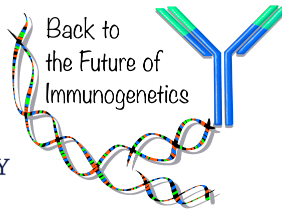
16th East–West Immunogenetics Conference

Final Programme and Abstract Book

25 – 27 February 2024
Van Swieten Saal, Van-Swieten-Gasse 1, 1090 Vienna, Austria

MEDICAL UNIVERSITY OF VIENNA



Welcome

Dear colleagues,

Welcome to the 16th East–West Immunogenetics Conference here in Vienna! It's an honour to have you join us, with participants from around the world, and share insights into the latest developments in our field.

Our programme offers a rich mix of expert lectures and ample networking opportunities during the breaks and evening events. We encourage you to take advantage of these valuable interactions to foster collaboration and advance our field.

The importance of immunogenetics can hardly be overstated. This conference provides an important platform for us to expand our collective knowledge and address new challenges within this realm.

We wish you an informative and inspiring meeting!

Yours sincerely,

Gottfried Fischer and Antonij Slavčev

Where & When, What else?

Van Swieten Saal
Van-Swieten-Gasse 1
A-1090 Wien
February 25-27, 2024

FEBRUARY 2024

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
28	29	30	31	1	2	3
4	5	6	7	8	9	10
11	12	13	14	15	16	17
18	19	20	21	22	23	24
25	26	27	28	29	1	2
3	4	5	6	7	8	9

Workshops
Keynote Lecture
Solid Organ Transplantation
Stem Cell Transplantation
Immunogenetics
Abstracts & Posters

Networking Event

26th February 2024
Heurigen s'Pfiff Rathstraße 4
1190 Wien

To get to the Heuriger s'Pfiff, take tram line 38 from Schottentor to the Hardtgasse station. Then, switch to bus line 35A towards Salmannsdorf, and get off at the Agnesgasse station in Vienna Neustift. From there, it's a 140 m walk to your destination.

Continuous Education

The programme of this event has been approved by the EFI Education Committee. For those wishing to record this event for personal CME-CPD, 1 EFI Educational Credit is awarded per hour of attendance at the event.
Submitted to the Austrian Medical Chamber for 27 continuing education credits

Organising Committee

Gottfried Fischer
Antonij Slavčev
Ingrid Faé
Sabine Wenda
Katarzyna Maria Bogunia-Kubik
Blanka Vidan-Jeras
Zorana Grubić

Scientific Programme

Day 1 25th February 2024

Time	Activity
07:30–20:00	Registration
	Preconference Workshops
09:00–12:00	GenDX Workshop hands-on NGS-Turbo: High-res HLA-sequencing in 3 hours Guest Speakers: *Milena Vraná* (Institute of Hematology and Blood Transfusion, Prague) and *Martin Danzer (Blutspendezentale Linz, Linz)*
12:00–13:00	Break There will be food and drinks.
13:00–16:00	Antibody screening and identification *Robert Liwski* (Dalhousie University, Halifax)
	Opening Chairs: Ann Margaret Little and Antonij Slavčev
17:00–17:15	Welcome *Gottfried Fischer* (MedUni Wien, Vienna) *Antonij Slavčev* (IKEM, Prague)
17:15–18:15	*Marcelo Fernández-Viña* (Stanford University, Stanford) **Update on the new WHO Nomenclature Committee for HLA factors**

Welcome Reception

18:15–20:00	There will be food and drinks.

Day 2 26th February 2024

Time	Activity
	Organ Transplantation Chairs: Ann-Margaret Little and Antonij Slavčev
09:00–09:30	*David Lowe* (Thermo Fisher Scientific, Waltham) **What does the CDC crossmatch tell us?**
09:30–10:00	*Ann-Margaret Little* (NHS Greater Glasgow and Clyde, Glasgow) **The UK approach to organ allocation and compatibility testing**
10:00–10:30	*Rainer Blasczyk* (Medizinische Hochschule Hannover, Hannover) **Next-Gen Transplants overcoming allorecognition**
10:30–11:00	*Rainer Oberbauer* (MedUni Wien, Vienna) **Non-HLA alloimmunity**
	Break/Poster Viewing
11:00–11:30	There will be coffee and fruits.
	Lunch Symposia
11:30–12:15	Lunch Symposium Thermo Fisher/BmT/Biomedica Chair: David Lowe *Maja Swoboda* **New Post-Transplant Monitoring Solutions – Accept cfDNA assay** *Mariusz Gronkowski* **One Lambda MagSort™**

12:15–13:00 Lunch Symposium Werfen, formerly Immucor
Christine Heylen
The Way to Future
Katerina Jaklova
Comparison of HLA-specific antibody analysis results with supplier A (Immucor) and supplier B

Lunch

13:00–14:00 Food and drinks will be provided.

