Transfusion Medicine and Hemotherapy

Contents

DGHO = Deutsche Gesellschaft für Hämatologie und Medizinische Onkologie e.V.; GSCN = German Stem Cell Network – Deutsches Stammzellnetzwerk e.V.; ARGE Plasmapherese = Arbeitsgemeinschaft Plasmapherese e.V.; DG-GT = Deutsche Gesellschaft für Gentherapie e.V.; GTH = Gesellschaft für Thrombose- und Hämostaseforschung e.V.; DGI = Deutsche Gesellschaft für Immungenetik e.V.

PLENARSITZUNGEN

PL-1
Joint session DPG – Alternative Blood Products

PL-1-2
Blood Pharming

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Remarkable advances have recently been made on the in vitro generation of blood cells and in their first steps into clinical application. Large-scale production of induced pluripotent stem cells (iPSCs)-derived Megakaryocytes represents a promising alternative to donated platelets. In addition, the possibility to generate genetically engineered megakaryocytes and platelets offers the possibility to increase their therapeutic potential and reduce off-target effects. We have developed a strategy to produce low immunogenic iPSC-derived Megakaryocytes in a bioreactor system. For this purpose, specific iPSC-lines were generated from selected donors and genetically engineered to silence HLA class I and II expression. Low immunogenic Megakaryocytes showed a typical phenotype, but in contrast to non-engineered Megakaryocytes they were incapable to trigger antibody-medicated cytotoxicity and T-cell activation and cytotoxicity in vitro. Furthermore, in a physiologically platelet transfusion refractoriness mouse model, HLA-silenced Megakaryocytes and Platelets were capable to evade allogeneic cellular and humoral immune responses. Worldwide innovative blood pharming technologies are emerging as promising platforms for cell therapies and a new era in transfusion medicine.

PL-1-3
Artificial Blood

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Introduction: Alternatives to blood are urgently needed as blood products are associated with side-effects, are of limited availability and require cooling chains. When researchers started to cover this need about 45 years ago, they had to painfully realize that replacing the natural product blood was not as easy as it might have been spotted at first sight. Blood accomplishes so many different tasks such as transport of respiratory gases (erythrocytes), coagulation (platelets, coagulation factors), immune defense (leukocytes), transport of lipophilic compounds (albumin, lipoproteins) and electrolyte supply. Some compounds are already clinically available such as crystalloidal or colloidal aqueous volume substitution solutions or coagulation factors. In contrast, all mentioned cell types are still difficult to replace.
Methods: Relevant databases such as Medline (https://www.ncbi.nlm.nih.gov/pubmed/?holding=ideudelib), ClinicalTrials.gov (https://clinicaltrials.gov), EU Clinical Trials Register (https://www.clinicaltrialsregister.eu), and Australian New Zealand Clinical Trials Registry (http://www.anzctr.org.au) were searched up to August 2023 using search terms related to artificial blood products in order to identify new and ongoing research over the last 5 years.
Results and conclusion: Important milestones and obstacles in the development of artificial blood products will be explained and linked to different application areas. Furthermore, the actual status (recent and ongoing research as well as clinical studies) of artificial erythrocytes, platelets and leukocytes will presented.

Until today no all-purpose product covering the numerous functions of natural whole blood is clinically available. Today researchers focus on the design of single components (artificial erythrocytes, platelets, leukocytes) instead of aiming to replace whole blood, which resulted in clinical approval of at least few artificial blood products in selected countries.

Disclosure Statements: *Miterfinderin in einer europäischen Patentanmeldung zum Schutz der "LENOX oxygen carrier". Die Patentanmeldung ist von Amtswegen noch geheim bzw. nicht offengelegt.*

PL-2
Immunotherapy: Primary T cells

PL-2-3
BK-specific T Cells to treat PML

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Progressive multifocal leukoencephalopathy (PML) is a rare but serious opportunistic viral infection of the brain that often leads to death. The disease is caused by the human polyomavirus 2 (HPyV-2) and particularly affects patients with a significantly impaired cellular immune defence. Especially in patients with an underlying haematological disease, the mortality rate of PML is almost 90%. Survival depends on reversal of the underlying immunosuppression, but immune reconstitution is not readily possible in most patients, especially those with lymphoproliferative disorders.

Rapid reconstitution of T cell-mediated immunity by infusion of allogeneic virus-specific T cells partially matched to human leukocyte antigen (HLA) in immunocompromised patients is currently the most promising route for successful treatment of PML. This presentation will give a detailed overview of the new experimental treatment approach.

Disclosure Statements: Thomas Skripuletz reports honoraria for lectures and travel grants from Alexion, Alnylam Pharmaceuticals, argenx, Bayer Vital, Biogen, Celgene, Centogene, CSL Behring, Euroimmun, Janssen, Merck Serono, Novartis, Pfizer, Roche, Sanofi, Siemens, Sobi, Teva, Viatris. His research is supported by the German Ministry for Education and Research (BMBF), Bristol-Myers Squibb Foundation for Immuno-Oncology, Claudia von Schilling Foundation for Breast Cancer Research, Else Kröner Fresenius Foundation, Hannover Biomedical Research School (HBRS), Alnylam Pharmaceuticals, CSL Behring, Novartis, Sanofi Genzyme, VHV Foundation.

PL-4
Blood Safety

PL-4-1
Pathogen inactivation

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The conventional approach of risk mitigation of pathogen transmission by blood components has relied almost exclusively on a combination of selection of low-risk donors and testing for selected pathogens. This strategy has its immanent gaps (e.g., bacteria and low-titre infections) and is increasingly challenged by changes in donor policies and occurrence of emerging pathogens. In addition, further donor-excluding measures introduced to increase blood safety may have the unintended consequence of decreasing blood availability.

Pathogen inactivation (PI) technologies for blood components are crucial for closing infection safety gaps not covered by regular blood donor screening programs. PI for plasma and platelet concentrates is already used in clinical practice. However, methods for red blood cells and whole blood, which are needed to achieve the full potential of this proactive protection strategy for blood safety, are still under development.

Loss of transfusion product efficacy post-PI treatment and potential side effects in transfused patients as well as significant expenses for blood services have been the major concerns for the use of PI technologies. However, clinical studies have shown that pathogen-reduced blood components have sufficient clinical efficacy and acceptable safety profiles, albeit the use of PI technologies for platelets may result in a greater number of transfusions. Indeed, PI technologies require additional working steps on-top of the established preparation methods, and their introduction therefore generally increases the workload and complexity of the existing blood manufacturing. However, technical advances and automation may facilitate the implementation of the PI manufacturing processes by reducing workload and costs. Recent risk-benefit studies and economic evaluations suggest that the use of pathogen-reduced plasma and platelets would significantly and cost-effectively reduce the risk of transfusion-transmitted infections in Western countries in the event a new blood-borne pathogen entered the blood supply.

Considering the spread of vectors and infectious agents driven by climate change and globalisation, PI offers a proactive solution to help safeguard the blood supply in terms of safety and availability.

PL-4-2
Hotblooded - blood borne emerging infections in times of climate change

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The currently observed climate change will inevitably change ambient temperature, rainfall patterns, and humidity on a local level but also globally. The effects are manifold and can have positive, neutral, or negative impact on natural hosts, vectors or parasites and the likelihood of human infections.

Therefore, a precise modelling of the effects of climate change on transfusion-relevant infections is difficult. What can already be observed is the fact that some previously absent vectors now have established populations in Germany. These include mosquitoes like Aedes albopictus or Aedes japonicus which can transmit chikungunya virus or dengue virus and ticks like Hyalomma marginatum which are known vectors for Krim-Kongo haemorrhagic fever. It could also be shown that climatic conditions - to date in sothern Europe only - are sufficient for the spread of triatominae, the vector for Chagas disease. Also, depending on the interaction between the rise in temperature and humidity, fairly recently introduced pathogens like the West-Nile-virus may spread at an increased rate. Climate change, including changes in temperature and precipitation, has also been proposed to increase the risks for visceral, cutaneous, and mucocutaneous leishmaniases globally, including Europe. Additionally, the burden of Plasmodium transmission, the pathogen causing malaria, is widely assumed to increase in a future warmer world.

The most important measure for blood establishments is therefore to cooperate with public health institutes in order to identify transfusion-transmitted disease risks early. Surveillance of these pathogens and infections is of utmost importance and includes veterinary and entomological data in a one-health approach. Blood establishment may even contribute to the surveillance as many residual blood samples can be analysed quickly in case of an emerging infection. Once identified, the need for additional testing can be evaluated as long as suitable pathogen reduction technologies are not available for all blood components. As climate change is already reality, existing contingency plans should be evaluated in view of potentially emerging infections and lessons learned from the COVID-19 pandemic integrated. Appropriate inter-disciplinary working groups should add these topics to their agenda and ideally collaborate with international colleagues.

PL-4-3
Blood supply on the battlefield

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In the event of a military conflict, injury patterns associated with life-threatening blood loss must be expected. This makes the stockpiling of blood products and the development of blood replacement procedures a necessity for the medical care of these patients. Possible logistic approaches and their limitations for the supply of blood products to the wounded are presented in relation to the nature of the potential conflict. Under the conditions of a disturbed logistic infrastructure this supply must be maintained or resilient alternatives to the classical blood substitute therapy must be prepared in order to keep the mortality of the mentioned clientele as a result of blood loss as low as possible. To this end, various forms of whole blood donation have become established in the international arena. In addition to the existing stockpiling possibilities with blood products, prerequisites for warm blood donation in compliance with national quality standards have been developed and established for the medical service of the German Armed Forces. This is intended to take account of special requirements in the event of disrupted logistics, insufficient stockpiling or special military conditions.

In a national context, all of these expenses must be managed in addition to the increasing demand for the supply of blood and blood products - with a simultaneous decline in the willingness to donate.

VORTRAGSSITZUNGEN

VS-1
Sektion Automation und Datenverarbeitung

VS-1-1
Status of digital patient records, electronic entry of organ donation in the register

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On the part of the Automation and Data Processing Section, the topic of digital patient records was presented in previous sessions. Many hospitals offer digital patient data exchange in the patient management system. After logging in to the portals, a system was created which also ensures data protection for patient data. In addition, many health insurance companies are responsible for the patient, the administration and digitization of doctor's letters in compliance with data protection. With the electronic patient record, patients can securely organize their health documents digitally. You can share these with service providers such as doctors' surgeries and have other documents posted. The digital exchange enables an easier overview of the health history and thus supports treatment. Equipping doctors' offices to access electronic health records has begun. As a result, the electronic patient file is slowly finding its way into everyday practice. There are still some practical hurdles to overcome, but the perspective is clear: applications such as the electronic patient record have great potential to simplify processes for medical practices and patients. The digital patient record in the hospital (electronic patient record — ePA) contains all of the patient's medical information in a structured manner. These include doctor's letters, findings, emergency data, nursing information, medication and anamnesis. The electronic patient record in many clinics makes it possible to automatically exchange the data with other systems. This includes the clinic patient portal, which allows patients to access their treatment information or book appointments from home or on their mobil devices. In addition, the data of the electronic patient record can be stored in the cross-institutional patient file of the telematics infrastructure (a legally regulated network for the exchange of medical data). There, the information is then available to all institutions involved in the treatment of the patient. The electronic patient record also includes all nursing information and supports the clinic staff during treatment. The electronic patient record increases the safety of drug therapy through the direct availability of all information such as allergies and ongoing medications at the time of prescription. Thanks to a centrally structured electronic filing system, all relevant information is available in real time

VS-1-5
Data processing and software validation for an in-house molecular blood group screening

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Extended donor blood group typing can provide compatible blood for patients with rare antibodies. A new in-house MALDI-TOF MS assay simplifies the identification process for new donors by testing 26 different blood group antigens. Data generated must be transferred to the laboratory information system of the blood bank using an in-house software tool, the AgenaImporter. The whole process has been successfully validated and is now implemented into the blood bank system of the DRK BSD West.

The in-house assay was designed by the online-/offline Agena Assay Designer software tool, identifying blood group alleles, primers and molecular masses for each blood group SNP. Parameters of the Agena HemoID software had to be adjusted for a correct assignment of determined molecular masses to blood group alleles and the deduced phenotypes. These phenotypes had to be imported into the BAS400, our current blood bank system, by an in-house software tool, the so-called AgenaImporter. It translates the initial information and automatically checks for errors during the transfer. Every step in this process was validated in a test environment for errors or loss of information by using samples with previously tested blood group antigens.

Each process step involved in the MASS ARRAY blood group typing was successfully validated. All blood groups, pre-determined either by serology or other molecular methods, were confirmed or discrepancies could be explained by limitations of serology. Parameter adjustment of the HemoID software successfully translated molecular masses into blood group alleles and phenotypes. Finally, the AgenaImporter was successfully validated. All information generated by the MassArray System was correctly translated and imported into the test environment. All errors, that deliberately had been added manually, were correctly detected preventing import of these data into the BAS400. Since then, nearly 15.000 donor samples have been successfully analyzed.

Conclusion: In-house molecular blood group typing by MALDI-TOF MS is a valuable tool for extended donor blood group genotyping. All downstream data analysis steps have been thoroughly examined. All critical errors were successfully identified by the software and not transferred to the blood bank system, thus reducing the risk for patients to a minimum. The whole process is reliable, and a big step towards automated data processing, definitely saving time and reducing the risk of man-made mistakes.

VS-1-6
IT and data processing requirements in a specialized laboratory with a broad range of examination methods

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Introduction: Medical laboratories require specialized IT and software solutions in addition to the laboratory information and management system (LIMS), depending on the spectrum of analyses and medical devices. Our laboratory offers patient and blood donor diagnostics, including HLA-/HPA- and blood group molecular typing, HLA-/HPA-antibody analysis, and stem cell diagnostics, applying both, CE IVD tests as well as in-house tests. Device control and test results must be verified and imported into the LIMS.

Methods: Results of patient HLA- and HPA-antibody testing are generated by Immucor Lifecodes software tools, but there is no automated data import into the LIMS (iLIS, an in-house developed user interface of BAS400). Complex antibody situations with more than 20 specificities must be entered manually to generate a patient record. The same applies to NGS HLA typing, where the results of the highly complex molecular typing are assigned by the GenDx NGSengine software with regular internet-based database updates but manual data transmission. Contrary, data of a molecular MassArray blood group screening are imported into the BAS400 batch-wise by an in-house developed software tool. Analysis of stem cell products requires excel algorithms.

Results: The wide range of different serological and molecular analyses with various automated evaluation software programs including software validation requires well-trained employees. Constant internet-based database updates for molecular analysis are associated with an increased effort due to high IT safety requirements. Concerning in-house blood group NGS typing by the Geneious bioinformatics software for sequence data analysis, implementation of different blood group allele references is on-going. However, manual entry of complex data into the blood bank system is error-prone and requires continuous verification using the four-eyes principle. This accounts for manual data entry of stem cell quality control, too.

Conclusions: Specialized laboratories offering a broad range of serological and molecular analyses need various software tools for data analysis. Especially molecular typing requires higher computing capacities and frequent database synchronization. Our blood service is currently implementing a new blood bank system and LIMS with the aim to use the advantages of the automated data evaluation for an improved patient and donor management, and the creation of reports as well.

Introduction of a LIMS in the quality control laboratory of the DRK-BSD West – the challenge of rethinking tried-and-tested structures

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Introduction: The documentation of the quality control results of the DRK-BSD West is currently still based on Excel spreadsheets. This approach is not state-of-the-art and is no longer accepted by the supervisory authorities. Therefore, the processes in the laboratory are to be mapped by the LIMS LAB+ by MAQSIMA. This change holds several challenges, as it requires rethinking structures that have grown over decades.

Implementation: Initially, the challenge was to question the tried and tested processes and adapt them to the requirements in a contemporary way so that they could be mapped in the new LIMS. This means relief in some regards, since a large part of the documentation is eliminated via the connection of laboratory equipment, while in other places a rigid IT system leads to a loss of flexibility and processes that can be done relatively easily using Excel have to be restructured to map them accordingly in the system.

Additionally, the project must be carried out together with the routine tasks, as there was no extra personnel capacity. Tasks such as participation in training courses and in the configuration of interfaces as well as the subsequent testing and validation of the processes required a high degree of participation by the employees. Moreover, the staff at the individual workstations changed weekly, leaving little room for the individual to deal with the project in a more intuitive way.

The Corona-related general shortage in personnel meant that the project often had to stand back behind the routine and progressed slowly. The requirements for the validation of IT systems are high (EU-GMP guideline Annex 11) and cost a lot of time. Autohorities have also been paying increased attention to validation for several years. Since the beginning of 2023, the system is running in parallel with the Excel spreadsheets for parts of the routine.

Conclusion: The introduction of a LIMS in the quality control laboratory turns out to be lengthier and more complex than initially thought. The high demands on the system by the laboratory and GMP guidelines, also in terms of validation, require a lot of time and commitment by the employees. The introduction of a new LIMS parallel to the laboratory routine and without additional human resources should not be underestimated.

Eurocode Technical Specification, Annex/Appendix German UPN specification - update

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Eurocode International Blood Labelling System e.V. (Eurocode IBLS e.V.) is a non-profit organisation founded in 1998 to create and maintain standard coding structures for the safe exchange of products of biological origin for application in human medicine, such as blood products, cells, tissues and organs. The top priority is to ensure a unique and internationally acknowledged code corresponding to EU Directive 2002/98/EC which can be employed for a minimum period of 30 years.

The Technical Specification defines symbology and data structures used within the Eurocode standard following the primary identifier "!".

The document is revised and developed continuously to meet current technical requirements and incorporate new regulations such as the Single European Code (SEC).

The final draft of the latest version 2.2 of the Technical Specification is presented: It includes editorial revision and clarification, changes to the Unique Product Identification Number (UPN-T) for organs, tissue and cell products and introduces a new Annex "German UPN Specification".

The Technical Specification explicitly describes the option to define national substandards for the unique product number. The new annex for Germany specifies length of the consecutive product number, possible variations for establishments with large numbers of donations and algorithms used for the check digit. The Annex considers previous agreements of German blood product manufacturers and the DGTI-ADV delivery file structure. The new version is presented to the community and discussed before being implemented by Eurocode's technical committee.

The technical committee also continuously advances the qualifier tables to enable the creation of new product codes requested by member institutions. Recent changes where necessary due to the decision of the Paul Ehrlich Institute of April 5th, 2023 (possibility to omit quarantine storage of frozen fresh plasma by adapting the donor screening NAT sensitivity). The committee therefore revised the definition of Eurocode qualifier Q "additional testing/quarantine". The updated definition of the qualifier Q1 will be discussed.

VS-2
Sektion Immunhämatologie und Immungenetik

Molecular Blood Group Testing in Poland

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Identification of antibodies to red blood cell, platelets and granulocyte antigens as well as precise antigen phenotyping of patients, pregnant women and donors is often challenging using standard serological methods. Modern immunohematology requires constantly development and implementation of new technologies and protocols beyond the routine testing. Especially in the reference laboratory there is a need to support the serological diagnostics of discrepant and complex cases with the newest genetic methods. The Laboratory of Blood Cell Genetics and Chimerism at the Institute of Hematology and Transfusion Medicine in Warsaw, Poland, is a country-wide reference genetic center for complicated serological cases related to red blood cell (RBC), platelet (HPA) and granulocyte (HNA) antigens, also including feto/maternal incompatibilities.

The laboratory, established in 1995, is a corelab responsible for RBC, HPA, HNA antigen genotyping of patients and blood donors using a wide range of standard and advanced commercially available tests and *in-house* molecular methods such as PCR-SSP, real-time PCR, crystal digital PCR, multiplex ligation-dependent probe amplification (MLPA), time-of-flight mass spectrometry (MALDI-TOF), classical sequencing, whole exon sequencing (WES) and blood group panel exon sequencing (ES). During the last 2 years over 1700 RBC, HPA and HNA genotyping tests were performed for patients with allo-, autoantibodies and after recent transfusion; for individuals with weak or null expression of antigens to predict their phenotypes and facilitate serological typing; for immunized pregnant women to predict fetal Rh, K and HPA-1a antigen genotype as well as for non-immunized RhD-negative women for targeted RhD immunoprophylaxis. Also the laboratory was involved in the screening of HPA-1a in pregnant women and rare blood group and platelet antigen donor screenings. Apart from the routine diagnostics, the laboratory has participated in research programs such as evaluation of HLA/HNA allo-immunization risk in a population of allo-exposed COVID-19 convalescent plasma donors; and the establishment of a donor registry to secure/provide antigen-negative compatible blood for alloimmunized patients with multiple alloantibodies.

VS-2-3
Full immunohematological workup despite treatment with an anti-CD47 monoclonal antibody

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Background: Magrolimab is a monoclonal antibody targeting CD47. As a checkpoint inhibitor, it blocks the "don't eat me" signal expressed by cancer cells to avoid macrophage mediated killing. As CD47 is highly expressed on the surface of red blood cells as well, this therapy interferes with immunohematological workup. MagroEx, a new diagnostic reagent, blocks this interference thus enabling full immunohematological workup despite treatment with Magrolimab.

Methods: Here, we present a case of a 74-year-old 80 kg male AML patient treated with a first course of Magrolimab in a clinical phase 3 study. Due to an expected need of packed red blood cells (pRBC), extended blood group typing and antibody screening were initiated prior to administration of study medication. Column agglutination technique (Bio-Rad) was utilized for standard serological testing as well as antibody screening and differentiation. During saturation with study medication, extended RBC antigen typing (Kell, Cellano) was performed in LISS/Coombs in addition to cold antibody (IgM) screening in saline.

Results: In indirect agglutination testing (IAT) utilizing standard MagroEx concentration no agglutination was observed in samples with low study medication titers. After maximum saturation with study medication, an alternative protocol employing higher MagroEx concentrations was required in order to inhibit the agglutinating effect mediated by the therapeutic antibody. This use of a larger amount of MagroEx resulted in complete inhibition of Magrolimab interference, even at maximal anti-CD47 levels. Thus, the exclusion of alloantibodies and the provision of compatible pRBC became possible. Subsequently the patient could be safely transfused even during Magrolimab therapy.

Conclusion: The anti-CD47 study medication impaired antibody screening as well as serological crossmatch right from the start of treatment. By applying MagroEx a reliable inhibition of Magrolimab was achieved, so that valid serological crossmatches and antibody screenings became possible. Prior to treatment, extended blood grouping and antibody screening should be initiated as early as possible, to maximize transfusion safety. Fortunately, AB0 blood group typing is possible until day 12 of treatment.

Disclosure Statements: R. B. is a shareholder of imusyn

VS-2-4
Immunohematological test reagent for pre-analytical depletion of anti-CD47 antibody from patient plasma

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Background: CD47 is an immune checkpoint that promotes tumor cell evasion as a "don't eat me" signal. Consequently, anti-functional antibodies against CD47 are being tested as immune checkpoint inhibitors in a variety of malignant indications. Since, besides tumor cells, erythrocytes also express CD47, these antibodies cause pan-agglutination in the indirect antiglobulin test (IAT) with most available test systems, thus interfering with antibody screening and differentiation.

Methods: Using lentiviral vectors, the murine suspension cell MEL was engineered to express CD47 at a density of approximately 700,000 molecules per cell. These cells will henceforth be referred to as "Magrosorb". Patient plasma was spiked with the anti-CD47 antibody Magrolimab, then subjected to pre-incubation as indicated, subsequently co-incubated with test erythrocytes in LISS buffer on conventional gel agglutination cards. Shown left to right are untreated plasma, plasma treated with parental cells (not CD47-transduced) and Magrosorb cells. Negativity of the right-most plasma for anti-RBC immune antibodies is apparent (Figure 1).

Results: Human plasma spiked with Magrolimab, currently the leading candidate as clinical CD47 antagonist resulted in 4+ positive reactions during antibody screening on conventional gel agglutination cards. Incubation of 1×10^7 Magrosorb cells in 25 μl of Magrolimab-spiked plasma and subsequent removal of Magrosorb cells by centrifugation depleted Magrolimab, resulting in a cleanly negative antibody screening test. In selected spiked plasma from antibody patients, these blood group antibodies became readily specifically detectable. We also demonstrate the possibility to stabilize the cells in fixating buffers, providing the possibility of intermittent batch production and distribution of test cells.

Conclusion: Medicinal antibodies causing pan-agglutination of test erythrocytes and thus obscure diagnostics provide a novel challenge for immunohematology testing. High antigen expression on non-human cells allows specific depletion of these medicinal antibodies from patient plasma without interfering with underlying specific immune antibodies. The system provides a readily adaptable tool for other medicinal or immune antibodies.
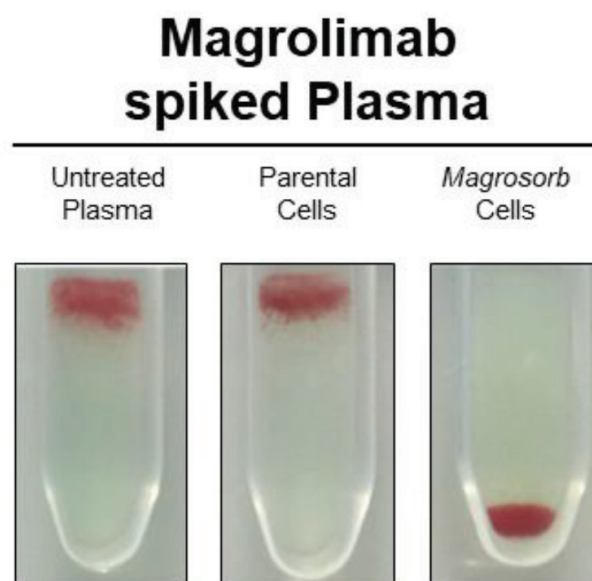

Magrolimab spiked Plasma

| Untreated Plasma | Parental Cells | *Magrosorb Cells* |

Fig. 1.

VS-3-1

Bioburden of postmortem bone donations depending on a collection time of more than 36 hours

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Background: To qualify for bone donation many criteria must be met. For recovery, a postmortem interval of 6 h until cooling of the deceased and a maximum recovery time of tissues within 24/36 h pm are mandatory. Expanding the recovery to donors with a longer pm-interval would be helpful e.g. to have more time to contact relatives for consent for donation. A loss of quality of bone tissues should not be expected in the early pm-interval, but the question of critical microbiological contamination remains.
Methods: According the study plan samples from the iliac crest should be recovered under standard recovery conditions between 48-54 hours (n=10, group 1) and 72-78 hours (n=10, group 2) postmortem. Samples were immediately frozen after recovery at <-18°C and sent to the tissue bank of the DIZG and the Charité. After preparation the samples were frozen again and sent to the contracted microbiological lab. Bioburden testing was there performed according the European Pharmacopoeia with the tissues samples after recovery (1), preparation (2) and CHCl3 treatment (3).
Results: As acceptance criterion a maximum amount of 10^5 CFU/g tissue was defined.

Currently samples from 10 of 10 donors from group 1 and 3 of 10 donors from group 2 were recovered, prepared and analyzed. All of the samples were within the defined limit with maximum values reaching $2{,}1 \times 10^2$ CFU/g. These values are in the range of bone tissues recovered under non-strict-aseptic recovery conditions within 24 respectively 36 hours and are within the capacity of the per-acetic acid sterilization process used by several tissue banks.
Conclusion: Preliminary results are very promising to expand the recovery time for bone tissue donation over the period of 24 or 36 hours. Therefore the outstanding 7 donors of group 2 (72-78 hours recovery time) have to be investigated.

VS-3-2

The new law to strengthen decision-making in organ donation - does the introduction of the online register endanger the supply of tissue?

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In March 2022, the law to strengthen the willingness to make decisions in organ donation came into force. This law applies also to tissue donation and it includes the establishment of a central electronic register to record the decisions made by individuals about their attitude towards donation. In the current state of the law access is restricted to hospital employees with an electronic health professional card, including transplantation representatives. Persons involved in donation and retrieval are denied access. Thus, tissue establishments must request the information stored in the registry from third parties in each potential donation case. This circumstance presents an obstacle to the donation process given the limited human and time resources on the clinic side and narrow time frames for performing tissue donation.

The DGFG held expert and background discussions with representatives in politics and medicine to explain the situation and processes of tissue donation and to talk about possible solutions. Based on the results of the discussions, the DGFG is working on a strategy to improve the situation in tissue donation on a legal level.

Our interlocutors share the concern that the introduction of a register, to which tissue donation establishments do not have access, could be detrimental to the donation processes. The practice, that those who do the donor screening must not be involved in the retrieval and further donation process, is proven for organ donation, as it prevents any conflict of interest in the sensitive area of brain death diagnostics. However, many experts were not aware that this separation of donation and retrieval does not exist in the case of tissue donation. Tissue donation always takes place after organ donation or, as in most cases, after cardiovascular death. This circumstance was not sufficiently taken into account in the regulation of the registry.

Contrary to the goal of the new law to improve the supply of patients with transplants, it currently puts a supply of tissues at risk. To ensure tissue donation and thus the sufficient and timely provision of tissue transplants to patients in future, tissue donation establishments must have immediate access to all donor-relevant information – regardless of the availability of third parties' personnel resources. Therefore, politicians should make appropriate changes in the law.

VS-3-3

Influence of timing of evaluation on corneal endothelial cell density in human donor corneas

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Introduction: The endothelial cell density of a donor cornea is the most important parameter in deciding its transplantability. The limit of ≥ 2000 cells/mm² is clinically established. The evaluation of endothelial cell density must be performed at least once at the end of cultivation. Due to newer concepts regarding a pre-disecting in the cornea bank or for an improvement of the predictability for the graft supply, intermediate evaluation during cultivation has been established.
Material and Methods: 100 donor corneas were subjected to endothelial cell microscopy between days 10 and 14 of organ culture and 16 to 36 hours after deswelling in culture medium containing 6% dextran. Endothelial cell density was determined with a computer program (Navis Nidek Technologies, Italy) on 3 digitized images at 200× magnification by an experienced investigator using the fixed frame method.
Results: In the intermediate evaluation, the mean endothelial cell density was 1966 cells/mm² (range 1539 to 2718, standard deviation 251), in the final evaluation after deswelling it was 1976 cells/mm² (range 1406 to 2800, standard deviation 324). The mean deviation of both endothelial cell densities was only 23 cells on average, but there was a high variation in a range from - 496 to + 570 cells/mm². 42 donor corneas showed an endothelial cell density below 2000 at the intermediate evaluation. 9 (21%) of them had a cell density above 2000 cells/mm² at the final evaluation. 58 donor corneas showed an endothelial cell density ≥ 2000 cells/mm² at the intermediate evaluation. 5 (9%) of them had a cell density below 2000 intermediate at the final evaluation.
Conclusion: The intermediate evaluation show a good correlation with the results of the final evaluation. Thus, it allows a reliable predictability of the transplantability of the donor cornea. For donor corneas with an endothelial cell density close to 2000 cells/mm² at the intermediate evaluation, a re-evaluation after deswelling is recommended, especially to avoid losing grafts due to an incorrectly low endothelial cell density.

VS-3-4
Shelf life extension of Tendon Allografts

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Background: Various types of human allograft tendons are used in sports medicine, foot and ankle as well as trauma surgery. The effects of sterilisation on tissue quality are varied (McGilvray et al. 2011), but peracetic acid (PAA) sterilisation has been demonstrated to preserve tissue biomechanics of bone-patella tendon-bone constructs (Scheffler et al. 2005). However, the influence of storage times on the biomechanical properties of PAA-sterilised tendons is currently unknown and will be evaluated here.

Methods: The tendons, tibialis anterior and posterior were processed and stored as finished medicinal products for either 0, 2 or 4 years (n=10, 10, 12 respectively). Tensile testing was performed on an InspektTableBlue (H&P, Germany). Samples were looped around bolts and thus secured on both ends using custom made cryoclamps while the mid-substance of the tendons remained free, adhering to material testing standards. Tendons received a 10-cycle preconditioning between 50 N and 250 N, a 5-minute load relaxation and a failure test with 300 mm/min speed. Tendons that did not suffer a mid-substance rupture (2-3 per group) but instead suffered a clamp failure were disqualified for the analysis of failure parameters.

Results: Compared to the control, tendons stored for 2 or 4 years did not display any significant differences regarding Young's modulus, stiffness and ultimate tensile strength. Four years stored tendons revealed an ultimate strength of 65.1±17.0 MPa (0 y: 50.9±18.8 MPa) and a Young's Modulus of 266.1±121.4 MPa (0 y: 316.8±119.9 MPa). Compared to short-term storage (0 y), a storage period of 2 and 4 years does not significantly affect the biomechanical properties of PAA-sterilised human tendon allografts.

Conclusion: Compared to short-term storage (0 y), a storage period of 2 and 4 years does not significantly affect the biomechanical properties of PAA-sterilised human tendon allografts.

Disclosure Statements: YG, DK, NK, VE, and JB were/are employees of the German Institute for Cell and Tissue Replacement (DIZG gemeinnützige GmbH), a non-profit provider of sterile allografts.

VS-3-5
Aspects of micobological safety of cardiovascular tissue preparations

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Introduction: To minimize the risk of infection in the recipient, cardiovascular tissue preparations are subjected to microbiological diagnostics. Usually, tissue and media samples are tested aerobically and anaerobically for microbial growth before and after antibiotic incubation.

Material and Methods: Twenty cryopreserved heart valves that were not or could not be used for clinical purposes and had therefore reached their expiration date after 5 years were thawed. The heart valves were rinsed with 0.9% NaCl solution and then cut in half under aseptic conditions. The two halves were incubated in 100 ml thioglycolate solution at 35°C and caso-boullion at 22°C, respectively, for 16 days and were checked for signs of microbiological growth daily.

Results: In two tissue preparations, bacteria were detected after cryopreservation. These two tissues already had abnormal findings before cryopreservation with detection of Streptococcus anginosus before antibiotic treatment in one case and with detection of Cutibacterium acnes before and after antibiotic treatment in the other case. All other 18 heart valves with negative microbiological findings after antibiotic treatment also showed negative findings in retesting after cryopreservation.

Conclusion: Retesting of the tissues showed complete agreement with the tests performed on reference samples before cryopreservation. The established concept of microbiological testing of cardiovascular tissue preparations appears sufficiently reliable.

VS-3-6
An update on allogeneic tissue transplants

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Background: Tissue engineering and cell therapy have become core elements of regenerative medicine. Allografts act as a promising vehicle for tissue regeneration. Different technologies are used to process them, enhancing their safety for clinical use. New technologies aim to make grafts more usable, increasing bone fusion, reducing immunogenic potential and minimizing disease transmission. Strict regulations ensure the safety of both tissue preparations and graft recipients.

Methods: Various technologies, such as high-precision CNC milling, allow for the development of allografts with unique properties that are tailored to specific tissue regeneration challenges, considering both the surgeon's needs and the patient's health. For example, a traditional acellular dermal matrix can be combined with a controlled milling process to form fibers capable of generating a stable axially vascularized free flap *in vivo* (Falkner et al., 2023).

Results: Fiberfill®, is a carrier graft consisting of cortical fibers and cancellous bone. It was specifically designed to provide high liquid absorption and high biocompatibility. It exhibits low immunogenicity, and is capable of forming new bone in an *in vivo* AV-loop model. High-precision CNC milling is the key process for the manufacture of allogeneic cortical bone screws with sufficient biomechanical properties to replace metal screws in certain indications, e.g. DIP arthrodesis. The intricately threaded design ensures a stable fixation in hand, elbow, foot and ankle surgery. In shoulder surgery, stability and high biomechanical requirements have to be met. Thus, a thick ADM with superior ultimate load and suture retention was developed.

Conclusion: New processing technologies and new transplant properties go hand-in-hand with each other. Hence, the surgeon's requirements serve as the foundation for the development of new allografts that are both usable and clinically relevant. New allografts cover a wide range of regenerative strategies including e.g. enhanced vascularization or improved absorption of functional liquids. Together, these grafts are a valuable addition to the regenerative medicine toolkit.

Disclosure Statements: VE, NA, and JB are employees of the German Institute for Cell and Tissue Replacement (DIZG gemeinnützige GmbH), a non-profit provider of sterile allografts.

VS-4
Sektion Präparative und therapeutische Apherese

VS-4-1
Therapeutische Apheresen bei neurologischen Erkrankungen

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Therapeutic Plasma Exchange (TPE) is a well-established Therapeutic Apheresis (TA) treatment and is safe in the therapy of acute progressive neuropathies with an immune etiology. The indication for Immunoadsorption (IA), a newer TA technique, depends on the availability and the experience of the physicians with that technology.

Most prevalent indications of TPE are acute progressive neuropathies with an antibody based immune etiology. The informed consent of the patient should carefully weight risk and benefit of the apheresis treatment considering adjunctive therapy. TA should improve the clinical outcome of patients reducing acute or chronic neurological symptoms. The frequency of TA depends on the improvement of the neurological symptoms. In neurological disorders, reduced muscle contraction or vascular tone could reduce the blood flow and may favor the indication for CVC for the

apheresis treatment. The replacement fluids of TPE are albumin or saline. Electrolytes (e. g. calcium) have to be carefully monitored and substituted. In intensive TPE protocols, depletion of plasma proteins may cause coagulation factor and immunoglobulin deficiencies which requires monitoring and adequate substitution in the treatment plan. The processed blood volume of 1.0–1.5 plasma volumes showed the best efficiency in TPE. The starting frequency of TA is recommended with 3–5 treatment procedures per week for a period of 1–3 weeks. The therapy control is mainly the improvement of neurological symptoms. TA alone or in combination with IVIG is appropriately used as short-term treatment in patients with life lifethreating symptoms in MG (i.e., MG crisis), in severe GBS, or in chronical neuropathies.

VS-4-2

Optimising leukocyte collection as starting material for ATMPs [Optimizing leukocyte apheresis as starting material for ATMPs]

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The arrival of autologous gene modified immune effector cells on the main stage confronts blood establishments with a new challenge, i.e. the generation, typically by leukapheresis, of autologous blood cells as starting materials for cell therapy manufacturing. The unexpected interest of immune effector cell manufacturers in leukapheresis stems from the observation that often the quality of the starting material is limiting – either manufacturing of the ATMP fails outright, or an in-spec product can be generate but the cells then do not engraft in vivo, engraft transiently, or fail to induce an optimal clinical response. In these cases, the cell processing laboratory or the clinician suspect the apheresis department's culpability for the inferior quality of the starting material as stakeholders outside the immediate field erroneously assume that apheresis can influence cell quality. Experience shows, however, that chronic and acute toxicity associated with the underlying disease and the frequently cytotoxic therapies there for affect effector cell quality and quantity, cell concentration in blood alone being a reasonable predictor of quality and hence, ultimate functionality in vivo. All the apheresis unit can do, within limits, is deposit an optimal number of target cells in the apheresis bag. Apheresis per se in these patients is fairly unremarkable except for the often lowly target cell concentrations in blood. Waiting with apheresis for peripheral blood cell counts to improve should be considered, the clinical situation of the patient permitting, as longer wash-out periods result not only in quantitative, but also in qualitative, functional improvement of the target cells, with immediate clinical benefit. If at all possible, wash-out periods of five half lives or more after qualitatively or quantitatively immunosuppressive medicines. Often, however, biological effects can linger far beyond the pharmacokinetics of a causal drug. If the decision to perform apheresis has been taken, rarely will a patient who is a candidate for immune effector cell therapy not be apheresis-eligible. Necessary and feasible process volumes to meet target doses should be calculated before apheresis, both to guide expectations of patient, clinical team and cell processing unit, to process adequate blood volumes, and to benchmark apheresis performance. This is particularly critical where patient size or venous access limit inlet flow and hence, total process volume. Suitable estimation tools have been proposed. Typically, relatively small process volumes are required. Minimal quality controls should include sterility testing and CBC as well as target cell concentration in blood and apheresis product to calculate total target cell dose and apheresis performance. In summary, observation of wash-out periods and hematopoietic regeneration before effector cell apheresis meaningfully improve cell quality as immune effector cell. Apheresis per se can only collect whatever quality of effector cell is represented in the blood stream. Stringent planning can guide apheresis duration, to achieve an improved patient experience and to protect limited resources.

Influence of the new substances of human origin (SoHo) regulation on preparative Apheresis

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While the current tissue & cell legislation has improved safety and quality in this field since it was implemented approximately 20 years ago, an evaluation carried out in 2019 identified a number of gaps and shortcomings. In particular, new infectious disease risks had emerged in the meantime and technologies for processing blood, tissues & cells have developed rapidly, with the legislation not keeping pace with these changes. In addition, it was concluded that donors were not given a high enough priority in terms of health protection and that the legislation was being applied in quite a variable way across the EU.

Therefore, the European Commission recently proposed a new Regulation for substances of human origin (SoHO), from blood, tissues & cells, but also breast milk or microbiota. More specifically, the new Regulation will aim at facilitating cross-border circulation of these critical health products. It will reinforce solidarity between public health authorities, while ensuring that the sector can manage the supply of critical substances and promoting innovation with the same high standards of quality and safety for all citizens that donate or are treated with these substances.

The proposal strikes a balance that protects donors from exploitation and from risks to their own health, while ensuring that they are not discouraged from donating. The approach builds on the principle of voluntary and unpaid donation and on published work by the Council of Europe (CoE) and it promotes the approach of ensuring "financial neutrality" for donors.

For cell collection by apheresis this means a better protection of volunteer cell donors but also of the recipients of such cells. Expert bodies as the European Centre for Disease Prevention and Control (ECDC) and the European Directorate for the Quality of Medicines & HealthCare (EDQM; CoE), to keep technical guidelines up to date are on board.

The Regulation, once adopted and implemented, replace the rules for safety and quality set out in two Directives (2002/98/EC, for blood & blood components and 2004/23/EC, for tissues & cells), and their implementing acts.

Currently, the proposal is examined and discussed, through the co-decision process, in the Parliament and the Council of the European Union until a final text is agreed.

VS-4-4

Process validation of platelet concentrates and plasma units – Evaluation of three different apheresis devices

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Background: To further ensure the supply of our oncological patients with platelet concentrates, three different apheresis machines were compared within the scope of a process validation. In addition to measurable product quality, donor feedback and employee handling of equipment are important process components. For this reason, major focus was placed on latter two aspects.

Methods: As part of the process validation, the critical process parameters (CPP) and critical material attributes (CMA) were used to determine the critical quality attributes (CQA). Besides these measurable quality attributes, questionnaires were used to evaluate the three different apheresis devices by the same donors and operators. It was planned to perform ten thrombocytapheresis and six plasmapheresis procedures with each device. Testing included platelet content, volume, residual cell count, pH, platelet aggregation, sterility, and swirling effect for the platelet concentrates. The following parameters were determined for the obtained plasmas: Residual cell counts, coagulation factor VIII, total protein and sterility.

Tab. 1.

	TRIMA Accel 7	AMICORE	MCS+
Donor feedback*	1	3	n.a.(early termination
Operator feedback*	1	2	due to multiple qc
			failings)
QC testing passed (%)	96.7	92.6	71.4

*grade from 1 (best) to 5 (failed) from internal evaluation score

Results: see Table 1

The total process time (including set-up times, donation time, post donation activities), the product yield (platelet per unit, single versus double units) and the possibility of plasma donation were identified as particularly relevant CPPs.

For the CMAs, the set configuration was identified, in particular the size of the primary label and other additional materials required.

Conclusion: Based on the results of the surveys and the measured quality control parameters, and taking into account our customer requirements, a clear preference was established from the three apheresis machines evaluated. Device qualification was performed after installation with a total number of 12 additional runs per device.

VS-4-5
Selection of exchange fluid for therapeutic plasma exchange (Leipzig Protocol)

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Background: Therapeutic plasma exchange (TPE) provides evidence-based clinical benefit in a variety of disorders (American Society for Apheresis, DOI: 10.1002/jca.21705). As exchange medium, recommendations reserve fresh frozen plasma for thrombotic thrombocytopenic purpura or to replace clotting factors (e.g. JPAC, United Kingdom; DGTI Section Preparative and Therapeutic Hemapheresis, Germany). Since clear rules are lacking, we initiated a protocol for standardized selection of exchange media in TPE.

Methods: Patients are categorized into a total of five groups as to liver status, fibrinogen (Fg) levels before TPE and recent (24h) bleeding (see figure 1). Principally, 100% Albumin±crystalline solution is used if Fg is > 1.5 g/l in patients with healthy liver. In patients with liver cirrhosis and if Fg is 1.5-2 g/l with no bleeding events <24h, 50%/50% FFP/Albumin or 100% Albumin plus 2g Fg post TPE i.v. are used. If bleeding is recorded the last 24h or if Fg is <1.5 g/l, 100% GFP is used. Bleeding is assessed according to in-house standards and SOPs.

Results: We treated a cohort of 26 patients with the following indications for TPE: acute autoimmune exacerbation (n=13), acute liver failure (n=7), suspected or confirmed TTP (n=3), imminent transplant rejection (n=2) and pretransplant conditioning (n=1), representing a total of 132 TPE procedures. The selection procedure for exchange media was applicable throughout and protocol adherence was nearly complete. No increased bleeding tendency or bleeding complications were observed in the patients. Preliminary data show a significantly lower consumption of donor plasma compared to historical controls. Further analysis in currently ongoing including the number of transfusion incidents, and will be further substantiated by ongoing TPEs.

Conclusion: Our protocol proved feasable and represents a novel instrument to standardize the use of colloids vs FFP in patients undergoing TPE procedures. It is expected to reduce transfusion incidents and immune sensitizations, without exposing patients to additional risks of bleeding

Protocol for Selection of Exchange Media in TPE

Category	Liver status	Fibrinogen (g/l) pre TPE	Bleeding	Albumin (%)	FFP (%)	Post TPE Fibrinogen
1	normal	≥ 1.5	No	100	-	-
			Yes	50	50	-
2	normal	< 1.5	Yes or No	100[1]		2g
				-	100[2]	2g
3	cirrhosis	≥ 2.0	No	100	-	
			Yes	100		2g
4	cirrhosis	1.5-2.0	No	50	50	2g
			Yes	-	100	-
5	cirrhosis	< 1.5	Yes or No	-	100	-

[1] age $\leq$ 60y; [2] age > 60y

Fig. 1.

complications. Further benefits lie in the preservation of donor plasma, which is a limited source for production of fractionation-derived products. Finally, it should be cost-effective.

VS-5
Sektion Stammzelltransplantation und Zelltherapie

VS-5-1
Alternative curative treatment options for sickle cell disease and transfusion dependent thalassemias

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Hemoglobinopathies (HGB), namely sickle cell disease (SCD) and transfusion-dependent thalassemias (TDT), are the most prevalent genetic disorders worldwide. Approximately 5% of the world's population are healthy carriers of a gene for SCD or TDT. Despite significant advances in the prevention and treatment of these HGB, the associated morbidity and mortality remain high. The only curative therapy for patients with HGB is a hematopoietic stem cell transplantation (HSCT). Event-free (EFS) and overall survival (OS) for HLA-identical HSCT, particularly before the age of 15 years now exceeds 90%. Due to the ethnic background of many HGB patients, the probability of finding a matching HLA-identical donor is less than 20%. Due to the available in vivo and ex vivo T-cell depletion strategies, haploidentical HSCT could be developed as a very efficient curative treatment option, which is also investigated in prospective studies (NCT04201210). In addition, gene therapy and gene editing are currently being tested in several trials, some procedures of which are close to approval. The most advanced cell therapy is a procedure to reactivate fetal hemoglobin (HbF) by ex vivo CRISPR/Cas9 gene editing of autologous CD34+ hematopoietic stem cells (HSPCs). Two studies have shown that a single dose of the gene product increases hemoglobin F (HbF) and total Hb to avoid red blood cell transfusions in TDT and vaso-occlusive crises (VOCs) in SCD. Conclusions: A variety of promising alternative therapeutic approaches are now available, leading to transfusion independence or cessation of VOCs in almost all patients with TDT/SCD.

VS-5-2

CRISPRing the Genome: Lessons Learnt from Evaluating On- and Off-Target Effects

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Genome editing with programmable nucleases such as CRISPR-Cas has ushered in a new era of personalized medicine. In recent years, several approaches for the treatment of congenital and acquired diseases have been developed and successfully transferred into clinical trials. The first CRISPR-based drugs for the treatment of transthyretin amyloidosis and hemoglobinopathies are expected to be approved in Europe this year. In my talk, I will explain the principles of genome editing, present some examples of successfully applied editing strategies, and conclude with diagnostic procedures that enable us to assess the risk of genotoxicity and to improve the safety of these new therapeutic approaches.

Disclosure Statements: T.Ca. ist als Berater von Cimeio Therapeutics, Excision BioTherapeutics und Novo Nordisk tätig. Er erhielt Honorare von CSL-Behring und Pfizer, kooperiert mit der Fa. Cellectis und ist Inhaber von Patenten zur Bestimmung der Off-Target-Aktivität von CRISPR/Cas-Nukleasen.

VS-5-3

Characterization of stem cell product quality in vitro and by in vivo function through hematopoietic engraftment

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Background: Stem cell transplantation is a quality-controlled key element in treatment of hematological disorders. Characterization of stem cell products follows internationally standardized criteria and is supervised by accreditation standards. We aimed to characterize the quality of all allogenic stem cell products obtained in our collection center produced within a 5-year period, and investigate stem cell function by engraftment criteria in two subcohorts of related donors transplanted in our hospital.

Methods: A total of 193 G-CSF mobilized peripheral blood (PBSC) and 74 bone marrow (BMSC) products collected 2018-2022 were analyzed for a total of 27 parameters including volume, CD34$^+$ and T cell content. Graft function was analyzed in a cohort of adult patients with malignancies receiving PBSC (n=24) and pediatric mainly nonmalignant patients receiving BMSC (n=12). Successful engraftment was defined as neutrophil (NEU, >0.5 G/l), platelet (PLT > 50 G/l) and complete donor chimerism (>90%) determined by STR analysis and absence of overt relapse. Median follow-up for patients in the subcohort of related transplantations was 321 days.

Results: Unrelated PBSC contained a median of 7.9×10^6 (range: 3.2–28.9) and related PBSC a median of 7.5 (range: 3.4–22.1) $\times 10^6$ CD34$^+$/kg. Unrelated BMSC contained median 3.3 (range: 1–22.2) and related BMSC median 5.7 (range: 1.6–20.7) $\times 10^6$ CD34$^+$/kg. In the related cohorts, median transplanted cell doses were 8.2 PBSC $\times 10^6$ CD34$^+$/kg (3.4–22.1) and 7.0×10^6 BMSC CD34$^+$/kg (1.58–12.32). Median time to NEU recovery in days was 17 (10–23; PBSC) and 22 (14–28; BMSC) and to PLT recovery 15 (12–37; PBSC) and 22 (15–37; BMSC). Complete donor chimerism was reached in 20/24 of PBSC and 12/12 of BMSC patients. In the PBSC group, 1 patient died before engraftment and 1 rejected the graft. In BMSC, high graft volumes were difficult to apply in most children.

Conclusion: Guideline-conform in vitro product quality was reached throughout. Engraftment analysis indicated a slightly slower NEU and PLT engraftment in the BMSC compared with PBSC cohort. All but 2 PBSC patients reached engraftment. Grade 3 and 4 GvHD was so far observed in 1 PBSC and 1 BMSC transplant recipient. Pediatric BMSC could be optimized by volume reduction. Our analysis demonstrates that conform in vitro transplant quality is associated with robust stem cell regenerative function in our center.

VS-5-4

Allogeneic cryoPBSC, DKMS-1: Banking of the world's first allogeneic undirected cryopreserved hematopoietic stem cell transplant

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Background: Cord blood transplants are a promising opportunity for patients with hematological diseases. However, application rates are declining, complicating the economic sustainability of public cord blood banks. The DKMS Stem Cell Bank has established high standard processes in terms of processing, cryopreservation, quality control, transport and quality management. These represent excellent conditions for the development, processing and storage of further cell products.

Methods: For the further development of a cord blood bank into a stem cell bank, existing processes, facilities and resources were used to expand activities. An existing quality management system and official approvals were built on. The technical requirements of the cord blood bank were adopted and expanded for the stem cell bank. The manufacturing process had to be implemented and validated, the quality control methods had to be adapted to the new cell product and re-validated. Further adjustments were made, e.g. with regard to the integration of apheresis centers, the expansion of the IT infrastructure, the addition of official approvals and the implementation of the PBSC listing via the DKMS Registry.

Results: In the new GMP clean room unit of the DKMS Stem Cell Bank, allogeneic undirected cryopreserved PBSC products are produced with 5% DMSO and a volume of 2×100 ml. The quality of each batch is verified using validated quality control methods. The documentation is carried out using GMP-compliant software. The high quality of the stem cell products was verified both before freezing and after thawing and after 18 months of storage as part of approval-relevant validations. In 2022, the manufacturing license of the DKMS Stem Cell Bank was extended to include allogeneic undirected cryopreserved PBSC preparations. In 2023, approval was granted for "Allogeneic cryoPBSC, DKMS-1", the world's first allogeneic undirected cryopreserved PBSC product.

Conclusion: The DKMS Stem Cell Bank has evolved from a pure cord blood bank to a stem cell bank. With "Allogeneic cryoPBSC, DKMS-1" the world's first allogeneic undirected cryopreserved PBSC product was approved. The advantages of this ready-to-use drug are a significant reduction of the time-to-transplant, the improved possibility of transplantation coordination and the previously known product specifications of the selected preparation.

VS-6
Sektion Sicherheit von Blutprodukten

VS-6-1

Babesiosis: Just "canine malaria" or also a risk to the safety of blood components?

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Babesiosis is the clinical disease named for infection with any of the members of the *Babesia* genus, a group of tick-borne, intraerythrocytic protozoan parasites. Over 100 species of *Babesia* infect vertebrate hosts, mostly attributed to *Babesia microti*. The latter gained attention with its recognition as a leading infectious risk to the United States (US) blood supply. *B. microti* is readily transfusion transmissible via any red cell containing product. *Babesia* infection may be subclinical or mild in immunocompetent adults; however, *Babesia* can persist for months to years in some infected individuals, thus escaping detection at the time of blood donation. For neonates, patients aged >50 years, asplenic, and immunocompromised patients (e.g. immunosuppressant therapy), babesiosis may be even fatal. These same populations are notably over-represented among transfusion recipients, the likely basis for the high all-cause mortality (~19%) associated with transfusion-transmitted babesiosis (TTB).

A 2014 seroprevalence study of Tyrolean blood donors (n=988) found that 2.1% of donors demonstrated IgG antibodies against the *B. divergens* complex and 0.6% were seropositive against *B. microti*. Recently we conducted a *Babesia* molecular surveillance study of blood donors in Tyrol, Austria. Testing was conducted using the cobas® *Babesia* assay (Roche Molecular Systems, Inc.), a commercial PCR assay approved for blood donor screening that is capable of detecting the 4 primary species causing human babesiosis (i.e., *B. microti*, *B. divergens*, *B. duncani* and *B. venatorum*). A total of 7972 donors were enrolled and screened. No positive cases of *Babesia* were detected. The findings suggest that the current risk to the local blood supply is low.

The discordance between serological findings from previous regional surveillance and our molecular testing results is unsurprising and echoes results from other studies. A high proportion (almost 90%) of infected individuals resolve infection (i.e., as reflected by negative molecular testing) within 1 year of an index positive test.

Despite our negative finding, tick-borne diseases —including babesiosis— appear to be on the rise. One possible reason is global warming, leading to shortened tick life cycles, higher birth rates and wider distribution of ticks in new areas. With regard to this aspect molecular surveillance seems to be an adequate tool to predict increasing risk for TTB.

VS-6-3

Quality of methylene blue/light treated plasma using a DEHP-free bag system

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Background: Di-ethyl-hexyl-phthalate (DEHP) is currently one of the major plasticizers used in blood bags. Due to its endocrine disrupting properties European regulators decided to ban its use in medical devices. Although, the final sunset date is not yet clear, DEHP-free blood bags will be essential in Europe within the next years. In the current study the quality of Methylene blue/light treated plasma using the DEHP-free version of the THERAFLEX MB-plasma system (Macopharma) was investigated.

Methods: Whole blood units (500 mL) were collected into 70 mL CPD anticoagulant solution in regular, DEHP-containing bags and kept at room temperature (22±2°C) overnight. After centrifugation plasma was separated and processed within 18 h after whole blood donation. Plasma units (n=8) were connected to the DEHP-free THERAFLEX MB-Plasma disposable (REF PROSDV1, Macopharma) and MB/light treatment was done according to the instructions of the manufacturer using the Macotronic B2 illumination device with a light dose of 120 J/cm². Plasma was then transferred through the Blueflex filter into the storage bag and frozen.

Results: Samples were taken before treatment (sample a), after Blueflex filtration (sample b) and after storage for 1 month (sample c) for the measurement of plasma factors showing a significant effect on the plasma factors (table 1). Coagulation times significantly increased (aPTT 7% and TT 13%) while the coagulation factors decreased significantly except for factors VII and protein S. Especially, fibrinogen (Clauss) (25%), factor V (10%), factor VIII (22%) and factor XI (18%) decreased. European guideline specifications however were hold for all plasma units. Factor VIII was >70% in untreated samples (82-170%) and >50% in MB/light treated samples (56-146%). The decrease of fibrinogen activity was <40% (17,1%-28,6%) after MB/Light treatment.

Conclusion: The plasma quality of MB/light-treated plasma using the THERAFLEX MB-Plasma disposable PROSDV1 without DEHP showed the expected increase/decrease of plasma factors. Data was comparable to published data obtained for the DEHP-containing disposables.

Disclosure Statements: Study was sponsored by Macopharma Productions (CS).

Tab. 1.

Parameter	Sample	Mean	SD	De- or increase [%]
aPTT [s]	a	34	2.9	
	b	37	2.3	7.4
	c	39	3.7	14.0
TT [s]	a	17.6	0.7	
	b	19.9	1.2	13.2
	c	19.7	1.1	12.0
Fibrinogen (Clauss) [g/l]	a	3.1	0.4	
	b	2.3	0.4	-24.9
	c	2.3	0.3	-23.7
Factor V [%]	a	105	20.3	
	b	94	19.3	-10.4
	c	83	17.1	-21.4
Factor VII [%]	a	96	23.5	
	b	94	22.3	-1.4
	c	106	24.5	10.5
Factor VIII [%]	a	107	30.7	
	b	84	28.9	-21.8
	c	72	20.5	-32.6
Factor X [%]	a	87	10.0	
	b	81	10.0	-6.9
	c	82	10.2	-5.3
Factor XI [%]	a	110	11.9	
	b	90	12.7	-18.4
	c	87	12.3	-20.7
free Protein S [%]	a	91	19.5	
	b	89	18.1	-2.5
	c	96	22.7	4.9

VS-6-4
Bacteria elimination from plasma by methylene blue/light treatment using a DEHP-free bag system

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[2]Macopharma, Tourcoing, France

Background: Di-ethyl-hexyl-phthalate (DEHP) is currently one of the major plasticizers used in blood bags. Due to its endocrine disrupting properties European regulators decided to ban its use in medical devices. Although, the final sunset date is not yet clear, DEHP-free blood bags will be essential in Europe within the next years. The bacteria elimination capacity by Methylene blue (MB)/light treatment of plasma using the DEHP-free version of the THERAFLEX MB-plasma system (Macopharma) was investigated.

Methods: Relevance of the THERAFLEX MB-plasma procedure steps (leukocyte depletion (Plasmaflex filtration), MB/light treatment, removal of MB and photoproducts (Blueflex filtration)) were examined for their impact on elimination of two different bacteria species (*Klebsiella pneumoniae* and *Brevundimonas diminuta*). Leukodepleted plasma was prepared from whole blood using standard blood banking technology (containing DEHP). Plasma units (n=3 for each bacteria strain) were spiked with bacteria suspension (≈ 106 CFU/mL). MB/light treatment was done according to the manufacturer's instructions (120 J/cm², Macotronic B2 illumination device) with the DEHP-free THERAFLEX MB-Plasma system PROSDV1. Bacteria titer was determined by plating on agar plates.

Results: Samples were taken after spiking, Plasmaflex filtration, illumination and Blueflex filtration and the log10 reduction was calculated. *K. pneumoniae* was reduced by ≥ 5.7 log steps by filtration through the Plasmaflex filter. A reduction factor of at least 5.9 log steps was achieved by the entire process. *B. diminuta* was reduced by ≥ 2.2 log steps by Plasmaflex filtration. A further reduction below the limit of detection was achieved by subsequent irradiation and Blueflex filtration so that an overall reduction factor of at least 4.7 log steps was achieved for the entire process.

Conclusion: In the current study it could be demonstrated that bacteria species *Klebsiella pneumoniae* and *Brevundimonas diminuta* were efficiently removed from plasma by using the THERAFLEX MB-Plasma System PROSDV1 (non-DEHP). Due to a higher spiking concentration log reduction factors achieved in this study are even higher than formerly published for DEHP-containing THERAFLEX MB-Plasma systems.

