma (cHL) and primary mediastinal large B-cell lymphoma (PMLBCL) are lymphoid malignancies with certain shared clinical, histologic, and molecular features. Primary cHLs and MLBCLs include variable numbers of malignant cells within an inflammatory infiltrate, suggesting that these tumors escape immune surveillance. Expression of certain immunoregulatory proteins in Reed Sternberg (RS) cells is modulated by pro-survival transcription factors, such as NF-κB and STATs. By integrating high-resolution copy number data with transcriptional profiles, we identified the immunoregulatory genes, PD-L1 and PD-L2, as key targets at the 9p24.1 amplification peak in HL and MLBCL cell lines. RS cells were also characterized by the overexpression of immunoregulatory lectin, galectin 1. In cHL and MLBCL, the extended 9p24.1 amplification region also included the Janus kinase 2 (JAK2) locus. Of note, JAK2 amplification increased protein expression and activity, specifically induced PD-1 ligand transcription. In line with the role of JAK/STAT and NF-κB signaling driving the expression of oncogenic PIM1/2/3 serine/threonine kinases, we found nearly ubiquitous expression of PIM1/2/3 in cHL and PMLBCL. PIM kinases in cHL modulate transcriptional activity of NF-κB and STATs, and genetic or chemical PIM inhibition induced RS cell apoptosis. PIM inhibition markedly decreased the expression of multiple molecules engaged in developing the immunosuppresive microenvironment, including galectin-1 and PD-L1. Taken together, our data indicate that cHL and PMLBCL cells feature multiple structural and functional mechanisms that foster immune evasion of tumor cells. As a consequence, PD-1 pathway and JAK2/PIM kinases represent complementary, rational therapeutic targets.

Application of *Ex-Vivo* Expanded $CD4^+CD25highCD127^-$ T Regulatory Cells in the Clinic

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T regulatory cells (Tregs) are potent tool in cellular therapy. Due to dominant immunosuppressive activity of these cells in the body, they are considered as a therapeutic agent in transplantation setting, autoimmune and allergic conditions. Here, we will describe our ongoing clinical studies in the Medical University of Gdańsk in patients after bone marrow transplantation, where cellular therapy with $CD4^+CD25^{high}CD127^-$ Tregs is administered as a prophylaxis or treatment of graft-versus-host disease and in diabetes type 1, where $CD4^{+-}CD25^{high}CD127^-$ Tregs are administered to prediabetic patients as a "diabetes vaccine". In addition, we will present a perspective of the local use of Tregs as immunosuppression after pancreatic islets allotransplantation. We will also present and discuss crucial aspects necessary to maintain high quality during sorting, expansion and administration of Tregs to patients.

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Studies on the Biological Properties of Olfactory Ensheathing Glial Cells and Their Application in the Treatment of Spinal Cord Injuries

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Olfactory ensheathing cells (OECs) represent unique type of the glial cells of the mammalian olfactory mucosa and the olfactory bulb. They aid in renewal and

regeneration of olfactory nerves, which was discovered in 1985 by Geoffrey Raisman from the University College of London. Since 2003 we have been conducting independent studies in cooperation with the Department of Neurosurgery of the Wroclaw Medical University on the OECs biology and possibilities of their application in the treatment of spinal cord injuries. The aim of our presentation is to summarize the results of these *in vitro* and *in vivo* investigations which finally enabled to develop techniques for OECs preparation for transplantation. We will also present the results of the OECs transplantation in patients with complete spinal cord damage at the thoracic level. Particularly, we will discuss results of the last surgery done in 2012 with the use of OECs isolated form olfactory bulb, which resulted in significant patient's improvement from A (complete spinal cord injury) to C (sensory and motor incomplete) stage according to ASIA score, suggesting the possibility of OECs supported regeneration of damaged neurons.