Stem Cell Transplantation

Chairs: Marco Andreani and
Esteban Arrieta Bolaños

14:00–14:30 *Esteban Arrieta Bolaños* (Universitätsklinikum Essen, Essen)
Immunopeptidomics: biological and clinical roles in alloreactivity and HCT

14:30–15:00 *Marco Andreani* (Ospedale Pediatrico Bambino Gesù, Rome)
Role of human leukocyte antigen evolutionary divergence after allogeneic hematopoietic stem cell transplantation from unrelated and haploidentical donors

15:00–15:30 *Milena Ivanova* (University Hospital Alexandrovska, Sofia)
Role of NKG2D ligands MICA and MICB in haematological malignancies and HSCT

15:30–16:00 *Antonia Müller* (MedUni Wien, Vienna)
Donor selection in the era of haploidentical hematopoietic cell transplantations

Break

16:00–16:15	There will be coffee and fruits.

Abstracts

Chairs: Ronit Pasvolsky-Gutman and František Mrázek

16:15–16:35	A.Hofmanová et al. (Prague, Czech Republic) *Rapid HLA typing by Nanopore sequencing for use in stem cell transplantations*
16:35–16:55	J.Novak et al. (Warsaw, Poland) *Analysis of the effect of inhibitory KIRs and their cognate HLA ligands on tumor progression-free survival after allogeneic hematopoietic stem cell transplantation*
16:55–17:15	A.Witkowska et al. (Warsaw, Poland) *Analysis of NK cell reconstitution and the occurrence of aGvHD in ALL patients*
17:15–17:35	K. Štingl Janković et al. (Zagreb, Croatia) *The distribution of risk associated HLA heterodimers among siblings of patients with coeliac disease*
17:35–17:55	M. Kirijas et al. (Skopje, North Macedonia) *Correlation of HLA-DRB1 shared epitope with response to methotrexate treatment in patients with rheumatoid arthritis*
17:55–18:00	Summary

Networking Event, @ s'Pfiff

19:30–23:00	There will be food and drinks.

Day 3 27th February 2024

Time	Activity
	Immunogenetics Chairs: Milena Vraná and Alicia Sanchez-Mazas
09:00–09:30	*Ronald Bontrop* (Biomedical Primate Research Centre, Rijswijk) **Evolution of the KIR region in primates**
09:30–10:00	*František Mrázek* (University Hospital Olomouc, Olomouc) **Current status of disease associations with the HLA system in clinical diagnostics**
10:00–10:30	*Alicia Sanchez-Mazas* (University of Geneva, Geneva) **HLA molecular variation: walking across the fields to understand diversity**
10:30–11:00	*José Manuel Nunes* (University of Geneva, Geneva) **Making sense of bioinformatics tools**
	Break
11:00–11:30	There will be coffee and fruits.
	Lunch Symposia
11:30–12:15	Lunch Symposium BAG Chair: Waldemar Böhm *David Elsnic* (BAG, Lich) **Detection of DSA in patients with humoral rejection after kidney transplantation using the Histo Spot™HLA AB System**

12:15–13:00	Lunch Symposium Omixon *Libor Kolesar* **NanoTYPE and EFI standards**

Lunch

13:00–14:00	Food and drinks will be provided.

Abstracts

Chairs: Milena Ivanova and Katarzyna Bogunia-Kubik

14:00–14:20	L. Jukić et al. (Zagreb, Croatia) *Case report: persistent HLA class II antibodies after liver re-transplantation*
14:20–14:40	P. Łacina et al. (Wroclaw, Poland) *HLA eplet mismatch and incidence of graft-versus-host disease after allogeneic haematopoietic stem cell transplantation – preliminary study*
14:40–15:00	D. Koren et al. (Vienna, Austria) *Antibody Screening Tests and a priori Immunisation in Lung Transplantation*

EFI Matters

Chairs: Blanka Vidan-Jeras and Zorana Grubić

15:00–15:10	Z. Grubić (Zagreb, Croatia) *News in Accreditation regions 5a*
15:10–15:20	I. Faé (Vienna, Austria) *News in Accreditation regions 5b*
15:20–15:30	M. Dutescu (Bucharest, Romania) *News in Accreditation regions 8*
15:30–15:40	B. Vidan-Jeras (Ljubljana, Slovenia) *Accreditation in Europe*

15:40–15:50	K. Bogunia-Kubik (Wroclaw, Poland) *HLA Proficiency Testing for Central and East Europe – the results of the 30th trial (2023)*
15:50–16:00	M. Vraná (Prague, Czech Republic) *Results Of External Proficiency Testing „Detection of HLA Alleles Associated with Diseases“*
16:00–16:10	G. Fischer (Vienna, Austria) *Results of the CET EPT exercise 2023*

Closure

16:10–16:15	Gottfried Fischer and Antonij Slavčev

Back to
the Future of
Immunogenetics

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Sponsors

We thank our sponsors!

We sincerely thank our sponsors for their generous support of this event. Please take the time to visit their booths and learn more about their valuable contributions to our field.