VS-7
Sektion Hämotherapie

VS-7-3
Leitlinie Polytrauma

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The guideline „S3-Leitlinie Polytrauma/Schwerverletzten-Behandlung" of the working group AWMF (Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften) was revised between 2020 and 2022. This contribution reviews the chapters of the guideline about coagulation management.

Persistent severe bleeding remains the most frequent cause of death after severe traumatic brain injury or severely injured polytrauma patients in general. The knowledge about factors influencing the coagulation system during severe trauma has improved since the last version of that guideline.

The method, a systematic literature review, was performed by the IFOM (Institut für Forschung in der Operativen Medizin), using MEDLINE (via Ovid) and EMBASE (via Elsevier) as literature source, and found more than 3,000 articles. The evidence criteria were prospectively determined by use of the PICOS („population, intervention, comparison, outcome, study design") format which led to three grades of recommendation (GoR), A, B or 0, including benefit-risk considerations, directness and the homogeneity of the evidence, as well as clinical expertise. "Mortality" is the most important patient-relevant endpoint. Studies which demonstrate a survival benefit led to a GoR rating.

Trauma induced coagulopathy (TIC) is an independent clinical picture and risk factor which impacts on patient's survival. Starting coagulation therapy thus is critical for patient's clinical outcome and should be started at least during shock room treatment. Most critical factors for TIC are hypoxia, endothelial cell and tissue damage, release of tissue plasminogen activator (tPA), platelet dysfunction, as well as hyperfibrinolysis. Early active bleeding control helps to prevent TIC and thus improves the clinical outcome of polytrauma patients.

VS-7-4
The transfusion committee as a tool for successful PBM, experiences of a university hospital

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Patient Blood Management (PBM) is a comprehensive approach and relies beside other components on training and education. We investigated the impact of information to the training provided for transfusion officers in our university hospital's Transfusion Committee (TraCo), on transfusion indication settings, with a special focus on Hb- triggers and single unit use Triggers and single unit use of erythrocyte units (RBCs) were presented during TraCo sessions held in Nov 21 (Dataset 1 (DS1); 8470 transfusion events (TrEvs) involving 17898 RBC units, Jan 21 -Oct 21) firstly, categorized by specialty and ward type. TrEvs were included in the analysis if the pre- and post-transfusion Hb values were available in the laboratory information system and could be assigned to the TrEvs. Multiple units transfused within a 6-hour interval were considered as a single TrEv and attributed to the specialty and kind of ward where the first transfusion occurred. The same information was presented in the TraCo session held in May 22 (Dataset 2 (DS2); Nov 21 to May 22; 9349 TrEvs with 18083 RBCs). DS1 and DS2 were then compared with Dataset 3 (DS3, Jun 22 to Apr 2023; 5856 TrEvs involving 11279 RBCs).

Overall, the mean Hb transfusion trigger decreased from 8.4 g/dl (DS1) to 8.1 in DS2&3 (median). The proportion of TrEvs with a pre-transfusion Hb level below 7 g/dl increased from 16% (DS1) to 17% (DS2) and 18% (DS3). Notably, the departments of internal medicine exhibited a decrease in the transfusion trigger (median: DS1 7.2 g/dl, DS2 6.9 g/dl, DS3 6.8 g/dl), as did the intensive care units (median DS1 8.5 g/dl, DS2&3 8.3). Single unit use increased significantly in the ambulatory setting (31% in DS1 vs. 37% in DS2&3), the ICUs (42% in DS1 vs. 48% in DS2&3), and the operating theater (33% in DS 1 vs. 38% in DS2&3). Surgery and hematology/oncology departments also showed an increase in the proportion of single unit use (37% in DS1 vs. 43% in DS2&3).

Our study demonstrates a notable decrease in the transfusion trigger within the hospital, along with an increase in the utilization of single unit donations during the study period. Although outstanding leaps were not observed, the findings indicate a continuous improvement trend. The comprehensive presentation of transfusion triggers and – success to the transfusion officers proves to be a valuable tool in achieving the goals of the anemia reserve pillar of PBM and can effectively support PBM as an accompanying measure.

Counting of transfused platelets in recipient blood by digital PCR of 7 mitochondrial markers – analytical qualities

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Background: Recovery and survival of transfused platelets can be assessed by digital PCR of mitochondrial markers. Therefore, we investigated the key analytical qualities of the droplet digital PCR of seven mitochondrial markers.

Methods: The SPEF1 gene served as internal control for the entire process and was tested for intra-and inter-assay variability with 20 replicates and 10 independent PCR runs, respectively. Limit of blank (LoB: mean copy number NTC + 1.645 × SD copy number NTC), limit of detection (LoD: LOB + 1.645 × SD copy number non-blank material) and limit of quantification (LoQ: lowest platelet concentration with a coefficient of variation ≤ 25%) were determined with 10different PCR runs and samples measured in duplicate. The linearity was evaluated by dilution of platelets (0.07 to 10 G/L) tested in quadruples. Determination of the assays" validity was done by comparison with flow-cytometry and hematology analyzers (Symex XS 800i and Advia 2120i).

Results: Assay linearity was demonstrated by R^2-values above 0.99, the slope varied in respect to the different markers between 0.738 and 1.382 and intercept ranged from -0.03 to 0.16. DNA was prepared from EDTA anticoagulated samples (n=10) on a daily basis and tested in the ddPCR assay. The maximal difference in platelet count between day one and day five of sample preparation was 0.5 G/L. DNA samples stored at -30°C showed mean differences to the initial results of 0.131± 0.5 (one month), 0.121 ± 0.5 (three months), 0.130 ± 0.2 (six month) and 0.126 ± 0.180 G/L platelets after 12 months" storage. Validity of ddPCR results was in the range of R^2 = 9.3 – 9.5. The calculated recovery of the internal control was 79.9%±7.93.

Conclusion: Quantification of endogenous and transfused platelets with ddPCR offers a sensitive and reliable tool to monitor platelets in patients.

Long-COVID patients show enhanced formation of COHb and changes in Raman spectra of Hemoglobin–associated vibrational modes

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Background: The Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was the cause of the global COVID-19 pandemic. Irrespective of the disease severity, a growing number of patients report a delayed state of exhaustion called chronic fatigue syndrome (CFS). At least 10% of all COVID-19 patients experience various persistent or new health complaints after SARS-CoV-2 infection lasting longer than three months. In addition to CFS, Long-COVID patients also suffer from dyspnea, anemia and others. Recently, we demonstrated that SARS-CoV-2-induced dysregulation in hemoglobin (Hb)- and iron-metabolism contributes to the severe systemic course of COVID-19. Changes in Hb structure may also be significantly involved in the development of Long-COVID symptoms. Therefore, we analyzed Hb- and iron-metabolism in Long-COVID-Patients.

Methods: We performed blood gas analysis of more than 20 Long-Covid-19 and control patients. Furthermore, we measured hematological parameters and Raman spectra of red blood cells (RBCs) from peripheral blood of Long-COVID patients and compared those with healthy donors.

Results: Whereas, no differences in the RBC count, Hb content and hematocrit were observed in the blood of Long-COVID patients compared to healthy donors, the amount of COHb was significantly increased. Furthermore, we found significant changes in spin state of the iron in Hb, which were partially comparable to packed RBCs stored for 42 days. In addition, BE- and BI values but not pH were diminished in Long-COVID patients compared to healthy donors, albeit still within the normal range. Lactate levels were also significantly elevated in the blood of Long-COVID patients. Thus, O2Hb was reduced in Long-COVID patients.

Conclusions: Our data shows drastic changes in Hemoglobin–associated vibrational modes in samples of Long-COVID patients. Together with the diminished Acid-base balance and enhance formation of COHb, we suggest an impaired erythrocyte functionality in Long-COVID patients, leading to diminished oxygen supply. This in turn could be an explanation for the CFS and dyspnea as well as anemia. The project was funded by the Roland-Ernst-Stiftung.

mt marker	LoB				LoD				LoQ			
	FAM copies/µL	plt (G/L)	VIC copies/µL	plt (G/L)	FAM copies/µL	plt (G/L)	VIC copies/µL	plt (G/L)	FAM copies/µL	plt (G/L)	VIC copies/µL	plt (G/L)
73	0.23	0.025	0.33	0.036	0.64	0.070	1.15	0.126	2.55	0.280	4.06	0.446
195	0.25	0.025	0.20	0.022	0.61	0.067	0.58	0.064	2.16	0.237	3.90	0.429
295	0.09	0.010	0.12	0.013	0.25	0.027	0.28	0.031	2.17	0.238	1.84	0.202
310	0.23	0.025	0.80	0.088	0.72	0.079	2.28	0.251	4.04	0.444	3.42	0.376
16069	0.21	0.023	0.15	0.016	0.54	0.059	0.64	0.070	2.68	0.294	5.27	0.579
16399	0.36	0.040	0.49	0.054	0.52	0.057	0.65	0.071	2.74	0.301	1.79	0.197
16519	0.46	0.051	0.30	0.033	0.79	0.087	0.63	0.069	1.60	0.176	2.86	0.314

Fig. 1.

VS-8
Sektion Hämostaseologie

VS-8-1
New treatment options for haemophilia

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Hemophilia A (HA) and hemophilia B (HB) are inherited blood clotting disorders in which either factor VIII (FVIII) or factor IX (FIX) is absent or has reduced activity. New therapeutic approaches in hemophilia focus on improving patient-relevant outcomes related to bleeding risk, joint health, and social participation.

Prophylactic therapy, in which patients are regularly administered clotting factors, is the standard of care to reduce spontaneous and traumatic bleeding. An alternative treatment option in HA with and without inhibitors to FVIII is the bispecific monoclonal antibody emicizumab, which is administered subcutaneously and mimics the function of activated FVIII. Phase 3 clinical trials are investigating other drugs that offer further half-life extension, such as efanesoctocog alfa, or interfere with the impaired coagulation system as non-factor therapies. These include monoclonal antibodies to block tissue factor pathway inhibitor (TFPI) and a small interfering RNA (siRNA) that suppresses antithrombin formation.

AAV-based gene therapy is approved for the treatment of HA and HB and offers potential for further improvement. By permanently increasing clotting factor levels, the risk of severe bleeding could be reduced and the development or progression of joint damage could be prevented. The long-term nature of gene therapy may also improve patients' participation in social life.

Disclosure Statements: Bayer, Biomarin, Biotest, CSL Behring, Chugai, Freeline, LFB, Novo Nordisk, Octapharma, Pfizer, Regeneron, Roche, Sanofi, Takeda/Shire, uniQure

VS-8-2
Mouse models in thrombosis and hemostasis research

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Da *ex vivo*-Analysen die komplexen biochemischen und zellulären Vorgänge der Thrombose und Hämostase nur unzureichend widerspiegeln, bleiben standardisierte Mausmodelle ergänzend zu funktionellen Analysen von Blutproben gesunder Probanden und Patienten für ein genaues Verständnis der an Thrombophilien und Hämophilien beteiligten Mechanismen unabdingbar. Mittels standardisierter Mausmodelle zur Untersuchung der Hämostase und der arteriellen und venösen Thrombose konnten die *in vivo* beteiligten Zell-Zell-Wechselwirkungen sowie die wesentlichen Signalmechanismen und Adhäsionsrezeptoren genauer definiert werden.

Als Blutungsmodelle der Maus werden das Schwanzblutungsmodell zur Analyse der Hämostase sowie ein Gelenkblutungsmodell zur Erforschung der Hämarthrose bei Hämophilien vorgestellt. Zur Untersuchung der an der arteriellen Thrombusbildung beteiligten Mechanismen wird das Ligaturmodell der *Arteria carotis* als ein Modell zur Quantifizierung der Thrombozytendeposition erklärt. Zur Untersuchung der an der tiefen Venenthrombose beteiligten Pathomechanismen wird das *Vena cava*-Stenosemodell erläutert. Zusätzlich wird das mesenteriale Ischämie-Reperfusions-Mausmodell zur Untersuchung des Ischämie-Reperfusionsschadens beim akuten Mesenterialinfarkt vorgestellt.

Die experimentelle Anwendung dieser Blutungs- und Thrombosemausmodelle und deren Bedeutung für das mechanistische Verständnis der Thrombose und Hämostase wird anhand ausgewählter Beispiele veranschaulicht.

Standardisierte Mausmodelle sind für die Erforschung der an Thrombophilien und Hämophilien beteiligten Pathomechanismen von enormer translationaler Bedeutung und sind für die Entwicklung und Optimierung therapeutischer Interventionsstrategien unerlässlich.

VS-8-3
Quantitative pharmacokinetic model to characterize and extrapolate long-term FVIII activity levels in patients with severe hemophilia A treated with valoctocogene roxaparvovec

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Background: Valoctocogene roxaparvovec delivers a B-domain deleted factor VIII (FVIII) coding sequence with an adeno-associated virus vector to reduce bleeding and FVIII concentrate use in people with severe hemophilia A. The aim is to characterize the long-term trajectory of transgene-derived FVIII activity using a linear mixed effects (LME) model to estimate mean and median FVIII activity levels 5 years post-infusion.

Methods: In GENEr8-1, an open-label, single-arm, multicenter phase 3 trial, 134 participants with severe hemophilia A received a single 6×10^{13} vg/kg dose of valoctocogene roxaparvovec. FVIII activity was assessed using the chromogenic substrate assay and one-stage assay.[i] Ln-transformed FVIII activity values from week 76 to 104 were fit to the LME model with random effects for participants on slope and intercept using a restricted maximum likelihood method with the lmer package in the R software. The precision of parameter estimates and model diagnostics were evaluated to confirm goodness-of-fit. The model and extrapolation approach was further qualified by comparing to observed FVIII activity at week 156.

Results: A previously published quantitative pharmacokinetic (PK) model[ii] was updated to extrapolate FVIII activity levels to 5 years post-infusion. The final LME model dataset included 928 observations from 120 participants. The long-term trajectory of FVIII activity was consistent with first-order elimination kinetics starting at week 76. Model parameter estimates were consistent with the previously published model as shown in **Table 1**; diagnostic plots showed no major deficiencies. FVIII activity was extrapolated to 5 years post-gene transfer (**Table 2**). Mean and median FVIII activity extrapolations at week 156 were consistent with observed values, confirming adequacy of the model.

Conclusion: Pharmacokinetic modeling indicates valoctocogene roxaparvovec-derived FVIII activity levels will remain in the mild hemophilia range for ≥5 years post-gene transfer for the majority of patients treated.

Disclosure Statements: Marco Stadler is an employee of BioMarin Pharmaceutical.

Tab. 1.

Table 1. Parameter estimates for the LME model

	Parameter	Typical value	SE (%)	Lower 2.5th	Upper 97.5th
Fixed effects	Intercept	3.29	4.67	2.98	3.59
	Slope	-6.03×10^{-3}	21.1	-8.52×10^{-3}	-3.52×10^{-3}
	Groups	**Name**	**Variance**	**SD**	**Corr**
Random effects	SUBJID	(Intercept)	1.839	1.36	
		Time (Weeks)	7.371×10^{-5}	8.59×10^{-3}	-0.78
	Residual		7.67×10^{-2}	0.277	

Corr, correlation; LME, linear mixed effects; SD, standard deviation; SE, standard error.

Tab. 2.

Table 2. Extrapolated FVIII activity for GENEr8-1 6×10^{13} vg/kg participants

	FVIII per CSA, IU/dL	
	Mean ± SD	Median (min, max)
Week 104	22.3 ± 29.9	11.2 (BLQ, 173)
Week 156	17.2 ± 25.4	8.8 (BLQ, 160)
Week 208	13.8 ± 22.9	6.4 (BLQ, 149)
Week 260	11.6 ± 21.3	5.0 (BLQ, 139)

BLQ, below the limit of quantitation; CSA, chromogenic substrate assay; FVIII, factor VIII; SD, standard deviation.

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VS-8-4

Prevalence of anti-PF4/Heparin antibodies after second COVID-19 vaccination in healthcare workers

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Background: Anti-platelet factor 4 (PF4) antibodies have been identified as the cause of vaccine-induced immune thrombotic thrombocytopenia following adenoviral vector-based COVID-19 vaccines. Previous studies have primarily focused on determining the frequency of anti-PF4 antibodies shortly after the first COVID-19 vaccination, leaving the rates of anti-PF4 antibody positivity after second vaccinations largely unknown.

Methods: In this study, we analyzed serum samples from healthcare workers who had received at least two vaccines, with the first vaccine being the AstraZeneca (AZ) vaccine. Anti-PF4/heparin IgG antibody levels were determined using a commercially available ELISA kit. An optical density (OD) greater than 0.5 was considered a positive result.

Results: A total of 444 study participants (356 (80%) females and 88 (20%) males) with a median age of 48 years (ranging from 21 to 67 years) were included in this study. Serum samples were collected a median of 158 days (range: 76 to 209 days) after the second vaccination. Of the samples analyzed, 27 (6%) were positive by ELISA with a median OD of 0.728 ranging from 0.5 to 2.8. Specifically, there were 5 of 93 cases (5.3%) in individuals who received two AZ vaccinations, 15 of 226 cases (6.6%) in individuals who received AZ-Biontech/Pfizer vaccinations, and 7 of 125 cases (5.6%) in individuals who received AZ-Moderna vaccinations. Investigations into the platelet activation properties of these antibodies are ongoing.

Conclusion: The observed frequency of positive cases is consistent with findings from previous studies conducted after the first vaccination. However, the presence of cases with high OD values and the time elapsed since the second vaccination suggest the need for further studies to fully evaluate the clinical significance of vaccine-induced anti-PF4 antibodies.

VS-9
Sektion Versorgungsforschung – Donor Management

VS-9-2

Awareness and mobilisation: first-time donor recruitment in post-Corona times

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Intro: The ageing of society associated with demographic change poses challenges for the mobilisation of blood donors. Currently, 64% of all blood donations at GRC blood-drives (BaWüHe & Nord-Ost) stem from donors 55+. This clientele will pass the age limit in the years to come. The 2022 share of future new donors (<18 yrs.: 16.9%) is lower than e.g. in 1990 (19.2%). Thus, measures that are suitable for encouraging young people to become donors are vital.

Campaigns: In the first 2 years of the pandemic, the 1st-time donor rate varied: While it hit 10.3% in HY1/20 (intensive press coverage), the rate dropped to 6.6% in Dec"21. This decrease mirrored the need for far-reaching new-donor campaigns in "22&23:

Q2: Competition of GRC branches. District associations were to call for donations in their regional networks.

Q3: Donors-recruit-donors. A cinema ticket as a booster for recruiter and recruited should encourage to donate in pairs.

Q4: Focus on social media. An amplifier advertised on Social Media only was meant to activate younger donors.

2023: Use of new channels (e.g. Google Ads), refine the message "It's a match".

Results: With 9.9% new donors, "donors-recruit-donors" was highly successful (max. impact in large cities: 18.3%). But: New donors recruited in this period were less likely to give a 2nd donation. If the GRC-branches-competition had been assessed on a quarterly basis, then the campaign would have been rated as successful (8.5%). In June, however, the action was accompanied by the successful nationwide MissingType campaign.

The exclusive social media approach (Q4) has not been successful. Especially in Hessia, the campaign was not accepted (6.8%). The Google-Ads campaign (= virtual advertising of selected blood-drives) used for the 1st time in 2023 got off to a promising start: a rate of 12.1% was achieved at the selected blood-drives (others: 7.9%).
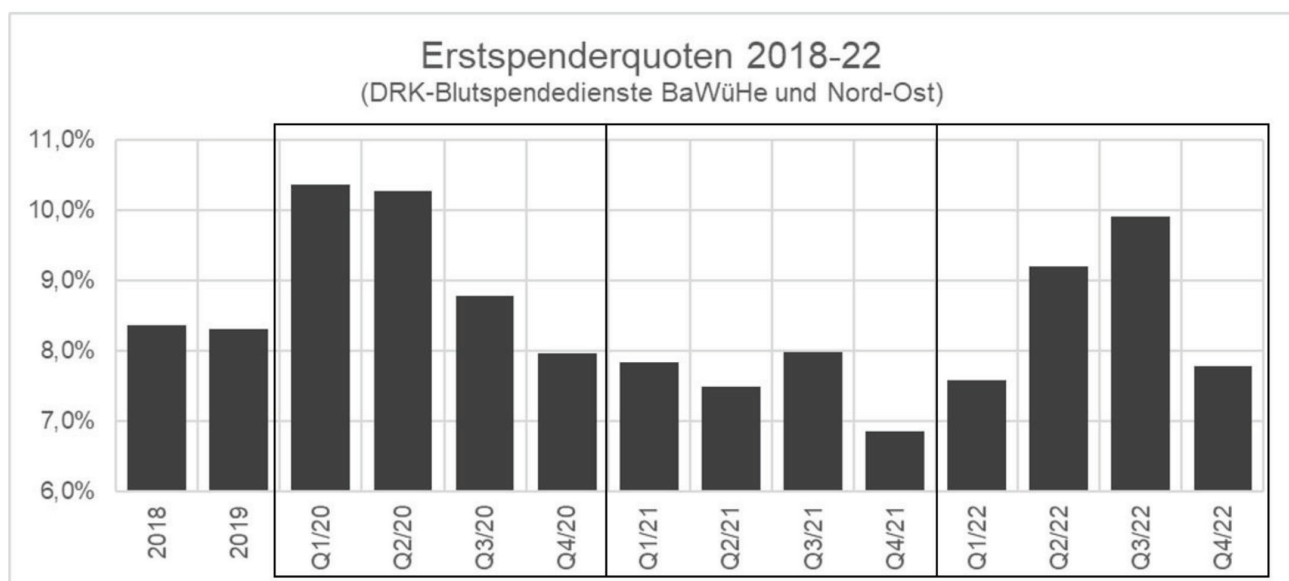

Fig. 1.

Conclusion: Some aspects to be considered in donor recruitment:
Blood donation is a social act that relies on personal contacts. A current survey show that 40% of new donors were brought to donate blood by "friends & acquaintances". This must be emphasized. The Q4-campaign showed a gap between donors' urge for digital networks and their use at the organization"s marketing. So, more expertise must be built up in this field.

Several publications (e.g. PwC 2020) point out that sustainability is very important for the genZ. Therefore, donor recruitment must be authentic sustainability-wise (incentives, communications).

Disclosure Statements: Es besteht kein Interessenskonflikt.

VS-9-3

Demographic behaviour of whole blood donors in a regional blood service – Implications for donor recruitment policies

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Background: Demographic changes in the general population have been shown to result in changes in the blood donor population and are a major challenge to ensure a sufficient and reliable blood supply both, currently and in the coming years. Moreover, different (social) media are increasingly used to enhance donor recruitment an maintenance. We aimed to characterize the dynamics within our whole blood donor reservoir, in order to elaborate potential checkpoints to optimize mobilization to donation.

Methods: Donor and Donation Data were obtained using Structured Query Language Data Extraction from our database (PC-Blut, MSSE, Schwäbisch Hall, Germany). Donors were personally invited using printed invitation cards, through our own telephone call center, or contacted via E-Mail. Invitation algorithm and invitation frequencies were not grossly altered over the observation periods. In 2020, a telephone call center was installed for incoming and outgoing donation requests and dates.

Results: The mean frequency of whole blood donations per donor decreased from 2.6 to 1.9/year between 2010 and 2022. In parallel, the percentage of first time donors remained relatively constant in the observation period (7.4% to 8.4 %), with only a modest outlier in 2020 (9.2%). First time donors in the 18-30y age group decreased from 64% (2010) to 58% (2022), whereas they increased from 14% to 21% in the 30-39y age group, whereas the other age groups remained relatively constant. Over all age groups, the number of donors who donated once a year increased by 41% and those who donated twice by 15%, whereas, donors donating 3 and more times per year dropped from 26 to 11%.

Conclusion: Our data show a higher donor reservoir, but more difficulty to mobilize donors to donation. First time donor mobilization seems to get less effective in the 18-29 year olds but more effective in the 30-39 year age group. The SARS-CoV2 pandemic situation did not recognizably result in
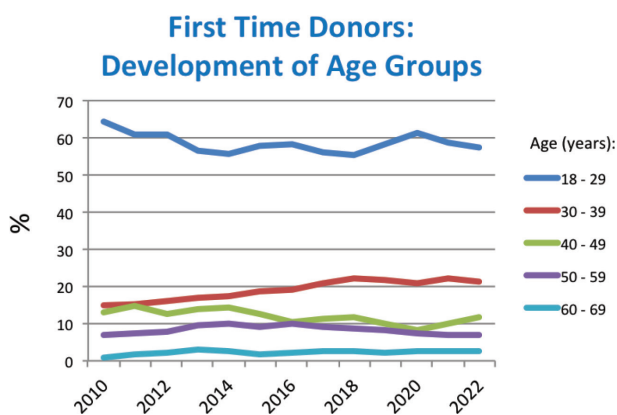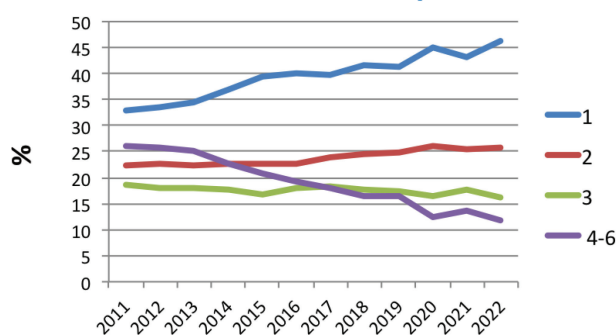
changes in these long-time trends. These data are valuable to optimize targeting age groups for new donor recruitment, and form a basis to better assess the available donor reservoir, e.g in case of blood shortages.

VS-9-4

Pretransfusional electronic crossmatch prevents transfusion to the wrong patient

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Background: At our institution, we recorded one ABO-mismatched transfusion to a wrong patient each in 2020, 2021 and 2022, representing a rate of about 1:25,000 detected transfusions to the wrong patient. As a corrective and preventive measure and to improve patient safety, the Transfusion Responsible Person with support by the Transfusion Committee and the Regional Physicians Association (Landesärztekammer) enforced the introduction of an electronic pretransfusional crossmatch into clinical practice.

Methods: Our bloodbank information system (BIS) was supplemented with a pretransfusion electronic crossmatch module. Instead of a data interface between the BIS and the patient information system (KIS), a direct web-surface based entry from patient case in KIS into the BIS was established using the Windows login and password of the transfusing physician (step i) and allocating the physician to the patient (ii). Next steps are entry of bed side test results and confirmation by Windows password (iii), scan of blood product barcode (iv), and the electronic crossmatch between patient, bed side test, blood group results, issued product and product scanned on site (v). This results in a GO - PROCEED WITH TRANSFUSION or STOP - DO NOT TRANSFUSE! (step vi).

Results: The pretransfusion e-crossmatch module was first tested in one small outpatient unit in June 2022 and then underwent minor adaptations which helped to establish a smooth process. Training about wrongly administered products was given to physicians in an E-learning module. The system started into clinical routine in October 2022. By now, the application is mandatory in 5 clinical units covering ca. 15% of all transfusions at our University Hospital. After about 3000 pretransfusionally e-crossmatched products, the first near miss was recorded. The physician was in the process to transfuse a patient to whom the product was not assigned; the transfusion was prevented through interference by the pretransfusional e-crossmatch module.

Conclusion: Establishment of a pre-transfusional e-crossmatch is feasable in a University Hospital environment. Operational simplicity of the method, reliability of day-to-day function, and understanding of the risk of transfusing a wrong blood product are important for acceptance by personnel. It allows to stop paper documentation and controls for 100% documentation according to Transfusion Law (TFG). The first case of a near-miss transfusion of a product to the wrong patient proved function of the system.

Fig. 2.

Fig. 1.

VS-10-1

Soluble components of the ECP product affect monocyte activation *in vitro*

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Background: Extracorporeal photopheresis (ECP) is an effective therapy for various T-cell-mediated diseases. However, its mechanism of action remains largely unknown. Most studies focus on fate and function of cellular components of ECP product and prove their contribution to ECP efficacy, whereas the cell free fraction of the ECP product has not been thoroughly studied. This study further investigated the composition of soluble factors of the ECP product and their functional effect on monocytes *in vitro*.

Methods: Human peripheral blood mononuclear cells (PBMCs) of healthy blood donors were incubated with 200ng/ml 8-methoxypsoralen (8-MOP) for 30 min and treated with 2 J/cm2 UVA *in vitro*. PBMCs treated with either 8-MOP, UVA or left untreated served as controls. Up to 48 hours later, cell free cell culture supernatant (ECP-SN) was harvested. Concentrations of several cytokines within the ECP-SN were analysed using a cytometric bead-based immunoassay. To address the functional effect of the soluble ECP product on immune cells, PBMCs were exposed to ECP-SN alone for 12h, 24h and 48h or exposed to ECP-SN for 24h and then stimulated with R848 or INF-γ for 24h. Survival and activation marker expression on monocytes were analysed via flow cytometry.

Results: ECP treatment of PBMCs affects the cytokine milieu in cell culture supernatant over time compared to controls: Concentration of IL-6 and IL-1β were increased and MCP-1 concentration was decreased in ECP-SN compared to controls within 48h, whereas IL-8 IL-10 and IL-2 were not affected. Regarding the functional analysis of the ECP-SN, addition of ECP-SN to PBMCs induced increases cell death within 48 hours in non-monocytes. Moreover, ECP-SN activates monocytes reflected by increased CD38 and CD83 expression and further increases CD86 expression induced by R848 or INF-γ stimulation.

Conclusion: These data suggest that soluble factors may play a role in the mechanism of action of ECP in addition to cell-based effects described in the literature. Further studies exploring additional soluble factors within the ECP product, or investigating its functional effects on other cell types can contribute to a better understanding of the immunological effects induced by ECP therapy. Finally, this could help to improve ECP therapy and to expand its medical scope.

VS-10-2

Survival of dendritic cell subsets is differentially affected by ECP treatment *in vitro*

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Background: Extracorporeal photopheresis (ECP) can induce both an activating as well as regulatory immune response depending on the clinical context. Current studies indicate that dendritic cells (DC) – key players in the regulation of T cell immunity – are critical for ECP efficacy. However, contribution of DC subsets to ECP efficacy is scarcely investigated. This study examined the direct effect of ECP treatment on plasmacytoid DC (pDC) and conventional DC type 1 (cDC1) and type 2 (cDC2) *in vitro*.

Methods: To address the effect of ECP treatment on DC subsets, human peripheral blood mononuclear cells (PBMC) of healthy blood donors were treated with 200ng/ml 8-Methoxypsoralen (8-MOP) for 30 min and then irradiated with 2J/cm² UVA *in vitro*. Untreated, UVA or 8-MOP treated PBMCs served as controls. After 24 and 48 hours quantity and quality of cell death of pDC, cDC1 and cDC2 was evaluated via flow cytometry and

compared to the survival of other leukocytes. To reliably identify cDC1 within PBMCs after *in vitro* culture, staining with XCR1, CLEC9A and CD141 was compared via flow cytometry.

Results: Staining of PBMC with CD141 was superior to CLEC9A and XCR1 to identify cDC1 after *in vitro* culture. Thus, after exclusion of CD45+Lin+CD14+CD16+ cells, CD123+HLA+ were defined as pDC, CD123-CD11c+HLA+CD1c+ cells as cDC2 and CD123-CD11c+HLA+CD141+ cells as cDC1. cDC1 and cDC2 were more sensitive to cell death due to *in vitro* culture compared to pDC and other leukocyte subsets over time. T cells, B cells and pDCs were similar susceptible to ECP treatment. Relative amount and absolute number of these populations was substantially reduced within 48h. In contrast, cDCs were less affected by ECP induced cell death.

Conclusion: The results of this study indicate that DC subsets respond differentially to ECP treatment. Thus, it seems to be worth to further investigate the role of pDC, cDC1 and cDC2 during ECP treatment in more detail. Such studies will help to get a better understanding of the immunological mechanism of action of ECP and therefore can contribute to improve this therapy.

VS-10-3

Vitamin C conditioning creates CAR-T cells with superior cytotoxic capacity and metabolic fitness to combat the immunosuppressive tumor micromilieu

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Background: Despite the promising results of CAR-T-cell therapy, poor *in vivo* persistence and low potency in the treatment of solid tumors are challenges that remain to be addressed. It has been shown that the efficacy of adoptively transferred and genetically modified T cells can be modulated by *ex vivo* culture conditions. Vitamin C (vitC) is a micronutrient that influences the immune system by mechanisms such as the regulation of epigenetic processes and reactive oxygen species-induced oxidative stress.

Methods: In this study, we investigated the impact of vitC pre-conditioning on the phenotype and functionality of CD19-targeting CAR-T cells (vitC-CAR19-Ts vs. CAR19-Ts) in co-culture with CD19+ Nalm-6 cells. We also generated CD19 knockout (CD19-) Nalm-6 cells using CRISPR/Cas9 as a negative control. Using multicolor flow cytometry, bulk-RNA transcriptomics, metabolic and multiplex assays, we analysed the activation and regulatory status as well as phenotypic characteristics and cytotoxic capacity of (vitC-)CAR19-Ts during generation and upon target cell encounter.

Results: Strikingly, vitC-CAR19-Ts had a prominent effector memory phenotype and displayed increased long-term mitochondrial fitness when compared to CAR19-Ts. Moreover, vitC-CAR19-Ts showed significantly enhanced cytotoxicity towards CD19+ but not CD19- Nalm-6 cells. In line, their granulysin release was significantly increased, which was confirmed to originate from both CD4+ and CD8+ T cells by intracellular staining. RNA sequencing of (vitC-)CAR19-Ts after target cell encounter revealed upregulation of GNLY (granulysin) as well as genes involved in T-cell activation, metabolism and epigenetic reprograming. Furthermore, vitC-CAR19-Ts showed increased cytotoxicity upon repetitive target cell encounter when compared to CAR19-Ts.

Conclusion: In conclusion, we could show in this proof-of-principle study that vitC pre-conditioning leads to CAR19-T-cell products with significantly enhanced cytotoxicity and fitness, which is potentially epigenetically imprinted. Therefore, vitC pre-conditioning is a promising strategy to generate improved T-cell products with superior long-term cytotoxic and persistence capacity.

VS-10-4

Evaluation of migratory and phagocytic properties of monocyte-derived dendritic cells from human umbilical cord blood

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Background: Umbilical cord blood (UCB) represents a unique source for stem cell transplantation while also harboring professional antigen-presenting cells such as monocytes. These may serve as a future source for monocyte-derived dendritic cells (MoDC) for clinical studies, yet the immunological properties of UCB-MoDC are still mostly unknown. This project aims to compare the functional properties of adult- and UCB-MoDC in terms of their migratory and phagocytic behavior.

Methods: Monocytes from adult healthy donors and UCB were magnetically enriched from PBMCs followed by their differentiation and maturation into MoDC. To assess endocytosis and pinocytosis, differentiated MoDC were exposed to FITC-Albumin and TITC-Dextran for 1 hour. Antigen uptake was evaluated by flow cytometry. Control groups were primed with respective inhibitors prior to antigen exposure. Migratory behavior was evaluated in the presence or absence of chemokines (MIP-3β, MIP-1α, SDF-1β). Maturated adult- and UCB-MoDC were added to 8μm transwell inserts, placed into well plates containing media with chemokines and incubated for 90 minutes at 37°C. Migrated MoDC were thereafter enumerated using a flow cytometer.

Results: UCB-MoDC presented macrophage mannose receptor (MMR)-mediated endocytosis of FITC-Dextran in comparison to controls, similar to adult MoDC in a concentration-dependent manner. UCB-MoDC sensitivity to MMR-blocking was similar to adult MoDC. Likewise, macropinocytosis-driven uptake of TITC-Albumin was unaltered in UCB-MoDC in comparison to their adult counterpart. Receptiveness to imipramine, an inhibitor of membrane ruffle formation, was similar between UCB- and adult MoDC. UCB-MoDC presented slightly elevated rates of MIP-3β- and SDF-1β-mediated chemotaxis. However, no significant differences could be detected in comparison to adult MoDC in terms of CCR7 (MIP-3β), CCR5 (MIP-1α) and CXCR4 (SDF-1β)-mediated transmigration.

Conclusion: UCB-derived MoDC present no significant differences compared to APB-derived MoDC in terms of antigen uptake and migratory behavior. These findings have important implications for the evaluation of UCB-MoDC suitability for future clinical applications.

VS-10-5

Evaluation of exosome-dependent NF-κB activation in head and neck carcinoma

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Background: Head and neck squamous cell carcinomas (HNSCC) are highly immunosuppressive and show increased NF-κB activation. The immunosuppressive effects are partially caused by tumor-derived exosomes (TEX) within tumor microenvironment (TME). TEX contain immunomodulatory molecules influencing the function of immune cells. In this study, we investigate the influence of patient-derived exosomes on the NF-κB signaling pathway.

Methods: Exosomes were isolated from plasma of HNSCC patients by size-exclusion chromatography. TEX were co-incubated with primary macrophages with and without NF-κB inhibitors to assess the effect on NF-κB and its downstream signaling pathways. NF-κB activation was measured by Western Blot and nuclear Translocation-Assay. Downstream effects were evaluated by qPCR for CCL5, CXCL10, CCL22, IDO, TNFα and IFNβ, chemokine ELISA and T-cell migration assays.

Results: TEX were internalized by macrophages and NF-κB activation was visible after 2h of coincubation. This activation was reversible by NF-κB inhibitors especially curcumin. In macrophages co-incubated with TEX, NF-κB-dependent downstream chemokines modulated the migration of cytotoxic CD8+ T-cells and Treg.

Conclusion: Exosomes from plasma of HNSCC patients interact with macrophages and can activate the NF-κB signaling pathway. This effect is reversible by NF-κB inhibitors and therefore shows potential to be considered as a future therapeutic target.

VS-10-6

A protein corona around human platelet-derived EVs promotes regenerative functions

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Background: Human platelet lysate (hPL) is currently used as efficient substitute for fetal bovine serum for clinical cell manufacturing. Furthermore, we have previoulsy observed accelerated skin organoid formation and in vivo wound healing by hPL. As shown by others and us, EVs bear a biologically active protein corona, depending on the mode of preparation and the protein milieu.

Methods: In this study we asked whether hPL-derived EVs or platelet-derived soluble factors mediate these trophic effects of hPL. We separated EVs from soluble factors of hPL to understand the mode of action during skin organoid formation and immunomodulation as model systems for tissue regeneration. EVs were concentrated from hPL by tangential-flow filtration (TFF-EVs) and further purified by size-exclusion chromatography (TSEC-EVs) separating EVs from (lipo-) protein-rich soluble factors (TSEC-sol.F). Samples were characterized by tunable resistive pulse sensing, western blot, tandem mass-tag proteomics and super-resolution microscopy, and functionally tested during organoid formation and immunomodulation.

Results: We identified three major protein clusters by proteomic principle component analysis separating TSEC-EVs from hPL clustering with TFF-sol.F and TFF-EVs clustering with TSEC-sol.F. TFF-EVs induced significantly improved skin-organoid formation and inhibition of T-cell proliferation, compared to TSEC-EVs or to TSEC-sol.F. Reconstituting the corona on TSEC-EVs with TSEC-sol.F re-established functionality comparable to TFF-EVs. Zeta potential and super-resolution imaging confirmed corona formation.

Conclusion: TFF is a permissive technology enabling scalable enrichment and separation of functional corona-bearing EVs and soluble factors. Depletion of the TFF-EV corona by SEC or ultracentrifugation abrogated functionality indicating a novel mode of action. The corona could be artificially reconstituted in add-back of sol.F showing similar effects compared to TFF-EVs. This enables EV engineering with selected corona proteins for specific purposes and therapeutic applications.

Overcoming immune escape in EBV-associated PTLD by understanding the two levels of immunosuppression: the tumor microenvironment and iatrogenic immunosuppression

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Background: Epstein-Barr virus (EBV) is one of the most common opportunistic pathogens causing morbidity in immunocompromised patients including the development of B-cell lymphomas (e.g. post-transplant lymphoproliferative disease, PTLD). Viral clearance requires the restoration or establishment of an EBV-specific T-cell response, which is impaired in PTLD patients due to the required immunosuppressive treatment (e.g. Tacrolimus, Tac) as well as the tumor microenvironment (TME).

Methods: Serving as _in vitro_ EBV-associated PTLD model, EBV-transformed B-lymphoblastoid cell lines (B-LCLs) were established from >15 healthy EBV-seropositive donors. At different time points of B-LCL generation, their immunomodulatory receptor expression and secretome were analyzed by flow cytometry and multiplex assays. B-LCL-conditioned medium (CM) was collected at selected time points and pooled from 8-12 donors to generate CM_{early} (B-LCL age 6-8 weeks), CM_{int} (12-14 weeks) and CM_{late} (>30 weeks) which were used to mimic the TME of PTLD in T-cell assays. EBV-specific memory T cells were expanded in presence of autologous B-LCLs and the effect of CM or Tac on their phenotype, activation level and functionality was analyzed by flow cytometry.

Results: Longitudinal analysis of immunomodulatory receptor expression by B-LCLs revealed significant upregulation of CD40, CD86 and PD-L1. Cytokine profile analysis revealed a remarkably high inter-individual variability. While the secretion of the pro-inflammatory IL-6 and IL-8 decreased significantly during B-LCL generation, IL-10 secretion was increased in a fraction of intermediate-aged B-LCLs. Due to its known immunosuppressive effect, two different CM_{int} were generated based on IL-10 concentration (CM_{int-hi}, CM_{int-lo}). In all co-cultures of $CD3^+$ T cells with irradiated autologous B-LCLs, central memory T cells were the main active subset. Activation and proliferation were markedly inhibited in presence of Tac as well as CM_{int} and CM_{late}.

Conclusion: Creating an immunosuppressive TME is a key mechanism of tumor immune escape. Our data indicate longitudinal changes in TME composition during tumor development. Moreover, T-cell activation and proliferation, both basal attributes of T-cell functionality, were reduced in presence of CM as well as Tac. Our results provide insights into how not only Tac but also the TME affects T-cell responses, thus supporting the development of therapeutic strategies to overcome tumor-mediated immune escape.

Discovery of the human cytomegalovirus-specific peptide repertoire naturally processed and presented by infected human antigen presenting cells

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Background: Infection with or reactivation of human cytomegalovirus (HCMV) remains a clinically problem in immunocompromised patients mainly caused by insufficient T-cell functionality. Knowledge of viral targets is critical to improve monitoring of high-risk patients and to optimize antiviral T-cell therapy. We aimed to identify naturally presented HLA-A*11:01-restricted HCMV-derived T-cell epitopes from HCMV-infected professional antigen presenting cells to expand the spectrum of immunogenic targets.

Methods: Monocyte-derived and genetically-engineered induced dendritic cells (iDCs) were generated to provide a stable platform for soluble (s)HLA-A*11:01 production. After infection of sHLA-A*11:01-secreting cells with wild type HCMV or a mutant lacking known immune evasion molecules (US2-6+11), sHLA-A*11:01-bound peptides were isolated by w6/32 immunoaffinity chromatography followed by mass spectrometric analysis. Identified peptides were screened for their viral protein origin and binding strength to HLA-A*11:01 using established databases. The highest scoring candidates were selected for _in vitro_ evaluation of immunogenicity, cytotoxicity, clinical relevance and suitability for immune monitoring.

Results: More than 50 naturally presented candidate peptides were identified. The immunogenicity of the 25 highest scoring HLA-A*11:01-restricted peptides was evaluated, with functional active CMV-specific T cells detected against five candidates in healthy CMV^+ donors by IFN-γ-EliSpot. Their complex stability was demonstrated by _in vitro_ peptide binding assays. Highly proliferative and cytotoxic memory T cells were detected after stimulation with the UL36-derived $A11_{SAL}$ and UL122-derived $A11_{SVS}$ peptides in healthy CMV^+ donors and HCMV-infected patients. Surprisingly, $A11_{SAL}$-specific memory T cells exhibited functional properties at levels comparable to those T cells against the known immunodominant pp65-derived $A02_{NLV}$ peptide.

Conclusion: Eliciting strong antiviral T-cell responses in healthy CMV^+ donors as well as HCMV-infected patients, the $A11_{SAL}$ peptide was identified as a new major target of the anti-HCMV immune response. Newly identified HCMV peptides expand the repertoire of immunodominant targets and will improve strategies for identification of high-risk patients, and enhancing therapeutic options using HCMV-specific T cells.

VS-11-1

Molecular characterization of the complement receptor 1 gene in patients with alloantibodies against antigens of the knops blood group system

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Background: The antigens of the Knops blood group system (KN; ISBT 022) are located on the complement receptor 1 (CR1), a large glycoprotein composed of 4 Long-Homologous-Repeats (LHR-A, -B, -C, -D). Most KN antigens are located LHR-D, except for KN11/KN12 and KNMB located in LHR-C. Antibodies to KN antigens are not clinically relevant but they are common in patients. Here, we sequenced the *CR1* gene in patients with suspected alloantibodies to KN antigens.

Methods: Serologic work-up identified 23 cases with suspected antibodies against a high prevalence antigen in the Knops blood group system. The ethnic background of the patients was European (15 cases) or African (8 cases). All cases were subjected to Massively Parallel Sequencing (MPS) of all 39 exons of the *CR1* gene included in the targeted panel of all blood group genes. Allele frequencies of the identifed gene variants in different populations were taken from the gnomAD database.

Results: The *CR1* sequence in 2 of the European cases showed homozygosity for the *KN*01.–05* allele (c.4223C>T; p.T1408M) indicating absence of the Yk(a) antigen. Homozygosity for the *KN*01.–08* allele (c.4828T>A; p.S1610T) indicating absence of the Sl3 antigen was identified in 2 European cases. In 8 cases we found heterozygosity for at least one known KN allele or rare *CR1* variant. The other 3 European cases showed no *CR1* variation. Two of the African cases were homozygous for *CR1* c.3290T>C (p.L1097P) and further serologic investigation showed that the variant defines a new high prevalence antigen named KNMB. Each of the other 6 African cases revealed heterozygosity for at least 2 known KN alleles or rare *CR1* variants.

Conclusion: MPS simplified the molecular analysis especially of blood group systems with large genes such as *CR1* for the KN system. The systematic case work enabled the identification of the new KN antigen KNMB. The significantly different frequency in African and European populations of *CR1* variants encoding KN antigens may result in an increased risk of alloimmunization evoked by blood transfusion.

VS-11-2

KNMB, a novel Knops blood group antigen located on LHR-C

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Background: The complement receptor 1 (CR1) carries the antigens of the Knops blood group system (KN; ISBT 022). They are located on the long homologous repeats (LHR) C and D of the CR1. Antibodies to Knops antigens are not clinically significant but they are common in patients. In a previously transfused female patient of Ethiopian origin with a severe COVID-19 pneumonia we found an antibody to a new high prevalence antigen which we suspected to belong the Knops blood group system.

Methods: Antibody identification was performed in IAT in gel technique (BioRad, CH) using different commercial panels as well as untreated and papain treated red cells negative for high prevalence antigens. Different recombinant blood group proteins (imusyn, Germany) and specially produced recombinant proteins for LHR-C, LHR-C_1097Pro, LHR-C_1100Gly and LHR-C_1097Pro-1100Gly were used for inhibition assays. Massive parallel sequencing (MPS) of a blood group gene panel including all exons of the genes encoding the blood group systems ISBT001 to 043 was performed on the patient and a single non-related African patient non-reactive with patient's plasma. Sequence-specific PCR (PCR-SSP) methods were established to confirm the mutations in CR1.

Results: The antibody was reactive with all test cells of the antibody identification panels and 29 test red cells negative for different high prevalence antigens. It was non-reactive with papain-treated red cells. Recombinant DACY protein inhibited, recombinant Kn(a) protein did not inhibit the antibody, indicating that the antigen is located on LHR-C. Molecular analysis revealed 2 unknown variants in CR1 exon 21: c.3290T>C (p.Leu1097Pro; rs200111726) and c.3298A>G (p.Arg1100Gly; rs202070239). Further assays showed inhibition of the antibody with LHR-C and LHR-C_1100Gly recombinant proteins, but not with LHR-C_1097Pro and LHR-C_1097Pro-1100Gly. This proved that the antibody is directed against p.1097Leu.

Conclusion: Using an antibody to a high prevalence antigen found in a previously transfused patient of Ethiopian origin we identified a new Knops blood group antigen located on LHR-C region of the Knops protein. The provisional antigen name (KNMB) was derived from the initials of the antibody producer. KNMB is defined by p.1097Leu and homozygosity for p.1097Pro caused the KNMB-negative phenotype. The CR1 variant rs200111726T>C is rare in the European population but frequent in the African population.

VS-11-3

Creation of an adequate structural model of the vel epitope with artificial intelligence

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Background: Vel is a clinically relevant blood group antigen known to be carried by the small integral membrane protein 1 (SMIM1). The presentation of the Vel epitope requires inter-molecular disulfide bonds and a functional GXXXG-motif, which reportedly supports the dimerization of membrane proteins. The exact structure of the Vel epitope, however, remains elusive so far.

Methods: A putative Vel epitope was modelled using artificial intelligence supported structure prediction of dimers of SMIM1 and SMIM1 variants.

Results: Two conditions were considered necessary for a model of the Vel epitope to be acceptable: firstly, the formation of an inter-molecular disulfide bond, and secondly, the correct orientation of the GXXXG-motif. Modelling a Vel dimer without constraints fulfilled neither the first nor the second condition. After an iterative process of adding other structural motifs and linkers, a model was obtained that fulfilled both conditions (Fig 1).

Conclusion: The dimerization of SMIM1 puts a certain apparent stress on the structure, which probably requires the disulfide bond to remain stable. This could explain why the disulfide bond is crucial for the presentation of the Vel antigen and anti-Vel antibodies seem to be mostly conformation dependent. The modelled Vel epitope still needs experimental validation.

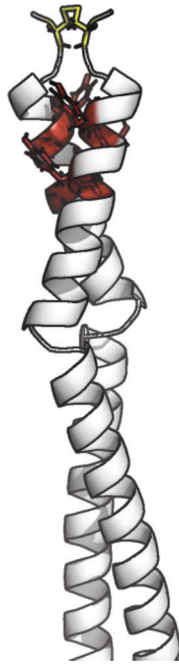**Disclosure Statements:** Angestellter bei imusyn GmbH & Co. KG

Fig. 1.

VS-11-4

Recombinant blood group antigens in patient testing

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Background: Antibodies to high-prevalence red cell antigens can be challenging in serologic patient workup. Hemagglutination inhibition assays using recombinant blood group antigens (rBGAs) can identify the specificity of the antibody and uncover additional, possibly significant, alloantibodies. Data of use in routine immunohematologic laboratories are still scarce. We report the use of rBGAs in patient testing regarding feasibility, effectiveness and limitations.

Methods: Patients (n=12) with suspected or known antibodies to high-prevalence antigens were tested with single soluble rBGAs (Ch, Rg, Kn^a, JMH, Yt^a, Do^b, CROM/DAF, Kell-Kp^b-Js^a, Cellano-Kp^b-Js^a, Lu^b/Au^b, Sc1; inno-train Diagnostic GmbH, Kronberg, Germany) according to the manufacturer's instruction. 2 µl rBGA solution was mixed with 25 µl patient plasma, incubated at room temperature for 30 min and tested in a gel agglutination system (BioRad ID-System) in the indirect antiglobulin test with untreated or papain-treated test cells. Inhibition of reactivity occurred if the corresponding antibody was present and additional antibodies could further be identified in routine test systems. Choice of rBGAs was dependent on preceding serologic results.

Results: In 9 (75%) of 12 patients antibodies to high-prevalence antigens were identified with rBGAs of the specificities Ch (n=3), Yt^a (n=3), Kn^a (n=2), and Lu^b (n=1). Single or multiple additional underlying antibodies of 6 different specificities (Wr^a, Fy^a, C, Jk^b, Fy^b, and S) were identified in 4 (44%) of these 9 patients. In 3 cases (25%) inhibition was not successful and explained by a warm-reactive autoantibody plus anti-E (n=1), nonspecific reactivity (n=1) and reactions to a high-prevalence antigen not related to the rBGAs used (n=1). Specificities of rBGAs and antibodies are detailed in Table 1.

Tab. 1.

Table 1: Cases with previously identified or suspected antibodies to high-prevalence antigens

Case	Previously identified antibody specificities	rBGAs used	Successful inhibition, specificity	Specificity of additionally identified antibodies	Remarks
1	Wr^a suspected	Ch, Rg, Kn^a, JMH, Yt^a	Kn^a	Wr^a	Additional unidentified antibody to low-prevalence antigen
2	-	Yt^a	Yt^a	Fy^a	
3	-	Kn^a, Yt^a	Kn^a	-	
4	-	Do^b, CROM/DAF, Kell-Kp^b-Js^a, Lu^b/Au^b, Yt^a, Sc1, LW^a, k-Kp^b-Js^a	-	n/a	Nonspecific reactivity
5	E, D (autoantibody)	Lu^b, Sc1, LW^a, Do^b, Yt^a, Kn^a	-	n/a	Warm-reactive autoantibody and anti-E; LW^a excluded
6	-	Ch, Rg, Kn^a, JMH, Yt^a	-	n/a	Undetermined specificity
7	-	Ch	Ch	-	
8	-	Rg, Ch	Ch	-	
9	Lu^b suspected	Lu^b	Lu^b	-	
10	Yt^a plus C, Jk^b	Yt^a	Yt^a	C, Jk^b, Fy^b	
11	Yt^a plus Fy^a	Yt^a	Yt^a	Fy^a, S	
12	Ch	Ch	Ch	-	

n/a = not applicable

Conclusion: Hemagglutination inhibition assays using rBGAs can easily be carried out in a routine testing laboratory without the need of rare test cells or antisera, or sometimes complicated and time-consuming procedures. In 75% of our cases the antibody specificity to a high-prevalence antigen were determined and all additionally identified single or multiple alloantibodies were clinically significant. Recombinant blood group antigens are a valuable tool to increase transfusion safety.

VS-11-5

A universal blood genotyping array tailored for transfusion services and validated in a pre-clinical study of a large, ethnically diverse cohort

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Background: Extended blood group matching substantially reduces sensitization to non-self antigens as side effect of transfusion. For broad application, affordable high-throughput typing of relevant antigens is required. Following an earlier proof-of-concept study, our consortium presents here the development of a tailored universal blood donor genotyping array, including the results of an international Pre-Clinical accreditation Study (PCS) comprising an ethnically highly diverse panel of samples.
Methods: The custom-designed Axiom array contains 50,000 probes tagging 20,000 variants relevant for blood services. It has a 384-sample format and runs on GeneTitan instrument, which can generate data for 3,000 samples/week. The array allows simultaneous typing of clinically relevant human erythroid (HEA), platelet (HPA), and leukocyte (HLA) antigens. For the PCS, DNA samples and clinical antigen typing data from 13,908 donors provided by seven blood services were analysed. Samples representing diverse ethnic groups (74% European, 11% African, 15% others) were genotyped at three blood services in the Netherlands, USA, and UK. Array-inferred antigen types were analysed for concordance with provided clinical antigen types for the first 6,953 samples.
Results: Reproducibility between sample results from the testing laboratories was outstanding for the 20k variants. The overall concordance for HEA, HPA, and HLA antigens with previous testing was very high. Of ~100,000 comparisons between blood service determined HEA types and array determined types, the concordance was 99.8%. Over 80% of the discordances can be resolved by simple algorithmic modifications and over half of the remaining ones were caused by incorrect serology. The results of the PCS proofed a robust validation of also non-European genotypes configurations. The 778 samples of African ancestry showed a HEA concordance rate >99.7%. Also HLA class I and II concordance level, which could be assessed for 1319 DNA samples, was excellent.

Conclusion: An affordable and comprehensive DNA-based test for automated high-throughput typing of donors and patients is reported here. The universal blood typing array is tailored for the needs of transfusion services and validated in an international, diverse cohort. Among others, the array represents a promising new tool to facilitate a broader application practise of extended blood matching to reduce sensitization rates and to identify rare donors.

VS-11-6

Adaptive sampling to resolve complex structural variants in targeted blood group genes by nanopore sequencing

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Background: Adaptive sampling is a software-driven enrichment method unique to Oxford Nanopore Technologies (ONT) and allows simultaneous sequencing of the entire blood group genome. Without any restrictions on read length, it can excel in resolving complex structural variants (SV) like hybrid alleles, which are a hallmark of some blood group systems like the RH. Here, we used adaptive sampling to tackle RHCE genotype-phenotype discrepancies unresolved by other methods and suspected to harbor a SV.
Methods: We reassessed three RHCc genotype-phenotype discrepancies, which we observed in routine high-throughput donor typing, by PCR-SSP, MLPA, and extensive serological analyses. Suspecting a SV, we employed high density MALDI-TOF MS analysis along the *RHCE* gene to get hints on copy number variation. As unresolvable, we took advantage of latest developments of ONT's adaptive sampling for sequencing the entire *RHCE* gene as single reads for one of the samples. The sequencing library was built with high-molecular weight gDNA. Beside *RHCE* we targeted the entire blood group genome (48 genes; ~8 Mb incl. 50 kb flanking regions) in adaptive sampling. Structural variant breakpoints in all three samples were confirmed by bridge-PCRs and Sanger sequencing.
Results: All three donors were serologically typed as C+c- but genotyped as *RHCE*Cc*. The high-density MALDI-TOF MS assay pointed towards an identical potential SV located near the end of the *RHCE* gene. The type or location of the SV, however, could not be determined. ONT sequencing of one sample exhibited high mean read length (N50 of >30 kb) with a median coverage of ~15x across all 48 genes. The availability of particularly long reads (20 reads >50 kb; max. 250 kb) spanning the RH locus allowed identifying a large deletion. Specifically, we identified a novel ~8.6 kb deletion spanning from intron 8 to intron 9 of *RHCE*. Bridge-PCRs and Sanger sequencing confirmed the exact breakpoint locations, with an identical deletion in the other two samples.
Conclusion: Adaptive sampling is a promising enrichment method for simultaneously targeting all blood group genes without restrictions on read length, which opens new avenues for identifying SVs. It has allowed us resolving long-tackled RHCE genotype-phenotype discrepancies by discovering a large novel deletion. Although SVs are a hallmark of RH, to our knowledge only two other large deletions have yet been reported for *RHCE*, suggesting the need for suitable approaches like the one presented here.

VS-12-1

Assessment of cellular and plasmatic determinants of the APC response to thrombin in factor V Leiden carriers using an endothelial cell-based ex vivo model

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Background: The thrombophilic factor V Leiden (FVL) mutation shows a highly variable clinical expressivity. We have shown in vivo that asymptomatic FVL carriers respond to extrinsic coagulation activation with higher activated protein C (APC) formation rates than those with a history of venous thromboembolism (VTE). Aim of this study was to investigate potential modulating factors of the APC response in an ex vivo model of the PC pathway using endothelial colony forming cells (ECFCs) and autologous plasma.

Methods: ECFCs were isolated from FVL carriers with (VTE+) or without (VTE-) previous VTE and healthy controls (n=7 each). After being overlaid with autologous defibrinated plasma, thrombin formation was initiated by addition of tissue factor (1 pmol/L). Thrombin and APC formation were measured over time using oligonucleotide-based enzyme capture assays (OECAs). Additionally, cross-over experiments with FVL and non-FVL cells/plasma were conducted as well as measurements of ECFC-dependent APC formation in HEPES buffer containing thrombin and the zymogen protein C (PC). Thrombomodulin and endothelial protein C receptor (EPCR) expression were quantified in cell-ELISAs. Inactivation kinetics of exogenously added APC were monitored in plasma by OECA.

Results: Thrombin formation rates did not differ, but APC formation rates in plasma were higher in the FVL VTE- cohort than in the FVL VTE+ cohort (P=0.011) and healthy controls (P=0.003) as indicated by the ratio between the area under the curve (AUC) of APC generation to the thrombin AUC. Cross-over experiments with non-FVL cells and FVL VTE+ plasma yielded increased APC formation in comparison to the autologous approach (P=0.009), indistinguishable from FVL VTE- plasma. The APC AUC of all other combinations and the thrombin AUC did not differ. APC formation in the purified system, APC inactivation kinetics, thrombomodulin and EPCR expression as well as plasma levels coagulation factors and inhibitors did not differ significantly between cohorts.

Conclusion: Consistent with previous in vivo experiments, the APC response to thrombin formation was impaired in FVL carriers with a history of VTE in comparison to asymptomatic FVL carriers. However, this difference disappeared when FVL cells were replaced by non-FVL cells in cross-over experiments, suggesting that endothelial cell-specific factors contribute to the APC response and thus to thrombotic risk in FVL carriers. Further studies are warranted to identify these factors.
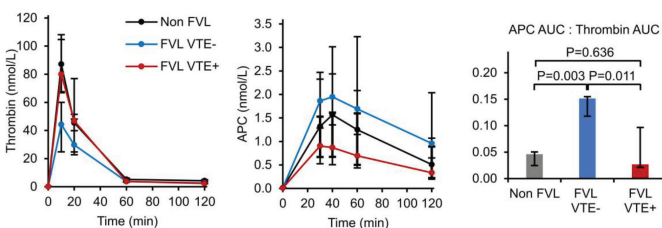
Fig. 1.