Isolation, Production and Transplantation of the Amniotic Stem Cells Culture According to Good Manufacturing Practice Regulations

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Amniotic stem cells have been by decision of Committee for Advanced Therapies classified as advanced therapy medicinal product, the preparation of which is subject to the rules of Good Manufacturing Practice. This work aims to standardize the isolation and culturing process of amniotic stem cells, as well as the selection of the optimal timing and method of application of cells graft in patients in accordance with Good Manufacturing Practice. In order to select the optimal cell isolation method we used three enzymes and one mechanical method. For primary cell cultures four media were checked. From primary culture stem cells were selected. We checked assumptions of quality control – minimal criteria for mesenchymal stem cells. We examined the maximum time of culturing of amniotic stem cells. We also standardized method of transferring cells into the wound. Cells should be isolated by using Dispasa or by tissue homogenization, for primary culture AmioGrow medium is recommended, as confirmed by the results of cell viability ($p<0.05$), the quantity of isolated cells ($p<0.05$) and population doubling ($p<0.05$). Based on the analysis of the population doubling, the aging population and the cell viability it was found that, cells to be applied between passage 3–6. The largest number of cells is transferred through the use of commonly used enzymatic method (87.8%), but this causes the disintegration of the sheet. Significantly high number of cells in form of sheet can be moved by lyophilized collagen or human skin and amniotic membrane.

MSC if an Excess Decrease the Angiogenetic Potential of Mononuclear Marrow Derived Cells Employed for Treatment of Patients with Critical Limb Ischemia

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Thirteen years of experience in the use of marrow derived mononuclear cells in patients with critical limb ischemia (CLI) lead us to the following conclusions: 1) Bone marrow cells harvested from the posterior iliac crest when enriched in mononuclear cells population using a COBE Spectra separator contained the cells characterized with the following phenotypic characteristics: $CD34^{-}CD45^{-}$ median 11.23% (4.45–24.35) and $CD45^{-}CD34^{-}CD90^{+}$ 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 of 0.5 mL portions of these cell population were injected cells into the calf muscles. 3) All except one or two pts 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 six years post implantation check point. 31% patients deteriorated after one year and seven had to have the leg amputated. 4) The novel aspect of this presentation relies on an observation that the treatment failure was associated with a higher proportion of $CD34^{-}CD45^{-}CD90^{+}$ (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 inoculums than it was seen in pts well responding to the treatment. In conclusion, a

higher proportion of cells with cells MSC phenotype do not facilitate the revascularization but in opposite they prevent the stable angiogenesis.

Amniotic Stem Cells Share Clusters of Differentiation of Fibroblasts and Keratinocytes, Influencing Their Wound Healing Ability

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The most advanced therapy in treatment of burned patients is transplant of autologous keratinocytes and fibroblasts. This method does not give satisfactory results due to low viability and population doubling of cells. Stem cells are alternative. The aim of this study is to verify ability of human amniotic mesenchymal stem cells (hAMMSCs) to share cluster of differentiation with fibroblast and keratinocytes and to verify impact of hAMMSCs on keratinocytes and fibroblasts ability to proliferation and wound healing. The study was performed on 18 amnia and skin cells from three patients. Similarity of cluster of differentiations such as CD26, CD13, CD10, Integrin alpha-1, CD39, FSP, bFGF, Vimentin, CD98, Keratinocyte Transglutaminase, KGF, Thrombomodulin, Pormin, TNFRSF5 was checked. In order to differentiate the cells different cell culture media, Boyden dishes and co-culture were used. Three periods of culturing time were checked. Also impact of amniotic stem cells on skin cells ability to close wounds in vitro and proliferation rate was examined. hAMMSCs have the panel of fibroblasts and keratinocytes tested cell markers. Analysis revealed lack of significant differences between the levels of surface markers depending on the method of differentiation compared to fibroblasts and keratinocytes ($p \geq 0.05$). hAMMSCs stimulate fibroblast migration and proliferation ratio. Stem cells also stimulate migration of kerationocytes, whereas there was no effect on the proliferation rate of keratinocytes ($p \geq 0.05$). hAMMSCs can be used as first-line transplantation or assist both allogeneic and autologous transplantations by stimulating population doubling in culture or migration of cells from wound borders.