Abstracts

O01 Rapid HLA typing by Nanopore sequencing for use in stem cell transplantations

Adéla Hofmanová, Marie Kroutilová, Milena Vraná
Institute of Hematology and Blood Transfusion, Prague, Czech Republic

High resolution HLA genotyping is essential for the selection of the best matching donors for hematopoietic stem cell transplantations. In some urgent cases, rapid typing is required. However, most of the quick typing methods generate only low resolution results. Nanopore sequencing, the 3rd generation method, has changed the field, providing high resolution typing in a short time-frame when compared to conventional NGS methods. Moreover, there is now an option for a very quick and easy library preparation. Until now we have typed 11 unique samples for HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DPB1, HLA-DQA1, HLA-DQB1 loci using NGS-Turbo® kit from GenDX company (Netherlands) in combination with Ligation Sequencing Kit V14 from Oxford Nanopore Technologies (United Kingdom). We used DNA isolated from peripheral blood by different techniques. We were facing an issue with alleles drop-out in the past, so we included some problematic samples to the current study. Most of our samples were diluted with ultra-pure water, but we also typed one sample, which already had the target concentration to see, if the dilution could have some effect. From DNA to the result, it took about 3 hours in average for one sample depending on sequencing time. The library preparation protocol for this kit is very quick and user-friendly compared to other NGS methods, although it is not possible to prepare more than one sample at a time so far. According to quantity of passed reads, each run can be stopped manually and that enabled us to experience various sequencing times. We have seen the lower quality of DNA correlated with lower quality of the result due to a higher noise rate, but the correctness of the result was not affected. Moreover, we have not encountered any false homozygous result caused by allele drop-out. In summary, our results indicate that NGS-Turbo® kit offers a great option for HLA HR typing

mainly of single statim samples due to its speed, reliability and simplicity.

O02 Analysis of the effect of inhibitory KIRs and their cognate HLA ligands on tumor progression-free survival after allogeneic hematopoietic stem cell transplantation

Jacek Nowak,[1] Agnieszka Witkowska,[1] Agnieszka Malinowska,[1] Elżbieta Graczyk-Pol,[1] Klaudia Nestorowicz-Kałużna,[2] Anna Flaga,[1] Urszula Szlendak,[1] Anna Wnorowska,[1] Aleksandra Wawiórko,[1] Agnieszka Gawron[1]

[1] *Department of Immunogenetics, Institute of Hematology and Transfusion Medicine, Warsaw, Poland*
[2] *Organization and Coordination Center for Transplantation "POLTRANSPLANT", Warsaw, Poland*

Transplantation of allogeneic hematopoietic stem cells of different HLA and KIR constellations can influence the activation/inhibition or education/resetting states of NK cells and change anti-tumor immunosurveillance including relapse and patient survival. In a group of 481 donor-recipient pairs, we set out to assess which donor inhibitory KIR receptors (iKIRs), recipient HLA cognate ligands, iKIR-HLA pairs, or post-transplant iKIR-HLA pair number variation have the greatest impact on progression-free survival (PFS). Among donor iKIRs, the strongest association with PFS was found for the KIR2DL2 molecule (134 [53%] vs. 92 [40.5%]; p=0.0066; OR=1.65; 95% CI 1.15-2.37). Among the KIR receptors for which ligands are unknown, a statistically significant adverse effect on PFS was demonstrated for KIR2DS2 (134 [52.1%] vs. 92 [41.6%]; p=0.022; OR=1.53; 95% CI 1.06-2.195) and KIR2DL5 group 2 (54 [56.8%] vs. 78 [44.1%]; p=0.045; OR=1.67; 95% CI 1.01-2.76]. Beneficial effect on molecular relapse and survival was demonstrated for KIR2DL4 del (97 [44.5%] vs. 34 [64.2%]; p=0.011; OR=0.45; 95% CI 0.24-0.83). There was no relationship between the presence of HLA-C1, -C2, and –Bw4 ligands in patients' environment and PFS. There was also no association between pairs of donor inhibitory KIR–-recipient cognate HLA ligand in the following post-transplant constellations: KIR2DL2/3-C1; KIR2DL2-C1; KIR2DL3-C1; KIR2DL1-C2; KIR3DL1-Bw4; KIR3DL1-ABw4 except A*25:01 and KIR3DL2-A3/11. While the presence of individual KIR receptors in donor or their cognate ligands in recipients, or even particular iKIR-HLA pair did not show a significant relationship with PFS, the decrease in the number of iKIR-HLA pairs after transplantation had a significant adverse effect on PFS as compared with the increase in the iKIR-HLA pairs (18/20

[90.0%] vs. 6/17 [35.3%]; p=0.0010; OR=13.09; 95% CI 2.82-60.67). Conclusion: Maintenance of immune surveillance against tumor after HSCT is mediated by NK cells via iKIR receptors and their cognate HLA ligands with the following patterns: i) NK cell function depends on receptor-ligand interactions rather than on the presence of the receptor or ligand itself; ii) NK cell function depends on a constellation of series of iKIR-HLA pairs rather than on any particular pair; iii) The change in the effectiveness of tumor immune surveillance with the change in the number of iKIR-HLA pairs after HSCT may indicate the existence of a mechanism for setting and resetting NK cell education.

