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Engineered T cells overcoming rejection by antibodies (CORA-T cells) through selective targeting of alloreactive B cells in solid organ transplantation

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Background: One major complication after solid organ transplantation (SOT) is antibody-mediated rejection (AMR) of the graft by anti-donor HLA antibodies. Modern immunosuppression mainly addresses T cell-mediated rejection, affecting the B-cell alloimmune response only indirectly. 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 are characterized by extracellular expression of respective B-cell receptors (BCRs). Using the anti-HLA BCR as target molecule, we redirected T cells towards alloreactive B cells by introducing a novel, CAR-like receptor comprising an HLA molecule fused to intracellular 4-1BB/CD3ζ signalling domains in order to generate T cells overcoming rejection by antibodies (CORA-T cells). As proof of concept, CORA receptors based on a modified truncated HLA-A*02:01 molecule were designed and transduced into primary T cells. Their ability to recognize and selectively eliminate HLA-A*02:01 specific B cells to limit their antibody release was tested in vitro.

Results: Upon cocultivation with a B-cell line expressing and releasing anti-HLA-A*02:01 antibodies, CORA-T cells were specifically activated (expression of CD25, CD69, CD137) and released pro-inflammatory cytokines (e.g. IL-2, TNF-α). Moreover, CORA-T cells exhibited cytotoxicity towards the B-cell line, secreted cytotoxic mediators and effectively reduced the amount of released HLA-A*02:01 antibody. An optimized linker in the CORA receptor allowed for ideal HLA presentation and selective modification of the HLA-A*02:01 α3-domain was shown to abrogate CD8 binding and T-cell sensitization towards the HLA domain of the CORA receptor.

Conclusion: Our results demonstrate that CORA-T cells are able to specifically recognize and eliminate alloreactive B cells, having the potential to selectively prevent the formation of anti-HLA antibodies. This suggests application of CORA-T cells as an innovative approach to specifically combat AMR to improve long-term graft survival in SOT patients while preserving the general 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-T cells.
ZF-1
Zentrale Klinische Fortbildung I
Prätransfusionelle Immunhämatologie

ZF-1-2
Blood Group Typing, Cross-Match and Antibody Testing: Complex Cases
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Invited talks abstract/summary: The German Guidelines, "Richtlinie zur Gewinnung von Blut und Blutbestandteilen und zur Anwendung von Blutprodukten (Richtlinie Hämotherapie)", define the state of the art of the pre-transfusion diagnostics and assure compatible and safe blood transfusions. The substantial parts of the diagnostics are the ABO, RhD and in some cases an extended blood group typing, the antibody-screening test and the cross-match of the patient’s plasma or serum with red cell units. The blood group typing, the antibody screening/identification and the cross-matches are commonly performed in the gel technique. Some other methods like the tube test, slide/plate test, microplate, solid phase and the lateral flow test are used also. In patients who recently received transfusions genetic blood group typing can be very helpful to interpret unclear serological results.

The antibody testing and the cross-matches have to be performed in the indirect antiglobulin test. They are valid for three days only because of the possibility of formation new antibodies by the patients after transfusion or during pregnancy. In case of a positive antibody screening the antibody identification and testing of additional patient’s blood group antigens are necessary.

Every planned transfusion of red blood cells requires a valid result of the blood group typing of the patient and valid negative results of the antibody screening test and cross-matches. If the patient’s plasma contains red cell antibodies a valid antibody identification and the provision of compatible blood units are mandatory. The identification of antibodies in patients with multiple antibodies or antibodies to high frequency antigens may be very difficult, time consuming and often requires special investigations in a reference immunohematology laboratory. In rare cases, undetected antibodies to low frequency antigens in pregnant women can be dangerous for the fetus.

The provision of compatible red cell concentrates to patients with clinically significant red cell antibodies is one of the greatest challenges in transfusion medicine and requires in some cases international cooperation. The correct interpretation of the results and the distinction between relevant and clinically not significant antibodies is an essential part of the pre-transfusion diagnostics.

Disclosure Statements: No conflicts of interests.
GMP-KURS

GMP
GMP: Neue EU-Blutdirektive, IVD-Verordnung

GMP-3

In-house manufacturing of IVD under IVDR

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Invited talks abstract/summary: In vitro diagnostic medical devices that are manufactured non-industrially in healthcare facilities and used only there are essentially exempt from the requirements of EU Regulation 2017/746 (IVDR). The - although no products are placed on the market - nevertheless applicable requirements of this regulation are formulated in Article 5, Paragraph 5 (IVDR). These include compliance with the general safety and performance requirements formulated in Annex I of the IVDR. In addition, a quality management and risk management system must be established that specifically addresses the respective manufacturing process - which is outside the scope of ISO 15189. In accordance with a postponement of the start of validity of some IVDR regulations decided at the beginning of 2022, only from 2028 onwards must there additionally be a declaration that in each case a possibly similar product on the market does not meet the given specific requirements for a required article. The implementation of this controversial "industry privilege" for commercial products versus self-manufactured products is controversially debated.

Disclosure Statements: No conflicts of interests.
Abstracts

VS-1
Sektion Automation und Datenverarbeitung

Status of automation and data processing according to GAMP5 and GMP Guide Annex 15 in the Automation and Data Processing section

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Background: The main focus and topics of the DGTI section meetings “Automation and data processing” are to be presented at the DGTI Congress in Mannheim.

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.

Methods: 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. The topics are stored in the online program of the DGTI Congress in Mannheim on September 21, 2022 for the Automation and Data Processing session, as well as the following image.

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.

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 Mannheim 2022 should be made available on the digital platform in the members’ area. The following topics were presented at the digital DGTI Congress in Vienna in 2021.

Introduction of the digital donation (electronic donor registration form)
– Critical IT infrastructure for blood donation services (KRITIS)
– Digital Patient Identification Eurocode

Conclusion: For the automation and data processing meeting at the 55th annual meeting of the DGTI in Mannheim, 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.