Biovital Skin Substitute Consisting of Transgenic Porcine Acellular Dermal Matrix with Human Skin Cells

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Genetic engineering methods enables to introduce into the genome of porcine human genes. The first aim of this study evaluating the clinical usefulness of skin from pigs transgenic was the choice of decellularization methods. Secondly, evaluation of the impact on human keratinocytes and fibroblasts was performed. Third aim was to create biovital transplant of human skin seeded on porcine acellular dermal matrix. Human skin was obtained from three donors. Porcined skin was derived from three animals with knockout of gen responsible for αGal cell surface protein creation. Keratinocytes and fibroblasts were obtained from three donors. For decellularization substances: Triton-X, SDS, tripsin, Dispase, 85% glycerol and 15% glycerol were tested. Histopatological examination of these biovital matrices was performed. In addition, extensibility and strength was analyses. The influence of dressings on human keratinocytes and fibroblasts was analyzed by numbers of living cells, dead and apoptotic, population doubling, cell cycle, cell antigens, including HLA-DR. Differences in efficiency of decellularization was confirmed by macroscopic and histopathological examination, as well as the ability to repopulate the matrix by keratinocytes was different. Trypsin is the best decellularization factor in porcine skin. Differences between strength and flexibility of both human and swine skin were identified ($p<0.01$). These results exclude the skin incubated in 85% glycerol. Incubation of cells with the human and transgenic porcine skin impacts the viability depending on decellularization factor, but it seems that acellular transgenic pig skin dressing is not worse than human skin.

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Heightened Sensitivity of Neutrophils in Familial Mediterranean Fever

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Familial Mediterranean fever (FMF) is an autoinflammatory disease characterized by seemingly unprovoked recurrent episodes of fever and localized inflammation. The causative *MEFV* gene encodes pyrin protein which plays critical role in apoptotic and inflammatory signaling pathways and leads to inappropriate neutrophil activation. We aimed to analyze molecular processes that contribute to the abnormal activation of FMF cells. We assessed mRNA levels of a broad range of inflammatory mediators in circulating neutrophils and neutrophils cultured with lipopolysaccharide (LPS) in patients with FMF and controls. Spontaneous and tumor necrosis factor (TNF)-α-, LPS-induced rates of neutrophil apoptosis *in vitro* were evaluated. The spontaneous apoptotic rates (baseline and 24 h) of neutrophils from FMF patients were higher compared to the cells from healthy subjects ($p<0.05$). Three hours exposure of FMF cells with LPS and TNF-α caused delay of apoptosis, while 24 h exposure – increased apoptotic rates in response to inducers. Baseline gene expression of c-FOS, IL-8, MMP9, and TLR2 was upregulated in FMF neutrophils. LPS had no significant effect on activation of diseased neutrophils compared to healthy cells. The most prominent differences in mRNAs (caspase-1, IL-1β, c-FOS, TLR2, TLR4, MMP9) between investigated diseased and healthy groups were obtained when neutrophils were cultured in LPS-free media. Taken together, observed phenomenon of heightened sensitivity of neutrophils from FMF patients towards *in vitro* conditions suggests that the host-derived stress signals could be responsible for the excessive activation of these cells. Increased neutrophil apoptosis in FMF may have a protective effect in preventing tissue damage due to excessive inflammation.

Epinephrine Differentially Modulates Innate Immune Response

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Although it is widely accepted that catecholamines, such as epinephrine (Epi), influence immunity and have consequences for health, their effect on innate immunity (e.g. monocytes and neutrophils) is still not well investigated. Design and method: our study was aimed to analyze the production of TNF-α, IL-1β, MCP-1 and IL-8 by the whole blood cells following short-term exposure (4 h) by Epi (10, 100, and 1000 μM) in the presence or absence of LPS (100 ng/ml). Simultaneously, we investigated their effect on β2 integrin (CD11b/CD18) and L-selectin (CD62L) expression by monocytes and neutrophils from the blood of 22 healthy subjects by flow cytometry. Results: In neutrophils, modest rise in CD11b expression ($p<0.05$) was observed after Epi exposure. Simultaneously, Epi suppressed LPS-induced expression of CD11b and CD18 ($p<0.05$). In monocytes, Epi was able to suppress LPS-induced expression of C11b ($p<0.05$). Epi has shown a potential to modulate the production of pro-inflammatory mediators. Its exposure resulted in significantly increased production of IL-8 in a dose-dependent manner. In opposite, dose-dependent suppression of LPS-induces production of IL-1β, IL-8, and MCP-1 by Epi was observed. In conclusions: thus, our results showed that Epi differentially modulates innate immune response and has a dual effect on cytokine modulation. The findings suggest that the observed immunoregulatory role of Epi may influence the outcome in endotoxin responses and can be critical to regulation of inflammatory responses.