VS-12-2

Bleeding, FVIII activity, and safety 3 years after gene transfer with valoctocogene roxaparvovec: results from GENEr8-1

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Background: Valoctocogene roxaparvovec (AAV5-hFVIII-SQ) provides endogenous factor VIII (FVIII) production to prevent bleeding in people with severe hemophilia A. The aim is to evaluate outcomes 3 years after receiving valoctocogene roxaparvovec.

Methods: The open-label, multicenter phase 3 GENEr8-1 trial (NCT03370913) evaluated 6x10^{13} vg/kg valoctocogene roxaparvovec in 134 adult men with severe hemophilia A (FVIII ≤1 IU/dL) without inhibitors. Bleeds and FVIII use were self-reported after regular prophylaxis ended (scheduled for week [W] 4) through data cutoff. Comparisons to baseline on FVIII prophylaxis were performed in 112 HIV-negative participants enrolling from a non-interventional study (rollover population). FVIII activity per chromogenic assay and quality of life (QOL) per Haemo-QOL-A were assessed in 132 HIV-negative participants (modified intent-to-treat [mITT] population). Safety was assessed in all participants.

Results: Median follow-up was 162 weeks (N=134); 131 participants completed W156. Over 3 years in 112 rollover participants, mean annualized treated bleeding rate was 0.8 bleeds/year, mean annualized rate of all bleeds was 1.3 bleeds/year, and mean FVIII utilization was 125 IU/kg/year (**Table**). During Y3, 73.2% of 110 rollover participants had 0 treated bleeds and 61.6% had no bleeds. At W156, mean and median FVIII were 18.8 and 8.4 IU/dL (mITT, N=132; **Figure**); Overall, 10/132 (7.6%) participants resumed prophylaxis. Mean Haemo-QOL-A Total Score change from baseline to W156 was +6.6 (n=122; P <0.0001). No new safety signals emerged. In Y3, 34/134 participants (25.4%) had alanine aminotransferase (ALT) elevation.

Conclusion: Valoctocogene roxaparvovec provided robust hemostatic efficacy relative to FVIII prophylaxis for 3 years, with QOL improvement and stable safety.

Tab. 1.

Table. Rates of bleeding and FVIII utilization over 3 years of follow-up post-treatment with valoctocogene roxaparvovec

	Rollover population (N=112)[a]				
	Baseline[b]	Year 1[c]	Year 2	Year 3[d]	All post-prophylaxis[e]
ABR (treated[f]), bleeds/yr					
Mean±SD	4.8±6.5	0.9±3.6	0.7±2.7	1.0±3.5	0.8±2.3
Median (Q1, Q3)	2.8 (0.0, 7.6)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.4)
Annualized FVIII utilization, IU/kg/yr					
Mean±SD	3961±1751	45±157	89±300	228±589	125±316
Median (Q1, Q3)	3754 (2799, 4707)	0 (0, 0)	0 (0, 22)	0 (0, 115)	11 (0, 74)
ABR (all), bleeds/yr					
Mean±SD	5.4±6.9	1.5±3.8	1.0±2.8	1.4±3.8	1.3±2.4
Median (Q1, Q3)	3.3 (0.0, 7.9)	0.0 (0.0, 1.6)	0.0 (0.0, 1.0)	0.0 (0.0, 1.0)	0.3 (0.0, 1.3)
Participants with 0 bleeds (treated[f]), n (%)	36 (32.1)	92 (82.1)	94 (83.9)	82 (73.2)	68 (60.7)
Participants with 0 bleeds (all[g]), n (%)	34 (30.4)	65 (58.0)	74 (66.1)	69 (61.6)	32 (28.6)

[a]In GENEr8-1, bleeding rates were analyzed in 112 participants in the mITT population who rolled over from study 270-902 (a prospective, non-interventional study of people with severe hemophilia A receiving prophylactic treatment with exogenous FVIII) to enable pre- and post-treatment comparisons.
[b]Baseline values are based on prospectively collected data over 6 months in study 270-902 while participants were receiving FVIII prophylaxis.
[c]Year 1 began at the latter of week 5 or prophylaxis discontinuation and continued to week 52.
[d]Two rollover participants discontinued the study prior to reaching year 3 and therefore were excluded from the calculation of ABR (treated, all) and annualized FVIII utilization.
[e]All post-prophylaxis values were from the period beginning at the later of week 5 or prophylaxis discontinuation and ending at the data cutoff date or when a participant discontinued from the study, whichever was earlier.
[f]Treated bleeds were defined as bleeds followed by use of plasma-derived or recombinant standard half-life or extended half-life FVIII products within 72 hours.
ABR, annualized bleeding rate; FVIII, factor VIII; mITT, modified intent-to-treat; Q1, first quartile; Q3, third quartile; SD, standard deviation.
[g]Excluding surgeries and procedures.

VS-12-3

Hijacking of the fibrinolytic system by B-cell acute lymphoblastic leukaemia and its therapeutic targeting

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Background: The fibrinolytic system consists of the proenzyme plasminogen and its active form, plasmin, a serine protease. The activation of plasmin is tightly regulated by activators such as tissue plasminogen activator (tPA) and various inhibitors. A role of this plasminogen activation system in the microenvironment of solid tumours is being unravelled, but a function of this pathway in the bone marrow microenvironment (BMM), where haematological cancers usually originate, has so far been elusive.

Figure. FVIII activity over 3 years post-treatment with valoctocogene roxaparvovec in the mITT population (N=132) and a subgroup of the mITT dosed ≥4 years (N=17)

Year 3 FVIII (N = 132)
CSA
Mean: 18.4 IU/dL
Median: 8.3 IU/dL
OSA
Mean: 29.7 IU/dL
Median: 16.2 IU/dL

Year 4 FVIII (n = 17)
CSA
Mean: 15.2 IU/dL
Median: 7.4 IU/dL
OSA
Mean: 22.1 IU/dL
Median: 13.2 IU/dL
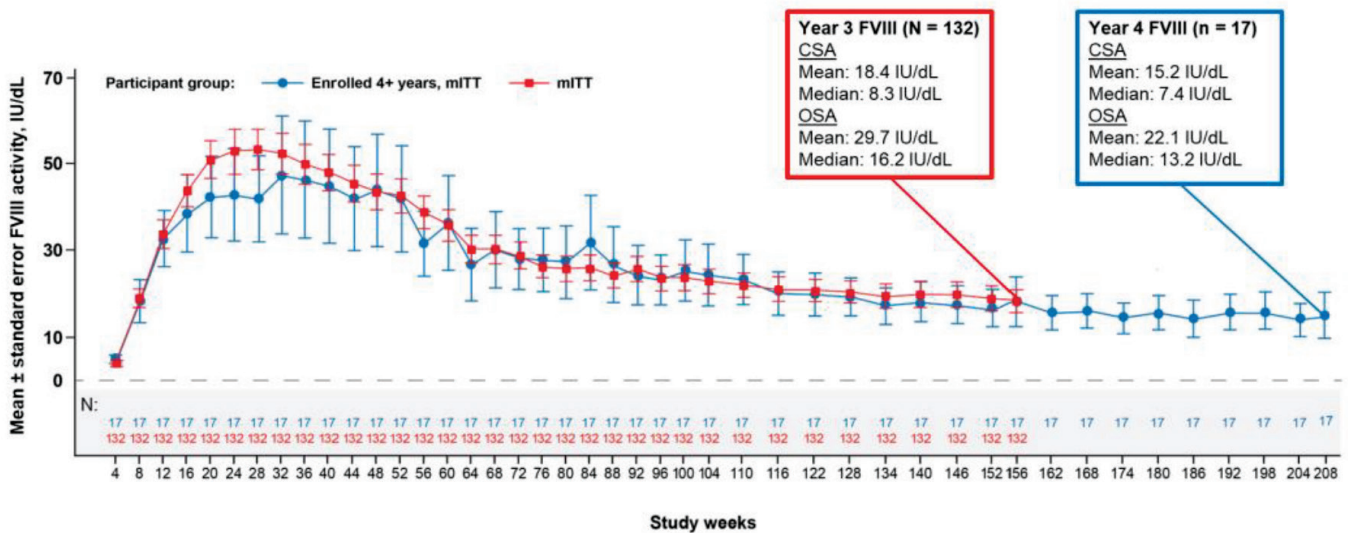
The mITT population included all HIV-negative participants who received valoctocogene roxaparvovec. For participants who discontinued the study, missing FVIII values post-discontinuation were imputed to be 0 IU/dL through the data cutoff date for the analysis. For participants with missing data who continued on study, missing values were imputed to be the smaller of the median value in the participant's last visit window prior to the evaluation week containing a valid observation and the median value in the participant's next visit window after the evaluation week containing a valid observation. If the value of the next visit window was unavailable (eg, the evaluation week was the last visit by the data cutoff date), the missing value was imputed through linear extrapolation using the median values in the last 2 visit windows prior to the evaluation week containing a valid observation, capped at the last non-missing value.
FVIII, factor VIII; mITT, modified intent-to-treat; SE, standard error.

Fig. 1.

Methods: We employed the retroviral transduction/transplantation model of B-cell acute lymphoblastic leukaemia (B-ALL), mice deficient for annexin A2 (ANXA2), a calcium-binding protein, which is also found in a complex with the plasminogen receptor protein, S100A10, and various in vitro assays including modeling of the extracellular matrix (ECM) to show the role of degradation of the ECM for B-ALL progression.

Results: ANXA2 was shown to be essential for plasmin activation and remodeling of the ECM in the BMM, thereby shortening survival in B-ALL. Induction of B-ALL in tPA-deficient mice led to fibronectin accumulation in the BMM and significant survival prolongation. Increased ECM density due to ANXA2 deficiency resulted in an entrapment of growth factors such as insulin-like growth factor 1, altering downstream signaling in B-ALL cells. Secretion of interleukin (IL)-6 by B-ALL cells increased hepatic generation of plasminogen/plasmin/tPA. Therapeutically, inhibition of plasmin activation by ε-aminocaproic acid (EACA), an anti-hemorrhagic drug, in murine models of B-ALL led to survival prolongation and fibronectin accumulation in the BMM.

Conclusion: Our data suggest that B-ALL cells condition their environment by increasing plasminogen/plasmin generation in the liver by IL-6, thereby, influencing the remodeling of ECM proteins in the BMM and, ultimately, B-ALL progression. We propose that inhibition of plasmin-mediated degradation of the ECM by EACA may be beneficial as an adjunct therapy for B-ALL.

VS-12-4
Antibody-induced platelet phosphatidylserine mediates increased prothrombotic potential in heparin-induced thrombocytopenia

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Background: Heparin-induced thrombocytopenia (HIT) is a severe adverse event caused by antibodies (Abs) reactive to platelet factor 4 (PF4) and heparin. While HIT Abs are well characterized to activate PLTs via PLT Fc-gamma-RIIA, the contribution of different Ab-induced PLT subpopulations to the prothrombotic environment in HIT remains elusive. In this study, we hypothesized that HIT Abs have the potential to induce a procoagulant PLT phenotype that contributes to the prothrombotic condition in HIT.

Methods: Using flow cytometry (FC), we analyzed whether HIT Abs have the capability to induce changes in the expression level of the PLT surface activation marker P-selectin (CD62p) and the negatively charged procoagulant membrane phospholipid phosphatidylserine (PS). To investigate whether Ab-induced procoagulant PLTs contribute to increased thrombin generation and thrombus formation, calibrated automated thrombogram (CAT) analysis and an *ex vivo* thrombosis model were utilized, respectively.

Results: HIT patient Abs induced a procoagulant (CD62p/PS double positive) PLT phenotype with increased thrombin generation potential. Most importantly, spike-in of whole blood samples with HIT Ab-induced procoagulant PLTs prior to perfusion through a collagen coated microfluidic system resulted in a significant increase of thrombus formation in a heparin- as well as PLT Fc-gamma-RIIA dependent manner. Interestingly, HIT Ab-induced thrombus formation was unaffected by inhibiting the interaction of PLTs with neutrophils via CD62p-glycoprotein ligand-1 (PSGL-1) axis. Contrary, increased thrombin and most importantly, HIT Ab-induced thrombus formation were significantly reduced in the presence of the specific PS blocking protein Lactadherin.

Conclusion: Based on our mechanistic studies we conclude that increased PLT PS rather than CD62p on the surface of HIT Ab-induced procoagulant PLTs might be an essential factor for increased prothrombotic conditions typically observed in HIT.

Treatment of Immune thrombocytopenia (ITP) with eltrombopag – Results of the 5th interim analysis of the study RISA

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Background: Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by isolated thrombocytopenia due to increased platelet destruction and decreased megakaryopoiesis. Eltrombopag (EPAG) is an oral thrombopoietin-receptor agonist, which is proved to be effective and safe in the treatment of ITP. Here we present data from the 5th interim analysis of the non-interventional prospective study RISA.

Methods: RISA is a prospective multicenter non-interventional study in Germany. It started in December 2015, enrollment stopped in December 2021, and the observational phase will end by December 2023. Dosage of EPAG and treatment corresponded to the Summary of Product Characteristics (SmPC) and routine of treating physicians. In addition to platelet counts and bleeding events, fatigue is also assessed by using the FACIT-Fatigue (FACIT-F) questionnaire. Statistical analysis is solely descriptive. Data cutoff for this 5th interim analysis was 11th Feb 2022

Results: 302 of the enrolled patients received at least one dose of EPAG and completed at least one post baseline visit. Mean duration of ITP at baseline was 5.4±7.5 years. Mean age was 62.8±17.4 years. Median treatment duration was 14 months. Treatment with EPAG was carried out at a median dosage of 50 mg daily. Median platelet counts increased from baseline 34.0x10⁹/L to 91.5x10⁹/L within one month and remained stable above 90x10⁹/L until the end of the observation period. The number of bleeding events per patient-year decreased, from 1.35 at baseline to 0.59 and 0.16 after one and two years, respectively. In contrast to the improvement in platelet count and incidence of bleeding events, no significant improvement in fatigue score was observed.

Conclusion: EPAG therapy in ITP effectively increases platelet counts and reduces bleedings. Our preliminary data show that about 82 % of patients responded to EPAG. This seems to be consistent with the results of the open extension study EXTEND [Wong RSM et al. Blood 2017; 130: 2527-36], in which a similarly high overall response rate (86%) was found. In general, treatment with EPAG was well tolerated, with no new safety signals from our data set. However, ITP-related fatigue needs further investigation.
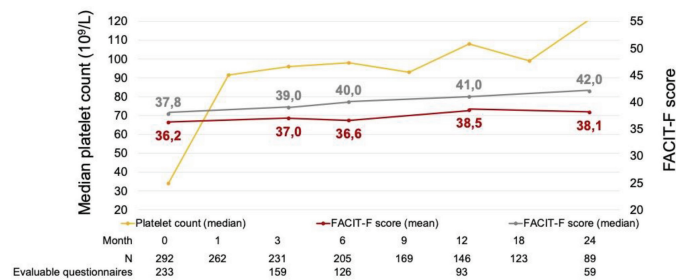

Fig. 1.

VS-12-6

Identification of prothrombotic variants in patients with Cerebral Vein Thrombosis using Next-Generation Sequencing

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Background: Prothrombotic hereditary risk factors for cerebral vein thrombosis (CVT) are underinvestigated but of clinical interest to better understand the underlying pathophysiology and stratify patients for the risk of recurrence. Our exploratory study aimed to identify hereditary prothrombotic risk factors in CVT patients using next generation sequencing (NGS).
Methods: In our outpatient clinic, 183 patients presented in the years 1999-2021 with a history of CVT. An initial screening identified a number of common prothrombic risk factors, including Factor V Leiden (rs6025) and Prothrombin G20210A (rs1799963). All patients without relevant findings (58 individuals) were invited to participate in a subsequent genetic analysis of 55 relevant genes using NGS. The prevalence of the identified variants in the study population was compared with a general European population, accessing the NCBI database of Genotypes and Phenotypes Release 2.
Results: Three intron variants (*ADAMTS13*: rs28446901, *FN1*: rs56380797, rs35343655) were identified to occur with a significantly higher frequency in the CVT patient cohort compared to the general European population. Furthermore, the combined prevalence of at least two of four potentially prothrombic single nucleotide polymorphisms (SNPs) [*FGA* (rs6050), *F13A1* (rs5985), *ITGB3* (rs5918), and *PROCR* (rs867186)] was significantly higher for five out of six possible combinations in the CVT subjects.
Conclusion: SNPs rs6050 and rs5985 affect fibrin characteristics, rs5918 enhances fibrin binding, and rs867186 reduces the profibrinolytic effect of APC. According to a higher frequency of at least two of these variants in CVT patients, altered blood clot properties combined with increased platelet binding/reduced fibrinolysis might increase the risk for thromboembolic events, such as CVT. Identification of hereditary prothrombic variants makes NGS a valuable tool for supporting standard diagnostics.

VS-13
Blood Components

VS-13-1

Platelet reactivity in platelet concentrates is promoted by short-term refrigeration

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Background: The use of cold-stored platelets is discussed to be beneficial for the treatment of acute hemorrhage. Recently, we could show that refrigeration for only 1-2 h was sufficient to increase reactivity of fresh platelets, rendering the opportunity to instantly generate platelets with a higher hemostatic capacity. In this study, we investigated the effect of cold storage for 1 h (CT) in stored apheresis-derived platelet concentrates (APC) after foregoing storage at room temperature (RT).
Methods: APC stored for 1 day or for 2 days at RT, were exposed to cold temperature for 1 h. APC continually stored at RT served as controls. Consecutively, platelet integrity was analyzed by different methods. Inhibitory signaling was explored by vasodilator-stimulated phosphoprotein (VASP) phosphorylation using flow cytometry and by measurement of cyclic nucleotide concentrations. Western Blot analysis was used to investigate activating signaling pathways. Light transmission aggregometry was performed with activators in threshold concentrations.
Results: After cold exposition, VASP phosphorylation levels were partially decreased in APC at storage day 1 and day 2, compared to continued RT storage. Additionally, induced cGMP and cAMP levels were reduced in CT-stored APC. Basal phosphorylation levels of ERK1/2 kinase or p38 MAP kinase were emphasized after cold storage. Aggregation values induced with threshold concentrations of adenosine diphosphate, collagen or TRAP-6 reached higher values in APC after refrigeration for 1 h. In general, cold-mediated effects were more pronounced in APC, stored for 2 days.
Conclusion: In APC at storage day 1 and at storage day 2, exposition to cold temperature for 1 h results in increased platelet reactivity, characterized by an attenuation of inhibitory signaling, by supported activating signaling pathways and by facilitated threshold aggregation responses. Compared to permanently RT-stored APC, short-term refrigeration may be an opportunity to manufacture APC with improved hemostatic capacity "on demand", favorable for the treatment of hemorrhage.

VS-13-2

Ex vivo investigations of thrombus formation using platelet concentrates

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Background: Platelet concentrates (PCs) are used to treat or prevent bleeding in patients with impaired platelet function or after injury. Hemostatic functions of PCs have been investigated intensively under steady state settings but until now no standard methods are available for platelet function tested under shear stress. Our aim is to establish an *ex vivo* model to evaluate the contribution of PCs in thrombus formation and to investigate the effect of different storage conditions on platelet functions.
Methods: First, microfluid channels were coated with collagen (0.1 mg/mL) overnight at 4°C and the next day blocked with HSA (human serum albumin, 1%, 1h, room temperature). Next, PCs stored for 24h at room temperature (RT) were incubated with Calcein (4 μM, 15 min, RT) and
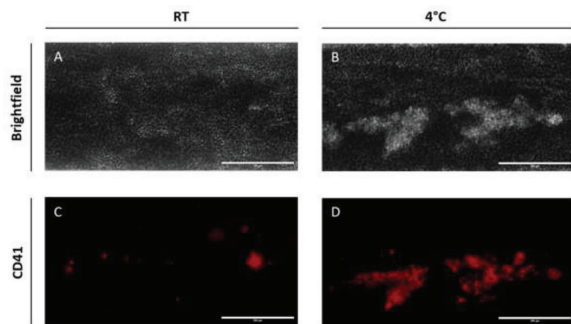
Figure 1: Ex vivo thrombus formation of RT- vs. 4°C-stored PCs.

Representative brightfield (upper panel) and immunofluorescence pictures (lower panel) of *ex vivo* thrombi formed on collagen-coated channels. Spike-in-PCs, stored at RT (A and C) or at 4°C (B and D) were perfused through microfluidic channels, upon TRAP stimulation, for 5 minutes. Prior to perfusion samples were stained with CD41 antibody. Red signal: CD41 antibody. Scale bar: 200 µm.

Fig. 1.

Tab. 1.

Table 1. Coagulation and immune parameters of control and treated plasma units (n=36, mean±standard deviation (SD)).

	Control units		Isoagglutinin depleted plasma units		p
	mean	SD	mean	SD	
Fibrinogen [g/l]	2.7	0.5	2.6	0.4	0.76
Factor II [%]	101.3	6.4	96.8	4.4	0.29
Factor V [%]	97.0	29.3	95.3	27.0	0.93
Factor VII [%]	98.0	16.5	97.0	18.5	0.97
Factor VIII [%]	75.4	19.1	76.0	19.9	0.97
Factor IX [%]	97.3	12.7	94.0	11.4	0.72
Factor X [%]	96.0	8.5	94.0	7.5	0.93
Factor XI [%]	86.3	10.8	85.5	12.2	0.93
Factor XII (%)	100.3	10.8	97.5	11.2	0.74
Factor XIII [%]	101.5	60.9	124.0	10.1	0.49
Protein C [%]	92.3	6.6	88.0	6.1	0.38
Protein S [%]	85.8	13.2	83.8	9.8	0.82
Anti thrombin	96.8	3.6	94.3	3.9	0.39
Activated Partial Thromboplastin Time [s]	28.5	1.7	29.0	2.2	0.73
Quick value [%]	81.3	14.6	83.3	15.8	0.94
vWF antigen [%]	123.5	14.0	117.8	11.9	0.55
vWF activity [%]	114.0	24.8	107.3	24.3	0.71
C3 complement [g/L]	0.9	0.1	0.9	0.1	0.66
C4 complement [g/L]	0.2	0.0	0.2	0.0	0.57
ADAMS 13 activity [%]	97.3	15.4	87.0	9.9	0.31
Lactate dehydrogenase [µkatal/L]	2.2	0.1	2.2	0.1	0.86
International Normalized Ratio	1.1	0.1	1.1	0.1	1.00

recalcified with CaCl (7.5 mM) and MgCl (3.75 mM). TRAP (thrombin receptor-activating peptide, 2.5 µM) was added to initiate thrombus formation. Finally, platelet-depleted whole blood samples from healthy donors were spiked-in with PCs and immunofluorescence pictures were taken randomly. Additionally, PCs were stored for 1, 4, 7 and 10 days either at RT or at 4°C. Samples were stained with CD41 antibody (0.5µg/ml), recalcified and applied to the *ex vivo* system.

Results: To establish the *ex vivo* model, platelets isolated from PCs were tested after 24h of storage at RT and cells from a healthy donor were used as control. Platelets from PCs, after reconstitution, showed stable thrombus formed upon incubation with TRAP. On the contrary, cells incubated with buffer did not form any thrombus (% Surface area covered, Buffer vs. TRAP 1.860±8.844 vs. 10.70±2.255, p=0.0172). To evaluate the effect of cold storage on platelet functions we analyzed the extent of thrombus formation of PCs stored at RT and 4°C upon CD41 staining. Our preliminary results indicate that cold-stored platelets tend to form larger clots under flow conditions after TRAP activation compared to PCs stored at RT (Figure 1).

Conclusion: The results indicate that our *ex vivo* assay, which simulates PC transfusion in thrombocytopenic patients, is suitable to test the hemostatic functions of PCs under physiological flow conditions. Moreover, it allows to investigate cold-induced effects on platelet functions during storage time.

VS-13-3

Towards marketing authorization of isoagglutinin depleted human plasma for blood group independent transfusion

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Background: Isoagglutinins in plasma require for ABO blood group compatibility. We developed a GMP conform procedure to deplete the isoagglutinin titer of fresh frozen plasma (FFP) units group A, B, and O. The method is based on isoagglutinin adsorption onto added red cells. A requirement for the approval of isoagglutinin depleted plasma from blood group A units was an extensive validation study to be able to assess the impact of the plasma treatment on coagulation and immune parameters.

Methods: We developed a bag system, which enables pooling of three FFP units, addition of the adequate RCC volume and subsequent removal of isoagglutinin red cell sediment in a closed system. After incubation at 20-24°C for two hours the isoagglutinin red cell sediment was removed from the plasma pool by centrifugation (4,000g, 10min) and separation. Within the validation study we compared plasma units of 12 production processes (=36 units) with or without the addition of RCC for isoagglutinin adsorption with regard to the following parameters: anti-B titer, free hemoglobin, residual red cells, factors II–XIII, fibrinogen, vWF antigen+activity, protein C + S, antithrombin, ADAMTS13, aPTT, Quick value, lactate dehydrogenase and complement activation.

Results: Starting from maximum values of 1:16 in the plasma pools, total anti-B titers were reduced to < 1:1 in all units of the treatment arm. Free hemoglobin was similar between the control and treatment group (24.8±19.7 µM vs 22.9±15.4 µM; p>0.5). No residual red cells were found by microscope counting. All other coagulation and immune parameters showed no significant differences between the control and treatment arms (Table 1).

Conclusion: We present a fully automated GMP conform procedure for the production of isoagglutinin depleted plasma without any impact on a large set of coagulation and immune parameters.

Disclosure Statements: Macopharma kindly provided parts of the bag system used.

VS-13-4

Indocyanine green labeled platelets for survival and recovery studies

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Background: Before being implemented in daily clinical routine, new production strategies for platelet concentrates (PC) must be validated for their safety. Besides in vitro testing, the establishment of new methods requires the labeling of platelets for in vivo studies of platelets" survival and recovery. Indocyanine green (ICG) is a FDA and BfArM approved near-infrared (NIR) fluorescent dye suitable for non-radioactive direct labeling of platelets for diagnostics.

Methods: Platelets from PC in storage solutions with different plasma concentrations were labeled with ICG up to concentrations of 200 µM. Whole blood was used as an ex vivo matrix to monitor the labeling stability of ICG-labeled platelets. The impact of labeling processes was assessed by the quantification of CD62P expression and PAC-1 binding as platelet function markers. Platelet aggregation was analyzed by light transmission aggregometry. ICG labeling efficiency and stability of platelets was determined by flow cytometry.

Results: Platelets from PC were successfully labeled with 10 µM ICG after 1 and 4 days of storage. The best labeling efficiency of 99.8% ± 0.1% (immediately after labeling) and 81% ± 6.2% (after 24 hours incubation with whole blood) was achieved by plasma replacement with 100% platelet

additive solution for the labeling process. The washing process slightly impaired platelet function, but ICG labeling itself did not affect platelets. Immediately after the ICG labeling process, plasma was re-added resulting in a recovered platelet function.

Conclusion: We developed a Good Manufacturing Practice compatible protocol for indocyanine fluorescent platelet labeling suitable for non-radioactive tracing in survival and recovery studies in vivo.

VS-13-5

Transcription factors STAT3 and MYC mediate human platelet lysate-induced cell proliferation

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Background: Human platelet lysate (HPL) has emerged as an efficient cytokine and growth factor providing medium supplement, replacing fetal bovine serum (FBS) for *ex vivo* clinical cell expansion. As growth promoting effects of HPL may be based on intracellular mechanisms, we hypothesized a specific influence on cell cycle target gene expression and aimed to identify the main molecular key players.

Methods: Cell cycle target gene expression of bone marrow-, umbilical cord- and white adipose tissue-derived stromal cells cultured in HPL- or FBS-supplemented media was compared using RT-qPCR and a cell cycle specific antibody array. As HPL is rich in growth factors that are ligands of tyrosine kinase receptor (TKR) pathways, we applied TKR inhibitors and analyzed cell proliferation. Additionally, genome profiling, RT-qPCR and Western blotting were used to identify downstream mediators of HPL-derived growth signals.

Results: RT-qPCR as well as cell cycle specific antibody array revealed significant upregulation of cell cycle genes in stromal cells cultured in HPL. Blocking specific TKR pathways lead to significantly reduced cell proliferation. In response to HPL we could detect an enhanced expression of the transcription factors signal transducer and activator of transcription 3 (STAT3) and MYC, both known as TKR downstream effectors and stimulators of cell proliferation. Furthermore, specific inhibition of STAT3 resulted in a significant reduction of HPL-induced cell proliferation and cell cycle gene expression.

Conclusion: Based on our results we suppose that HPL-promoted cell proliferation is induced by enhanced TKR signaling and thus elevated expression of the TKR downstream effectors STAT3 and MYC. The enhanced expression of STAT3 and MYC might in turn stimulate the expression of cell cycle promoting target genes.

VS-13-6

Artificial oxygen carriers optimized for *ex situ* organ perfusion

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Background: Normothermic machine perfusion relies on sufficient O_2 supply, while availability of blood by the donor is limited. Artificial oxygen carriers (AOC) based on perfluorocarbons (PFC) can replace blood, as they are synthetic, fully halogenated alkanes, physically dissolving respiratory gases & are of unlimited availability. PFCs must be emulsified for use in aqueous media, which rises stability issues.[1]
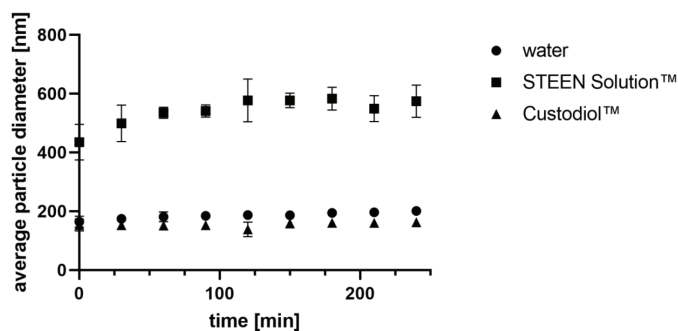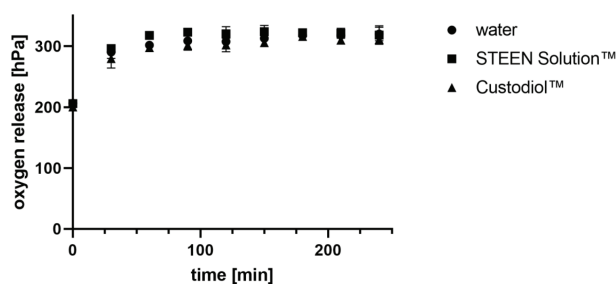
Methods: A new type of PFC-based AOC was developed by using an emulsifier combination of albumin & lecithin. A special high pressure homogenizer (Microfluidizer™) with ice cooling was used to synthesize the novel **le**cithin-modified **n**anoscale **ox**ygen carriers with a perfluorodecalin core (LENOX).[2] LENOX were synthesized in water, Custodiol™ and STEEN Solution™. The applicability of LENOX for machine perfusion was tested by circulating LENOX in an *ex situ* perfusion circuit without organ at 37 °C for 4 h. Samples were taken at regular intervals and analyzed by e.g. dynamic light scattering (size), rheometry (viscosity) & a blood gas analyzer (pH, O_2 release, free ions). Error bars shown in the figures represent standard deviation of n=3 experiments.

Results: LENOX could be synthesized in all three media and formed a sufficiently stable emulsion. The particle size analysis showed a similar behavior for LENOX in water & Custodiol™. LENOX in STEEN Solution™ revealed significantly larger mean particle diameter than the other media. However, LENOX in all 3 media were stable over 240 min & small enough for use in machine perfusion settings (Fig. 1).

All three variants of LENOX showed the same amount of O_2 release, which, due to non-existent consumption, reached a maximum of about 320 hPa (240 mmHg) after 60 min. The different perfusion media depicted no influence on the functionality of LENOX (Fig. 2).

Conclusion: The novel AOC LENOX revealed high stability despite the difficult requirements of organ perfusion. Compatibility with clinically relevant perfusion media such as Custodiol™ or STEEN Solution™ makes LENOX an interesting alternative to blood for organ perfusion.

Disclosure Statements: Three of the named authors are listed as inventors in the given patent

Fig. 1.

Fig. 2.

VS-14
Joint session DGHO – Therapeutic Antibodies – Update

VS-14-3
Therapeutic monoclonal antibodies directed against CD38 or CD47: interference with immunohaematology testing

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A few years ago, monoclonal antibodies against CD38 were introduced to treat multiple myeloma. In addition to myeloma cells and B cells, erythrocytes also carry CD38 antigen. Immediately after administration of the antibody the patients' erythrocytes are also loaded with anti-CD38, but after a few days the direct antiglobulin test (DAT) becomes negative again in most patients. A mild haemolysis of CD38 highly expressing erythrocytes or a shedding of the CD38 molecules are discussed as possible mechanisms. Reagent red cells for antibody testing do not have this possibility of adaptation; they bind the anti-CD38 antibodies and cause positive reactions when the plasma of treated patients is tested in indirect antiglobulin tests (IAT). These reactions can mask any alloantibodies, which is why various methods for mitigating the interference have been developed. One possibility is the treatment with dithiothreitol (DTT) of test cells and of red cells for crossmatching. This substance cleaves disulfide bonds and thereby destroys the CD38 antigen and eliminates the interference in antibody testing and crossmatching. The disadvantage of this method is that some blood group antigens of the systems KEL, DO, YT, JMH, KN, LW, IN are also destroyed and antibodies against these antigens cannot be detected with DTT-treated reagent cells. An alternative is the use of a commercially available inhibitor which mitigates the interference without destroying blood group antigens. Anti-CD38 does not interfere with ABO blood grouping or with typing of other blood group antigens.

Monoclonal antibodies directed against CD47 for the treatment of malignant diseases are currently being investigated in studies. CD47 is highly expressed on erythrocytes. The antibodies lead to a persistently reactive DAT in the patients and to a strong interference with the IAT. In contrast to anti-CD38, high concentrations of anti-CD47 also interfere with ABO blood grouping (both, forward and reverse) and the determination of other blood group antigens. Treatment of test cells with enzymes or with DTT does not destroy the CD47 antigen, inhibitors are not yet available. The antibodies are subclass IgG4, which is why in some cases the use of an antiglobulin serum that does not recognize IgG4 appears to give evaluable results in antibody screening and crossmatching.

Disclosure Statements: Frühere Mitarbeit im Advisory Board von Janssen (> 3 Jahre).

VS-15
Joint session GTH – Hemostaseology – Update

VS-15-2
Platelets and viral infections

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Platelets are blood cells that lack a nucleus and play a vital role in maintaining hemostasis, the process of blood clotting. While platelet activation and high platelet counts (thrombocytosis) are linked to an increased risk of thrombosis, low platelet counts (thrombocytopenia) and certain platelet function disorders elevate the risk of bleeding. Recent evidence suggests that platelets, in their activated state, can also influence innate and adaptive immune responses. Viral infections often trigger platelet activation and cause hemostatic problems. Moreover, low platelet counts are indicative of poor prognosis in many infectious diseases. The dysfunction of platelets during viral infections stems from a complex interplay of various factors. Some viruses directly interact with platelets and/or megakaryocytes to modify their function, while immune and inflammatory responses indirectly promote platelet activation. Consequently, platelet activation leads to increased consumption and degradation, contributing to thrombocytopenia in affected patients. The role of platelets in viral infections often follows a biphasic pattern. Initial hyperactivation is followed by platelet exhaustion and/or decreased responsiveness, which, when coupled with low platelet counts, heightens the risk of bleeding events. Consequently, infectious diseases not only raise the risk of blood clotting but also increase the likelihood of bleeding, or both, which represents a significant clinical complication. Treatment options for these patients are limited, necessitating urgent development of new therapeutic strategies to prevent adverse outcomes.

VS-15-3
Diagnosis and treatment of acquired hemophilia A

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Acquired hemophilia A (AHA) is a rare autoimmune bleeding disorder with an estimated annual incidence of 1–2 cases per 1 million. While most patients present at an advanced age, women may develop AHA during pregnancy or the puerperium. AHA has been associated with other medical conditions such as autoimmune diseases, malignancies, or infections, but in 50–60% of cases, no underlying disorder is identified. Occurrence of extensive cutaneous and soft-tissue hematomas in a patient with an unremarkable previous bleeding history in combination with an isolated prolongation of the activated partial thromboplastin time (aPTT) is highly suspicious for AHA and should prompt further diagnostic work-up. A decreased plasma factor VIII (FVIII) activity in the presence of a functional FVIII inhibitor as shown by the (Nijmegen modified) Bethesda assay confirms the diagnosis. In clinical practice, demonstration that a 1- to 2-hour incubation of patient with normal human plasma at 37°C fails to correct the aPTT may be sufficient to initiate treatment. However, presence of a lupus anticoagulant or acquired von Willebrand syndrome should be considered. Treatment of AHA incudes bleeding control and inhibitor eradication. In patients with low-titer inhibitors, human FVIII concentrates may be efficacious, while the safety and efficacy of desmopressin in a generally elderly patient population with frequent cardiovascular comorbidities are questionable. Approved hemostatic agents comprise recombinant activated FVII, activated prothrombin complex concentrate, and recombinant porcine FVIII. Tranexamic acid may be used as an adjunct. Recent evidence from individual case reports, smaller cases series, and prospective studies indicates that the bispecific FVIII-mimetic monoclonal antibody, emicizumab, confers convincing bleeding protection in patients with AHA. Agents such as corticosteroids, cyclophosphamide, and rituximab are used to eradicate the inhibitor. However, individualized risk stratification is required to tailor the intensity of immunosuppressive therapy and avoid increased morbidity and mortality due to excess toxicity.

Disclosure Statements: Persönliche Honorare für Berater- oder Referententätigkeit: Chugai, Novo Nordisk, Roche, Takeda.

VS-16
Plasma – Update

VS-16-1
Antibody diversity in intravenous and subcutaneous immunoglobulin preparations as inspiration for novel immunotherapeutic drugs

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Intravenous and subcutaneous immunoglobulin preparations contain the broad antibody repertoires of the donor population. While antibody replacement therapy in immunodeficiencies benefits from classical functions of antibodies in host defense, other non-canonical functions have been described that contribute to immunoregulatory effects of polyclonal immunoglobulins as a high-dose therapy. Furthermore, pooled IgA preparations might shape orthobiotic microbial colonization in the gut. The better understanding of human antibody repertoires and polyclonal immunoglobulin mechanisms will inspire the development of novel drugs.

Disclosure Statements: The laboratory of Stephan von Gunten receives research funding from CSL Behring AG, Bern, Switzerland.

VS-17
How to start a company?

VS-17-3
Models of IP-Transfer including Virtual Shares

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The transfer of high-tech patents originating in academic institutions to spin-off companies founded by the inventors is far from being the simple or straightforward process it should be. It is often the most important legal step after the start-up's formation and critical to its success. Still, lengthy and exhausting transfer negotiations are not uncommon and are a significant burden for both the University and the startup. Built-in roadblocks are usually the result of unclear responsibilities and lack of methods allowing to value the technology.

For example, the university often tries to base its valuation on market-based prices, but these often cannot be determined at all, since the market for that new innovative product may even not yet exists at all. All these tough tasks have to be done by typically understaffed legal departments of the universities that by no means can be competent in all aspects of the relevant markets and values in all areas of research conducted at their university. Their negotiation partners are often academic inventors with excellent knowledge of their technology, but no sales experience and without a magic ball to predict the future. Consequently, models that avoid valuation and circumnavigate the efforts and responsibilities of a direct shareholder, but still guarantee profit sharing in the event of success of the startup, would remove a major obstacle to the success of the spin-off. One such model which now is pursued by a growing number of academic institutions is based on virtual shares.

Disclosure Statements: Director, Department of Biotechology, TU Braunschweig, Co-founder/Shareholder of Yumab GmbH, mab-factory GmbH, Abcalis GmbH, Mabswitch Inc. (recombinant antibody engineering companies) and Norden Vaccines GmbH (Vaccine development company). Managing Dirstor, Centre for Molecular Engineering within iTUBSmbH, Scientific Board Member Ichnos

VS-20
Joint Session German Stem Cell Network

VS-20-1
Restoration of immune function in a model of familial hyperinflammation syndrome by editing of hematopoietic stem cells

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Familial hemophagocytic lymphohistiocytosis (FHL) is a group of hereditary immunohematologic disorders caused by hyperactivated T cells and macrophages, leading to severe inflammation and multi-organ damage. Given that current treatment protocols, including allogeneic hematopoietic stem cell (HSC) transplantation, still have high mortality, new treatment options are urgently needed. We have developed gene and base editing strategies to alter the disease-causing mutations in the HSC compartment and investigated them in a preclinical model of FHL type 3 as proof of concept. To this end, we engineered multiple CRISPR-Cas9 nucleases and base editors to alter the FHL3 underlying mutation in the Unc13d locus. After electroporation of HSCs to transfer the editors, approximately 70% of the mutant alleles were modified in either T cells or stem cells. Transplantation of the edited cells into busulfan-conditioned recipient mice reestablished the cytolytic activity of the T cell compartment, restored the ability of the immune system to clear viral infections, and prevented hyperinflammation. On the other hand, high-throughput sequencing-based CAST-Seq and rhAmp-Seq analyses of the edited cells revealed unexpected genotoxicity, including chromosomal translocations and mutations at off-target sites. The results of the in vivo experiments as well as a thorough evaluation of off-target effects will be presented.

Disclosure Statements: T.Ca. ist als Berater von Cimeio Therapeutics, Excision BioTherapeutics und Novo Nordisk tätig. Er erhielt Honorare von CSL-Behring und Pfizer, kooperiert mit der Fa. Cellectis und ist Inhaber von Patenten zur Bestimmung der Off-Target-Aktivität von CRISPR/Cas-Nukleasen.

VS-20-2
Toward clinical application of leukocyte counts based on targeted DNA methylation analysis

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Background: Cell-type specific DNA methylation (DNAm) can be employed to determine the numbers of leukocyte subsets in blood. In contrast to conventional methods for leukocyte counts, which are based on cellular morphology or surface marker protein expression, the cellular deconvolution based on DNAm levels is applicable for frozen or dried blood. Here, we further enhanced targeted DNAm assays for leukocyte counts in clinical application.

Methods: DNAm profiles of 40 different studies were compiled to identify CG-dinucleotides (CpGs) with cell-type specific DNAm using CimpleG. DNAm levels at these CpGs were then measured with digital droplet PCR in venous blood from 160 healthy donors and 150 patients with various hematological disorders. Deconvolution was further validated with venous blood (n=75) and capillary blood (n=31), that was dried on Whatman paper or on Mitra microsampling devices.

Results: In venous blood, automated cell counting or flow cytometry correlated well with epigenetic estimates of relative leukocyte counts for granulocytes (r=0.95), lymphocytes (r=0.97), monocytes (r=0.82), CD4 T cells (r=0.84), CD8 T cells (r=0.94), B cells (r=0.96), and NK cells (r=0.72). Similar correlations and precisions were achieved for dried blood samples. Spike-in with a reference plasmid enabled accurate epigenetic estimation of absolute leukocyte counts from dried blood samples, correlating with conventional venous (r=0.86) and capillary (r=0.80) blood measurements.

Conclusion: The advanced selection of cell-type specific CpGs and digital droplet PCR assays improved accuracy of epigenetic blood counts. Analysis of dried blood facilitates self-sampling with a finger prick.

VS-20-3
HLA-silenced megakaryocytes evade allogeneic T-cell immune responses

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Background: Clinical demand on platelets (PLTs) to treat thrombocytopenic patients is rising. Transfusion of in vitro produced megakaryocytes (MKs) may be used as an alternative to donor PLTs. HLA class I-silenced MKs effectively produced PLTs in the circulation of a refractory mouse model. However, their capacity to upregulate HLA class II may lead to harmful allogeneic immune responses. Hence, we investigated the potential beneficial effect of producing HLA class I and II-silenced MKs.

Methods: HLA class I or HLA- class I and II-silenced iPSCs were generated using lentiviral vectors encoding short-hairpin RNAs (shRNA) targeting β2-microglobulin (shβ2m) and CIITA (shCIITA) respectively. Nontransduced or with a nonspecific shRNA (shNS) encoding vector transduced iPSCs were used as control. Expression levels of β2m, CIITA and HLA class II were evaluated by RT-qPCR. MKs and PLTs were differentiated and phenotyping was performed by flow cytometry. Allogeneic CD4 and CD8 T-cell responses were assessed in CPD-based proliferation assays followed by flow cytometric analysis. Granular T-cell mediated cytotoxicity was measured by quantifying Granzyme B levels by ELISA. MK production was upscaled in an Eppendorf DasBOX Mini bioreactor.

Results: Gene expression levels of β2m and CIITA in HLA class I or class I and II-silenced iPSCs were significantly reduced (p<0.01). HLA-silencing had no effect on their differentiation capability. After incubation of primed T-cells with MKs, both HLA class I or HLA class I and II-silenced MKs led to significantly (p<0.001) lower CD8- and CD4 T-cell proliferation compared to the controls. T-cell granzyme B secretion levels were significantly reduced by 25% (p<0.01) after exposure to HLA class I-silenced MKs. Remarkably, release of Granzyme B-containing granules was even more pronouncedly reduced (52 %, p<0.01) when HLA class I and II-silenced MKs were used. In addition, MKs were successfully produced in a bioreactor.

Conclusion: This study reports the effect of silencing HLA class I and II antigens to prevent allogeneic T-cell-mediated responses towards allogeneic iPSC-derived MKs. Furthermore, the feasibility of upscaling MK production in bioreactors, to meet clinical needs was shown. Use of in vitro manufactured HLA-universal MKs may open new frontiers in the management of highly sensitized thrombocytopenic patients.

Deciphering enucleation: Analysis of RNA sequencing data for investigating differences in differentiation behavior between HSCs and erythroblast cell line "imBMEP"

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Background: An erythroid progenitor cell line capable of efficient in vitro production of mature red blood cells (RBCs) represents a promising alternative for transfusion medicine. Since an erythroblast line previously established in our lab (imBMEP) showed limitations in efficient maturation, knockout (K.O.) of enucleation inhibitor miR-30a-5p was performed. Although this modification led to a significant promotion of terminal erythropoiesis, insufficient enucleation remains a major challenge.

Methods: To systematically investigate possible causes of the inhibited final maturation a transcriptome analysis was performed. Samples for RNA Sequencing were obtained from imBMEP cells and hematopoietic stem cells (HSCs), acting as control. Extracted RNA was derived at different time points during erythroid differentiation in biological triplicates. RNA quality and concentration were determined. The following enrichment of mRNA was performed via ribosomal RNA depletion with RNAse H. After reverse transcription barcoded cDNA libraries were prepared. The following next generation sequencing was executed paired-ended. After read alignment to a human reference genome and quality control, differential gene expression (DGE) analysis was carried out.

Results: DGE analysis revealed high variance between imBMEP cells and HSC controls. Comparison of raw counts of the different RNA biotypes showed a significant elevation of lncRNAs during differentiation compared to corresponding HSC controls (8,4±0,5% vs. 14,4±1,2%). A notable number of cancer related lncRNAs are among the RNAs with the highest differences in expression between imBMEPs and HSCs. Furthermore, dysregulation of previously described factors involved in terminal erythropoiesis and enucleation, like HDAC5, FOXO3 or GATA2, was observed in imBMEP cells.

Conclusion: RNA Sequencing is a powerful tool for analysing differential gene expression. Comparison of sequencing data between imBMEP cells and HSCs gave first insights in dysregulated processes and possible candidates causing differences in differentiation behaviour. Planned ingenuity pathway analysis (IPA) and pathway enrichment analysis should now help to infer the underlying reasons of observed differences, predict downstream effects and find key candidates for further modifications of the cell line.

Mesenchymal stromal cells-derived secretome rescues cisplatin-induced injury of proximal tubular epithelial cells while dampening macrophage activation in an indirect co-culture system

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Background: The interplay between renal proximal tubular epithelial cells (PTEC) and macrophages is important in the progression of kidney injury, caused by a chemotherapeutic drug, cisplatin. Upon injury, PTEC attract macrophages, where macrophages can either promote injury resolution

or inflammation. Mesenchymal stromal cells (MSC), having pro-regenerative capacity, might rescue the injury by acting on PTEC and macrophages. We hypothesize MSC can modulate PTEC and macrophage crosstalk upon the injury.

Methods: MSC secretome was harnessed by producing MSC conditioned medium (CM). First, the effect of CM was tested on PTEC and macrophages upon cisplatin injury separately. To evaluate CM and cisplatin effect on PTEC, apoptosis, gene expression and reactive oxygen species of PTEC were assessed. On macrophages, the surface markers for M1 and M2 polarization, and their phagocytosis capacity were measured. Lastly, the interplay between PTEC and macrophages was investigated using an indirect co-culture system. The PTEC injury was evaluated by measuring their apoptosis, nuclei fragmentation, and TNF-α, while macrophage polarization was assessed by phagocytosis assay. The crosstalk of ciPTECs and macrophages was interrogated by LUMINEX assay.

Results: CM rescued cisplatin-induced PTEC death via gene expression modification and oxidative stress amelioration. On macrophages, CM promoted phagocytosis and the expression of M2-associated surface markers CD163 and CD206, while suppressing M1 markers CD86 and HLA-DR. In the co-culture system, CM suppressed PTEC death by inhibiting apoptosis and nuclei fragmentation. CM also downregulated pro-inflammatory response of PTEC, by lowering TNF-α release. While cisplatin inhibited macrophage phagocytosis, PTEC, and CM, to a greater extent, enhanced it. CM dampened macrophage cytokine secretion triggered by PTEC. Of note, despite CM presence, cisplatin caused macrophage death in long-term.

Conclusion: CM rescued cisplatin injury on PTEC and promoted M2 polarization of macrophages, individually. However, combining PTEC and macrophages did not boost CM amelioration of injury on PTEC. We conclude that MSC-CM overrules the fine-orchestrated crosstalk between PTEC and macrophages, at least in vitro.

VS-21
Hematopoetic Stem Cells

VS-21-1

Outcomes of haploidentical stem cell transplantation with post-tx-cyclophosphamide in comparison with 10/10 MUD in Germany – An observational analysis of German registry data

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Background: Allogeneic stem cell transplantation (alloHSCT) is the best curative treatment modality for many benign or malignant haematological disorders. In the absence of a matched related donor (MRD), matched unrelated donors (MUD) and haploidentical donors (Haplo-SCT) are the most important sources of stem cells. Despite the increasing number of Haplo-SCT in recent years, multicentre real-life data to compare 10/10 MUD transplantations with Haplo-SCT is still limited.

Methods: In this registry based study, we compared the outcomes of alloHSCTs from 10/10 MUD with conventional graft versus host disease (GvHD) prophylaxis (n=10740) versus haploidentical donors using post-transplant cyclophosphamide as a component of the immunosuppressive regimen (n=515) in adult patients with haematological malignancies. These transplantations were performed between 2010 and 2020. Outcomes for overall survival (OS), disease free survival (DFS), acute GvHD/relapse free survival (GRFS), non-relapse mortality (NRM), relapse, as well as acute GvHD (aGvHD) and chronic GvHD (cGvHD) incidences were analysed. Cox proportional hazard models and competing risks regression models were built to compare the outcomes of the groups.

Results: The stem cell source was more often PBSC in the 10/10 MUD group (96% vs. 75.5%) and 81.2% of the patients in 10/10 MUD group had received ATG. 5-year OS and DFS was seen slightly lower in PT-CY Haplo patients compared to 10/10 MUD (42.8% vs. 45.9%, p=0.032; 34.3% vs. 38.4%, p=0.043, retrospectively). 5-year GRFS was significantly worse in PT-CY Haplo patients (22.6% vs. 32.0%, p<0.001). Multivariate analysis was consistent. Additionally, aGvHD grade II-IV and NRM was found to be increased in the PT-CY Haplo group, whereas cGvHD was higher in the 10/10 MUD group. Interestingly when analyzed for severe aGvHD (grades III-IV) no differences were present between both groups. Also, no difference was observed for relapse incidence.

Conclusion: Our findings suggest that 10/10 MUD transplantation in the lack of MRD remains a better alternative than haploidentical transplantations in terms of better OS, DFS, GRFS and lower incidence of NRM and aGVHD.

VS-21-2

Predictive factors for successful hematopoietic stem cell collection in healthy allogeneic donors

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Background: Collection of peripheral blood stem cells (PBSC) from healthy donors after mobilization with G-CSF is performed routinely. We aimed to identify factors predictive of successful CD34+ PBSC collection and established a formula capable of predicting the CD34+ cell yield.

Methods: We retrospectively evaluated 588 healthy adult donors (median age 29 years, range 18–69 years) at our institution from 2017 to 2022. The predicted minimal number of CD34+ cells was calculated as follows: [(peripheral CD34+ cells/μL × adjusted collection efficiency of 30%] × total liters processed. This formula was further modified taking the donor and recipient body weight (BW) into account.

Results: Total CD34+ collection was in median 8.0×10^6 CD34+ cells/kg of recipient BW (range $1.0–47.1 \times 10^6$ cells/kg BW) with 522 donors (89%) collecting $\geq 5.0 \times 10^6$ cells/kg of recipient BW. A second leukapheresis (LP) session the following day was required in 49 donors (8%). Female donors (p<0.001), donors with higher age (p=0.003), donors with WBC count <30 $\times 10^9$/L after 5 days of G-CSF stimulation (p=0.004), and a donor/recipient weight ratio <1 (p<0.001) had a higher risk for a second LP session. Predictive factors for optimal LP were peripheral blood CD34+ cell count >50/μL (p<0.001), male donor (p=0.04), and a donor/recipient weight ratio >1 (p=0.001).

Conclusion: Of the routinely monitored indicators in PBSC donors, CD34+ cell count in peripheral blood is the most important factor in predicting G-CSF-induced PBSC yields. Risk factors for suboptimal mobilizers were higher age, female sex, WBC <30 $\times 10^9$/L, and a donor/recipient weight ratio <1. We applied a formula that has shown successful and consistent performance in the prediction of key outcome measures.

Fig. 1: Predicted versus actual collected CD34+ cells x10⁶ /kg body weight of the recipient in 588 leukapheresis sessions

Equation: $\log10(\text{actual CD34+ cells collected}) = 0.932 \times (\log10(\text{predicted CD34+ cells to be collected})) - 0.237$
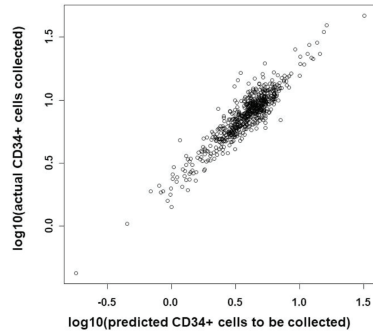

Fig. 1.

VS-21-3

Plerixafor vs placebo for stem cell mobilization in patients with multiple myeloma optimize collection results in moderate mobilizers (Optimize)

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Background: This study evaluates the safety and efficacy of plerixafor (PLER), a CXCR4 antagonist, in mobilizing hematopoietic progenitor cells for autologous hematopoietic cell transplantation (HCT) in multiple myeloma (MM) patients.

Methods: This is a phase IV, multicenter, randomized (1:1), double-blind, placebo-controlled study. Patients with MM scheduled for 2 autologous HCTs mobilized with granulocyte colony-stimulating factor (G-CSF; 2×5 µg/kg) s.c. daily for up to 5 days were eligible. On day 4 of G-CSF, peripheral blood (PB) CD34+ count was analyzed and patients were enrolled if they had 15-40 CD34+ cells/µL. They received either PLER at a dose of 240 µg/kg or placebo 4-12 hours before start of apheresis. The primary endpoint was to collect 6×10^6 CD34+ cells/kg in one apheresis, thus enabling 2 autologous HCTs. In addition, CD34+ and lymphocyte subsets, colony forming units (CFU) and engraftment kinetics were analyzed.

Results: The study was designed for 90 patients but was stopped after 21 patients due to approval of daratumumab also for first line therapy in MM, which led to insufficient mobilization (i.e. <15 CD34+ cells/µl in the PB) in the predefined patient cohort. With PLER pre-apheresis CD34+ cell counts were significantly higher than with placebo (median 93,3/µL vs. 29,34; p-Value 1.71E-08). Ten of 21 (48%) patients received PLER and 11 (52%) placebo. All patients in the PLER group met the primary endpoint with a median of 7.6×10^6 (range 7.03 to 13.18) CD34+ cells/kg compared to none in the placebo group (median 2.52×10^6 CD34+ cells/kg, range 1.40 to 5.27×10^6 CD34+ cells/kg). PLER was tolerated well with only rare cases of gastrointestinal disorders.

Conclusion: In contrast to G-CSF alone, PLER + G-CSF allowed to collect $>6 \times 10^6$ CD34+ cells/kg for 2 autologous HCTs in only one apheresis session in moderately mobilizing MM patients (i.e. 15-40 CD34+ cells/µl). This not only reduces apheresis procedures and workload for operators and technicians but also facilitates planning of apheresis and increases patient's comfort.

Therefore, PLER should be considered not only in cases of imminent mobilization failure but also in patients with moderate mobilization.

VS-21-4

BluStar – A project to recruit non-Caucasian hematopoietic stem cell donors resulting in more than 9,100 donors and 12 PBSC, one bone marrow and three donor lymphocyte aphereses so far

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Background: 19 million non-Caucasians live in Germany. These people have genetically different HLA antigens compared to the most common HLA frequencies in Central Europe. In severe hematological diseases, allogeneic stem cell transplantation offers the only curative therapy. However, finding HLA-compatible hematopoietic stem cells (HSC) donors in case a related donor is lacking, is a major challenge as there are only few unrelated donors with a similar genetic background registered in HSC donor registries.

Methods: The "BluStar" project was initiated to recruit non-Caucasian donors of HSC. The non-Caucasian donors were registered and typed by the Westdeutsche SpenderZentrale (WSZE), Ratingen, Germany. Several dedicated recruiting events, multi-lingual flyers, and intensive interactions with relevant social stakeholders have been employed to attract potential non-Caucasian HSC donors. We analysed the gender and age distribution of donors recruited in the BluStar project. Besides, we documented the "work-ups" (i.e. requests for a potential HSC donation) resulting out of the BluStar cohort per country and compared the apheresis probability and cancellation rate of work-ups in the BluStar cohort with that in the non-BluStar WSZE donor cohort.

Results: More than 9,100 non-Caucasian HSC donors have been recruited. Gender distribution is equal: 4,583 (50%) donors are male (m) and 4,550 (50%) donors are female (f). The age distribution is as follows: < 30 years: 2,937/3,168 (32%/35%, m/f); <40 years: 962/627 (11%/7%, m/f); <50 years: 551/589 (6%/6%, m/f); >50 years: 133/166 (1%/2%, m/f). 29 work-ups were initiated. 13 work-ups were cancelled; however, 12 PBSC, one BM and three DLI were collected so far. The PBSC/BM/DLI were delivered to Germany, USA, France, Spain, UK, Poland and Australia. Apheresis probability is twofold higher in the BluStar donor cohort (0.18% vs. 0.07%). In contrast, the cancellation rate of work-ups in the BluStar cohort is almost twice as high (45% vs. 25%).

Conclusion: Within the BluStar project more than 9,100 non-Caucasian donors were registered as unrelated HSC donors. The twice-fold apheresis probability in the non-Caucasian donor cohort pinpoints the lack of non-Caucasian HSC donors. However, the high cancellation rate might indicate higher logistic hurdles in this cohort. Obviously, there is serious medical need to increase the number of non-Caucasian HSC donors to enable more allogeneic stem cell transplantations in the non-Caucasian patient population.

Disclosure Statements: There is no conflict of interest to declare. The European Development Fund 2014-2020 (ERDF) and the European Union funded this project.
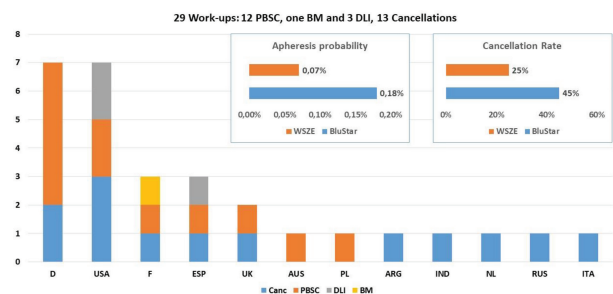

Fig. 1.