O03 Analysis of NK cell reconstitution and the occurrence of aGvHD in ALL patients

Agnieszka Witkowska,[1] *Beata Krzymieniewska,*[2] *Agnieszka Malinowska,*[1] *Anna Flaga,*[1] *Aleksandra Wawiórko,*[1] *Barbara Nasiłowska-Adamska,*[3] *Jacek Nowak*[1]
[1] *Department of Immunogenetics, Institute of Hematology and Transfusion Medicine, Warsaw, Poland*
[2] *Department of Hematological Diagnostics, Institute of Hematology and Transfusion Medicine, Warsaw, Poland*
[3] *Hematopoietic Cell Transplantation Clinic, Institute of Hematology and Transfusion Medicine, Warsaw, Poland*

GvHD is a common complication after HSCT and has a significant mortality rate. The recipient's tissues are attacked by the immunocompetent cells of the transplant, such as NK cells and T lymphocytes. HLA-G does not participate in the presentation of antigens, but has immunomodulatory properties. It binds to surface receptors such as ILT2, ILT4, KIR2DL4, NKG2D found on NK cells, T and B lymphocytes, dendritic cells, macrophages, monocytes. These are receptors that inhibit the cytotoxicity. HLA-G simultaneously inhibits the cytotoxicity of T lymphocytes, causes their differentiation towards Treg regulatory cells. In the case of dendritic cells, it inhibits their differentiation. A number of actions triggered by HLA-G affect the production of cytokines and promote the preservation of an anti-inflammatory environment. HLA-G exists in two forms, membrane bound and plasma soluble. Both higher expression of the membrane form and higher concentration of the soluble form have been shown to be associated with a protective effect against graft rejection. So far we tested 17 patients who received a hematopoietic stem cell (HSC) transplant. During the preliminary analysis of data for 10 patients,

statistical significance was found in the Chi^2 test for NK after 60 days after HSCT. Namely, on the 60th day after transplantation, in people with II-IV aGvHD, the percentage of activated CD85j+ %z NK cells is higher (more often above the median) than in people who did not develop II-IV aGvHD (75% vs. 0%, p=0.048 , OR=16.33, 95% CI 1.02-261.1). This is confirmed by the trend in the results of activated CD85j+ % zNK cells for patients with III-IV aGvHD (100% vs.60%; p=0.16; OR=6.43; 95%CI 0.47-88.07) and the opposite trend for resting 60_CD85j+% zNK cells in aGvHD patients (40% vs. 100%; p=0.06; OR=0.08; 95%CI 0.01-1.09). It seems that a lower percentage of activated NK cells may be associated with a lower incidence of GvHD. On the other hand, a very large reduction in this percentage may have a negative effect in the form of systemic infections. It seems useful to perform early characterization of the NK population in order to control the occurrence of GvHD after transplantation and to consider the implementation of treatment prior to the possibility of systemic infection.

O04 The Distribution Of Risk Associated Hla Heterodimers Among Siblings Of Patients With Coeliac Disease

Katarina Stingl-Jankovic, Kristina Kopic, Natalija Martinez, Danijela Čleković, Matea Vuković, Zorana Grubic, Renata Zunec
Tissue Typing Centre, Clinical Department for Transfusion Medicine and Transplantation Biology, University Hospital Centre Zagreb

Coeliac disease (CD) is an autoimmune disease affecting primarily the small intestine. The disease occurs in genetically susceptible individuals, with HLA-DQA1 and -DQB1 genes being the main genetic predisposing factors. The aim of the study was to investigate the presence of three HLA heterodimers which displayed the strongest association with CD: HLA-DQ2.5 (HLA-DQA1*05, -DQB1*02), HLA-DQ2.2 (HLA-DQA1*02, -DQB1*02) and HLA-DQ8 (HLA-DQA1*03, -DQB1*03:02), among siblings of patients with a confirmed diagnosis of CD. The study included 75 individuals, all of whom were a sibling of a patient with CD, who were referred to our laboratory for HLA typing in the period June 2020 - December 2023. The HLA typing of HLA-DRB1, -DQA1 and -DQB1 loci was performed using the PCR-SSO method (Immucor Inc., Stanford, CT, USA) followed by the PCR-SSP method (CareDx, Stockholm, Sweden). The results were as follows: 36 individuals (48.0%) carried only HLA-DQ2.5 heterodimer, with 10 of them being homozygous for HLA-DQ2.5 heterodimer. Ten subjects carried both HLA-DQ2.5 and DQ2.2 heterodimers, with