Population Genetics

Genetic History of Armenians: Answered and Pending Questions

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The history of Armenians is difficult to reconstruct due to several reasons. The vast majority of historical evidence has been destroyed or lost during numerous invasions and natural disasters. The modern political division of historical Armenia among neighbouring states has made archaeological and anthropological research a sensitive and difficult task. Though most of historical Armenian provinces were forcibly depopulated during the last few centuries, fortunately, the descendants of these refugees retain information providing the possibility of reconstructing the genetic legacy of their ancestors. Our studies show that the Armenian paternal genetic pool displays immense diversity of lineages, indicating a large number of "founding fathers". The vast majority of Y chromosomes belongs to the haplogroups originated and expanded during or following the Neolithic. The modern Armenian gene pool consists of traces of an origin from a mixture of various populations taking place from 3000 to 2000 BCE, with dramatic decrease of admixture signals after 1200 BCE. Armenians had no significant mixture with other populations also in their recent history. Of the primary reasons that have impeded genetic contact of Armenians with foreigners, the highland geography, early adoption of Christianity, strong ethnic and cultural identity can be considered the most likely. Recently the origins of Armenians have been examined based on ancient DNA. A strong genetic continuity between the Bronze Age population and modern Armenians has been revealed. Further aDNA studies will cover time span from the Neolithic to the Middle Ages while addressing other principal questions on the Armenian genetic history.

Whole Exome Sequencing for Diagnosis of Rare Diseases: 3 Year Experience

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In 2013 Department of Medical Genetics (Warsaw Medical University) has acquired Illumina HiSeq 1500 which allowed to establish whole exome sequencing (WES) as method for both research and diagnostic purposes. Since then we have performed >600 WES analyses, most of which aimed at finding diagnosis in patients suspected to suffer from rare disorders with a genetic basis. We also established bioinformatics infrastructure and pipeline which allow efficient analysis of WES data generated in other laboratories. During the lecture selected findings will be presented illustrating possibilities and challenges of WES application in medical genetics. In particular, problems caused by multiple mutations, mutations with unclear effects and mutations with "spurious" pathogenicity associated with erroneous data from existing literature will be discussed.

HIV-1 Phylodynamics in Poland

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Phylodynamic, sequence data based reconstructions for the surveillance of the geographic spatial spread are a powerful tool in molecular epidemiology. Also, reconstruction of HIV transmission links allows to trace the spread and dynamics of infection and guide epidemiological interventions. For subtype B transmission networks among HIV-1 infected patients and the region of infection origin for patients with the history of travel-related HIV transmission was analysed using phylogeographic approach. Maximum likelihood trees based on the sets of country-annotated reference sequences were inferred, region of sequence import was assigned using on the highest approximate likelihood ratios. For subtype B, transmission networks were more common among men-who have-sex-with-men (MSM) (234 sequences, 24.2%) compared to other infection routes (injection drug use: 28 (2.9%) and heterosexual transmissions: 59 (6.1%) cases, respectively [OR: 3.5 (95%CI: 2.6–4.6); $p<0.001$]. In multivariate models clustering was associated with both MSM transmission route [OR: 2.24 (95%CI: 1.38–3.65); $p<0.001$] and asymptomatic stage of HIV infection [OR: 1.93 (95%CI: 1.4–2.64); $p<0.0001$]. For non-B variants, import of novel clades, including recombinant forms previously rarely found in Poland and Europe is frequent among travellers. Reconstruction of the HIV-1 subtype B transmission patterns reveals increasing degree of clustering and existence of interregional networks among Polish

MSM. Dated phylogeny confirms the association between onward transmission and recent infections. High transmission dynamics among Polish MSM emphasizes the necessity for active testing and early treatment in this group. For non-B clades, observed founder events result in the heterosexually-driven introduction of the novel HIV-1 variants into the population.