Disclosure Statements: None

VS-1-1
MYLA® software introduction at BACT/ALERT® blood culture system

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Background: The software of the blood donation services OBSERVA is no longer supported by bioMérieux and will be replaced by a new MYLA® software. The new software was originally developed for the clinical area and adapted for the industry. In practice, however, the blood donation services represent a kind of hybrid between clinic and industry, since they carry out both patient and drug investigations. The presentation will show how the adjustment was made in our laboratory during routine operation.

Methods: The existing evaluation unit on the BACT/ALERT® blood culture system was replaced and installed by FA bioMérieux with a new evaluation computer with the latest Microsoft Windows 10 PC version and the new MYLA® software. bioMérieux accompanied all phases of the GMP-compliant qualification with complex IQ, OQ and PQ.

Results: bioMérieux was able to fully implement the physical installation of the MYLA® data management software. But the transformation of the old report templates from OBSERVA to MYLA® is currently not possible without compromises. There is an option to choose between clinical and industrial termination. A user-defined adjustment as in the old system is unfortunately not possible and requires a reassignment of familiar terms and the restructuring of laboratory processes.

Conclusion: MYLA® is a modern software with enriching functions. Further adjustments for a user defines termination would be desirable

Disclosure Statements: No conflicts of interests.
It seems that it is impossible to find the perfect donor of tomorrow. With the increase in the need for high-quality transplants, the question must be asked whether donor shortage is a home-grown problem in industrialised countries. What developments need to be initiated to counter the trend toward donor shortage? Could greater flexibility at the medical and/or regulatory level be a solution? The presentation aims to address these and other questions and would like to discuss this with the experts.

Disclosure Statements: The authors are employees of the DFG gGmbH

VS-2-5
Carrier grafts and new developments in 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. They are processed using different technologies in order to increase their safety for clinical use. New technologies aim to make grafts into more usable forms, enhancing bone fusion, reducing immunogenic potential and minimizing disease transmission. Strict regulations ensure the safety of both tissue preparations and graft recipients.

Methods: Different technologies such as high-precision CNC milling and antimicrobial coating processing techniques allow for the development of classical grafts with a "twist". These allografts can be tailored for different therapeutic uses while considering the needs of both orthopaedic surgeons and patients. Traditional grafts can be combined with plastic surgery techniques yielding pedicled bone allografts (Struckmann et al., 2017) and acellular dermal [VE1] matrices can be made more resilient using graft defence peptides (Kasetty et al., 2015).

Results: A newly developed graft consisting of cortical fibers has been designed as a high absorption carrier-graft. These fibers have interstitial and matrix-linked absorption capabilities with the ability to retain therapeutic solutions for intended target sites. A novel graft from cortical bone is the allogenic bone screw developed utilizing high precision CNC milling technology. A fine threaded design makes this bone screw suitable for osteosynthesis in hand, elbow, knee, and foot surgery. Arthroscopic interventions are simplified by flexible cancellous bone blocks that can be introduced through portals.

Conclusion: New processing technologies have provided more usable and clinically relevant graft solutions. The developed grafts support osteosynthesis, cortical fibers carrier-grafts harness their high matrix absorption capacities providing answers for the lasting delivery of therapeutics despite natural processes. Allogenic grafts take many forms varying from flexible bone to vascularized soft and hard tissues. Altogether these grafts are a valuable addition to the regenerative medicine toolkit.

Invited talks abstract/summary: [PAPERTEXTS]

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-2-3
Influence of specific donor selection criteria on the quality of tissue transplants
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Invited talks abstract/summary: The development of cornea transplants in Germany shows that the number of keratoplasties performed has steadily increased in recent years and has more than doubled since 2011. The resulting increase in demand for transplants is reflected by the numbers of donated corneas and delivered transplants the DFGF network.

In parallel, new surgical methods are being developed that change the requirements for a graft. The introduction of DMEK is a huge improvement for patients but requires stricter tissue selection criteria. In the meantime, more than 60% of all keratoplasties in Germany are lamellar. The numbers of corneas supplied from our network for these techniques already account for over 66% of surgeries.

This leads to the need to provide an increasing number of corneas with higher quality requirements. At the same time, however, the potential donors are getting older due to demographic change. This is accompanied, for example, by a decrease in endothelial cell density, which continues to play a very decisive role in the acceptance of a transplant by surgeons. Further, more donors are undergoing pre-operative treatment, so the demand for a good transplant may also become increasingly difficult to meet.
VS-2-6
Significance of corneal endothelial cell density for corneal transplantation

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Background: With the introduction of isolated posterior corneal transplantation techniques for selective replacement of the diseased posterior corneal endothelium, the number of these operations has increased significantly. This leads to the wish of the ophthalmic surgeons for a longest possible graft survival and a high endothelial cell density of the corneal graft, if possible more than 2300 cells per mm2 centrally.

Methods: To assess the importance of endothelial cell density for the success of corneal transplantation and graft survival, a literature search and an evaluation of published studies were performed. In addition, the own data of transplantable donor corneas of the cornea bank of the Charité - Universitätsmedizin Berlin were evaluated.

Results: The studies published so far do not show a sole influence of corneal endothelial cell density of the corneal graft on the long-term success of endothelial keratoplasty. Additionally, the intensity of graft manipulation during surgery and postoperative graft detachment with consecutive surgical treatment (“rebulbing”) play a significant role. The central endothelial cell density of the available corneal grafts is on average significantly lower than the often required 2300 cells/mm2 in donors older than 60 years.

Conclusion: A corneal graft from a preferably older donor with a high endothelial cell density is unrealistic, especially against the background of an aging society and thus an increasing proportion of people undergoing cataract surgery. Endothelial cell density is not the all-decisive factor for the long-term success of endothelial keratoplasty.

Disclosure Statements: No conflicts of interests.

VS-3-3
Sektion Präparative und therapeutische Apherese

Impact of different collection rates on the collection efficacy of peripheral blood stem cells

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Background: The collection of peripheral blood cells has meanwhile become the clinical standard for harvesting stem cells, although the procedure itself is relatively new. In the last years, a new collection machine (Spectra Optia) has become available. We investigated the influence of the collection rate (adjustable collection of target cells in mL/min) on the collection outcome (total cell yield and collection efficiency). Part of a PhD thesis.