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VS-21-5

Automatic generation of alloreactivity-reduced donor lymphocytes and hematopoietic stem cells from the same mobilized apheresis product

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Background: In vitro or in vivo depletion of alloreactive T-cells can facilitate haplo-identical hematopoietic stem cell transplantation (HSCT). We established and validated a TCRαß/CD19 depletion module for automatic cell-processing on CliniMACS Prodigy, and investigated quality attributes of the HSPC products. The process was combined with aCD45RA depletion module as such a product could serve as allo-reactivity attenuated donor lymphocyte infusion (DLI) after transplantation.

Methods: We evaluated 6 apheresis products from G-CSF-mobilized volunteer donors which were split, one portion each depleted of CD45RA+ or of TCRα/ß+ and CD19+ cells. Products were assessed for recovery of HSPCs and certain mature subsets, as well as depletion of targeted cells using flow cytometry. Effects of apheresis and product age post 48 h storage at 4 °C as well as freeze-thawing on product viability and recovery of WBC and CD34+ cells were assessed by flow cytometry.

Results: Ten sequential depletions were technically uneventful, proceeding automatically with minimal hands-on time. Depletion of CD45RA+ resp. TCRα/ß+ and CD19+ cells was nearly complete, at least equally to previous reports, achieving mean depletions of 4 log of targeted cells for both products. HSPC products retained TCRγ/δ+ and NK cells and even were passively enriched. 48 h storage of apheresis product was associated with the expected modest loss of HSPCs, but depletions proceeded efficiently. Depleted products were stable until at least 72 h after apheresis with stem cell viabilities > 90 %. Freeze-thawing resulted in loss of NK cells; post-thaw recovery of viable CD45+ and HSPCs was > 70 % and in line with expectation.

Conclusion: A closed, GMP-compatible process on CliniMACS Prodigy generates two separate medicinal products from the same mobilized apheresis product. The CD45RA-depleted products contained functional memory T cells, whereas the TCRαß/CD19-depleted products included stem cells, TCRγ/δ+ and NK cells. CD45RA-depleted DLI and/or TCRαß/CD19-depleted HSPC products are predicted to be effectively depleted of GvH-reactivity while providing immunological surveillance, in support of haplo-identical HSCT.

VS-21-6

Hematopoietic stem cell transplantation and its influence on the bone structure and marrow cells

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Background: In most malignant hematological diseases the preferential treatment is a hematopoietic stem cell transplantation (HSCT). Initially, lymphopenia and diminished immunity is associated with HSCT. Several studies proved a higher risk of fractures and osteoporosis after HSCT. However it is rarely studied what happens on the cellular level within the bone directly after transplantation. This project aims to investigate the underlying proceedings responsible for changes in the bone after HSCT.

Methods: Within our group, a mouse model for HSCT has been developed to investigate bone and changes within during graft versus host disease (GVHD). Two groups were investigated a) syn = control without GVHD after HSCT and b) allo = a group that developed acute GVHD. 20, 40 and 60 days after cell transfer animals were sacrificed and bones were harvested. Bones underwent radiological and histological analyses. Consecutively, primary human mesenchymal stromal cells and osteoblasts were stimulated to differentiate and analyzed with metabolic assays and specific stainings in a GVHD simulating in vitro setting. During this stimulation, media immunized with immune cells were added to examine their potential to influence bone homeostasis.

Results: Femora of mice developing a strong GVHD after allogeneic transplantation showed a larger medullary area and thinner cortical bone in the diaphyseal as well as an increase in bone volume and trabecular number in the epiphyseal region revealing differences in the whole bone structure. Also, no osteoblastic lining cells were detectable and osteoid formation was absent, indicating an impaired bone formation capacity due to the GVHD. Furthermore, deviating B cell numbers and distributions were seen. The in vitro analyses demonstrated the involvement of immune cell signaling seen in impaired – T cells or improved – B cells osteogenesis.

Conclusion: Impaired recovery of the immune system after allogeneic HSCT not only leads to an increased risk of severe infections. By examining bone structure and stroma cells we could prove that the imbalance of
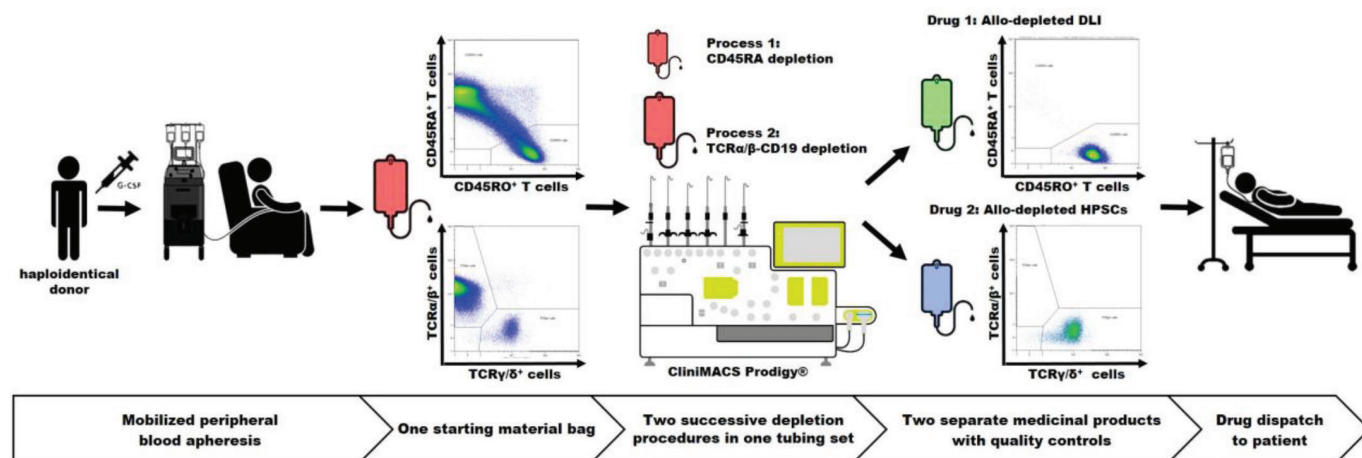

Fig. 1.

immune cells of the adaptive immune system impedes the bone structure. This is caused by a decreased capacity of osteogenic differentiation due to the lack of B cells. This discovery may be significant for the prognosis and treatment of long-term effects on the musculoskeletal tissue after HSCT.

VS-22
Immunotherapy 2 – Engineered Cells

VS-22-1
Engineered T cells selectively depleting alloreactive B cells and resisting immunosuppressive treatment as tool to combat AMR following solid organ transplantation

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Background: One major complication after solid organ transplantation (SOT) is antibody-mediated rejection (AMR) by anti-donor HLA antibodies. However, the B-cell alloimmune response is only indirectly addressed by modern immunosuppression. Unselective B-cell depletion protocols are inefficient in preventing AMR and associated with an increased infection risk, emphasizing the need for a more precise targeting of alloreactive B cells.
Methods: B cells with anti-donor HLA specificity are uniquely characterized by expression of the corresponding B-cell receptors (BCRs). Using BCRs against a distinct HLA molecule as target, we redirected T cells towards alloreactive B cells by introducing a novel chimeric receptor comprising the respective HLA molecule fused to intracellular 4-1BB/CD3ξ signaling domains to generate T cells overcoming rejection by antibodies (CORA-Ts). As a proof-of-concept, CORA-Ts based on an HLA-A*02 molecule were generated and further modified to abrogate T-cell sensitization and confer resistance to immunosuppression. Their ability to recognize and selectively eliminate anti-HLA-A*02 B cells to limit antibody release was tested *in vitro* as well as *in vivo*.
Results: Upon co-cultivation with B-cell lines expressing and releasing anti-HLA-A*02 antibodies, CORA-Ts were specifically activated (expression of CD25, CD69, CD137), released pro-inflammatory molecules (e.g. IFN-γ, granzyme B), and exhibited strong cytotoxicity resulting in an effective reduction of the anti-HLA-A*02 antibody release. In a mouse model, CORA-Ts significantly reduced growth of an anti-HLA-A*02 hybridoma B-cell line. Modification of the HLA-A*02 α3-domain abrogated T-cell sensitization against the CORA receptor by prevention of CD8 binding. Additionally, CRISPR/Cas9-mediated knockouts of selected binding proteins endowed CORA-Ts with the ability to resist immunosuppressive treatment.
Conclusion: Our results demonstrate that CORA-Ts are able to specifically recognize and eliminate alloreactive B cells, and thus selectively prevent formation of anti-HLA antibodies even under immunosuppressive conditions. This suggests CORA-Ts as a potent novel approach to specifically combat AMR and to improve long-term graft survival in SOT patients while preserving their overall B-cell immunity.

Disclosure Statements: The authors declare no conflict of interest, except that authors A.C.D., C.F., R.B. and B. E.-V. submitted a patent application on CORA-Ts.

VS-22-2
Zebrafish as a novel in vivo model to assess CAR-NK cell efficacy against metastatic breast cancer

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Background: NK cells are attractive effectors for adoptive immunotherapy of cancer. Results from first-in-human studies with chimeric antigen receptor (CAR)-engineered primary NK cells and NK-92 cells are encouraging in terms of efficacy and safety.
Methods: To further improve treatment strategies and to test the efficacy of CAR-NK cells in a personalized manner, high-throughput preclinical screening assays using patient-derived tumor samples are needed. Here, we established a flexible *Danio rerio* (zebrafish) larvae *in vivo* xenograft model and tested the efficacy of PD-L1-targeting CAR NK-92 cells (PD-L1.CAR NK-92) against the PD-L1-expressing breast cancer cell line MDA-MB-231. In addition, we used transgenic zebrafish models with labeled blood vessels to study CAR-NK migration in the vasculature.
Results: We have shown that MDA-MB-231 GFP cells injected into zebrafish larvae at 2 days post fertilization (dpf) are viable and migrate to peripheral parts of the zebrafish body, including the tail region. PD-L1. CAR NK-92 cells injected 2.5 hours later could also migrate to the zebrafish periphery and eliminate cancer cells throughout the body as early as 24 hours, in contrast to parental NK-92 or uninjected controls. Residual cancer cells were further eliminated at later time points, with 48 hours post-injection (hpi) being the best time point for analysis. Confocal live-cell imaging *in vivo* demonstrated intravascular migration and real-time interaction of PD-L1.CAR NK-92 with MDA-MB-231 cells, resulting in cytotoxicity.
Conclusion: Our data thus suggest that zebrafish larvae can be used for rapid assessment of CAR-NK cell potency *in vivo* to predict patient response to therapy.

VS-22-3
Enhanced NK-cell cytotoxicity by combining genome editing and RIG-I activation

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Background: Natural killer (NK) cells can recognize and eliminate cellular targets without MHC-restriction, rendering them particularly amenable to off-the-shelf cellular immunotherapy. Nonetheless, potential target cells employ diverse molecular signals to limit NK-cell recruitment and evade cytotoxicity. Both innate immune activation and checkpoint inhibition on NK cells are promising approaches to improving the infiltration and cytotoxicity of NK-cell immunotherapy.
Methods: We examined the effect of the modulation of the immune checkpoint axis NKG2A/HLA-E on NK-cell tumor toxicity both alone and in combination with targeted activation of the innate immune receptor Retinoic-acid Inducible Gene I (RIG-I). Using CRISPR/Cas genome editing, we ablated the NKG2A-ligand HLA-E in diverse human tumor cell lines. Moreover, we developed a robust protocol for the targeted genome editing of primary and immortalized NK cells, allowing us to reduce NKG2A levels in effector cells. We then analyzed the activity and cytotoxic potential of these *NKG2A-/-* NK cells *in vitro* both in the presence and

absence of HLA-E expression on tumor cells as well as alone and in combination with stimulation with a specific RIG-I agonist (3pRNA).

Results: All tumor cell lines tested demonstrated enhanced susceptibility to NK-cell killing after ablation of HLA-E on their targets. In line with this, *NKG2A-/-* NK cells also demonstrated greater degranulation, cytotoxicity towards target cells and cytokine induction, all of which occurred in an HLA-E dependent manner. Activation of RIG-I in tumor cells also enhanced NK cell activity overall but concurrently induced the expression of HLA-E in target cells. However, combining RIG-I activation with *NKG2A-/-* counteracted the effect of HLA-E induction and thus resulted in the highest cytotoxicity against target cells.

Conclusion: Altogether, our data demonstrate that combining *NKG2A-/-* NK cells with RIG-I activation is an effective approach to boosting allogeneic antitumor responses *in vitro*. Moreover, by establishing an efficient system for genome editing of primary NK cells, we provide an approach to targeting other promising candidate genes during NK-cell based therapies. Further studies will be needed to examine further NK checkpoints in this context and to investigate *NKG2A-/- /RIG-I* combination therapy *in vivo*.

Disclosure Statements: G.H. is an inventor on a patent covering synthetic RIG-I ligand and was a co-founder of Rigontec GmbH. All of others have no conflict of interest.

VS-22-4

Next generation LMP2A-targeting TCR-recombinant T-Cells with inducible expression of Il-18 as promising tool to treat various EBV-associated malignancies

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Background: Epstein-Barr virus (EBV) infects >90% of the population and remains in B-cell compartments life-long, passing through several latency stages. In latency stages II and III, latent membrane protein 2A (LMP2A) is expressed and therefore associated with post-transplant lymphoproliferative disorder (PTLD) as well as with various lymphomas and carcinomas. Recently, a clinically protective TCR recognizing an LMP2A-derived peptide (CLG) in context of HLA-A*02 (A*02/CLG) was identified (LMP2A_TCR).

Methods: We developed TCR-engineered T cells based on the LMP2A_TCR (LMP2A_TCR-Ts) and further equipped these with an inducible cassette for locally restricted IL-18 release (LMP2A_iIL18_TCR-Ts) to enhance T-cell functionality. Engineered TCR-Ts were generated by lentiviral transduction and their memory phenotype, replicative capacity, activation and exhaustion state during generation and after target cell encounter were analyzed. Their cytotoxicity towards HLA-A*02+ SPI-801 cells loaded with the CLG peptide and HLA-A*02+ EBV-transformed B-lymphoblastoid cell lines (B-LCLs) were analyzed using flow cytometry-, microscopy- and impedance-based assays. Furthermore, cell avidity (z-MOVI) as well intracellular Ca2+ signaling were evaluated.

Results: LMP2A_TCR-Ts and LMP2A_iIL18_TCR-Ts recognized HLA-A*02+ SPI-801 cells loaded with different concentrations of CLG in a dose-dependent manner. No signs of HLA cross-reactivity or recognition of an irrelevant HLA-A*02-restricted peptide were observed. Specificity and activation capacity of LMP2A_iIL18_TCR-Ts was confirmed by Ca2+ signaling analysis. Release of IL-18, which was shown to convert

T cells into pro-inflammatory effector cells and reshape the immunosuppressive tumor milieu, resulted in significantly increased cytotoxicity when compared to LMP2A_TCR-Ts. Of note, LMP2A_iIL18_TCR-Ts but not LMP2A_TCR-Ts recognized and efficiently eliminated HLA-A*02+ B-LCLs serving as PTLD model and described to produce an anti-inflammatory milieu.

Conclusion: Our data indicate that LMP2A_iIL18_TCR-Ts specifically recognize A*02/CLG, leading to effective elimination of EBV+ HLA-A*02+ target cells. The release of IL-18 improved the functionality of the engineered T cells. In conclusion, *ex vivo* isolated, protective TCRs could be redirected into T cells from third-party donors with the potential to attract innate immune cells and alter the tumor environment, thereby widening the applicability of T-cell therapy to refractory viral infections.

VS-22-5

4th generation CAR-T cells with inducible cytokine expression targeting CD176 to overcome the immunosuppressive tumor milieu in lung cancer

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Background: In the development of effective CAR-Ts against solid tumors, both choosing an appropriate tumor-associated target and overcoming the immunosuppressive tumor microenvironment (TME) remain major challenges. The oncofetal carbohydrate CD176 (Thomsen-Friedenreich antigen), hidden in adult benign tissues by sialylation or prolongation with carbohydrates, is unmasked in 90% of carcinomas, predicting low "on-target/off-tumor"-toxicity.

Methods: To target CD176 and overcome the immunosuppressive TME, we developed 4th-generation CD176-specific CAR-Ts, also known as T cells redirected for universal cytokine-mediated killing (TRUCKs). In addition to the constitutive CD176-CAR expression, these cells were engineered with an inducible cassette expressing either IL-12 (iIL12_CD176_TRUCKs), IL-18 (iIL18_CD176_TRUCKs), or EGFP as a control (iEGFP_CD176_TRUCKs). Functionality of CD176_TRUCKs was tested by co-cultivation with CD176+ cell lines and human lung cancer tissue. To characterize activation and cytotoxicity of CD176-TRUCKs following target recognition, multicolor flow cytometry, confocal microscopy, multiplex assays and bulk-RNA transcriptomics were performed.

Results: Following co-culture with different CD176+ lung carcinoma cell lines, all CD176_TRUCKs increased NF-κB activity, became activated, released effector molecules (e.g. IFN-γ), and mediated effective cytotoxicity. They did not react towards CD176− control cells. The inducible cytokines IL-12 and IL-18 were released by respective TRUCKs in a target-specific manner and clearly improved particular effector functions in comparison with iEGFP_CD176_TRUCKs. Precision-cut lung sections (PCLS) were generated from explanted human lung adenocarcinoma tissue and shown to express CD176. Using PCLS as *ex vivo* model, specific cytotoxicity of CD176_TRUCKs against tumor tissue but not against healthy tissue of the same lung was demonstrated.

Conclusion: CD176_TRUCKs equipped with inducible cytokines were shown to be highly functional, suggesting them as promising strategy to overcome the TME. Based on the tumor-selective expression of CD176, CD176_TRUCKs have a high potential to effectively control lung carcinoma while minimizing "on-target/off-tumor"-toxicity.

Efficient CAR T cell generation starting with leukoreduction system chambers of thrombocyte apheresis sets

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Background: During plateletpheresis, the leukoreduction system chamber (LRSC) reduces the leukocyte amount within the subsequent platelet concentrate through saturated, fluidized, particle bed filtration technology. Normally, the LRSC is discarded after apheresis is completed. To explore if those retained leukocytes are attractive for research purposes, we isolated CD3+ T cells from the LRSCs via density gradient centrifugation in order to manufacture CD19-targeted chimeric antigen receptor (CAR) T cells.

Methods: Immunophenotyping of the LRSC derived mononuclear cells (MNCs) was performed via flow cytometry. T lymphocytes were isolated using the Pan T Cell Isolation Kit and stimulated with IL-2 and paramagnetic beads. Upon lentiviral transduction with concentrated self-inactivating viral vector supernatants, CD19 CAR T cells were further enriched through anti-CD19 CAR MACS, co-incubation with irradiated CD19+ Daudi cells, or through a vector containing an EGFP-T2A-puromycin resistance gene and a one-shot 2 day puromycin treatment. CAR T cell cytotoxicity was assessed using Luciferase labeled cell lines. *In vitro* CAR T cell expansion to clinically relevant numbers was achieved.

Results: Compared to peripheral blood, LRSC yields a 10-fold MNC concentration. Immunophenotypic characterization revealed viable and normal CD4+ and CD8+ T cell populations, with low CD19+ B cell counts. T cells showed low expression of exhaustion markers and a normal memory subpopulation distribution. Robust CD19 CAR cell surface expression on transduced T cells was confirmed by flow cytometry. Anti-CD19 CAR MACS resulted in 80% CAR+ cell populations, whereas puromycin selection achieved >90% CAR T cell populations. To prove functionality, CAR T cells were co-incubated with the human CD19+ B cell precursor leukemia cell lines Nalm6 and Daudi. Compared to unmodified T cells, CD19 CAR T cells effectively eradicated Nalm6 and Daudi cells.

Conclusion: Stable transduction rates of the isolated T cells could be achieved by lentiviral transduction. Puromycin selection or anti-CD19 CAR-MACS can be used for further purification. These CAR T cells can be successfully expanded and show superior cytotoxicity against target cells compared to unmodified T cells. Taken together, we can show that lymphocytes isolated from LRSCs of plateletpheresis sets can be efficiently used for the generation of functional CAR T cells for experimental purposes.

VS-23
Immunohematology 2 – Non Red Cells

Autoimmune neutropenia of infancy is almost exclusively caused by IgG antibodies

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Background: The relevance of IgM antibodies in autoimmune mediated haemocytopenias remains a matter of debate. For autoimmune neutropenia, conflicting results were published. This study was performed as part of the Giessen Neutropenia Registry which propectively included children with suspected autoimmune neutropenia.

Methods: Children with suspected autoimmune neutropenia were prospectively enroled and data central to their suspected diagnosis were collected over 3 years. Sera were tested for the presence of autoantibodies against neutrophils by granulocyte immunofluorescence test (GIFT) and granulocyte agglutination test (GAT). The presence of IgG and IgM antibodies was assesed in a subgroup of patients with positive initial GIFT in whom spontaneous remission of their neutropenia was documented.

Results: A total of 406 children were included in the study. A remission was reported for 114 patients during the study period. Of those, 96 had an initial positive GIFT. Their median age at diagnosis was 11 months (7-17.5), and their mean neutrophil count was 293 (±267)/μL, ranging from 0 to 975. In total 75/96 (78%) of children had at least one documented infection at diagnosis. In 29/96 (30%), a bone marrow aspirate was available. At the documented time point of remission, children were 27 months (18-41) old. In the initial sample send for diagnosis, IgG antibodies against neutrophils were detected in 94/96 children (98%), and IgM antibodies were detected in 2/96 (2%). We did not observe children with autoantibodies of both Ig classes.

Conclusion: To our knowledge, this is the first study to demonstrate the contribution of IgG or IgM antibodies in a well-defined clinical cohort with a typical course including, spontaneous disease remission. IgG antibodies were present in 98%. Only very rarely, IgM antibodies can be detected. We conclude that in general, screening for IgG autoantibodies is appropriate as a first diagnostic step. In cases with strong clinical suspicion but negative GIFT with anti-IgG, anti-IgM testing may be considered.

The impact of antibody subclasses on anti-HPA-1a mediated platelet phagocytosis

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Background: Accelerated clerance of maternal IgG antibody (anti-HPA-1a)-opsonized fetal platelets by phagocytes represents the major mechanism in cases of fetal and neonatal alloimmune thrombocytopenia (FNAIT). Until today, limited studies examining the impact of IgG antibody subclasses have been conducted. Such study is mainly hampered by the absence of good standards for anti-HPA-1a antibody subclasses and reliable platelet phagocytosis assay. In this study, both limitiations are carefully addressed.

Methods: Different IgG subclasses (IgG1, -IgG2, -IgG3 and -IgG4) of recombinant monoclonal antibody anti-HPA-1a (moAb 26.4) and effector-silent moAb 26.4 (IgG1-LALAP) were produced. Binding of antibody subclasses onto platelets was tested by flow cyometry using fluorescence labelled subclass-specific and Fcγ-specific secondary antibodies. The clearance of anti-HPA-1a sensitized platelets was analyzed by Whole Blood Platelet Phagocytosis Assay (WHOPPA). Platelets were labelled with pH sensitive dye (pHrodo-SE), opsonized with different moAb 26.4 subclasses and subjected to WHOPPA. The rate of pHrodo entgulfed platelets by monocytes was measured by flow cytometry.

Results: All 26.4 IgG subclasses did not interact with HPA-1bb, but bound equally to HPA-1aa typed platelets when tested with Fcγ-specific secondary antibodies by flow cytometry. In contrast, IgG subclass bound moAb 26.4 could only be detected by subclass specific secondary antibodies; bound moAb 26.4 IgG1 only reacted with anti-IgG1, but not anti-IgG2, -3 or -4. This specific reaction was also found with IgG2, -3 and -4. Analysis by WHOPPA showed that except effector-silent 25.4-IgG1 LALAP, all 26.4 IgG sublasses induced significant platelet phagocytosis by monocytes, but not by neutrophils. Highest phagocytosis rate was observed with 26.4-IgG3 (~78.7%), followed by IgG1 (~51.1%), IgG4 (~35.4%), and IgG2 (~19.1%).

Conclusion: Here, we enable reliable analysis of platelet phagocytosis caused by anti-HPA-1a IgG subclass using different subclasses of moAb 26.4 as standard. Our observation that moAb 26.4 IgG3 subclass induced platelet phagocytosis most strongly is interesting and is in line with the high capability of IgG3 to induce effector functions, most probably via

high affinity FcγRI receptor on monocytes. The question wheather anti-HPA-1a IgG3 abs are dominated in maternal sera with severe FNAIT is intriguing.

VS-23-3
NAIT due to a presumably new alloantibody against Gp IIb

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Background: Neonatal alloimmune thrombocytopenia (NAIT) is a disorder of the newborn due to fetomaternal incompatibility of platelet antigens. Polymorphisms associated with platelet alloimmunisation are primarily located in *ITGB3* & *ITGA2B*. The latter one encodes for the α chain of Gp IIb/IIIa and to date, nine variants with corresponding alloantigens are known. The rare variant rs547581737 of *ITGA2B* (estimated frequency <10-4) leads to p.Arg774Trp. So far, no alloimmunisation is described for this variant.

Methods: Here, we present a case of a male newborn with a suspected NAIT and a presumably new alloantibody. Blood samples of all direct family members were analysed. Antibody detection was performed by indirect MAIPA (in-house assay). Serological analysis included a crossmatch between paternal platelets and maternal serum. Initial genotyping was performed by commercial SSP-PCR (BAGene HPA-TYPE, BAG Diagnostics). For sequencing of *ITGA2B* and *ITGB3* a direct taq-cycle method was chosen.

Results: No maternal platelet specific alloantibody could be detected by indirect MAIPA using a routine platelet panel. For HPA-1, -2, -3, -4, -5, -6, -9, -15 systems, no incompatibility between mother and newborn was found by SSP genotyping. However, a crossmatch between maternal serum and paternal platelets was strongly positive (Gp IIb/IIIa). Thus, the existence of an antibody against a low-frequency antigen was assumed and sequencing of both eligible genes (*ITGA2B* and *ITGB3*) was initiated. The rare variant rs547581737 with a nucleotide exchange in exon 23 (c.C2320T) was found in the father and the newborn, but not in the mother. At GnomAD, an allele frequency of 4.6x10E-5 is given for this variant and no homozygote sample was found so far.

Conclusion: In Europeans, NAIT is caused by anti-HPA-1a in the majority of cases, though several cases with antibodies to low-frequency antigens have been described. Thus, the rare variant of *ITGA2B* in our case is probably also responsible for maternal alloimmunisation and neonatal thrombocytopenia. Further serological investigations are needed to prove this antibody specificity. However, our case demonstrates once again that a serological crossmatch is essential for detecting antibodies to novel antigens.

Dose-dependent elimination of HPA-1a platelets by subcutaneous RLYB212, a monoclonal antibody to prevent fetal and neonatal alloimmune thrombocytopenia

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Background: Fetal and neonatal alloimmune thrombocytopenia (FNAIT), based on a rare development of maternal alloantibodies against fetal platelets, may result in potentially catastrophic bleeding. No preventative FNAIT treatments are currently available. Prophylactic treatment with a monoclonal human platelet antigen (HPA)-1a antibody (RLYB212) is intended to drive rapid phagocytosis of fetal-derived HPA-1a antigen in maternal circulation to prevent alloimmunization to HPA-1a.

Methods: Following approval by the University Hospital Ethics Committee, participant informed consent was obtained. We assessed the dose-effect relationship of subcutaneous monoclonal anti-HPA-1a antibody (RLYB212) to eliminate HPA-1ab platelets transfused to HPA-1bb participants. Participants (HPA-1bb/human leukocyte antigen [HLA]-A2–negative healthy males) received a single dose of RLYB212 (0.09 mg or 0.29 mg) or placebo on day 1, followed by transfusion with 10 X10^9 HPA-1ab/HLA-A2–positive platelets on day 8. The proportion of HPA-1ab platelets in circulation over 7 days was determined by flow cytometry using HLA-A2 antibodies.

Results: RLYB212 rapidly eliminated HPA-1ab platelets, with a mean reduction in platelet elimination half-life of ≥90%, thus meeting proof-of-concept criteria. Platelet elimination kinetics of RLYB212 were dose-dependent and biphasic, with a mean lag phase of 4.8 h (0.09 mg) and 2.0 h (0.29 mg) vs 15.0 h for placebo; mean terminal half-life was 5.8 h (0.09 mg) and 1.6 h (0.29 mg) for RLYB212 vs 71.7 h for placebo.

Conclusion: Subcutaneous RLYB212 treatment resulted in dose-dependent and rapid elimination of HPA-1ab platelets in HPA-1bb participants. Platelet elimination kinetics were consistent with those of RhD(+) erythrocytes transfused to RhD-negative individuals after intramuscular administration of anti-RhD, which is known to prevent RhD alloimmunization when administered within 72 h of a suspected fetal-maternal hemorrhage. Our data support the potential use of subcutaneous RLYB212 as a prophylactic for FNAIT.

VS-23-5

Evaluation of knowledge about fetal and neonatal alloimmune thrombocytopenia among gynaecologists and midwives in Germany: A survey-based study

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Background: Fetal and Neonatal Alloimmune Thrombocytopenia (FNAIT) is a potentially life-threatening condition characterized by maternal alloantibodies against fetal platelets, which can lead to severe fetal and newborn thrombocytopenia. Recognizing clinically relevant FNAIT is important for the management of subsequent pregnancies, but little is known about the knowledge of midwives and gynaecologists on this subject. This study aimed to assess the knowledge of these professions regarding FNAIT.

Methods: We designed a questionnaire with qualitative and quantitative questions for use among midwives and gynaecologists working in clinical and ambulatory pregnancy, childbirth and postpartum care. The survey consisted of questions regarding the etiology, clinical manifestations, diagnosis, and management of FNAIT. Participants' responses were collected online via SurveyMonkey and analyzed using descriptive statistics.

Results: Preliminary results show, that 40% (12/32) of midwives and 19% (11/57) of gynecologists questioned, were aware of the most common signs and symptoms of FNAIT, while the majority of midwives and gynecologists would refer children with symptoms to further diagnostics based on typical symptoms. Clinically less significant symptoms relating to extracranial bleeding were more often ignored than more severe symptoms like intracranial hemorrhage or severe thrombocytopenia in laboratory testing. The survey is ongoing.

Conclusion: We designed a questionnaire with qualitative and quantitative questions for use among midwives and gynaecologists working in clinical and ambulatory pregnancy, childbirth and postpartum care. The survey consisted of questions regarding the etiology, clinical manifestations, diagnosis, and management of FNAIT. Participants' responses were collected online and analyzed using descriptive statistics.

VS-23-6

Frequency of antiplatelet antibodies in patients with thrombocytopenia after SARS-CoV-2 vaccination

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Background: Thrombocytopenia has been reported as a complication following vaccination against SARS-CoV-2. We analyzed clinical data and antiplatelet antibodies of patients who developed de-novo or recurrent thrombocytopenia in temporal relationship to SARS-CoV-2 vaccination.

Methods: Sera of patients with thrombocytopenia (platelet count < 150 Gpt/L) sent to the Greifswald laboratory after SARS-CoV-2 vaccination between March 1st 2021 and March 31st 2022 were tested for antiplatelet antibodies against glycoprotein (GP) IIb/IIIa, GPIb/IX/V and GPIa/IIa by the indirect monoclonal antibody-specific immobilization of platelet antigens (MAIPA) assay. Anti-platelet factor 4 (PF4) antibodies were tested by IgG specific ELISA. Clinical features (age, sex, vaccine type and date of vaccination, bleeding symptoms) of patients were extracted from the laboratory submission form.

Results: We identified 156 patients (68 females, median age: 57 years) with thrombocytopenia who tested negative for anti-PF4 antibodies: after vector-based (n=74), mRNA-based (n=72), or a heterologous, first vector-based + second mRNA-based vaccination (n=10). Median platelet
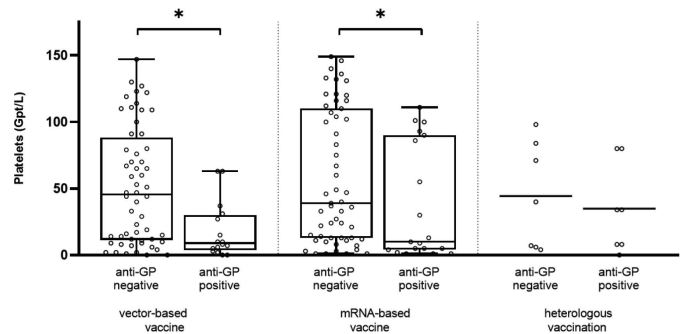
Fig. 1.

count was 33 Gpt/L and symptom onset was 5 days post-vaccination. Antiplatelet antibodies were found after different vaccination schemes to a similar extend: vector-based vaccines 25.7%; mRNA-based vaccines 22.2%; and heterologous vaccinations 30%. Patients testing positive for antiplatelet antibodies had lower platelet counts (Fig.1) and reported more often bleeding symptoms.

Conclusion: In general, vaccination against SARS-CoV-2 is a safe measure to control the COVID-19 pandemic, but patients who experience new bleeding symptoms a few days after vaccination with low platelet counts might be screened for antiplatelet antibodies. In this study, we found antiplatelet antibodies in about 20-30% of patients, which suggests a similar pathogenesis but independent of the type of vaccine.

VS-24
Blood Safety

VS-24-1

HEV incidence in the German blood donor population – 1 in 1000 is RNA positive

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Background: Transfusion transmitted HEV infections pose a risk of serious illness especially to immunocompromised recipients. In Germany, screening of blood donations for HEV-RNA is mandatory since 2020 for all non-virus-inactivated packed red cells, platelet concentrates and plasma. A detection limit of 2000 IU/ml HEV-RNA must be ensured. This is the first nationwide analysis of HEV incidence in the German blood donor population.

Methods: Data from the national blood donor surveillance system were used to estimate sex, age and regional stratified incidences of acute asymptomatic HEV infections and compared to data from mandatory reports according to the Infection Protection Act on symptomatic infections.

Results: Altogether 3507 HEV infections were reported in 2020 in 3,437,039 donors (102/100,000 donors). Male donors were 1.8 times more affected than females (127 vs. 71/100,000). HEV incidence was twice as high in donors >44 yrs as in donors <25 yrs (150 vs 74/100,000). Regional differences were found with highest incidence in the state of Brandenburg (169/100,000) and lowest in Baden-Wurttemberg (74/100,000). Reported symptomatic infections showed a similar pattern but incidences were 20 to 30 times lower. The overall incidence was 5.2/100,000 in the

general population (18-78 yrs) and was also age dependent (44 yrs: 2.5 vs. 6.5/100,000). The difference between males (6.0/100,000) and females (4.3/100,000) was less pronounced. The regional spread of infections among donors was also observed in symptomatic infections.

Conclusion: In 2020 1 in 1000 donors was detected positive for HEV RNA. This is a much larger incidence than any other infection tested for. According to recent viral load analyses [1, 2] the current obligatory test sensitivity could result in further 3000-10,000 undetected infections. While it is assumed that most are not infectious due to a low viral load, the large number of undetected infections may pose a residual risk of infectious donations that should not go unnoticed. Therefore, hemovigilance data should be carefully monitored to check whether the required sensitivity should be amended.

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VS-24-2

Hepatitis E and blood donation: Status quo after 3 years of mandatory testing

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Background: The testing of blood and stem cell donations for the hepatitis E virus (HEV) was introduced as mandatory for Germany on 01.01.2020. Since then, numerous donations have been found to be HEV-positive and have not been marketed. The positive test result was associated with a traceability procedure as defined in Votum 48 of the Working Group on Blood. We report on our experience with testing in the period from 01.01.2020 to 31.12.2022.

Methods: Sample material from each donation was tested for the presence of HEV genome using NAT. Routine HEV nucleic acid test (NAT) donor screening of minipools ≤ 96 was performed on the fully automated cobas 8800 system using the cobas HEV test kit (Roche Diagnostics). Minipools and subpools for pool resolution are generated by MicroLab Star IVD liquid handler (Hamilton) using 100 µl aliquots EDTA-anticoagulated plasma of each donor sample. Initially reactive minipool resolution has been performed by "chessboard subpooling" testing, finally confirming putative HEV positive sample(s) by individual NAT.

Results: 1734 donations were identified as HEV-positive, 1613 from repeat donors and 121 from first-time donors. In the look-back procedure, the pre-donations of 25 donors were identified as HEV genome positive. All donors were clinically healthy at the time of donation.

Conclusion: Donors cannot be identified as possibly infected with HEV on the basis of the donor questionnaire because the infection is usually not a sexually transmitted disease. The infestation of our donor population is high compared to other pathogens tested. In contrast to the other mandatory donor tests, a significantly higher group of repeat donors is affected for HEV because, unlike other test parameters, the prevalence of the infection is always reflected in the group of multiple donors.

VS-24-3

Malaria testing: NAT or infectious serology or both?

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Background: Potential donors who have a malaria risk are currently examined by means of NAT before collecting a first donation in the DRK BSD West. According to a recommendation of the WGB, it is preferable to be tested with an infectious serological test. In 2022 we identified two potential donors who had plasmodia genome in the NAT. Only one case showed a positive result in an infection serological test for antibodies against plasmodia.

Methods: In accordance with GL, nucleic acid testing (NAT) by PCR is performed at our facility after a retention period of 4 years using the AltoStar Purification Kit 1.5 combined with RealStar Malaria PCR Kit 1.0 and Alto Star AM 16 system (altona Diagnostics). In addition to the validated molecular biological testing, an infectious serological examination by means of an ELISA, which is approved for donor testing in Germany, shall be introduced. Therefore, the NovaLISA malaria immunoassay and the ETI-Max 3000 analyser will be used. The results of the two methods will be compared.

Results: Still pending.

Conclusion: The results are to be discussed against the background of the WBG recommendation. The question will be raised whether PCR tests are dispensable or whether the combination of infection serological and NAT tests should be preferred.

VS-24-4

Implementation of a UVC-based pathogen reduction treatment for apheresis platelets at a regional blood service in Germany

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Background: Until now, pathogen reduction treatment for platelet concentrates (PCs) is not mandatory in Germany. Nevertheless, the Bavarian Red Cross Blood Service started to implement the THERAFLEX UV-Platelets System (Macopharma) as effective and preventive measure to increase blood safety. The UVC-based system for PCs was validated as required for manufacturing license and marketing authorization approval.

Methods: Six double apheresis PCs were collected and split into 12 single units. Six PCs were treated with the THERAFLEX UV-Platelets System, while the other six corresponding PCs were left untreated and served as control. Pathogen reduction of PCs using the THERAFLEX UV-Platelets System was performed under routine-like conditions. PCs were stored under agitation at 22 +/- 2 °C and samples were collected on day 2, day 6 and day 9. Platelet in vitro quality parameters were tested according to the local standard operation procedures. Manufacturing steps of the THERAFLEX UV-Platelets procedure were validated, and UVC treatment was monitored in the platelet units using a mitochondrial DNA multiplex real-time polymerase chain reaction inhibition assay.

Results: Platelet content per unit was between $3,12 \times 10^{11}$ and $3,75 \times 10^{11}$ (mean $3,43 \times 10^{11}$) in UVC-treated PCs and between $3,25 \times 10^{11}$ and $3,91 \times 10^{11}$ (mean $3,56 \times 10^{11}$) in control units. Pathogen-reduced and untreated PCs showed similar result in swirling and hypotonic shock reaction until day 9 and were tested negative for anaerobic and aerobic bacterial growth. During time of storage glucose concentration decreased and lactate concentration increased in all units. Glucose consumption was higher in UVC-treated units than in untreated units; however, glucose was still present on day 9. The mitochondrial polymerase chain reaction analysis correctly discriminated between UVC-treated and untreated PCs.

Conclusion: UVC treatment of apheresis PCs is a fast and easy procedure and was successfully integrated into the local manufacturing processes. All UVC-treated PCs fulfilled the specifications of the THERAFLEX UV-Platelets technology and met the requirements of the German guidelines for pathogen-reduced PCs.

VS-24-5

Virus inactivation of plasma by methylene blue/light treatment using a DEHP-free bag system

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Background: DEHP is a widely used plasticizer in blood bags. Due to its endocrine disrupting properties European regulators decided to ban its use in medical devices. Although, the final sunset date is not yet clear, DEHP-free blood bags will be essential in Europe within the next years. Inactivation capacity of model viruses varying in structure and biophysical characteristics by Methylene blue (MB)/light treatment of plasma using THERAFLEX MB-plasma system (DEHP-free, Macopharma) was investigated.

Methods: Leuko-depleted plasma was prepared from whole blood using standard blood banking technology (containing DEHP). Plasma units were spiked with virus suspension (10% v/v; Suid Herpes Virus (SHV-1), Bovine Viral Diarrhea Virus (BVDV), Feline Calici Virus (FCV), Vesicular Stomatitis Virus (VSV)). MB/light treatment was done according to manufacturer"s instructions (Macotronic B2 illumination device) with the DEHP-free THERAFLEX MB-Plasma system PROSDV1. Samples were taken after spiking and after illumination with different light doses (30, 60, 90 J/cm^2: n=3 and 120 (standard) J/cm^2: n=6) The virus titer were determined as tissue culture infective dose (TCID$_{50}$) by endpoint titration Large volume plating was done to improve the detection limit.

Results: Log$_{10}$ reduction factors of ≥ 5.89 and ≥ 5.50 log steps were achieved for **BVDV** at a light dose of 120 J/cm^2 in the respective experiments with and without intermediate sampling. Log$_{10}$ reduction factors of ≥ 4.29 and ≥ 4.17 log steps were achieved for **FCV**, ≥ 4.96 and ≥ 4.92 log steps for **SHV-1** and ≥ 5.35 and 4.77 log steps for **VSV** with and without intermediate sampling.

Conclusion: All of the viruses tested in this study were inactivated with reduction factors ≥ 4 log steps at the final illumination dose of 120 J/cm^2 using the DEHP-free bag disposable PROSDV1 and the illumination device Macotronic B2. Reduction factors are comparable to data achieved in the past for the DEHP-containing THERAFLEX MB-Plasma system.

Disclosure Statements: Study was sponsored by Macopharma Productions (CS).

VS-24-6

Virus inactivation by UV-C irradiation of two plasma proteins, trimodulin (IgM concentrate) and fibrinogen

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Background: To ensure high safety margins with respect to adventitious viruses for therapeutic proteins purified from human plasma, different physico-chemical technologies are available to inactivate/remove viruses during manufacturing. Choices are limited for inactivation/removal of non-enveloped viruses which are typically more robust and smaller. Furthermore, when proteins are very large and also sensitive towards harsh treatments, no universally applicable inactivation/removal technology for non-enveloped viruses may be available.

Methods: Protein solutions were treated with Ultraviolet C (UV-C) irradiation at 254 nm with low-pressure mercury lamps. Inactivation of viruses was measured by cell-based infectivity assays (i.e. virus titration)

Results: We show effective inactivation of Hepatitis A virus (HAV) and porcine parvovirus (PPV), a model for human parvovirus B19 (B19V), after irradiation with 200 J/m². Inactivation of lipid-enveloped viruses at this dose showed mixed results. At 100 J/m², inactivation was still effective for PPV.

Conclusion: UV-C irradiation is a valuable virus inactivation tool for proteins from human blood or plasma when other methods cannot be employed for various reasons.

Disclosure Statements: Beide Autoren sind Angestellte von Biotest AG, ein kommerzieller Produzent von Arzneimitteln aus humanem Plasma.

VS-25
Blood Donation

VS-25-1

Comparison of different strategies for a generalized risk assessment of blood donors for sexual transmittable diseases independent of donor and partner gender

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Background: Current German guidelines differentiate in risk assessment for sexual transmittable diseases (STD) according to donor and partner sex. This differentiation is currently revised. A recent FDA draft guidance recommends deferral of donors with a new sexual partner or more than 1 partner in the past 3 months only, if the donor had had anal sex. In Austria, only donors with more than 3 partners in the last 3 months or unprotected sex with a new partner are deferred.

Methods: All 21,570 donors with known email address were asked to fill in an anonymous online survey on risk factors for STD and on their attitude towards questions about sexual practices as part of donor risk assessment. Donors were allowed to skip single questions. Presumed eligibility was determined according to (A) current guidelines, (B) FDA draft guidance, (C) considering ineligible all donors with a new or more than 2 partners during the last 3 months or (D) donors with unprotected sex with a new partner during the last month or more than 2 partners during the last 3 months, or (E) donors with more than 2 partners or with more than one partner and anal sex or with anal sex with a new partner during the last 4 months.

Results: 6387 donors (29.6%) answered the survey. The proportion of ineligible donors was about 1% for strategy B, 2% for strategies A, D and E, and 8% for C. 15% of donors aged 18-29 years reported a new sexual partner during the last 4 months (6% had unprotected sex and 1% anal sex with the new partner).

1950 donors (28.3% of all donors) disliked questions about anal sex, and 371 (5.4%) would stop donating if those were asked. 372 donors (5.4%) reported that they would not answer truthfully about having anal sex. This proportion was increased in donors with many partners (e.g. 15.4% of donors with 3 or more partners during the last 3 months) and in donors having anal sex or unprotected sex with a new partner in the last month (12% and 10%).

Conclusion: A generalized deferral of donors with new partners would significantly decrease the number of eligible donors. Detailed questions about sexual activities should be omitted or restricted to subgroups of donors with increased risk (e. g. new or multiple partners) to avoid unnecessary deterrence of other donors. Extensive measures to convince donors of the necessity and effectiveness of such questions would be needed, as especially donors with high risk for STD tend not to answer truthfully.

VS-25-2

Securing the blood supply – demographic aspects

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Background: The population in Germany is aging and so is the donor population. Already, most whole blood donations are made by individuals older than 45 years with 9.3% of donations given by donors aged 65+ in 2021. Due to these demographic changes, recruiting and retaining blood donors becomes increasingly challenging. Detailed knowledge of the demographic profile and donation behavior of donor populations are therefore essential to secure an adequate blood supply.

Methods: Surveillance data from the national donor surveillance system and population data from the federal states statistical offices were used to assess changes in demography and donation frequencies of whole blood donors aged 18-68 between 2012 and 2021.

Results: From 2012 to 2021, the general population aged 18 to 68 increased by 2%. At the same time, the number of first time and repeat whole blood donors dropped by 13% and 36%, respectively. In 2021, 2.5% of the population aged 18-68 was active as repeat whole blood donors (2012: 4.3%). The highest proportion of donors was observed in 2021 among those aged 18 to 24 (4.5%). As the age structure of whole blood repeat donors roughly resembled that of the general population, the greatest number of donors were 55-64 years of age in 2021 (45-54 years in 2012). While in 2012 48% of all whole blood donations came from new and repeat donors younger than 45 years, this proportion dropped to 44% in 2021. As observed in previous years, the number of donations per year (2.0 on average) increased with age in both male and female donors: from 1.9 and 1.6 respectively among the youngest donors (18 to 24 years) to 2.6 and 2.1 for donors 65 years and older.

Conclusion: The substantial drop in whole blood donors and the demographic shift in the age structure of the current donor population is alarming. With only 1 in 40 potentially eligible individuals donating strong efforts must be made to increase the pool of regular and repeat donors to overcome seasonal fluctuation or other effects which might negatively influence the blood supply. A coordinated and collective approach including all stakeholders should be pursued. The National Advisory Committee (AK Blut) has initiated a working group focusing on donor recruitment as part of an integrative concept to ensure the future blood supply in 2022. Societal recognition of blood donations must be increased, barriers to donation lowered and use of blood must always be appropriate to secure the high standard of care for patients.

VS-25-3

Impact of demographic changes on whole blood donations between 2005 and 2020

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Background: The German reunification in 1990 resulted in a decline in birth rates, particularly in the Eastern federal states. On the other hand, the baby boomer generation (born 1955-1969), the largest contributor to whole blood donations (WBD) during the last decades, is aging and will exit the donor pool in the coming years. To analyze the impact of these demographic changes on blood supply, we perform a prospective, longitudinal study on all WBD in Mecklenburg-Western Pomerania since 2005.

Methods: The four blood donation services in Mecklenburg-Western Pomerania (German Red Cross, Haema, and the university blood donation services Greifswald and Rostock) provided data on date of the donation, sex, and age of the donors for all WBD in the years 2005, 2010, 2015, 2019 and 2020. Donation rates per 1,000 inhabitants were calculated based on the population registry of Mecklenburg-Western Pomerania. The population prognosis of the Federal Statistical Office was used to build projections of blood supply for the year 2030, assuming constant age-related blood donation rates as in 2020.

Results: The absolute number of WBD (Figure 1A) continuously declined from 118,419 WBD in 2005 to 83,871 WBD (-29.2%) in 2019, and 76,912 WBD (-35.1%) in 2020. The decline in 2020 might have been slightly amplified by an effect of the SARS-CoV-2 pandemic (83,871 WBD in 2019 vs. 76,912 WBD in 2020, -8.3%). The donation rates per 1,000 inhabitants (Figure 1B) decreased by 31.3 % between 2005 (69.4/1,000) and 2020 (47.7/1,000). Assuming constant age-related donor frequencies as in 2020, the projected number of WBD in 2030 will further decrease to 67,724 WBD (-11.9% compared to 2020).
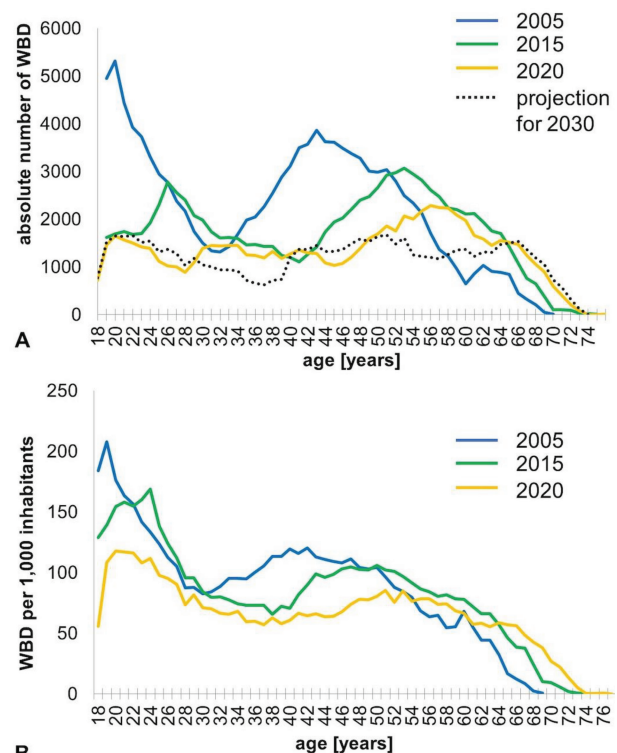

Fig. 1.

Conclusion: The demographic change after 1990 caused a pronounced decrease in WBD in Mecklenburg-Western Pomerania over the last 15 years. This decline continues but seems to slowly stabilize. However, as the baby-boom generation enters the age groups with higher blood demand, ensuring an adequate future blood supply remains a major challenge. Blood donation numbers and blood transfusion demand should be regularly monitored to recognize trends towards a supply-demand mismatch over time.

VS-25-4

Does hemoglobin analysis taken from venous blood of the previous plasmapheresis ensure donor safety at the next plasmapheresis?

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Background: According to the German Guideline "Hemotherapy" the blood donor hemoglobin (Hb) is a pre-requisite to ensure donor safety and should be analysed within the first 15 minutes of donation. Gold standard for Hb analysis is the measurement of venous blood using automated blood cell analyzer. Since 2008, in France, Hb measurement prior whole blood donation is not required if values of the previous donation meet the given specification.

Methods: Four plasma centers assessed Hb of venous blood using Sysmex XP300 within 15 minutes after start of donation. 382,461 Hb values (43% female vs 57% male) from 13,983 donors (50% vs 50%) between 2020-2022 were exported in a calculation tool, sorted by gender. The difference between two consecutive Hb values were calculated to analyze whether the value prior the previous donation was comparable with the result of the next donation. Differences with the same result were grouped, unplausible high differences were followed up in several cases. Additionally, these

Tab. 1.

Hb-difference	female		male	
	values	in %	values	in %
all values	165.776	100,0%	216.685	100,0%
>0,9	878	0,5%	1.369	0,6%
> 0,6 bis ≤ 0,9	4.345	2,6%	6.304	2,9%
> 0,3 bis ≤ 0,6	18.709	11,3%	25.252	11,8%
> 0 bis ≤ 0,3	45.751	27,6%	59.192	27,3%
=0,0	27.173	16,4%	32.883	15,2%
> 0 bis ≤ -0,3	45.234	27,3%	58.402	27,0%
> -0,3 bis ≤ -0,6	18.341	11,1%	24.850	11,5%
> -0,6 bis ≤ -0,9	4.304	2,6%	6.505	3,0%
>-0,9	1.041	0,6%	1.655	0,8%

results were sorted by days between two donations to analyze whether the timeframe between two donations had an influence on Hb levels.

Results: In 90.0% (female) vs 93.8% (male) the second Hb values lied above the acceptance limit; the values were equal or higher, or, if lower compared to the previous one, they did not fall below the limit. In 2.9% vs. 1.3% of the donors the results were under the limit. In 6.1% vs 4.8% the consecutive value was under limit particularly with negative differences more than 0.6 mmol/l. The follow up of those differences showed that the consecutive values were higher again and comparable to other values of the donors. The timeframe between two donations showed no influence on Hb difference in the first 6 months.

Conclusion: Most results demonstrated that an actual Hb should not be a prerequisite to ensure donor safety. Alert limits to control Hb before donation should be higher than exclusion limits to avoid donation with low Hb. The new approach to allow plasma donation with Hb from a previous donation was efficient, and a safe practice. Further avoid the automated measurement in a central lab pre-analytic and handling failures compared to the practice today with a decentral single measurement.

VS-25-5

How to reduce the disposal rate of platelet concentrates – A single canter experience of the LMU university hospital

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Background: The short life period of platelet concentrates (PC) is one of the biggest challenges for Blood Bankers. An exaggerated stockpiling will in most cases lead to higher disposal rates. The challenge here is to "walk the line", secure the supply for patients and reduce the disposal rate. Therefore, we developed new and more effective management techniques for PC logistics.

Methods: In addition to the in house PC donation, PC resupply is provided by a third party donation center. In May 2022 we changed the order frequency, from monthly to weekly and opposed the donor appearance to the estimated need of PC. We estimated a donation dropout (nonappearance, discontinuation) of 15%. The weekly order was supported by a responsive digital order form that displayed the estimated daily stock. The primary goal was a reduction of the disposal rate. We report the 2-years data before and 1-year after the intervention (2020 to 2022 vs. May 2022 to April 2023). The period from January 2022 to May 2022 is excluded due to changes in progress so the data is not representable.

Results: The donor compliance of 2833 donations planned in 2022 was 88%. Donation layers were planned with one to seven donors (on average 4.3 donors per layer) performed on Monday until Thursday. Nonappearance occurred in 200 layers (one donor less) and 54 layers (two donors less), respectively. There was no significant difference regard to the day of the week or the time of the day. On average, 22.8 PC were consumed in our hospital daily, with a significant lower consumption on Sundays and Mondays (18.4 vs. 24.5; p<0,0001). PC consumption did not differ during holidays. After optimization, the monthly disposal rate dropped from 144 PC to 64 PC on average without a change of the supply situation in general.

Conclusion: A more rigid, algorithm guided weekly planning of the supply chain for platelet concentrates can lead to lower disposal rates without a noticeable decrease of the supply situation. In comparison with the historic situation, we estimate a total saving of approximately 1600 PC annually. A long time follow up is warranted to see if the effect will persist. The developed Excel tool made the algorithm very easy to use (Picture 1 and 2).

Voreinstellungen	geschätzter täglicher Verbrauch TK	22
	Ausfallquote Eigenherstellung TK in %	15
	Jahr	2023
	Kalenderwoche	20
Eigenherstellung - Spenden (TK-Anzahl geplant)		
	Montag	32
	Dienstag	22
	Mittwoch	17
	Donnerstag	30
	Freitag	0
	Samstag	0
	Sonntag	0

Picture 1

benötigte TK in einer Woche	154	
geplante Menge Eigenherstellung	101	
erwartete freigegebene Menge Eigenherstellung	86	
damit Verbleib Bestellung Suhl:	68	

verteile diese Menge auf die Wochentage für die Bestellung
Empfehlungen: am Montag 18. Am Samstag 18.

0	sind noch zu verteilen
68	bereits verteilt

freigegebene TK unter Berücksichtigung der Ausfallquote

0	Montag	18
27	Dienstag	10
19	Mittwoch	6
14	Donnerstag	6
26	Freitag	10
0	Samstag	18
0	Sonntag	0

Picture 2

MB developed the Excel Tool. JL developed the algorithm.

Fig. 1.

VS-25-6

The supply of blood in fragile times – A comparison of international approaches based on interviews and literature study

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Background: Due to its important role in treatment, it is particularly important to ensure the availability of blood in emergency care. However, shortages of blood products becomes increasingly common even in normal times. In exceptional situations (e.g. alliance/defence emergencies), an acute and sharp increase in demand for blood (products) can be expected. At present, only isolated and mostly uncoordinated concepts among the actors in Germany for securing the blood supply in special situations exist.

Methods: A research project funded by the Federal Office for Civil Protection and Disaster Management (BBK) aims to develop a concept for ensuring the supply of blood and blood products in the event of an alliance or defence situation in coordination with the blood donation services. The results presented here are based on an intensive literature search in relevant journals and databases (Pubmed, Ebsco, etc.) as well as on interviews

with authorities of the producing companies and public administration in Germany and other countries.

Results: The literature review did not identify any policies that address coping strategies of blood and blood product shortages in alliance and defence situations. It was possible to identify instructions for action that deal with blood shortages in disasters. From international measures, plans and the armed forces, measures can be derived that seem worth to be included into a German concept discussion – (including legal aspects). These cover clear structures, contact persons and information channels. In addition, there should be continuous planning, exercises, and close cooperation with the authorities and responsible actors at federal and state level. Direct link of IT systems of hospitals and blood transfusion services will be essential.

Conclusion: Product-specific characteristics such as short shelf-life and storage requirements (e.g. plasma -40°) do not allow large stocks and blood transfusion services depend on an existing infrastructure at all stages of production, storage and distribution. There is no coordinated approach to cope blood shortages yet. Staff for the service might be on duty for other healthcare issues. It is therefore essential to develop solutions among all steps of the supply chain – including international actors.

Postersessions

PS-1
AI, Automation and Digitalization | Biobanking and Tissue Banking | Quality Management

PS-1-1
State of Automation and Data Processing at the 56th annual meeting of the DGTI in Berlin 2023

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Background: The state of automation and data processing for transfusion medical facilities in connection with patient care clinics will be presented at the 56th Annual Congress of the DGTI in Berlin.

New practical solutions are used to show examples of GAMP5 validations and GMP Guide Annex 15 for routine qualification for hospitals, transfusion facilities and medical facilities on the new Internet website in the DGTI members' area.addition.

Methods: The state of automation and data processing techniques will be discussed for selected topics.

With the involvement of external speakers, institutions, authorities, solution examples for the implementation of GAMP5 and GMP Guide in the routine for hospitals, transfusion facilities, and medical facilities are to be recorded, taking into account legally binding guidelines.

In order to implement the specifications for the validation of methods, the qualification of devices according to GMP Guide Annex 15, and for risk-assessing decisions according to GAMP, the following topics should be presented in the automation and data processing session.

Results: These sample qualifications and validations are to be published from last year on the previous DGTI platform. The results are to be made available on the new DGTI website with results for sample qualifications and validations. Both the main topics presented in the meeting of the Automation and Data Processing section at the digital DGTI Congress in Vienna in 2021 and the topics presented in Berlin 2023 should be made available on the digital platform in the members' area. The following topics were presented at the digital DGTI Congress in Mannheim in 2022.

Status of digital patient file, electronic entry of organ donation in the register

Critical IT infrastructure for blood donation services (KRITIS)

– Environment of digitization IT sector

Conclusion: For the automation and data processing meeting at the 56th annual meeting of the DGTI in Berlin, stipulations on the setting of qualification and validation solutions on the DGTI website are to be adopted for rapid implementation. For this purpose, the new DGTI website should be approved by the board for the section work.

Fig. 1.

Fig. 2.

ReMeDi:Blut – A future registry for medical data and antigenic properties of blood products

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Background: Currently, packed red blood cells are allocated according to relatively few patient-specific criteria - the AB0 blood group, rhesus type, and, in some instances, the Kell blood group system. Effective hemotherapeutic treatment with few side effects requires the selection of the most suitable blood product for each recipient. To date, there is no registry in Germany that systematically compiles clinical data on administered blood transfusions.

Methods: ReMeDi:Blut ("Registry for medical data and antigenic properties of blood products") aims to initiate a national registry for the centralized collection of data on transfusions performed in Germany. Using an evidence-based approach and artificial intelligence (AI)-driven assessment, the properties of blood products and clinical data of recipients will be accessed and connected to the central register via local bridgeheads at each participating treatment site.

