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TWENTY YEARS BULGARIAN ASSOCIATION OF CLINICAL IMMUNOLOGY

Abstract. Two decade have passed since the establishment of BACI, during which we worked as a team, with dedication and faith in what we do. Moreover, we have made the development and establishment of Clinical Immunology in Bulgaria our cause. And we can boldly say that our work has had meaning. Over the past years, thanks to the efforts of all BACI members, coordinated by the Executive Committee of the Association, immunology has turned from a little-known field into a major medical specialty with an interdisciplinary character. We are ambitious to make Clinical Immunology even more recognizable and attractive to young specialists and to attract a larger number of like-minded people. It is very important to continue to encourage students and colleagues from various specialties to join our association and publish on the pages of the BACI journal. Clinical immunology is not a limited area of activity for medical doctors, on the contrary – it is closely dependent on the achievements of fundamental immunology and a sought-after field for the implementation of results from various scientific and applied research in the field of experimental immunology, immunogenetics, molecular biology and microbiology, bioinformatics. The traditional interaction of BACI with colleagues from various areas of fundamental science with interests in immunology must be developed and strengthened. This will make us stronger, more effective and more ambitious.

THE BEGINNING

The visionary idea of a professional organization uniting those working in the field of clinical immunology has been discussed many times over the years. The first step towards its official establishment was the decision taken on July 8th, 2005 by the Expert Council of immunology to establish a non-profit society “Association of Clinical Immunology“. The honor of being the founders fell to academician Bogdan Petrunov, prof. Elissaveta Naumova, prof. Iskra Altankova, prof. Marta Bal-eva, prof. Fani Martinova, prof. Hristo Taskov, assoc. prof. Petrunka Petrova, assoc. prof. Svetoslav Balev, assoc. prof. Tsvetan Lukanov. The Statute was adopted, the Executive Committee and the President of the Association were elected. The association was legally registered on 25th of August 2006. The General Assembly of the Association from 26th of November 2008 decided to add the word „Bulgarian“ to the name of the Association and from 21st of January 2009 the official name became „Bulgarian Association of Clinical Immunology“ (BACI). Ambitious goals were set, which

defined the role of BACI as a unifier, driver and mediator.

UNIFIER

BACI unites and supports its members immunologists to achieve their common goals and objectives

According to Art.10. /1/ “*The association can be joined by medical doctors and specialists with non-medical education working in the field of clinical immunology*“. Over the past twenty years, our membership from 33 people at the beginning reached 115 people in 2025. The growing interest in clinical and laboratory immunology during the years is visible from the distribution of members: medical doctors with a specialty in clinical immunology – 64 (56%); medical doctors working in the field of clinical immunology and with another medical specialty – 13 (11%); biologists and chemists with a specialty in laboratory immunology – 11 (9%); biologists and chemists working in the field of laboratory immunology – 17 (15%); residents in clinical or laboratory immunology – 10 (9%).

DRIVER

BACI promotes innovations in the field of immunology and contributes to the development of clinical immunology in Bulgaria.

Over the past two decades, the Executive Committee of BACI put efforts to create conditions for the professional development and realization of its members, and to promote innovations in the field of clinical immunology. This was achieved by regularly providing a forum for the presentation and discussion of cutting-edge clinical and fundamental scientific research, as well as their practical applications in patient care. In addition, scholarships are awarded for the participation of young scientists in international and national scientific forums. It should be noted the significant number of scientific conferences, congresses, webinars, schools and workshops organized and held by BACI on a variety of topics and with the participation of specialists from various medical and biological disciplines. This contributed to increasing the knowledge and qualifications of the participants, provided a platform for the appearance of young scientists and stimulation of scientific research work. The best presentations were awarded with diplomas and awards.

Last but not least, our interaction with other medical specialties has significantly improved.

The annual celebration of the International Day of Immunology – April 29, associated with the World Week of Primary Immunodeficiencies (April 22-29) and the World Immunization Week, has become a tradition. Their celebration with various events throughout the country makes BACI part of the global immunological community.

BACI participates in the work of the European Union of Medical Specialists (UEMS). The result of this cooperation is the modernization and harmonization of training programs in the specialty „Clinical Immunology“ in accordance with the European Directive and the UEMS Blue Book.

In 2018, a memorandum of cooperation was signed between BACI and the European Federation for Immunogenetics (EFI) in the field of immunogenetics and transplantation.

In 2023, a memorandum of cooperation was signed between BACI and the Universal Scientific Education and Research Network (USERN). Its expression was the active Bulgarian participation in the 9th International Congress of USERN in Plovdiv

in 2024. BACI is also a full member of the Union of Bulgarian Medical Societies.

Our cooperation with the “Immunology” Section of the Bulgarian Union of Scientists, the Medical Society of Hematology, the Bulgarian Pediatric Association, the Institute for Rare Diseases and Orphan Drugs, and the Alliance for People with Rare Diseases is particularly fruitful.

As a result, the following documents were created:

- consensus recommendations for immunophenotyping by flowcytometry in oncohematology;
- criteria for initiating and continuing treatment with human gammaglobulin and C1 esterase inhibitor;
- algorithm for treatment of patients with primary immunodeficiencies (PID);
- algorithm for immunomodulatory treatment of organ transplant recipients;
- program for determining reference values in Bulgarian women for the percentage and absolute number of NK-cell subpopulation CD3-CD16+CD56+ in peripheral blood, published in the BACI yearbook, 2010.

The publication activity of BACI is traditional. The BACI Yearbook was published annually in 2007-2018 period, which reflected the activities of the Association in the respective year, Conference reports, consensuses, information on workshops and scientific forums were published. In 2020, the first issue of the Bulgarian Journal of Clinical Immunology (BJCI), a successor of the BACI Yearbook, was published. The journal aims to take a worthy place as a forum for presenting new scientific achievements in the field of immunology and sciences related to it. Since 2020, BJCI has been included in the National Reference List of NACID for contemporary Bulgarian scientific publications with scientific review. This is a prerequisite for its popularization among a wider range of specialists. As a result of all this, **clinical immunology was recognized and established as a major medical specialty with a constantly increasing role and importance in human medicine.**

MEDIATOR

The Executive Committee of BACI has made serious efforts to support those working in the field of clinical immunology in their dialogue and relationships with local and central authorities, as

well as with the Bulgarian Medical Association (BMA) and the National Health Insurance Fund (NHIF). Key problems faced by medical doctors and scientists in the field of immunology, as well as its interrelationship with other medical disciplines and healthcare in general, are regularly discussed. It can be now stated that the immunological community has already been recognized by the Ministry of Health (MH), BMA and NHIF. This is supported by achievements such as the inclusion of a clinical pathway „Treatment of proven primary immune deficiencies“ (2013) in the Regulation for determining the package of health activities guaranteed by the NHIF budget. And subsequently the Outpatient procedure for the diagnosis of primary immune deficiencies was approved. The inclusion of human immunoglobulins and C1 esterase inhibitors in the Positive Drug List in 2013 allowed treatment for patients with primary immune deficiencies. As a result of close collaboration with the National Program for Rare Diseases, joint criteria for PID Expert Centers in our country have been developed. The entry of these centers in the register of the Ministry of Health through the Commission for registering rare diseases, according to Regulation No. 16/2014, allows their inclusion in the European Reference Networks for diagnosis, consultation and therapy.

Ensuring quality in laboratory and clinical practice is a priority for BACI. In this regard, the Medical Standards „Clinical Immunology“ and „Immunological Preparation for Organ, Tissue and Cell Transplantation“ were updated. Rules for Good Medical Practice in Clinical Immunology were also developed, published on the BACI website, as well as Pharmacotherapeutic Guide for the Treatment of Immune-Mediated Diseases was prepared. The laboratory comparative control for flow cytometric determination of lymphocyte populations performed in 2004 marked the beginning of an organized program for External Proficiency Testing (EPT) in immunology in Bulgaria. In 2005, several EPT schemes for parameters of the humoral immune response began to be conducted. Over the years, the EPT in immunology has been updated and supplemented with new components and schemes. Today, the National EPT system in immunology covers almost all routine and some highly specialized immunological tests, which improved the quality of immunological diagnostics

and allowed immunological units to conclude contracts with the NHIF.

The mediatory role of BACI would be difficult without the use of the Internet. In 2006, a WEB page was created, which, with the development of communication technologies, had to be updated with an English version (<https://www.acibg.org/>).

We did not stop there! Under the initiative of the Bulgarian Association of Clinical Immunology and the Expert Council of Clinical Immunology, as well as with the support of the Ombudsman of the Republic of Bulgaria, the Institute of Rare Diseases and the Alliance for People with Rare Diseases, in 2019 the need to develop a National Strategy for Protecting the Immune Health of Children and Adults in the Republic of Bulgaria was discussed and approved. The reasons for this are directly related to the need for urgent and consistent actions to protect the immune health of Bulgarian citizens, in connection with the postulate enshrined in the Constitution that the health of the population should be a national priority. In this regard, in order to implement targeted policies to improve the immune health of the population, a National Program for the Protection of Immune Health and Prevention of Immune-Mediated Diseases was developed and submitted for consideration to the Ministry of Health. It was included in Priority 1: „Protection of the Health of the Population“ of the National Health Strategy 2030. The Program focuses on the following main areas:

- raising public awareness about immune health;
- preventive testing of immune competence;
- evaluation of the results of mandatory vaccination;
- early detection of severe combined immune deficiencies (SCID) through national neonatal screening;
- early detection of immune-mediated diseases;
- replacement therapy for secondary hypogammaglobulinemia with human immunoglobulins;
- outlining the patient's route from symptoms to diagnosis and treatment of immune-mediated diseases.

Information from SCID neonatal screening, the mandatory prophylactic testing of immune indicators in children, adolescents and adults, as well as the presence of autoantibodies, is a condition for

early diagnosis of immune-mediated diseases and their timely therapy. Last but not least, together with other clinical and laboratory data from the individual electronic file, the same information can be actively

used to create machine algorithms for assessing immune competence and early diagnosis of immune-mediated diseases – a rapidly developing area in human medicine.

**HAPPY ANNIVERSARY,
dear colleagues, and remember that only together we can achieve heights!**

Prof. Elissaveta Naumova, MD, PhD, DSc
Founder and President of BACI

Academician Bogdan Petrunov, MD, PhD, DSc
Founder and Past Member of the Executive Committee of BACI

Prof. Marta Baleva, MD, PhD, DSc
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Current Member of the Executive Committee of BACI

Assoc. Prof. Emiliana Konova, MD, PhD
Current Member of the Executive Committee of BACI

**NATIONAL CONGRESS ON IMMUNOLOGY
WITH INTERNATIONAL PARTICIPATION
October 2-5, 2025**

ABSTRACTS

I. ORAL PRESENTATIONS

**CANCER NICHE-TARGETING CANNABINOID BASED NANOTHERAPEUTIC
PLATFORMS***

Petek Korkusuz

*METU MEMS Center & Hacettepe University Faculty of Medicine,
Dept of Histology & Embryology, Turkiye*

Conventional cancer treatment protocols often result in extensive side effects and demonstrate limited efficacy. Our research concentrates on the endocannabinoid system (ECS), which plays an essential role in the tumour microenvironment by regulating the proliferation and dissemination of malignant cells. We have identified that ECS can selectively target and eliminate specific cancer cells by inducing apoptosis via CB1 and/or CB2 receptors in laboratory and animal models. We examined the potential of ECS constituents as anti-cancer therapeutics, given their capacity to activate intrinsic cell death pathways at low doses without inducing psychoactivity. Our investigations have demonstrated real-time anti-neoplastic effects across various solid tumours, including endometrial, laryngeal, non-small cell lung cancer (NSCLC), and osteosarcoma. We developed and validated innovative targeted nanoscale biomaterial-based ECS platforms as prospective nano-chemotherapeutic agents for the treatment of diverse solid malignancies. One such advancement, ACPA-PCL nanoparticles, which inhibit the Akt/PI3K pathway, glycolysis, TCA cycle, amino acid biosynthesis, and the urea cycle while concurrently activating the JNK pathway, has been evaluated in an ectopic NSCLC xenograft model (PCT/TR2020/050618). ACPA also reverses the transformation of healthy lung fibroblasts into cancer-associated fibroblasts through the use of NSCLC-derived exosomes within the tumour microenvironment. In summary, this presentation reviews our published preclinical trials, emphasising personalised ECS-based nanoparticle systems for tumour targeting, and introduces novel therapeutic concepts for various solid cancers. High-quality translational studies are essential to supplement existing clinical treatments.

**Hacettepe University (#TSA-2023-20427 and #TSA-2025-21763) and TUBITAK (#321S058) funded.*

INNOVATIVE 3D MODELS TO STUDY (UN)SUCCESSFUL HUMAN IMPLANTATION

Tanya Dimova

Institute of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences – Sofia, Bulgaria

Human embryo implantation is a unique process and a key event that largely determines how it will proceed and whether there will be a pregnancy at all. Although this process has been defined for a long time, it has not yet been fully studied both as a purely biological phenomenon and as a special type of immunological interaction between two genetically different organisms. Due to ethical considerations, scientific experiments with human embryos are highly restricted and 3D surrogates derived from human cell lines that mimic the behavior of the human embryo in the peri-implantation period are welcome. Through this wonderful, modern, non-invasive, ethical approach, the science gives us an effective research model. We constructed, standardized and determined the functionality of Sw71 blastocyst-like surrogates (Sw71 BLS) as a model of a human peri-implantation embryo. Similar to the implanting embryo at the blastocyst stage, our surrogates undergo compaction, have a cavity, and are similar in morphology and size to those of an expanded human blastocyst. Moreover, they attach to epithelial endometrial cells, have the ability to invade the extracellular matrix, and migrate between endometrial stromal cells. The trophoblast cells of our models have a phenotype of extravillous trophoblast cells (EVT), which in vivo participate in two very important processes for the construction of a functional placenta: 1) EVT induce an immune tolerance from the maternal immune cells towards the embryo and 2) EVT remodel the uterine spiral arteries to ensure adequate bloodflow to the site of implantation/placenta. It should be emphasized that these are highly invasive cells similar to cancer cells, but whose invasion and migration are under tight control. To induce immune tolerance, EVTs possess a specific expression profile of major histocompatibility complex molecules, which helps them to be recognized and accepted by the maternal immune system. These cells are still enigma, which also makes it difficult to create 3D models that mimic their behavior in the earliest stages of implantation. By an assembling of the Sw71 spheroid with endometrial organoids, we simulate in vitro the maternal-fetal interface in the peri-implantation period. In addition to these models of normal implantation, we also created two in vitro 3D models of compromised implantation – due to detrimental microenvironmental factors and due to genetic alterations in the trophoblast. The 3D models are a useful tool for studying molecular mechanisms of trophoblast differentiation and its interaction with maternal stromal and immune cells, as well as the influence of various factors on trophoblast differentiation and function (attachment, migration and invasion). The usage of cell lines allows for standardization of the models, which is not applicable when using primary tissues. However, each of the components of these in vitro models is validated by replacing it with a primary culture.

FUNCTIONAL ANALYSIS OF THE INTERACTION BETWEEN HUMAN C1Q AND HUMAN GALECTIN-3

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Aim. C1q serves as a key initiator of the complement system and it interacts with a wide variety of molecular ligands with its globular head domains (gC1q). Currently, no experimental data

exists on the molecular mechanism of interaction between C1q and the proteins from the galectin family. Galectin-3 is known to control intercellular and extracellular molecular interactions during immunological responses. Thanks to its carbohydrate binding domain Galectin-3 can interact with ligands as a protein or/and a lectin.

Methods. We investigated the recognition between C1q and Galectin-3 by ELISA, hemolysis and fluorescence spectroscopy. Additionally, molecular docking and molecular dynamics simulations were performed to further identify binding site amino acids.

Results. The data showed that C1q and Gal-3 had a pronounced affinity for interaction and supramolecular binding. When the active AAs of the two proteins interacted, electrostatic attraction, aided by a plethora of hydrogen bonds, was dominant for the stabilization of the complex. The complex between them was stabilized mainly by Van der Waals interactions and fewer but stronger hydrogen bonds. Fluorescence spectroscopy gave quantitative assessment with Kd value of 0,04 μ M. We registered significant increase of the C1q-IgG binding and hemolysis activation upon treatment of C1q with Galectin-3.