Determination of HLA-A-B-DRB1 Allele and Haplotype Frequencies in the Croatian Population Based on a Family Study

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In the present study HLA allele and haplotype frequencies among Croatian families were investigated. A total of 350 families which have been typed for the purpose of hematopoietic stem cell transplantation were included. All individuals were tested using PCS-SSO or PCR-SSP methods for HLA-A, -B, and -DRB1 alleles. The HLA-A-B-DRB1 haplotypes were determined by segregation and directly counted. A total of 30 HLA-A, 54 HLA-B, and 38 HLA-DRB1 alleles and 716 different HLA-A-B-DRB1 haplotypes were identified. Of these, the three most frequent alleles at HLA-A, -B, and -DRB1 loci, respectively, were A*02:01 (30.39%), A*11:01 (13.37%), A*24:02 (10.91%); B*51:01 (12.48%), B*18:01 (8.35%), B*08:01 (8.06%); DRB1*03:01 (11.20%), DRB1*01:01 (9.84%), DRB1*16:01 (9.63%). The following HLA alleles were detected only once: A*02:09, A*24:03, A*24:04, A*24:07; B*07:04, B*15:07, B*15:08, B*39:04, B*39:10, B*39:24, B*40:04; DRB1*08:03, DRB1*11:06, DRB1*13:32, DRB1*14:05. Five most frequent haplotypes were: A*01:01-B*08:01-DRB1*03:01 (5.34%), A*02:01-B*18:01-DRB1*11:04 (1.57%), A*02:01-B*27:02-DRB1*16:01 (1.50%), A*02:01-B*27:05-DRB1*01:01 (1.42%) and A*02:01-B*44:02g-DRB1*16:01 (1.28%). The haplotype frequencies based on family study were compared with the frequencies from Croatian Bone Marrow Donor Registry and similar results were obtained for all except HLA-A*26:01-B*38:01-DRB1*04:02 haplotype. Significantly higher frequency (p=0.0017) of this haplotype was observed among family individuals. Nine haplotypes were unique and data about their frequencies does not exist in current databases. In conclusion, the data obtained in this study could be useful for anthropology, transplantation and disease association studies.

The New HLA Alleles in the Russian Population

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Sequence based typing was used to identify human leukocyte antigen (HLA)-A, -B, -C, -DRB1 alleles from 27,932 recruited volunteers in 14 regions from Russia, for unrelated hematopoietic stem cell (HSC) registry. In the course of the research identified 49 new alleles. As of January 29, 2016 31 new alleles were register on The WHO Nomenclature Committee for Factors of the HLA System: 10 – locus A, 12 – locus B, 4 – locus C, 4 – at locus DRB1, 1 – at loci DQB1. To confirm the new alleles were used pyrosequencing technology (Roche), NGS (GenDx), monoallelic sequencing (PROTRANS). It is interesting to note that some of these alleles were found a few people who are not relatives: A*26:106, B*57:78, B*27:133, A*24:314, identified by two, two, two and fifteen cases respectively. This testifies not to random mutations in the gene, and the circulation these alleles among Russian populations. Another 11 new alleles are at various stages of registration and another for seven new alleles laboratory is not able to obtain a sequence of new alleles separately from those already known. Studies have shown the relevance of further study the diversity of HLA- alleles in populations of representatives of various Russian populations and expanding the geography of recruiting potential donors of HSCs, which will increase the effectiveness to matching donor of HSCs to Russian clinics patients, which need of allogeneic transplantation of HSCs.