Methods: Retrospective analysis of 340 cases of autologous and allologic stem cell apheresis collections at a university clinic. Descriptive statistics of collection parameters (height, body weight, processed blood volume, total blood volume, concentrate volume, ACD volume, heparin, problems during apheresis, erythrocytes, platelets, leukocytes, mononuclear cells, stem cells, vitality of leukocytes or stem cells, CFU, collection rate, collection efficiency) were done. Correlation testing was done concerning the collection efficiency, followed by a regression analysis, if applicable.

Results: A higher collection rate led to a larger product volume. The collection efficiency ranged from 24% to 478%. The pre apheresis leukocytes predicted the efficiency for the overall collective and both genders best, with an inverse correlation. In contrast, separation time was the decisive factor for the different types of donation (longer separation time led to a higher stem cell yield). No relevant impact of the collection rate on the collection efficiency was found.

Conclusion: The collection rate showed no correlation with the collection efficiency. A smaller collection rate can be used for apheresis, without the fear of a disproportionate amount of cell loss. This is especially relevant in the case of cryoconserved concentrates, since a smaller concentrate volume leads to a lower DMSO exposition of the patient. Our data also supports the thesis that a high concentration of stem cells before apheresis allows for the anticipation of a high collection efficiency.

Disclosure Statements: No conflicts of interests.

VS-3-4
Post-COVID conditions (PCC)/long COVID and chronic fatigue syndrome (CFS): Practical experience in the treatment with immunoadsorption (IA) and plasma exchange (PE)

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Background: Many people have been infected by SARS-CoV-2 virus. A vast number of individuals complain about continuing breathlessness and fatigue even months after the onset of the disease. This overwhelming phenomenon has been called “post-COVID syndrome” or “long-COVID”. There are striking similarities to myalgic encephalomyelitis (ME) / CFS linked to a viral and autoimmune pathogenesis. In both disorders neurotransmitter receptor antibodies against β-adrenergic and muscarinic receptors may play a key role.

Methods: We found similar elevation of these AAB in both patient groups. Eligible patients with PCC and CFS have receive IA or PE. Patients in both groups received 5 treatments on 5 days. In the IA group, the 2-fold individual total plasma vol (TPV) was processed on each day. In the PE group, 2.5 L of plasma (corresponding to the 1-fold individual TPV) were removed each day and substituted by 5% human albumin solution. The primary endpoint was the removal of AAB. Side effects like hypotension, citrate reaction occurred but did not lead to cessation of the session. Due to only minimal loss of both IgA and IgM there is no need to isolate patients during the course of IA. Also 20-30% of IgG remains available to protect the patients against infections.

Results: Between January 10, 2022, and March 31, 2022, 26 patients (w =11, m = 15), were screened for eligibility, most of them between 20 and 50 years old (24 = 20–50 y, 2 = >50). 26 patients have received 132 treatments with IA (n = 24) or PE (n = 2). Blood was drawn immediately before and after the apheresis sessions. Results showed lowered values for IgG: 72.10% (average pre 4.47 g/l vs. post 1.24 g/l), IgM: 30.16% (pre 0.930 g/l vs. post 0.684 g/l), IgA: 27.95% (pre 1.38 g/l vs. post 1.045 g/l). Treatment resulted in lowering of plasma IgG to levels below 2 g/l. 20 patients reported a subjective improvement after the treatment.

Conclusion: Aggressive immunoglobulin elimination with appropriate IA or PE can be beneficial in cases of PCC/CFS. IA and PE seem to be effective and safe in reducing pathogenic antibodies in a significant way clearly improving the debilitating symptoms of patients with long COVID/CFS. Therefore, IA and PE may provide a promising therapeutic option for patients with PCC. This method will also be effective when other hitherto unknown antibodies and inflammatory mediators are involved.

Disclosure Statements: No conflicts of interests.
Background: Therapeutic HER2-specific antibodies, such as trastuzumab, dramatically improved the outcome for patients with HER2+ breast cancers, where natural killer (NK) cell-mediated antibody-dependent cell-mediated cytotoxicity (ADCC) acts as a key effector mechanism. While ICAM-1/LFA-1 binding was shown to be required for directed FcR-mediated degranulation towards the immunological synapse, the role of ICAM-1 in the efficacy of trastuzumab-mediated ADCC against breast cancer cells is poorly understood.

Methods: Here, we used high-affinity FcR transgenic NK-92 cells (haNKTM) and primary NK cells as well as tumor cell lines to test whether ICAM-1 downregulation contributes to escape from trastuzumab-mediated ADCC.

Results: Genetic or pharmacological blockade of ICAM-1/LFA-1 interaction resulted in significantly reduced killing of breast cancer cell lines MDA-MB-453 and MCF-7 by haNK and primary NK cells plus trastuzumab, and in decreased production of anti-tumor cytokines TNF-α and IFN-γ by haNK cells. In contrast, retargeting of NK-92 cells (NK-92/5.28.z) or primary NK cells with HER2-specific chimeric antigen receptor (CAR) was unaffected by the reduced expression of ICAM-1. We found that the lack of ICAM-1 did not affect cell-cell adhesion and conjugate formation during ADCC but rather resulted in decreased signaling through the Pyk2 pathway, which was restored in CAR-mediated targeting.

Conclusion: Taken together, we identified downregulation of ICAM-1 expression on breast cancer cells as a significant tumor escape mechanism from ADCC. Hence, the efficacy of therapeutic antibodies for cancer therapy could be augmented by upregulation of ICAM-1 on tumor cells. Our studies also showed that CAR-mediated tumor cell targeting overrides any negative effect triggered by low ICAM-1 expression on cancer cells or by immune checkpoints engagement.