HLA-DQ2.5 being in either a cis (9 cases) or a trans position (1 case). Five individuals carried both HLA-DQ2.5 and HLA-DQ8 heterodimers. Conversely, four subjects were positive for HLA-DQ2.2 heterodimer only, while only HLA-DQ8 heterodimer was found in six cases. In total, 51 individuals (68.0%) carried HLA-DQ2.5 heterodimer, 14 subjects (18.7%) were positive for HLA-DQ2.2 heterodimer, and HLA-DQ8 heterodimer was identified in 11 cases (14.7%). Finally, 14 subjects (18.7%) were negative for all three risk associated HLA heterodimers. The distribution of these HLA heterodimers in general population was then investigated on a sample of 202 healthy subjects. Among them, HLA-DQ2.5, HLA-DQ2.2 and HLA-DQ8 heterodimer was carried by 23.7%, 14.9% and 16.3% of individuals respectively, while 53.5% of subjects were negative. The increase in the frequency of HLA-DQ2.5 among family members of patients with CD in comparison to general population was highly significant (P<0.0001) as opposed to the difference observed for the frequency of HLA-DQ2.2 and HLA-DQ8 heterodimers, which did not reach significance. The difference between two tested groups in the percentage of individuals negative for any of the investigated HLA heterodimers was also highly significant (P<0.0001). A significant difference was also observed for the percentage of individuals carrying two HLA-DQ heterodimers among sibling of patients in comparison to healthy controls (34.7% vs. 11.4%, P=<0.0001). A the individual HLA-DQ heterodimer combinations level, the individuals homozygous for HLA-DQ2.5 heterodimer were significantly more present among CD patients' siblings (13.3% vs. 0.5%, P<0.0001), as well as persons carrying both HLA-DQ2.5 and HLA-DQ2.2 heterodimers (13.3% vs. 5.4%, P=0.0392). In conclusion, the results confirm the high predictive value of a negative result obtained for the HLA-DQ heterodimer presence. Furthermore, the observed prevalence of HLA-DQ2.5 heterodimer among siblings of patients with CD in comparison to population in general, undoubtedly justifies the need to perform the HLA-DQ typing of these individuals for screening purposes and identification of individuals at a higher risk for developing CD.

O05 Correlation of HLA-DRB1 shared epitope with response to methotrexate treatment in patients with rheumatoid arthritis

Meri Kirijas,[1] Anzelika Karadzova Stojanoska,[2] Maja Bojadziovska,[2] Teodora Brnjarchevska,[1] Gorjan Milanovski,[1] Tamara Savevska,[1] Sanja Kajevikj,[1] Kristina Stamatovska,[1] Olivija Efinska Mladenovska,[1] Aleksandar Petlichkovski[1]
[1] *Institute of Immunobiology and Human Genetics, Medical Faculty – Skopje, North Macedonia*
[2] *University Clinic for Rheumatology, Skopje, North Macedonia*

Background. Rheumatoid arthritis (RA) is complex autoimmune disease in which the genetic factor makes significant contribution. Strongest relationship was reported with HLA-DRB1 shared epitope (SE), 5 amino acid sequence motifs coded by several HLA-DRB1 alleles: HLA-DRB1*04 allele group, *01:01, *14:02 and *10:01. Aim. The aim of our study was to determine the correlation between HLA-DRB1 shared epitope and the response to methotrexate treatment in patients with rheumatoid arthritis. Methods. We analyzed 36 patients with active rheumatoid arthritis treated at the University Clinic for Rheumatology in Skopje, North Macedonia. They received de novo methotrexate therapy 15 mg once a week orally without other DMARDs. Disease activity was assessed by the 28-joint disease activity score (DAS28) and the EULAR response was evaluated at 24 weeks. According to the presence of shared epitope allele, the patients were divided in 2 groups: shared epitope positive (SE+) and shared epitope negative (SE-). Results. We analyzed 32 women and 4 men with rheumatoid arthritis, with mean age 58.5±10.8 years. Twenty patients (56%) were SE+ and 16 (44%) were SE-. HLA-DRB1*01:01 was detected in 11 patients (55% from the SE+ group) which is in concordance with the age group of our patients (onset of the disease >60 years). There was no statistical difference between the EULAR clinical response to the therapy between the two groups. Negative effects of the therapy were detected in 6 patients, 5 in SE+ group (83,3%) and one in SE- group (6,25%) with no statistical significance (p=0.1962). Conclusion. The role of SE+ alleles in the pathogenesis of RA is well established but it's role on the effectiveness of therapy and the presence of negative effects should be analyzed on a bigger group of patients.

O06 Case report: persistent HLA class II antibodies after liver re-transplantation

Lucija Jukić, Marija Burek Kamenarić, Magdalena Svetec, Katarina Štingl Janković, Renata Žunec

Tissue Typing Centre Zagreb, Clinical Department for Transfusion Medicine and Transplantation Biology, University Hospital Centre Zagreb, Zagreb, Croatia