HLA Gene and Haplotype Frequencies in Russians from Rostov-on-Don Region and Comparisons with Other Eurasian Populations

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HLA-A, -B, -C, -DR frequencies were determined in 123 unrelated Russian, living in Rostov-on-Don region

(Southern Russian Federal District). Typing was performed by PCR-SSP at the allele family level. For population genetics analysis Arlequin v3.1 software was used. The most frequent HLA alleles of Russian, living in Rostov-on-Don region, were: A*02, 01, 03, 24 (gf>0.106), B*35, 07, 18, 44, 08 (gf>0.069), C*07, 06, 12 (gf>0.106), DRB1*11, 13, 15, 07, 04, 01,(gf>0.1). Rare alleles (gf<0.02) were A*30, 29, 32, 66; B*37, 39, 41, 45, 47,50,52,55,56,73; C*15, 16; DRB1*09, 10. No examples of A*34, 36, 43, 69, 74, 80; B*42, 46, 48, 53, 54, 58, 78 were found. The five most common haplotypes were: A*01-B*08-C*07-DRB1*03 (3.66%), A*02-B*18-C*07-DRB1*11 (3.66%), A*03-B*07-C*07-DRB1*15 (2.85%), A*03-B*35-C*04-DRB1*01 (2.85%), A*02-B*18-C*12-DRB1*15 (2.44%), A*02-B*57-C*07-DRB1*07 (2.85%). Among the most frequent alleles and haplotypes of South Russian population the large fraction of them is typical for the western and eastern Slavs populations (A*01-B*08-C*07-DRB1*03, A*03-B*07-C*07-DRB1*15). Haplotypes A*02-B*18-C*07-DRB1*11, A*02-B*18-C*12-DRB1*15 are more common in South Slavs (Bulgarians, Serbs, Albanians, etc.).

HLA DRB*04 Allele Frequencies in Russians and Tatar of Chelyabinsk Region, Russia

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The aim of the study was to estimate the frequency of HLA DRB1*04 alleles among the Russian and Tatar donors from Chelyabinsk Bone Marrow Registry. HLA DRB1*04 alleles distribution was analyzed of 185 Tatar donors and 300 Russian donors from BMR of Chelybinsk region. Typing was done by SSP-PCR, at the second field level. The frequency of gene DRB1*04 was similar in Russian (11.68%) and Tatar (11.47%) populations. The most frequent DRB1*04 alleles in Russian population were DRB1*04:01 (24.12%) and DRB1*04:04 (12.96%), in Tatar population DRB1*04:01 (11.47%) and DRB1*04:13 (7.8%). The lowest frequent DRB1*04 alleles in Russian population were DRB1*04:06 (0.76%) and DRB1*04:07 (0.76%), in Tatar population DRB1*04:08 (1.26%). No statistically significant difference of HLA-DRB1*04 alleles between Russian and Tatar populations of Chelyabinsk region. Our results of typing alleles HLA-DRB1*04 of Russian and Tatar donors from Chelyabinsk Bone Marrow Registry provides genetic information for effective selection of future donors for bone marrow transplantation.

CCR5DELTA32 Allele Frequencies in Russians, Bashkirs and Tatars of Chelyabinsk Region, Russia

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The aim of the study was to estimate the frequency of CCR5delta32 alleles among the Russian, Bashkir and Tatar donors from Chelyabinsk Bone Marrow Registry. CCR5delta32 alleles distribution was analyzed in 300 Russian, 118 Bashkir and 91 Tatar donors from BMR in the Chelyabinsk region. Typing was done by RT-PCR using a kit manufactured by "DNA technology" (Russia). In Russian population of Chelyabinsk region the CCR5delta32 allele frequency was10.83%, the frequency of genotype CCR5delta32/delta32 was 1.67%. The Bashkirs of Chelyabinsk region have allele frequency 6.36% and genotype's frequency – 0.85%. The CCR5delta32 allele frequency in Tatar population was 7.14%, the frequency of genotype – 1.10%. All donors with the genotype CCR5 delta32/delta 32 have the most common alleles of HLA. Comparison of allele frequencies in Russian and Bashkir populations showed presence of significant differences [p=0.05]. No significant difference of CCR5delta32 alleles between Russian and Tatar [p>0.05], Bashkir and Tatar populations [p>0.05] of Chelyabinsk region was found. We have determined the frequencies of the mutant allele variant CCR5delta32 among the potential bone marrow donors on the bone marrow donor registry the Chelyabinsk State Hemotransfusion Station in three ethnic groups. We have established the highest frequency of alleles CCR5delta32 and genotype frequency of the CCR5 delta32/delta 32 to be present in the Russian population. Identification of the frequency of CCR5 in a cohort of bone marrow donors allows one to create a category of donors to choose from for an increased chance of a positive outcome in bone marrow and stem cell transplantation to HIV-infected patients.