Conclusion. Galectin-3 is a newly discovered ligand of C1q. It is a protein-protein interaction between gC1q and the backside of the lectin domain of Galectin-3. Also, Galectin-3 appears to promote classical complement activation via its interaction with C1q.

Acknowledgement. This study was financially supported by Grant KP-06-N41-9 of the Bulgarian NSF.

COMPLEMENT SYSTEM ACTIVATION IN HER2+ BREAST CANCER: FROM PROGNOSIS TO THERAPY

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Study aim: Breast cancer profoundly affects women's health worldwide. Activation of the innate immune complement system occurs in the breast tumor microenvironment, similarly to its activation to eliminate pathogens, yet it is still unknown if this is a mechanism to eliminate tumor cells or creates chronic, tumor-promoting inflammation. This study aimed to investigate the patterns of complement activation and its association with the stage of Her2+ breast cancer patients.

Material and methods: Tumor tissues from 28 Her2+ breast cancer patients were studied, categorized into non-metastatic and metastatic. Methods included immunohistochemistry, C4d ELISA, hyperplex immunofluorescent imaging on selected tumors (38-plex Comet Lunaphore) and gene expression analyses (TCGA cohort).

Results: Overexpression of most of the classical complement pathway genes correlated with favourable prognosis in Her2+ breast cancer patients. Complement activation was detected by hyperplex imaging, with colocalization of C1q/C1r/C1s/C4/C3/FB/C5b-9 on IgG-deposits on viable tumor cell surface. C4 expression by tumor cells (cytoplasmic staining), membranous C4-activation fragments deposits on tumor cells and circulating C4d frag-

ments, were more common in early-stage versus metastatic Her2+ patients. Correlation between C4-activation fragments and C4-positive tumor cells suggests local complement production and activation.

Conclusion: Our findings reveal full-blown complement activation in early stage Her2+ breast cancer patients, in part related to local complement production, correlating with favorable outcome. Current anti-Her2 therapeutic antibodies Trastuzumab and Pertuzumab do not activate complement alone. Our data supports the use of Trastuzumab+Pertuzumab which activate complement and the development of anti-Her2 antibodies with enhanced complement activation as a mode-of-action to improve the therapeutic outcome.

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INNATE LYMPHOID CELLS: IN HEALTH AND DISEASE PATHOGENESIS

Günnur Deniz

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Innate lymphoid cells (ILCs) have emerged as crucial players in the immune system, bridging the gap between innate and adaptive immunity. They are rapidly responding effector cells that provide the first line of defense against pathogens and contribute to tissue homeostasis. Unlike T and B lymphocytes, ILCs do not undergo somatic recombination during their development and therefore lack antigen-specific receptors. Despite this fundamental difference, they display remarkable similarities to T cells with respect to transcriptional programs and cytokine expression patterns. Based on these characteristics, ILCs are classified into three major groups: ILC1, ILC2, and ILC3, each mirroring the functional properties of Th1, Th2, and Th17 cells, respectively.

ILCs are preferentially enriched in mucosal tissues, such as the respiratory and gastrointestinal tracts, where they play an essential role in maintaining barrier integrity and local immune balance. By producing a wide array of cytokines, they not only shape early immune responses but also regulate subsequent adaptive immunity. Accumulation of ILCs at sites of inflammation highlights their role in the pathogenesis of chronic inflammatory and allergic diseases, where their dysregulated activity can exacerbate tissue damage.

Recent studies have also linked ILCs to the development of autoimmune conditions, cancer, and metabolic disorders, underscoring their broad impact on health and disease. Understanding the mechanisms governing ILC activation, plasticity, and tissue-specific functions is therefore critical for identifying new therapeutic targets.

In this talk, I will highlight the contribution of ILCs to immune regulation in health and explore their emerging roles in the pathogenesis of diverse diseases.

ALGORITHMS AND PROGRAMS FOR EARLY IDENTIFICATION OF PATIENTS WITH PID

Pere Soler Palacin

Spain

Inborn errors of immunity (IEI) are rare diseases associated with frequent, severe infections, inflammatory and autoimmune diseases and/or cancer. Because of the variability in presentation, undiagnosed IEI patients can be encountered by many different medical specialists. A lack of awareness of and the rarity of PI can lead to delayed diagnosis particularly among primary care physicians and non-immunology specialists. These delays can lead to irreversible sequelae, decreased quality of life and premature mortality. In this presentation, we describe two approaches designed to decrease the time to diagnosis in PI patients: 1) the expert-driven PIDCAP project conducted in Spain to promote early diagnosis in the primary care setting, and 2) a multi-modal data-driven approach using artificial intelligence and machine learning to identify individuals at high risk for PI. Both approaches aim to create widely available tools to promote early diagnosis and treatment of PI. Initial results have been positive. Future directions include larger studies and potentially combining expert-driven and data-driven approaches.

THE CLINICAL SPECTRUM OF PRIMARY IMMUNE DEFICIENCIES – SINGLE-CENTER EXPERIENCE

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We present our single-center experience in the diagnosis and treatment of primary immune deficiencies in childhood at Division of Pediatric oncohematology, Department of pediatrics for the period 2019-2025.

Twenty-eight patients are enrolled, distributed in the major subtypes of primary immune deficiencies as follows: defects in the humoral immunity, including common variable immune deficiency: 13 patients, severe combined immune deficiency, including leaky SCID: 5 patients, syndrome complexes, associated with immune deficiency (Nijmegen syndrome and Ataxia teleangiectasia): 4 patients, defects of the innate immunity: 6 patients. We report the major clinical signs at the time of the diagnosis and those presenting during follow-up, summarized in three major groups: infections, autoimmune diseases and malignancies.

The improvement of the survival rate of the primary immune deficiencies in the last decades by reduction of the rate of infectious complications after the introduction of regular subcutaneous immunoglobulin replacement and the antibiotic prophylaxis and the increased life expectancy of the affected patients have turned out the autoimmune and malignant disorders as a clinical problem of increasing frequency. The therapeutic problems in such a clinical scenario are discussed, based on the own experience of the authors.

THE ROLE OF NATIONAL PID CENTER FOR THE BETTER MANAGEMENT OF PATIENTS WITH IMMUNODEFICIENCY

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Inborn errors of immunity (IEI) are a large group of genetically inherited human disorders that affect various components of the immune system and comprise more than 500 distinct disorders (disease syndromes). For better management of patients with primary immunodeficiency (PID) in Bulgaria an Expert Centre was established at the University Hospital “Alexandrovska”-Sofia in 2016. As a result the delay in diagnosis in our country has decreased significantly for the severe forms of PID (less than 12 months of age) as well as for the patients with the most common clinically manifested immune deficiency – CVID (up to 4 years). Furthermore, 62% of patients in the National PID Registry underwent genetic testing, and 89% of them had a genetically confirmed diagnosis. Within the CVID group 70% underwent genetic testing and we found a CVID related genetic defect in 43% of them. Interestingly, after genetic testing, confirmation of the initial phenotypic diagnosis was achieved in 88.5% of all PID patients with an identified genetic defect, and a change in diagnosis occurred in 11.5%.

This talk will present our recent progress on the diagnosis and management of PID patients. Additionally, we will focus on the potential for further genetics, functional genomics and immunopathological studies in IEI. This complex approach may help improving the diagnosis and development of targeted therapies. Long-term follow-up of PID patients also remains a challenge to detect late-onset complications and guide current clinical practice and patient management.

CLINICAL SPECTRUM OF IMMUNE DYSREGULATION IN FAMILIAL MEDITERRANEAN FEVER

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Introduction: Familial Mediterranean Fever (FMF) is a periodic fever syndrome classified as auto-inflammatory primary immunodeficiency (PID). Clinical findings and genetic testing could help for diagnosis. Because of inter- and intra-familial genetic variability detailed additional data from patients with FMF phenotype would contribute to better understanding the type and degree of immune dysregulation. Aim: To summarize the information about patients from the registry of the Bulgarian PID Expert Center regarding FMF. From overall 198 subjects, 17 males and 7 females (average age 26.02 y.) were tested positive for variants in MEVF gene. 18 were found to be heterozygous carriers, 2 patients were compound-heterozygous, 2 were homozygous and in 2 patients no genetic mutation was found. Although it is accepted that FMF is an autosomal recessive disorder (MedGen UID: 45811), single variants may contribute to risk for recurrent fevers (MedGen UID: 341987, PMID: 23844200). In our subjects we detected overall 12 variants in MEVF gene, 7 pathogenic and 5 VUS. Only 8 patients, in

which the mutations were in heterozygous state had typical clinical presentation, in one subject the only pathology was evaluated as autistic spectrum disorder, 1 patient was with asymptomatic macroscopic hematuria, 3 patients were with other forms of PIDs and in the rest the genetic finding was an occasional finding. Genetic evaluation contributed to shift in diagnosis in one subject. All of the individuals with FMF related symptoms responded to colchicine and NSAID, In conclusion there is no clear association between MEVF variant zygosity and pathogenicity and individual complains. Carrying one disease causing variant is not likely to be the only cause of FMF, thus other genetic, epigenetic and environment factors seems to play additional role in immune dysregulation.

EXPRESSION PROFILING OF IMMUNOLOGY RELATED GENES IN INDIVIDUALS WITH IMMUNOLOGICAL SENESENCE

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Aim of the study. Immune senescence refers to the gradual deterioration of the immune system associated with aging. This process affects both the innate and adaptive immune responses, leading to increased susceptibility to infections, reduced vaccine efficacy, chronic inflammation, and age-related diseases. The aim of the study was to analyze the gene expression of immunology related genes in healthy individuals with immune senescence compared to ones without it.

Material and methods. 55 clinically healthy individuals are divided by their immunophenotypic profiles. Individuals with signs of immunosenescence (16) were analyzed vs those without (39) by means of RT2 Profiler PCR array comprising 84 genes of human innate and adaptive immune responses.

Results. The study identified 48 genes with significant changes in expression: the majority of the genes were upregulated (fold change > 2) while 3 genes were downregulated (fold change < -2). The genes identified with highest expression were CCL4, BCL6 and IL1 β .

Conclusion. Immunosenescence is associated with chronic low-grade inflammation (inflammation). Some gene expression changes suggest an adaptive protective response against oxidative stress and tumorigenesis. These gene markers may serve as early indicators of preclinical degenerative changes and support preventive strategies for premature immune aging.

PD-1/PD-L1 CHECKPOINT INHIBITORS AND AUTOANTIBODY FORMATION

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The PD-L1/PD-1 interaction is a key mechanism used by the immune system to block normally existing autoreactive lymphocytes. The same pathway is also exploited by various tumors

to suppress the immune system, aiding tumor survival. To counteract this process, innovative therapies in immuno-oncology include a range of PD-L1 or PD-1 inhibitors, and the results of such immunotherapy are highly encouraging. However, blocking the PD-L1/PD-1 checkpoint allows for the release of autoreactive lymphocyte clones that were normally suppressed, potentially leading to the development of autoreactivity, autoantibodies, and eventually autoimmune diseases.

Using indirect immunofluorescence, immunoblot, and ELISA, we investigated 30 patients with lung cancer at the 1st, 3rd, and 6th month of their therapy with PD-1 or PD-L1 inhibitors for the presence of 76 types of autoantibodies. The autoantibodies tested are associated with: autoimmune thyroiditis, type 1 diabetes, systemic connective tissue diseases, autoimmune liver diseases, and autoimmune neurological conditions. This study may shed light on the potential side effects related to pathological autoreactivity and autoimmunity caused by anti-checkpoint inhibitor immunotherapy.

IMMUNOTHERAPY RE-CHALLENGE IN THE EVENT OF SEVERE IMMUNE-RELATED SIDE EFFECTS AND IN THE EVENT OF FIRST PROGRESSION AFTER SUBSEQUENT NON-IMMUNOTHERAPY TREATMENT – CONTROVERSIES AND STANDARDS

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The widespread use of immunotherapy with checkpoint inhibitors in oncology has significantly changed the prognosis and quality of life of many patients with malignant solid tumors. Although the introduction of immunotherapy has influenced the treatment of patients with different tumor locations, the greatest effectiveness has been observed so far in patients with malignant melanoma and lung carcinoma.

Re-challenge of immunotherapy with checkpoint inhibitors after recovery and treatment of severe immune-related side effects is questionable in terms of its efficacy and safety. Re-challenge of immunotherapy with checkpoint inhibitors after disease progression and subsequent different treatment is also controversial in terms of efficacy and safety.

The report analyzes published data up to 2025 on the issue of both the re-administration of checkpoint inhibitors after first progression and subsequent treatment other than immunotherapy, and the re-entry of checkpoint inhibitors after the occurrence of more severe immune-related side effects. In the latter cases, current treatment guidelines from the European and American Cancer Societies advise permanent discontinuation of immunotherapy treatment.

Only retrospective studies are available, as well as a series of published clinical cases in which re-treatment with checkpoint inhibitors has benefited patients with the same immune-related side effects ranging from 18% to 42%.

Several meta-analyses on the issue are also reviewed, which emphasize that reintroduction of immunotherapy in both clinical situations is justified only after informing the patient in extremely selected patients.

IMBALANCED TH1/TH17/TREG CYTOKINE GENE EXPRESSION IN PERIPHERAL BLOOD OF RHEUMATOID ARTHRITIS PATIENTS

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Objective: Recently, we reported significantly elevated serum levels of pro-inflammatory cytokines, including TNF- α , IL-6, IL-17A, IL-23, IL-12p40, IL-18, alongside the immunoregulatory cytokine IL-10, in patients with rheumatoid arthritis (RA). In line with this, we aimed to investigate the mRNA expression of pro-inflammatory cytokine genes TNFA, IL6, IL23, IL12B, IL18, and immunoregulatory genes FOXP3, TGFB1, IL10 in the peripheral blood of RA patients concerning disease characteristics.

Methods: Total RNA was isolated from the peripheral blood of RA patients and healthy controls. Gene expression of the eight target cytokines and regulatory genes was quantified using TaqMan-based quantitative PCR (qPCR) assay. The qPCR data were analysed using the comparative $\Delta\Delta C_t$ method. Clinical data from RA patients were recorded, and disease activity was assessed.

Results: Of the eight genes analysed, four showed significant differential expression between cases and controls. Our results demonstrated predominant overexpression of IL23A and downregulation of IL12B, IL6, and FOXP3. IL23A was upregulated (1.7-fold), whereas IL6 (5-fold), FOXP3 (4-fold), and IL12B (2.56-fold) were downregulated in patients compared to controls. No significant changes were detected in IL18, TNFA, TGFB1, or IL10 mRNA levels overall. Additionally, we analyzed gene expression levels in relation to disease characteristics and activity. Active RA was associated with an elevated IL10 combined with lower TNFA and IL12B expression.

Conclusion: The altered cytokine gene expression in peripheral blood reflects the imbalance in the Th1/Th17/Treg immune response in RA. Furthermore, the expression patterns of IL10, TNFA, and IL12B may serve as molecular indicators distinguishing between different RA disease activity states.

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PREVALENCE OF VIRAL HEPATITIS B IN BULGARIA – ACHIEVEMENTS AND CHALLENGES IN LIGHT OF THE GLOBAL ELIMINATION GOALS BY 2030

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Sixty years after the discovery of the hepatitis B virus (HBV), it remains one of the most significant human pathogens, attacking the liver and causing conditions ranging from acute hepatitis

to a wide spectrum of chronic diseases, liver cirrhosis, and hepatocellular carcinoma. Once considered an infectious disease with severe consequences and no prospects for control, HBV is now manageable due to the availability of highly effective vaccines, positioning it among the diseases under epidemiological control.

As of 2022, global data indicate approximately 254 million people chronically infected and 1.1 million deaths, with around 1.2 million new cases each year. At the same time, 86% of those infected are unaware of their status, and 97% are not receiving treatment. The World Health Organization has set an ambitious target to eliminate the infection and disease by 2030: a 90% reduction in chronic HBV cases, a 65% reduction in HBV-related deaths, and reducing the prevalence of HBV (HBsAg+) among children under five years old to below 0.1%.