Disclosure Statements: TT and WSW are named as inventors on patents in the field of cancer immunotherapy owned by their respective institutions. Other authors declare that they have no competing interests.
An oncogenic transcription factor exploits the hematopoietic master regulator CEBPa to expand human CD34+ stem-/progenitor cells

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Background: Chromosomal rearrangements of the mixed-lineage leukemia gene (MLL-r) are strong drivers of acute leukemia with poor prognosis, especially in childhood cancer. During the last years, the first inhibitors have been developed targeting MLL-r oncprotein complexes. Here, we identified a highly upregulated core gene set in human CD34+ blood progenitors expressing the t(11;19) fusion product MLL-MLLT1, with the intention to uncover further vulnerabilities in MLL-MLLT1 driven transformation.

Methods: Defined human primary cell models are highly relevant to study disease mechanisms and potential target structures. MLL-MLLT1 was introduced into human CD34+ blood progenitors from healthy donors via retroviral transduction. Upon ex vivo expansion and outgrowth of transduced mononcytic progenitors, gene expression profiling was performed with total RNA via the Nanostring technology. We accomplished a top 30 list of upregulated genes in MLL-MLLT1 progenitors, including that of the transcription factor CEBPa. To investigate cell biological consequences of CEBPa disruption in MLL-MLLT1 progenitors, two approaches were taken: genetic downregulation of CEBPa through lentiviral shRNA and pharmacological inhibition with small molecules.

Results: Among upregulated genes, a MYC signature (MYC, FLT3, PRDX3, CCR2, and FASN) was identified together with high CEBPa expression levels. Interestingly, in MLL-r patient cohorts, CEBPa appears un-mutated. At the protein level, high CEBPa expression was verified by Western blotting. Of note, specific degradation of MLL-MLLT1 triggered CEBPa downregulation and growth arrest. Vice versa, upon shRNA-mediated knockdown of CEBPa, MLL-MLLT1 progenitor cells gradually lost proliferation capacity indicating a cooperative role in progenitor cell transformation. Interestingly, a combination of the MLL inhibitor MI-503 and the CEBPa blocking compound OICR-9429 induced strong anti-proliferative effects on the investigated human MLL-MLLT1 precursors.

Conclusion: Our study sheds light into the oncogenic mechanisms of MLL-MLLT1 activity in human CD34+ primary blood progenitor cells suggesting cooperativity of MLL-MLLT1 and CEBPa in AML transformation. As CEBPa comprises several biological functions in cellular differentiation, blood cell lineage priming, self-renewal and metabolism, the identified CEBPa upregulation might be an attractive target structure to inhibit the aggressive tumor promoting effects of MLL-MLLT1 in human leukemia.

Disclosure Statements: No conflicts of interests.

Dual targeting of CAR-NK cells to PD-L1 and HER2 facilitates specific elimination of cancer cells of solid tumor origin and overcomes immune escape by antigen loss

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Background: Retargeting of natural killer (NK) cells with chimeric antigen receptors (CARs) can be a powerful approach to overcome NK-cell resistance of tumor cells. However, targeting a single tumor-associated antigen may be insufficient for some tumors to trigger effective NK-cell activation or result in the selection of antigen-loss variants and tumor immune escape.

Methods: To overcome this hurdle, here we generated CAR-NK cells carrying two CARs that target the tumor-associated antigens PD-L1 and HER2 (ErbB2), respectively. NK-92 cells were transduced with lentiviral CAR constructs, and their cytotoxicity against cancer cell lines of different solid tumor origins was compared to that of parental NK-92 and corresponding single-target CAR variants.

Results: Dual targeting significantly increased in vitro cytotoxicity against PD-L1 and HER2 double-positive tumor cell lines including breast, ovarian, pancreatic and gastric cancer cells when compared to single-target CAR variants. These results were also confirmed with 3D spheroid tumor models. Off-target cytotoxicity was not observed. On a molecular level, this enhanced cell killing may be explained by synergistic activation of PLCγ and MAPK pathways. Incubation of cancer cells with IFN-γ further improved killing efficacy due to upregulation of PD-L1 expression. Furthermore, blocking experiments revealed that dual PD-L1/HER2-CAR NK-92 cells can overcome immune escape based on loss or inaccessibility of a single target antigen.

Conclusion: Altogether, we showed that dual targeting of PD-L1 and HER2 improves efficacy of CAR-NK cells against otherwise difficult to treat tumors, and counteracts potential resistance and immune escape mechanisms of cancer cells.

Disclosure Statements: TT and WSW are named as inventors on patents in the field of cancer immunotherapy owned by their respective institutions. Other authors declare that they have no competing interests.

CAR-effector cell-mediated depletion of hematopoietic stem cells as conditioning prior to allogeneic stem cell transplantation – in vitro proof of principle analysis

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Background: Allogeneic hematopoietic stem cell (HSC) transplantation treats refractory malignant and non-malignant bone marrow and blood disorders, to establish donor-derived hematopoiesis. Conventional conditioning is associated with high toxicity, making it unsuitable for very...
young and old people, as well as for chromosomal breakage syndrome patients. An alternative chemo- and radiotherapy-free conditioning aims to deplete HSCs by chimeric antigen receptor (CAR)-modified NK92 cells targeting c-kit.

**Methods:** Second-generation CARs were constructed with scFvs derived from the hybridomas 2B8 (anti-mouse, gift from I. Weissman, Berkley, CA) and SR1 (anti-human). The NK92 cell line was lentivirally transduced with the CAR constructs. For murine target cells, the human cell line MDA-MB-468, naturally not expressing murine c-kit, was transduced for over-expression of murine c-kit. In the naturally human c-kit expressing cell line HEL, c-kit was knocked-out via CRISPR/Cas9 for a negative-control. Functionality of CAR-constructs was tested with a Europium-release (EU) assay and with CFU-C assays. For the latter, primary murine bone marrow cells were incubated with CAR-NK92 cells for 20 hours prior to seeding into semi-solid cytokine replete media.

**Results:** Using flow cytometry with the antibody from which the scFV was derived, the MDA-MB-468 cells were shown to specifically express murine c-kit at lower, similar and higher levels compared to primary murine HSCs. c-kit knockout was shown in HEL cells at the genomic level by sequencing and at the protein level by flow cytometry and Western blot. EU-assays showed specific killing for murine target cells that was dependent on both antigen dose and effector-to-target ratios. Further, co-incubation of primary murine bone marrow cells with CAR-NK92 cells showed a reduction of 83.21% in CFU-C assays. EU-assays with HEL cells and the human CAR-construct showed specific killing of the parental HEL cells.