Current HLA Typing Standards

Full Length HLA Gene Characterisation for Submission of New and Confirmatory Alleles using a Dual Redundant Sequencing Strategy

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The DKMS Life Science Lab has to date genotyped more than 2 Mio samples in high throughput using a short amplicon Next Generation Sequencing (NGS) approach. In addition we developed a system to characterise the whole gene from 5' to 3'UTR for the HLA loci A, B, C, DQB1 and DPB1 for submission to the IMGT/HLA database. Our dual redundant sequencing strategy is based on shotgun sequencing on an Illumina MiSeq instrument and Single Molecule Real Time (SMRT) sequencing on a PacBio RS II. Both approaches are used on each sample independently and provide high quality consensus sequences with very low error rate. The integration of the data from both technologies using DR2S (Dual Redundant Reference Sequencing, DKMS Life Science Lab) yields reference quality sequence data. For submission of the sequences to ENA and the IMGT/HLA databases we developed the TypeLoader software. In the IMGT release 3.22 (October 2015) only 4.3% (587) of all known HLA alleles were fully characterised including intronic sequences. We have applied our dual redundant sequencing strategy to characterise and submit 600 alleles so far, thereby doubling the number of fully characterised alleles in the database. Given the increasing application of NGS for full gene characterisation in clinical practice, improving the data quality of the HLA database is much needed. Therefore, we propose the Dual Redundant Sequencing strategy for submission of novel alleles and characterisation of sequences that are as yet incomplete.

Two Million Samples Typed by Next Generation Sequencing – Establishing a Successful High-Throughput Workflow for Registry Typing

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DKMS Life Science Lab successfully switched its high-throughput workflow from Sanger based to NGS based typing in 2013. Since then over two million samples have been typed with next generation sequencing (NGS). Efficiency, stability and scalability of the workflow allowed the introduction of additional loci (CCR5delta32, ABO, Rh, and KIR) to the standard typing profile for newly recruited donors at very little additional costs. The workflow is based on direct sequencing of short amplicons on Illumina MiSeq and HiSeq instruments. For HLA loci A, B, C, DRB1, DQB1 and DPB1 Exons 2 and 3 are sequenced to achieve high resolution genotyping (G group level or higher). KIR genes are typed for absence/presence, ABO and Rh are typed as blood groups, CCR5 is typed as wildtype/Delta32. Adapters for the NGS reaction and tags to identify the samples are incorporated during a second PCR. PCR products of up to 5000 samples (HiSeq)/384 samples (MiSeq) are pooled. To provide highly automated and accurate data analysis we use the in-house developed software neXtype, which requires several fold less manual analysis time in comparison to the analysis of Sanger sequencing data. Applying this workflow for registry typing we achieved 99% high resolution genotyping for the HLA genes at an error rate below 0.05%. The remaining errors are mainly due to imbalanced amplification between alleles. After two million samples typed our NGS based workflow has proven to be a stable, reliable approach for cost efficient high throughput typing.

EFI – Standards Version 6.3 in Respect to NGS Technology – Additional HLA Typing Resolution Levels

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Next Generation Sequencing (NGS) is increasingly used in laboratories accredited for high resolution typing. Version 6.3 of the EFI Standards provides, for the first time, NGS related standards. They are positioned in section L10 under the title "Sequencing based Typing (SBT)". These standards cover the whole NGS procedure, starting with the initial PCR and library production. I will discuss the following aspects: a) Purification of the PCR products, b) normalization and pooling methods; c) possible PCR artefacts pertinent for the routine interpretation; d) short amplicons vs. by long range amplification strategies and subsequent shotgun sequencing; and e) fragmentation, barcoding and fragment sizes in case shot gun strategies are applied. At present there are several detection devices on the market, e.g. from Illumina, Ion Torrent, PacBio. Standards are valid for all of them. An essential part of this new technology is the data analysis and there are standards. I will discuss the following issues: a) acceptance of the sequences; b) minimum amount of reads; c) adequate depth of coverage; d) a sufficient overlap of the sequences; e) validation of accurate allele calls; and f) algorithms for modification of raw sequence reads (e.g. sequence trimming and quality filtering). NGS brings about a new category of resolution as covered in paragraphs D1.4 to D1.5 of the standards. High resolution typing is defined resulting from polymorphism located within exons 2 and 3 for HLA class I loci and exon 2 for HLA class II loci and the exclusion of null alleles. For the new category, allelic resolution, it is necessary to define a single allele by a DNA based typing method. HLA alleles must be identified at the level of resolution which defines all of the fields according to current WHO nomenclature and all currently known ambiguities must be solved. Finally I shall address the issue of the prerequisites for allelic resolution.