This review addresses various aspects of HBV infection in Bulgaria in the context of the global elimination goals, highlighting both achievements and challenges. Available national data show a declining trend in acute incidence (from 33 per 100,000 in 1983 to 2.22 per 100,000 in 2022), but there is a lack of recent seroepidemiological studies assessing HBsAg levels in the general population, which are crucial for monitoring trends.

The prevalence of chronic HBV infection is estimated at approximately 4% (about 270,000 chronically infected individuals), yet according to data from the National Health Insurance Fund, only 14,392 patients were treated between 2016 and 2021. The national immunization program has covered newborns since 1992 and maintains a relatively high coverage rate (92.3% in 2023), but there is no targeted program for high-risk groups.

Additional measures to reduce the risk of transfusion-transmitted hepatitis B were introduced at the end of 2019, including molecular methods for HBV detection in donor blood. The National Program for Prevention and Control of Viral Hepatitis (2021–2025) offers an opportunity for early detection of chronic HBV infection through the introduction of population-based screening (targeting individuals aged 40–60 years).

DYSREGULATION OF T-CELL IMMUNE RESPONSE IN CHRONIC FATIGUE SYNDROME AND LONG-TERM COVID-19

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Aim: Chronic fatigue syndrome (CFS) and long COVID-19 (LC) are post-infectious conditions with similar clinical features and supposedly common pathogenetic mechanisms, among which impaired T-cell functions associated with altered immune metabolism and autoimmune manifestations stand out. To comparatively assess T-cell dysfunction in both conditions, we examined 17 CD4 T effector and 12 regulatory subpopulations, along with cytokine profile data.

Materials and methods: Isolated peripheral blood mononuclear cells (PMNCs) from donors with CFS (n=15) and LC (n=15), and age- and sex-matched healthy controls (HC, n=11) were studied. 15-color combinations of monoclonal antibodies against CD3, CD4, CD25, CD15s, CD197, CD183, CD194, CD185, CD161, CD127, CD45RA, CD196, PI16, CD38, CD39, CD147, CD71, CD161, CD73, GARP и HLA-DR and a standard labeling protocol were applied. The concentrations of IFN- γ , TNF α , IL-2, IL-6, IL-17, IL-4 and IL-10 were determined by flow cytometry. The analysis was performed with a FACSAria III flow cytometer FACSDiva v. 9.0.1, FloJo v.10.10.0, and BD CBA software.

Results: The CD4 T cell Th1, Th2, Th17, Th22, ThG, Tfh, Tfh1, Tfh2, Tfh17 subpopulations, together with naive, memory, activated, and induced Treg, Th1Treg, Th2Treg, Th17Treg, Th22Treg, TfhTreg, were analyzed. Donors with LC were characterized by an increased proportion of Th22 and ThG CD4 T (average 39% vs. 21 and 22%), and those with CFS – by increased Th17 (45% vs. 33 and 34%), Treg Th17 (53% vs. 40 and 42%) and Tfh17 (61% vs. 56 and 44%) subsets, at the expense of reduced Th1, Th1Treg and Th9. Phenotypic changes were associated with different profiles of cytokine imbalance resulting in persistent immune inflammation.

Conclusion: Phenotypic analysis of effector, regulatory and transient CD4 T subpopulations reveals similar yet specific disorders of T-cell functions in the studied syndromes.

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CHRONIC HIV INFECTION, ANTIRETROVIRAL THERAPY, AND CYTOKINE IMBALANCE

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In the modern era, people living with HIV (PLWH) are reaching significantly older ages compared to the early years of the HIV-1 pandemic, primarily due to the effectiveness of contemporary combination antiretroviral therapy (cART). However, both chronic HIV-1 infection and increased longevity among PLWH contribute to persistent immune dysfunction, which is associated with premature and elevated rates of morbidity and mortality from non-AIDS-related conditions, even in the presence of sustained viral suppression. The complex interaction between HIV-1 infection, inflammation, immune cell metabolism, and immunosenescence remains incompletely understood. In this context, biomarkers of immune activation and inflammation have emerged as promising tools for assessing immune status and predicting the risk of aging-related comorbidities, both in the general population and among PLWH. Given the critical role of cART in sustaining prolonging survival, it is essential to elucidate the impact of modern cART on immune inflammation and activation. Moreover, potential differences in immunologic outcomes across various antiretroviral agents and regimens require investigation. This study focuses on the identification and evaluation of specific biomarkers indicative of immune activation and inflammation in PLWH, with a particular emphasis on comparing the effects of different contemporary cART regimens. Among the 13 biomarkers analyzed – GM-CSF, IL-1 β , IL-12p70, IL-2, IL-4, IL-5, IL-6, IL-13, IL-18, D-dimer, CRP, TNF- α , and sCD14 – significant associations with the cART regimen were observed for IFN- γ , IL-12p70, IL-18, IL-5, TNF- α , and sCD14. The results indicate that despite effective viral suppression, cART does not completely normalize immune activation and inflammation. The results of the current study do not provide hard evidence favoring a particular cART regimen, although they show a less favorable regimen profile containing a protease inhibitor or lacking nucleoside reverse transcriptase inhibitors. Addressing the multifactorial nature of aging in PLWH will likely require a comprehensive approach involving

a panel of biomarkers to accurately characterize immune status and predict clinical outcomes. Future researches into immune activation and inflammation markers are expected to play an increasingly important role in optimizing long-term care for this population.

COMPARATIVE ELISA PROFILING IN CHRONIC FATIGUE SYNDROME AND LONG COVID-19

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Aim of the study: Chronic Fatigue Syndrome (CFS) and Long COVID (LC) share clinical features, such as persistent fatigue and cognitive impairment. To explore potential common pathogenetic mechanisms and identify distinguishing biomarkers, we assessed a panel of inflammation, immune response and oxidative stress mediators.

Materials and methods: Serum samples from donors with CFS (n= 85), and LC (n=52), together with sex- and age-matched healthy controls (HC, n=55) were studied. Concentrations of Dickkopf-related protein1 (DKK-1), indoleamine 2,3-deoxygenase (IDO), catalase, superoxide dismutase1(SOD-1),Toll-like receptor4, malondialdehyde (MDA), nitric oxide synthase3 (NOS3), glutathione peroxidase1 (Glut-1), and haptoglobin(Hp) were measured using quantitative ELISA.

Results: In CFS the levels of MDA, NOS3, and GPX-1 were importantly increased as compared to LC and HC (mean 341vs. 59 and 16 ng/ml; 769 vs. 603 and 249 pg/ml; 47vs11 and 10.7 pg/ml, p<0.001 for all) while catalase, TLR4, and Hp were decreased (186.8 vs 248 and 250 kU/Lp<0.01; 30 vs. 22 and 18 ng/ml, p<0.05). SOD1 and DKK1 were increased in both pathologies as compared to HC, though without statistical significance. LC was distinguished with the highest Hp levels (201.6 vs 135 and 151 in CFS and HC, p<0.001). In addition, SOD1 and IDO correlated strongly with DKK1 only in CFS (r=0.7, p<0.05)

Discussion: We report a pronounced oxidative-stress signature, combined with discordant compensatory response in CFS. While LC shared several aberrations with CFS (increased MDA, NOS3, SOD1, they were less pronounced and combined with preserved regulatory and immune response mechanisms (IDO, Hpt, TLR4). These results support partially overlapping yet distinct pathogenic mechanisms in LC and long-standing CFS.

BIOMARKERS OF IMMUNOSENESCENCE

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Aim of the study: Persistent low-level immune activation is an acknowledged mechanism of premature immune ageing, that may result from misbalanced immune stimulatory and inhibitory signals.

We analysed the cytokine profiles in healthy individuals of active age exhibiting phenotypic markers of immunosenescence.

Methods: Peripheral blood samples from 89 clinically healthy individuals (33 males and 56 females), mean age (mean $\pm$ SD) 39.7 ($\pm$ 7.2) were analyzed. The shares of naïve (CD45RO-CD27+), memory (CD45RO+CD27-) and effector (CD45RO-CD27-), activated (HLA-DR+CD38+), exhausted (TIGIT+), and proapoptotic (CD57+CD27-) CD4 and CD8 T cells were analyzed by multicolor flow cytometry (FACSCanto II, Diva v.6.1.2). Cytokine concentrations were determined in PHA-stimulated samples (ProCartaPlex, Luminex200X) to evaluate: macrophage-mediated signalling, M1 (IL-1b, IL-6, TNFa), Th1 (IL-2, IL-12, IL-18, IFNg), Th17 (IL-17, IL-22), Th2 (IL-4, IL-13), and Treg (IL-10) responses. Z-unit weighted composite scores were calculated for M1/(Treg + Th2); Th1/(Th2+Treg); (Th1+Th17)/(Th2+Treg).

Results: In 14 donors (group B, 39.8 $\pm$ 6.7 years), a significantly increased expression of CD57+CD27- and CD57+TIGIT+ T cells were established as compared to the rest 75 (group A, 38.7 $\pm$ 9.2 years): (mean $\pm$ SD) 31.8 $\pm$ 7.9 vs. 9.1 $\pm$ 5.8, p <0.0001 and 26.26 $\pm$ 3.75 vs. 12.69 $\pm$ 7.09, p <0.001. While in group A the immunoregulatory ratios tended to zero (0.00, 0.03 and 0.04, respectively), group B was characterized by negative M1/(Treg + Th2) ratio (-0.18), and positive Th1/(Treg+Th2) and (Th1+Th17)/(Th2+Treg) ratios (0.26 and 0.25).

Conclusions: Elevated expression of immune exhaustion phenotypic markers in healthy people is associated with decreased capacity for induction of Th1 responses, and prevalence of proinflammatory cytokines as early indicators of premature immunosenescence.

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IMMUNOLOGICAL ASPECTS OF EXTRAPULMONARY INFECTIONS OF MYCOPLASMA PNEUMONIAE – CONTRIBUTION WITH TWO CLINICAL CASES

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Aim. To analyze two clinical cases of Mycoplasma pneumoniae (MP) infection with extrapulmonary manifestations, caused by immunological mechanisms of damage.

Material and methods: The first case is of a hospitalized 14-year-old boy with detected mycoplasma pneumonia by positive serological tests for anti-MP IgM, IgG and PCR from sputum. A few days later he developed myalgia, arthralgia, headache and decreased NK cells. The therapy ended with favourable outcome. The second case is of a child with hemolytic anemia after MP infection.

Results and discussion. In the first case it was assumed that the extrapulmonary symptoms followed inflammatory reaction with a mixed type of damage, and the high values of serum creatine kinase corresponded to rhabdomyolysis. In the second case, the hemolytic anemia was probably caused by cold cross-reacting IgM autoantibodies to a carbohydrate antigen on the surface of red

blood cells and cells of the respiratory epithelium. Extrapulmonary complications of MP are due to 3 mechanisms – 1) direct type with production of proinflammatory cytokines from pathogenic bacterial factors; 2) indirect type – from an autoimmune reaction through antibodies against MP, also acting as autoantibodies that cross-react with human cells, and 3) vascular occlusion type, a consequence of vasculitis and/or thrombosis with/without systemic hypercoagulation induced by MP
Conclusion: Extrapulmonary manifestations of MP are frequent and they require knowledge of the mechanisms of damage and recognition of the symptoms in order to make the correct diagnosis in a timely manner and choose the most appropriate treatment, associated with prognosis.

CURRENT IMMUNOTHERAPY IN REPRODUCTIVE FAILURE

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Reproductive failure, including recurrent pregnancy loss (RPL) and repeated implantation failure (RIF), remains a complex and often frustrating challenge in reproductive medicine. Immunological dysfunction – particularly involving dysregulation of maternal immune tolerance to the embryo – has been increasingly recognized as a contributing factor in a subset of these cases. Immunotherapeutic interventions such as intravenous immunoglobulin (IVIG), corticosteroids, intralipid infusions, lymphocyte immunization therapy (LIT), biologic agents immunosuppressive drugs etc. have gained traction in clinical settings. However, evidence supporting their efficacy is mixed, and standardized treatment protocols are lacking. Clinicians must balance potential benefits with the limited high-quality data, particularly in patient selection and outcome predictability. This presentation summarizes current immunotherapeutic options, their proposed mechanisms, and relevant clinical trial findings, offering practical insights into how these approaches may be integrated into personalized treatment strategies for reproductive failure. Clinical case data from successful treatment protocols will be presented.

MOLECULAR MECHANISMS OF INTRALIPID THERAPY

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Bulgaria

Aim. To identify the molecular mechanisms by which Intralipid suppresses elevated levels of NK cells in women with recurrent pregnancy loss.

Materials and methods. Peripheral blood samples collected from women with elevated NK cell levels and a history of recurrent miscarriages, both before and after Intralipid infusion. NK-92 cell line. Flow cytometric analysis of surface markers in peripheral blood cells. Cell culturing. Long read nanopore-based transcriptomics

Results. Two weeks after Intralipid infusion, a statistically significant reduction was observed in the total lymphocyte population within the peripheral blood leukocyte pool, with a increase in the proportion of monocytes. There were also changes in T-cell subpopulations, including an increase in CD4+ and Treg cells, accompanied by a reciprocal decrease in CD8+ and $\gamma\delta$ T-cells. NK cells were reduced both as a proportion of lymphocytes and in absolute count. The reduction was mainly due to the CD56bright population, which is believed to primarily target the endometrium. Additionally, there was an increase in CD56-CD16+ NK cells.

Transcriptomic analysis of the NK-92 model system revealed changes in the expression of numerous structural genes, as well as several regulatory elements, including long non-coding RNAs – with 65 being upregulated and 32 downregulated.

As expected, many of the affected genes were associated with lipid metabolism and cellular energetics. Components of the cytoskeleton involved in polarization and granule transport in NK cells were also affected, along with regulatory pathways such as RAS/MAP/ERK, “Cytokines and inflammatory response (WP530)”, and various membrane proteins involved in signal transduction.

Conclusion. The effect of Intralipid on NK cells is multifaceted: it directly influences gene expression and cellular behavior, indirectly modulates responses via bioactive derivatives of Intralipid components, and alters the broader immune environment by affecting other immune cell populations.

THROMBOPHILIA AND PREGNANCY

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The term „thrombophilia“ refers to an increased tendency to form pathological intravascular venous or arterial thromboses, as a result of the interaction of multiple hereditary (genetic) and/or acquired predisposing factors. Hereditary (genetic) thrombophilia is defined as a genetic predisposition to venous thromboembolism (VTE), due to genetic deletion or alteration of a functional protein in the coagulation cascade. The most common and widely studied genetic factors for VTE are: deficiency of natural anticoagulant proteins – antithrombin III, protein C, and protein S; as well as mutations in Factor V Leiden (FVL G1691A); methylenetetrahydrofolate reductase (MTHFR), and the prothrombin gene (F2, G20210A). Normal pregnancy is associated with numerous significant changes in hemostasis, the final result of which is a predominance of procoagulants and a pro-thrombotic state, the indicators of which become more pronounced as gestational age increases. Changes in hemostasis begin at the moment of conception, gradually intensifying, reaching the highest level of hypercoagulability during the third trimester and childbirth, and persist with a slow regression back to baseline levels at the earliest 8 weeks after birth. The association and frequency of the most common genetic risk factors with pregnancy losses are presented, along with the use of anticoagulant and antiplatelet therapy during pregnancy, as well as the options for its monitoring.

KILLER CELL IMMUNOGLOBULIN-LIKE RECEPTORS (KIR) AND HUMAN LEUKOCYTE ANTIGEN-C (HLA-C) ALLO-RECOGNITION PATTERNS IN WOMEN WITH MISSED ABORTIONS

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Aim of the study: KIRs play a key role in regulating placentation, and hence in reproductive success. This occurs through a mechanism of maternal allogeneic recognition involving interac-

tion between KIRs on maternally derived uterine NK and T cells with HLA molecules on the embryonic trophoblasts. We aimed to investigate the frequency distributions of KIR genotypes and HLA-C group among women with missed abortions (MA) vs healthy pregnant women in 1st trimester. The inhibitory KIR (iKIR) expression by T cells and the cognate HLA-C ligand at maternal-fetal interface (MFI) were analyzed as well.