**Conclusion:** The initial in vitro data regarding targeting of c-kit on HSCs by CAR-NK92 reveal the effectiveness and specificity of the newly constructed murine and human CAR constructs. Killing was dependent on both antigen dose and effector-to-target ratios. CAR-NK92 are efficient against primary HSCs and might thus constitute a safe, universal off-the-shelf product for myelosuppression providing access to allogeneic HSC transplantation for patients for whom conventional conditioning is not an option.

**Disclosure Statements:** The authors declare no conflict of interest.

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**VS-5-6**

**Understanding and targeting the cellular machinery sensing the chemical milieu in the leukaemic bone marrow microenvironment**

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**Background:** 99% of the body's calcium is stored in bone, and calcium is released during bone remodeling. The calcium sensing receptor (CaSR) plays a role in the localization of normal haematopoietic stem cells in the bone marrow microenvironment (BMM). However, the role of this receptor and its associated pathways for leukaemia development, therapy success and whether modulation of this receptor may be beneficial therapeutically, is not known.

**Methods:** Hypothesizing that the CaSR contributes to development, progression and response to therapy in leukaemia, we employed various in vitro assays, in vivo microscopy, leukaemia induction and in vivo treatment assays to test this question.

**Results:** The local calcium concentration forms a gradient in the BMM with highest calcium concentrations close to the endosteam. The calcium concentration in the BMM, CaSR expression on leukaemia cells and CaSR sensitivity to extracellular calcium differs between leukaemia types. CaSR acts as tumor suppressor or an oncogene in different leukaemias. In acute myeloid leukaemia (AML), limiting dilution transplantation of CaSR-deficient AML-initiating cells revealed a 7-fold reduction of leukemic stem cells. Downstream of CaSR, we implicated filamin A and other proteins as important signaling molecules. Treatment of mice with a CaSR agonist or antagonist differentially impacted myeloid leukaemias.

**Conclusion:** In summary, our results suggest that the CaSR and possibly calcium ions from the BMM strongly and differentially influence leukemia progression, with filamin A playing an essential role in AML. As an adjunct to existing treatment strategies, targeting of CaSR with specific pharmacologic agents may be beneficial in different leukaemias.

**Disclosure Statements:** No conflicts of interests.

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**VS-5-7**

**WNV in Germany – here to stay?**

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**Invited talks abstract/summary:**

**Background:** West Nile virus (WNV) is an arthropod-borne virus. It circulates in an enzootic cycle between mosquitoes as vectors and avian host species for amplification but humans can be accidental hosts. WNV is also transmissible by horizontal transmission (TTI). In most individuals, mosquito-borne WNV infection remains silent while 20% develop mild symptoms of West Nile Fever and only 1% develop neuroinvasive disease. TTI often have a more unfavourable course due to the underlying medical conditions of the recipients. Human WNV cases have been identified in southern and eastern Europe for more than 20 years but until 2018, Germany was considered to be a non-endemic country. This changed when in the exceptionally warm summer of 2018 conditions for viral replication were ideal and the first WNV cases among birds and horses were identified. The widespread domestic Culex mosquitoes are efficient vectors for WNV. These circumstances lead to mandatory donor deferral or testing after a stay in an affected area within Germany.

**Methods:** Data for human infections from the mandatory reporting according to the infection protection act (IfSG) and the transfusion act (TFG) and data from the animal surveillance were analysed.

**Results:** The first WNV cases in animals were detected in 2018 in central eastern Germany. Autochthonous mosquito-borne WNV infections in humans were reported in the same area: In 2019, 5 cases, in 2020 30 cases and in 2021 5 cases were notified according to IfSG and TFG. Of these, 13 displayed neuroinvasive symptoms and one patient died. None of the WNV-positive blood donors developed severe symptoms and no TTI occurred. So far, no clear expansion of the affected areas is discernable but may develop. From 2020 onwards, roughly 80% of all blood establishments in Germany tested their donations for WNV using nucleic acid amplification techniques in the transmission season.

**Conclusion:** Human mosquito-borne WNV infections have been reported for three consecutive years and WNV is likely to become endemic in Germany. As long as effective and affordable pathogen reduction is not available for all blood components, WNV testing or donor deferral will be essential to ensure blood safety. In order to timely identify affected areas, combined results of human and veterinary surveillance are needed. Partnerships between public health experts, transfusion medicine specialists, veterinarians and entomologists, should be strengthened to ensure a One Health approach.

**Disclosure Statements:** No conflicts of interests.
Using a mathematical simulation model to predict the impact of changes in platelet concentrate shelf life

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Background: Platelet concentrates (PC) are used with different shelf lives (4 to 7 days), yet platelets in vivo lifespan is 8 to 12 days. Unlike other transfusion transmitted infections, donor sample screening will not help identify bacterial contamination (mostly caused at blood collection / processing). A pathogen reduction technology (PRT) at end of PC production could increase shelf life. This is expected to reduce discarded products and improve supply management. Yet, this effect is not easy to predict.

Methods: We developed a Monte Carlo Inventory Management Simulation mathematical model in Microsoft Excel based on our production and delivery data of 52 weeks to simulate the effect of different shelf lives for platelet concentrates (PC).

A 1,000 weeks were simulated per PC type (blood type, rhesus factor, CMV status) in order to assess the average daily stock, overproduction, expiry rates and specific PC type shortages, overall and by day of the week.

Results: We simulated different overproduction rates and shelf lives of PC, using day 4, 5, 6 and 7 as end of storage.

A change from 4 to 5 days of shelf life increases average PC age at delivery from 2.76 days to 3.1 days. Extending to 7 days leads to an average PC age of 3.8 days at the time of delivery.

Percentage of platelets needed, but not produced due to suitable whole blood shortage reduces from 3.28% (day 4) to 1.0% (day 5 ) and 0.3% (day 7).