New Insights into *HLA-G* Promoter Genotyping

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Human leukocyte antigen (HLA)-G is a non-classical human leukocyte class I antigen with immunomodulatory functions. The most polymorphic region of *HLA-G* gene is located in the promoter of the gene. The –725C/G/T polymorphism is suggested to have an influence on its expression and it has been associated with many pathological conditions. *HLA-G* genotyping in position –725 C/G/T was conducted by the TGGE method in above 2100 samples that came from research on the *HLA-G* association with different diseases in the Polish population. From them, randomly selected 351 samples were genotyped also using a 7300 Real Time PCR System (Applied Biosystems). Software dedicated for allelic discrimination allows to recognize basically three genotypes for typical biallelic SNPs. In our method, triallelic SNP genotyping is also possible. In order to confirm the correctness of genotyping we conducted direct sequencing. Concordance of genotyping by using TGGE method, TaqMan probes and the sequencing was complete. Our implemented Real-Time PCR method will guarantee a correct and fast genotyping of the –725 *HLA-G* triallelic SNP for future studies and clinical purposes.

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Results of the Last External Proficiency Testing Exercise for Central Europe Transplantation

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In 2015, as often before, the Central Europe Transplantation (CET) External Proficiency Testing (EPT) exercise was organised from Vienna. During the year before, ten DNA samples from the routine work have been labelled as "interesting" and appropriate for EPT. They were sent out in June to 45 laboratories in Central and Eastern Europe, and additionally, in Kazakhstan, Iran and Israel. Forty-one of them responded in due time. The reference typing comprised HLA-A, -B, -C, -DRB1, -DRB3/4/5, -DQB1, -DQA1, and -DPB1 at low and high resolution, the latter performed by whole-gene next generation

sequencing. The results were analysed according to the EFI EPT standards. In general, there were 1–4 discrepancies per locus with the organiser's typing. The resolution was allocated based on the ambiguity list of the IMGT/HLA database, version 3.22. Successful participation in EPT is a prerequisite for EFI accreditation. Those participating laboratories, who are not yet accredited are supposed to apply for accreditation in a foreseeable future.

HLA Proficiency Testing for Central and East Europe – Outcomes of the XXI Trial

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The HLA Proficiency Testing for Central and East Europe is organized under the auspices of the Polish Society for Immunogenetics. Currently, the workshop covers serological typing of HLA class I antigens and DNA typing at the low and high resolution level of HLA class I (A, B, C) and class II loci (DRB1, DQB1 and DPB1) and has over 40 HLA laboratories participating every year. There were 42 laboratories from 14 countries participated in the last XXI trail. The participants were provided with blood samples taken on heparine for serology and/or with DNA samples for genotyping. The results of serological and DNA typing were submitted by 15 and 39 laboratories, respectively. Some inconsistencies were observed with respect to both serological and DNA typing results, including erroneous typing and improper reporting of genotyping results. This time HLA typing at the high resolution level associated with the highest grade of discrepancies. The results of one laboratory did not comply with requested consensus of typing of HLA-A, B, HLA-C and HLA-DQB1 alleles, while two other laboratories were unsuccessful with respect to HLA-C typing at the high resolution level. Only one laboratory did not successfully complete the HLA class II low resolution typing. These data confirm the usefulness and effectiveness of the proficiency testing exercises and imply that there is a need for the continuation of the workshop as some doubtful results are still reported.