Materials and methods: The study included 30 pregnant women in three groups: with missed pregnancy, with elective termination of normal early pregnancy and with full-term pregnancy. KIR and HLA-C genotype analyses were performed by PCR-SSP method. iKIR expression and HLA-C detection were analyzed by FACS and immunohistochemistry, respectively.

Results: The two groups showed no significant differences in the KIR genotypes and in HLA-C1 and HLA-C2 frequencies. HLA-C was expressed by extravillous trophoblasts at MFI early but not in late normal pregnancy. In line, the iKIRs+ $\gamma\delta$ T cells predominantly populated MFI early in pregnancy. However, their number was significantly decreased in MA patients.

Conclusions: Although we did not reveal an association between KIR polymorphisms and HLA-C genotypes with missed abortions, the decreased presence of iKIR on decidual $\gamma\delta$ T cells could compromise the immune tolerance early in human pregnancy.

EFFECT OF TROPHOBLAST EXOSOMES ON HUMAN IMPLANTATION

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Aim of the study: Exosomes are extracellular nanovesicles of endosomal origin, containing various signaling molecules and participating in the intercellular communication. In the present study, we analyzed the effect of trophoblast-derived exosomes on human implantation.

Materials and methods: As a 3D model for studying human implantation we used a Sw71 blastocyst-like structure (Sw71-spheroid) as a surrogate of human peri-implantation embryo. We isolated exosomes from serum-free conditioned medium of primary extravillous trophoblast cells (EVTs) and EVT-like Sw71 trophoblast cells. The primary trophoblasts were obtained from 1st trimester-chorion of healthy pregnant women directed to elective abortion and were differentiated into EVT. We characterized the morphology and size of the exosomes (TrExo) by TEM and DLS, and their phenotype by immunogold-electron microscopy and FACS. The migration of Sw71 surrogates was monitored by time-lapse imaging system after adding or not of TrExo. The percentage of migrated surrogates and the size of their migration zone were determined.

Results: We confirmed the EVT phenotype (CK7+/Vim+/HLA-G+/HLA-C) of primary and Sw71 EVT and demonstrated that they both produce spherical, intact exosomes with size of 30-100 nm and a hydrodynamic diameter of 40 ± 10 nm. TrExo expressed the classical exosome marker CD63 and were positive for the tolerogenic HLA-G and HLA-C molecules. The presence of TrExo improved the migration of Sw71 surrogates by increasing the percentage of migrated spheroids but without significant change of their migration zone (100% vs. 96% and 1.979 ± 0.797 mm² vs. 1.652 ± 0.787 mm², $p > 0.05$).

Conclusion: Trophoblast exosomes containing HLA-G and HLA-C molecules promote human implantation. The exosome-based therapy could be a promising approach for immunotherapy in women with reproductive failures.

ENDOMETRIAL PROGENITOR CD34+ NK CELLS AND ENDOMETRIAL MICROBIOTA IN WOMEN WITH REPRODUCTIVE FAILURES

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Introduction: The role of CD34+ NK precursor cells in successful implantation and placenta-tion is a subject of growing scientific interest. It has been established that there is a correlation between elevated levels of these cells in the endometrium and successful implantation in pa-tients with endometriosis. However, the impact of endometrial microbiota on tissue infiltration has not yet been investigated.

Aim: The objective of this study was to ascertain potential therapeutic approaches for women diagnosed with RIF and RPL by assessing the significance of the endometrial microbiota in en-dometrial CD34+ NK cell infiltration.

Materials and methods: The study included 200 women with a mean age of 34.27 ($\pm$ 4.21) years, conducted between December 2022 and December 2024. Endometrial biopsies were tak-en during the mid-luteal phase of the menstrual cycle. A molecular diagnostic method (REAL-TIME PCR, Femoflor 16®) was applied for the analysis of endometrial microbiota, and a flow cytometric method (FACS Calibur, Becton Dickinson) was used to determine endometrial im-mune cells.

Results: The results indicated statistically significantly higher levels of progenitor NK cells in women with normal endometrial microbiota (*Lactobacillus* spp. above 90%) who achieved preg-nancy. The findings of this study lend support to the hypothesis that CD34+ NK precursor cells play a significant role in reproductive success, thereby underscoring the importance of probiotic therapy in this demographic.

Conclusion: An imbalance in the microbiota, characterised by a reduced presence of lactobacilli and the presence of dysbiosis, can lead to local inflammation that affects the development and activity of endometrial progenitor NK cells. This study supports the hypothesis that CD34+ NK cells play a role in reproductive success and that probiotic therapy is important for these women.

NON-COMMERCIALLY MANUFACTURED CHIMERIC ANTIGEN RECEPTOR T-CELLS FOR TREATMENT OF AUTOIMMUNE AND OTHER DISEASES

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Germany

Aims: The current state of the Chimeric Antigen Receptor (CAR) T cells manufacturing and ap-plication is summarized. Details about the treatment of single patients with different underlying refractory/relapsed diseases with such gene modified T-cells as a Point of Care (PoC) strategy at the Hannover Medical School is presented.

Materials and methods: The manufacturing of the corresponding anti-CD19 or CD19/CD20 CAR T-cells was carried out by the university own production site, the Cellular Therapy Centre.

Autologous lymphaphereses were performed on five patients so far: a 39 years old (yo) male with acquired haemophilia A, a 57 yo male with DLBCL, a 65 yo male with ANCA vasculitis, a 62 yo female with systemic sclerosis, and a 54 yo male with Richter transformation. The generation of CAR T-cells was initiated with 1 Billion CD4 and CD8 cells using the CliniMACS Prodigy® device and corresponding materials from Miltenyi Biotec Germany including 2nd generation self-inactivating lentiviral vectors. All products were cryopreserved. Standard in vivo lymphodepletion with Fludarabine und Cyclophosphamide was conducted prior to CAR T-cell infusion.

Results: Altogether, between 0.8 and 1.6×10^9 CAR T-cells were available after cryopreservation. The patients received $1-3 \times 10^6$ CAR T-cells/kg. No major side effects were observed. All but the DLBCL patients reached remission. This patient was treated with dual CD19/CD20 CAR-T-cells because of CD19low/CD20high expression of the lymphoma.

Summary: The local production of CAR T-cells for the treatment of patients, who are not eligible for the application of commercially available CAR T-cell products and have no other curative options, may represent a feasible option.

IMMUNOTHERAPY IN SOLID MALIGNANT TUMORS – IMMUNE CHECKPOINT INHIBITORS

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The relationship between cancer development and the tumor-specific immune response has been investigated for decades. The discovery of immune checkpoints regulating antitumor responses has fundamentally changed the concept of cancer treatment. Immune checkpoint inhibitors (ICIs) restore T-cell-mediated antitumor activity by blocking suppressive feedback mechanisms induced by tumor cells. Currently, the most widely used ICIs are monoclonal antibodies targeting CTLA-4, PD-1, and PD-L1, which have demonstrated clinical efficacy in various malignancies, including lung cancer, melanoma, urothelial carcinoma, renal cell carcinoma, and cholangiocarcinoma. This presentation reviews the mechanisms of action of ICIs, their therapeutic outcomes, and the major challenges related to resistance, immune-related toxicity, and the absence of reliable predictive biomarkers. The potential of combination strategies with chemotherapy, radiotherapy, and targeted agents is also discussed. Recent data from pivotal clinical trials are presented, outlining future directions in immuno-oncology.

EXPRESSION PATTERNS OF IMMUNE CHECKPOINT MOLECULES IN ACUTE MYELOID LEUKEMIA – CLINICAL RELEVANCE

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Aim: To study the impact of immune checkpoint (IC) molecules expression on major lymphocytic subtypes and blasts in acute myeloid leukemia (AML) to develop predictive classifiers for overall survival (OS) based on integrative analysis.

Materials and methods: A total of 60 AML patients: 31 male and 29 female (mean age 61.05 ± 14.78 years), were included. CD279(PD-1), CD152(CTLA-4), CD366(Tim-3), and CD273(PD-L1),

CD274(PD-L2), CD80(B7-1), CD86(B7-2), CD66a(CEACAM1), were analyzed by multiparameter flow cytometry on major lymphocytic subtypes and blasts, respectively. Therapeutic response and survival were assessed. Cluster analysis was performed by the WARD method.

Results: IC ligands demonstrated a wide range of expressions on leukemic cells: CD66a (3.1%-87.4%), CD273±CD274 (0.2-6.2%), CD80±CD86 (0.8-71%). Heterogeneous patterns were observed regarding IC receptors on major lymphocytic subpopulations. CD273±CD274 high expression was associated with shorter OS (median 51.9 months vs 31.4 months, log-rank test $p=0.03$). Certain patterns contributed further to unfavorable outcomes incl. high expression of: CD152 on NK and/or T-cells, CD80±CD86 on blasts; CD279 on NK and/or T-cells and CD274±273 on blasts. Cluster analysis defined 2 clusters with distinct IC expression patterns. Cluster 1 demonstrated a significantly lower proportion of CTLA-4(+) NK($0.1\pm0.3\%$ vs $4.0\pm5.7\%$, $p<.001$) and CD8(+) T-cells subpopulations and significantly worse OS compared to Cluster 1 (median 5.4 ± 1.6 months vs. not reached, log-rank test $p=0.029$).

Conclusion: Expression patterns of IC molecules in AML correlate with clinical outcome. A prognostic cluster model may provide potential information for risk assessment and emphasize the importance of investigating new immune-related biomarkers in AML.

T-CELL CLONALITY – FLOW CYTOMETRIC DETERMINATION

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Discriminating between malignant and benign mature T-cell proliferations is often difficult and insufficient based solely on evidence of an aberrant immunophenotype.

Aim of the study: Our aim was to investigate the applicability of a flow cytometric approach for the study of clonality as a surrogate for the probable malignant nature of TCRab(+) mature T-cell lymphoproliferations by surface labeling with antibodies against TCRβC1 (JOVI-1) and TCRβC2, two mutually exclusive β-chain constant regions of the T-cell receptor (TCR).

Materials and methods: Between April 2023 and April 2025, a total of 144 peripheral blood samples were examined from patients with absolute lymphocytosis (3.86 ± 1.35 G/L). The analysis was performed using a panel containing CD3, CD4, CD8, CD2, CD5, CD7, CD26, CD27, CD19, CD20, CD56, CD16, TCRα/β, TCRγ/δ, TCRβC1, TCRβC2, and 12-color flow cytometry (FACSLyric BD). An 85% threshold of positive or negative expression of TCRβC1 or the recommended ratio of TCRβC2:TCRβC1=1.6 [1.3-2.0] in the analyzed T-cell population was accepted as an indicator of T-cell monotypy.

Results: Clonotypic T-cell populations were identified in 36% (N=52) of the subjects, respectively 38% (N=20) CD4+T-lymphocytes, 48% (N=25) CD8+ T-lymphocytes, 9% (N=5) CD4+CD8+ and 4% (N=2) separate clonotypic CD4+ subpopulations. The group included both patients with proven T-cell neoplasms and those with T-cell clones of uncertain significance (T-CUC). In the remaining 64% (N=92), T-cell proliferations were determined to be polyclonal. In our cluster analysis, patients with populations defined as monotypic fell completely into a common cluster, while those with polytypic distribution clearly formed a separate cluster ($F=407.6$, $p<0.001$).

Conclusion: The inclusion of markers allowing TCR typisation can easily and reproducibly prove the clonal T-cell nature and synergistically with the defined phenotype and morphology support the integrated diagnosis.

FLOW CYTOMETRIC DETERMINATION OF PNH CLONES WITH HIGH SENSITIVITY

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Paroxysmal nocturnal hemoglobinuria (PNH) is a rare clonal disease affecting the hematopoietic stem cells.

Aim of the study: Our aim was to investigate a highly sensitive ($<0.01\%$) flow cytometric approach for the detection of erythrocytes, neutrophils, and monocytes with PNH phenotype in peripheral blood.

Materials and methods: Between January 2024 and April 2025, a total of 83 peripheral blood samples were examined from 77 patients suspected of PNH, aged 5 to 83 years (mean 48 years), of whom 47% were women and 53% were men, and a control group of 12 healthy individuals. The analysis was performed with a panel containing FLAER, CD15, CD24, CD157, CD64, CD14, CD45, CD235a, CD59, CD71 CD55, and 12-color flow cytometry (FACSLytic BD), in accordance with the current recommendations of the ICCS/ESCCA.

Results: Based on the control group, the analytical sensitivity metrics of the method were calculated: Limit of Blank and Lower Limit of Detection, with a detection sensitivity of PNH-phenotype cells $<0.01\%$ in neutrophils, monocytes, and erythrocytes. In 10 patients, PNH phenotype was detected in $>60\%$ of the three lines. All of them were characterized by clinical manifestations of PNH. In 5 patients, PNH phenotype was detected in 1-10% of the three lines, and in 2 patients in 0.1-1%, with 3 of them having proven bone marrow failure and 1 with DAT(-) hemolytic anemia. In 13 patients, the PNH phenotype was found only in erythrocytes, which does not reflect the existence of a true clone.

Conclusion: Flow cytometric determination of cells with PNH phenotype allows precise and reproducible determination of the size of the PNH clone, which, together with the clinical and laboratory constellation, allows an integrated diagnosis and serves as a starting point for therapeutic intervention.

KIDNEY PAIRED DONATION IN THE CZECH REPUBLIC – RESULTS, INTERNATIONAL COLLABORATION AND PERSPECTIVES

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Kidney pair donation (KPD) is an effective strategy to improve the chances of receiving a kidney transplant for patients with an HLA incompatible living kidney donor. The Czech KPD program was officially started in the Institute for Clinical and Experimental Medicine (IKEM), Prague, in April 2011. A central database was established and the program quickly grew to become one of the most successful in Central Europe. Within the first 3 years, more than 60 transplants were completed through paired donation. Computerized algorithms were adopted for matching (the software TXMatching), allowing chains and more complex swaps, not just 2-pair exchanges. Non-directed altruistic donors were also incorporated, which opened up the possibility of donation chains benefiting multiple patients. Czech Republic is involved in international collabora-

tion in KPD with Austria (since 2016) and Israel (since 2019), where the Prague transplant center is the coordinating center for these exchanges. Today, the Czech program is recognized as one of the most efficient KPD systems in Europe relative to population size. IKEM remains the leading transplant center, responsible for a large share of all kidney transplants in the country (over 300 annually). Paired donation contributes a significant proportion of living-donor transplants (sometimes up to 25–30%).

In **conclusion**, the program has markedly reduced waiting times for sensitized patients and improved access for those with ABO or HLA incompatibilities. Czechia is currently recognized as having one of the most efficient KPD programs in Europe (relative to its population) and regularly achieves multi-pair chains with excellent outcomes.

TRANSPLANTATION ACTIVITY AND GVHD FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: EXPERIENCE OF THE VARNA CENTER (2017–2024)

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Aim: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a standard curative approach for high-risk hematologic diseases. This study aims to present the activity of the Varna transplant center, focusing on donor types, indications, immunological complications, and survival.

Materials and methods: A total of 114 allo-HSCT procedures were analyzed (2017–2024), including 108 adult and 6 pediatric cases. Donors included 67 related (39 haploidentical) and 47 unrelated. Data on diagnosis, age, donor type, incidence of acute and chronic GVHD, and survival were evaluated.

Results: Mean age was 43 ± 7 years. Main indications included AML (n=68), ALL (n=21), AA (n=7), lymphomas (n=9), and others (n=9). Acute GVHD grade $\geq$ II occurred in 36 patients (31.6%), chronic GVHD in 26 (22.8%). Three-year overall survival was 54%, progression-free survival – 52%.

Conclusion: The Varna center demonstrates consistent transplant activity with acceptable GVHD rates despite a high proportion of haploidentical transplants. These findings highlight the need for personalized GVHD prophylaxis and long-term monitoring of immune recovery.