Results: ReMeDi:Blut is intended to be a research registry with freely accessible interfaces for various hospitals to participate in. In the future, the acquired data may be used to gain important insights through AI-driven operations. This process could make more specific properties of blood products available, avoid incompatibilities and identify rare donors through big data. A more precise characterization of blood products, including molecular genetic blood typing, e.g. based on real-time PCR or next-generation sequencing (NGS), may allow for significantly more blood group antigens to be considered in the selection process of blood products. Overall, ReMeDi:Blut may help prevent the overuse, underuse and misapplication of blood products.

Conclusion: By implementing a national registry for the collection of data on transfusions performed in Germany, ReMeDi:Blut could make healthcare provision more efficient by distributing transfusions in a more targeted fashion. This could avoid the unnecessary administration of blood products and enable a comprehensive, AI-driven analysis of the factors that influence clinical outcomes.
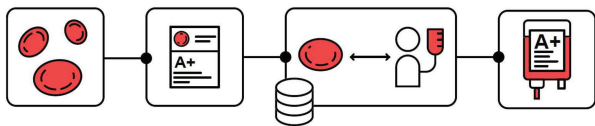
Fig. 1. Diagram showing the general ReMeDi:Blut project design. Detailed information regarding each blood transfusion will be transmitted from local sites to a central registry, where they will undergo an AI-based assessment to analyze antigenic properties and clinical data, improving the selection of the most suitable blood product for each recipient.

Introducing the AutoPiLoT Monitor 2.0: A real-time dashboard for efficient blood bank inventory management

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Background: In times of declining blood donation numbers, blood banks must manage labile blood products like red blood cell concentrates (RBC), platelet concentrates (PC), and fresh frozen plasma (FFP) efficiently to strike a balance between wastage and product shortages. Current software solutions, however, mostly offer manual inventory queries.

Methods: The AutoPiLoT Monitor is a web-based tool that provides historical and real-time data on blood product inventory. It uses a RESTful FHIR API interface to communicate with a FHIR-server which integrates various hospital information systems. The previous version served primarily as a reference point for physicians to gauge blood product inventory, but was constrained by computer-only accessibility. The AutoPiLoT Monitor 2.0 has undergone significant improvements, including the development of a new overview page. This page is continuously displayed on a large screen in the distribution area of the Institute for Transfusion Medicine at the University Hospital Essen, ensuring round-the-clock availability to all blood bank staff.

Results: The new overview page shows the number of RBCs in stock, segregated by crossmatch status and further subdivided by AB0 and Rhesus groups. Additionally, the explicit display of RBCs that will expire within the next three days facilitates timely utilization. Stock levels of pooled and apheresis PCs and the number of units approaching their expiration dates are visualized. Moreover, the dashboard displays PC consumption during the past week and integrates a machine-learning model to forecast PC consumption for the next seven days. Current FFP in stock and past FFP consumption, both itemized by AB0 type, are also pictured. Lastly, the dashboard refreshes every minute, ensuring that the displayed information remains up-to-date and reliable.

Conclusion: The updated AutoPiLoT Monitor 2.0 provides a comprehensive and user-friendly overview of blood product inventory. It delivers real-time updates and insights into past consumption, current and future stock levels as well as predicted platelet demand. Consequently, the AutoPiLoT Monitor 2.0 enables proactive decision-making to promote optimal resource utilization and waste reduction, ensuring the availability of vital blood products.
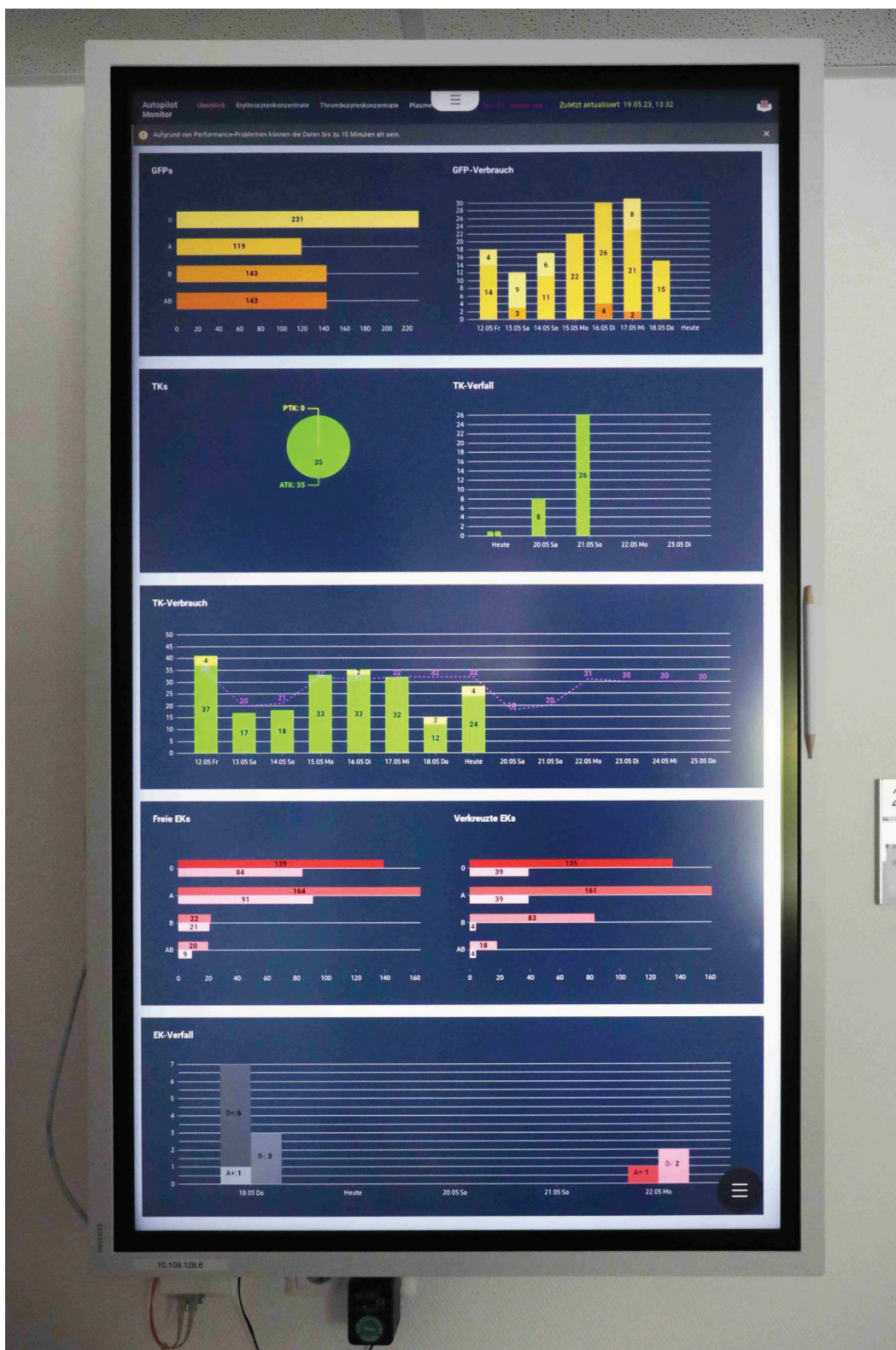
Fig. 1. The AutoPiLoT Monitor 2.0 overview page displayed at the Institute for Transfusion Medicine of the University Hospital Essen, providing real-time updates and comprehensive oversight of blood product inventory.

Analyzing the protein content in human milk for the effects of different pasteurization methods

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Background: In the setup of human milk banking, donated human milk (HM) is frequently pasteurized to reduce potential pathogens and to ensure the safety of premature infants. However, it is known that heat treatment can affect the protein composition of HM. In this work, alterations of selected whey proteins were investigated after the application of three different pasteurization approaches.

Methods: Breast milk samples (n = 15) were Holder-pasteurized (62.5 ± 0.5 °C for 30 min) using either water bath (WB-HoP) or dry temperature (DT-HoP) treatment or were sujected to High Temperature Short Time Treatment (HTST; 62 °C for 5 sec). To enable protein analysis, samples were pretreated by filtration and centrifugation. In the resulting whey, secretory immunoglobulin A (sIgA) and lactoferrin (LF) concentrations were determined by commercially available enzyme-linked immunosorbent assays before and after heat treatment. Alkaline phosphatase activity (ALP) was measured via enzyme activity assay (BioVision, Milpitas, CA, USA).

Results: Both HoP methods resulted in almost a complete decrease of ALP activity (WB-HoP = 0.3 ± 0.4 %, DT-HoP = 0.5 ± 0.4 %), whereas after HTST 52.8 ± 12.2 % was retained (all p < 0.001). The sIgA retention was significantly higher after WB-HoP (73.2 ± 13.5%) and after HTST (80.4 ± 22.7 %) than after DT-HoP (57.0 ± 14.4%, all p < 0.01). In terms of retention of LF, the two HoP methods did not differ significantly (WB-HoP = 47.0 ± 40.0 % vs. DT-HoP = 25.0 ± 9.7 %). Compared to both HoP methods, HTST showed significantly higher retentions of LF (69.9 ± 41.8 %, all p < 0.01).

Conclusion: Holder pasteurization by dry tempering (DT-HoP) seems to have a stronger impact on the quality of human milk than the other two approaches (WB-HoP, HTST). In terms of the protein retention, HTST seems to be a good alternative to the current gold standard HoP.

Application of Far-UVC for disinfection of corneal tissue

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Background: The two main criteria limiting the use of cornea as a transplant are the endothelial cell density, and the requirement for the absence of microorganisms. Resistance to the antibiotics within culturing media limit the microbiological safety of corneal transplants. We aimed to test a new disinfection method using Far-UVC radiation showing a low penetration depth into the cell resulting in killing bacteria (given their small size) while leaving human cells intact.

Methods: Porcine and human corneas were irradiated with far UVC for bacterial decontamination using a krypton-chloride excimer lamp emitting 222 nm. Corneas were irradiated half-sided for 15 and 60 seconds at 1 mW/cm², in order to adjust for an irradiation intensity not influencing the integrity of corneal endothelial cells. ANOVA was used for statistical analysis.

Results: Application of up to 60 mJ/cm² did not influence the integrity of corneal endothelial cells.

Conclusion: In the next step we are objecting corneal tissue to microbial contamination followed by irradiation at 222nm for 60 seconds aiming at decontaminating the corneas.

Provision of allogeneic bone grafts for orthopaedic surgery: A post pandemic update

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Background: Bone allografts have been used for surgical treatment of large bone defects, especially in endoprothesis replacement and spinal surgery. From patients undergoing primary total hip arthroplasty (THA), their femoral heads and necks were collected in the operation theatre. They were stored fresh frozen unchanged or converted into bone chips aseptically by the Bone Bank. In order to meet the demand for bone allografts we analysed the entire procedure for potential of improvement.

Methods: We analysed key data from the bone bank´s registry and the hospital administration between January 2019 and December 2022 with regard to the harvest of fresh frozen allogenic femoral heads and necks, the manufacture of bone chips and their discard rates and reasons over time: donor history, microbial growth, histology, IDM testing, others. Criteria for donor eligibility and product release were assessed in accordance with EU and national legislation.

Results: 890 femoral heads and necks were harvested during 1,664 THA surgeries. Thanks to the relaxation of Covid-19 restrictions, equal amounts of bone donations were recovered in 2022 and in 2019, 41% of these yielded the bulk material for bone chips. The discard rate for bone chips remained below 1.5%, but increased from 14.7% to 16% for bone donations. Analysis of the reasons for discard revealed donor history as the major cause over time. The increase from 5.6% in 2021 to 8.4% in 2022 had occured despite some improvement measures e.g. clarification of inconclusive diagnoses and a revised donor questionnaire. Thus, other factors may also contribute, such as the inclusion of increasingly older bone donors and staff turnover.

Conclusion: Providing allogeneic bone grafts of high quality and in sufficient quantity is a challenge. Therefore, it is of utmost importance to regularly review the registry data in order to adjust the procedures if necessary and possible. In this complex task other factors, such as donor characteristics and structural changes should also be considered.

Correlation of storage conditions and DNA degradation

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Background: Molecular analyses, including RBC typing, epigenetic studies and screening for disease-associated gene alterations, are widely used in blood banks and hospital laboratories. Human genomic DNA (gDNA) isolated from blood samples is the primary material used for these analyses. Improper storage conditions can result in DNA degradation, which can lead to diminished amplification efficiency and even false-negative results, thus compromising the accuracy and reliability of subsequent genotyping.

Methods: For middle-term storage, a total of 100 gDNA samples derived from 5 blood donors were stored at different temperatures (RT, 4°C, -20°C, -20°C with a single thawing process, and -20°C with thawing every month) for eight months. The level of DNA degradation was assessed by agarose gel electrophoresis, medium-range PCR (1,650 bp), and Agilent Bioanalyzer System.

For long-term storage, each 5 EDTA blood and gDNA samples were stored at -20°C for 3, 5, 10, and 15 years. DNA integrity was compared by agarose gel electrophoresis, medium-range PCR (1,650 bp) and RBC genotyping. All extractions and purifications were done by magenetic bead-based technology.

Results: gDNA samples stored at 4°C exhibited degradation and decreased amplification efficiencies after eight months. Contrary, high DNA integrity was identified in samples stored at RT and -20°C. Additionally, both EDTA blood and gDNA samples stored at -20°C for 3, 5, 10, and 15 years revealed good DNA recovery and prevented DNA degradation.

Conclusion: In summary, we recommend short and middle-term storage of gDNA samples at RT or -20°C instead of 4°C, as the latter can result in decreased DNA integrity and compromised amplification efficiency.

For long-term storage, a temperature of -20°C is suggested to prevent DNA degradation in EDTA blood and gDNA samples for up to 15 years. These results may have implications for molecular diagnostic laboratories, where DNA integrity is crucial for additional typing requests and retrospective studies.

PS-1-9
BloodTrain: Results of the first phase of regulatory systems strengthening in Sub-Saharan Africa

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Background: The GHPP BloodTrain project at PEI seeks to ensure availability, safety and quality of blood products in Africa through regulatory systems strengthening, focusing on haemovigilance, licensing and inspection of blood establishments, and IVDs in five partner countries (Ghana, Nigeria, Tanzania, Zambia, Zimbabwe). The first phase of the project started 2018 and ended in December 2022. Here, we present the achievments from the first phase and give an outlook to the recently started second phase.

Methods: The BloodTrain Project works to strengthen the regulatory capacity in sub-Sahara Africa with the focus on Blood and Blood products. Based on the results of a benchmarking using the Global Benchmarking Tool of the WHO, we have been pursuing a three-tiered approach to

- provide specialized technical support to build regulatory structures;
- perform capacity-building activities like workshops;
- support continental technical working groups to foster the harmonization of regulatory requirements in Africa.

During the first phase of the project, the activities were maintained during the Covid-19 Pandemic by using a self-developed eLearning platform to perform virtual workshops, and online meetings to advance regulatory documents.

Results: We progressed on all three tiers and achieved important milestones, which will enable our further progress in the currently ongoing second phase of the program:

- Our technical support activities lead to the development of numerous regulatory documents, including a unified haemovigilance framework and guidelines in Ghana, launched in October 2022.
- For capacity building we performed numerous workshops including participants from our five partner countries and as well as from other interested countries. This was especially possible during the online courses (see Table in Abb.1).
- Concerning the effort to harmonise regulation we supported the development of three Guidance documents by the African Blood Regulators Form.

Conclusion: Together with NRA and NBTS of our partner countries, we supported the strengthening of the regulatory systems, measured by the number of performed workshops and trained participants and the number of developed regulatory system documents.

These results are the basis for the continuing work in the second phase to further strengthen the capacities of our partner countries' NRAs so that they can perform trainings of regulators from neighboring countries in the regulation of Blood Products.

PS-1-10
Implementation report: ISO 15189 accreditation of a blood bank

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Background: Since the new EU-regulation (2017/746) for in-vitro-diagnostics came into effect, many healthcare institutions especially laboratories challenge to make their workflows ISO 15189 conform.

Blood banks often use "in house-tests" for donor or product testing, therefore they have to follow this special standard for medical laboratories to fulfill aspects of the EU-regulation. We aimed to implement the requirements and passed accreditation by national authority in 2023.

Methods: The implementation project of the ISO 15189:2012 standard started in August 2020. The project-members were all in-house employees of the laboratory, without external support.

The identified key-milestones of the project were: 1) kick-off 2) gap-analysis and implementation of appropriate measures 3) application for accreditation 4) inspection by national authority 5) resolve nonconformities 6) receipt of accreditation confirmation.

Employees of the laboratory were included in all phases of project work to internalize the relevant requirements for their units. The project leader was responsible for planning the project, conducting the application and was the contact person to the authority during the whole project.

Results: Net project time to implement the ISO 15189 was 30 months. 4 laboratory units and over 80 procedures where successfully accredited.

Tab. 1.

Year	Date	Activity	Venue	Number of participants	Number of countries
2019	4-22 March 2019	Training Fellowship - Regulatory Placement	Langen, Germany	8	4
	20-24 May 2019	Inspection of Blood Establishments	Harare, Zimbabwe	26	7
	20-22 August 2019	Implementation of Blood and Blood Product Regulation	Johannesburg, South Africa	33	16
	29-31 October 2019	Haemovigilance	Lusaka, Zambia	27	7
2020	20-23 October 2020	BloodTrain-ISBT-WHO Haemovigilance Workshop	Online	87	5
2021	05-08 July 2021	Authorisation and Licensing of Blood Establishments	Online	170	19
	09-09 September 2021	EuBIS-BloodTrain-AfSBT Training Workshop	Online	265	31
	27-30 September 2021	Assessment of Technical Files for Blood Screening IVDs	Online	107	12
	09-10 November 2021	AfSBT Educator Refresher	Online	19	18
2022	21-23 March 2022	Authorisation and Licensing of Blood Establishments - Zambia	Lusaka, Zambia	20	1
	05-08 April 2022	Authorisation and Licensing of Blood Establishments - Zimbabwe	Nyanga, Zimbabwe	18	1
	10-12 May 2022	Authorisation and Licensing of Blood Establishments - Tanzania	Arusha, Tanzana	23	1
	28-30 June 2022	Authorisation and Licensing of Blood Establishments - Ghana	Cape Coast, Ghana	20	1
	30 May - 01 June 2022	Assessment of Technical Files for Blood Screening IVDs	Victoria Falls, Zimbabwe	17	2
	06-08 June 2022	AfSBT Assessor/Educator Refresher	Kigali, Rwanda	24	18
	12-14 October 2022	EuBIS-BloodTrain-AfSBT Training Workshop	Cape Town, South Africa	34	16

Employees of the laboratory internalized new ISO 15189 requirements by continuously taking part in project work and they could transfer this knowledge instantly into routine. The advantages of an internal project leader are the increase of acceptance of the project and the minimization of external costs. Additionally, the expertise of the accreditation procedure itself remains in the organization.

Most important aspects are to take enough time to do the gap-analysis and to examine all documents recommended by national authority before starting implementation of the standard.

Conclusion: One successful way to implement the ISO 15189 in blood banks is through project work. Expected duration of such a project is medium term. The key to success is to involve internal staff from the beginning to use and retain know-how.

Blood banks have high quality standards, as they are obliged to implement the requirements of e.g. parts of the good practice guidelines of the EDQM guide or national law. This could be an advantage in the implementation of the ISO 15189.

PS-1-12
Traceability beyond borders: Development of a global standard for the labeling of tissue-engineered products

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Background: ICCBBA develops ISBT 128 as an international code for substances of human origin, and brings together clinical, scientific, technical, and informatics experts, professional societies and observers from regulatory authorities and industry. Several Technical Advisory Groups (TAGs) contribute to the global implementation of ISBT 128. The Regenerative Medicine TAG (RMTAG) developed a terminology for tissue-engineered products (TEPs) and is now pursuing the development of a global labeling standard.

Methods: With the aim to develop an ISBT 128 standard for the labeling of TEPs with the involvement of facilities experienced in TEP manufacture, a survey was conducted among RMTAG members from September 19 to October 20, 2022. Acknowledging that TEPs can be regulated as Advanced Therapy Medicinal Products (ATMPs), biologics or medical devices, the survey included information on two labeling options: a labeling standard compatible with biologics regulations, and a labeling standard compatible with medical device regulations.

Participants were asked to provide background information on their facilities (products manufactured/received and applicable regulations) and which of the two proposed standards they would recommend for the labeling of TEPs.

Results: A total of 10 survey responses were obtained. Although participants affirmed that TEPs can be regulated as biologics, medical devices, or drugs, the majority (9 out of 10) representing TEPs manufacturers, blood, cellular therapy, and tissue facilities recommended developing a labeling standard compatible with biologics regulations.

Conclusion: Initial input from stakeholders indicates strong support for developing a labeling standard compatible with ATMP and biologics regulations rather than a labeling standard compatible with medical device regulations. The RMTAG is actively reaching out to stakeholders from academia, industry and across geographical regions to ensure that the resulting standard truly meets users" expectations and effectively enables the traceability of TEPs worldwide.

PS-2-1
In-vitro quality of platelet concentrates, x-ray-irradiated, at the beginning or end of storage

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Background: X-ray-irradiation of blood product is used to inactivate lymphocytes to prevent TA-GvHD, similar to γ-irradiation. Compared with γ-irradiation, X-ray-irradiation is preferable from an operational point of view, as the radiation emission is easier to control and thus safer. Only limited data is available from literature on the quality of x-ray-irradiated platelet concentrates (PC), which we evaluate here In-vitro, compared to untreated controls, dependent on the storage time before treatment.

Methods: Plasma-reduced PC in SSP+ were derived from pools of 4 buffy coats after overnight storage of whole blood. PC were used in two sets of experiments (n=6 each). In a pool and split design, two PC were merged and separated, with one bag remaining untreated, while the other was x-ray-irradiated at 25 to 50 Gy using an X-ray blood irradiator RADGIL2 (Gilardoni, Italy). Bags were irradiated either directly after PC production on day 2 (set 1) or on day 5 (set 2) at the end of storage. PC were stored under agitation at $22 \pm 2°C$ and sampling to determine platelet quality was done on day 6. The quality parameters observed for this study were PLT number, pH, presence of swirling, collagen-induced aggregation, CD62P exposition and Annexin V binding.

Results: PCs were in accordance with the German guidelines with respect to platelet content ($>2 \times 10^{11}$/unit)) and pH (pH between 6.4 to 7.8). When comparing untreated controls with the corresponding X-ray-irradiated PCs no significant differences were detected for collagen-induced aggregation (table). Annexin V binding and CD62P exposition were slightly but significantly elevated in the x-irradiated bags. There was no significant difference in the quality of PCs x-ray-irradiated directly after production compared with those x-ray-irradiated at the end of storage (unpaired t-test, $p < 0.05$).

Tab. 1.

Table 1: Summary of platelet quality parameters.

	Set 1: Irradiation day 2		Set 2: Irradiation day 5	
	Control [Mean ± SD]	**X-Ray** [Mean ± SD]	**Control** [Mean ± SD]	**X-Ray** [Mean ± SD]
PLT/unit [x10[11] PLT/unit]	2.57 ± 0.22	2.66 ± 0.19	2.35 ± 0.10	2.39 ± 0.09
pH	7.34 ± 0.03	7.31 ± 0.03	7.35 ± 0.02	7.33 ± 0.02
Collagen-induced Aggregation [%] 10 µg/mL collagen	78.50 ± 6.17	78.64 ± 7.43	79.04 ± 2.49	80.63 ± 3.62
Collagen-induced Aggregation [%] 2 µg/mL collagen	7.13 ± 5.08	4.67 ± 3.05	4.71 ± 1.49	5.50 ± 2.26
Annexin V [%]	5.63 ± 1.08	7.2 ± 1.40*	5.68 ± 0.42	7.18 ± 0.48*
CD62P (Sample) [%]	40.32 ± 4.45	43.38 ± 4.47*	42.43 ± 4.18	44.18 ± 4.10*
CD62P (TRAP activated control) [%]	97.47 ± 1.09	97.74 ± 0.28	97.43 ± 0.47	97.39 ± 1.02

* $p \leq 0.05$ compared to untreated control of the same set (paired t-test)

Conclusion: Platelet quality is well preserved after x-ray radiation with only minor differences in platelet activation (CD62P and Annexin V) between X-radiated PCs and the corresponding untreated controls at the end of storage. The time point of irradiation, immediately after production or at the end of shelf life, had no effect on the platelet quality parameters we examined in this study.

PS-2-2

In vitro quality of pediatric and standard units of packed red blood cells after x-ray irradiation

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Background: Irradiation of blood products is most commonly used for reducing the risk of the transfusion-associated graft-versus-host disease (TA-GvHD) by preventing T-cell proliferation. In recent times, there have been strong efforts by governmental authorities in many countries to replace radioactive irradiators by x-ray irradiators for reasons of safety and sustainability. However, there is still limited data available on the quality of x-ray irradiated standard and pediatric units of red blood cells.

Methods: Red blood cells in additive solution PAGGS-M (Macopharma) were produced from whole blood (500 ml) in CPD anticoagulant (70 ml) by centrifugation and subsequent leukodepletion. RBC were x-ray irradiated using the RADGIL2 (Gilardoni) with > 25 Gy according to German guidelines. Pedriatric units were prepared by splitting a regular unit into four pediatric units after irradiation. Non-irradiated units served as controls. Relevant quality parameters were evaluated in x-ray irradiated and non-irradiated standard and pediatric units (n=12 each) of packed red blood cells at different time points of storage

Results: As expected, extracellular potassium was considerably elevated in irradiated packed red blood cells. The level of extracellular potassium was equal in standard and pediatric units and it was comparable (or slightly lower) in x-ray irradiated compared to gamma irradiated units (61.0 ± 1.9 mmol/l versus 69.5 ± 4.0 mmol/l) at the end of shelf life.

Hemolysis rate was increased in pediatric units but when irradiation is performed within 7 days after donation there is no difference between irradiated and control units up to 14 days after irradiation. All units met the criteria of German guidelines (hemolysis < 0.8%) at the end of the shelf life.

Conclusion: Extracellular potassium is of major concern in irradiated packed red blood cells. Potassium levels were similar in x-ray and gamma irradiated units, showing that x-ray is a good alternative to gamma irradiation. Early irradiation and short storage after irradiation are favorable, especially in pediatric units to reduce levels of extracellular potassium and rate of hemolysis.

PS-2-3

Optimization of the pooling time during the preparation of platelet concentrates in octopus configuration with a new sterile multiple connection device

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Background: Maconnect is the new sterile connection device designed by Macopharma that allows to perform up to 6 connections simultaneously. This device was implemented in our production site and is used for the Buffy-Coat (BC) pooling with an octopus configuration during the preparation platelet concentrates (PCs).

A comparative study was performed to evaluate the effectiveness of the pooling step using a multiple or simple sterile connection device.

Methods: Time measurements were performed by several operators during the BC pooling step in octopus configuration (pooling of 5 BCs and 1 SSP+ solution) using either a multiple (Maconnect, Macopharma) or a simple (TSCD, Terumo) sterile connection device.

The time-to-prepare between 1 and 6 BC-pool in series was assessed for both devices: the time measurement started when the first 5 BC were taken to be pooled and ended when the group of prepared BC-pools were packed and ready to be centrifuged. A total of 78 BC-pools was prepared with the Maconnect and 69 with the TSCD. In both cases, 9 different operators performed the pooling and time measurement.

Results: The pooling step (time-to-prepare 1 BC-pool) was significantly faster (t-test) with the multiple sterile connection device (7min 21s ± 1min 36s) than with the simple one (8min 53s ± 1min 24s).

Pooling time for 1 BC-pool obtained from the preparation of small series of BC-pools (1 to 2 BC-pools) were also compared to the one obtained from the preparation of larger series of BC-pools (3 to 6 BC-pools): it was increased with both devices for larger series. It can be explained by a small delay between the preparation of each pool. However, the time difference between Maconnect and TSCD was similar for small and large series (1min 48s and 1min 40s, respectively) which suggest no further time saving than the connection time with Maconnect.

Conclusion: In conclusion, the Maconnect allows time saving during the BC pooling in Octopus configuration. The introduction of this multiple sterile connection device in the production thus contributes to the optimization of the PC preparation from BC and of the workflow in the routine environment.

Disclosure Statements: The co-author Massimo Benzoni is an employee from Maco Pharma.

PS-2-4

Validation of a pneumatic tube system for transport of blood products (EC, PC, TP)

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Background: Blood products like platelet concentrates (PCs) or erythrocyte concentrates (ECs) are highly sensitive to shifts in temperature and mechanical stress. Due to reconstruction of the internal transport system, a new pneumatic tube system (PTS) – Sumetsberger, Vienna - links the blood bank with the wards. Analogous to the evaluation of transporting PCs with the previously existing PTS, we have set out to validate the transport of PCs, ECs and therapeutic plasma (TP) with the new PTS.

Methods: Rate of speed at the PTS was set to 3 m/sec. Irradiated PC and EC (> 21 days old) and thawed TP (each n=6) were transported as single products by the new PTS. Multiple transports per product (TP 1x, PC 2x, EC 5x) were carried out on the longest possible distance between the blood bank and wards. Temperatures as well as mechanical stress (acceleration and vibration) were monitored by data loggers. Further, we recorded transport duration and visual control of the products. Rate of hemolysis (rh), hematocrit (hct) and potassium (K+) were analyzed in EC, pH value and CD62P activation in PC as well as coagulation parameters in TP. Product samples were analyzed before start and after each bidirectional transport.

Results: Visual control was overall inconspicuous. EC: rh, hct and K+ were within specifications of < 0.8%, < 70% and < 80mmol/l, respectively with low coefficients of variation (CV; max. 2.9%). PC: The pH-value (median pH 7.56, CV 0.4%) remained within specification of pH 6.4 - pH 7.8. The platelet activation marker CD62P displayed 24.4% (median, CV 18.1%). TP: Quick, INR and PTT showed no relevant changes between pre- and post- transport. Regarding FVIII, all TP complied with the specification of >70%. The temperature of EC (n=4, 2x transport) was 6.4°C to 8.6°C, for PC and TP it was 22°C-26°C. Longest possible transport time of EC was up to 30 min at 37°C (worst case scenario).

Conclusion: Transport by PTS did not cause any impairment or loss of product quality. The temperature in the PTS-case during transport of PC and TP complied with the specifications defined in the guideline of hemotherapy. The required temperature for EC of 2-10°C was achieved with limited transport duration of 30 min.

The prospective transport validation of blood products as platelet and erythrocyte concentrates and therapeutical plasma with the PTS was completed successfully.

PS-2-5

Ensuring the stability of serum eye drops

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Background: Since 2009 we provide serum eye drops for the treatment of ocular surface diseases. In Austria serum eye drops are considered as medicinal products, therefore a stability program is mandatory to monitor product compliance with specifications and shelf life. Our stability program consisted in measuring growth factors EGF, PDGF-BB and Fibronectin as surrogate markers. In 2021, total protein was validated and is used since then. In addition, sterility testing is performed on each batch.

Methods: Three batches of serum eye drops are manufactured according to SOP. Serum samples are tested before freezing and then thawed at 3, 6,

9 and 12 months of storage. Growth factors as well as total protein concentration proof the stability of the product, sterility verifies the integrity of the applicators, both are therefore used as quality parameters. Additional in-use stability tests are performed after 3 months of storage and 24 and 48 hours of usage. Growth factors are tested with ELISA, total protein with CMIA. Sterility testing was performed by direct inoculation, changing to BacTAlert 3D mid 2020. A loss of growth factor, respectively total protein concentration of no more than 50% is accepted and sterility testing must be negative.

Results: From 2017 to 2021, EGF and Fibronectin passed the acceptance criteria in the 6 and 12 months testing, for PDGF-BB two results were OOS (-55.9 % and -54.2 % after 12 months). Overall, there are notable batch variations in initial and follow-up growth factor values. In contrast, total protein (in use since 2021) has very stable initial values and a deviation of -5.8 % to 3.0 % for 6 months and -4.2 % to 3.0 % for 12 months storage. Sterility was performed before freezing and after 6 and 12 months and was negative for all batches. In-use stability testing was done at 24 and 48 hours. All results passed the acceptance criteria, except one for sterility where human failure and contamination was likely.

Conclusion: Serum eye drops stability can continuously be demonstrated for 12 months at -20°C. Furthermore, total protein concentration is an efficient and preferable surrogate marker for serum eye drops with minimal deviations. Serum eye drops quality can also be retained in the opened applicator for 24 to even 48 hours at 2-8°C and the application is safe, provided that the patient handles it carefully (e.g. without touching the applicator tip).

PS-2-6

Over 6 years experience in allogenic serum eye drop manufacturing

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Background: Serum eye drops have been increasingly used world wide for years as an experimental therapy for severe forms of dry eyes. In cases where autologous blood donation is not possiblefor various reasons, allogeneic serum eye drops are used. At the University Hospital of Cologne, treatment with allogeneic serum eye drops has been possible since 2017 as part of an individual healing attempt.

Methods: In a retrospective database, relevant clinical and epidemiological information of the patients was acquired. Production in general has already been reported elsewhere in 2016.

Results: In the period between 2017 and 2022, 556 allogeneic preparations were produced for 142 patients. Most of the patients needed the eye drops because of keratoconjunctivitis sicca. As underlying disease, 63(44.3%) patients had an occult GvHD, 20(14.1%) neurotrophic keratitis, 9(6.3%) Sjörgens syndrome. In 7(4.9%) cases the eye drops were used to support corneal healing, e.g. after a trauma. 59 (41.5%) patients could not donate autologous blood due to adverse health conditions, 20(14.1%) due to anemia, 20(14.1%) due to contraindicated comorbidities, and 15(10.6%) due to weight. 7(4.9%) patients were given allogenic serum eye drops based on non-response to autologous blood eye drops.

Conclusion: With an expertise of 6 years, we could show that allogenic serum eye drops are an alternative to autologous eye drops for patients with contraindications for auto- donation. Future subjective effectivity trials are in the pipeline.

PS-2-7

Investigation of a Perfluorodecalin nanoemulsion as an artificial oxygen carrier in whole blood and 3D-printed blood vessels

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Background: Blood components such as erythrocyte concentrates remain in high demand. Artificial oxygen carriers in the form of a perfluorodecalin (PFD)-based nanoemulsion with an albumin shell (A-AOCs) offer a promising alternative. In prior experiments our group verified pertinent oxygen transport capacity of A-AOCs. [1-2] When injected into patients, A-AOCs come into contact with their erythrocytes. In this study it was evaluated if human erythrocytes remain intact and functional after contact with A-AOCs.

Methods: A-AOCs were synthesized by high-pressure homogenization of bovine serum albumin (BSA) along with PFD. These were mixed with human whole blood (local ethics approval no.21-10314-BO) at a clinically relevant ratio for up to 24 h at 37 °C and static conditions. Spectroscopic photometry was used to detect the free plasma hemoglobin concentration. Hematocrit and osmolality were determined along with glucose and lactate levels by using a blood gas analyzer. In addition, viscosity was measured utilizing a rheometer. To test A-AOCs in a physiologically relevant in vitro set-up, an artificial blood vessel was manufactured with a bio-3D printer using gelatin-methacryloyl and poloxamer 407.

Results: When mixing human whole blood with A-AOCs in a 1:1 ratio, the viscosity increased as indicated by photometric hemoglobin determination, which was caused by hemolysis of the erythrocytes. This phenomenon could be attributed to a combination of control solution (BSA) and A-AOCs as it was already increased in the control group (1:1 blood/BSA). In a more diluted (4:1 ratio) formulation, erythrocytes stayed intact for 24 h and viscosity was not impaired. These positive results were corroborated by a rise in lactate levels and a concomitant drop in glucose levels (Fig 1.) indicating, that functional erythrocytes were still producing energy through anaerobic glycolysis. The 3D-printed blood vessels could be integrated in a perfusion circuit.
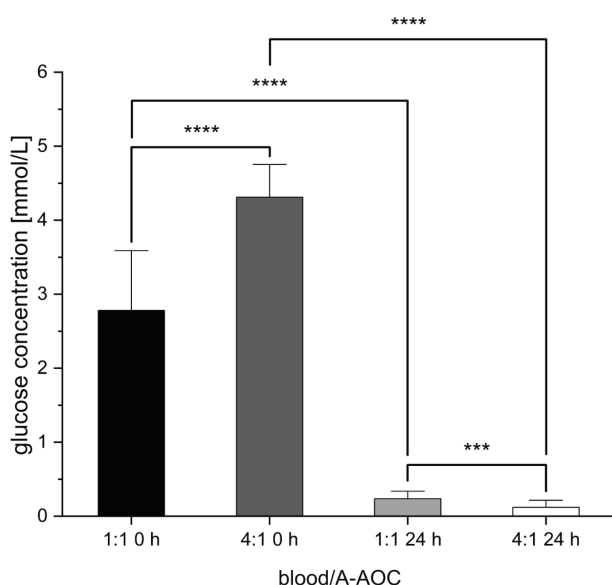
Fig. 1.

Conclusion: To summarize, the study demonstrated that in presence of low amounts of A-AOCs in a 4:1 ratio, erythrocytes retained their functionality. Future research examining blood compatibility and usage of 3D-printed blood vessels in conjunction with endothelial cells will aid in determining the clinical utility of perfluorodecalin nanoemulsions as an artificial oxygen carrier.

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PS-2-8

Production of metabolically improved Red Cell Concentrates under an new GMP Protocol under Hypoxia

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Background: Recent work has demonstrated that Red Blood Cells (RBCs) can be stored under hypoxia, resulting in enhanced glycolysis, generation of adenosine triphosphate (ATP) and 2,3-diphosphogycerate, as well as increased glutaminolysis, glutathione pools and post-transfusion recovery (Transfusion 60:786-798, 2020). We aimed to investigate the feasability of the process and product quality under GMP production conditions.

Methods: A total of 17 whole blood donations (495 ml) were separated by 5000g centrifugation and subsequent buffy coat depletion of platelets and leukocytes. RBCs were suspended in PAGGSM storage solution. Units were processed using the HEMANEXT ONE® RBC processing system (HemaNext, Lexington, MA, USA) by transfer into a separate oxygen reduction bag and subsequent storage in a PVC storage bag. Hematocrit (HCT), total Hemoglobin (Hb), ATP, O_2 saturation, hemolysis were determined on days 9, 15, 23, 30 and 42 of storage.

Results: HCT and Hb remained constant during the 42d storage with mean+SD 62.3±2.4 % and 63.1±2.3 % (HCT) and 53.5±4.7 g/dL and 53.6±4.5 g/dL (Hb) on days 0 and 42, respectively. O_2 saturation was 16.6±4.9 % (day 0) to 13.9±5.0 % (day 42). Hemolysis was 0.11+0.02% on day 0 and increased to 0.25+0.07% on day 42. ATP concentration increased from 4.07+0.59 µmol/g Hb to 4.36+0.71 µmol/g Hb on day 30, and fell to 3.36+0.58 µmol/g Hb on day 42. Irradiation did not detectably influence quality parameters within 24h.

Conclusion: GMP production of Deoxygenated RBCs is feasible, results in improved ATP levels and decreased hemolysis compared with historical controls at normoxia. Criteria suffice to apply for a manufacturing license for RBC at the responsible Länder Authority. The data form a basis for subsequent clinical evaluation of deoxygenated RBCs in order to evaluate the safety and efficacy of the novel product in chronically transfused patients.

PS-2-9

Implementation of a perfluorocarbon-based artificial oxygen carrier in normothermic porcine heart perfusion

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Background: Normothermic machine-perfusion (NT-MP) for organ preservation still depends on perfusates spiked with erythrocytes and thus is limited by donor blood shortage. Perfluorodecalin-based albumin-derived artificial oxygen carriers (A-AOC) display a promising blood alternative. _In vitro_ experiments in cardiomyocytes confirmed the A-AOCs' ability to attenuate ischemia-elicited damage. The aim of this project was to establish a physiological model in which A-AOCs can be tested as blood surrogate.

Methods: NT-MP was chosen as physiological model for testing the functionality of A-AOCs in isolated perfused porcine hearts. Parameters such as oxygen partial pressure, pH-value, level of electrolytes and metabolic parameters of the perfusate were studied by a blood gas analyzer (BGA, ABL 815) and photometry (respons 920).

Results: After first pilot experiments, the perfusion system was successfully built with a heater, a perfusate reservoir, an oxygenator as well as pH- and pO$_2$-sensors. Preliminary experiments in the established perfusion apparatus showed that the perfusion with a preservation solution was able to maintain a contracting heart for at least one hour. Furthermore, the level of Ca^{2+}, K$^+$, Na$^+$ and lactate as well as oxygen, pH, lactate dehydrogenase and aspartate aminotransferase were successfully monitored throughout the perfusion experiments

Conclusion: After successful establishment of the experimental set-up for perfusing porcine hearts, we can investigate the A-AOCs' potential to reduce ischemia-reperfusion injury (IRI) in an *ex vivo* heart perfusion. Parameters for IRI (i.g. apoptosis, oxidative stress, Troponin I) will be investigated in the future. Our group previously demonstrated a successful application of A-AOCs in a Langendorff-perfused rat heart. Therefore, an overall improvement of organ viability is expected for NT-MP with A-AOCs.

PS-2-10

More than 6 years experience with the collection of granulocytes using modified fluid gelatin 4%

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Background: Patients with impaired granulocyte function or sustained neutropenia after chemotherapy suffering from severe bacterial or fungal infections are routinely administered granulocyte concentrates. Ten years ago, we initiated the establishment and optimization of granulocytes apheresis using modified fluid gelatin 4% (MFG 4%) instead of hydroxyl ethyl starch (HES). This retrospective study is to present our compiled data since 2017.

Methods: 796 granulocyte concentrates were obtained from 393 collections from male donors after mobilization with a single subcutaneous dose (480 μg) of granulocyte-colony-stimulating factor (G-CSF) and 8 mg of orally administered dexamethasone 10-12 hours before apheresis. Granulocyte collections were performed using a cell separator Spectra Optia IDL (Terumo BCT, PMN program) with MFG 4% as a sedimentation agent combined with ACD-A as an anticoagulant. Apheresis lasted around 150 min aiming to harvest at least 1x10^{10} or preferably 2x10^{10} granulocytes as the latter also implicates the possibility of splitting the product. Data were collected and analyzed retrospectively to show the effectiveness of MFG (4%) in granulocyte apheresis.

Results: 393 granulocyte apheresates (GAs) with a mean concentration of 4,5×10^{10} (min. 1,0×10^{10}; max. 12,9×10^{10}) granulocytes per bag were collected from 01.01.2017 until the 30.04.2023. Interestingly, while 100% of the GAs have more than 1,0×10^{10} granulocytes per bag, 87,5% of the GAs contain more than 2,0×10^{10} granulocytes per bag. According to the amount of harvested granulocytes and considering the number of patients to be treated with granulocytes, we were able to finally obtain 796 granulocyte products (GP) with a mean concentration of 2,2×10^{10} (min. 1,0×10^{10}; max. 4,9×10^{10}) granulocytes. Moreover, MFG (4%) did not induce any side effects in either donors or patients.

Conclusion: Our results clearly show that granulocytes can be collected successfully with MFG (4%), which represents an available alternative to classical HES. High-yield granulocyte collections allowed the splitting of the GAs; whereby 796 products were obtained from collections with MFG (4%) and transfused to their determined patients. The possibility of GAs splitting allows us to serve more than two fold of the patients. In addition, MFG provides a good safety profile for both donors and recipients.

PS-2-11

Purified granulocyte concentrates from buffy coats with extended storage time

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Background: Granulocyte concentrates (GC) have limited clinical applicability due to rapid quality deterioration caused by excessive lactate production from erythrocytes, leading to decreased pH within 24 hours. Buffy coat-derived granulocytes are an alternative to apheresis donations. We have developed a system compatible with standard blood banking technologies to remove erythrocytes and platelets and extend the storage time.

Methods: Five to six AB0 blood group-matched buffy coats were combined to form a pool preparation. Subsequently, the erythrocytes were sedimented using HES solution. The resulting leucocyte-rich supernatant was washed twice with saline to remove the majority of platelets and then resuspended in AB0-matched donor plasma. This leucocyte concentrate was transferred to a platelet storage bag and stored for 72 hours. Cell count and viability, pH, blood gases, phagocytosis and oxidative burst activity were monitored daily.

Results: Applying this approach, erythrocyte and platelet concentrations were reduced to 0.4 % and 6.1 % of the baseline levels. A total of 49.8% of the originally present leucocytes could be extracted. This corresponds to an absolute granulocyte count of about 3.37 x 10^9 cells. In the course of 72 hours of storage, there were no significant changes in cell counts. The viability exceeded 98% during the entire period. The rate of granulocytes performing phagocytosis and oxidative burst remained above 95% anytime.

Conclusion: Designed to obtain a leukocyte concentrate from buffy coats, our technique provides a precious alternative source to obtain granulocytes. Purification extends the maximum shelf life of GC from 24 h to 72 h and may therefore facilitate the availability for clinical purposes. For a clinically usable GC with > 1 x 10^{10} granulocytes about 15 to 20 buffy coats need to be pooled.

PS-2-12

Assessment of purified granulocyte preparations in multiple environmental settings

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Background: Dysfunctional immune cells contribute to sepsis-related immune paralysis. Transfusing healthy donor immune cells, like granulocyte concentrates (GC), can cause tissue damage via neutrophil activity. Prior trials utilized standard GC in an extracorporeal therapy system, separating patient and donor cells with plasma filters, resulting in positive effects with reduced side effects. Our ex vivo study demonstrates the efficacy of purified granulocyte preparations in diverse environmental conditions.

Methods: Standard GCs (sGCs) were sedimented, washed twice with 0.9% sodium chloride (NaCl), and resuspended in blood group-identical fresh frozen plasma. The resulting purified GCs (pGCs) were then stored in platelet bags at a cell concentration of about 5 × 10^7 ± 1.8 × 10^7 leukocytes/ml without agitation at room temperature for multiple days. Cell count and viability, pH, blood gases, phagocytosis, and oxidative burst were monitored daily. Furthermore, we simulated prolonged circulation times by

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subjecting the pGC to extended extracorporeal therapy simulations up to 24 h. Following the prolonged circulation, we analyzed the cytokine profile to determine any alterations in cytokine activity.

Results: Cells were viable throughout the study period and exhibited well-preserved functionality and efficient metabolic activity. Course of lactate dehydrogenase and free hemoglobin concentration yielded no indication of cell impairment. The capability of the cells to secret various cytokines was preserved after prolonged circulation. Of particular interest is preservation in performance of the cells after 5 days of storage, demonstrating the sustained shelf life and performance of the immune cells in the purified GCs.

Conclusion: Results demonstrate the suitability of a simplified extracorporeal system. Furthermore, granulocytes remain viable and highly active even beyond a 6 hour treatment even after storage of over 3 days supporting the treatment of septic patients with this system in advanced clinical trials.

Disclosure Statements: Steffen Mitzner, and Jens Altrichter are employees or shareholders of ARTCLINE GmbH. All others have no conflict of interest.

PS-2-13

Demographic changes of first-time donors over the last 20 years in Upper Austria

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Background: An aging population, as well as a shift toward more selfishness instead of altruism, poses challenges to the availability of blood and blood products. Although restrictive transfusion policies and patient blood management have been introduced in the last decade, consistent and constant efforts must be taken to recruit new donors in order to compensate for the loss of long-time regular donors.

Methods: The objective of this retrospective study was to identify patterns and changes in first-time donors in Upper Austria between 2002, 2012 and 2022. A descriptive analysis using data from 138.841 donors were compared with demographic data from Statistik Austria. Blood donors were categorized and evaluated based on the following criteria: first-time donors, repeat donors, gender, and age. Furthermore, the donors were assigned to the 15 political districts (rural area) and 3 statuary cities (urban area).

Results: The average age of the population in Upper Austria was under 41 in 2002 In 2022, this was more than 43 years.Between 2002 and 2022, the ratio of first-time donors increased. (+28.8%), with this is mainly due to female first-time donors (proportion of first-time donors - 2002: 14.5%, 2012: 15.7%, 2022: 18.6%). With regard to the age of first-time donors, it became apparent that this has changed significantly over the last 20 years: see Table 1.

Although the proportion of first-time donors in urban areas decreased by about 15% between 2002 and 2022, the overall number of blood donors in urban areas increased from 6.7% (2002) to 18.3% (2022).

Tab. 1.

	2002	2012	2022
	%	%	%
18-25 years	71.2	71.7	44.9
26-35 years	13.4	15.0	25.7
36-50 years	12.7	10.4	21.1
>50 years	2.7	2.9	8.3

Conclusion: Over the past 20 years, the age of first-time donors in Upper Austria has increased continuously. Numerous measures such as holding educational events, guided tours at the blood bank, digitalization of the process such as the introduction of an app are being made. These efforts help to raise awareness among more young people about the topic of blood and voluntary blood donation in order to be in a position to guarantee 100% supply security for our patients in the future.

PS-2-14

Influences on blood donor return rate and donation interval

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Background: Maintaining a motivated pool of eligible blood donors is the most important basis to provide the health care system with the required blood supply. Society and individual commitment has changed in the last years. The pandemic has accelerated these factors; in contrast it had a different but temporary impact on blood donation behavior.

The recruitment of new donors is an extensive task; therefore it is significant to have donors with a constant donation frequency.

Methods: Insights into motives that modulate donation frequencies can help influencing them without much effort. From 2017 to 2022, 125,917 individual persons accounted for 334,175 whole blood donations (including adverse reactions) and 47,241 deferrals in our institute. For these donations, we analyzed the last and next contact with the particular person and if these had a certain abnormality. This donation interval was tested for correlation with demographic characteristics as age, local factors (urban vs. rural) and reasons for deferral.

The presented data does not include donations from platelet and plasma apheresis, since these donation appointments are actively managed.

Results: 38.5 % of donors are one-time contacts that did not donate a second time. 50 % of returning donors do this within 246 days after a successful donation. After deferral, this increases to 356 days and 263 if they had a negative experience (e.g. fainting). Covid pandemic temporarily decreased the interval (presumably, because blood donation was exempted from restrictions). Donors return sooner if deferral was due to travelling or low hemoglobin compared to other reasons (medical condition or treatment, risk behavior). In 2017, there was a clear difference of age groups with a significant shorter interval for older persons (56+); this difference has converged in 2022. Overall, the interval has dropped and fewer donors need to donate more often.

Conclusion: The changes in our donor pool demand new strategies to keep donors motivated. Considering that, 42.2 % of donors younger than 29 requires new and valuable incentives for their second donation.

There need to be more focus on donors that had negative experiences during their donation. Deferred donors tend to have longer comeback rates and 46.5 % do not come back at all. Distinguished contact approaches could help to mitigate disappointment and reactivate potential.

PS-2-15

Case report: Blood donation with a contraceptive stick on one arm

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Background: A 29 year old woman claimed her contraceptive stick (Implanon NXT®) in her left arm broke due to the blood donation she had done. She reported that the cause of the breakage had been the preasure of the sphygmomanometer used during the donation. It is advised to remove and replace the implant when damaged to avoid the risk of migration and reassure a consistent hormone release and thus contraceptive efficacy. A physical and imagery examination was done.

Methods: Systematic analysis of case reports and studies from the database of the national library of medicine.

Results: Implants have gained popularity throughout the years since their release in the early 1990´s, with an estimated 492,00 women using them for contraception as of 2012 in the United States alone. There are currently no statitics in Germany.

The clinical examination from a gynaecologist, as well as the sonographical and the radiological one, showed no damage of the device. Although breakage of the Implanon can occur through local trauma, this remains a rare phenomenon, as publications of case-reports throught the years show.

Conclusion: A contraceptive implant can be broken at the time of insertion, at the time of removal, or while in situ. In order to avoid any risks and unnecessary stress, the woman was advised to not do future blood donations with the arm where her contraceptive stick lies, in her case with the left one.

PS-2-16
Impact of positive testing for Hepatitis E virus on donor return

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Background: Since the introduction of mandatory testing for hepatitis E virus (HEV), blood collection services have been confronted with an increasing number of deferred blood donors due to positive test results, which is assumed to bear a high risk of non-return. Usually it is difficult to measure this effect. However, the possibility of a large number of donors tested positive for HEV and the possibility of donor re-admission after 4 months with a negative test result allow this effect to be measured.

Methods: Until introduction of voluntary HEV genome testing in 2015, all positive tested blood donors were retrospectively investigated regarding their return to blood donation depending on their donor status (first-time or permanent donor, whole blood and/or plasma donor). In total, 731,657 blood donations were screened, including red blood cell, apheresis platelets and therapeutic plasma donations.

Results: In total, 497 donors were tested HEV RNA positive in the period from January 2015 until December 2022. Of these, 58 donors were first-time donors (11.6 %). In total, 339 of the positively tested donors returned for donation (68.2 %). In the group of first-time donors, 19 donors (32.8 %) returned, whereas 320 of the permanent donors (72.9 %) returned. Splitting the permanent donor group into red blood cell or plasma donors, 78.7 % of plasma and 74.8% of red blood cell donors returned.

Conclusion: The HEV deferral resulted in higher non return rates in the group of first time donors compared to permanent donors. No differences were observed among permanent plasma or red blood cell donors. Therefore, it could be assumed that previous positive experiences with blood donation result in a lower deterrence of positive infectious disease testing.

PS-2-17
"Disposal Approach" to meet the challenges in provision of high volume donations for diagnostic industry in Europe

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Background: The global shortage of available blood is not only a major challenge for transfusion medicine and the pharmaceutical industry, but also for diagnostics manufacturers worldwide. The political situation during and after the pandemic with the current Ukraine war and the open conflict over the island of Taiwan leads to a partial separation of the global markets, especially between the USA, Europe and China.

Methods: Should the next president of the USA declare *"America First"* again, this could jeopardize the supply of blood products from the USA to Europe. While Europe is making great efforts to increase the amount of blood available in the region, this does not include the diagnostic industry. With a steady growth of more than 10% even after the pandemic in the diagnostics industry, the demand for blood products will also lead to increased parallel activities in the field of proprietary blood product collection worldwide. Competition between national blood donation systems and suppliers to diagnostic manufacturers must and can be prevented.

Results: To ensure sufficient quantities of plasma and serum for diagnostics in Europe, a *"90-to-10 Approach"* is being discussed internationally, which should secure 10% of the available blood products for diagnostics. Approximately 1 million liters of blood products collected throughout Europe would be made available annually for diagnostics, from today's perspective a significantly too large quantity.

Of all the blood components collected, approx. 1% are not suitable for use in transfusion medicine for a variety of reasons (infectology, cooling process, quality of blood bulks, etc.). This quantity would mean an extensive coverage of the European demand for diagnostics and would also be ensure production during crisis situations.

Conclusion: Our *"Disposal Approach"*, as a milder, preliminary version of the *"90-to-10 Approach"* requires national regulations to ensure that all blood components to be discarded are offered to the suppliers of the diagnostics industry, thus preventing the ethically difficult destruction of high-quality clinical material.

PS-2-18
2 Years of experience in HEV Screening of plasma donors

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Background: Testing for HEV RNA has been mandatory for all medical products by the Paul-Ehrlich Institute (PEI) since 2019. Since 2020, Haema AG also tests all source plasma donations. It is shown that low HEV RNA concentrations in plasma pools have been detected [1] and no transmission of HEV RNA through plasma derived medicinal products has been reported to date [2]. Dreier et al. estimated a general infectious dose of 50,000 IU/ml and 7,050 IU/ml as a minimum dose [3].

Methods: From 2021 to 2022, we have analyzed over 2.24 million donations for HEV RNA in Germany to PEI specifications, the test method used for the HEV-NAT must reliably detect a HEV RNA concentration of 2,000 IU/ml based on a single blood donation for medicinal products. Therefore, HEV RNA was tested in a pool of 96 samples – also for plasma donations. We used the Roche cobas 6800® with the PCR method (Polymerase Chain Reaction) and a sensitivity of 18.6 IU/ml (ID-NAT) and the Grifols Procleix Panther with the TMA method (Transcription-Mediated Amplification) and a sensitivity of 7.89 IU/ml (ID-NAT). We determined the virus concentration for one quarter (semiquantitative) by using a standard curve for HEV RNA.

Results: Since the start of HEV testing, the number of HEV positive donations has continually increased from 203 donations in 2021 up to 424 donations in 2022 to 127 donations in the first quarter of 2023 (Tab.1). We detect most of the HEV positive donations from plasma donors (78.3%), as most donors are repeated donors and are therefore tested frequently. 54% of the HEV RNA positive plasma donations had a low virus viral load

Tab. 1.

Table 1: Evaluation of HEV positive donations in 2021 - 2022 including prevalence and seroconversion.

	Number of donations	Number HEV pos. donations	Proportion [%]	Prevalence of first-time donors			Seroconversion rate repeated donors		
				Amount of first-time donors (FD)	FD HEV pos.	Positive per 100.000 donors/month	Amount of repeated donors (RD)	RD HEV pos.	Positive per 100.000 donors/month
Total 2021	1.107.853	307	0,028	26.810	9	33,57	84.823	298	351,32
Total 2022	1.141.001	424	0,037	30.906	28	90,60	88.133	396	449,32

Tab. 2.

Table 2: Distribution of HEV positive plasma donations regarding the viral load [IU/ml] in the first quarter of 2022 by using the Roche cobas® 6800.
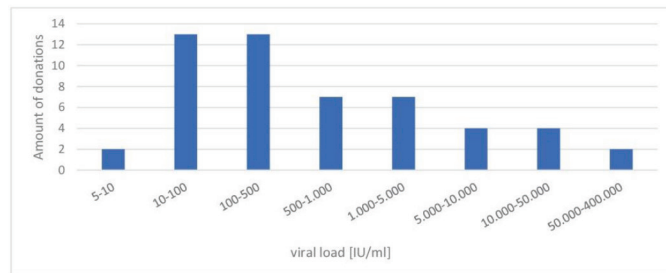
(50 to 500 IU/ml), 42% had a viral load in the range of 500 to 50,000 IU/ml and 4% of the donors were detected with a viral load >50,000 IU/ml (Tab.2). The low viral load in a large number of donations could assume that more early-stage infections are detected.

Conclusion: According to the PEI, an 80% risk reduction was achieved by using a 96 pool with an analytical sensitivity of 20 IU/ml. Based on this, not all infections could be detected particularly in the early stage of infection. Blood donors had a significantly lower virus load than symptomatic patients [4]. Due to the increasing number of HEV positive donors, viral load might become more important.

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PS-2-19
Impact of hepatitis E notification on donor return

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Background: Among blood donors in Germany, the most frequently positive virus parameter leading to a donation ban is Hepatitis E. Although most donors with Hepatitis E viremia are asymptomatic, the detection of HEV triggers notification of local health authorities and a temporary donation ban. Rarely, donors complained about these actions. We reasoned that non-complaining donors might also be upset and refrain from future donations. Therefore, we investigated the impact of HEV notification on donor return

Methods: Donations were tested for HEV using the AltoStar HEV RT-PCR Kit 1.5 (altona Diagnostics). HEV positive donations between 01-Mar-2020 and 28-Feb-2021 were identified. Donations from the same donation date and nearby donation number (if possible index number + 5) were used as controls. Donors with more than 6 donations per year were excluded from analysis. The number of donation attempts within 18 months before the index donation and within 18 months after the donation ban were determined and the difference calculated. The differences of HEV positive donors and control donors was evaluated. Statistical comparisons were calculated using Students t-Test.

Results: From 01-Mar-2020 and 28-Feb-2021, 625 HEV positive donations were identified. After exclusion of frequent donors, 606 HEV positive donors and 610 controls remained. The mean number of donations 18 months before the HEV positive donations was 2.64 ± 1.94 (mean ± SD). After the donation ban, there were 2.71 ± 2.33 donations within 18 months (p>0.5). Donations numbers of controls were 2.55 ± 2.01 donations before and 2.75 ± 2.24 donations after the index donation. The difference between donation number before and after the event was similar for HEV positive

donors (0.07 ± 2.23) and controls (0.20 ± 2.06; p>0.3). Donation attempts during ban were 0.05 ± 0.44 (controls 0.49 ± 0.61; p<0.001).

Conclusion: Notification on HEV infection and the accompanying discomfort did not relevantly impact on donation frequency of the donors. Most donors respected the temporary donation ban. The slight increase in the mean number of donations after the index date was likely a result of the inclusion of donors without prior donation. Obviously, the vast majority of donors is able to cope with an information on HEV infection in a rational manner.

PS-2-20
Evaluation of a new PCR-based CMV assay for blood donor screening

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Background: According to the German Guidelines, the use of leucocyte-depleted blood components is an effective strategy for reducing the risk of transfusion-transmitted cytomegalovirus (CMV) infections. However, there is a residual risk of transfusion-transmitted infections by non cell-associated CMV. We tested a new PCR-based CMV assay developed for high-throughput blood donor screening.