Overproduction reduces from 15% to 5% w/o reduction of average units in stock though a change from 4 to 5 days of shelf life. If overproduction is kept at the 15% level (blood transfusion services common practice) and extend shelf life to 7 days, shortages of rare blood groups are eliminated.

Conclusion: Using simulations to model different over production rates and PC’s shelf life lengths may enable a better insight about possibilities to improve blood transfusion services operations and adequate clinical supply. This might be very helpful to estimate the medical and economic impact of major changes like the introduction of pathogen inactivation methods.

Disclosure Statements: No conflicts of interests.

West Nile virus is efficiently inactivated in platelet concentrates by UVC light using the THERAFLEX UV-Platelets system

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Background: West Nile virus (WNV) infection is a vector-borne disease caused by an enveloped ssRNA virus belonging to the family of Flaviviridae. WNV is known to be transfusion-transmittable and thus, donor testing was implemented in several European countries. This study aimed to investigate the efficacy of the THERAFLEX UV-Platelets system to inactivate WNV in platelet concentrates (PCs).

THERAFLEX UV-Platelets system (Macopharma) uses UVC light alone, without any additional photoactive substances.

Methods: Plasma reduced PCs from 4 buffy coats (35% plasma in additive solution SSP+) were spiked with virus suspension (10% v/v). PCs (n=3, 350 mL) were then UVC-irradiated on the Macotronic UV illumination machine (Macopharma) and samples were taken after spiking (load and hold sample) and after illumination with different light doses (0.05, 0.1, 0.15 and 0.2 (standard) J/cm2). The titer of WNV (Lineage 1, Isolate N799, Genbank Accession # MZ6605381) was determined as tissue culture infective dose (TCID50) by endpoint titration and large volume plating in microtiter plate assays on BHK-21 cells (Friedrich-Loeffler-Institute, Isle of Riems).

Results: The infectivity assays demonstrated that UVC-irradiation inactivates WNV in a dose-dependent manner. After spiking, a WNV titer of 6.54 ± 0.10 log10 TCID50/mL was obtained in the PCs. At a UVC dose of 0.2 J/cm2 the titer was reduced to 2.95 ± 0.49 log10 TCID50/mL, resulting in a log10 reduction factor of 3.6 ± 0.4.

Conclusion: Our results demonstrate that the THERAFLEX UV-Platelets procedure is an effective technology to inactivate WNV in contaminated PCs.

Disclosure Statements: UG (and sy) received grants from the Research Foundation of the German Red Cross Blood Services (Deutsche Forschungsgemeinschaft der Blutspendendienste des Deutschen Roten Kreuzes) and Macopharma for the development of the UVC-based PI technology for platelets.

Criminal and civil responsibility of the donor in a case of transmission of malaria by a blood transfusion in a non-endemic country

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Background: The blood donation process is strictly regulated by law. The process includes the necessity of a medical history taken to determine if a donor is suitable to donate, which shall guarantee both donor and recipient safety. Supplementary screening for a limited number of infections is performed by antibody (HIV, HCV, Syphilis) or antigen (HBV) tests or NAT (HIV, HCV, HBV).

Methods: Case Presentation: With regard to a possible infection due to (sexual) risk behavior or foreign travel, blood donation facilities are predominantly dependent on information provided by the donor. This is especially true for malaria in non-endemic areas such as Europe.

Results: A transmission of malaria by a blood transfusion in a non-endemic country that led to fulminant septic shock and death of the patient happened.
Conclusion: Discussion: From a legal perspective, the donor thus assumes a high level of responsibility with regard to accuracy and completeness of the information provided on the donor questionnaire. Incorrect information may, as in this Austrian case reported, result in civil or criminal responsibility of the donor.

Disclosure Statements: None

VS-5-5

Risk of blood bag lesions induced by standard transfusion devices

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Background: At the bedside leakage of a platelet concentrate was observed before start of transfusion and the defective platelet concentrate returned (after a futile attempt to repair the leakage with a plaster). The bag membrane had been punctured by the transfusion device. The tip of the transfusion device protruded the puncture resistant tubing of the blood bag by 2-3 mm (Fig. 1A). The blood bag was a MCS+ apheresis set bag (Ref: 997CFE; Haemonetics; Munich, Germany).

Methods: Transfusion devices and blood bags are highly standardized. DIN EN ISO 1135-4 states the length of the thorn of the transfusion device with 28 ± 1 mm while DIN EN ISO 3826-1 states that the port must be compatible with a thorn according to DIN EN ISO 1135-4 without puncturing the bag membrane. The norms do not consider the “stopper” at the end of the thorn that is supposed to prevent the thorn from being pushed too far into the bag (Fig. 1C). The diameter of the stopper of the transfusion device used (Ref: VH-26-EGA; CareFusion) is only 0.6 mm larger than the diameter of the thorn (Fig. 1B) and the force required to overcome that barrier is negligible.

Results: The issue of poor compatibility of blood bags and transfusion devices has previously been raised, mostly regarding the force which is needed for insertion and the stability of the connection. Punctured blood bags present a high risk for bacterial contamination of blood bags. While macroscopical lesions are easily detected and the blood bag then returned, non-leaking microscopical punctures are less likely to be detected and might present an equal risk for contamination.

Conclusion: In a field where massive and cost-intensive efforts have been implemented to minimize the risk of pathogen transmission, compatibility of transfusion device and blood bag should be a basic requirement. We therefore recommend an adaptation of the DIN standard, to also include the diameter of the stopper.

Disclosure Statements: AG is an advisor for MacoPharma and received funding from MacoPharma for another Project.
Analysis of Platelet Product Utilization at a University Hospital Reveals Optimization Potential in Guideline Adherence

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Abstract

Background: Platelet transfusions (PTx) are an essential part of treatment for bleeding and of bleeding prevention due to thrombocytopenia. PTx are aimed to be applied ABO-identical. Transfusion success is often controlled using absolute platelet (PLT) increases as surrogate marker at 1 or 24 hours. Here we aimed to study the transfusion success of the different platelet products and setup a database to correctly diagnose and treat potential refractoriness.