HEMATOPOIETIC STEM CELL TRANSPLANTATION – THE EXPERIENCE OF THE SPECIALIZED HOSPITAL FOR ACTIVE TREATMENT OF HEMATOLOGICAL DISEASES

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Introduction. Since the establishment of the hematopoietic stem cell transplantation (HSCT) program at the Specialized Hospital for Active Treatment of Hematological Diseases in 2003,

more than 700 HSCTs (both allogeneic and autologous) have been performed. In recent years, safer conditioning regimens, better supportive measures, and the increase in healthy volunteer unrelated donors have led to a significant increase in the number of HSCTs worldwide, as well as in Bulgaria.

Materials and methods. We have retrospectively collected and analyzed data from patients who underwent autologous and allogeneic HSCT from January 2023 until May 2025.

Results. A total of 263 patients underwent HSCT during the study period (184 autologous and 79 allogeneic). Median age at transplantation was 55 years for the autologous and 45 years for the allogeneic group. Main indications for autologous HSCT were MM, HL, and NHL. Among patients who received allogeneic HSCT, 52% (n=41) had AML, 32% (n=25) had ALL, and 16% (n=13) had other hematological malignancies. In the allogeneic group, 96% received peripheral blood stem cells, whereas 4% received bone marrow as the graft type. More than half of the patients (55%) had a matched unrelated donor. Transplant-related mortality (TRM) was 1.8% for autologous and 7.9% for allogeneic HSCT.

Conclusion. HSCT remains the only curative treatment modality in a variety of hematological malignancies. Our data are consistent with international experience and point toward an increase in transplant procedures, an increase in unrelated donor availability, and a decrease in TRM.

TRANSPLANTATION OF HEMATOPOIETIC STEM CELLS IN CHILDREN

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The transplantation of hematopoietic stem cells as a second-line therapeutic line for a number of malignant and severely progressing benign diseases has undergone exceptional development over the last 60 years. After its introduction in the late 1950s, the number of transplanted children and adults has continuously increased, the indications have been refined, especially in childhood, the sources of stem cells have been improved, the choice of the most suitable donor for the allogeneic transplantations has been enhanced, and the conditioning regimens and post-transplant supportive care and complication prevention have been optimized.

In October 1997, the first autologous transplantation of hematopoietic stem cells was performed in Bulgaria, and in 2000 the first allogeneic, both in children, patients of the department of pediatric hematology and oncology in Sofia. For more than 25 years, the transplantation activity of our department has been increasing and improving according to world standards.

We present the requirements and conditions for this activity in childhood, including indications, sources of stem cells, selection of donors, types of conditioning regimens, and post-transplantation care, as well as the results of transplantations in patients of the department of pediatric hematology and oncology in Sofia.

GENETIC DIVERSITY IN THE BULGARIAN BONE MARROW DONOR REGISTRY: IMPLICATIONS FOR GLOBAL MATCHING

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Aim: To evaluate HLA data quality, allele and haplotype frequencies, and population-specific characteristics of the Bulgarian Bone Marrow Donor Registry (BBMDR), and assess its relevance for global donor matching.

Methods and results: We analyzed HLA data from the BBMDR, focusing on typing resolution, allele diversity, and haplotype structure. Approximately 46% of donors are typed at high resolution for A-B-C-DRB1-DQB1-DPB1 loci – exceeding WMDA global averages. Demographically, the registry is skewed toward donors over 35 years, with only 4% aged 18-25, compared to 10% in WMDA, and a female predominance in this younger group.

The most prevalent alleles – A*02:01, B*51:01, C*04:01, DRB1*11:04, DQB1*03:01 – reflect a Southern European genetic profile, but differ slightly from Northern and Western Europeans, where alleles like DRB1*15:01 and DQB1*06:02 are more prevalent. HLA-B~C haplotypes show a more even distribution, while HLA-DRB1~DQB1 were highlighted by the presence of a few highly prevalent combinations like DRB1*16:01~DQB1*05:02 and DRB1*03:01~DQB1*02:01, both exceeding 10%. The high frequency of several haplotypes, particularly the 8.1 ancestral haplotype (26%), pointed to potential historical events or selective forces shaping the population's HLA profile.

The registry includes a significant proportion of Common and Well-Defined alleles, which supports the reliability and clinical utility of the registry for donor matching on a global scale. The presence of Rare and Very Rare alleles highlights the genetic diversity of the Bulgarian population and its relevance to global searches, especially for patients with uncommon HLA genotypes.

Conclusion: BBMDR demonstrates high-quality data of its donors, haplotype conservation, and strong genetic diversity, confirming its value for international donor registries and underscoring the need for focused recruitment strategies.

DONOR KIR HAPLOTYPE IS ASSOCIATED WITH CLINICAL OUTCOMES OF HAPLOIDENTICAL HEMOPOIETIC STEM CELL TRANSPLANTATIONS

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Haploidentical donor is an increasingly used alternative for hematopoietic stem cell transplantation (haplo-HSCT). A number of transplant centers include killer immunoglobulin-like receptors (KIR) and their HLA ligands (KIRL) in the protocols for identification of donors with optimal NK-mediated alloreactivity, but there is still no routinely accepted selection algorithm.

The aim of our study is to assess to which extent KIR and KIR ligands are associated with clinical outcomes in haploidentical HSCT.

Material and methods: Sixty-three haploidentical stem cell transplantations (conditioning regimen: MAC-69.3% and RIC-30.7%), performed in Bulgaria, were retrospectively analyzed. Patients were with various hematological diseases (AML – 54.7%; ALL – 24.0%; others – 21.3%) and PTCy prophylaxis (PTCy+CSA+MMF in 46.7% and PTCy+Tac+MMF in 30.7%) of graft-versus-host disease (GvHD). Following PCR-SSP KIR genotyping, donors were categorized as neutral, better, and best (KIR Donor B-Content Calculator). KIR ligand compatibility/incompatibility in GvH and HvG direction was defined using KIR Ligand Calculator.

Results: The mean age of donors was 38 years, with 59.7% of haplo-transplants being from a sex-matched donor. No significant difference in survival was observed between sex-matched and sex-mismatched transplants. Donors with a neutral (61.9%) KIR-B haplotype predominated. Donor KIR-B haplotype did not influence the development of acute GvHD, but was associated with chronic GvHD ($p=0.039$). Recipients transplanted from a better/best donor had better overall survival (OS; $p=0.019$), while KIRL incompatibility/compatibility did not affect OS.

In **conclusion**, our data suggest that donor KIR genotype information can be used in routine practice for pre-transplant assessment of KIR-driven NK cell alloreactivity in allo-HSCT setting.

METHODS FOR ASSESSMENT OF ALLERGEN-SPECIFIC ACTIVATION AND DEGRANULATION OF BASOPHILS (BAT) AND MAST CELLS (MAT)

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IgE-mediated immediate-type allergic reactions remain a diagnostic challenge, primarily due to the lack of highly sensitive and specific assessment methods, as well as the limited range of approved test allergens. Provocation tests – considered the “gold standard” for diagnosing hypersensitivity – carry significant risks of inducing systemic reactions. Skin prick tests (SPT), although widely used, have notable limitations: low sensitivity and specificity, particularly in early childhood, and a strong dependency on ongoing therapy.

These limitations of in vivo diagnostic methods have led to the development and rapid clinical implementation of several in vitro approaches. The two flow cytometric tests for measuring basophil degranulation (BAT) and mast cell activation (MAT), reproduce in vitro the main pathogenetic mechanisms of immediate-type allergic reactions, rely on the remarkable sensitivity of modern flow cytometers with respect to “rare events” and allow the use of a wide range of standardized allergens for in vitro stimulation.

Nowadays, BAT and MAT are gaining increasing significance in the comprehensive diagnosis of IgE-mediated allergies. Based on our clinical experience, BAT offers distinct advantages in confirming drug hypersensitivity, particularly in cases where no validated alternative tests exist or where the sensitivity of skin prick testing is insufficient.

Acknowledgements: *The study was funded by the Bulgarian National Science Foundation under project KP-06-N73/6 “Signal transduction and molecular mechanisms of cell signaling in IgE-mediated activation of mast cells by various allergens”*

ATOPIC DERMATITIS: IMMUNOLOGICAL IMBALANCE OR SKIN BARRIER DEFECT – WHICH COMES FIRST?

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Atopic dermatitis (AD) is a chronic inflammatory skin disease that commonly begins in early childhood and is often associated with other atopic conditions such as asthma and allergic rhinitis. A central question in dermatological research is whether the primary cause of AD lies in immunological imbalance or in a defective skin barrier. Skin barrier defects, including filaggrin gene mutations, lead to increased transepidermal water loss and easier penetration of allergens and microbes. On the other hand, immune dysfunction – particularly the overactivation of Th2 cells and the production of interleukins such as IL-4 and IL-13 – plays a crucial role in driving inflammation. This lecture explores the complex interplay between these two mechanisms, emphasizing that neither can be considered in isolation. Understanding the chronological sequence – whether immune dysregulation precedes barrier dysfunction or vice versa – is key to early diagnosis and personalized therapy. The presentation also highlights recent therapeutic strategies aimed at restoring skin barrier integrity and modulating immune responses.

SALIVARY SPECIFIC IgE TO D1 AND G6 IN PATIENTS WITH RHINITIS WITH OR WITHOUT *S. AUREUS* COLONIZATION

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Globally, allergic rhinitis impacts roughly 25% of children and 40% of adults. Immunoglobulin E (IgE) is crucial in allergic inflammation, with two sources: spontaneously produced IgE, and IgE stemming from reactions to environmental allergens. The damaging effects of *S. aureus*, as well as Staphylococcal enterotoxins, have been proven in numerous airway illnesses. As superantigens, they generate intense Th2 inflammation, with 70-80% of IgE being locally synthesized. Our study aimed to determine if any correlation existed between local and systemic specific IgE responses in rhinitis patients – both treated and untreated. Furthermore, we sought to pinpoint significant disparities in serum allergy-specific IgE levels between *S. aureus* positive and negative patients.

Results: From 70 patients with a relevant rhinitis history spanning at least two years, we found that in our rhinitis cohort, 36 were sIgE-negative for d1 in blood samples but positive in saliva samples ($\chi^2 = 19.76$, $\alpha = 0.181$), while 25 tested negative for g6 in blood samples but positive for g6 in saliva samples ($\chi^2 = 6.89$, $\alpha = 0.86$). No significant difference emerged between serum allergy-specific IgE levels in *S. aureus* positive and negative rhinitis patients ($\chi^2 = 0.38$). Similar results were noted within the saliva samples. However, mucosal-specific IgE levels were lower among patients receiving active therapy ($p < 0.001$ for both d1 and g6).

Conclusion: There is no correlation between mucosal-specific IgE levels and systemic-specific IgE levels or *S. aureus* carriers. We observed that salivary-specific IgE levels were lower in patients undergoing active treatment compared to untreated patients.

ANALYSIS OF GUT MICROBIOME COMPOSITION IN CHILDREN WITH ASTHMA COMPARED TO HEALTHY CONTROLS

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Introduction: Asthma is a complex and multifactorial disorder, with severe public health implications. The knowledge on human gut microbiota has expanded and allowed us to understand its crucial role in the development of many diseases including asthma.

The aim of the study was to compare the composition of the human gut bacteria among allergic asthmatic children, non-allergic asthmatic children and healthy children without asthma.

Patients and methods: In the study were included 12 allergic asthmatic children, 16 non-allergic asthmatic children and 24 healthy children. Gut bacterial composition of participants' fecal samples were analyzed by 16S rRNA gene sequencing. The bioinformatics analysis of the received data was done by alpha diversity (diversity of microbes in particular group) and beta diversity (dissimilarity between microbes in the studied groups). Linear Discriminant Analysis of effect size (LEfSe) was used to discriminate the taxa with statistical and biological difference.

Results: The composition of the gut microbiota of asthma patients was reduced compare to healthy controls. Alpha diversity (Shannon index) was significantly lower in non-allergic asthma group ($p < 0.05$), also lower in allergic asthma group although not significantly, evidence of gut bacteria depletion in asthma patients. The comparison between all studied groups by beta diversity, did not showed any significant differences ($p > 0.05$). Both allergic and non-allergic asthmatics showed a higher relative abundance of *Succinivibrio*, *Ketobacter* and *Brevundimonas* bacteria belonging to Proteobacteria and lower abundance of potential beneficial bacteria such as *Sutterella*, *Odoribacter*, *Lawsonibacter* and *Faecalibacterium*.

Conclusions: The study showed alterations in the gut bacteria composition of patients with asthma compared to healthy controls.

CIRCULATING FOLLICULAR T CELLS IN ADAPTIVE IMMUNITY AFTER INFECTION AND VACCINATION

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T follicular helper (T_{fh}) cells are of key importance for the generation of protective antibodies that recognize highly specific pathogen-associated molecules and are the basis of the post-vaccinal immune response. They are key drivers of immunoglobulin isotype switching, antibody affinity maturation and the humoral direction of immunological memory. The processes in the germinal centers and in particular the relationship between T_{fh} and B cells, including their regulation, have stimulated an increased scientific and medical interest. In methodologi-

cal terms, the study of Tfh cells is challenging due to their specific localization. However, the fact that Tfh cells are positioned not only in secondary lymphoid organs, but are a dynamic population, has allowed the investigation of circulating Tfh (cTfh) cells. A positive correlation between cTfh and antibody response to various vaccines has been established. It is important how age-related changes in the immune system affect Tfh-B cell interactions, given the observations during the COVID-19 pandemic regarding the immunological basis of differences in clinical presentation between children and adults. Our studies show significant qualitative and quantitative differences in the population of circulating Tfh cells in asymptomatic to mildly ill individuals ages 2-45 years. The enthusiasm in studying Tfh cells is also justified by their potential as an immunotherapeutic approach.

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BIOMEDICAL APPLICATIONS OF RECOMBINANT ANTIBODIES

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Anti-idiotypic antibodies recognize the variable domains of immunoglobulins specific to a certain antigenic molecule. Thus, by their antigen-binding site, i.e. the conformation of their CDR regions, the anti-idiotypic antibodies structurally resemble the recognized epitopes of that same antigenic molecule. Not all anti-idiotypic antibodies will be structural analogs of antigenic epitopes, since the immunoglobulin idiotype includes the variable domains as a whole, not just the conformation of the antigen-binding site. However, it is expected that some of the anti-idiotypes are conformationally complementary to the antigenic site of the immunoglobulin, thus appearing as structural analogues of the epitope recognized by this antigenic site. By utilizing this approach we generated recombinant anti-idiotypic scFv antibodies as structural analogues to one of the lupus autoantigens, namely the Complement protein C1q. As serum protein, C1q lacks intrinsic immunogenicity and the appearance of anti-C1q antibodies is still poorly understood. We searched for the putative immunogen through structural resemblance by studying the autoepitopes of C1q. One of our anti-idiotypes, scFv A1 confirmed the presence of a cross-reactive epitope within the globular domains of C1q with anti-dsDNA autoantibodies suggesting that anti-C1q autoantibodies are a consequence rather than a trigger for the autoimmune disease. Another anti-idiotypic, clone F9, exhibited structural resemblance to another globular autoepitope of C1q with higher inhibitory capacity.

C3 is another autoantigen in lupus. In contrast to C1q, anti-C3 autoantibodies are of lower affinity and are difficult to isolate in sufficient quantity for selection of recombinant antibodies. We approached the characterization of C3 by generating anti-C3 scFv which can be used as molecular tools for localizing C3 autoepitopes.