Donor-specific antibodies (DSA) against mismatched (MM) HLA antigens between recipient and the donor are a well-known risk factor for humoral rejection and lower graft survival after kidney transplantation. On the other hand, the role of DSA after liver transplantation (LTx) remains debateable as liver, an organ with the capability to adsorb DSA, is more resistant to antibody mediated rejection. For that reason, most of liver transplant centres still do not take HLA matching nor screening for HLA antibodies into account in their allocation algorithms. However, many studies have shown that in some cases DSA can contribute to complications after LTx. Here, we present a case of a 66-year-old patient that has undergone three ABO compatible LTx from deceased donors in a period of four years (2011-2014). The CDC retrospective crossmatches were done after each LTx and were negative. Neither CDC nor Luminex screening were performed prospectively before any of the three transplantations. In 2023 he was referred for the first time to HLA-DSA monitoring with suspected graft rejection. Luminex testing resulted with the detection of HLA class I and HLA class II antibodies with high mean fluorescence intensity (MFI) up to 20 000. Among positive HLA class I and class II specificities, DSAs against all three donors were present. The time of formation of each DSA and other antibody specificities could not be determined due to the lack of sampling and DSA monitoring before and after each LTx. Only pre-transplant sera sample from the third LTx was available and retrospective testing revealed that patient underwent a 3rd LTx with the pre-transplant DSAs against HLA class II antigens (HLA-DQ5 and -DQA1*01) of high MFI values which persisted until today. HLA class I antibodies became positive after 3rd LTx with specificities against 2nd donor MM antigens, while HLA class II analysis revealed that patient developed de novo DSA HLA-DR51 against the 3rd donor. Even though there is not enough evidence for clinical importance of DSA screening in liver transplantation, this case emphasises its potential benefit. Patients with detectable persistent DSA, especially HLA class II antibodies, can be at higher risk of transplanted liver rejection. For that

reason, closer monitoring and increased screening for HLA antibodies may be useful for patients with liver re-transplantation.

O07 HLA eplet mismatch and incidence of graft-versus-host disease after allogeneic haematopoietic stem cell transplantation – preliminary study

Piotr Łacina, Jagoda Siemaszko, Katarzyna Bogunia-Kubik
Laboratory of Clinical Immunogenetics and Pharmacogenetics, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland

HLA mismatches are a major risk factor for complications after allogeneic haematopoietic stem cell transplantation (HSCT), including graft-versus-host-disease (GvHD). Both HLA class I and class II mismatches can increase risk of complications and mortality in post-HSCT patients. Despite prophylaxis, HSCT may still result in post-transplant complications. Using high-resolution genotyping, it is possible to determine the number of mismatches in eplets – small amino-acid sequences on the surface of HLA molecules that may be relevant for transplantation outcome. In the present study, we analysed high-resolution HLA-A,-B,-C,-DRB1 and -DQB1 genotyping data from 112 donor-recipient pairs to find the number of mismatched eplets. Eplet mismatches were identified by HLA Fusion Software 4.6.0. Number of mismatched eplets was analysed in the context of post-transplant complications such as acute GvHD, chronic GvHD, and cytomegalovirus infection. A logistic regression analysis revealed that the number of mismatched eplets was associated with acute GvHD occurrence (p=0.013). A multivariate analysis additionally including the type of conditioning and gender of the donor/recipient (female donor to male recipient) suggested that the number of mismatched eplets may be an independent factor of acute GvHD (p=0.061). However, we did not observe any such associations between the number of mismatched eplets and occurrence of either chronic GvHD or cytomegalovirus infection. Our preliminary results show that the number of mismatched eplets may be of importance in predicting acute GvHD in patients after allogeneic HSCT.

This work was supported by National Science Centre (Poland) project No. 2018-/31/B/NZ2/03065 and was conducted in collaboration with the following Polish clinical centres: Department of Hematology, Blood Neoplasms and Bone Marrow Transplantation, Wroclaw Medical University; Department of Bone Marrow Transplantation and Hematology-Oncology, Maria Skłodowska-Curie Memorial Cancer Centre and Institute of Oncology, Gliwice Branch; Institute of Hematology and Blood Transfusion, Warsaw; Department of Hematology, Transplantation and Internal Medicine, Medical University of Warsaw; Department of Hematology and Transplantology, Medical University of Gdansk.

O08 Antibody Screening Tests and *a priori* Immunisations in Lung Transplantation

Daniela Koren, Ingrid Faé, Daniela Kriks, Sabine Wenda, Gottfried Fischer
Department of Transfusion Medicine and Cell Therapy, Medical University of Vienna

It is well known that organ transplant candidates who have been exposed to allogeneic cells can develop HLA antibodies, which are considered a risk factor for organ rejection. Even if these patients can be transplanted by temporarily removing the antibodies, they still have a higher risk of complications. If patients do not have donor-specific antibodies at the time of transplantation, this is usually regarded as a low risk. A special group of patients are women who have given birth. In a significant proportion of these women, HLA antibodies can be detected by single-antigen testing just a few days after transplantation. Typing of the children shows that these antibodies are directed against paternal HLA antigens. Often these immune responses are not allele specific, but show a broad reactivity. If an HLA antigen of the children happens to be the same as that of the transplanted person, this antigen could be considered a repeat mismatch. In conclusion, patients who are considered unimmunised prior to transplantation because of negative antibody screening tests should not necessarily be considered immunologically risk-free. Transplantation may stimulate the patient's immunological memory. Previously undetected HLA-specific antibodies may become visible a few days after transplantation and the post-transplant course of the graft may be more complicated than expected.