Methods: CMV detection by nucleic acid amplification testing (NAT) was performed in pools of up to 48 samples of EDTA-plasma (0.1 ml per sample). Sample preparation was performed by the *PoET Instrument* with the PoET CMV-Kit (GFE). Analytical limit of detection (95%) is specified with 12.5 IU/ml, while the limit of detection (LoD) in pools with 48 samples is 600 IU/ml per tested sample. For evaluation of the assay, CMV reference material (1st WHO IS for human CMV 09/162) using three different concentrations (12.5 IU/ml, 25 IU/ml and 50 IU/ml corresponding to 1-fold, 2-fold and 3-fold-LoD, respectively) was tested. In addition, samples provided for proficiency testing and samples from whole blood donations were tested in a routine-like setting.

Results: Of the CMV reference material diluted with human negative plasma, all samples (N=48) were tested positive at concentrations of 25 IU/ml and 50 IU/ml, whereas only 45 of 48 samples were tested positive at a concentration of 12.5 IU/ml. Proficiency testing samples were all correctly tested. Of the 1,138 whole blood donor samples, which were tested in a total of 24 minipools, one pool was positive with the PoET CMV assay. However, positivity of this pool could not be confirmed by repeat testing. The other 23 pools were all tested negative.

Conclusion: PoET CMV on the *PoET Instrument* is a new and sensitive method for CMV infectivity screening of blood donors.

PS-2-21
Routine blood donor screening on a new NAT platform

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Background: According to the German guidelines all blood donations must be screened for transfusion-relevant viruses by nucleic acid amplification testing (NAT). In June 2022, a new high-throughput, fully automated NAT platform called *PoET Instrument* (GFE) running CE-IVD PCR-based assays was introduced. In this study, we used this new platform to test minipools of whole blood and apheresis donor plasma samples for the presence of HCV, HBV, HIV-1/-2, HAV and B19V.

Methods: NAT screening of the blood donations was performed in minipools of up to 96 EDTA-Plasma samples. On the *PoET Instrument* three-times 1.3 ml plasma of each pool was extracted followed by PCR amplification (real-time RT-PCR) and detection. CE-IVD PCR assays (PoET HCV, PoET HBV, PoET HIV, PoET HAV, PoET B19V) were used.

Analytical sensitivity (95% LoD) according to manufacturer information for detection of HCV, HBV, HIV, HAV and parvovirus B19 is 9.1 IU/ml, 1.6 IU/ml, 15 IU/ml, 0.9 IU/ml, and 8.6 IU/ml, respectively. In a routine setting, up to 1,440 donor samples per run were tested for 5 viruses within 3.5 hours. Result evaluation was performed by the system (Calliope Management Software).

Results: From June 2022 to April 2023, a total of 525,276 donations in 7,174 pools were tested. 97.4 % of the runs were successfully completed. In valid test runs, 99.8 % of the test results were valid. Inhibition rates for each PCR test were between 0.01% (PoET HBV) and 0.1% (PoET HIV). Rates of initially positive, not confirmed pools were between 0% (PoET HCV, PoET HAV) and 0.24% (PoET HBV).

Conclusion: Our experience with a total 10 months of testing demonstrates that the new *PoET* screening platform is a sensitive and robust system for routine infection screening of blood donors.

PS-2-22
Reduction in the initial reactive rate by a new HBsAg assay version

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Background: In August 2022, we changed HBsAg testing for blood screening from the Alinity i HBsAg Qualitative II assay to a newer version, the Alinity i HBsAg Next assay on the Alinity i fully automated system. According to the manufacturer, the new assay is offering a higher analytical sensitivity. While a higher sensitivity is desired, the specificity of a screening assay is critical as well, since false reactive results will lead to an unnecessary rejection of donations and an increased workload.

Methods: The analysis timeframe covered all year 2022, samples were tested with the Alinity i HBsAg Qualitative II assay (Abbott GmbH, Germany) from January 1, 2022 until August, 2022, replaced by the Alinity i HBsAg Next assay (Abbott GmbH) until the end of the year. In addition to the initial and repeat reactive rate of both assays, we also analyzed the distribution of sample to cut-off (S/CO) values of the negative results as a measure to determine the discrimination power of the assays.

Results: Over 200.000 samples were tested in 2022, with more than 100.000 using the Alinity i HBsAg Qual II assay and more than 90.000 samples using the Alinity i HBsAg Next assay. We observed a reduction in the overall initial reactive rate for the Alinity HBsAg Next compared to its predecessor in the investigated time frame. Especially in the low positive range of the assay (1-10 S/CO), we detected a reduction in initial reactive rates. However, we did not observe a change in the rate of high-positive samples (>1000 S/CO).

Conclusion: Overall, the data demonstrated that the new Alinity HBsAg Next assay has a better specificity and sensitivity compared to the comparator assay Alinity i HBsAg Qual II in our blood screening setting. Despite the reported higher sensitivity, the new assay design has lowered the initial reactive rate. This reduced the number of repeated testing substantially and is leading to an improved workflow and a reduction of repeat testing in our setting.

Health-related quality of life in Iranian adult men with severe hemophilia

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Background: Health-related quality of life (HRQOL) assessment should be part of the regular clinical assessment of persons with hemophilia. This study assessed quality of life of severe hemophilia patients (Type A) in Tehran comprehensive hemophilia care center.

Methods: This cross-sectional study was done in 2016 and 84 men aged over 20 years with severe hemophilia (Type A) were assessed. All patients have been treated over 10 years at the comprehensive hemophilia care center, Iran. THE HRQOL assessment includes the A36 Hemophilia-QOL questionnaire, sociodemographical and clinical characteristics.

Results: The overall HRQOL mean (WSD) in the present sample was 69.1W26.0 (A36Hemofilia-QoL Global Index). The mean (WSD) scores according the domains were: physical health (15.0W5.7), daily activities (6.7W3.8), joint damage (4.2W2.9), pain (3.0W1.9), social activities and relationships (10.7W5.8) and emotional function (9.2W5.0) classified moderate-to-poor state. In contrast with treatment satisfaction (4.8W1.6), treatment difficulties (9.5W3.4) and mental health (6.2W3.3), those were all in moderate-to-good state. Patients with higher education levels had better quality of life in the area of anxiety (PU0.034), mental health (PU0.007), social activities and personal relationships (PU0.002). In general, poor quality of life of patients was 13.1%, 42.9% weak-to-moderate, 38.1% moderate-to-good and 6% good.

Conclusion: It was shown that over 50% of patients had low-to-moderate HRQOL in the domains, such as physical health, daily activities, joint damage, pain, social activities and personal relationships. Efforts are necessary to improve their HRQOL.

Two novel *VWF* gene mutations associated with von Willebrand Disease type 1

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Background: Von Willebrand disease (VWD) is the most frequent hereditary bleeding disorder in man. Its prevalence is about 1 %. The von Willebrand gene consists of 52 exons and spans about 180 kb of the genome. Several genetic variants have been identified causing quantitative deficiencies (type 1 and type 3) and qualitative deficiencies (type 2). Nevertheless, new genetic variants are constantly being found. We describe two variants whose causal relationship to VWD type 1 has not yet been established.

Methods: In 2 patients with mild bleeding history, von Willebrand factor antigen (VWF Ag), ristocetin cofactor activity, and the activity of factor VIIIc were measured on STA R Max3, Stago, France. Multimer analysis was performed using SDS-agarose gel electrophoresis (Hydrasys 2, Sebia, France). In vitro bleeding times were measured using the platelet function analyzer PFA-200 (Siemens, Germany). *VWF* gene was analyzed via next generation sequencing (MiSeq, Illumina). The characterization of variants was performed with in silico evaluation tools, including varSEAK Online.

Results: A 45-year-old woman presented with VWF Ag of 0.57 U/ml, RistoCoF of 0.42 U/ml, F VIIIc of 1.08 U/ml and bleeding times of 250 and 163 s. *VWF* gene sequencing identified a heterozygous variant in exon 45 (c.7666delG; p.Val2556SerfsTer8), resulting in the deletion of G at the 7666th position in the coding sequence, that causes a frameshift and a premature stop codon. A 32-year-old woman presented with VWF Ag

Gene	Genotype	Human Genome Variation Society Nomenclature	NCBI-Transkript	dbSNP-Reference
Case 1				
VWF	heterozygous	c.7666delG; p.Val2556SerfsTer8	NM_000552.4	—
Case 2				
VWF	heterozygous	c.7082-3_7084delCAGCCT	NM_000552.4	—

Fig. 1.

of 0.46 U/ml, RistoCoF of 0.35 U/ml, F VIIIc of 1.01 U/ml and bleeding times of 255 and 212 s. *VWF* gene sequencing identified a heterozygous deletion of 6 bp at the junction between intron 41 and exon 42 (c.7082-3_7084delCAGCCT) likely leading to aberrant splicing and subsequently to a non-functional VWF antigen.

Conclusion: Genetic analysis of the *VWF* gene is useful in addition to in vitro analysis by laboratory experiments and common diagnostic tests, multimer analysis, phenotypic studies of patients and co-segregation analysis within families to classify the type of VWD.

PS-2-25
A *SERPINE1* gene mutation possibly associated with intraoperative bleeding

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Background: The activity of plasminogen activators (PAs) is regulated by a complex process that involves regulation of PA gene expression by hormones, growth factors and cytokines, interactions with fibrin, and PA activity regulation by specific PA inhibitors (PAIs). Among these, PAI-1 is the most kinetically efficient. Here, we describe a case with a *SERPINE1* gene variant with possible causal relationship to intraoperative bleeding.

Methods: A 15-year-old patient underwent minimally invasive pectus excavatum (MIRPE) repair with two 11-inch rods for funnel chest correction. Intraoperatively, an unusual bleeding tendency occurred, which, however, could be controlled without blood transfusions. Global tests and procoagulant factors were measured on STA R Max3, Stago, France. Multimer analysis was performed using SDS-agarose gel electrophoresis (Hydrasys 2, Sebia, France). In vitro bleeding times were measured using the PFA-200 (Siemens, Germany). The sequencing of 55 genes (complete coding sequences and adjacent intron regions) was performed via next generation sequencing (MiSeq, Illumina). For interpretation, we used in silico evaluation tools, including PolyPhen-2 and SIFT.

Results: In this case, genetic analysis of numerous relevant genes (for details see ijms24097976) in a patient who would otherwise have been found hemostaseologically unremarkable led to the probable explanation of a bleeding tendency observed intraoperatively. Postoperative detailed coagulation examination including examination of platelet aggregation according to Born and in impedance aggregometry revealed no abnormalities. However, *SERPINE1* gene sequencing identified a mutation in exon 2 (c.43G>A; p.Ala15Thr).

Conclusion: For the identified *SERPINE1* variant, which leads to the amino acid exchange Ala15Thr in the PAI-1 signal peptide, only two other case reports exist to date, in which individual members of affected families also exhibited a bleeding tendency occurring exclusively intra- or postoperatively (PMIDs: 15650551 and 22993578). Further studies are needed to ensure or exclude with greater certainty any influence of this variant on the coagulation system.

Gene	Genotype	Human Genome Variation Society Nomenclature	NCBI-Transcript	dbSNP-Reference
SERPINE1	heterozygous	c.43G>A; p.Ala15Thr	NM_000602.4	rs6092

Fig. 1.

Structural characterization of B domain and full-length of coagulation factor VIII

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Background: Full-length factor VIII protein (FL-FVIII) is structurally well characterized containing a heavily glycosylated B domain. Current structural information is skewed toward the B domain deleted FVIII (BDD-FVIII), leaving most of the B domain to be structurally unresolved. We aim at the structural importance of the B domain by applying biophysical techniques on FL-FVIII and BDD-FVIII and correlating the experimental observations to computational studies performed on FVIII structural models.

Methods: Our sources of FL-FVIII protein were recombinant and plasma-derived concentrates which were subjected to processing on the Krios G4 platform. The data was processed using cryoSPARC software. Air and liquid AFM was performed on high pure rFVIII protein. Comparative CD spectrometry for rFL-FVIII and rBDD-FVIII was performed. Computational studies were performed by glycosylating and cleaving at the furin cleavage site of the alpha fold model of FL-FVIII and BDD-FVIII. The models were subjected to all atomic molecular dynamic simulations and the simulation-equilibrated models were analyzed with the cryo-EM and AFM data. Comparative studies for the binding of interaction partners with FL-FVIII and BDD-FVIII computational models were performed.

Results: Cryo-EM analysis of rFVIII showed conformational and particle heterogeneity whereas pdFVIII showed particle homogeneity thus yielding low-resolution cryo-EM maps. Spatial positioning of the B domain identified by rigid fitting appeared to be scattered around the central core of the FVIII protein. Similar observations of the B domain wrapping around the core globular domains were made in the AFM images. Similar orientation and conformational variability of the B domain were reflected well in our simulation equilibrated models. Qualitative and quantitative differences in secondary structure between FL-FVIII and BDD-FVIII were observed. Surface area accessibility for the binding of interacting partners differed between FL-FVIII and BDD-FVIII.

Conclusion: Owing to the low resolution of the maps, the B domain was not resolved to an atomic level. Biophysical analysis suggests conformational variability and heterogeneity in the FL-FVIII structure indicating high disorderness of the B domain. Glycosylation stabilizes the orientation of the B domain. Differences in secondary structures between FL-FVIII and BDD-FVIII indicated that the binding of FVIII interacting partners or neutralizing antibodies might structurally, and functionally differ.

PS-2-27

SpikeIn studies for the diagnosis of coagulation disorders using Thrombelastography

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Background: Thrombelastography measures in real time coagulation parameters in whole blood samples. The method serves as a useful point-of-care test to diagnose and manage patients with coagulation disorders. The advantage of thromboelastography is the rapid overview of all phases of coagulation and fibrinolysis. However, this method is limited by the need for immediate testing and difficulty in dissecting the exact mechanism of the dysregulation.

Methods: In this study we aim to modify the method to enable assessment of specific aspects of coagulation, even retrospectively. In particular, we investigated whether thromboelastographic studies can be performed using spike-in experiments from frozen plasma samples to measure fibrinolysis parameters (maximum of lysis, lysis time).

First, fifteen poor platelet plasma samples (PPP) were spiked with three donor samples each. Both SpikeIn and whole blood were measured and compared in Ex-, FIB- and TPA-test in thrombelastography (TE). Additionally, PPP of 20 probands was investigated in TE.

Results: SpikeIn and whole blood are measurable in TE. It was possible to perform thrombelastografic assays with fresh PPP and PPP which has been frozen before. Further experiments are following and we expect to correlate SpikeIn and whole blood samples to allow retrospective diagnosis of defects in fibrinolysis system. Furthermore, supplementary investigations to determine plasminogen activator inhibitor-1-, plasminogen- and thrombin-activatable fibrinolysis inhibitor-activity and lysis timer are performed.

Conclusion: Thrombelastografic assays can be used to investigate ability of the plasma factors in frozen samples to lyse clots. The modified method can be useful for laboratories with no immediate access to thromboelastography to retrospectively analysis the fibrinolysis system in patients with bleeding or thrombosis disorders of unknown origin.

PS-2-28

Platelet-activating factor promotes activation of neutrophil granulocytes in conjunction with the formation of platelet-neutrophil complexes

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Background: Platelet-activating factor (PAF) is an inflammatory mediator involved in many pathophysiologies such as sepsis or allergies. PAF has the ability to activate both neutrophils and thrombocytes. In the present work, we first describe the response of neutrophils elicited by PAF, then examine how the PAF-induced neutrophil response is dependent on platelet-neutrophil complex (PNC) formation, and finally evaluate potential strategies to modulate PNC formation.

Methods: Following ethical approval (#459/18) and informed written consent, blood was taken from healthy human volunteers (24±3 years). Whole blood was stimulated with buffer control or PAF (1μM) and pharmacological agents as indicated for 15 min. Neutrophil activity and the formation of PNCs was analyzed by flow cytometry. Data are reported as mean ± SD from at least six independent donors.

Results: PAF induced neutrophil activation as shown by an upregulation of CD10, CD11b, and CD66b expression as well as in a rise in phagocytic activity and production of radical oxygen species. Additionally, PAF significantly increased the appearance of PNCs. Anti-CD62P (p-Selectin), Iloprost (a prostacyclin analogue), and Ketanserin (5-HT$_2$ antagonist) prevented the formation of PNCs. When PNCs and neutrophils without platelet satellitism (NT⁻) were compared, it became evident that PNCs had significantly higher phagocytotic activity and produced more radical oxygen species in unstimulated blood and after the addition of PAF than NT⁻. However, there was no discernible difference between PNCs and NT⁻ when comparing the analyzed activation markers.

Conclusion: PAF stimulates neutrophil activity, partially in a platelet-dependent manner. Further studies need to elucidate the discrepancy regarding cellular effector functions and activation markers and confirm the findings with other methods and stimuli. Moreover, pharmacological interventions that limit the formation of PNCs might modify an acute inflammatory response, which may have therapeutic value. Likewise, activated platelets might be a tool to enhance neutrophil activity in neutropenic patients.

PS-2-29

The impact of different platelet subpopulations on platelet-neutrophil interaction

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Background: Activated neutrophils and neutrophil extracellular traps (NETs) were observed to play pivotal roles in thromboinflammatory diseases. Increasing evidence suggests that platelets (PLTs) activated by neutrophils amplify different prothrombotic disorders. However, the contribution of different PLT subpopulations in activating and modulating neutrophil response remains elusive. In this study, we aim to characterize the impact of distinct PLT phenotypes on PLT-neutrophil interplay.

Methods: PLTs were stimulated with different agonists (TRAP-6, thrombin, convulxin and combinations) to induce different PLT phenotypes including activated P-selectin single pos. [CD62p⁺] and CD62p⁺/phosphatidylserine [PS]⁺ procoagulant PLTs, and incubated with isolated neutrophils. Afterwards, samples were labelled with anti-CD41-APC and anti-CD16-PE for flow cytometric detection of PLT-neutrophil aggregates (PNAs). Using an immunofluorescence microscopy-based assay, the impact of different PLT phenotypes on NET formation was investigated. Briefly, neutrophils were allowed to attach on fibrinogen and incubated with PLTs which was followed by staining with Sytox green and anti-CD41-APC for the detection of extracellular DNA and PLTs, respectively.

Results: Treatment of PLTs with TRAP-6 and thrombin resulted in the formation of activated CD62p⁺ (mean percentage [%]±SEM: 79.0±11.3 vs. 80.4±10.7, respectively) while procoagulant [CD62p⁺/PS⁺] PLTs were only induced by convulxin, and dual TRAP-6/convulxin or thrombin/convulxin stimulation: 41.4±9.5 vs. 44.4%±8.6; and vs. 56.3±12.9, respectively. Interestingly, PNA formation seems to show agonist dependency as TRAP-6 activated PLTs induced only a low amount of PNA. Contrary, thrombin activated and procoagulant PLTs induced a significant increase in PNA formation in vitro. A finding that was further confirmed as higher amounts of extracellular DNA were induced by thrombin activated and procoagulant PLTs compared to TRAP-6 and buffer treated PLTs.

Conclusion: Our findings indicate that different PLT subpopulations harbor the potential to induce increased PLT-neutrophil interplay with subsequent formation of PNAs as well as NETs. Further dissection of the precise mechanisms in PLT-neutrophil crosstalk and identification of potential pharmaceutical targets could help to decipher the role of different PLT surface proteins and their relevance in PLT-neutrophil interactions.

PS-3-1
Immunoadsorption as a method of antibody donation during the Covid-19 pandemic

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Background: In the fight against the Covid-19 Pandemic, administration of plasma from convalescent donors containing anti-SARS-CoV-2 antibodies, though promising according to case reports, failed to show a clear benefit in a greater number of trials. We hypothesize that instead of collecting whole plasma units, convalescent donors could donate solely immunoglobulins by undergoing immunoadsorption, a mode of therapy regularly applied in autoimmune diseases.

Methods: Immunoadsorption using an affinity column adsorber pair (TheraSorb, Miltenyi Biotech) was performed on 6 convalescent plasma donors (4 male and 2 female) that had been tested positive for COVID-19 prior to antibody donations. The resulting eluate contained IgG, IgA and IgM dissolved in a glycine buffer. For bioburden reduction, a Tangential Flow Filtration System (Centramate LV, Pall Corporation) was used to concentrate the eluate and exchange glycine for 0.9% NaCl using diafiltration. The final antibody concentrate was finally sterile filtered, before storage at 4°C or freezing at -80°C.

Results: Immunoadsorptions were very well tolerated with no side effects. Collected and neutralized eluates were concentrated using Tangential Flow Filtration. The concentrates contained three times more IgG, IgA and IgM as well as specific SARS-CoV2 N and S antibodies compared to the peripheral blood of the donors. The specific SARS-CoV2 virus neutralization capacity was not diminished but rather increased in all but one donation. All ACs passed sterility tests at the collection day and could be stored at -80°C and 4°C with minor loss of function.

Conclusion: Immunoadsorption proved to be a feasible method of antibody donations for the collection of IgG, IgA and IgM as well as SARS-CoV2 N and S specific antibodies. The antibody concentrates could be produced within one day and final antibody neutralization titers in the ACs were found to be comparatively high. All ACs were sterile, free of hazardous glycine levels and could possibly be administered as a potential therapeutic approach.

Disclosure Statements: Jannik Rothenburg is employed by PALL Corporation
Silke Rink-Baron is an employee of Miltenyi Biotec.
Lisa Mueller, Philipp Niklas Ostermann, Johannes C. Fischer, Derek Hermsen, Johannes Stegbauer and Anja Moldenhauer have nothing to disclose.

PS-3-2
Reactivation of Epstein-Barr-Virus is not causative for post-COVID-19-syndrome

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Background: Post-COVID-19-Syndrome (PCS) frequently occurs after an infection with severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and is a long-lasting health impairment with a broad range of symptoms. Currently the causative mechanisms remain elusive. Aim of this study was to investigate the PCS rate of SARS-CoV-2 seropositive blood donors as representatives of supposedly healthy adults (n = 400) and to examine whether Epstein-Barr-Virus (EBV) is reactivated in individuals reporting PCS.

Methods: To ensure that a SARS-CoV-2 infection was experienced, SARS-CoV-2 anti-N total antibodies, which are produced after an infection only, were monitored by an electrochemiluminescence immunoassay (ECLIA). Participants had to fill in questionnaires regarding the time and course of the infection and persisting symptoms, allowing the determination of the PCS rate. Applying ECLIA, the levels of EBV viral capsid-antigen (VCA) IgM, IgG and EBV nuclear antigen 1 (EBNA) IgG were determined. In addition, we performed qRT-PCR to detect EBV DNA. Furthermore, we examined the amount of neopterin, a prognostic marker for a pro-inflammatory immune status, by an enzyme-linked immunosorbent assay (ELISA) and compared it to pre-pandemic values.

Results: Our data reveal that 18% of SARS-CoV-2 infections result in PCS, with symptoms lasting for up to one year. Quantity and functionality of specific SARS-CoV-2 antibodies were declining in individuals with and without PCS over time, excluding unrecognized reinfections with SARS-CoV-2. All individuals reporting PCS were screened negative for EBV DNA. Furthermore, antibody profiles of VCA IgM, IgG and EBNA IgG revealed past EBV infections in all individuals screened but did not indicate an acute phase of EBV infection after a SARS-CoV-2 infection. Additionally, no elevated levels of neopterin could be found, indicating no pro-inflammatory immune status or enhanced macrophage-related antiviral immune response compared to individuals without PCS.

Conclusion: Our data did not reveal detectable levels of EBV DNA, a specific antibody response, pro-inflammatory immune status or macrophage-related immune response in healthy adults reporting PCS. Therefore, our study indicates that PCS in immunocompetent adults may not be explained by a reactivation of EBV or by persisting inflammatory processes. Further examination is required to identify the cause of PCS.

PS-3-3

SARS-CoV2 mRNA vaccine-specific B-, T-, humoral responses in pediatric stem cell transplant recipients – Importance of a booster vaccination

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Background: Stem cell transplant recipients (SCTR) suffer from increased COVID-19-associated risks and higher mortality rate than determined for the general population, supporting the need for effective vaccination strategies. Data on immunogenicity of SARS-CoV2 mRNA-based vaccination is limited and it remains largely unaddressed whether and how SARS-CoV2-vaccination-specific humoral and cellular immunity is altered in stem cell transplant recipients, particularly in pediatric cohorts.

Methods: In this observational study we comprehensively analyzed mRNA vaccine induced humoral and cellular responses after second and third vaccination in pediatric SCTR aged 2-19 years and age-matched healthy children 5 weeks post-vaccination. Vaccine-specific IgG, IgA and Omicron variant neutralizing capacity was assessed by ELISA together with extensive quantification and functional characterization of spike protein-specific B- and T cells by flow cytometry. Spike-specific CD4+ T cells were identified according to the co-expression of CD137 and CD154 after peptide mix stimulation and vaccine-specific B cells were detected by co-staining with fluorescently labeled recombinant receptor binding domain protein and recombinant full spike protein.

Results: After third vaccination, SCTR reached similar levels of vaccine-specific IgG, IgA and neutralizing antibodies against omicron variant as controls. Although patients showed an increase in frequencies of SARS-CoV2 specific B cells after this booster vaccination, overall frequencies were still fourfold reduced compared to controls. While the majority of individuals enrolled mounted SARS-CoV2 Spike protein-specific CD4+ T helper cell responses, SCTR were characterized by elevated frequencies of vaccine-specific CD4+ T cells compared to controls. Functional analysis revealed significantly diminished portions of specific CD4+IFNγ+ T cells along with an increase in IL-2 producers after the second and third dose in patients compared to controls.

Conclusion: Our data show multiple quantitative and functional impairments of SARS-CoV2 specific cellular immunity in pediatric SCTR. Moreover, they underline the need of a third vaccine dose for SCTR for mounting sufficient humoral responses, particularly with respect to development of neutralizing antibodies. On the background of vaccination hesitancy amongst parents of SCTR, transplant pediatricians are urged to provide COVID-19-related education and information to protect this vulnerable patient group.

PS-3-4

Validation of a modified rapid antibody test to identify COVID-19 patients with low neutralizing antibody titers in an outpatient setting

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Background: Primary aim of the presented project was to establish an assay, which allows rapid identification of patients with low anti-SARS-CoV-2 IgG titers in an outpatient setting. Such patients may be eligible for recruitment into the COVIC-19 study and may benefit from treatment with high-titer COVID-19 convalescent plasma.

Methods: For the validation, the rapid test from Canea for qualitative detection of neutralizing antibodies against SARS-CoV-2 in human serum, plasma or whole blood samples was used. The test cohort included 80 samples from 35 SARS-CoV-2-positive patients. Whole blood (EDTA) and corresponding serum samples were acquired before and on days 3, 14 and 28 after convalescent plasma administration. Modification of the test included a defined pre-dilution of whole blood samples with D-PBS and the introduction of a 5-level grading system to visually evaluate the test results. The main acceptance criterion was a negative predictive value of > 90% to detect a serum antibody concentration of ≤ 700 BAU/ml in the samples.

Results: With 94.6%, the negative predictive value met the required main acceptance criterion. The overall concordance rate between quantitative anti-spike IgG titers and the results of the modified Canea COVID-19 rapid test was 95%. Furthermore, the analysis showed a highly significant correlation between the rapid test results (5-level grading) and the quantitative antibody measurements (BAU/ml), thereby confirming the semi-quantitative validity of the modified rapid test system (**Fig. 1**, Pearson, *** p < 0.0001).

Conclusion: The modified test system from Canea was successfully validated and allows patients with anti-SARS-CoV-2 IgG titers < 700 BAU/ml to be recognized with minimal equipment using whole blood samples. Based on this test method, potential subjects benefiting from a treatment with high-titer convalescent plasma can be easily identified in an outpatient setting.
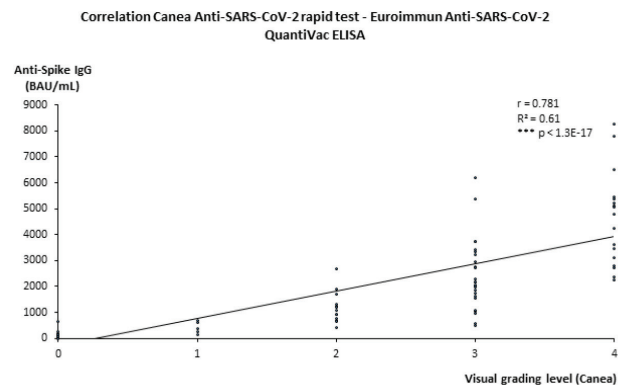
Fig. 1.

Is immunoapheresis a therapeutic option for post-COVID patients? Data presentation in the treatment of ME/CFS, Post Covid, Post Vaccine (Long Covid syndrome) with immunoadsorption

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Background: Infection-triggered disease onset, chronic immune activation and autonomic dysregulation in CFS point to an autoimmune disease directed against neurotransmitter receptors. Autoantibodies (Aab) against G-protein coupled receptors were shown to play a pathogenic role in several autoimmune diseases.

Therapeutic approaches can be based on the principle of reduction of agonistic Aab. These principles could therefore lead to an improvement in the quality of life of Long Covid patients.

Methods: All eligible Patients with long COVID (n=) and ME/CFS (n=) received IA (primary device: Spectra Optia, Terumo BCT, Inc. Lakewood, USA/TPE; secondary device: ADAsorb medicap clinic, Ulrichstein, Germany TheraSorb Ig omni 5 Adsorber Miltenyi Biotec B.V. & Co. KG, Bergisch Gladbach, Germany). Patients received 5 treatments on 5 consecutive days. The 2.0-fold individual total plasma volume was processed on days 1–5. Blood was drawn immediately before and after the IA session from an antecubital vein. Patients were followed up directly after last apheresis. The primary endpoint was to remove the autoantibodies and to lower plasma IgG to levels below 2 g/l after the last treatment.

Results: Between January and December 2022, 99 patients (w=54, m=45), were screened for eligibility, most of them between 30 and 50 years old (< 30 (n=25), 30-39 (n=30), 40-49 (n=24), 50–70 (n=20)). The removal of IgG was successfully high (IgG lowering about 90 %). The symptom descriptions of the patients before and after IA suggest that solely using the evaluation of the Bell score does not reliably reflect the predominantly positive development after IA treatment, since patients have other main problems beside exhaustion! Our specially developed questionnaire shows improvement for most of the treated patients; e.g: How have your symptoms changed since your treatment at the DHZ? -> About 71% improved, 15,5% unchanged, about 14% deteriorated.

Conclusion: IA is a safe and efficient method for the removal of (auto-) antibodies. A clinical benefit could be demonstrated in most of the patients, but larger trials are needed. Ideally, the free and bound Aab are measured after treatment (antibody screening/biopsy). Maintenance therapy could be a further indication to prevent relapses caused by trigger factors (e.g. infection, vaccination, stress). The treatment decision of the involved physician should be used for a follow-up treatment after IA.

Change in Symptoms		Quantity	Percent
Greatly improved	7	7	12.1
	6	12	20.7
	5	22	37.9
Unchanged	4	9	15.5
	3	4	6.9
	2	3	5.2
Greatly deteriorated	1	1	1.7
Sum		58	100.0
Missing		41	

= 70.7 %

Table 3 How did your symptoms change since the treatment?

Fig. 2.

Prevalence of wildtype and vaccination induced SARS-CoV2 antibodies in a blood donor population

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Background: SARS-CoV-2 (=COVID19) has been a new challenge for health care systems around the world. Blood donation centers were also affected. At some point, vaccination against the virus was possible. In order to examine the prevalence of infections in vaccinated and non-vaccinated donors we performed a prospective observational, single-center study for antibodies against COVID19 ("wildtype" and "vaccination induced") from February until May 2022.

Methods: After their written informed consent participants were asked to complete a questionnaire about the number of vaccinations against SARS-CoV2 and any known COVID-19 infection. In addition, antibodies against the N-antigen ("nucleocapsid" of the wildtype virus) and the S-antigen ("spike antigen", vaccination induced) were detected via enzyme immune-essays (ECLIA-testing system, Cobas, Roche Diagnostics Mannheim). Laboratory results were compared with the provided information in the questionnaire with a special focus on unrecognized infections.

Results: 2157 donors were tested between February and May 2022. 92% of them were vaccinated, 4% were not and 4% declined providing information on their vaccination status. Most of the donors (60-80%, depending on the number of vaccinations) were immunized by mRNA-vaccines such as Comirnaty or Spikevax (no matter whether it was the first, second,

Bell Score	Quantity	Percentage
10	1	2.5
20	5	12.5
30	12	30.0
40	10	25.0
50	1	2.5
60	2	5.0
70	1	2.5
90	1	2.5
NA's	7	17.5
Total	40	100.0

Table 1. Bell Score pre treatment

Bell Score	Interval				Total
	[1,30)	[30,60)	[60,90)	[90,365)	
20	5	3	0	0	8
30	4	7	2	3	16
40	5	5	2	5	17
50	4	0	6	4	14
60	2	3	0	4	9
70	0	0	3	1	4
80	0	0	2	0	2
90	0	0	2	0	2
100	0	0	0	2	2
Total	20	18	15	21	74

Table 2 Bell Score post treatment

Fig. 1.

patient data	wildtype strain and vaccinal strain	vaccinal strain only	wildtype strain only	No detectable strain	Sum of patients
Vaccinated (as per questionnaire)	343	1666	0	0	2009
no vaccination (as per questionnaire)	0	0	27	45	72
no data (as per questionnaire)	16	30	12	18	76
summary	359	1696	39	63	2157

Fig. 1.

third, or the fourth vaccination). 398 donors were infected despite being fully vaccinated (18 %). These infections occurred mostly after at least 50 days after the last dose. There were no significant differences between the immunization schedules of the donors. 79 infections with SARS-CoV2 had been unrecognized and were only detected due to this study.

Conclusion: This single center study showed the success of vaccinations as well as the prevalence of seropositive donors from February until May 2022. By offering antibody testing, the blood donation center was able to address many first-time donors, so besides the study results, this study turned to a successful advertising campaign as well. Interestingly, approximately 24,7 % of infections with SARS-CoV2 had been unrecognized by the respective donor.

Disclosure Statements: The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there are no financial interests to report. We certify that the submission is an original work and is not under review at any other publication.

PS-3-7

Immune adsorption as an individual experimental therapy in two patients with multiple autoantibodies and post-COVID-19 syndrome

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Background: Autoantibodies (AABs) against G-protein-coupled-receptors (GPCRs) may underlie the symptom complex of cognitive and physical deficits in patients with post-COVID-19 syndrome (PCS). Based on this assumption, the hypothesis arose that an AAB removal therapy, e.g. immunoadsorption (IA), could causally lead to symptom relief. To investigate this, we performed IA in two patients using the LIFE21 system and adsorbers Omni 1 and 5, respectively.

Methods: Two patients (f28; m36) with PCS and pre-known AABs against GPCRs each received one cycle IA (5 treatment days) using the LIFE21 system (Miltenyi Biotec, Germany) with the Omni 1 adsorber (f28) and the Omni 5 adsorber (m36). Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) was evaluated by the Bell fatigue scale before and after completion of the IA cycle, and immunoglobulins (IgG, IgM, IgA, IgE) were analyzed (Cobas ProRoche) before and after each IA session. At time points before and after each completed IA cycle, relevant pre-existing AABs against β1-/ β2- adrenergic and M3-/ M4- muscarinic acetylcholine

receptors and angiotensin II type I receptors (ELISA, IMD laboratory, Berlin) were determined.

Results: IA sessions with the adsorbers Omni 5 (m36, 5x IA over 13 days) and Omni 1 (f28, 5x IA spread over 44 days - interrupted for 35 days after day 1 due to SARS-CoV-2 re-infection) were clinically well tolerated, and mean plasma volumes treated were 1.3-fold for m36 and 1.8-fold for f28. For each IA, average IgG reduction was 57.1% (m36) and 70.2% (f28). When comparing baseline to final, IgG was reduced by 88.4% (m36) and 90.4% (f28). Elevated AABs (pre-treatment: m36: ß1, ß2, M3, M4, ATII-T1; f28: ß1, ß2, M3, ATII-T1) fell below their reference values in both patients after the IA cycle. Bell fatigue scale did not change when comparing results before/ after the IA cycle and about 4 weeks after (f28: 20/20/10; m36: 50/50/50).

Conclusion: The Omni 1 and Omni 5 adsorbers are highly capable of removing immunoglobulins non-selectively in a safe and tolerable setting. In both patients, IA was technically successful without the need to treat extraordinary high plasma volumes. However, Bell fatigue scale indicated no improvement in ME/CFS in both patients. Hence, the present investigation does not support a causal relationship between elevated AABs against GPCRs and ME/CFS symptoms.

Disclosure Statements: Technical equipment and consumable material was provided by Miltenyi Biotec, Germany.

PS-3-8

Autoantibodies against G-protein-coupled receptors can be detected in post/long-COVID-19 patients with fatigue syndrome (ME/CFS) as well as in healthy blood donors

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Background: Several providers of therapeutic apheresis hypothesize that autoantibodies (AABs) against G protein-coupled receptor (GPCRs) play a crucial role in the development of post-COVID Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS). Their detection is used as an indication for multiple sessions of immune adsorptions as experimental treatment. We investigated whether such AABs also occur in healthy subjects, such as blood donors, in addition to patients with post-COVID ME/CFS.

Methods: Serum samples from 41 healthy platelet donors and 8 employees of the department for Transfusion Medicine were tested for 5 different AABs against GPCRs: ß1-, ß2-adrenergic receptor-AABs, M3-, M4-muscarinic acetylcholine receptor-AABs and anti-AT1R. The frozen samples (-80°C) were sent to a special laboratory (IMD Labor Berlin). The IgG type AABs were determined using commercial ELISA kits (CellTrends GmbH, Germany) according to the manufacturer's instructions. The experimental procedure has been approved by the university"s ethics committe.

Results: In 29 of 49 healthy subjects (59.2%) at least one and in 16 persons (35%) all five of the AABs examined were detected above the cut-off. Anti-M3-AABs (61%) and anti-ß2-AABs (59%) were detected most frequently. The mean age of the AAB-positive subjects was 30.9 years (20-55y), with 68.75% being younger than 35 years of age. The COVID-19 history indicated that 62.5% had both recovered and been vaccinated, while 31.25% were vaccinated without ever having experienced confirmed SARS-CoV-2 infection. 6.25% of those testing AAB positive reported being neither vaccinated nor infected with SARS-CoV-2. The gender distribution was: 27 (55%) men, 22 (45%) women. None of the people examined stated to have ever had symptoms of ME/CFS.

Conclusion: The fact that GPCR-AABs can be detected in a high proportion of healthy individuals suggests that there is no direct causal relationship between AABs and ME/CFS and also raises the question of whether these laboratory findings are indeed clinically relevant. The understanding and significance of the mere detection of AABs as the main indication for immunoadsorption should be seriously reconsidered until causality between them and post-COVID ME/CFS is based on sufficient evidence.

PS-3-9

SARS-CoV-2 vaccination of convalescents boosts neutralization capacity against Omicron subvariants BA.1, BA.2 and BA.5 and can be predicted by anti-S antibody concentrations in serological assays

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Background: COVID-19 convalescent plasma remains a potential therapy of COVID-19, e.g. for new variants resistant to monoclonal antibodies and for patients with impaired immune response. Significant immune evasion by Omicron has raised concerns that antibody-based therapies may no longer be effective. We assessed whether superimmunized individuals, i.e. vaccinated convalescents, have sufficient cross-neutralization capacity against Omicron to be considered as plasma donors for passive immunotherapy.

Methods: We report the *in-vitro* neutralization capacity against SARS-CoV-2 variant B.1 and the Omicron subvariants BA.1, BA.2 and BA.5 of sera from convalescent individuals with and without boost by vaccination. The study included 313 serum samples from 155 individuals with a history of SARS-CoV-2 infection, divided into subgroups without (n=25) and with SARS-CoV-2 vaccination (n=130). We measured anti-SARS-CoV-2 antibody concentrations by serological assays (anti-SARS-CoV-2-QuantiVac-ELISA (IgG) and Elecsys Anti-SARS-CoV-2 S) and neutralizing titers against B.1, BA.1, BA.2 and BA.5 in a pseudovirus neutralization assay.

Results: Sera of unvaccinated convalescents did not effectively neutralize Omicron sublineages BA.1, BA.2 and BA.5. Neutralizing titers against B.1, BA.1, BA.2 and BA.5 were significantly higher in vaccinated compared to unvaccinated convalescents (p<0.0001) with 52.7-, 210.7-, 141.3- and 105.4-fold higher geometric mean of 50% neutralizing titers in vaccinated compared to unvaccinated convalescents. The increase in neutralizing titers was already achieved by one vaccination dose. Neutralizing titers were highest in the first 3 months after the last immunization event. Concentrations of anti-S antibodies in the anti-SARS-CoV-2-QuantiVac-ELISA (IgG) and Elecsys Anti-SARS-CoV-2 S assays predicted neutralization capacity against B.1 and subvariants.

Conclusion: These findings confirm substantial immune evasion of the Omicron sublineages, which can be overcome by vaccination of convalescents. This informs strategies for choosing of plasma donors in COVID-19 convalescent plasma programs that shall select specifically vaccinated convalescents with very high titers of anti-S antibodies.

PS-3-10

A randomised open-label trial of early, very high-titre convalescent plasma therapy in clinically vulnerable individuals with mild COVID-19 as model of early treatment in a pandemia with a new pathogen: Experience from collection of very high-titer plasma from superimmunized individuals

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Background: COVID-19 convalescent plasma (CCP) remains a potential therapy of COVID-19. The new CCP trial COVIC-19 takes into account lessons learned from previous trials, including the trial CAPSID, and combines them into a novel approach:
- CCP with very high concentrations of SARS-CoV-2 antibodies (≥4,000 BAU/ml) from donors with previous SARS-CoV-2 infection and vaccination
- treatment of vulnerable persons early after symptom onset
- Sequencing and cross-neutralization analyses to study viral evolution

Methods: We report the initial experience of collection of very high-titer plasma units for this COVIC-19 trial. We recruited 686 potential donors with previous SARS-CoV-2 infection (inf) and vaccination (vax) who passed initial eligibility check. Anti-SARS-CoV-2 antibody concentrations were measured by anti-SARS-CoV-2 QuantiVac ELISA (Euroimmun) and neutralization capacity was assessed in the GenScript Surrogat neutralization assay and in a pseudovirus neutralization assay. High S-Ab in the QuantiVac assay correlated with high neutralizing capacity.

Results: The average antibody concentration of those willing to donate was 4,298.58 BAU/ml. S-Ab was ≥4,000 BAU/ml in 41,1% of the individuals and did not significantly differ by gender or ABO type, but were higher among those who had received 3 vax (median 3,746 BAU/ml) or 4 vax (median 4,166 BAU/ml). Highest S-Ab were observed in those with a breakthrough infection after 2 vax, followed by a booster (median 5,159 BAU/ml, 64% ≥4,000 BAU/ml) or breakthrough inf after 3rd or 4th vax (median 3,846; 48% ≥4,000 BAU/ml). 158 qualified individuals ultimately donated CCP with anti-SARS-CoV-2 IgG concentrations ranging from 4,105 BAU/ml to 22,923 BAU/ml with a mean concentration of 8,019 BAU/ml.

Conclusion: Taking into account all eligibility criteria 31.9 % of the individuals screened could provide plasma units meeting the criteria for high-titer plasma for COVIC-19, however, only 23% actually donated. Collection of very-high titer plasma from super-immunized individuals with previous infection and vaccination is feasible, but requires substantial donor selection and rapid screening and start of apheresis to take advantage of the short period of very high mAb.

COVIC-19 is supported by BMBF.

PS-3-11

Effective neutralizing and cellular immunity against SARS-CoV-2 after mRNA booster vaccination is associated with the activation of B cells and pDCs

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Background: Recently, we demonstrated that heterologous vaccination regimes against COVID-19, which consisted of a combination of the vector vaccine ChAdOx1 from AstraZeneca and either the mRNA vaccine BNT162b2 from BioNTech or Spikevax from Moderna, resulted in significantly enhanced serological and cellular immune responses against SARS-CoV-2 after second vaccination.

Methods: In the present follow-up study, we continued to analyze anti-SARS-CoV-2 antibody titers, neutralization capacities against the wildtype virus and the variant of concern (VOC) Omicron BA.1, as well as IFN-g responses against the spike protein up to 6 months after booster vaccination. In addition, applying FACS analysis and cell culture studies using SARS-CoV-2-specific peptides (Novavax), we investigated the potential role of two important antigen-presenting cell types, which circulate in the peripheral blood (plasmacytoid dendritic cells (pDC) and B cells), for the immune response after vaccination.

Results: One month after booster vaccination, up to 91% of all donors from vaccination regimes containing two or three mRNA doses exhibited detectable Omicron neutralization capacity (**Figure 1**). Furthermore, we found a stable frequency and expression of antigen-presenting and co-stimulatory molecules on CD19+ B cells up to 3 months after booster vaccination, regardless of the vaccination regime. In pDCs in contrast, we observed a trend for increasing expression of MHC class II and CD40L even after booster vaccination, suggesting an ongoing role in antigen presentation. Performing functional pulsing studies with pDCs and SARS-CoV-2 peptide (Novavax), we demonstrated that pDCs are involved in the presentation of these peptides to T cells (**Figure 2**).
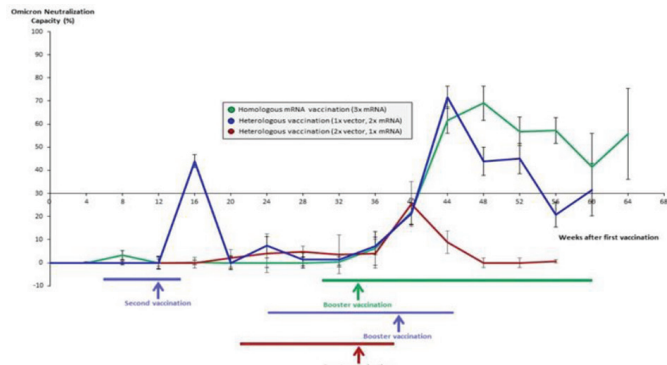
Fig. 1.
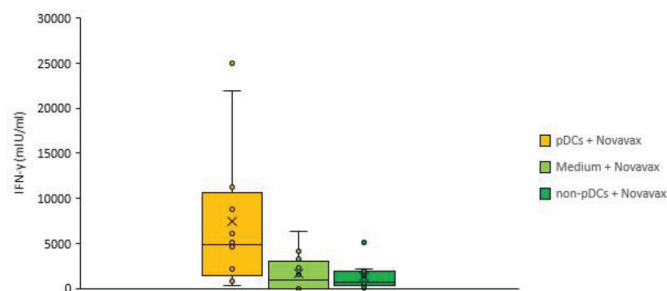
Fig. 2.

Conclusion: In summary, our follow-up study demonstrates that effective neutralizing and cellular immunity against SARS-CoV-2 is associated with the activation of both B cells and pDCs, and that pDCs are functionally involved in antigen presentation after vaccination. Furthermore, our follow-up data up to 6 months after booster vaccination suggests no significant differences in serological and cellular response parameters between vaccination groups, as long as two or three mRNA doses are included.

PS-3-12

SARS-CoV-2 immunity in blood donors during the Omicron BA.5 infection wave – the COVIMBA-study

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Background: (Re-)Infections of pre-immunized vaccinated or convalescent individuals were the main players in SARS-CoV-2 infection waves with the different omicron strains. There is still a lack of detailed data on how different immunization schemes, exposition risks and antibody tests could predict the probability of (re-)infection. This population-based approach aims to understand the underlying mechanisms of infection and immunization, which is relevant for any future pandemic.

Methods: To address this issue we selected blood donors who donated blood in June or July 2022 at the beginning of the omicron BA.5 wave. We sent a digital COVID-19 REDCap survey including 170 data fields on SARS-CoV-2 exposure, previous immunizations, and acute infection in the timespan between July and October 2022 to these donors. In close collaboration with data protection supervisors, we thoroughly planned the digital study information and consent forms and permission was granted by the local Ethics committee. Statistical analyses of survey data were performed via "SAS" and "Prism".

Results: Of 52.512 blood donors invited for study participation via E-mail, 20% completed the questionnaire. Thus, we recruited a cohort of 10.532 individuals for the investigation of detailed anamnestic data. Analysis revealed that full vaccination with monovalent vaccines significantly reduced infection rates with non-Omicron variants and still had effects on infections with Omicron strains before BA.5 but did not significantly reduce infection rates with the BA.5 strain compared with not fully vaccinated individuals. Carefully partitioned subgroup analysis showed that various combinations of vaccinations and previously experienced infections with different SARS-CoV-2 strains led to significantly varying infections rates during the BA.5 wave.

Conclusion: REDCap database approach is a suitable method for digital clinical research including anamnestic and serological data. Study concepts based on digital consent forms and questionnaires are convenient
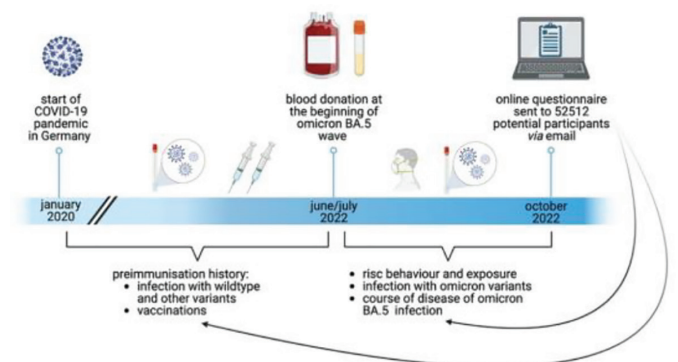
Fig. 1.

for study participants and lead to high response rates. As many countries recently reduced their SARS-CoV-2 surveillance systems and as monitoring does not cover self-testing, digital surveys are a helpful way to capture reliable data on (re-)infection rates keeping evidence up to date.

PS-4
Hemotherapy | Immunohematology

PS-4-1
Peripartum blood component management of severe IgA-deficient patient

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Background: Allergic transfusion reactions (ATRs) occur during or shortly after blood derivatives application and are the leading cause of death associated with transfusion. Triggers for ATR are multifactorial. One factor predisposing to ATRs discussed in literature is the severe IgA-deficient patient (sIgAd, IgA levels below 0.01g/L) with a positive anti-IgA antibody (anti-IgAab) status a./o. a history of ATRs. SIgAd patients are susceptible to respiratory infections a./o. allergic reactions e.g. to drugs.
Methods: We present the peripartum management of a 39-y-old, sIgAd patient (G2P1, 34w+0d, fetus: breech position). SIgAd was diagnosed after a history of multiple allergic reactions (AR, grade II-III) following NSAID intake. Laboratory reevaluation of the IgA levels confirmed prior findings with IgA<0.01g/L. A low probability of the presence of anti-IgAabs was assumed (no prior transfusions) and confirmed with negative anti-IgA-ab testing (2.1 U/mL). With iron suppl. ferritin, haemoglobin levels were normalized prior to delivery. Due to the patient´s allergic susceptibility, the potential immunisation risk, it was interdisciplinary agreed to reduce the risk of IgA exposure. Sponateous vaginal delivery (SVD): IgAd donor RBCs. C-Section: washed RBCs.
Results: A C-section (38w+5d) was indicated (breech position) with moderate bleed risk. Tools of patient blood management (tranexamic acid, uterotonics, cell salvage) were applied. Finally, the blood loss was moderate (400 mL) and no allogenic blood transfusion was required. However, the patient developed an allergic reaction (AR, max. grade II,

Flow chart: Diagnostic and therapy plan.

‡ Limited shelf-life of 24 hours.
* Total IgA concentration: Immunoturbimetric assay. Normal range (adults): 0.7 to 4g/L
# Adequate IV-lines, anti-allergic medication (corticoids H1/2-inhibitors, volume, epinephrine) available
§ Prophylactic use of: Corticoids, H1/2-inhibitors
$ Rare donor program. Residual IgA content of fibrinogen concentrates (information provided by the manufacturer)
Y yes, N no, RBC red blood cell concentrates, AR allergic reaction

Fig. 1.

due to immediate anti-allergic treatment), which was most likely due to the administration of an intravenous opioid. Generally, patients requiring blood transfusion are often exposed to additional drugs concomitantly, thus it is challenging to reliably confirm the causality between the AR and the provoking allergen. (s. Figure 1: Diagnostic and treatment plan)
Conclusion: The mode of delivery impacts blood product management: The timing of SVD is difficult to predict, even with labor induction, and can outlast the shelf life of washed RBCs. Here, blood products from IgAd donors can be favourable. In elective situations, manufacturing and provision of washed products can be planned. In case of emergencies transfusion should not be delayed. If available products with low plasma content should be considered e.g. platelets in additive solution.

PS-4-2
Ramipril-induced shock during therapeutic leukapheresis

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Background: When chronic leukemia progresses to an acute leukemia, therapeutic leukapheresis might be necessary: if the patient shows signs of leukostasis and if the required chemotherapy does not lead to a satisfactory and fast improvement, therapeutic leukapheresis can lower the leukocyte count fast, by collecting (and subsequently discarding) as many leukocytes as possible.
Methods: Our patient presented with acute leukostasis, after starting chemotherapy for chronic lymphatic leukemia. An acute kidney failure, due to the leukostasis, led to an emergency hemodialysis and to the request for a leukocyte reduction apheresis.
Results: During apheresis, the patient showed signs of circulatory collapse, which could initially be treated sufficiently. After 100 minutes of apheresis, the patient went into shock, requiring emergency intubation. The apheresis was discontinued right away. After extensive intensive care and within 24 hours, the patient had completely recovered. At that time, the chemotherapy had become effective, leading to a falling leukocyte count, so no further apheresis was necessary.
Conclusion: The patient had been taking Ramipril for high blood pressure. The last application had taken place only a few hours prior to apheresis. Adverse reactions after Ramipril-intake and lipid-lowering apheresis have been documented. To our knowledge, we present the first case of ramipril-induced shock during leukocyte apheresis.

PS-4-3
Red blood cell usage in a tertiary care hospital during the COVID-19 pandemic

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Background: The COVID-19 pandemic has led to a social and economic crisis with far-reaching effects on the health care system as well. The pandemic has affected the blood supply, whereas there is limited data on changes in red blood cell requirements and clinical usage patterns during the pandemic.
Methods: The University Hospital Erlangen is a 1400-bed tertiary care hospital. Here, all relevant data for interpreting patterns in the use of blood components are continuously collected since 2010. We are able to integrate the clinical data warehouse components of RBC recipients' personal data, German Diagnosis Related Group (G-DRG) system codes of inpatients,

ICD codes, OPS codes, and RBC component data from the blood bank IT system. The obtained DRGs, IDC codes, and OPS codes are associated with the data of the blood component consumption on an individual basis. We analyzed red blood cell usage patterns in 2018 and 2019, the two years before the pandemic, and 2020 and 2021, the first two years of the pandemic.

Results: In 2020, there was no significant change in red cell usage patterns throughout the whole hospital (15,378 RBC units vs. 15.468 units in 2019 and 15.189 units in 2018). Among pre-MDC DRGs for ventilated patients, there was no enhancement in RBC usage. In 2021, there was a 8.2 percent decline in RBC usage in comparison to 2020. Interestingly, the number of RBCs per transfusion recipient declined from 4.42 in 2019 to 4.19 and 4.06 in 2020 and 2021, respectively. Overall, the long-term trends of previous years with decreasing use of RBCs in internal medicine and increasingly cautious indications continued during the pandemic.

Conclusion: The COVID-19 pandemic has impacted the blood supply, resulting in unprecedented blood shortages, particularly during the summer months of the last two years. Obviously, this exacerbated the trend toward a reduction in RBCs actually consumed per patient in need of transfusion more than changes in clinical demand.

PS-4-4
TIMMY (Transfusionsmedizin-Training mit Dummys) – a novel concept for practical education in transfusion medicine using blood product dummys

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Background: Practical knowledge in transfusion medicine is essential for physicians to safely administer blood products to patients and to navigate the legal and ethical considerations involved. The "Arbeitskreis Blut" of the Robert-Koch institute claimed to intensify teaching of practical skills in medical education to reduce the rate of transfusion-associated errors or mistaken identity. Therefore, a training session was implemented into the curriculum of the medical degree course (8th semester).

Methods: In line with the national catalogue of learning objectives, a structured curriculum was developed to provide training in blood transfusion skills focussing on red blood cell concentrates bearing the highest risk of serious adverse events in case of handling mistakes. In addition, it was an important prerequisite to base the training on clinical cases and to provide authentic material required for the performance of blood transfusions.

Results: A protocol has been established for the preparation of "red blood cell concentrates" using artificial blood-like fluids to ensure an adequate supply of "blood products". In a new 90-minute training session called TIMMY ("Transfusionsmedizin-Training mit Dummys"), students learn how to order blood products using an original order sheet and how to supply a fictitious patient with red blood cell concentrates. The practical aspects included the comparison of the accompanying sheet with the blood product, the performance of the bed-side test and the fixation of the transfusion system. Medical technologists supervised the students in addition to physicians.

Conclusion: The novel practical lesson provides the opportunity for all students in human medicine to work with original material and to obtain first experience in basic rules of blood transfusion. The use of dummys avoids limitations caused by the shortage of real blood products and reduces the risk of infection. The concept is regularly evaluated by the students for amendments and designed for the transfer to other universities.

Disclosure Statements: The project is supported by the foundation "Stiftung Innovation in der Hochschullehre", referring to the announcement "Freiraum 2022" (project number FRFMM-430/2022).
The authors have no other conflicts of interests to declare.

Autologous transfusion of cryopreserved platelets in a CD36-negative patient after allogeneic stem cell transplantation

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Background: CD36 is a transmembrane glycoprotein which is present in a variety of cell types, including platelets, and haematopoietic progenitors. CD36-deficiency can lead to CD36 iso-immunizations e.g. from allogeneic platelet transfusion. Platelet cryopreservation can be used as an alternative to room temperature storage of platelet concentrates. Especially, in the absence of compatible platelet products, the cryopreservation of autologous platelet concentrates can be crucial for patient care.

Methods: We report the case of a 21-year-old patient with acute myeloid leukemia and a type I CD36-deficiency. Autologous platelets were collected at three different days by apheresis for cryopreservation prior to the conditioning therapy for an allogeneic transplantation of stem cells from a CD36-positive donor. Platelet concentrates (PC) were irradiated and cryopreserved with a final concentration of 5% DMSO, automatically frozen and stored at -170°C. Platelet concentration was measured with a haematology analyser and swirling was assessed before and after cryopreservation. To estimate platelet activation, the P-selectin (CD62P) degranulation assay and the delta granules mepacrine-uptake and release assay were carried out with ADP and TRAP6.

Results: PC concentrations showed no significant decrease after cryopreservation. Without stimulation, an increased degranulation assessed by surface expression of CD62P was observed before and even higher after cryopreservation. Additionally, degranulation was less induced with ADP and TRAP6 after cryopreservation. After TRAP6 stimulation of mepacrine loaded platelets, cryopreserved PC show only minor release of delta granules compared to fresh platelet concentrates. Swirling and lack of aggregates was observed after thawing PC. Three out of eight autologous platelet concentrates were given at day eight and nine after allogeneic hematopoietic cell transplantation and showed an adequate increment of platelets with no clinical signs of bleeding.

Conclusion: Transfusion of platelet concentrates is a supportive prophylactic therapy for patients with hematologic diseases. With no available compatible platelet products, the cryopreservation of autologous platelet concentrates is a feasible supportive therapy option. In case of a CD36-deficency with CD36 iso-antibodies, autologous platelet concentrates can be applied after previous allogeneic hematopoietic stem cell transplantation from a CD36-positive donor.

Invited talks abstract/summary: [PAPERTEXT5]

Disclosure Statements: The authors declare that no conflict of interest exist.

Successful erythrocytapheresis in patients with hereditary hemochromatosis

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Background: The standard therapy for hereditary hemochromatosis (HH) is the removal of red blood cells to achieve serum ferritin target levels of <50μg/L. Phlebotomy as the classic gold standard and therapeutic erythrocytapheresis (TE) as a highly effective apheresis technique represent two possible treatment methods. We investigated the effectiveness of TE and analyzed the required number of treatment cycles in five patients with hemochromatosis.

Methods: Between 04/2020 and 04/2023, five patients with HH (39 to 66 years, body weight 85kg to 125kg)] were treated with TE (Spectra Optia, Terumo BCT, exchange set, erythrocyte depletion technique). One patient was newly diagnosed while 4 patients were pretreated and had undergone phlebotomy previously but required a switch to TE due to limited venous access. Erythrocyte volumes for depletion were set in proportion to the total blood volume (TBV) and according to the hematocrit: 205 to 793 mL of RBCs were withdrawn per single treatment procedure. Hemoglobin, hematocrit, and serum ferritin levels were analyzed before and after treatment. Depending on these results we established further TE intervals.