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DYNAMICS IN POST-VACCINE ANTIBODY IMMUNE RESPONSE TO POLYSACCHARIDE ANTIGENS AND THE SUBCLASSES OF IgG

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The assessment of immune competence during different periods of individual development and the early detection of impaired immune response is the basis of the personalized vaccine program. Our **aim** is to study the post-vaccination antibody status after administration of conjugated polysaccharide vaccines and its correlation with IgG subclasses in different age groups and depending on the clinical condition of the individuals. 424 individuals were included, from five age groups, consistent with the periods of the immunization schedule: 0-4 years, 4-6 years, 6-12 years, 12-17 years, 17-50 years, and in two clinical groups: healthy and individuals with frequent infections. IgG subclasses were determined by turbidimetry (SPAplus; Binding Site, UK). Pneumococcal antibodies (PCP-IgG and PCP-IgG2) and antibodies to Haemophilus influenzae (Hib-IgG) were determined with ELISA kits (VaccZyme™, Binding Site, UK). The results show age related dynamics in the levels of IgG subclasses and specific antibodies to polysaccharide antigens. IgG1, IgG3 and IgG4 reach the highest mean serum levels between 12-17 years age (IgG1-5.93 g/L, IgG3-0.64 g/L, IgG4-0.74 g/L). The mean serum levels of IgG2 gradually increase and reach maximum values in adults over 17 years (4.48 g/L). PCP IgG antibodies reach the highest mean values between 6-12 years (96.38 mg/L), and PCP-IgG2 and Hib-IgG are highest in individuals over 17 years. We found a positive correlation between the subclasses IgG1, 2 and G3 and PCP-IgG and PCP-IgG2 antibodies. We found a lower mean level of PCP-IgG and PCP-IgG2 antibodies in sick individuals compared to healthy individuals (healthy individuals: PCP IgG-104.04 mg/L, PCP IgG2-31.86 mg/L and individuals with infections: PCP IgG-65.55 mg/L, PCP IgG2-16.55 mg/L, $p < 0.001$), while for Hib-IgG no such difference was found ($p = 0.066$).

In conclusion, our data provide information on the level of immunity to polysaccharide antigens among vaccinated individuals in Bulgaria and allows for the identification of persons suspected of having an immune deficiency. Additional investigations are needed in order to provide reliable recommendations for the national vaccine program and personalized vaccinations.

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HERPES VACCINES: CHALLENGES AND SOLUTIONS

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Herpesviridae is a large family of more than 150 dsDNA viruses that cause infections and certain diseases in animals (both vertebrate and invertebrate) and humans. Human herpesviruses (HHV) are widespread and affect a large part of the population, it is believed that most people are in-

ected with at least one of their representatives. They can cause lifelong infection, characterized by alternating periods of latency and reactivation. There are 9 known HHVs: herpes simplex virus 1 (HSV1/HHV1) and HSV2/HV2, varicella zoster virus (VZV/HHV3), Epstein-Barr virus (EBV/HHV4), cytomegalovirus (CMV/HHV5), HHV6A, HHV6B, HHV7 and Kaposi's sarcoma virus (HHV8).

Herpesviruses have a wide range of host cells, including epithelial cells, neurons, fibroblasts and leukocytes. However, different human herpesviruses and even individual strains of the same virus may exhibit a preference for certain cell types.

Herpesviruses often cause mild or asymptomatic infections, but they can lead to serious health complications, especially in immunocompromised individuals or newborns. These include encephalitis, meningitis, neonatal herpes, and disseminated herpes infection. HHVs have also been implicated in the development of autoimmune diseases, neoplasia, and neurodegenerative conditions.

To date, only one licensed, commercially available vaccine is available against varicella-zoster virus (live attenuated varicella vaccine; live attenuated and recombinant subunit herpes zoster vaccines).

The development of herpes vaccines is challenging because of the ability of these viruses to induce latent infection, their complex life cycles, and their sophisticated mechanisms for evading the immune response. Their creation is the subject of ambitious scientific efforts, using various technologies (including subunit, mRNA and DNA vaccines). Candidate vaccines (prophylactic, therapeutic) against some human herpesviruses are in various phases of clinical trials.

SHOULD WE IMMUNIZE CHILDREN WITH CHRONIC DISEASES?

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Children with chronic medical conditions represent a high-risk group for developing severe complications from infectious diseases, many of which are vaccine-preventable. In Bulgaria, both medical and organizational barriers exist in the implementation of immunization practices within this vulnerable population. Vaccination in chronically ill children is often accompanied by hesitancy and logistical challenges, stemming from both healthcare providers and caregivers. These concerns are primarily related to the perceived safety of vaccines in the context of underlying health conditions.

This report addresses the activities related to the implementation of the “Methodological Guidelines with Recommendations for Immunizations in Children with Chronic Diseases”, approved in February 2024. This document was developed in accordance with Ordinance No. 15 on Immunizations in the Republic of Bulgaria and was prepared by Bulgarian specialists. It summarizes the recommendations of leading international health organizations concerning the immunization of children with malignant, cardiovascular, rheumatologic, gastroenterological, endocrine, pulmonary, and neurological diseases, as well as children with immune deficiencies or those who have undergone organ or hematopoietic stem cell transplantation.

VACCINE PROPHYLAXIS IN PEOPLE OVER 65 YEARS OF AGE

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The World Health Organization (WHO) defines healthy aging as "the process of developing and maintaining the functional ability that enables wellbeing in older age." The proportion of older people is rapidly increasing worldwide, and WHO's focus in the period 2015-2030 is on the processes of healthy aging. With advancing age, changes occur at the systemic, cellular and molecular levels, which are a natural biological process. The immune system also ages, leading to a gradual decrease in the strength of immunity. The antibody response to polysaccharides and proteins is limited, the quality (affinity, isotype) of antibodies is limited, the antibody response to proteins is short-lived. The TCR repertoire and the induction of T-cell responses are limited, the reservoir of IgM+ B memory cells is reduced, and differentiation into plasma cells is impaired. Successful vaccination of older people is a major preventive strategy against important infectious pathogens and a growing public health priority. However, vaccination of the elderly population differs in efficacy and safety compared to that of other population categories. Our knowledge of the mechanisms of aging and how they affect the quantity and quality of the immune response to vaccines allows the design of new vaccines for the elderly that improve the effectiveness of protection against vaccine antigens.

SENOLITICS AND IMMUNITY – NEW OPPORTUNITIES

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Cellular senescence is characterized by block of cell division, morphological changes and resistance to apoptosis. Senescent cells exhibit a specific phenotype known as senescence-associated secretory phenotype (SASP). Secreted mediators support chronic inflammation, tissue degeneration and metabolic dysfunction.

Immunological senescence manifests itself in the gradual dysfunction of the immune system induced by the natural progression of age. During this process, all elements of the immune response are affected, with adaptive immunity being more severely inhibited than innate immunity. The most significant feature is the so-called Inflammaging - age-related immune dysfunction and persistent proinflammatory status.

Senolytic agents are natural substances (food senolytics) that slow, prevent or even reverse the aging process by selectively eliminating senescent cells without affecting normal ones. They induce apoptosis by activating various intracellular pathways. Senomorphic agents are synthetic substances that act as suppressors of SASP. They inhibit I κ B kinase (IKK) and nuclear factor NF- κ B, scavenge free radicals, and inhibit Janus kinase (JAK).

The lack of specificity and the side effects of senolytics still limit their widespread use. However, the established mechanisms of action in animal models of senolytic therapy reveal new opportunities to modulate chronic inflammation, immune dysfunction and oncogenesis. Clinical trials are underway with a combination of senolytic and senomorphic drugs that target T cells, metabolic pathways and signal transduction.

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II. POSTERS

SELECTION OF HIGH AFFINITY ANTI-HUMAN C3 RECOMBINANT ANTIBODIES

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Aim: C3 is the key protein in the activation of the Complement system. C3 is also a target for autoantibodies associated with Systemic Lupus Erythematosus. In order to study the molecular aspects of C3 autoantigenicity we aimed to select a high affinity anti-C3 antibodies from the "Griffin 1" phage display library expressing human scFv antibodies.

Methods: We screened the "Griffin 1" library in four rounds of selection for anti-human C3 scFv antibodies. We applied a new approach with decreasing the amount of antigen twice in every successive round. The selected anti-C3 phages were used to transfect a non-suppressor E.coli HB2151 cells followed by induction and expression of soluble monoclonal anti-C3 scFv antibodies which were analysed by ELISA for their affinity and by immunoblot for their epitope specificity against C3 and its smaller fragments C3b, C3c and C3d.

Results: Four rounds of phage selection yielded a phage suspension with a titer of 2.76×10^4 pfu/ml. Forty clones of recombinant antibodies recognized C3 molecule and five clones, designated B11, F8, F9, F10 and F11, were found to have the highest binding affinity to the tested antigen. Four clones B11, F8, F10 and F11 recognized C3, C3b and C3c, but not C3d.

Conclusion: Antibody phage display is a successful approach to overcome the low immunogenicity of evolutionary conserved proteins like C3. The selection approach with decreasing amounts of antigen resulted in high affinity monoclonal anti-C3 scFv antibodies with established epitope specificity within C3c.

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Sw71 TW-KO MODEL FOR IMPAIRED EMBRYO IMPLANTATION

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Aim: Bioconstruction of 3D blastocyst-like model for impaired embryo implantation.

Materials and methods: We genetically modified Sw71 human trophoblast cells by deletion of the Twist1 gene using the CRISPR/Cas9 method to obtain Sw71-Tw-KO trophoblast cells. The expression of HLA-G and HLA-C molecules in both type of cells was analyzed by immunocytochemistry. Sw71-TW-KO spheroids and Sw71 control spheroids were generated in parallel and their morphology and differentiation time were compared. The functionality of the Sw71-TW-KO surrogates was tested by migration and invasion assays. Migration was analyzed

in two culture media, Opti-MEM and supplemented serum-free (sf) DMEM/F12, as a percentage of the migrated spheroids and migration zone measurement. The three-dimensional invasion of Sw71-TW-KO spheroids was monitored on extracellular matrix in two media, OptiMEM and supplemented DMEM/F12 with 10% fetal calf serum.

Results: Sw71-TW-KO cells did not express the transcription factor Twist1 and showed reduced expression of HLA-G and HLA-C molecules compared to normal trophoblast cells. Sw71-TW-KO spheroids differentiated similarly to controls with comparable morphological parameters. However, they had impaired migration ability as 63% of the Sw71-TW-KO spheroids (vs 980% of the normal Sw71 spheroids) migrated in OptiMEM, showing a smaller migration zone. In sfDMEM/F12 media, only 19% of the Sw71-TW-KO spheroids (vs 94% of the controls) migrated, again with a smaller migration zone. We did not detect invasion by Sw71-TW-KO spheroids in any of the tested media, in contrast to the normal invasion of control Sw71 spheroids.

Conclusion: 3D blastocyst-like Sw71-TW-KO surrogates have a preserved capacity for differentiation, but show impaired migration and are unable to invade. These could be used as an in vitro model to study signaling pathways responsible for compromised trophoblast cell invasion.

ANALYSIS OF REGULATORY T CELLS – BETWEEN THE CANONICAL AND THE ISOFORM OF FOXP3

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Aim of the study: In response to environmental stimuli, cells undergo changes in gene expression, which can lead to the production of isoforms with similar or different characteristics from the original protein variant. This is the case with the key transcription factor for regulatory T cells, FOXP3, which is represented by the full-length canonical version (FOXP3FL), as well as by a version lacking exon 2 – Δ2. Both variants play an important role in maintaining the Treg population, but also for its proper functioning in the control of inflammatory and autoimmune reactions.

The aim of the present study was to investigate FOXP3Δ2 isoform in the population of activated Tregs, but also those at rest.

Materials and methods: PBMCs, isolated from the peripheral blood of healthy individuals, were labeled with a multicolor panel for the detection of FOXP3FL and FOXP3Δ2 within the Treg subpopulations.

Results: The data analysis showed that in both activated (CD45RA⁺FOXP3⁺) Tregs and resting (CD45RA⁺FOXP3⁺) Tregs, there can be two subsets: one that expresses both variants simultaneously and the other – only FOXP3Δ2. The presence of both isoforms is associated with elevated levels of T cell activation markers, but with different values between individual Treg subpopulations.

Conclusions: The results obtained offer a new perspective into the control of FOXP3 isoforms in Treg activation, which may lead to a better understanding of their function.

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CHALLENGES IN ESTABLISHING DIAGNOSIS OF FAMILIAL MEDITERRANEAN FEVER IN CHILDHOOD – CLINICAL CASE

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Familial Mediterranean fever is a congenital autoinflammatory disease, which is most often caused by mutations in the MEFV gene and is inherited autosomal recessive or autosomal dominant with incomplete penetrance of the gene and variable expression.

Description of the clinical case: A twelve-year-old girl with the onset of the disease after the onset of menarche with subfertility, severe fatigue, inflammatory activity, anemic syndrome and echographic evidence of pericarditis. After numerous consultations and examinations to exclude autoimmune, infectious and malignant diseases of the blood, treatment was carried out with antibiotics, intravenous and oral corticosteroids with a satisfactory clinical and laboratory effect. After stopping the corticosteroids, the symptoms and inflammatory activity recurred. Immunological studies showed no evidence of cellular and humoral immune deficiency, highly elevated CRP, ESR, Serum amyloid and cytokines (GM-CSF, IL-18, IL-6). Treatment with Colchicine was started with a good response to the clinical symptoms and laboratory indicators. A genetic study was conducted at the Clinic of Clinical Immunology with a stem cell bank at the University Hospital „Aleksandrovska“ by Real Time PCR of the MEFV gene, which revealed a pathogenic variant K695R in a heterozygous state.

Conclusion: The case is presented as an example of an autoinflammatory disease in a child with persistent high inflammatory activity and not fully developed clinical symptoms, typical of familial Mediterranean disease.

HETEROGENEOUS CLINICAL AND IMMUNOLOGICAL MANIFESTATIONS IN PATIENTS WITH TACI MUTATIONS – OUR EXPERIENCE

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Tumor necrosis factor receptor 13B, also known as transmembrane activator and calcium-modulating cyclophilin ligand interactor (TACI) plays a key role in humoral immunity, being expressed on the surface of B cells and controlling T-cell-independent antibody responses, isotype switching and mediating immunoglobulin production. TACI mutations are associated with autosomal recessive or autosomal dominant inheritance with variable expression of clinical manifestations and account for about 10% of patients with common variable immunodeficiency (CVID).

Objective: To evaluate clinical symptoms and immunological changes in patients with mutations in the TNFRSF13B gene.

Material and methods: We present eleven patients from the Bulgarian registry for primary immunodeficiencies with mutations in the TNFRSF13B gene, of which 7 children and 4 adults. The genetic analysis was performed with NGS technology in Invitae and Veritas laboratories.

Results: In 5 of the patients (45%), the mutations are pathogenic, heterozygous. In 5 patients (45%), hypogammaglobulinemia is present, but only 4 of them currently meet the criteria for CVID. In 36% (4 patients), T/NK cell deficiency is present, and one patient (9%) is without significant immunological abnormalities. Three of the children were diagnosed with an autoinflammatory disease, and three of the adults had a CVID phenotype, one of them with an autoimmune disease.

Conclusion: Patients with TACI mutations included in the PID registry have heterogeneous clinical manifestations and immunological changes. TNFRSF13B mutations rather induce a predisposition to disease, rather than being a direct etiological cause of manifestation, which supports the early diagnosis of complications in PID patients.

20 YEARS EXPERIENCE IN MANAGEMENT OF CHILDREN WITH PRIMARY IMMUNE DEFICIENCY

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Within the framework of an international project (J-Project) in the University Hospital „Alexandrovska“ in the year 2005 we developed and introduced a program for management of children with primary immune deficiencies (PID).

For a period of 20 years, we investigated the immune status of 385 children (214 boys, 171 girls) with an average age of 5.75 ± 3.847 years. Of these children in 49 no significant abnormalities or a slight disturbance of the immunobiological balance between the immune cells and other components of the immune response was described. The remaining children have some deficiency in any part of their immune system (combined or humoral immune deficiency). Patients with severe impairment of immunobiological balance and decreased T cells predominate. Some of the patients had also genetic analysis for confirmation of the diagnosis. Appropriate immune therapy (substitution with immunoglobulins and/or immunomodulators) with good effect was started in all children. Some children receive additional biologic therapy.

The efforts of pediatricians and clinical immunologists during all these years were focused on early detection of children with immune deficiencies, outlining the patient's path – from pre-hospital to specialized care and timely inclusion of specific therapy. As a result, the time from the initial symptoms to the diagnosis was shortened and appropriate health care was provided for children with PID in Bulgaria, including free access to substitution therapy.