O09 HLA Proficiency Testing for Central and East Europe – the results of the 30th trial (2023)

Katarzyna Bogunia-Kubik
Hirszfeld Institute of Immunology and Experimental Therapy Polish Academy of Sciences, Wrocław Poland

To fulfill the requirements and standards of the European Federation for Immunogenetics (EFI) and national accreditation bodies, laboratories whose activities are associated with HLA testing are obliged to participate in external proficiency testing (EPT) exercises covering all the techniques used in the laboratory to obtain typing results. Our Wrocław EPT – HLA Proficiency Testing for Central and East

Europe, which was initiated in 1999, belongs to the EPT providers from EFI Region 5. Currently it covers serological typing of HLA class I (A, B, and C loci) as well as DNA typing at the low (A, B, C, DRB1, DRB3/4/5, DQA1, DQB1) and high (A, B, C, DRB1, DRB3/4/5, DQA1, DQB1, DPA1, DPB1) resolution levels. Participants are provided with blood and/or DNA samples to test HLA class I antigens by serology and/or to perform genomic assessment of HLA class I and class II alleles at the low or high (two fields allele assignment) level. In 2023, our EPT exercise was organized for the 30th time. There were 31 participating laboratories of our EPT in 2023. The vast majority of participants employed DNA-based methods, 29 and 17 submitted the results of low and high resolution typing, respectively. There was a group of 6 laboratories that provided HLA typing results obtained by serological methods, but in general all of them except one also use genotyping techniques. In total ten discrepancies were observed: four in serological typing, and four and two in DNA typing results at the low and high resolution level, respectively. Two laboratories did not fulfil the criteria for successful performance: one due to the discrepancies in the HLA class I low resolution results while the other because of inconsistency with requested consensus of the high resolution HLA-DQB1 typing. The activity of EPT providers, including our Wrocław EPT, is of interest for immunogenetics community, leading to the detection and elimination of inconsistent results and thus considerable improvement of the quality of HLA typing and reliability of histocompatibility testing.

O10 Results Of External Proficiency Testing „Detection Of HLA Alleles Associated With Diseases“

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EPT program “DETECTION OF HLA ALLELES ASSOCIATED WITH DISEASES“ was organized by our HLA department in 2023 same way as in previous years. Program started in March, samples were distributed in two parts for variant CD (alleles predisposing to coeliac disease) and all in one for variant B*27 and DQB1*06:02. Results were evaluated in terms of correct determination of predisposing alleles/allelic groups and the clinical interpretation. All together 34 labs from 6 countries (Czech Republic, Austria, Slovakia, Poland, Bosnia and Herzegovina, Italy) participated in 2023 EPT. Overall performance was success-

ful in B*27 and DQB1*06:02. This result was same as in previous years. Overall performance was poor in CD variant, only 21 of 31 participating laboratories pass the EPT successfully. New EPT program starts in March 2024. Details are available on our webpage: https://www.uhkt.cz/laboratories/external-proficiency-testing/detection-of-hla-alleles-associated-with-diseases. All HLA laboratories willing to participate in EPT are welcome. Please contact the guarantor Milena Vraná (milena.vrana@uhkt.cz) or our assistant Barbora Kinská (barbora.kinska@uhkt.cz).

P01 Comparative analysis of two PCR-SSP methods: real-time based QTYPE and endpoint fluorescence detection based FluoGene, for the molecular typing of 11 HLA loci

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The main objective of the present study was to compare the results and performance of the two commercial assays/techniques for HLA molecular typing: QTYPE 11 (CareDx, Stockholm, Sweden) and HLA-FluoGene assays (Inno-train Diagnostik GmbH, Kronbrg, Germany). A comparative analysis was performed to evaluate the real-time PCR method QTYPE11, which is currently being introduced in our laboratory practice. Both techniques use sequence-specific priming (SSP) to determine the alleles present in a DNA sample. The HLA-FluoGene assay on the FluoVista analyzer (Inno-train Diagnostik GmbH, Kronberg, Germany) provides low-resolution HLA typing results, whereas the QTYPE results obtained using LightCycler 480II (Roche Diagnostics, Mannheim, Germany) real-time PCR instrument have low/medium resolution. We analyzed a total of 10 DNA samples provided from an external quality assurance (EQA) scheme for the molecular typing of HLA-A, -B, -C, -DRB1, -DRB3, -DRB4, -DRB5, -DQA1, -DQB1, -DPA1 and -DPB1 loci using both methods. HLA typing analysis was performed using FluoGene software v1.8.0.0/IPD-IMGT/HLA 3.52.0 and SCORE 6 software v6.2.1.0/HLADB_3.52.0. The results of the HLA typing using these two methods were matched at the first field level for all 11 loci. For HLA-A, -DRB1, -DQA1, -DQB1, and –DPA1 loci there were no ambiguities at the first field level in the QTYPE 11 assay. For the HLA-DPB1 locus, ambiguities were observed in both methods for all samples. Am-

biguities at the first field level were more prevalent for all HLA loci tested using the FluoGene assay. The decisions on typing results were based on the published catalog of common or well-documented (CWD) HLA alleles (DOI 10.1111/tan.12956). QTYPE real-time PCR-based technology for typing 11 HLA loci in a single 384-microtiter plate offers the advantages of time consumption and more accurate testing than the endpoint fluorescence detection-based FluoGene method.