Results: We performed 75 TEs in 4 men and 1 woman. Prior to our treatment, serum ferritin levels ranged from 1614ng/mL to 152ng/mL, with lower serum ferritin levels measured in two of the pre-treated patients. The average number of required treatments to decrease serum ferritin levels below 50µl was 11, ranging from 3 to 19 with an average of 23 days between treatments. Hematocrit levels before treatment ranged from 31,8% to 49,1% and after treatment from 28,7% to 42,7%. After reaching a serum ferritin level of ≤50µg/L, another TE was required on average every 66 days.

Conclusion: All five patients with HH were successfully treated with TE and achieved optimal serum ferritin levels, although two had difficult venous conditions and low hematocrit limiting phlebotomy. Also for TE, a low hematocrit represents an important limiting factor that restricted TE frequency. While phlebotomy is more accessible for patients with HH, TE can provide the required serum ferritin levels in a short time interval in difficult clinical situations and is also well tolerated.

PS-4-7
Characteristics of red cell transfusion dependent outpatients: a single center experience

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Background: Little is known about the optimal transfusion strategy in outpatients, where the goal of treatment is often to maintain quality of life. Our hypothesis is that an individualized approach is required in the outpatient setting, which may result in significant deviation of suggested transfusion triggers and volumes from established guidelines. The aim of this retrospective analysis is to describe real-world transfusion practice of red blood cell concentrates (RBC).

Methods: Data on outpatient RBC transfusions performed in the year 2022 are retrospectively collected in a single center were n=2943 red cell units were transfused. We assume that we perform at least 30-40% of outpatient RBC transfusions in our region as our blood service delivered 3460 red cell units to outpatient facilities. A total of n= 1557 RBC units given in 860 episodes are included in this interim analysis. Most patients (90%) came from an area with a travel distance of

Results: In total 1287 visits with 860 transfusion episodes in 340 patients (43% women) were analyzed. The median age was 64 (74; 83) years. No RBC was given in 217 visits with Hb levels of 9.2 (8.7;10.0) g/dl, and 1 unit in 165 episodes at 8.4 (8.1;8.6)g/dl and 2 in 691 episodes at 7.5 ±6.9;7.9 g/dl (p<0.001). The mean increase of Hb was 1.1±2.0 g/dl after 1 unit without additional transfusion of a platelet concentrate (PC) and 0.5±0.6 g/dl with additional PC transfusion, and 1.9 (±1.0) g/dl after 2 RBC units without PC and 1.5±0.8 g/dl with PC transfusion. The pre-transfusion Hb level was the same (7.6± 0.9 g/dl) in patients with or without a history of coronary heart disease and were not associated with age, travel distance or Pro-NT-BNP-levels.

Conclusion: Pre-transfusion hemoglobin values did not deviate from the suggested trigger of 7-8 g/dl in chronic red cell transfusion dependent outpatients. Prospective studies are urgently needed to better define the optimal dose in these patients.

PS-4-8
Intrauterine blood transfusion for maternal red-cell alloimmunization – a single center experience

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Background: Maternal red-cell alloimmunization is frequent cause of fetal anemia, mainly due to Rhesus antibodies. Monitoring of infants for anemia potentially receiving intrauterine transfusion (IUTs) to prevent hydrops is a common tool. We report our experiences with the management of fetal anemia including intrauterine fetal transfusion and neonatal transfusion requirement.

Methods: Between 2017 to 2022 29 pregnancies were managed of intrauterine transfusion for severe fetal anemia by 59 IUTs. Indication and timing for the IUT were based on doppler measurement of peak systolic velocity in the fetal middle cerebral artery to predict severe fetal anemia.

Results: 12 out of 29 patients had red-cell alloimmunization causing fetal anemia, 17 patients received IUT for viral anemia or other causes. 7 out of 12 red-cell allo-immunized mothers delivered in our hospital. 4 childs out of these 7 childs managed in our hospital had additional transfusion requirement 4-8 weeks after discharge from the hospital. Free maternal antibodies were still detectable in all postpartal samples from the affected neonates. Possibly hyporegenerative anemia is caused following persisting maternal antibodies.

Conclusion: Anti-D remains the most common antibody in fetuses requiring intrauterine transfusion. Intrauterine transfusion seems to be an effective and save procedure to treat fetal anemia. Infants receiving IUT for fetal anemia following maternal alloimmunization could be at risk for hyporegenerative anemia first 3 months after birth.

PS-4-9
Influence of corona pandemic on error frequency in hemotherapy

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Background: The corona years from 2020 to 2022 led to increased stress for the nursing and physician professions. On one hand, a very high number of sick leaves resulted in a massive workload. On the other hand, there were uncertainties in the private sector such as child day care, elderly care, health safety and job safety of partners. In this study we investigated the impact of the corona pandemic on the frequency of near miss events in hemotherapy.

Methods: At our institution, all near miss events are evaluated semi-annually and reported in the transfusion safety board meetings. This systematic analysis is based on a standardized reporting form to be completed by the person responsible for the mistake. This retrospective study includes all detected near miss events that occurred in the context of hemotherapy from 01/2017 to 04/2023.

Results: Near miss events increased during the years of the corona pandemic (Figure 1). In 2023, 18 events have been reported in the first 4 months, which could resemble a slight decreasing tendency. The most common near miss event was wrong blood in tube correlating with a false identification of the patient while drawing the blood. At our institution, nurses and doctors receive continual training once per year. In addition, a central transfusion medicine training is mandatory for every new clinician. Therefore, a lack of knowledge doesn`t seem to be the main reason for the increasing number of mistakes. In our opinion, lack of accuracy due to overstressing might be the real cause.

Figure 1: near miss events from 2017 to 2023

Conclusion: The corona pandemic had a massive impact on the incidence of near miss events at our institution. For the clinicians it is important to protect the staff for work overload and to strengthen the resilience of nurses and doctors.

near miss events
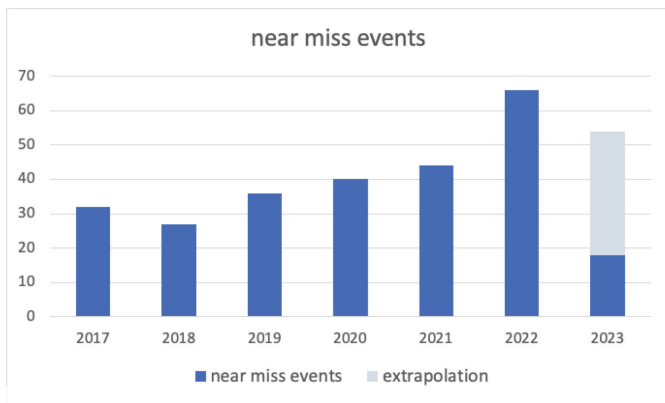
Fig. 1.

PS-4-10

Use of the Spectra Optia RBC exchange module to induce experimental severe normovolemic anemia

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Background: To assess solely effects of red blood cell (RBC) components or efficacy of artificial oxygen carriers in relevant clinical models, it is necessary to induce severe normovolemic anemia. Since phlebotomy and volume replacement with crystalline fluids introduces additional variables such as platelet loss, loss of clotting factors and loss of oncotic pressure, we sought to establish severe anemia by selective removal of RBCs, using apheresis.
Methods: The method was established using three independent pools of ABO-identical buffy coats (BC pool) with a combined total volume of 1768ml (1115-1781) in a Spectra Optia BMP bag. This volume is equivalent for big animal model (sheep/pigs). Complete blood counts (CBC), especially haemoglobin (Hb) (median 8,7g/dl; range 8,2-10,9), provided a reasonable approximation of e.g. sheep blood (8-12g/dl). The Spectra Optia TEA with normal saline as replacement fluid was used for RBC depletion. To avoid a prefill, variables yielding a fictitious total blood volume of 2600ml were entered. 55% was entered as haematocrit of the replacement fluid. BC pools were processed with an inlet flow of 20-25ml/min, with manual mixing every five and CBC every 15 minutes
Results: Our targeted Hb of 4-6g/dl was reached in all runs after 45 minutes. We continued the run for a total of 60 minutes to ascertain the limits of the method. Hb dropped in a highly reproducible, near-linear fashion over time. As expected, given that true Hb in the patient is not supervised by Spectra Optia TEA, no alarms prevented the essentially limitless
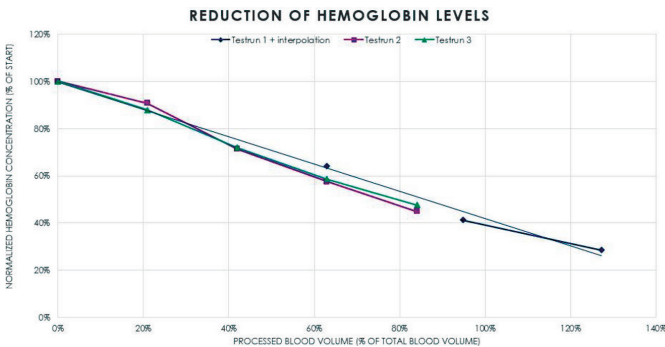
Fig. 1.

reduction of the Hb. With respect to blood volume, normovolaemia was preserved, as intended (99%; -27- -9ml). Platelet loss was considerable (43%; 42-60) and could not be reduced by entering a false-low Hb.
Conclusion: With the mentioned modifications and using crystalline replacement fluid, Spectra Optia TEA can be harnessed to reproducibly establish profound normovolemic anemia within an adequate period of time. The model experiments do not account for release of blood components from spleen that can occur in vital big animal models such as sheep or pigs. As a next step, we will seek to corroborate the method in vivo in a big animal model.

PS-4-11

Molecular testing for Rhesus D antigen (RhD) in patients with ambiguous results in serological testing for RhD: implications for transfusion strategy

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Background: According to the Guideline "Hemotherapy", patients should be tested for Rhesus D antigen (RhD) by two different monoclonal IgM antibodies which cannot detect the D category VI. In case of an ambiguous reaction, a patient is preliminary declared as RhD-negative and transfusion of RhD-positive red blood cells (RBC) should be avoided before clarification. This approach is time-consuming and presumably results in urgent transfusion of RhD-negative RBC´s to RhD-weak positive patients.
Methods: Patient samples were tested for RhD by manual tube testing (IgM Anti-D Mono-Type, Grifols, Barcelona, Spain) and automated by two monoclonal IgM antibodies with a solid-phase or (since May 2022) a column agglutination assay (Erytype S ABO+D, BioRad, Dreieich, Germany or DG Gel ABO/Rh, Grifols). Samples showing a discrepant result between tube and automated testing or weak reaction with at least one monoclonal antibody were considered ambiguous and forwarded to molecular RhD-testing by PCR (RBC-Fluogene Dweak/variant and RBC-Fluogene CDE, Inno-Train, Kronberg, Germany). Patients were transfused with RhD-negative RBC until the PCR result was available. RhD type of transfused RBC was determined by database analysis.
Results: Between September 2019 and September 2022, 119 patient samples showed ambiguous results. Molecular RhD-testing revealed that 71 (59.7 %) were weak D type 1, 16 (13.4 %) had type 2 and 14 (11.8 %) type 3. Two (1.7 %) patients were type 4.0 and seven (5.9 %) were normal D. Four patients (3.4 %) were dd. One (0.8 %) patient each was type 4.2, type 15, type 45.1, partial D DVII and DAR-E. In 33/119 (27.7 %) patients, 118 RBC`s were transfused, 21 of those patients received 89 RhD-negative RBC´s as the result of molecular RhD-typing was pending. 9/21 had weak D type 1, two type 2, two type 3, one type 4.0 and another two had a normal D. Two patients were dd and one each weak D type 15, type 4.2 and partial D DAR-E.
Conclusion: The majority of patients (110, 92.4 %) had a normal RhD or weak D type 1 – 4.0 and thus could be safely transfused with RhD-positive RBC´s. Only a minority of patients should precautionary be declared as RhD-negative, as transfusions of RhD-positive RBC´s might induce anti-D formation. Urgent transfusions with RhD-positive RBC`s in patients with ambiguous results in the serological RhD-testing is reasonable, safe in the majority of patients and avoids wastage of RhD-negative RBC´s.

PS-4-12

Development of multiplex TaqMan probe PCR Assays for typing of up to 6 genetic markers per reaction

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Background: TaqMan probes and fluorescent endpoint detection for allelic discrimination is commonly used for genotyping of Single Nucleotide Variations (SNVs). Assays are usually desigend for typing one di-allelic SNV per reaction using two fluorescent dyes. Recent systems for quantiative PCR enable the detection of up to 6 colors. We aimed to develop multiplex assays for up to 3 di-allelic SNVs in one reaction and used Human Platelet Antigens (HPA) and Human Neutrophil Antigens (HNA) as model.

Methods: Primers and probes were designed for the SNVs coding for HPA-1a/b, -5a/b, -15a/b and HNA-3a/b, -4a/b, -5a/b according to reference sequences. Probes were labeled with FAM (HPA-1a, HNA-3a), HEX (HPA-1b, HNA-3b), TAMRA (HPA-5a, HNA-4a), TexasRed (HPA-5b, HNA-4b), Cy5 (HPA-15a, HNA-5a) or Cy5.5 (HPA-15b, HNA-5b). Multiplex assays for HPA and for HNA were used to genotype DNA samples from blood donors and the results were compared with reference data in the donor files. PCR amplification was performed in standard cyclers and fluorescence was measured after PCR by using the QuantStudio 5 system (Thermo Fisher Scientific).

Results: Re-typing of HPA-1, -5, -15 and HNA-3, -4, -5 in 475 blood donor samples revealed 100 % concordance with typing data in the donor files previously obtained by using monoplex TaqMan assays.

Conclusion: We were able to develop multiplex assays for time and cost efficient genotyping of 3 SNVs per reaction. The assays are used in the ongoing blood donor typing of HPA and HNA at our institute. Additional multiplex assays will be developed and also tested in digital PCR for the use in noninvasive prenatal diagnosis.

PS-4-13

Molecular blood typing of patients with Sickle Cell disease in Germany

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Background: Because of acute and chronic symptoms patients with Sickle Cell Disease (SCD) require frequent blood transfusion. The ethnic background of most SCD patients is African or Arabian, whereas, the ethnicity of most blood donors in Germany is European. Since the prevalence of antigens is significantly different in the populations we aimed to identify SCD patients with high risk for alloimmunization against blood from European donors by molecular boold typing of the most relevant blood group antigens.

Methods: DNA samples from 249 SCD patients were subjected to typing of the genetic markers for the blood group antigens D, C/c, E/e, K/k, M/N, S/s, Fy(a/b)/Fy0 and Jk(a/b) according to international recommendations. Genotyping was performed by using inhouse validated PCR-based methods and a commercial kit for RHD/RHCE (RBC-FluoGene CDE eXtend; inno-train GbmH, Kronberg, Germany). Allele frequencies in the SCD patients were compared with data for the African and European population in the gnomAD database.

Results: As expected, DAU alleles were the most frequent _RHD_ variant alleles in SCD patients and 15 % were homo- or hemizygous for a DAU allele associated with a high risk for alloimmunization against D. The Fy0 allele was frequent in SCD patients (0.79) with 62 % being homozygous as known for Africans. From the genotyping data we could assume that 82 % of the SCD patients were negative for the Fy(b) antigen. The Fy(b) antigen is also negative in 20 % of our blood donors which can be considered for Fy(b)-matched transfusion. In summary, the allele frequencies observed in SCD patients corresponded to the allele frequencies reported for the African population.

Conclusion: For the transfusion of SCD patients without alloantibodies it is recommended to use blood matched for ABO, D, CcEe and K. If patients have clinically relevant alloantibodies the transfused blood should be additionally matched for Fy(a/b), Jk(a/b) and S/s. Based on the genotype data we were able to identfy the SCD patients with a high risk for allo-immunization against blood group antigens such as D, Fy(b) and others.

PS-4-14

Compound heterozygosity for five different CD36 mutations induces CD36 type I deficiency in three members of a family of Arabian origin

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Background: CD36 type 1 deficiency is characterized by missing CD36 expression on both, platelets and monocytes and enables iso-immunization. A broad variety of mutations has been identified as underlying molecular reason. We describe the molecular basis of compound heterozygosity that silence CD36 expression in a family of Arabian origin with three affected members. Additionally, we analyzed the CD36 type 1 deficiency in an unrelated donor whose platelet concentrate was transfused to the index patient.

Methods: CD36 expression on platelets and monocytes was tested by flow cytometry using anti-CD36 FITC (FA6-152, Stem Cell Technologies, Vancouver, CAN), anti-CD42b PE (HIP1, Biolegend, San Diego, CA, USA), and anti-CD14 PE (M5E2, Biolegend). The presence of CD36 iso-antibody in individuals with type 1 deficiency was tested by Luminex PAK Lx (Immucor Lifecodes, Dreieich, D) and crossmatch between the patient"s serum donor platelets was performed by the MASPAT assay (Sanquin, Amsterdam, NL). Sanger sequencing on an ABI Prism 310 (Applied Biosystems, Weiterstadt, D) was applied for further molecular analysis. Custom primers specific for _CD36_ were used for amplification and sequencing reactions of _CD36_ exons 2-14 including short flanking intron regions.

Results: The 15 years old patient with a glioma and severe thrombocytopenia, his mother and his paternal grandmother had CD36 type 1 deficiency combined with isoantibody formation in the patient and his mother. Compound heterozygosity for 5 variations on 4 different alleles caused the deficiencies (Genbank OQ909049-..56; fig.1)

- _CD36*c.451insTCAA_ (p.164X), *c.824T>G (p.I275S)
- _CD36*c.1202_1205del_ à _fs_ (p.404X)
- _CD36*c.1200-7_1208dup_ à _fs_ (p.422X)
- _CD36*c.1156C>T_ (p.R386W)

An unrelated CD36 negative donor was identified earlier with a homozygous _CD36*c.649G>A_ (p.G217R) exchange. He and the patient"s grandmother showed a negative MASPAT crossmatch. The patient could benefit from their platelet concentrates with platelet rises from 1 to 30x10⁹/L.

Conclusion: A broad variety of mutations is responsible for CD36 deficiency on platelets and monocytes, thus aggravating rapid identification by SNP specific PCR-methods. Instead, search for CD36 negative platelet donors is most promising by flow cytometric screening amongst individuals from African, Arabian or eastern Asian descent. Sanger sequencing or NGS can add further information on the molecular background. This strategy can help to supply immunized patients with CD36 isoantibodies.
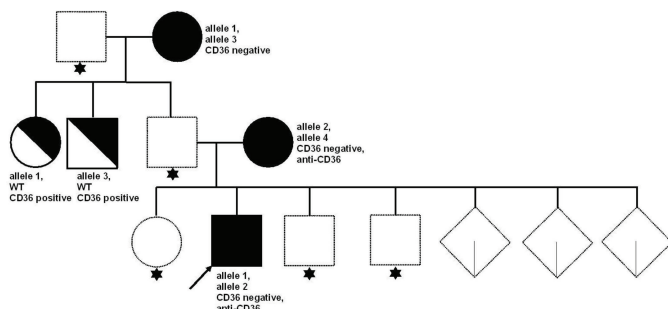
Figure 1: Segregation of *CD36* alleles within an Arabian family.
Circles: Females; squares: males; black circles and squares: CD36 negative individuals with two mutated alleles; divided circle and square: CD36 positive individuals with only one affected allele; arrow: index patient with anti-CD36; asterisks: individual not tested; rhomb: miscarriage; WT: wild type allele

Fig. 1.

PS-4-15

A p.Leu319Pro missense mutation in *ABO* likely results in a non-deletional O allele

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Background: ABO is the most important blood group system in transfusion medicine. A glycosyltransferase attaching N-acetyl-galactosamine or galactose to specific sugar chains of glycoproteins and glycolipids is responsible for the expression of antigen A or B, respectively. Typical for the null phenotype is a single base deletion in the corresponding *ABO* gene (c.261delG). In addition, several missense mutations are known that also lead to an O phenotype; often referred to as non-deletional *O* alleles.

Methods: Here, we present a case of a male patient with a discrepancy between forward and reverse typing of ABO. Antigen determination was performed by hemagglutination with both, a column technique (Ortho Biovue) and a gel card technique (Bio-Rad ID-System). Commercially available red blood cells were used for detection of isoagglutinins (i.e. anti-A and anti-B). Initial genotyping was performed by commercial SSP-PCR (ABO subtype, innotrain). For sequencing of *ABO* a direct taq-cycle method was chosen. To detect weakly expressed antigens, a combined adsorption-elution test was performed: adsorption with a human anti-A at 4 °C, followed by heat elution. Additionally, antigen A expression on red blood cells was measured by flow cytometry.

I-1:
ABO*A1.01(L319P) /
O.01.01

I-2:
ABO*B.01 /
O.01.02

II-1:
ABO*01.01 /
O.01.02

II-2 (index patient):
ABO*A1.01(L319P) /
O.01.02

II-3:
ABO*B.01 /
O.01.01
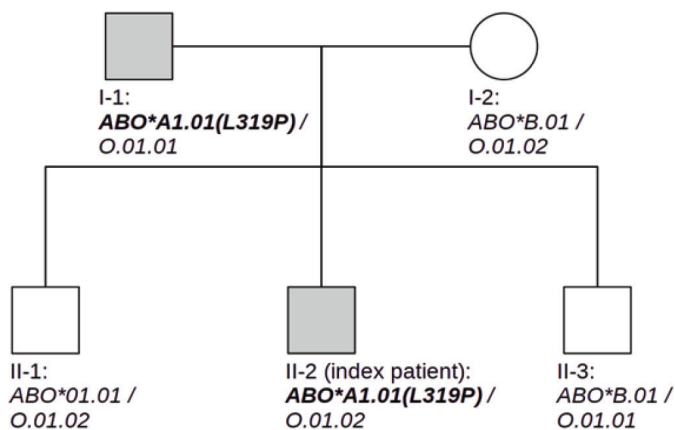
Fig. 1.

Results: Forward typing of the patients own red blood cells (RBC) revealed no expressed A or B antigen, respectively. The serum of the patient contained a strongly reacting anti-B and a weak anti-A1. Reverse typing with A2 RBC was negative. Genotyping by SSP-PCR suggested an A1 phenotype of the patient"s RBC. However, haplotype specific sequencing of *ABO* revealed a deletional O allele (*ABO*O.01.02*) and an *A1*-Allel with an additional nucleotide exchange in exon 7 (c.956C) leading to an amino acid substitution (p.Leu319Pro). An AEL phenotype was excluded by a negative result in a combined adsorption-elution test. Flow cytometry confirmed these findings: anti-A binding was comparable with well known RBC of the O2 phenotype. Figure 1 shows a pedigree.

Conclusion: Typically, RBC of individuals with an O phenotype do not express any A antigen. However, there is evidence that non-deletional *O* alleles have a residual activity of glycosyltransferase A. In our case we could not elute any potentially adsorbed anti-A. But, anti-A1 and anti-A in the serum were strongly diminished; a typical result for non-deletional *O* alleles. Interestingly, the predicted aminoacid exchange p.L319P is not in close proximity to direct enzyme activity or substrate binding sites.

PS-4-16

Routine testing for RHD*01W.1, RHD*01W.2 and RHD*01W.3 subtype alleles is dispensable in Switzerland

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Background: Alleles *RHD*01W.1*, *RHD*01W.2* and *RHD*01W.3* are defined by single nucleotide variants (SNVs) which lead to reduced RH1 antigen expression. Anti-RH1 alloimmunization has not been reported for individuals carrying these alleles. Subtypes with additional SNVs have been described and may cause a risk for alloimmunization. Therefore, we developed sequence specific primer (SSP)-PCRs to genotype individuals with known *RHD*01W.1/.2* and .3 alleles to elucidate the frequency of these subtypes.

Methods: Blood donor and patient samples from 2019-2022 (four years) showing a weak RH1 phenotype were genotyped using the RBC-FluoGene D weak/variant kit (inno-train, Germany) identifying SNVs c.809T>G (*RHD*01W.1*), c.1154G>C (*RHD*01W.2*) and c.8C>G (*RHD*01W.3*). In-house developed multiplex SSP-PCR assays were used to further screen for subtypes defined by the presence of the SNVs c.52C>G (*RHD*01W.1.1*), c.712G>A (*RHD*01W.1.2*), c.667T>G (*RHD*01W.1.3*), c.455A>C (*RHD*62*), c.301T>A (*RHD*01W.2.1*), c.916G>A (*RHD*01W.2.1*) and c.178A>C (*RHD*01W.3.1*). The *Human Growth Hormone* gene *(HGH)* served as DNA extraction and amplification control. Amplicons were analyzed by capillary gel electrophoresis (Qiagen, Germany).

Results: A total of 714 samples carried c.809T>G of which two samples revealed the presence of the *RHD*01W.1.1* defining substitution c.52C>G. Two samples belonged to the subtype *RHD*62* due to c.455A>C. One sample showed the presence of c.667T>G indicative of *RHD*01W.1.3*. However, further analyses revealed that this SNV was detected in addition to c.602C>G rendering the presence of alleles *RHD*01W.1* and *RHD*09.03.01* more likely, rather than the much less probable combination *RHD*01W.1.3 and RHD*01W.40*. Furthermore, we identified 274 and 157 samples carrying the SNVs for *RHD*01W.2* and *RHD*01W.3*, respectively. In none of these samples the screen revealed any additional SNVs.

Conclusion: Subtypes of alleles *RHD*01W.1*, *RHD*01W.2* and *RHD*01W.3* are extremely rare. Only four out of 1145 samples (0.35 %) carried additional SNVs, all of them associated with the SNV c.809T>G. Moreover, alloimmunization in individuals carrying the investigated subtype alleles has not been reported to the best of our knowledge. Considering the rareness of subtypes of alleles *RHD*01W.1, RHD*01W.2* and *RHD*01W.3*, screening for these subtypes will not be proposed to be performed on a routine basis.

PS-4-17

Characterization of two novel ABO splice site variations preventing blood group A and B expression

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Background: The ABO locus is highly polymorphic with almost 200 alleles that encode altered phenotypes. Gene mutations result in functionally modified glycosyl transferases that can change the expression of ABO antigens. Rare mutations can push the limits of conventional serology or PCR-SSP methods. Analysis by Sanger sequencing can help to resolve complex cases. In this work, we analyzed the molecular background of two novel splice site variations, which prevent the expression of blood group A and B.

Methods: ABO phenotypes were determined by standard blood group serology (BioRad, DiaMed GmbH, Cressier, Schweiz). Genotyping was carried out by PCR-SSP (RBC Ready Gene ABO Subtype, Inno-Train, Kronberg). Sanger sequencing on an ABI Prism 310 (Applied Biosystems, Weiterstadt) was applied for further molecular analysis. Custom primers specific for ABO were used for amplification and sequencing reactions of all seven exons including short flanking intron regions.

Results: Blood samples of two patients showed inconsistent results between blood group and reverse typing. Further analysis with PCR-SSP could not explain the aberrant serological results. For safety reasons, both patients were assigned to blood group O. Sanger sequencing determined a heterozygous *ABO*204 -2g>a* located two nucleotides downstream of exon 1 in patient one and a heterozygous *ABO*c.28+3ins t* three nucleotides upstream of exon 5 in patient two. Both variations qualified as splice site mutations that affect the transcription of the antigen. Since serological testing revealed a weakened A, respectively B, expression in the two samples, it can be inferred that the mutations are located on the alleles coding for *ABO*A1.01* and *ABO*B.01*.

Conclusion: Both mutations led to the detection of altered blood group A and B expressions, resulting in Ax/Ael and Bweak/Bx/Bel phenotypes. Sanger sequencing allowed the identification of these novel mutations and therefore still is beneficial in advanced analysis of atypical serological results. Neither of the mutations is listed in the ISBT allele tables thus far. The nucleotide sequences of the new mutations have been submitted to the GenBank data base (GenBank accession numbers OQ469492, OQ595206).

PS-4-18

First report of the rare RhCE-depleted D--phenotype in sixteen people of Iranian origin

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Background: In transfusion medicine, it may be a challenge to acquire compatible blood for patients who have clinically important alloantibodies to high-prevalence antigens. This is the first report of the cases evaluated by the IBTO and family studies of the D-- proposita in Iran and possibly the first attempted comprehensive study in the current transfusion-related literatures.

Methods: This retrospective cross-sectional study was carried out on 6720 pregnant women and individuals with difficult positive pretransfusion testing referred for ABO/Rh(D) typing and antibody screening during a period of 8 years from 2008 to December 2016 in the Immunohematology Reference Labo ratory of the Iranian Blood Transfusion Organization, Tehran, Iran.

Results: During 2008 to December 2016, 16 persons from ten families were detected to have rare D-- phenotype. Anti-Rh17 and anti-c were identified in plasma of the 11 persons, including 10 females with a history of multiple unsuc cessful pregnancy and the total number of 24 abortions and one male with history of blood transfusion vs. 5 individuals, including an unmarried single woman, 1 person with a history of first-time pregnancy and 3 persons with a history of mul tiple pregnancy, who showed no alloimmunization. Based on these collective find ings, we interpreted these results as being confirmed as D-- phenotype (0.23%).

Conclusion: Irrespective of Rh (D) group a serological antibody screening test is recommended to be required in a National prenatal testing guideline.

Table 1 Data on Patients with D— rare blood type

No	Diagnosis	Age	Sex	N.ch	ABO	ABS	Antibody ID	Frozen autologous	Hx of Transfusion	No of abortion	G. Area
1	Multiple abortion	34	F	1	O	Positive	Anti-Rh17 titre 256	2	No	4	Borujerd
2	Kidney transplant	52	M	NA	O	Positive	Anti-Rh17, titre nt	1	Yes (anaemia Hg 6.8 g/dl) NA	Mashhad	
3	Multiple abortion	37	F	1	O	Positive	Anti-Rh17 titre 512	1	No	3	Tehran
4	Multiple abortion	35	F	1	O	Positive	Anti-Rh17 titre 512	0	No	4	Ahvaz
5	Multiple abortion	44	F	1	A	Positive	Anti-Rh17 titre, titre nt	0	Yes (anaemia Hg 6 g/dl)	3	Tehran
6	Multiple abortion	29	F	2	A	Positive	Anti-Rh17 titre 256	0	Yes (infant transfusion)	1	Tehran
7	Sibling	36	F	3	A	Negative	None, titre nt	0	No	1	Shiraz
8	Sibling	57	F	5	A	Negative	None, titre nt	0	No	2	Shiraz
9	Multiple abortion	35	F	1	A	Positive	Anti-Rh17 titre 512	0	No	3	Urmia
10	Multiple abortion	24	F	1	A	Positive	Anti-Rh17 titre 2048	0	Yes (infant transfusion)	2	Tehran
11	Multiple abortion	30	F	2	B	Positive	Anti-Rh17 titre 256	0	Yes (infant transfusion)	0	Tehran
12	Sibling	19	F	None	B	Negative	None, titre nt	1	No	0	Mashhad
13	Multiple abortion	41	F	1	AB	Positive	Anti-Rh17 titre 1024	2	Yes (IUT)	6	Zahedan
14	Sibling	36	F	None	AB	Negative	None, titre nt	0	No	0	Gorgan
15	Sibling	32	F	1	AB	Negative	None, titre nt	0	No	0	Gonabad
16	Sibling	20	F	1	A	Positive	Anti-Rh17, anti-c, titre 64,16	0	No	0	Tehran

F, Female; M, Male; N.ch, number of children; G.Area, geographic Area; nt, not tested; NA, not applicable.

Fig. 1.

PS-4-19
The first comprehensive study of H-deficient phenotypes in Iran

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Background: The lack of correct blood grouping practices can lead to missing of the rare Bombay Oh phenotype and subjecting patients to the risk of severe hemolytic transfusion reaction. In the absence of blood donor registry, transfusion management of patients is a challenge. We performed this study in order to estimate the prevalence of the Bombay blood group (Oh) in Iran and to determine whether consanguinity plays a role in the prevalence of Oh group.

Methods: This is a descriptive study in the Immunohematology Reference Laboratory of the Iranian Blood Transfusion Organization (IBTO) Tehran, Iran, over a period of 7 years. All donor blood samples showing blood group O and a strong initial reaction with blood group O RBC control cells were tested with anti-H lectin. Also blood samples from blood group O patients were tested with anti-H lectin if all cells on both antibody screening tests and antibody identification panels were reactive with negative auto control test. Specialized tests like adsorption/elution technique and inhibition assay for determination of secretor status were performed on Oh cases

Results: Analysis of the results of over 7 million first-time blood donors in Iran showed that the most common ABO blood group was O, with 2,520,000 (36%) subjects. 56 Oh individuals' (donors and patients) phenotypes (0.0008%) were detected. Consanguinity was observed in 50 cases (89%).

Conclusion: This study shows that the prevalence of Bombay blood group in the general population of Iran is relatively high (0.0008%) and associated with consanguineous marriage. Thus, consanguinity is still an important risk factor present.

PS-4-20
First report of known rare rhnull phenotype individuals in Iran

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Background: The Rh-deficiency syndrome is a rare genetic disorder of red blood cells (RBCs) with a reported frequency of approximately 1 in 6 million individuals, transmitted via an autosomal recessive mode generally via consanguineous genealogy. It results in the same clinical syndrome characterized by chronic hemolysis of varying severities, with stomatocytosis, spherocytosis, increased osmotic fragility, altered phospholipids asymmetry, altered cell volume, defective cation fluxes, and elevation in Na+/K+ ATPase activity

Methods: The Patients extended phenotyping showed that they were negative for D, C, E, c, e RBC antigens, indicating they were strongly suspicious of being the rare Rhnull phenotype. Adsorption and elution studies in Anti-human globulin did not reveal the presence of D, C, E, c, e, RBC antigens in the blood obtained from both patients. We performed

Rh phenotyping with two sources of antisera. Positive and negative control tests were performed for each antigen according to the manufacturer"s recommendations. Based on these collective findings, we interpreted these results as being strongly suggestive of the Rhnull phenotype with a clinically significant anti-Rh29 identified in serum of the female patient

Results: Rhnull phenotype is a rare blood group with a frequency of approximately 1 in 6 million individuals, transmitted via an autosomal recessive mode. It is characterized by the weak (Rhmod) or lack (Rhnull) of expression of all Rh antigens on the red cells. The clinical significance of its assessment is that such patients with Rhnull syndrome are associated with chronic hemolytic anemia of varying degrees. Another clinical importance is that such subjects readily form alloantibodies when exposed to Rh antigens. We report herein a rare Rhnull phenotype in a sibling, which was detected as a part of the difficult sample work-up for red cell antibody screening and identification.

Conclusion: Availability of a National Rare Donor Program and support of well-trained reference laboratory personnel in a close collaboration with relevant medical team help to manage a patient"s need to a very rare Rhnull phenotype in an acceptable turnaround time. In this study, the patient"s life was saved by her brother"s compatible Rhnull blood. After consultation with his brother, he consented to be added to the list of National rare donor registry database in Iran.

PS-4-21
Maternal red blood cell alloantibodies identified in blood samples obtained from Iranian pregnant women: the first population study in Iran

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Background: Alloimmunization of red blood cells in pregnant women is still a challenge to clini cians. Maternal immunoglobulin G anti bodies are the main cause of fetal RBC hemolysis by targeting fetal RBC antigens. we aimed to determine the frequency of alloimmuniza tion in pregnant women who were referred to the Iranian Blood Transfusion Organization. Before 2015 there was no national protocol for testing of pregnant women for the detection and identification of RBC antibodies.

Methods: Thiswasa prospective cross-sectional study, which was carried out in the immunohematology reference laboratory of the Iranian Blood Transfusion Organization in Tehran, Iran, in 2008 to 2015. Screening and identification of red blood cell (RBC) alloantibodies was done on the sera of 7340 pregnant females using the standard tube method and gel column agglutination technique.

Results: Alloantibodies were identified in the serum of 332 of the 7340 (4.5%) pregnant women. A total of 410 antibodies were detected in 332 positive maternal serum samples with no previous history of blood transfusion. Anti-D was the most common antibody accounting for 70.5% of all the antibodies formed in D– women. The incidence of specific alloimmunization other than Rh group was 14.4%.

Conclusion: In Iran, like other developing countries, alloimmunization screening tests are performed only to detect anti-D in pregnant D– women. This high rate of alloimmunization, quite possibly, is due to the fact that the majority of blood samples came from pregnant women known to have previous obstetric problems. However, we suggest that RBC antibody screening tests should be extended to all D1 women.

PS-4-22

Red blood cell immunization and contributing factors in 685 Thalassemia patients

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Background: An analysis of red blood cell alloimmunization in patients with thalassemia can help to devise specific strategies to decrease the allo-immunization rate. This study explored the frequency and specificity of alloantibodies and autoantibodies against red blood cell (RBC) antigens in patients with thalassemia referring to the Iranian Blood Transfusion Organization (IBTO) Immunohematology Reference Laboratory (IRL) in Tehran.

Methods: This study first examined the laboratory records of 23,113 patients suffering from different diseases referring to IBTO's IRL for pre-transfusion testing in the 2008-2015 period. ABO and Rh(D) typing and antibody screening tests were performed for all 23,113 patient records and 685 (2.97%) beta-thalassemia patients with positive pre-transfusion test results (antibody screening and/or DAT) were selected for further investigation.

Results: The antibody screening test was positive in 640 out of 685 thalassemic patients (93.4%). DAT was performed for 529 patients, 226 (33%) of which showed positive results. Meanwhile, 161 out of 685 beta-thalassemia patients (23.5%) had positive auto control test results, reflecting the possible presence of allo- and/or autoantibodies. The most common antigen-specific alloantibodies were directed against K and E RBC antigens with a frequency of 25% (Anti-K) and 11.91% (Anti-E), respectively. The development of two antibodies (double antibodies) in one patient was observed in 80 individuals (11.46%).

Conclusion: Extended red blood cell phenotyping should be considered as an essential procedure for expected multi-transfused thalassemia patients before blood transfusion. Considering the high frequency of anti-K and anti-E observed in this study, it is recommended that thalassemia patients in Iran are tested through phenotyping of RBC units for K and E antigens before transfusion.

PS-4-23

Titer progression of two blood group alloantibodies under massive antibody-reducing chemo- and apheresis therapy

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Background: Some blood group alloantibodies develop and persist for life, while others - as the ones subsequently mentioned – typically fall under the detection limit after a few months. Here we report titer progression of alloantibodies anti-FY(a) and anti-Jk(b) in a 26y female patient with sickle cell disease over 16 weeks. During this period the patient received therapy primarily intended for HLA antibody reduction and conditioning chemotherapy (cCTX) before planned allogeneic stem cell transplantation.

Methods: The alloantibodies anti-Fy(a) and anti-Jk(b) - already existing for 2.5 months before initiation of the B-cell- and plasma-depleting immunochemotherapy (iCTX) containing (Rituximab 375mg/m2; Fludarabine 40mg/m2; Dexamethasone 25mg/m2; Velcade 1,3mg/m2) in 02/2023 - were determined automatically using appropriate red cells with the ORTHO VISION® MAX Analyzer (QuidelOrtho). Titer measurements were performed from EDTA plasma at several intermittent
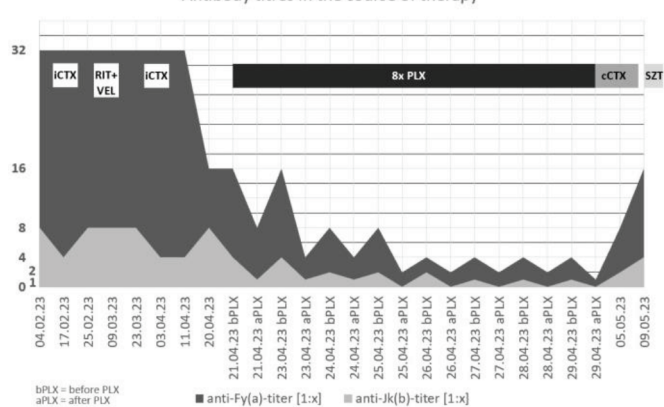
Fig. 1.

time points during the course of 2 cycles of iCTX plus 1 additional dose of Rituximab and during a phase of 8 plasma exchanges (PLX) (8x PLX, means: 1.29-fold plasma volume; 3171mL; 65mL/kg bw) each) before and after each therapy and after cCTX (incl. ATG+Treosulfan).

Results: Antibody titers before initiation of iCTX on 02.02.23 were 1:32 [anti-Fy(a)] and 1:4 [anti-Jk(b)]. After this first cycle, the antibody titers stayed unchanged (16.02.23: anti-Fy(a) 1:32; anti-Jk(b) 1:4). In the period before the 2nd iCTX cycle, anti-Fy(a) was 1:32 and anti-Jk(b) was 1:8 (20.02.23; 09.03.23; 23.03.23). After the 2nd iCTX cycle and a 3rd Rituximab administration on 06.04.23, anti-Fy(a) and anti-Jk(b) remained 1:32 and 1:4, respectively. Finally, after a total of 8 PLX therapies (20.04.23 - 29.04.23), titers dropped from 1:16 to 1:1 [anti-Fy(a)] and 1:8 to negative [anti-Jk(b)], relapsing to 1:16 [anti-Fy(a)] and 1:4 [anti-Jk(b)] after cCTX (09.05.23).

Conclusion: Anti-Fy(a) and anti-Jk(b) proved largely refractory to iCTX over 12 weeks. Only heavily forced antibody-reducing PLX therapy led to a significant reduction by 4 titer levels. Remarkably, even after four PLX therapies, a constant rebound by one titer level was still detectable, which may indicate a high antibody content in tissues or persistence of antibody producing cells. Furthermore, no reducing effect on titer levels was observed in the short term after cCTX.

PS-4-24

Experience with a triple exon RHD-NIPT and detection of maternal and fetal RH variants

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Background: Since mid-2021, RhD-negative pregnant women in Germany have the option of fetal RHD typing using a non-invasive cell-free fetal DNA (cffDNA) prenatal test (RHD-NIPT). The investigation is covered by the statutory health insurance. The molecular prerequisite for the RHD-NIPT is homozygosity for the complete deletion of the RHD gene (RHD*01N.01). We present our overall experience with the RHD-NIPT as well as in the presence of non-deletional maternal RHD alleles or fetal RH variants.

Methods: The RHD NIPT was performed using the FetoGnost® Kit RHD (Ingenetix), which detects exons 5, 7 and 10 of the RHD gene. Indications of maternal RHD sequences were low CT values or a small CT difference (<4) between the RHD-specific reaction and the internal positive control (δIPC). When maternal RHD gene sequences were suspected, Rh blood group antigens were determined serologically (ID-Card System, BioRad), followed by maternal RHD genotyping (ERY Q® Weak D, BAG

Tab. 1.

Table 1 - Sample results of RHD-NIPT in serologically RhD-negative pregnant women with RHD variants or RHD/RHCE hybrid alleles
(Ct value *in italics - usual values in RHD-positive foetuses*; **in bold - indication of maternal RHD sequences**).

maternal allele	Rh phenotype		RHD Exon 5			RHD Exon 7			RHD Exon 10		
RHD*08N.01 (RHD*Ψ)	ccddee	Ct	*37,0*	*37,5*	*36,8*	**31,7**	**31,7**	**32,0**	**31,7**	**31,8**	**31,8**
		δIPC	*8,1*	*8,5*	*7,7*	**2,9**	**2,7**	**2,9**	**2,8**	**2,8**	**2,7**
RHD*03N.01 [(C)de^S] **(RHD-RHCE(4-7)-RHD)**	C^{Var}cddee	Ct	*37,4*	*37,0*	*38,0*	*37,9*	*37,8*	*38,4*	**32,4**	**32,6**	**32,8**
		δIPC	*8,1*	*7,7*	*8,5*	*8,7*	*8,6*	*8,9*	**3,2**	**3,3**	**3,3**
RHD*11	CcD*11.ee	Ct	**29,1**	**29,4**	**29,6**	**30,9**	**31,3**	**31,1**	**32,0**	**32,2**	**32,1**
		δIPC	**-0,1**	**0,1**	**0,2**	**1,7**	**2,0**	**1,7**	**2,9**	**2,9**	**2,7**
RHD*15	ccD*15.Ee	Ct	**32,2**	**32,2**	**32,1**	**32,8**	**33,0**	**33,0**	**33,1**	**33,0**	**33,0**
		δIPC	**2,3**	**2,2**	**2,0**	**2,9**	**3,0**	**3,0**	**3,2**	**3,1**	**2,9**

Figure 1 – Molecular structure of the maternal alleles detected. Exons within the boxes are examined in the RHD-NIPT.
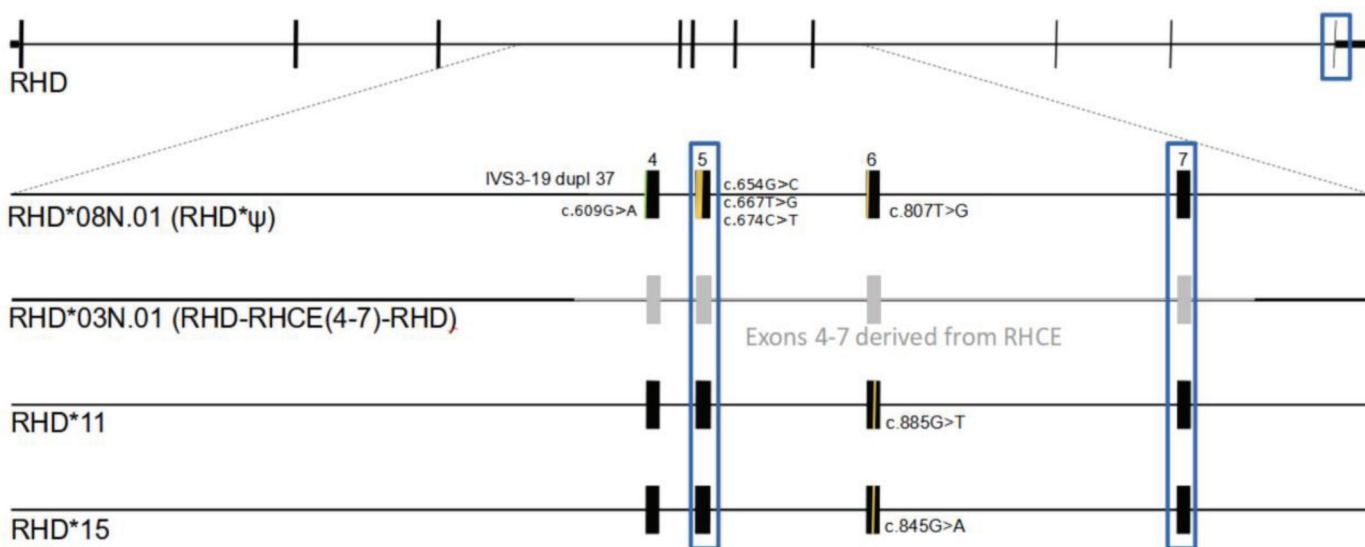
Fig. 1.

Diagnostics). Samples yielding negative results with the latter assay were forwarded to specialized laboratories for further molecular genetic testing. Due to the relatively small amount of cffDNA, samples with possible fetal RH gene variants could not be further investigated.

Results: Between July 2022 and April 2023, we detected maternal RHD sequences in 7 out of 870 (approx. 0.8%) samples of pregnant women, who were serologically RhD-negative by routine recipient testing. Further genotyping revealed RHD*08N.01 (RHD*Ψ) in four cases as well as RHD*11, RHD*15 and RHD*03N.01 ((C)de^S) once each. The observed patterns of exon amplification corresponded to the detected maternal RHD genotypes. With regard to the fetal RHD status we clearly detected 537 positive (approx. 62.4%) and 323 negative (approx. 37.6%) fetal RHD genotypes. Furthermore, a variant at the fetal RH locus was suspected in three cases (aprox. 0.3%). One of them with a suspected fetal RHD*08N.01 was subsequently serologically typed RhD-negative after birth.

Conclusion: If serological RhD negativity is not due to RHD*01N.01 homozygosity, the RHD NIPT may be confounded by the presence of maternal sequences. The same applies to other RHD variants that are considered RhD-negative in the recipient setting. In these cases, the maternal genotype can be further identified. Upon suspicion of fetal RHD variants, a definite prediction of the serological status is not possible and rhesus prophylaxis needs to be administered to the pregnant woman.

PS-4-25

Transmission of anti-CD38 (Daratumumab) by autologous stem cell transplantation causes interference in immunohematological diagnostic

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Background: A 53 year old female patient with multiple myeloma (IgG-kappa) presented with unexpected positive reactions in the indirect anti-globulin test (IAT) after autologous stem cell transplantation (HSCT) 3 days prior to the blood draw. With enzyme treated test cells no reactions were noticed. The auto control was negative. Diagnostic tests with blood taken directly prior the HSCT had been negative. She had not received further therapy between the two samples, no blood products had been given before.

Methods: Blood samples that were obtained before and after HSCT were screened in the IAT with and without pre-treatment of test cells with the Fab-fragment of anti-CD38 (DaraEx®) assuming, that the recently given autologous stem cell product could have contained daratumumab.

Results: Samples taken during therapy with anti-CD38 (daratumumab) (last dose was given 3 weeks before stem cell collection) and the sample taken by the stem cell collection showed low- but panreactive antibody screening in IAT, negative auto control but negative antibody screening with DaraEx®.

The initial samples during the current hospitalization including a sample taken in the morning of the autologous stem cell transplantation were unremarkable.

The next sample sent in 3 days later was panreactive in IAT with a negative auto control but was negative with DaraEx® again.

The phenomenon was visible in three samples sent in during the next ten days, on day 13 the antibody screening in the IAT without DaraEx® was negative again.

Conclusion: This case shows a presumable transmission of daratumumab by HSCT which caused interferences in immunohematological diagnostic several months after last doses of daratumumab.

It had to be kept in mind that events which had taken place before a collection of stem cells for autologous transplantation might cause unexpected results in immunohematological diagnostic after the transplantation.

For further confirmation, measurement of anti-CD38 in archive samples is intended.

ABO splice-site variants encoding weak A and B phenotypes complicate pre-transfusion ABO blood group diagnostics

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Background: Genetic variations in the *ABO* gene can cause discrepancies in routine blood group typing such as weak ABO antigen expression that need further investigations. This study aimed to characterize two novel mutations located at exon-intron junctions of the *ABO* gene resulting in weak A and Bel phenotypes in patients.

Methods: For serologic antigen typing and reverse grouping standard gel matrix techniques (Bio-Rad) were used and adsorption-elution studies of the patients' red blood cells were done using monoclonal and human antisera. Genomic DNAs were isolated by a column-based method on an automated DNA isolation system (Qiagen). The seven exons of *ABO* and its› regulatory regions, as well as *FUT1* and *FUT2* genes were amplified and sequenced by Sanger sequencing (AB3500). Total RNA was isolated (Qiagen) and cDNA synthesis was performed using Superscript III First-Strand Synthesis System and oligo-(dT) primers (Invitrogen). The transcripts detected were further investigated by allele-specific sequencing and translated by the in-silico translation tool ExPASy.

Results: Serologically sample 1 showed a weak A antigen expression with two distinct cell populations. Allele-specific sequencing identified a missense variant at the junction of intron 1 and exon 2 (c.28+12978T>C) compared to the *ABO*A1.01* allele. Sample 2 was negative for A and B antigens indicating an O phenotype. Reverse grouping resulted in positive agglutination reactions with A1 and A2 test erythrocytes and a lack of anti-B. An insertion of an additional T nucleotide at the 5´-end of intron 1 (c.28+3-4insT) resulting in an *ABO*B1*-like allele was found. Both alleles were inherited with *ABO*O.01.01* alleles in trans. Aberrant *ABO* transcript variants were detected and changes in the translated transferases were predicted.

Conclusion: The two novel mutations located at the splice-sites of *ABO* disrupt RNA splicing and result in the synthesis of aberrant *ABO* gene transcripts. Altered glycosyltransferase activity may be responsible for weak phenotypes as observed. Analysis of *ABO* mRNA can help to explain serological ABO blood grouping discrepancies found in routine testing. The gene variants characterized in this study add to the growing blood group gene databases and may help to improve pre-transfusion blood group diagnostics.

The strong potential of the weakly positive DAT for alloantibody elution

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Background: The direct anti-human globulin test (DAT) detects coating of red blood cells (RBC) with immunoglobulins (Ig) and/or complement factors. A positive DAT can be harmless as it may occur in healthy donors. If clinical significance is suspected and IgG coating is found, an elution is recommended. The value of weakly positive DAT for alloantibody elution in transfused patients was found using the classical tube testing. We reevaluated this in the era of the more sensitive gel card DAT method.

Methods: Elution procedures of 107 samples (of 90 patients) were evaluated for a period of 6 months in a university hospital. Acid elutions (BAG, Germany) were performed for either (A) first time diagnosis of warm autoantibodies; (B) detection of RBC-bound new alloantibodies within 14 days since the last transfusion, or (C) in DAT negative samples when haemolysis was evident or reported within 14 days since the last transfusion. Eluates were analysed by antibody screening (gel centrifugation) and differentiation as well as isoagglutinin screening where appropriate. DAT results were classified according to the agglutination strength in negative (n=7), weakly positive (up to 2+; n=71), or strongly positive (3+ or 4+; n= 27).

Results: 36 eluates from 30 patients (33%) were reactive. 24 patients had autoantibodies. One patient had a passenger lymphocyte syndrome caused by Anti-A after an allogeneic haematopoietic stem cell transplantation (recipient AB, donor B). This and two novel Anti-K and Anti-Fy(a) antibodies were found exclusively in the eluates and not in the plasma (informative eluates). Three more patients had new alloantibodies (Anti-Fy(a), Anti-Cw, and Anti-Lu(a), respectively) in the plasma, with similar or incomplete reaction pattern in the eluate. Reactive eluates were found in 16 (59%) of the strongly positive, 19 (27%) of the weakly positive and in 1 (14%) negative DAT. All six RBC-bound new alloantibodies or isoagglutinins had weakly positive DAT.

Conclusion: Our study confirmed that weakly positive DAT rather than strongly positive DAT have the potential to reveal new alloantibodies. Therefore, elution of weakly positive DAT remains useful even in the gel card era. The study also confirmed that a reactive eluate is most likely when the DAT is strongly positive. The efforts of elution in weakly positive DAT samples should be focussed on promising cases. As a conclusion, we advise clinicians to specifically consider and request an elution if needed.

Neutralization of Anti-CD38 in patient samples by a soluble CD38 protein to allow alloantibody detection procedures

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Background: Monoclonal antibody treatments for cancer therapy, such as daratumumab (Anti-CD38), cause panagglutination of Reagent Red Blood Cells and, thus, interfere with immunohematology (IH) testing. As previously shown (Binda et al.: 2018, Transfus Med Hemother), pre-incubation of plasma with a patented soluble CD38 (sCD38) was able to neutralize Anti-CD38 when samples were spiked with the oncological drug (Darzalex, Janssen, Horsham, USA).

Methods: To further prove efficient neutralization, sCD38 has been used to pretreat 53 patient samples (52 having received Daratumumab and 1 Isatuximab). The blood samples were collected between 0 and 245 days after the last infusion. Evaluation has been carried out with different

Tab. 1.

	Complete neutralization	Incomplete neutralization	Total
IAT Gel technique	17	4	21
IAT Tube method	10	0	10
X-match Gel technique	20	2	22
Total	47	6	53

Table 1: results per method

Tab. 2.

Antibody	Titer Anti-CD38	Complete neutralization	Incomplete neutralization
Isatuximab	1:16	1	0
Daratumumab	1:1	2	0
	1:512	3	0
	1:1024	14	0
	1:2048	22	3
	1:4096	5	2
	1:8192	0	1
Total		47	6

Table 2: results per Anti-CD38 titer in sample

immunohematological methods, *i.e.* antibody screening or crossmatching and using tube method or DG Gel technique (Medion Grifols Diagnostics, Düdingen, Switzerland; Diagnostic Grifols, Parets del Valles, Spain). Pre-treatment consisted in adding up to 4µl of sCD38 per 25µl of plasma. As none of those plasma naturally contained alloantibodies, 16 patient samples were then spiked with identified polyclonal antisera covering 13 different specificities and tested again.

Results: In 46 patients receiving Daratumumab and 1 receiving Isatuximab, sCD38 was able to completely neutralize the Anti-CD38 contained in plasma tested in the different IH methods (Table 1). Partial neutralization was observed with samples that had high Anti-CD38 titer and were collected within 28 days after last infusion (Table 2). Increasing even further the dose of sCD38 might improve the blocking of the interference. Except for Anti-Fyᵃ and Anti-Fyᵇ, all alloantibodies namely Anti-D, -C, -c, -E, -e, -K, -Jkᵃ, -Jkᵇ, -S, -s spiked in the patient samples could be detected with no significant loss in reaction strength. Investigation to clarify the reason for the interference with Duffy antibodies is ongoing.

Conclusion: The results of this study show that, at the doses used, sCD38 can completely neutralize Anti-CD38 in 88.7% of the patient samples. sCD38 has been designed as high affinity epitope, which makes it potentially a universal and broad solution for neutralization of any Anti-CD38. This hypothesis seems to be confirmed by the successful inhibition of Isatuximab in patient sample. In addition, as pre-treatment with sCD38 is done on plasma, different IH methods and techniques can be used afterwards.

Disclosure Statements: T. Bise, J. Hall, C. Brown, D. Yee, J. Melton Witt, L. Amniai and A. Caesar are employees of Grifols S.A.

PS-4-29

Case report: Anti-U caused mild haemolytic disease of the newborn

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Background: In pregnancy, antibodies directed against blood group antigens may be transmitted diaplacental and may cause haemolytic disease of the foetus and newborn (HDFN). For most of the common specificities, the risk is now well known. The risk assessment for rare specificities often is poor due to the lack of data. The DGTI Working Party Rare Blood Groups therefore had called to report cases of pregnancies with rare antibodies. We here report on the postnatal course of a newborn with a maternal anti-U.

Methods: Antibody identification and determination of the titres were done with the antiglobulin test in column agglutination technique (Bio-Rad and Grifols). Bilirubin was tested either with Siemens ADVIA Chemistry XPT (laboratory) or with Radiometer ABL90 FLEX (point-of-care device, POC). Haemoglobin was tested with the Sysmex XN-1000 device (laboratory) or with the Radiometer ABL 90 FLEX (POC). Haptoglobin and lactate dehydrogenase (LDH) were tested with Siemens ADVIA Chemistry XPT.

Results: At 24th week of pregnancy, anti-U (MNS system) was detected, titre was 32. At delivery, the titre was 128. The newborn's red cells were tested positive for the antigen U and were reactive in the DAT (4+). Anti-U was eluated, it was also detectable in the newborn's plasma (titre 1). Doppler of the middle cerebral artery did not indicate foetal anaemia at any time. Postnatal haemoglobin decreased from 15.6 g/dl to 12.9 g/dl on day 4 (Tab. 1). On day 8, it reached the nadir of 11.2 g/dl, but increased continuously thereafter without therapy. Bilirubin increased to 24.6 mg/dl on day 4. Intensified phototherapy for 18 h on day 4 and phototherapy for 24 h on day 7 lowered bilirubin to 15.5 mg/dl. Thereafter it decreased without further therapy.

Conclusion: In Germany, it is extremely difficult to provide U-negative red cell units, most often support of foreign blood services is required. In the case reported here, HDFN due to anti-U was relatively mild and did not require transfusion therapy, provision of U-negative units was not necessary. This case may not be representative, but the more cases are reported, the better the assessment of the HDFN risk due to anti-U and due to other rare blood group antibodies.

Tab. 1.

Table 1. Haemolytic disease of a newborn caused by anti-U: Laboratory tests and phototherapy

Date	day 1 < 2h	day 1 12 h	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10
Total bilirubin mg/dl (Cutoff: < 8.8) laboratory/POC*	< 2.0*	5.9	14.4	24.6	15.7*	16.9*	20.8	15.0	16.9*	15.5*
Haemoglobin g/dl (Ref. 15.0-24.0) laboratory/POC*	14.4	15.6	n.d.	12.9	12.4*	12.5*	11.6	11.7*	12.7*	12.5*
Phototherapy				18 h intensified			24 h			

Haptoglobin on day 1: < 0.6 mg/dl (ref: 2-300); LDH on day 4: 587 U/l (cutoff: 250); n.d. = not done

PS-4-30

Management of delivery of a woman with antibodies against high-prevalence red blood cell antigen Lan (Langereis) and von Willebrand disease

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Background: Anti-Lan (Langereis) is a rare antibody against a high-prevalance red blood cell antigen. It has been described as having variable clinical significance for hemolytic transfusion reactions. Only few cases of hemolysis disease of the fetus and newborn caused by anti-Lan are reported yet. Von Willebrand Disease (VWD) is the most common human inherited bleeding disorder due to a defect of Von Willebrand Factor (VWF), which a glycoprotein uniquely involved in key aspects of hemostasis.
Methods: The disease's principal manifestations are spontaneous bleeding from mucous membranes, excessive bleeding from wounds, and menorrhagia. Various bleeding phenotypes are due to different types of VWD and severity of a defect of VWF. *Case report:* A 30 years old woman presented in the 33rd gestational week was referred to our hospital for differentiation of alloantibodies against red blood cells. Serological testing revealed an anti-Lan titer of 32. Ultrasound and fetal blood samples were not suggestive for hemolytic disease in utero. External medical records indicated a Willebrand disease of unknown type. The patient reported mild bleeding symptoms. The ISTH-Bleeding Score was 8 of 56 points.
Results: Interestingly, at the age of ten years she had a gastrointestinal bleeding of unknown origin with anemia requiring blood transfusion. After laparotomy, there was a pronounced abdominal wall hematoma. Because of the positive bleeding history and due to the difficult transfusion medical supply situation, we recommend prophylactic administration of FVIII-containing von Willebrand factor medication at delivery to minimize the risk of bleeding. In addition, blood donation by a compatible family member should be considered in these rare cases.
Conclusion: The availability of Lan-negative blood units is very rare and only a few products are frozen in specialized laboratories. Fetus of women with Anti-Lan have to be screened regularly for hemolytic disease. For the management of Anti-Lan positive patients, hemostaseological optimization is recommended.