CHURG-STRAUS THE UNKNOWN DISEASE

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Syndrome Churg-Strauss, also known as allergic granulomatosis or allergic angiitis /EGPA/ is an inflammation of small blood vessels. The most common feature of this syndrome is asthma. Syndrome itself can lead to many problems such as hay fever, rashes, bleeding in various parts of the digestive system, severe pain and arthritic symptoms of upper and lower limbs. Many symptoms and their similarity to the symptoms of other diseases, making this syndrome very difficult to find. Syndrome Churg-Strauss is rare and is not curable. To copy symptoms using various drugs, such as corticosteroids, immunosuppressant, biological drugs intravenous immunoglobulin, etc.

CHITINASE-LIKE PROTEINS AS POSSIBLE BIOMARKERS IN RHEUMATOID ARTHRITIS

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Rheumatoid arthritis (RA) is an autoimmune disease with joint deformity and disability. There are great expectations for biomarkers that would predict the response to treatment. CHI3L1 and CHI3L2 are chitinase-like proteins (CLPs) which lack enzymatic activity. CHI3L1 is expressed by a variety of cells, while reports on CHI3L2 are limited.

The **aim** of the study is to evaluate gene and protein levels of CHI3L1 and CHI3L2 before and after treatment of patients with RA and to search for correlations with ultrasonography and conventional laboratory parameters.

Twenty-four newly diagnosed RA patients were enrolled in the study. Fourteen were treated with tofacitinib (TOFA) and 10 – with methotrexate (MTX) for 24 weeks. Conventional biochemical and immunological markers were examined at the start of the treatment and after the follow-up period. The activity of RA was assessed via Disease Activity Score 28 (DAS28). Gene expression (qPCR) and protein analysis (ELISA) were performed.

Ultrasonographic and clinical laboratory parameters showed improvement after therapy in both groups. A decrease in plasma levels of CHI3L1 ($p=0.04^*$) and CHI3L2 ($p=0.03^*$) was found after treatment with TOFA. Strong correlations between CRP, GUS7 and CLPs were also established. No changes in CLPs protein levels were detected after MTX therapy. Gene expression of CLPs were not affected after treatments with both therapeutics.

The dynamics of CLPs levels in naïve RA patients and the distinct association of their serum concentrations with disease-related parameters after treatment suggest that both proteins may serve as possible biomarkers for monitoring the therapy in RA.

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CHITINASE-LIKE PROTEINS AND PRO-INFLAMMATORY CYTOKINES IN PATIENTS WITH SYSTEMIC SCLEROSIS

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Aim: Systemic sclerosis (SSc) is an autoimmune disease of the connective tissue with complex pathophysiology. The Chitinase-like proteins (CLPs) of interest are YKL-40, a pro-inflammatory glycoprotein that affects multiple processes, and YKL-39 which role in SSc is still elusive. The aim of our study was to analyze CLPs in parallel with pro-inflammatory cytokines and to evaluate their association with the inflammatory process in SSc.

Material and methods: Serum and white blood cells (WBCs) were obtained from 32 patients with SSc and 12 healthy individuals. RNA was isolated from WBCs and gene expression was assessed by qPCR. Protein levels of CLPs, IL-6, IL-12 and TNF- α were evaluated by ELISA.

Results: Our results show that serum concentrations of all molecules were elevated in SSc patients, compared to healthy controls with YKL-40 being statistically significant. Gene expression of YKL-39 was found to be increased. Positive correlations between protein levels of YKL-40/IL-6, YKL-40/IL-12, IL-6/IL-12 and mRNA expression YKL-39/IL-12 and YKL-40/TNF- α were detected.

Conclusion: The high levels of CLPs in SSc suggest that they are related to the inflammatory process. YKL-40 might have a more prominent role in the pathophysiology of the disease compared to YKL-39

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COMPARISON BETWEEN ANA SCREENING (IIF) AND ANA IMMUNOBLOT: A RETROSPECTIVE ANALYSIS IN PATIENTS WITH AUTOIMMUNE RHEUMATIC DISEASES

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Objective: Antinuclear antibodies (ANA) are a significant biomarker in the diagnosis of both systemic and some organ-specific autoimmune diseases. Two major methods used in the algo-

rithm for detecting ANA are indirect immunofluorescence (IIF) of Hep2 cells (the gold standard for initial detection of ANA) and the solid-phase method, specifically immunoblot, used to detect some of the specific ANA. This retrospective study aims to compare the diagnostic advantages and disadvantages of the two methods and to correlate them with the diagnosis, clinical presentation, and therapeutic plan of the patients.

Materials and methods: Retrospective analysis of sera of patients tested for ANA screening by IIF (ANA-HEp2, Biosystems) and ANA immunoblot (Euroline ANA Profile 3 plus DFS70 (IgG), EUROIMMUN). Data were analyzed using GraphPad Prism 8.0 and IBM SPSS Statistics 27.0.

Results: The study, conducted from April 2024 to April 2025, analyzed data from a total of 156 patients from the Rheumatology Clinic of the University Hospital “St. Ivan Rilski” in Sofia. A total of 61% of patients were positive for ANA (IIF) and 49% for ANA immunoblot. In 24 (15%) of the patients, discrepancies between the methods used were found: 4 patients with a positive ANA screening and ANA pattern AC-1 (according to ICAP) were without detection of specific antibodies from the immunoblot test, 18 patients had a positive ANA screening and AC-4 pattern, without specific autoantibodies detected from the immunoblot test; 2 patients had a positive ANA immunoblot and a negative ANA screening.

Conclusion: The discrepancies obtained were analyzed in terms of clinical symptoms, the diagnosis made and the therapeutic plan at the time of the antinuclear antibody test, which also determines the correct interpretation and supports the significance of ANA as an indicator not only for the diagnosis of autoimmune conditions but also as a marker for chronic inflammatory processes.

PRIMARY ANTIPHOSPHOLIPID SYNDROME WITH SEVERE PULMONARY VASCULAR INVOLVEMENT AND SUBSEQUENT RIGHT VENTRICULAR DECOMPENSATION: A CLINICAL CASE WITH FATAL EVOLUTION

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Antiphospholipid syndrome (APS) is a systemic autoimmune disease occurring in approximately 1–5% of the general population, characterized by arterial and venous thrombosis, obstetric complications in the presence of antiphospholipid antibodies. Pulmonary manifestations, including thromboembolism and pulmonary hypertension, are observed in over 40% of patients and are among the most severe complications.

We present a case of a 35-year-old woman with primary APS. On admission, the patient presented with hypoxemia (SpO₂ 60–65%), cyanosis, and negative T-waves in the precordial leads. Imaging studies (cardiopulmonary angiography) revealed massive thrombosis of segmental and lobar branches of the pulmonary artery, an area of infarction in the right lung, and bilateral parenchymal changes. Echocardiography revealed right ventricular dilatation, significant pulmonary hypertension, and high-grade tricuspid regurgitation. The medical history included deep venous thrombosis, spontaneous abortion, and preeclampsia.

Immunological diagnostics demonstrated the presence of anti-cardiolipin and anti-β₂-glycoprotein I antibodies with a negative antinuclear profile.

Treatment was performed with heparin, direct oral anticoagulant, corticosteroids, and antibiotics. Despite short-term clinical stabilization, the patient was readmitted with decompensated right ventricular failure, cardiogenic shock, and fatal outcome.

The case illustrates the aggressive pulmonary course of APS and emphasizes the importance of early diagnosis for timely, comprehensive treatment in patients with thromboembolic complications and an autoimmune profile.

ALTERED TH17 AND TREG SUBPOPULATIONS IN PATIENTS WITH IgA NEPHROPATHY

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Aim of study: IgA-nephropathy is an organ-specific autoimmune disease. Dysfunction of MALT plays a key role in the development of the disease. Changes in IgA synthesis and clearance are the first steps of the complex pathogenesis. The aim of our study was to evaluate differences in Th17 and Treg lymphocyte subpopulations in peripheral blood of patients with IgA-nephropathy with a possible role in the immunopathogenesis.

Materials and methods: We evaluated 19 patients with biopsy proven IgA-nephropathy who had not received corticosteroid therapy in the past two months. We measured Th17 subpopulations (CD3+CD4+CD161+CD196+) and Treg subpopulations (CD3+CD4+CD25+CD127-) through flowcytometry.

Results: Out of 19 patients, 4 have higher than 15% Th17 (our range is 10-15%), 11 patients have lower than 10% and 5 are within range. As for Tregs, 3 patients have higher than 10% (our range is 5-10%), 4 patients have lower than 5% and 12 patients are within range.

Conclusion: Changes in Th17 and Treg subpopulations may be useful in elucidating the intimate mechanisms involved in the pathogenesis of IgA nephropathy, but further data are needed.

HUMORAL AND MEMORY B CELL RESPONSES FOLLOWING SARS-CoV-2 INFECTION AND mRNA VACCINATION

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Aim: To evaluate the dynamics and duration of the humoral and B cell immune memory responses following natural SARS-CoV-2 infection or vaccination with the mRNA vaccines BNT162b2 (Pfizer) and mRNA-1273 (Moderna).

Materials and methods: The study included 285 participants divided into three groups: individuals who had recovered from COVID-19, those vaccinated with BNT162b2, and those vaccinated with mRNA-1273. Participants were assessed at four time points: T0 (baseline for vaccinated individuals), T1 (1–2 months), T2 (6–7 months), and T3 (12–13 months), either post symptoms onset or after the second vaccine dose. Serological tests were performed to determine SARS-CoV-2-specific RBD-IgG using ELFA (Enzyme-Linked Fluorescent Assay) and virus-neutralizing antibodies by ELISA. Enzyme-linked Immunosorbent Spot (ELISpot) was conducted to assess specific antibody-secreting cells, and multiparameter flow cytometry was used to evaluate B cell subpopulations, including S1-specific memory B cells.

Results: Both mRNA vaccines induced a stronger humoral and B cellular response compared to that following natural infection. Antibody titers and the frequency of memory B cells peaked around T2, then declined by T3 but remained significantly above baseline levels. The mRNA-1273 vaccine elicited a more robust and sustained immune response than BNT162b2, likely due to the higher mRNA dose and longer interval between doses. Vaccinated individuals exhibited significantly higher levels of class-switched memory B cells, including S1-specific cells. Convalescent individuals developed immune memory with greater heterogeneity.

Conclusion: The results suggest that mRNA vaccination provides a more durable immune response than natural infection, particularly in terms of memory B cell generation.

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EPSTEIN-BARR VIRUS REACTIVATION IN AUTOIMMUNE THYROIDITIS AND CHRONIC FATIGUE SYNDROME IN PATIENTS FROM THE BULGARIAN POPULATION

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Autoimmune thyroid diseases are the most common autoimmune conditions, affecting approximately 2% of the female population and 0.2% of the male population during adulthood. Viral infections are known to play a role in the triggering and pathogenesis of autoimmune reactions. Epstein-Barr virus (EBV) remains latent after primary infection and can undergo lytic reactivation. Such reactivation is related to a number of conditions associated with immune depletion, such as chronic fatigue syndrome (CFS). We aimed to investigate the relationship between EBV lytic activity and the presence of autoimmune thyroiditis (AIT), as well as the manifestation of CFS. We studied 113 individuals: 41 healthy controls (10 males and 31 females; mean age 36.3 years (SD-2.55), 49 patients with AIT (4 males and 45 females; mean age 45.7 years (SD-1.64)) and 23 patients with CFS (6 males and 17 females; mean age 42.2 years (SD-2.44)). We used RT-PCR to quantitatively measure EBV in buccal mucosa and saliva samples. The mean EBV loads in the separate groups were as follows: 69,977 copies/ml in healthy controls (0-2,786,498 copies/ml); 401,418 copies/ml in patients with AIT (0-10,381,800 copies/ml); 2,318,922 copies/ml for patients with CFS (0-38,827 324 copies/ml). The results revealed a statistically significant difference as follows: patients with AIT had a considerably higher mean load compared to healthy controls ($p=0.002$). Patients with CFS also had a statistically significantly higher mean load compared to healthy controls ($p=0.003$). A comparison between the two patient groups

did not show significant differences. In conclusion: High-level EBV reactivation is present in individuals with AIT and CFS and probably plays an important role in the pathogenesis of these two conditions.

INFLAMMATORY MARKERS (NLR AND PLR) IN CANCER PATIENTS FOLLOWING COVID-19 INFECTION: A SINGLE-CENTER RETROSPECTIVE ANALYSIS

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Background: Cancer patients are at increased risk of complications from COVID-19. Inflammatory indices such as the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are established prognostic markers in both oncology and infectious diseases. This study aims to assess the dynamics of NLR and PLR before and after COVID-19 infection in patients with solid tumors and to explore associations with vaccination, disease stage, and clinical outcomes.

Methods: We retrospectively analyzed data from 83 patients with solid malignancies and confirmed SARS-CoV-2 infection. Information on tumor type, stage, vaccination status, hospitalization, and blood parameters before and after infection was collected. Paired t-tests, Wilcoxon signed-rank tests, regression models, and cluster analysis were performed.

Results: No statistically significant changes in NLR or PLR were observed after COVID-19. Vaccination emerged as the only significant factor independently associated with lower post-COVID NLR levels ($\beta = -0.97$, $p < 0.05$). Neither tumor stage nor hospitalization correlated with inflammatory changes. Cluster analysis identified five distinct inflammatory phenotypes with moderate internal consistency.

Conclusion: Inflammatory markers NLR and PLR did not show universal elevation after COVID-19 in cancer patients, but vaccination was significantly associated with more favorable post-infection profiles. While these indices are validated in breast, colorectal, and lung cancer, their prognostic role was not confirmed in our heterogeneous metastatic cohort. This may reflect the distinct immunologic effects of SARS-CoV-2 in advanced oncologic settings. Nonetheless, blood-based indices – especially when combined with clustering – remain a promising avenue for patient stratification.

IN SITU EXPRESSION OF PROINFLAMMATORY CHEMOKINE RECEPTOR CXCR3 DURING NORMAL AND PATHOLOGIC PREGNANCY

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Aim of the study: The mild proinflammatory microenvironment in the uterus is necessary for the success of embryo implantation and early human pregnancy, but the excessive inflammation

could provoke complications. In this study we aimed to investigate the *in situ* expression of the proinflammatory chemokine receptor CXCR3 in gestational tissues from normal and pathological pregnancies as well as in luteal phase non-pregnant endometrium.

Materials and methods: Gestational tissues from women with missed abortion, in healthy early and full-term pregnancy and non-pregnant endometrial tissue were examined. The CXCR3 receptor was visualized by immunohistochemical staining.

Results: We found CXCR3 expression in the decidua and chorionic villi of early and full-term pregnant women. The signal intensity, however, decreased with advancement of pregnancy. In the decidua, we found a reaction for CXCR3 on decidual stromal cells, immune cells, extravillous trophoblast cells, as well as in the uterine glands. The syncytio- and cytotrophoblast of the chorionic villi were positive for CXCR3 as well. In contrast to normal pregnancy, at the maternal-fetal interface in missed abortions, we found a much stronger expression of CXCR3. The receptive luteal phase endometrium showed CXCR3 intensity comparable to that of the decidual tissue in normal pregnancy, showing no effect of embryo presence.

Conclusion: The chemokine receptor CXCR3 plays an important role in the formation of the pro-inflammatory immune microenvironment during peri-implantation and early pregnancy. Its intense expression in missed abortions corresponds to the strong immune cell infiltration accompanying the excessive inflammation.

DETECTION OF MEASURABLE RESIDUAL DISEASE IN PEDIATRIC B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA: DIAGNOSTIC SIGNIFICANCE OF NEW IMMUNOPHENOTYPIC MARKERS

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Introduction: The assessment of measurable residual disease (MRD) in pediatric acute lymphoblastic leukemia (ALL) is crucial for predicting relapse and guiding treatment. Multiparameter flow cytometry (MFC) improves detection but faces limitations due to insufficient leukemia-associated immunophenotypes (LAIPs) and antigen modulation.