Methods: A database of >3000 patients was set up within a period of 3 months. In a first analysis, we included 72 patients hematological and oncological patients who received a total of 270 PCs manufactured by our blood bank within the time period of 15th–30th of November 2021. We classified the products into several categories including ABO-identical/ non-identical, Pooled vs apheresis concentrates and success control at 1h, 24h or both. The data was extracted from the SAP/KIS and PC-Blut software. Graphics and statistical analysis were performed using GraphPad Prism 7 with p<0.05. Absolute platelet increase was employed as a measurement of the transfusion success.

Results: The mean absolute PLT increase of all ABO-matched PTx was 32.1 (1h, n = 36) and 16.1 (24h, n = 104) Gpt/L, and of ABO-unmatched PTx 24.7 (1h, n = 6) and 13.5 (24h, n = 11) Gpt/L which was not statistically significant (1h p=0.9956; 24h p=0.999). PLT increase from ABO-matched pooled products was 23.20 (1h, n = 15) and 10.23 (24h) Gpt/L and from apheresis 38.38 (1h, n = 21) and 20.91 (24h, n = 57) Gpt/L (1h p=0.8862, 24h p=0.6853). After 24h, 79.4% (n = 77) of the ABO-matched and 70% (n = 7) of ABO-unmatched PTx reached a PLT increase >5 Gpt/L. Of all evaluable PTx, success was controlled within 24h in 46.6%, within 1h in 7.2%, and at both time points in 15.9%. Thus, in 30.3% of PTx, transfusion success was not controlled at all.

Conclusion: Based on the absolute PLT increase, we observed expected mean PLT increase values after 24h and 1h. No significant differences were so far observed between pooled and apheresis products. About one-third of PTx were not controlled for success. Further analysis is ongoing to optimize identification and handling of PLT refractoriness in patients who are at risk of bleeding.

Disclosure Statements: No conflicts of interests.

Extracorporeal therapy with purified granulocyte concentrates in patients with septic shock – Start of the randomized controlled clinical trial ReActIF-ICE

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Background: Immune cell dysfunction is a crucial aspect in sepsis and septic shock. Granulocyte concentrate (GC) transfusions, as the only available immune cell concentrates, potentially induce tissue damage via local effects of neutrophils. Therefore, using donor immune cells purely extracorporeally is an attractive option. Clinical trials with standard GC in an extracorporeal plasma treatment achieved beneficial effects. Here, purified GC with longer storability will be used in an extracorporeal circuit.

Methods: We describe a prospective, phase II, multicenter, randomized controlled parallel-group clinical trial in patients with septic shock. Subjects suffering from septic shock according to Sepsis-3-Definition who additionally require norepinephrine at a dose of ≥ 0.2 mcg/kg/min (and/or vasopressin at any dose) for a minimum of 6 hours will be randomized to standard of care therapy or extracorporeal immune cell therapy on top of standard of care, in a 1:1 ratio. In the verum group plasma is continuously filtered from the patient’s extracorporeal blood circuit and transferred into a closed-loop “cell circuit”, where the patient’s plasma is in direct contact with the human-donor immune cells from the purified granulocyte concentrate.

Results: A total of 120 evaluable patients will be enrolled at 4 sites within Germany. Primary endpoint is safety and tolerability consisting of new onset of serious adverse events. A key secondary endpoint is showing recovery from immune dysfunction after extracorporeal immune cell treatment. This study has been submitted to independent ethics committees and responsible agencies in Q4/2021. Approval of ethics committees has been granted in May 2022 and is expected for Yuval-Ehrlich-Institute on June 10th, 2022 immediately followed by starting the clinical trial. At DGTI 2022 the study layout as well as first results will be presented.

Conclusion: The extracorporeal immune cell plasma perfusion therapy may on one hand provide immune support and may avoid unwanted local side effects on the other hand. Recovery from immune dysfunction is a prerequisite for avoiding secondary infections and ultimately also for recovery from sepsis.

Disclosure Statements: TW, FD, SD, SM, JA are employees or shareholders of ARTCLINE GmbH. All other authors declare that they have no competing interests.
Mobile electronic documentation of transfusion – this boot is finally walking

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Background: Improving transfusion safety by electronic identification systems is an idea of old. But an initial surge in field studies and single hospital experiences never resulted in nationwide implementation. Since 2017, the high demands of the EU’s medical device regulation make the development of such a system outside an commercial setting highly improbable. Therefore in 2020 we formed a development partnership for a mobile transfusion application with the provider of our LIS (SWISSLAB, NEXUS AG).

Methods: To create a viable application, we agreed on the following requirements:
- Easy to use
- Mobile
- Runs on all common operating systems
- No data storage in the app itself
- No need for additional barcodes on blood products
- Scans all common barcode formats
- Ensurance of professional continuity/maintenance
- In agreement with german transfusion guidelines
- Includes documentation of bedside test, transfusion date/ time and transfusing physician
- Transfer of information document to the hospital information software (HIS)
- Connection between end device and database is SSL encrypted using a certificate.

A prototype was built, implemented in the test server of the laboratory information system and thoroughly vetted by selected clinicians.

Results: The application is browser-based and runs on all common mobile devices. The transfusing physician identifies via login or barcode-scan. Verification checks are distributed over three consecutive windows. Only a successful check of one step allows for the next. Discrepancies result in red warning windows. Correct patient-product-relation is checked by scan each bag, thereby reducing the risk of error (failure to scan) by the operator.

Conclusion: The main technical development of the application was surprisingly fast and only took about three months. Usability decisions often proved to be more difficult. Our main objective was always to provide for a high level of transfusion safety while not overcomplicating the application.

Disclosure Statements: None
**VS-7**
Sektion Hämostaseologie

**VS-7-3**
**Structural investigations into coagulation factor VIII full-length and B domain**

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**Background:** Factor VIII is a coagulation protein that contains a unique large heavily glycosylated but highly disordered B domain apart from five other globular domains. Due to high-level disorderliness within the B domain, it evades structural characterization by traditional structural determination techniques. Our study aims to structurally characterize the FVIII B domain and the full length FVIII (FL-FVIII) structure using integrated hybrid methodology (Figure 1).