P02 MICA and NKG2D polymorphism in multiple myeloma patients

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NK cells play an important role in the development of haematological malignancies. Their suppressor activity against cancer cells has been recognized and confirmed in many diseases, including multiple myeloma (MM). Various NK cell dysfunctions and changes were observed in MM patients. NK cell activity is normally fine-tuned by a large array of activating and inhibitory receptors. These include NKG2D, a major activating receptor, whose ligands are MHC class I chain-related protein A and B (MICA and MICB). Previous studies showed that expression of NKG2D and its ligands may be impacted in MM patients, which suggests their role in MM development. In the present study, we aimed to analyse single nucleotide polymorphisms (SNPs) in the genes coding for NKG2D (rs1049174, rs1154831) and MICA (rs1051792, rs1063635) in a group of multiple myeloma patients. Genotyping was performed on DNA samples from 251 patients using the LightSNiP assay. Data were analysed in the context of survival, disease progression and other clinical parameters. We found that patients with genotype MICA rs1051792 AA were characterized with shorter progression-free survival (p=0.002). Allele MICA rs1051792 A was more commonly detected in patients with more advanced disease (stages II-III according to the International Staging System; p=0.033). Additionally, patients with MICA rs1051792 AA also tended to have higher beta-2-microglobulin level (p=0.053). We also observed that patients with genotypes NKG2D rs1049174 CC

and NKG2D rs1154831 CC tended to have lower CRP levels than patients without these genotypes (p=0.035 and p=0.069, respectively). These findings suggest that MICA and NKG2D SNPs may have role in MM development, although further studies on a larger cohort would be needed.

P03 Rabbit Xenoantibody anti-HLA-A3 Detected In Patients Treated With Anti-T-Lymphocyte Globuline Before Stem Cell Transplantation

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Fourty-three patients underwent allogeneic hematopoietic cell transplantation (HSCT) in our Institute between June and December 2023. Seven out of these 43 patients were treated with rabbit anti-T-lymphocyte globulin (ATLG; Grafalon, 3 days from D-3 (testing dose, 100 mg) to D-1 (30mg/kg) before HSCT) as GvHD prophylaxis. Anti-HLA class I antibodies with a similar pattern of reactivity were detected by Luminex in all seven patients treated with ATLG. A specific anti-HLA-A3 antibody was subsequently found in six of these patients. None of these six patients or their donors bear the HLA-A3 antigen. Only one patient and his donor bear HLA-A3. This patient had a weakly positive screening for anti-HLA class I antibodies, but the identification test was negative. We hypothesize that the anti-HLA-A3 antibody could be saturated on the patient´s and donor´s A3 antigens. No serious reactions or graft rejection were noted in this patient. During the period from D-21 to the time of sampling for anti-HLA antibody testing (max. D+11), all seven patients treated with ATLG were transfused (average 10.1 units of platelets and 7.4 units of erythrocytes). Screening for anti-HLA antibodies performed before administration of ATLG showed a negative result in all seven monitored patients. Due to intensive hemotherapy, we couldn´t exclude patient´s immunization by transfusions. However, only 7 out of 43 transplanted patients (16.3%) were screened positive for anti-HLA class I antibodies by Luminex in the pre-D-3 sample. Any patient with positive anti-HLA class I antibody before D-3 wasn't treated with ATLG and no specific anti-HLA-A3 antibody was detected. Since anti-HLA antibody monitoring is important because of the risk of antibody-mediated graft rejection, the results of anti-HLA antibody testing should be interpreted carefully, considering the possible presence of therapeutic anti-HLA-A3 rabbit IgG xenoantibodies that are likely to

cross-react with detection antibody against human IgG used in Luminex kits.

P04 Pre-transplant anti-HLA antibodies in immunized patients awaiting kidney transplantation – single center experience

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Kidney transplantation is a treatment procedure dedicated to patients with advanced renal diseases. The presence of antibodies against human leukocyte antigen (HLA) molecules in kidney transplant recipients is a known risk factor for organ rejection and graft loss. The aim of this retrospective study was to determine and characterize anti-HLA antibodies in serum of 164 patients from the Lower Silesia and Opole regions in Poland referred for organ transplantation in 2023 tested in accordance with the standard qualification protocol for transplant candidates at our Laboratory. Pre-transplantation panel reactive antibody estimation was performed using complement dependent cytotoxicity test (PRA-CDC). Presence of circulating anti–HLA-A, -B, -C, -DR, -DQ, and -DP antibodies was analyzed in serum using SAB assays (One Lambda, Inc., Canoga Park, CA) on a Luminex platform. Sensitized patients (>5% PRA) comprised 19.5% of all analyzed patients. The analysis showed a disproportion in the frequency of antibodies between highly sensitized patients (>50% PRA) and potential recipients with PRA in the 5-50% range. It was found that the group of highly sensitized patients characterized with a prevalence of anti-HLA-A1 (71% vs 32%, p=0.0906), A2 (71% vs 16%, p=0.0101), A68 (85% vs 32%, p=0.0265), A24 (100% vs 52%, p=0.0288) antibodies. These results suggest that the presence of those specific anti-HLA class I antibodies may be a factor increasing the waiting time for transplant.

Notes