Aims: The present study investigates new markers to improve MFC-based MRD detection in B-cell precursor ALL (BCP-ALL).

Methods: Expression patterns of the following markers were investigated: CD22, CD34, CD38, CD44, CD304, CD73, CD86, CD123, CD99, CD58 in 143 samples with leukemic blast cells from sixty-one pediatric patients with BCP-ALL and in hematogones from 20 non-leukemic bone marrow (BM) samples using fourteen-color MFC.

Results: The highest relative frequencies of LAIPs, counting for 82.50%, were reported for CD99 and CD58, followed by CD44 (81.10%), CD73 (76.20%), CD22 (73.40%), CD304 and CD86 (68.50%), while the lowest relative frequency was reported for CD123 (44.40%). The differential expression of CD58, CD304, and CD73 in diagnostic samples was highly signifi-

cant ($P < 0.01$) between pre-B-I, pre-B-II, immature B-cells, and BCP-ALL blast cells. In MRD-positive samples, CD73 showed significantly high ($P < 0.01$) differential expression between all stages of hematogones and residual blast cells, followed by CD304, CD58, and CD22.

Conclusion: CD58, CD73, and CD304 were identified as the most reliable among the tested markers for distinguishing both diagnostic and MRD blast cells from normal B-cell precursors.

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IMMUNOTHERAPY IN PLASMA-CELL DISCRASIAS – ESTABLISHED AND NOVEL TREATMENTS – A SINGLE CENTRE EXPERIENCE

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Introduction: Plasma cell dyscrasias are clonal neoplasms that comprise approximately 10% of all hematological malignancies. Despite significant therapeutic advances these diseases remain incurable. Immunotherapy occupies a key place in modern therapeutic algorithms, especially in patients with relapsed and/or refractory disease. The last decade has seen a significant extension of progression-free survival, thanks to innovative approaches, including monoclonal antibodies, bispecific T-cell-engaging antibodies (BiTEs), and CAR-T cell therapy.

Objective of the study: Analysis of established and innovative immunotherapeutic strategies in patients with multiple myeloma and light chain amyloidosis for Multiple Myeloma and Light Chain Amyloidosis for the period 2019-2024, in the SHATHD, Sofia, Bulgaria.

Materials and methods: Evaluation of induction, consolidation and maintenance regimens including immune-mediated therapies in patients with multiple myeloma and light chain amyloid during a 5-year follow-up period (2019-2024, SHATHD, Sofia).

Conclusion: The modern therapeutic paradigm includes induction with triple and quadruple regimens combining immunomodulators (ImiDs), proteasome inhibitors (PI), monoclonal anti-CD38 antibodies and alkylating agents, followed by autologous transplantation and maintenance therapy as a standard of care based on our experience. Bispecific antibodies (targeting BCMA and GPRC5D) and CAR-T therapies have demonstrated high efficacy and are gradually being integrated into earlier stages of treatment, transforming the established approach to these heterogeneous diseases.

RESULTS OF APPLICATION OF IMMUNOTHERAPY IN PATIENTS WITH CHOLANGIOCELLULAR CARCINOMA

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Aim: To explore the effect of the application of immunotherapy alongside standard chemotherapy in patients with locally advanced and metastatic cholangiocellular adenocarcinoma (CCA) based on conducted following imaging studies, determining the best achieved response as well as the conversion of the disease from unresectable to resectable.

Materials and methods: Patients with unresectable CCA (intra- and extrahepatic) received immunotherapy treatment from January 2024 to May 2025. Patients were monitored with imaging studies (CT, PET/CT, MRI) after undergoing between 2 and 4 treatment cycles, then after 6th-8th cycle (for those who reached that time point), and at 3-6 months thereafter.

Results: The study included 6 patients who underwent at least one control imaging examination. Three of the patients had intrahepatic CCA, while the other three had extrahepatic CCA. In all patients, the first response to the disease was stable disease or partial remission. The best response achieved by four of the patients is a partial response (a reduction in the volume of target lesions by at least 30%), while two had stable disease (no progression or partial remission). The duration of the first line of treatment varied from 3 to 17 months, with two of the patients having progressive disease – one after 13 months of progression-free period and one after five months. One of the patients underwent radical surgical treatment.

Conclusion: Immunotherapy is a new but promising option for patients with unresectable CCA. The introduction of this group of drugs has led to an increase in overall survival and durable responses, becoming a bridge to radical surgical treatment.

BASELINE EOSINOPHILS PREDICT THE ONSET OF IMMUNE-RELATED ADVERSE EVENTS

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Introduction: Immunotherapy has changed the prognosis of metastatic non-small cell lung cancer (mNSCLC). Baseline eosinophil levels are being investigated as a predictive biomarker for immunotherapy efficacy and for the occurrence of immune-related adverse events (irAEs).

Methods: A retrospective analysis was conducted on patients with mNSCLC treated with immunotherapy at the Department of Medical Oncology, Military Medical Academy, Sofia, between May 2016 and May 2025. Patients received immunotherapy alone or in combination with chemotherapy as first, second, or subsequent lines of treatment. Three patient groups were defined for the purposes of the study: 1) No observed irAEs (28 patients), 2) Mild to moderate irAEs, grades 1-2 (15 patients), 3) Severe irAEs, grades 3-5 (9 patients). Eosinophil levels were categorized as low (<130 cells/ μL) and high (≥ 130 cells/ μL), with the threshold of 130 cells/ μL chosen based on literature data. The Kruskal-Wallis test was used with $p < 0.05$.

Results: Of the 52 patients included in the analysis, 53.8% had no irAEs, 28.8% (15 patients) developed mild to moderate irAEs (grades 1-2), and 17.3% (9 patients) experienced severe irAEs (grades 3-5). The results showed a statistically significant difference in baseline eosinophil levels between the three patient groups ($p = 0.026$). Patients with high baseline eosinophil counts (≥ 130 cells/ μL) had a higher likelihood of developing irAEs. Comparison of the No irAEs vs Grade 1-2 and Grade 3-5 groups revealed statistically significant differences ($p = 0.034$, $p = 0.019$, respectively).

Conclusion: A high baseline eosinophil count before initiating immunotherapy in patients with mNSCLC is associated with a higher frequency of irAEs of all grades.

PROOF OF T-LYMPHOCYTE ACTIVATION AFTER TREATMENT WITH CHECKPOINT INHIBITORS – MISSION POSSIBLE

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Activation of naive T-lymphocytes requires two different signals: the first from the interaction of the T-cell receptor with the antigen-MHC complex on antigen-presenting cells and the second - a costimulatory signal provided by the interactions of molecules on antigen-presenting cells with their receptors on T-cells (the B7/CD28 interaction).

However, given the complexity of the immune system and the need for strict control of its work, coinhibitory molecules – CTLA4, PD1, PDL1, PDL2 – also participate in the regulation of the above interaction. They prevent severe immune inflammation and prevent the manifestations of autoimmunity. But they can be the cause of the survival of tumors. And that is why modern immunotherapy – checkpoint inhibitors – is directed against them.

When suppression is inhibited, activation of T lymphocytes follows. And it can be proven with their activation markers – CD69 (earliest) CD25 (intermediate) HLA DR (latest). Flow cytometrically they can be proven on CD3 lymphocytes. What happens to activated T lymphocytes? They enter a state called blast transformation – they acquire characteristics of younger forms and begin to divide intensively. Usually, about 10% of lymphocytes are in a state of blast transformation. In cancer patients, especially in those undergoing treatment with cytostatics, the percentage of dividing cells should be low. And it should increase after treatment with checkpoint inhibitors.

A conclusion about the proliferation of T lymphocytes is made by using radioactively labeled thymidine to determine cell growth – by quantitative determination of newly synthesized DNA.

HISTOLOGICAL FINDINGS OF COMPLETE PATHOLOGIC RESPONSE TO PEMBROLIZUMAB IN PATIENTS WITH METASTATIC COLORECTAL CANCER

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Aim: To describe the histologic result of surgical specimen after treatment with Pembrolizumab in metastatic colorectal cancer (mCRC) with microsatellite instability (MSI) and to compare it to pretherapeutic pathomorphological material.

Materials and methods: Two patients were treated with Pembrolizumab 200 mg fixed dose as first line therapy for mCRC with MSI after oxaliplatin- and fluoropyrimidine-based adjuvant treatment. Application of immunotherapy was started in August 2022 and May 2023 respectively and continues as of June 2025 and after the surgical removal of metastatic lesions.

Results: In the case of the first patient after disease free survival (DFS) of 34 months a retroperitoneal recurrence and a solitary hepatic metastasis were found and were surgically removed after 11 cycles of Pembrolizumab. In the second patient after DFS of 17 months progression of disease was diagnosed with PET/CT data for appearance of two paraaortic lymph nodes with increased metabolic activity which were resected after 14 cycles of Pembrolizumab. Postoperative histological report in both patients demonstrates “soft tissues with acellular mucinous nests

with “cell shadows” without residual tumour tissue” in contrast with primary pathomorphological result moderately differentiated colon carcinoma.

Conclusion: Introduction of immunotherapy is a therapeutic opportunity in a limited number of patients with mCRC and MSI. Due to the lack of standards for therapeutical approach in resected metastatic disease for these patients only few clinical cases are present for surgically removed metastases after treatment with immunotherapy and, respectively, the histological findings of complete pathological response.

LIFETHREATENING AUTOINFLAMMATORY HEMORRHAGIC PERICARDITIS IN MYELOFIBROSIS: DIAGNOSTICS AND SUCCESSFUL TREATMENT WITH COLCHICIN AND JAK2-INHIBITOR

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Myelofibrosis is an oncohematological myeloproliferative disease, caused by activating somatic genetic variants in JAK2, CALR or NPM1 genes, occurring in hematopoietic stem cells.

Description of clinical case: A 63 years old male is with established diagnosis of primary myelofibrosis according to WHO 2016 criteria since April 2025. Two months later the patient is hospitalized with febrility and during hospitalization increasing leukocytosis and progressive increase in CRP was established. A therapy regimen with wide specrum antibioics was established with slight temporary clinical improvement for several days, after which a progressive deterioration with febrility, strong fatigue, lowering physical capacity, progressive leukocytosis, inflammatory activity and echocardiographic signs of pericarditis. From the additional cousulting and laboratory tests there was no evidence of autommune or infectious diseases, with establishment of hemorrhagic pericardial effusion with mature neutrophil granulocytes with concentration $\sim 1 \times 10^6/L$ and activated phenotype (low expression of CD14 and expression of CD64), level of IL-6 in the pericardial effusion $\sim 55\,000\text{ ng/mL}$ and level in serum $\sim 5\text{ ng/mL}$, leukocytosis $23 \times 10^6/L$, serum CRP 133 mg/L and serum procalcitonin 2 ng/mL . After starting treatment with colchicine and third generation JAK2 inhibitor (fedratinib) full clinical remission was established for a period of 6 days.

Conclusion: The clinical case is presented as an example of a very rare life-threatening autoinflammatory condition in myelofibrosis, a disease where the usual autoinflammatory component is usually underestimated.

CORRELATION BETWEEN VIRTUAL CROSSMATCH, FLOW CYTOMETRIC CROSSMATCH AND DONOR-SPECIFIC ALLOANTIBODIES IN PRETRANSPLANT EVALUATION FOR HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Donor-specific HLA antibodies (DSAs) are a significant risk factor for graft rejection after HLA-mismatched hematopoietic stem cell transplantation (HSCT). The results of HLA antibody status – presence of DSAs and flow cytometric crossmatch (FCXM) play an increasingly important role in donor selection.

The **aim** of this study was to evaluate the correlation between virtual XM (VXM), physical FCXM (PXM) and DSAs for assessing alloimmune reactivity before HSCT.

Material and methods. Standard (donor lymphocytes with recipient serum), reverse (patient lymphocytes with donor serum) and auto-FCXM were performed. HLA antibodies specificities were determined using Luminex technology (Single Antigen Beads Assay – SAB) and accepted as positive at MFI (mean fluorescence intensity) >1500. VXM was determined based on the donor HLA and recipient DSAs.

Results. Out of 340 PXM, 267 were negative and 73 were positive. Comparing positive PXM with virtual ones, a concordance was observed in 28 samples (38.4%). In 45 (71.6%) of the samples, PXM was positive, without detected DSAs. In the negative PXMs the concordance was 90.6% (242 VXM vs. 267 PXM). In 25 (9.4%) samples the negative PXM did not correspond to the positive VXM. DSAs were mainly against HLA class II (n=17), with MFI <5000.

Conclusion. The correlation between VXM and physical FCXM depends on the MFI value and the locus specificity of DSAs. VXM could be taken into consideration in the process of donor search. The decision to transplant should be based on DSAs and a physical FCXM in order an individual risk evaluation for a specific recipient/donor combination.

IMMUNE RECONSTITUTION AFTER ALLOGENEIC STEM CELL TRANSPLANTATION – SINGLE-CENTER EXPERIENCE, VARNA, BULGARIA

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Aim: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a potentially curative treatment for hematological malignancies. To assess the dynamics of immune reconstitution following allogeneic hematopoietic stem cell transplantation and to evaluate the impact of diagnosis, donor characteristics, conditioning regimens, and transplant-related complications on lymphocyte subset recovery.

Methods: We retrospectively analyzed 89 patients who underwent allo-HSCT between 2017–2023. Immune reconstitution was assessed by measuring absolute lymphocyte count (ALC) and specific subsets (CD3+CD4+, CD3+CD8+, NK, and CD19+ cells) on days +100 and +180. Associations with clinical variables were evaluated.

Results: The most common diagnosis was AML (60.6%), followed by ALL (18%) and NHL (5.6%). Activated CD3+CD8+ HLA-DR+CD38+ T cells were significantly higher in AML vs. ALL and NHL on day +100 ($p=0.028$), as were CD19+ B cells ($p=0.021$). Related donors were associated with superior CD3+CD4+ and CD19+ cell counts compared to unrelated and haploidentical donors ($p=0.001$ and $p=0.003$, respectively). Male donor sex and myeloablative regimens (BuCyATG, TBF-ATG) were linked to improved recovery. In contrast, complications such as GVHD, infections, relapse, and non-infectious events correlated with lower immune cell levels, particularly on day +100. Patients with CD3+CD4+ $<375/\mu\text{L}$ had significantly lower 3-year survival (12.9% vs. 69%, $p<0.001$).

Conclusions: Immune reconstitution after allo-HSCT is significantly modulated by patient and transplant characteristics. Early CD3+CD4+ and CD8+ T-cell recovery strongly predicts survival, underscoring the importance of individualized immune monitoring and optimization strategies to improve outcomes in transplanted patients.

COMPREHENSIVE ANALYSIS OF MONOCLONAL ANTIBODY POLYREACTIVITY USING ANTIBODY REPERTOIRE (IgOme[®]) GRAPHS

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Antibody repertoire description at the system level can be approached in two ways: high-throughput B cell receptor sequencing and high-throughput binding experiments using phage display random peptide libraries (IgOme) or antigen arrays. The latter offer insights into the specificities of mixtures of antibodies (e.g., in serum) where the reactivities of individual clones are convoluted. To understand such a complex image of the repertoire, we first need to understand the IgOmcs of single antibodies.

Using IgOme data from Ashkenazy et al. (J. Mol. Biol. 433, 167071 (2021)), we analyzed the structure of the repertoire of four different monoclonal antibodies: three anti-Gp120 HIV antibodies and Herceptin. We constructed graphs where nodes represent 7-mer peptide mimotopes, and edges indicate shared subsequences of at least five amino acids. With the help of spectral embedding and the density-based spatial clustering of applications with noise (DBSCAN) algorithm, we were able to extract the specificity spectra of the antibodies and build motifs, which were then compared to the epitopes of each antibody.

Our analysis revealed that each antibody has affinity to 16 to 45 different mimotope groups, representing distinct specificities. The specificities of each monoclonal appear clustered in the sequence space. Lastly, the comparison of the motifs to the known epitopes indicated that the specificity to the epitope is spread across one to four mimotope clusters.

Our approach provides a framework for dissecting the complete range of antibody specificities of single clones, helping to understand how they shape the human antibody repertoire.

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