PS-4-31

Advantages of targeted antenatal anti-d prophylaxis in RHD negative pregnant woman

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Background: The discovery of cell-free fetal DNA (cfDNA) in maternal plasma has led to the development of non-invasive methods to determine the fetal *RHD* genotype. This enables targeted antenatal anti-D prophylaxis only to woman with presumably RhD positive babies and is introduced in Slovenia from 2018 (1). After almost five years of introduction we were interesting in advantiges and disadvantages of targeted antenatal prophylaxis in our region of northeastern Slovenia.
Methods: Between 2018 and 2023, we received 2216 samples of pregnant women in 25- 26 weeks' of gestation. Fetal *RHD* genotype was determined in Blood Transfusion Center Ljubljana with real time PCR using the GenAmp PCR System 9700. The presence of intron 5, exon 7 and 10 on the *RHD* gene and *SRY* gene are assesed. After genotyping RhD we phenotyped only samples of cord blood presumably RhD positive newborns. We looked retrospectively at the results, medical documentation and analyzed how many pregnant women were treated according to the accepted

Predicted fetal phenotype RhD

	RhD-positive	RhD-negative	RhD-inconclusive	
2018 (1. 10. - 31. 12.)	22	25	0	**47**
2019	304	224	3	**531**
2020	253	186	7	**446**
2021	332	208	4	**544**
2022	290	193	1	**484**
2023 (1. 01. - 30. 04.)	96	65	3	**164**
	1297 (58 %)	**901 (41 %)**	**18 (1 %)**	**2216 (100 %)**

Fig. 1

principles of targeted protection, how many really needed protection and how many units of IgG anti-D were saved.
Results: From 1 October 2018 to 30 April 2023, we received 2216 samples of RhD negative pregnant women. According to the *RHD* genotyping, 1297 (58 %) fetuses were RhD-positive, so we advised protection with anti-D immunoglobulin at 28 weeks of pregnancy, and 901 (41 %) fetuses were RhD-negative, so pregnant women did not need protection. In 18 (1 %) cases, the predicted RhD phenotype of the fetus was inconclusive, therefore we advised protection with anti-D imunoglobulin as recommended. With the introduction of the national programme pregnant women receive imunoglobulin anti-D targeted protection according to the predicted foetal RhD phenotype. During the mentioned period, the use of anti-D immunoglobulin was reduces by at least 901 units.
Conclusion: With the introduction of targeted antenatal anti-D prophylaxis we confirmed that predicted phenotype of the fetuses in 41% of RhD-negative pregnant women is negative. Side effects of the drug are avoided in these pregnant women. The rational use of anti-D immunoglobulin is important due to the limited source of obtaining, as the immunization of male voluntary donors is ethically controversial in many countries.

Disclosure Statements: T Dovč Drnovšek, et all. Reliable Determination of Fetal RhD Status by *RHD* Genotyping from Maternal Plasma. Transfus Med Hemother. 2013 Feb; 40(1): 37–43.

PS-4-32

Monocytes subsets are responsible for anti-HPA-1a mediated platelet phagocytosis in blood

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Background: Clearance of anti-HPA-1a antibody-opsonized fetal platelets by phagocytes represents the major mechanism of fetal and neonatal alloimmune thrombocytopenia. According to the general idea, opsonized fetal platelets are cleared in the spleen by tissue-resident macrophages via Fc-dependent mechanism. However, previous studies indicated the role of blood phagocytes on platelet clearance. In this study, anti-HPA-1a mediated platelet phagocytosis by blood phagocyte subsets was investigated.
Methods: Platelet phagocytosis caused by anti-HPA-1a standard sera was analyzed by Whole Blood Platelet Phagocytosis Assay (WHOPPA) using pH sensitive dye (pHrodo-SE) labeled platelets. PHrodo opsonized

platelets were incubated with whole blood and the rates of platelets engulfed by neutrophils and monocyte subsets were measured by flow cytometry using gating strategy as previously described (Fujimoto et al., 2000). The expression of FcγRs and HLA-DR on neutrophils and monocytes subsets was analyzed using anti-FcγRI, -II, III and anti-HLA-DR specific monoclonal antibodies.

Results: Anti-HPA-1a mediated platelet phagocytosis was only observed in monocytes, but not in neutrophils. In the control experiments, monocytes and neutrophils engulfed pHrodo labelled *E. coli* bacteria. Analysis of monocyte subsets showed that not only the most abundant conventional monocytes (CD16-; around 85%), but also other monocytes subsets (CD16+; around 12%) including non-classical, intermediate monocyte subsets were able to engulf anti-HPA-1a opsonized platelets. Interestingly, shifted monocyte subset (around 5%) with high phagocytosis rate was found. Furthermore, analysis of FcγRs and HLA-DR on monocytes showed significant decreased of FcγRI and increased of HLA-DR surface expression.

Conclusion: In this study, we showed by WHOPPA that monocyte subsets are the most dominant phagocytes in blood cells responsible for the clearance of anti-HPA-1a opsonized platelets in vitro. The significant expression changes of FcγRI and HLA-DR, formation of previously unknown shifted monocyte subset underline the importance of FcγRI and FcγRIII in platelet phagocytic process and subsequent immune response. This assay may allow us to study on antibody mediated platelet phagocytosis in ex vivo conditions.

PS-4-33
Soluble CD177: A new allele for an old antigen

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Background: Recently a missense mutation c.787A>T truncating CD177 protein has been introduced to regulate the absence of CD177 protein on the neutrophil surface of CD177-null individuals. Despite this, not all CD177-null individuals are homozygous for c.787T.

Here we investigated if c.1291G>A polymorphism by de-stabilizing CD177 GPI-anchor sequence, converts membrane-bound CD177 to a soluble form and leads to the absence of CD177 from the neutrophil surface in heterozygote c.787T CD177-null individuals.

Methods: To prove this hypothesis, stably transfected cells HEK293F cells expressing CD177 wildtype (c.1291G) and mutant c.1291A were produced.

The expression of CD177 in both forms in transfected cells was analyzed by immunoprecipitation and immunoblot. The membrane expression of CD177 in both allelic forms was evaluated in flow cytometry. The reactivities of CD177 alloantibodies with immobilized CD177 (in both forms) were assessed in ELISA.

In confocal laser scanning microscopy, co-transfected COS-7 cells expressing fluorescence labeled CD177 (wildtype and c.1291A mutant) and PR3, the co-localization of both CD177 allelic forms with PR3 was evaluated.

Results: Cell lysate analysis detected CD177 protein in cytoplasm of both transfected cells. However, flow cytometry analysis detected CD177 protein only on the surface of CD177 wildtype expressing cells but not c.1291A mutant.

Using anti-V5 mab, CD177 in both forms was precipitated from the cell culture medium. In ELISA comparative reactivity of anti-HNA-2 alloantibodies with both immobilized CD177 forms was detected.

In confocal laser microscopy analysis of COS-7 cells co-transfected with fluorescence-labeled CD177 (wildtype and c.1291A mutant) and PR3, CD177 (in both allelic forms) were detected with PR3 in the cytoplasm. However, on the cell surface PR3 was detectable only when cells were co-transfected with CD177 wildtype.

Conclusion: Here we describe a new allele of CD177 protein encoded by CD177 c.1291A. In comparison to CD177 wildtype, c.1291A allelic form showed decreased membrane stability and increased solubility. However, in ELISA, a comparable reactivity of CD177 alloantibodies with both proteins was detected indicating a similar conformational structure for both proteins. This data explain the absence of CD177 on the neutrophil surface of CD177-null individuals heterozygous for c.787T allele who carry c.1291A allele.

PS-4-34
Neonatal platelets loaded with anti-HPA-5b but no fetal/neonatal alloimmune thrombocytopenia (FNAIT): a case report

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Background: A recent systematic review showed that neonatal platelet counts do not appear to differ between suspected cases of FNAIT with and without HPA-5b antibodies (Alm J et al, Br J Haematol 2022). Given the high prevalence of anti-HPA-5b antibodies in unselected pregnant European women, the likelihood of incidental detection is high. Here, we report the natural course of HPA-5b-incompatible pregnancy in a 22-year-old woman with HPA-5b antibodies who was not treated with intravenous immunoglobulins.

Methods: All platelet antibody detections and crossmatch procedure (paternal platelets and maternal serum) were performed by monoclonal antibody immobilization of platelet antigens assay (MAIPA) as described by Kiefel et al. 1987, using monoclonal antibodies against glycoprotein (GP) IIb/IIIa, GPIa/IIa, GPIb/IX, CD109, CD36, PECAM-1 and HLA Class I-antigen. Acid elution treatment of newborn platelets was performed as described previously (Hotchkiss AJ et al, Blood 1986).

Results: The newborn was delivered at 40th week of gestation (3,050 g body weight, 49 cm body length) after an uneventful pregnancy. His platelet count at birth was 285 G/l. He had no signs or symptoms of bleeding. His was genotyped as HPA-5ab. Anti-HPA-5b antibodies were present in the maternal blood, and a crossmatch between maternal serum and paternal platelets showed positive reactions with GP Ia/IIa exclusively. A direct MAIPA performed with neonatal platelets showed elevated IgG bound to GP Ia/IIa but normal levels for IgG bound to GP IIb/IIIa and GP Ib/IX. An eluate performed from neonatal platelets revealed the presence of anti-HPA-5b antibodies on the newborn"s platelets.

Conclusion: In line with current evidence, we did not observe an effect of anti-HPA-5b antibodies in an untreated, second pregnancy of an anti-HPA-5b immunized mother. Although the antibodies were transferred through the placenta and could be eluated from the neonatal platelets, they apparently did not induce platelet destruction.

We believe that anti-HPA-5b does usually not induce FNAIT. Larger prospective studies are needed to determine its clinical relevance.

PS-4-35

Fetal and neonatal alloimmune thrombocytopenia: no evidence of systemic inflammation as a modulator of disease severity, but placental inflammation could be key

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Background: In fetal/neonatal alloimmune thrombocytopenia (FNAIT), maternal alloantibodies against paternal human platelet antigens (HPA) cross the placenta and lead to platelet destruction. The extent of thrombocytopenia varies among neonates, and inflammation may constitute an important trigger

Methods: A set of stable inflammatory markers was measured in serum samples from 100 neonates with low platelet counts, of which n=50 were diagnosed with FNAIT due to anti-HPA-1a antibodies and n=50 were thrombocytopenic without detectable maternal HPA antibodies.

Results: Concentrations of C-reactive protein, soluble CD14, procalcitonin, and sFlt-1 did not differ between the two cohorts. There was no correlation between C-reactive protein or soluble CD14 and the platelet count, but a negative correlation was observed between procalcitonin concentrations and the neonatal platelet count in both cohorts. sFlt-1 concentration and the platelet count were correlated in FNAIT cases exclusively. None of the inflammatory markers was statistically different between cases with and without ICH.

Conclusion: We were unable to identify systemic inflammation as a relevant factor for thrombocytopenia in FNAIT. The antiangiogenic enzyme sFlt-1 did correlate with the platelet count in the FNAIT cohort. Our findings may give rise to the hypothesis that placental inflammation rather than systemic inflammation modulates disease severity in FNAIT.

PS-4-36

Alpha-Methyldopa-induced autoimmune hemolytic anemia diagnosed after delivery

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Background: Methyldopa is an antihypertensive medication the use of which is limited to pregnant women. Alpha-methyldopa commonly induces warm reactive IgG autoantibodies but only rarely leads to symptomatic autoimmune hemolytic anemia with an incidence of approximately one case per million. We report a case of methyldopa-induced autoimmune hemolytic anemia in a 36-year-old primipara woman.

Methods: The patient started methyldopa in the second trimester of pregnancy. Two months later she developed a positive direct antiglobulin test (DAT) and her plasma tested positive for free warm reactive autoantibodies without anemia. The patient continued the drug after delivery and six months later she presented to the emergency unit with jaundice, fatigue and dark urine. Laboratory evaluation on admission revealed low hemoglobin at 6,2 g/dl, elevated lactate dehydrogenase and bilirubin and low haptoglobin. Serologic workup showed a 4+ direct antiglobulin test with anti-IgG only, no complement fixation, and warm reactive autoantibodies in her plasma and in an eluate prepared by acid elution.

Results: Withdrawal of methyldopa and corticosteroid therapy resulted in rapid increase of the hemoglobin level within a few days. At birth the newborn had shown a positive DAT without maternal ABO-incompatibility, a bilirubin level near the upper and hemoglobin level near the lower reference interval. Since there were no clinical signs of erythroblastosis further treatment was not required.

Conclusion: Diagnosis of the drug independent subtype of drug induced autoimmunhemolytic anemia can not be made by serologic tests alone. It requires detailed medication history, clinical presentation and course in combination with serologic findings. Thus obstetricians, family doctors and immunohematologists providing care for pregnant women should be aware of this possibly hazardous phenomenon.

PS-4-37

World Human Neutrophil Antigens (HNAs) Investigation Survey (WHIS)

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Background: Human neutrophil antigens (HNA) are categorized into five subgroups (HNA-1 to -5). Given the importance of neutrophils in immunity, we sought to create awareness of the role of HNA diagnostic services in managing immune neutropenia and TRALI. To provide health communities all around the world with access to these services we conducted a survey to create a directory of these HNA diagnostic services.

Methods: An Excel table-based survey was created to capture information on the laboratory"s location and was emailed to 55 individuals with known or possible HNA investigation activity. The collected data were then summarized and analyzed.

Results: Of contacted laboratories, the surveys were returned from 23 (38.2%) labs; 17 have already established HNA diagnostic (Elabs; of them 12 were regular participant of International Granulocyte Immunobiology Workshop (ISBT-IGIW)), four labs were in the process of establishing their HNA investigation (LiD) and the remaining two responder labs, did not conduct HNA investigations. In Elabs, investigation for autoimmune neutropenia (infancies and adults) was the most frequently requested, and antibodies against HNA-1a and HNA-1b the most commonly detected.

Conclusion: The directory of survey respondents provides a resource for health professionals wanting to access HNA diagnostic services. The present study offers a comprehensive picture of HNA diagnostics (typing and serology), identifying weak points and areas for improvement for the first time. Identifying more laboratories involved in HNA diagnostics with limited access to international societies in the field will globally improve HNA diagnostics.

PS-4-38

Noninvasive fetal genotyping of red blood or platelet antigens using next generation sequencing – Experience of the last 8 years

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Background: Hemolytic disease of the fetus and newborn and fetal/ neonatal alloimmune thrombocytopenia are caused by maternal antibodies against paternal inherited fetal alloantigens on red blood cells or platelets. Noninvasive fetal genotyping is required to determine whether the fetus is at risk. Next-generation sequencing (NGS) has shown to be a reliable method for the noninvasive determination of fetal blood groups for diagnostic purposes.kein

Methods: Cell-free DNA was isolated from plasma of pregnant women with different gestational age and proven irregular alloantibodies against red blood cell antigens or platelets. A primer panel was designed to target sequences flanking single-nucleotide polymorphisms (SNPs)/exonic regions of *GYPA* (MNS), *GYPB* (MNS), *RHD*, *RHCE*, *BCAM* (LU), *KEL*, *ACKR1* (FY), *SLC14A1* (JK), *SLC4A1* (DI), *AQP1* (CO), *ITGB3* (HPA-1), *ITGA2* (HPA-5), CD109 (HPA-15), SRY and autosomal SNPs for internal control. All samples were sequenced using next-generation sequencing.

Results: In all samples, sequencing of polymorphic regions coding for common blood group antigens, SRY, and of anonymous SNPs allowed quantification of the fractional fetal DNA concentration. Non-maternal sequences were correctly determined in all pregnancies with a fraction of cell free fetal DNA that reached the pre-defined cut-off value of 4 %. In some pregnancies, the fetal fraction was below 4% and thus follow-up testing was recommended. In most cases, typing results were verified by confirmatory typing after birth.

Conclusion: Next generation targeted sequencing is a sensitive and specific tool for non-invasive prenatal diagnosis of fetal blood groups.

PS-4-39

Passenger lymphocyte syndrome – A rare cause of immunohematological problems and hemolysis after transplantation

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Background: Passenger lymphocyte syndrome (PLS) is a typical complication of solid organ or hematopoietic stem cell transplantation (HSCT). It is caused by donor-derived B-lymphocytes producing antibodies to antigens on the recipient's red blood cells, which can lead to hemolysis or even graft failure. PLS is considered rare, but the true incidence is unknown because many cases go undetected. We report three cases of PLS at our institution.

Methods: One patient each was studied after HSCT, donor lymphocyte infusion (DLI) and after heart-lung transplantation. All three patients were diagnosed with PLS by serological abnormalities or unexplained antibodies during routine blood bank testing.
Serological studies including antibody screening test in the indirect antiglobulin test (IAT) and papain test, as well as direct antiglobulin test (DAT) were performed using the standard gel technique (Biorad, Cressier sur Morat, Switzerland and Grifols, Barcelona, Spain). Red blood cell (RBC)-bound antibodies were eluted using the acid method (BAG, Lich, Germany).

Results: The clinical and serological data of the patients are shown in Table 1. PLS occurred between 10 and 20 days following transplantation. Patients 1 and 2 developed hemolytic anemia (Hb minimal 6.2 / 8.8 g/dl and LDH 1664 / 431 U/l, respectively and haptoglobin below detection limit), but only patient 1 needed therapy (RBC transfusion, i.v.Ig, prednisolone). Patient 3 had already changed to the donor's blood group (A RhD-) before transfusion of the DLIs, therefore hemolysis did not occur. Of the irregular RBC antibodies, only the donor anti-D from patient 1 was known before transplantation. In patient 2, the PLS was noticed mainly by a positive crossmatch with RBCs of type B, anti-B on the recipients RBCs and a negative antibody screening test

Conclusion: The diagnosis of PLS can be difficult, and the clinical and serologic picture varies. PLS typically presents 2-3 weeks following transplantation with or without hemolysis in combination with a (newly diagnosed) positive DAT and/or detection of antibodies against the recipient"s blood type. The latter must not be confused with autoantibodies as they are donor-derived alloantibodies, and antigen-negative blood must be provided in cases of anemia requiring transfusion.

Disclosure Statements: The authors declare no conflicts of interest

PS-4-40

Case report of resolving a KEL1 genotype-phenotype discrepancy with full-gene haplotype sequencing by Oxford Nanopore Technologies

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Background: Since the KEL1 antigen of the Kell blood group system is very immunogenic, it is usually determined in routine donor typing. At our blood service, KEL1 is determined by both serology and high-throughput genotyping. Genotype-phenotype discordances are normally resolved by laborious Sanger sequencing of all 19 exons without haplotype phasing capability. Here, we present an alternative protocol relying on Oxford Nanopore Technologies (ONT), which enables the generation of full-gene haplotypes.

Methods: Expression of KEL1 antigen was measured by standard serological techniques. Four variants within the *KEL* gene were part of our MALDI-TOF MS based high-throughput blood group genotyping routine, including c.578C>T determining KEL1/2 expression.

Tab. 1.

Table 1: Clinical and serological findings

Pat. no	Age	Transplantation	Recepient BG	Donor BG	Antibody	DAT	Onset PLS*	Hemolysis
1	43	HSCT	B RhD+, Jk(a+)	AB RhD-, Jk(a-)	anti-D, -Jk(a)	IgG4+, C3d 3+	16	yes
2	15	heart-lung	B	O	Anti-B	IgG, C3d	15	yes
3	72	DLI	O RhD+	A RhD-	Anti-D	negative	20	no

*days after transplantation, BG: blood group

Genotype-phenotype discrepancies were reassessed by commercially available PCR-SSP kits (inno-train, Germany) and extensive serological confirmation. To resolve a discrepancy in one donor, the entire *KEL* gene (~21 kb) was amplified in two long-range PCRs (fragments of 12.7 and 14.3 kb, respectively), exhibiting a large overlap (~4.4 kb) essential for haplotype phasing. Amplicons were sequenced on a Flongle flow cell (ONT) and detected exonic variants were confirmed with Sanger sequencing.

Results: We identified by routine donor typing a heterozygous *KEL*01/02* blood donor with a K-k+ phenotype. This discrepancy pointed to a potential null allele (*KEL*01N*). One hour of ONT sequencing already yielded ~450 reads covering both long-range PCR fragments, which is a high read-depth for variant calling. A heterozygous variant in the overlap sequence allowed complete gene haplotype phasing. In exon 11, we identified a missense variant c.1241C>A (Thr414Lys, rs13842342704), which was phased to the *KEL*01* allele. The variant is yet undescribed, despite the over 100 alleles collected by the ISBT, and was confirmed by Sanger sequencing. Adsorption-elution experiments for in-depth classification of true null or very weak expression are underway.

Conclusion: Using ONT sequencing, we resolved a genotype-phenotype discrepancy within short turnaround time and discovered a novel putative *KEL*01N* allele. The long reads allowed phasing detected variants to the respective *KEL*01/02* background and even constructing full-length *KEL* haplotypes. The use of a protocol and flow cell optimized for single-sample analysis kept time and expenses competitive. Overall, our approach proved very promising for resolving genotype-phenotype discrepancies.

PS-4-41
Case report of a rare JMH negative patient and detailed characterization with serological and molecular methods

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Background: In a patient with known nummulation who awaited elective surgery an antibody with 100% panel reactivity was detected. Initial screening approaches by IAT gelcard technique with or without enzyme or DTT and inhibition with recombinant blood group antigens focused suspicion onto a JMH HTLA-antibody. Further analysis by extended blood group serology and DNA sequencing should identify the antibody specificity and answer the question if the antibody is relevant with respect to transfusion reactions.

Methods: Extended testing by IAT gel technique under different conditions using specialized differentiation panels (BioRad, CH; DRK-BAD, DE) as well as Capture-R Ready-Screen (Immucor, GA, USA), and rBGA JMH, Yt(a) and Kn(a) for neutralization (Innotrain, DE) were used for antibody differentiation. Additionally, three JMH negative test cells were included. Extended genotyping by in-house PCR-SSP and mass spectrometry covering a wide range of blood group alleles should support definition of the patient's own blood groups. Because of a suspected JMH negative phenotype, DNA-sequencing should identify the molecular basis of the deficiency. Custom primers specific for JMH were used for amplification and sequencing reactions of all 14 exons.

Results: The patient's plasma showed continuous moderately positive reactions in the IAT gel technique and capture assay. The papain IAT and DTT treatment were negative. The auto control and eluate of the patient's RBC were negative too, but the DAT showed a moderately positive reaction. Missing serum reactivity with three JMH negative cells identified an anti-JMH. The patient's JMH antigen was tested negative as well.

Neutralization with rBGA did not result in a negative test, but further clinically relevant antibodies were mostly excluded. Sanger sequencing is currently conducted to determine possible mutations in the SEMA7A gene, which consists of 14 exons. First amplification reactions yielded sufficient PCR-products for downstream analysis.

Conclusion: Extended blood group serology and molecular typing identified a rare anti-JMH in a patient before elective surgery. Even although no clinically relevant hemolysis has been described for this antibody, laboratory analysis and crossmatch should be performed timely if urgent blood transfusion should be needed. Additionally, autohaemotherapy should be considered. In such complex cases extended molecular typing can help to identify the patient's blood groups and the molecular basis of the deficiency.

PS-5
Immunotherapy | Stem Cells

PS-5-1
Prolonged thrombocytopenia and severe transfusion reaction after ABO-incompatible allogeneic hematopoietic stem cell transplantation in a patient with chronic myelomonocytic leukemia

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Background: Major ABO-incompatible allogeneic hematopoietic stem cell transplantation (allo-HCT), is a common practice and represents a challenging transfusion scenario. Prolonged thrombocytopenia with increased platelet transfusion needs is one of its reported adverse effects, and this has been linked to the persistence of recipient anti-donor isoagglutinins.

Methods: A 55-year-old male patient, O, Rh(D)-positive, with chronic myelomonocytic leukemia (CMML) underwent major incompatible allo-HCT from a A, Rh(D)-negative donor. He suffered prolonged thrombocytopenia and multiple transfusion reactions after A, Rh(D)-negative platelet transfusions.

Results: A transfusion reaction work-up was initiated. The minor compatibility test was negative. No eluate was performed due to the absence of detectable IgG on the posttransfusion direct antiglobulin test (DAT) sample. Microbiological evaluation of the remaining platelets revealed no bacterial contamination. HLA-work-up revealed HLA-antibodies class I versus HLA B76 in weak reaction strength. Due to the rarity of the HLA trait, HLA-matched platelet transfusions were not possible. An additional platelet work-up was performed. No platelet-specific antibodies were detected. Finally, anti-A1 IgM titers in the patient were assessed showing a titer of >1:2048. Based on this result, platelet transfusions were restricted to O Rh(D)-negative donors.

Conclusion: In case of transfusion reactions against platelet products in major ABO-incompatible allo-HCT patients, isoagglutinin monitoring should be considered and a change in the platelet transfusion protocol maybe beneficial in patients presenting high isotiters against recipient's blood type.
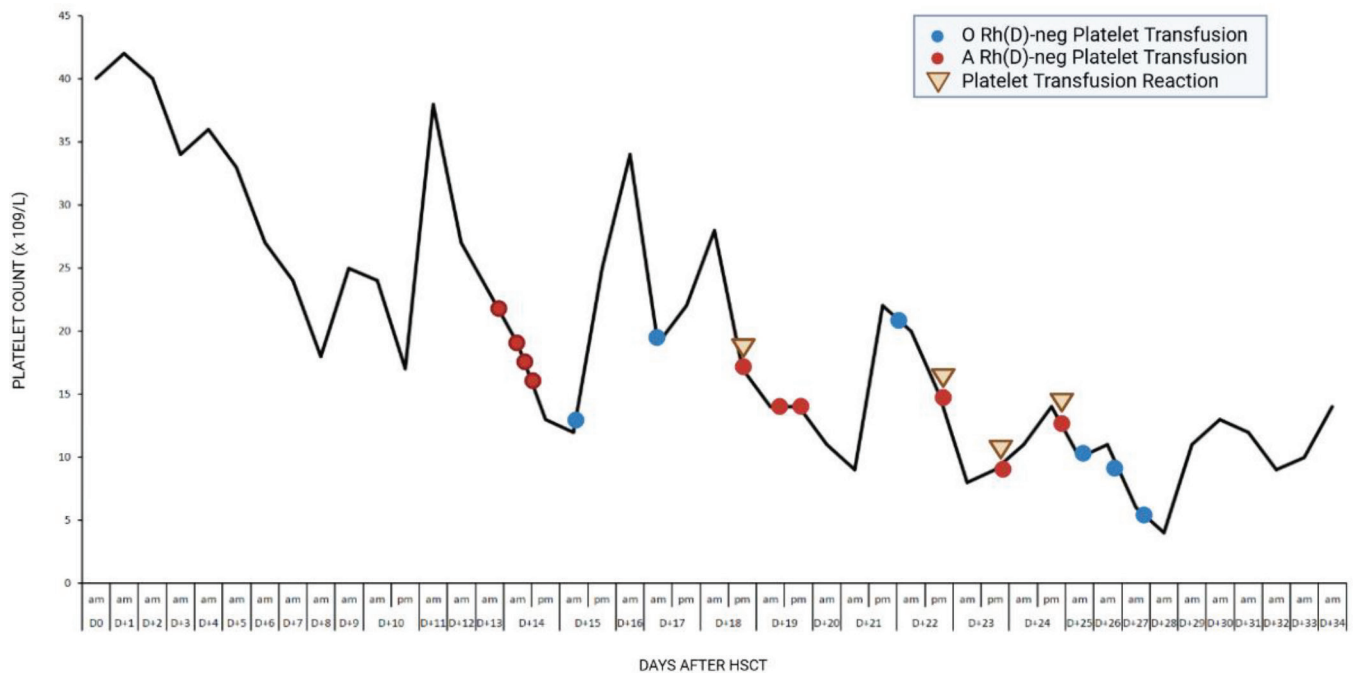
Fig. 1.

PS-5-2

Phenotype characterization of monocyte-derived dendritic cells from human umbilical cord blood

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Background: Umbilical cord blood (UCB) describes a valuable resource of stem cells for pediatric and ethnic minority stem cell transplantations. Monocytes, as part of the innate immune system, are able to generate dendritic cells (MoDC), and thus play an important role in T-cell activation and cellular therapies. However, UCB-MoDC have not been extensively characterized in previous studies. This study aims to characterize the phenotype of UCB-MoDC to assess possible applications in cellular therapies.
Methods: In order to characterize UCB-derived monocytes and MoDC, CD14^{+} monocytes were magnetically isolated from healthy adult donors and UCB-derived PBMC (purity >95%), differentiated with IL-4 and GM-CSF for 5 days followed by their maturation (IL-1β, IL-6, TNFα, IFNγ, PGE$_2$) for additional 2 days. Characterization and survey of monocyte and MoDC viability and surface marker expression for activation, migration and differentiation were analyzed on d0, d5 and d7 by flow cytometry.
Results: Freshly isolated UCB CD14^{+} monocytes displayed significantly lower frequencies in CD11c, HLA-DP,DQ,DR, and Fas-L expression in comparison to adult CD14^{+} monocytes. Comparison between adult and UCB-MoDC cultures revealed no significant differences in vitality and cell recovery. Expression of key MoDC markers such as CD209 and CD40 were similar between adult and UCB-MoDC. However, UCB-MoDC displayed significantly lower frequencies for CD11c and CD80 expression while expression levels were significantly reduced for CD11c, CD83, CD274 (PD-L1) and CCR7 in comparison to adult MoDC cultures. Simultaneously, frequencies of CD14 and CD16 expressing UCB-MoDC were significantly greater than in adult MoDC cultures.
Conclusion: UCB provide a viable source of monocytes for the generation of MoDC. Further investigations will clarify if the observed differences in co-stimulator and chemokine receptor expression translates to functional differences in comparison to adult MoDC in order to evaluate their potential for potential future clinical applications.

Disclosure Statements: The authors have no conflict of interests to declare.

PS-5-3

A "multimorphic" variant in IRF4 causes human autosomal dominant combined immunodeficiency

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Background: Interferon regulatory factor 4 (IRF4) is a transcription factor and key regulator of immune cell development and function.
Methods: To determine disease causing genetic variants whole-exome and Sanger sequencing of patient and family members' DNAs was performed. A plethora of cellular and molecular methods (e.g. flow cytometry, CyTOF, RNA-seq, ChIP-seq and EMSA) were used to characterize the immunological phenotype of the patients and to reveal the underlying pathomechanism of their diseases.
Results: We report a recurrent heterozygous mutation in IRF4, causing an autosomal dominant combined immunodeficiency in 7 patients from 6 families. The patients exhibited profound susceptibility to opportunistic infections and presented with agammaglobulinemia. Patients' B cells showed impaired maturation and defective plasma cell differentiation. The IRF4 variant T95R maps to the transcription factor's DNA-binding domain and results in a simultaneous "multimorphic" combination of loss-, gain- and new-functions for IRF4.
Conclusion: An IRF4 mutation with "multimorphic" impacts on DNAbinding specificity and activity reveals a new disease-causing mechanism in humans.

Disclosure Statements: No conflics of interests to declare.

PS-5-4

Antibodies inhibiting cell-to-cell spread of herpes simplex virus type 1 protect against viral reactivations: a retrospective study

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Background: Herpes simplex viruses (HSV) cause ubiquitous human infections. For vaccine development, knowledge concerning correlates of protection is essential. Therefore, we investigated (I) if humans are in principle capable producing cell-to-cell spread inhibiting antibodies against HSV and (II) whether this capacity is associated with a reduced HSV-1 reactivation risks.

Methods: We established a high-throughput HSV-1-ΔgE-GFP reporter virus-based assay and evaluated 2,496 human plasma samples for HSV-1 glycoprotein E (gE) independent cell-to-cell spread inhibiting antibodies. Subsequently, we conducted a retrospective survey among the blood donors to analyze the correlation between the presence of cell-to-cell spread inhibiting antibodies in plasma and the frequency of HSV reactivations.

Results: In total, 128 of the 2,496 blood donors (5.1%) exhibited high levels of HSV-1 gE independent cell-to-cell spread inhibiting antibodies in the plasma. None of the 147 HSV-1 seronegative plasmas exhibited partial or complete cell-to-cell spread inhibition, demonstrating the specificity of our assay. Individuals with cell-to-cell spread inhibiting antibodies showed a significantly lower frequency of HSV reactivations compared to subjects without sufficient levels of such antibodies.

Conclusion: This study contains two important findings: (I) upon natural HSV infection, some humans produce cell-to-cell spread inhibiting antibodies and (II) such antibodies correlate with protection against recurrent HSV-1. Moreover, these elite neutralizers may provide promising material for immunoglobulin therapy and information for the design of a protective vaccine against HSV-1.

Disclosure Statements: No conflicts of interest

PS-5-6

Follow-up of serotype-specific antibodies after sequential vaccination with two pneumococcal vaccines in kidney transplant recipients

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Background: To reduce the morbidity and mortality from invasive pneumococcal disease, vaccination against Streptococcus pneumoniae is recommended in kidney transplant recipients, using sequential vaccination with the 13-valent pneumococcal conjugate vaccine Prevenar 13 (PCV13) and the polysaccharide vaccine Pneumovax 23 (PPSV23). However, there are currently no data on serological responses after sequential vaccination in this cohort.

Methods: In the current study, we sequentially vaccinated 46 kidney transplant recipients with PCV13 and PPSV23 and determined serotype-specific and global anti-pneumococcal antibody responses in the year following vaccination.

Results: After vaccination, serotype-specific and global antibody concentrations were significantly higher compared to baseline. We observed that serotype-specific antibody responses varied by serotype (2.2- to 2.9-fold increase after 12 months). The strongest responses after 12 months were detected against the serotypes 9N and 14. Global IgG2 antibodies against pneumococci revealed the highest increase, IgM antibodies the lowest. Sequential vaccination with both vaccines achieved higher antibody levels in comparison with a historical cohort studied at our institute, that was vaccinated with PCV13 alone. During the 12-months follow-up period, none of the patients developed pneumococcal-associated pneumonia or vaccination-related allograft rejection.

Conclusion: In conclusion, we strongly recommend sequential vaccination over single immunization in kidney transplant recipients.

PS-5-7

Unstimulated autologous and allogenceic mononuclear cell apheresis

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Background: Autologous lymphocytes can be used therapeutically for the production of CAR-T cells or other cellular therapeutics. The dose of lymphocytes could be important in this context. We present data on allogeneic and autologous mononuclear cell apheresis (MNC-A) to obtain starting material for CAR-T cells, donor lymphocyte infusions (DLI) or allogeneic lymphocytes for research (RL). We hypothesise that there are differences in cell dynamics between allogeneic and autologous MNC-A.

Methods: All MNC-A procedures between 10/2009 and 04/2023 were extracted from our electronic records (n=1312). MNC-A was performed with the Cobe Spectra or the Spectra Optia. We started with MNC-A for CAR-T-cells in 2019 using exclusively the Spectra Optia cell separator. The absolute lymphocyte count (ALC) was obtained before and after apheresis. Collection efficiency 2 was calculated as follows: CE2=separated total blood volume * CD3+ cell count in peripheral blood /collected CD3+ cells. Data is presented as median with interquartile ranges. A Mann-Whitney test or a Wilcoxon matched pairs test was used to test for significance.

Results: For CAR-T-cell-MNC-A (n=48), ALC was 690/µl (60; 1480/µl) and CD3+ cell count was 432/µl (268; 915/µl) before MNC-A. The total body blood volume was processed (PTBV) 2.0-fold (1.8; 2.1 fold). CE2 for CD3+ cells was 76.9% (67.3; 87.6%). After CAR-T-Cell-MNC-A, ALC decreased to 520/µl (370; 940/µl) (75% (59; 98%)). For all allogeneic MNC-A (DLI and RL, n=1260), ALC was significantly higher than in CAR-T-cell-MNC-A: 1550/µl (1310; 1860/µl) (p= 0.0086) and decreased to 87% (78; 96%). Comparable DLI-MNC-A (n=66) with respect to PTBV (1.9 (1.85; 1.9) fold) were extracted from the database. These matched DLI-MNC-A again showed significantly less reduction in post-MNC-A lymphocytes to 90% (81; 102%) than observed in CAR-T-cell-MNC-A (p= 0.0079).

Conclusion: Lymphodepleted patients had a significantly lower pre apheresis lymphocyte count than healthy donors and showed a significantly reduced capacity to mobilize lymphocytes into blood indicating a severe impairment of the body lymphocyte pool.

PS-5-8

Hyperglycaemia and dyslipidaemia synergistically amplify TLR-mediated inflammatory macrophage responses

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Background: Hyperglycemia (HG) is a critical factor in the initiation of diabetic complications. Macrophages are key innate immune cells that regulate inflammatory responses which are responsible for the development of micro- and macrovascular complications. Increased expression of toll-like receptor 4 (TLR4) has been inked to type 2 diabetes (T2D). Here for the first time, we systemically addressed the role of hyperglycemia in the regulation of TLR system in primary human macrophages.
Methods: Expression of TLR 1-9 was examined in primary human monocyte-derived homeostatic M(NS), inflammatory M1(IFNgamma) and healing M(IL4) macrophages in normoglycemic and hyperglycemic (HG) conditions by RT-PCR and flow cytometry. Cytokine secretion was quantified by ELISA.
Results: HG induced upregulation in expression of TLR1 and TLR8 in M0 macrophages, TLR1, TLR2 and TLR6 in M1, and TLR4 and TLR5 in M2. HG potentiated TLR4-mediated response of M2 to LPS and significantly enhanced production of IL1beta. In M (IL4), HG in combination with PAM3CSK4 (PAM3), synthetic triacylated lipopeptide, ligand for TLR1/TLR2 amplified expression of TLR4, enhanced production of IL1beta, and supressed production of IL10.
Conclusion: We found that hyperglycaemia alone enhances inflammatory potential of homeostatic, inflammatory, and healing macrophages by increasing specific profiles of TLRs. In combination with dyslipidaemic ligands, hyperglycaemia can switch the inflammatory program in healing (M2) macrophages towards supporting vascular inflammatory complications in diabetic patients.

PS-5-9

The HLA specific IgG panel from recipient CD38+/138+ B cells represents a resilient surrogate marker for safe solid organ transplantation

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Background: HLA specific antibodies in peripheral blood leads to the exclusion of donors carrying the corresponding HLAs while the absence of DSAs leads to the acceptance of donors, even in the presence of repeated HLA mismatches. As a secondary immune response might remain unseen the need to provide a robust method for immune monitoring before SOT becomes obvious. The existence of circulating antibodies cannot be translated into reliable information on the memory B cell compartment.
Methods: We generated an artificial secondary immune response *by* re-expose recipient B cells to repeated HLA mismatches *in vitro*. Utilizing soluble HLA technology, recombinant HLA class I or II molecules were expressed and affinity purified. From whole blood of recipients polyclonal B cell activation was performed using the TLR7/8 agonist Resiquimod and IL-2 to generate memory B cell-derived plasma cells. A sophisticated ELISPOT assay has been developed using recombinant HLA molecules as targets for secreted IgGs from CD19+/27+/138+/38+ B cells. Autologous recombinant HLA antigens served as negative controls.
Results: This artificial secondary immune response allowed unequivocal detection of memory B cells against a specific HLA antigen. We could detect HLA specific memory B cells in recipients where no DSAs following a documented immunization record have been specified. In other cases, the emerge of a secondary immune response following acceptance of a

repeated mismatch could be virtually excluded, when no HLA specific memory B cells could be detected.
Conclusion: The ELISPOT enables the quantitative and qualitative detection of HLA-specific memory B cells and to dissect donor/recipient incompatibilities that would remain unseen with accepted screening methods. In living kidney Tx, this method has been applied to adjust donor selection to prevent humoral immune responses. This detection of a secondary immune response has the potential to be used in routine diagnostics, as it provides robust and incontrovertible data on the individual immunization status.

PS-5-10

HLA-B*57:01/small molecule interactions induce inflammatory processes and trigger the outcome of Steven Johnson Syndrome post CBZ administration

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Background: Extensive clinical trials prior to license of a drug could not prevent certain cellular drug-mediated severe immune reactions. Dependent on the genotype, Carbamazepine (CBZ) hypersensitivities cause diverse clinical pictures as MPE, DRESS (HLA-A*31:01) or more fatal diseases SJS or TEN (HLA-B*15:02). Recently, the association between HLA-B*57:01 and CBZ administration could be demonstrated to result in SJS/TEN.
Methods: Genetically engineered human B-LCLs expressing sHLA-B*57:01 molecules were treated with CBZ or its metabolite carbamazepine-10,11-epoxide (EPX). The incapability of these B-LCLs to metabolize CBZ makes them an ideal system for analyzing the influence of CBZ and EPX orthogonally. HLA/drug complexes were purified and the availability of CBZ or EPX was monitored by mass spectrometry. The cellular alteration through HLA/drug interdependence was measured by full proteome analysis.
Results: The data reveal significant stronger engagement of B*57:01 to the metabolite EPX than to CBZ. Subsequent full proteome analysis of engineered B*57:01+ cells following CBZ or EPX treatment uncovered an unknown mechanism; HLA-B*57:01/EPX complexes introduced drastic proteomic alterations as the induction of inflammatory processes through the upstream kinase ERBB2 and the upregulation of NFκB and JAK/STAT pathway implying a pro-apoptotic, pro-necrotic shift in the cellular response. Simultaneously, anti-inflammatory pathways and associated effector proteins were downregulated.
Conclusion: Fundamental knowledge of drug-susceptible HLA molecules, their drug antagonist and the disequilibrium of the proteomic content will certainly facilitate personalized and safe medication.

PS-5-11

Sulfated glycans prevent platelet-related cancer progression

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Background: Cancer growth requires physical contact between platelets and tumour cells. This extracellular supply of growth factors is essential for cell growth. All dividing cells have receptors for platelets. Blocking them inhibits cell growth and prevents metastasis and drug resistance. Heparins and even non-anticoagulant highly sulfated relatives can effectively block these receptors, offering a well-tolerated and cost-effective treatment that may reduce cancer drug doses and minimise side effects.
Methods: Heparins can prevent cancer-associated thrombosis in tumour patients and slow down tumour progression. High platelet counts cause

more rapid tumour growth. These observations are related. All primary cell cultures from tumours and normal tissues require a supplement, usually foetal bovine serum, to thrive. This supplement contains growth factors necessary for cell division. These are not synthesised in cancer cells but in megakaryocytes in the bone marrow and are transported in our circulation in platelets and released from platelets during serum production. Receptors for growth factors enable cells to take up these. A mechanism must therefore exist in vivo which allows cells to acquire these factors.

Results: All cells in the cell cycle, malignant and benign, possess platelet receptors. After docking to these receptors, platelets fuse with the cell membrane and release their contents into the cytoplasm. Proliferating cells now receive growth factors and the molecular machinery for making the same.

The anticoagulant effect of heparins and their blockade of the platelet receptor are independent of each other. Blocking the physical contact between cells and platelets inhibits cell growth. This is the mode of action of the heparins on tumour growth. Remarkably, not only is cell proliferation thereby blocked competitively but it also prevents metastasis. Such adjuvant treatment also prevents resistance to anticancer drugs.

Conclusion: Modified non-anticoagulant heparins and highly sulfated heparans, devoid of anticoagulatory activities, also effectively block these receptors. In contrast, fondaparinux does not affect the platelet binding to cells. Platelets thus assume a new, hitherto little-appreciated role in regulating cell growth.

The dosage of anticancer drugs can be reduced through such adjuvant treatment. Adverse side effects will become rarer, and metastases and chemoresistance will be a thing of the past.

Disclosure Statements: Der Autor verneint Interessenkonflikte. Er ist CVO (Chief Visionary Officer) des kleinen Unternehmens Cell Receptor GmbH, und dessen Muttergesellschaft, welche gegründet wurden, um eine klinische Studie zum vorstehenden Thema zu ermöglichen und wundert sich darüber, dass einfache und effektive Verfahren zur Verbesserung onkologischer Ergebnisse nicht die ihnen gebührende Beachtung finden.

PS-5-15

Ten years of manufacturing MSC at the IKT Ulm – perspectives for autologous MSC therapies?

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Background: Application of mesenchymal stromal cells (MSCs) as advanced therapy medicinal product (ATMP) is very versatile due to their regenerative and immunomodulatory properties. Approximately 90 therapeutic doses have been produced for 9 phase 1 - 3 non-commercial clinical trials at IKT Ulm. Autologous non-cryopreserved MSC were used within 24 after production for bone augmentation, treatment of fractures, osteonecrosis, osteoarthritis and multiple sclerosis.

Methods: MSC used in 9 clinical trials are manufactured in a xenogenic free system established at 5 different manufacturing centers in Europe. Heparinized bone marrow (BM) is received within 24 hours and seeded in alphaMEM supplemented with platelet lysate (50.000/cm²). Medium exchange is performed 2x per week for 10 or 14 days and harvested MSC (MSCP0) are reseeded at a density of 4000 cells/cm2 for 5 or 7 days. The investigational medicinal product (IMP) is packaged in syringes in physiological saline supplemented with or without 5% human serum albumin and immediately shipped after release to be applied to the patient within 24 hours or 48 hours using an accompanied transport service of a qualified courier company.

Results: All trials have proven the feasibility and safety of MSC so far. There were no adverse events. Consolidation after one year was >90% in ORTHO1 trial (non-union bone fractures), >70% in ORTHO2 trial (osteonecrosis of femoral head) and 100% in MAXILLO1 trial (bone augmentation prior to dental implant)

The use of autologous, non-cryopreserved MSC may be critical. Due to yet unknown factors, failure of manufacturing clinical doses occurred for some patients. Factors that may influence the manufacturing process are e.g. transport time, experience of the physician performing the small volume BM collection, but also patient variation and costs for manufacturing autologous MSC for individual therapy are very high.

Conclusion: The use of autologous MSC is safe, effective but expensive. Failure of MSC manufacturing cannot be excluded. Cost reduction could be performed by using automated, large scale bioreactor systems for manufacturing of huge amounts of cells from one or few donors for allogeneic applications. A product from the shelf could be transported frozen, without the request for an accompanied transport. We have tested different bioreactor systems for their suitability to isolate and/or expand MSC.

Disclosure Statements: The authors declare no commercial, proprietary or financial interest. The manufacturing of MSC and the clinical trials were financed by projects within the European Union's Seventh Framework Programme and the European Union's Network HORIZON2020. The materials presented and views expressed here are the responsibility of the authors only. The European Union Commission takes no responsibility for any use made of the information set out.

PS-5-16

Qualitiy of cord blood derived stem cells after 15 years of storage in the vapour phase over liquid nitrogen

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Background: Umbilical cord blood (UCB) is a valuable source of hematopoietic stem cells. So far, no end of shelf life has been defined for such stem cell products stored cryopreserved in the vapour phase over liquid nitrogen. We present cell content and viability measurements of UCB derived stem cell concentrates and attached sampe tubes thawed after 15 years of storage.

Methods: A total of 13 cord blood derived stem cell concentrates and 25 pilot tubes were thawed for further measurements and investigations. For each individual sample, the relative content of CD34+ cells, CD45+ cells, mononuclear cells (MNC) and viability of CD34+ cells and CD45+ cells were determined by flow cytometry. In addition, CFU tests were performed with each sample. Based on these values the absolute cell counts and recovery rates (CD34+, CD45+, MNC, CFU) were calculated.

Results: The recovery rate of CD34+ cells of the UCB concentrates was 78.6 +/- 20.1 % (mean +/- standard deviation). Viability of CD34+ cells was 88.4 +/- 5.3 %. The CFU recovery rate of UCB concentrates was 78.6 +/- 39.6 %. Cell viability, relative and absolute cell content, and the recovery rates of the different cell types and CFU were significantly lower in the corresponding pilot tubes compared to the UCB concentrate bags. However, there were significant correlations of the absolute number of different cell types in the PRB concentrates compared to the corresponding pilot tubes.

Conclusion: The quality of UCB stem cell concentrates after 15 years of storage is good. A high recovery rate of CD34+ cells and good viability were found. CFU testing showed very good clonogenic potential of the UCB products. These results are in line with published research. Cord blood can be used for transplantation after 15 years of storage. Pilot tubes are representative for the respective cord blood unit. However, quality of the pilot tubes after long-term storage is worse than the PRB concentrates.

Effect of expansion media on functional characteristics of bone marrow-derived mesenchymal stromal cells

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Background: Therapeutic application of mesenchymal stromal cells (MSC) implies their *ex vivo* expansion in growth media that can affect MSC characteristics. Human platelet lysate (PL) is a xenogeneic-free (XF) cell culture supplement but its mostly unknown composition is a big drawback. Hence, defined serum- and xenogeneic-free (SF/XF) media were developed, though, most of them led to poor cell growth. We expanded MSC in either XF or SF/XF media or in mixtures of both and analyzed functional characteristics.

Methods: MSC were expanded in aMEM+8%PL (XF, media 1), StemMACS (Miltenyi Biotec; SF/XF, media 13), or in mixtures of both media (media 4, 7, 10; for composition see Table 1). Proteome of media and cells was only analysed for XF and SF/XF approaches while further analyses were performed for all media. Specific growth factors, cytokines, chemokines, hormones and other factors were determined in media and MSC conditioned media. Consumption of growth factors or secretion of functionally relevant factors was analysed. Expression of surface antigens related to metabolism, adhesion, differentiation and other cellular processes was investigated. Differentiation potential and migratory capacity of MSC were determined as functional cell characteristics.

Results: Comparison of proteome of XF and SF/XF media identified various proteins exclusive for each media. Respective cells showed significant

Tab. 1.

	media					
	1	4	7	10	13	
αMEM+8%PL [%]	100	95	50	5	0	
MSC-Brew [%]	0	5	50	95	100	
	relative secretion (as compared to cells of other media)					P value < 0.05 (cells of media 1 against media X)
DKK1	++	+++	++	+	+	media 10
FGF-2	+					media 13
follistatin	+	+	++	++	++	media 7
fractalkine				+	+	media 10
GROα			++++	+++	++	
HB-EGF		+				
HGF	+	+	+++	+++	+	media 10
IL-6	+	+++	++	+	+	
IL-8		+	++++	+++	+++	media 7, 10
leptin				+	++	
MCP-1	+	++	++	++	+	media 4
MCP-3	+	++	+	+		
OC		++	++		+	
OPG	++	+++	++	+	+	
OPN			++	++	+	media 7
PDGF-AA					+	
RANTES				+		
sCD40L					+	
TGFβ	++					media 4
TSG-6	+	++	+++	++++	++++	
TSP-1	+++	+++	+++	++	+	

Table 1: Relative secretion levels of different functionally relevant factors by MSC grown in media 1, 4, 7, 10 and 13.

DKK1: Dickkopf-related protein 1, FGF-2: fibroblast growth factor-2, GROα: growth-regulated oncogene α, HB-EGF: heparin-binding epidermal growth factor-like growth factor, HGF: hepatocyte growth factor, IL-6: interleukin-6, IL-8: interleukin-8, MCP-1: monocyte chemoattractant protein-1, MCP-3: monocyte chemoattractant protein-3, OC: osteocalcin, OPG: osteoprotegerin, OPN: osteopontin, PDGF-AA: platelet derived growth factor-AA, RANTES: regulated and normal T-cell-expressed and secreted, sCD40L: soluble cluster of differentiation 40 ligand, TGFβ: transforming growth factor β, TSG-6: tumor necrosis factor-inducible gene 6, TSP-1: thrombospondin-1

alterations in protein expression involved in several cellular pathways. Analysis of additional factors showed higher concentrations in XF media except for fibroblast growth factor-2 and insulin. Significantly different consumption of growth factors like platelet derived growth factor was observed and confirmed by significant differences in receptor expression. Secretion of functionally relevant factors could be identified and varied significantly for cells of different media (see Table 1). Cells of all media retained their differentiation potential but migration of cells was impaired by SF/XF conditions.

Conclusion: Metabolism of cells varied for XF and SF/XF media as shown by proteome analysis. This may rely on availability of nutrients as indicated by divergent consumption and secretion pattern of cells. Impaired migratory potential of cells grown in SF/XF media may be unfavorable for MSC homing towards sites of injury. Since therapeutic applications require different MSC characteristics, growth media may be utilized as priming approach for specific purposes.

PS-5-19

Approval process for allogeneic undirected cryopreserved HSC transplants – regulatory and procedural challenges

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Background: Hematopoietic stem cell transplants are able to give patients a second chance of life. A significantly reduced time-to-transplant, an improved possibility of transplantation coordination and previously known product specifications of the selected transplant - these are the advantages of an inventory of undirected cryopreserved PBSC. "Allogeneic cryoPBSC, DKMS-1" was approved in April 2023 by the Paul Ehrlich Institute (PEI)as the world's first allogeneic undirected cryopreserved PBSC product .

Methods: For approval project, structural and procedural precautions were carried out. A GMP clean room unit has been built and qualified. Appropriate production and quality control processes had to be validated. DKMS SCB applied for extension of the manufacturing authorization. . A registration dossier was created for the PEI including relevant details on production, quality control methods, end product specifications, quality and shelf life data as well as a clinical and an analytical expert report. Premises and processes were inspected by the Saxony State Directorate. Moreover, collection centers that provide the undirected apheresis product as starting material also require a manufacturing license and an approval.

Results: In May 2019, the first scientific advice with the PEI for the approval of "Allogeneic cryoPBSC, DKMS-1" took place. Regulatory, structural and procedural preparations were required in preparation of the acceptance inspection by the Saxony State Directorate in November 2020. 19 months have passed between inspection to manufacturing authorization.,PEI requested rectification and additional information, in a majority focused on donor ethics and end product specification. 14 months have passed from submission of the approval dossier to the final approval. In April 2023, manufacturing authorization was given to the DKMS Collection Center.

Donor selection criteria has to be documented and assessed for 4 years after approval using real life data.

Conclusion: An approval process is time-consuming - for the applicant and also for the responsible authorities. Active communication between both parties and the early involvement of the authorities minimizes effort, misunderstanding, and simplifies the approval process. In case of "Allogeneic cryoPBSC, DKMS-1", the world's first allogeneic undirected cryopreserved PBSC product, process from scientific advice to the final approval took 4 years.

PS-5-20

miRNA profiling to assess protective effects of MSC-conditioned medium on cisplatin-induced injury in proximal tubule epithelial cells

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Background: Aberrant expression of microRNAs (miRNAs) appears associated with the progression of renal disease. Mesenchymal stromal cells (MSCs) exert a protective effect in damaged renal cells through the release of cytokines, growth factors and extracellular vesicles. We speculate that MSC-conditioned medium or EVs contained within it exert a protective effect, by modulating miRNA expression in injured renal cells and by this post-transcriptional gene regulation.

Methods: To mimic in vitro injury and MSC-mediated protection, conditionally- immortalized proximal tubule epithelial cells (ciPTECs) were treated with cisplatin for 1h and then treated for a further 23h with cisplatin plus adipose-derived Stromal Cells (ASC)-derived conditioned medium (CM-ASC). Viability, metabolic activity and migratory capacity in a scratch wound healing assay were then assessed. Further, mRNAs were prepared from ciPTECs treated with/without cisplatin and with/without MSC-CM and assessed by small-RNASeq.

Results: Cisplatin-treated ciPTECs showed reduced viability and metabolic activity, ameliorated by ASC-CM. Further, cisplatin significantly reduced the migration in the scratch wound assay, CM levelled it up even exceeding control levels. EVs failed to reproduce the results obtained by the CM. Small-RNASeq analysis identified differentially expressed miRNAs in all groups, particularly in the cisplatin-ASC-CM group compared to the ASC-CM group. Some of these miRNAs are known to regulate apoptosis and cellular fitness, while others have not been described in this context yet and are subject to further investigation now.

Conclusion: Our results demonstrate that MSC-CM per se exerts a protective effect reducing cisplatin cytotoxicity in renal proximal tubule cells. miRNA profiles are changed, suggesting that MSC-CM affects post-transcriptional gene regulation.

Dynamics in Apheresis of human hematopoietic Progenitor Cells

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Background: The yield of CD34+ cells obtained by apheresis (HPC-A) crucially depends on the success of CD34+ cell mobilization in combination with the efficiency of CD34+ cell collection. The *collection efficiency 2* (CE2) describes the collection process under the assumption of a steady state of CD34+ cell count in peripheral blood throughout the whole procedure. We state the hypothesis that the capacity to maintain the steady state varies between different donors.

Methods: All HPC-A procedures between 27AUG2009 and 14APR2023 were extracted from our electronic records (n=5780, autologous: n=1922, allogeneic: n=3858). HPC-A was performed with the Cobe Spectra or the Spectra Optia. Patients were usually mobilized with G-CSF and chemotherapy. Plerixafor could be used in addition for poor mobilizers. Allogeneic donors were mobilized with G-CSF alone. As the collection efficiency 1 cannot always be calculated from routine data, in addition to CE2 we introduced a *process efficiency* (PE) calculated by (Number of collected CD34+ cells/ (total blood volume[l] *CD34+ in peripheral blood [cells/l])*100%. Data is given as mean ± S.D.. A Mann-Whitney test was used to test for significance.

Results: CE2 was higher in allogenic than autologous HPC-As (62±34% vs. 58±25%). Using the Spectra Optia the difference was 69±38% (allogenic, n=2727) vs. 58±24% (autologous, n = 1434). Males had higher CE2 in allogeneic (63±38% vs. 60±25%) and autologous HPC-As (60±27% vs. 56±19%). CE2 was slightly higher in multiple myeloma (MM) patients than in other lymphomas (60±20% vs. 57±34). PE was higher in allogeneic than in autologous HPC-As (142±60% vs. 136±70%). There was no difference between MM and lymphoma patients (134±62% vs. 135±81%). For all differences p< 0.001. Linear regressions showed a slight decrease of CE2 on the processed blood volume (PBV) (-2.8%/PBV). PE increased with PBV (58.4%/PBV) or time (0.55%/min).

Conclusion: Different parameters contribute to the success of HPC-A: gender, allogeneic and autologous collection as well as the type of disease influence the effiency of CD34+ cell collection. Multivariate analysis will be provided to better characterize the contributing properties to HPC-A.

Improvements in unrelated donor search for hematopoietic stem cell transplantation over the last 10 years – Experience of the search unit Ulm

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Background: Hematopoietic stem cell transplantation is a curative treatment for various high-risk diseases of the hematopoietic system. Transplantation from unrelated donors is the most widely used transplant source as many patients lack a matched sibling donor. The search unit of the Institute for Clinical Transfusion Medicine and Immunogenetics Ulm conducts unrelated donor searches for 23 clinics across Germany. In this analysis, we summarize the trends and improvements over the last 10 years.

Methods: The search database of the search unit in Ulm was queried for all searches between 01.01.2013 and 31.12.2022. We generated descriptive statistics with the aim to identify trends and changes over time using RStudio and R for Windows Version 4.1.3.

Results: A total of 11035 searches were performed. Search numbers increased from 1085 (2013) to 1196 (2022), which leads to an average increase of 1.4% per year (determined per linear regression). The proportion of highly urgent searches decreased from 25.5 to 17.1% per year. Match rates showed almost constant improvement from 2013 (10/10: 73.1%, 9/10 24.8%, 8/10 2.1%) to 2022 (10/10: 82.3%, 9/10: 16.8%, 8/10: 0.9%). In all years search success rates were greater than 95% (defined as at least one donor found that met minimum requirements of the transplant center). Search duration defined as time from initiation of the search until first donor is identified improved significantly between 2013 (mean 34d, median 15d) and 2022 (mean 17d, median 13d).

Conclusion: Increasing numbers of upfront high-resolution HLA-typed donors are available worldwide. The larger donor pool and the high quality of HLA-typing in registry donors led to substantial improvements in donor search, manifesting in better match rates and shorter search duration. This benefits patients in urgent need of an allogenic stem cell transplantation in Germany.

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