**Methods:** The FL-FVIII was extracted from commercially available plasma concentrates, recombinant products and from expression done in HEK293T, HEK293F cells. Highly pure FL-FVIII obtained, was deglycosylated (PNGase-F) and then crosslinked using DSSO crosslinker. Cryo-EM of FL-FVIII was done on Glacios and Krios G4 Cryo-Transmission Electron Microscope (cryo-TEM). Air and liquid AFM (Atomic force microscopy) imaging of FL-FVIII was performed on Multimode 8, Nanoscope V (Bruker) and Dimension 3100, Nanoscope V (Bruker). The alpha-fold FL-FVIII model was subjected to all-atom MD simulation. Atomic fitting/ docking/ model building was done for the cryo-EM and AFM images with respect to the simulation equilibrated alpha-fold database FL-FVIII model.

**Results:** Successful crosslinking of the highly pure FL-FVIII with DSSO was achieved and is in the process of mass spectrometry analysis to further yield modelling restraints. The negative stains of FL-FVIII showed heterogeneous particles with different classes of size and shapes illustrated by 2D classification. Glacios and Krios G4 Cryo-TEM analysis also yielded the similar results as the negative stains, but predominantly crescent shaped particles were observed. AFM analysis in air and liquid mode also showed multiple conformational possibilities of FL-FVIII. The simulation equilibrated alpha fold FL-FVIII model showed conformational variability which fit the AFM and cryo-EM maps of FL-FVIII.

**Conclusion:** Our results show major conformational variability in FL-FVIII in both structural microscopy images as well as the FL-FVIII model. The conformational variability in structural microscopy images can be attributed to the following two possibilities: 1) The FL-FVIII is unstable while processing and therefore what we observe is a mix of heavy chain, light chain and also intact FL-FVIII and 2) The highly disordered B domain results in significant number of conformational possibilities.

**Disclosure Statements:** The authors declare no conflicts of interest. This work is funded by IIR grant awarded to PD Dr. Arijit Biswas and Prof. Johannes Oldenburg from Takeda (formerly Shire).
Efficacy and safety of valoctocogene roxaparvovec gene transfer for severe hemophilia A: Results from the GENER8-1 two-year analysis

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Background: GENER8-1 is a phase 3, single-arm, open-label, sponsor-blinded trial evaluating safety and efficacy of adeno-associated virus (AAV)-based gene transfer with valoctocogene roxaparvovec in severe hemophilia A (NCT03370913). We report results from the year two analysis of the ongoing GENER8-1 study.

Methods: Mean ABR decreased by 4.1 treated bleeds per year (p<0.0001; N=112; median follow-up 110 weeks), an 85% reduction from 4.8 (median 2.8) at baseline. Mean AFR was reduced by 133 infusions per participant (p<0.0001), a 98% reduction from 135.9 (median 128.6) at baseline. Mean FVIII activity at week 104 was 23.0 (median 11.8) IU/dL by chromogenic substrate assay, an increase of 22.0 IU/dL (p<0.0001; N=132). By one-stage assay, mean FVIII was 36.1 (median 21.6) IU/dL at week 104. No new safety signals emerged and no treatment-related SAE were reported during year two.

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Conclusion: A single treatment with valoctocogene roxaparvovec was well-tolerated and led to stable and durable annualized bleed control superior to prior prophylaxis through two years post gene transfer.

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FP: honoraria: Grifols, Sanofi, Takeda; advisor: Sanofi, Roche
KJ, HY, TR, WW: BioMarin employees

Veno-occlusive disease in pediatric patients after stem cell transplantation is associated with impaired fibrinolysis

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Background: Hepatic veno-occlusive disease (VOD) is a life-threatening complication of hematopoietic stem cell transplantation (HSCT) with a high incidence in pediatric patients. Early diagnosis and prompt initiation of defibrotide, the only approved therapy, is important for a successful treatment because of the severity of VOD. Currently, no established predictive biomarker exists for monitoring disease progression or therapy responsiveness.

Methods: In this prospective study, we systematically analyze the viscoelastic changes in coagulation system with a focus on plasmin resistance in HSCT patients. 57 pediatric hemat-oncological patients receiving HSCT and a control group of 55 non-HSCT patients were included in this study. Whole blood samples, grouped into different chronologic categories were investigated using a viscoelastic test system to assess the coagulation and fibrinolysis systems.

Results: 5 out of 57 (9%) HSCT patients developed VOD. Significantly elevated resistance to clot lysis and longer time of lysis after adding tissue plasminogen activator were observed in blood samples from VOD patients compared to non-VOD HSCT patients 1 and 3 weeks after HSCT (304±57 vs. 244±67, p-value 0.04; and 330±67 vs. 245±56, p-value <0.01, respectively). Most importantly, defibrotide improved thromboelastographic parameters in VOD patients. In addition, impaired fibrinolysis parameters were associated with clinical risk factors for VOD after HSCT.

Conclusion: Our data support the hypothesis that VOD is associated with impaired fibrinolysis. Thromboelastography could be useful to diagnose VOD in patients after HSCT and monitor defibrotide therapy. Further investigations are needed to determined cut-off values and investigate a larger cohort.

Disclosure Statements: None declared.

Sektion Versorgungsforschung – Donor Management

The effectiveness of telephone surveys on the retention of first-time donors – A randomized controlled trial

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Background: The supply of blood products relies critically on voluntary donations. The donor pool declines due to demographic change. Retention of first-time donors is key in optimizing blood product supply and economic costs. A monocentric, parallel-group, randomized controlled, open-label trial was conducted to investigate the effectiveness of a telephone survey on the retention of first-time donors (FTDs).

Methods: The study population consisted of first-time whole blood donors who met blood donation criteria, tested negative for infectious diseases, and gave their consent to be contacted by telephone. A randomization list with blocks of 10 was used to allocate FTDs to two groups, the telephone

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survey group (TG) and the control group (CG). In the TG men were contacted 8 and women 12 weeks after their first donation. They were asked about their satisfaction, and their intention to return, and were offered an appointment. The primary outcome was the return rate within 6 months after the first donation.

**Results:** In an Intention-to-treat analysis, 418 FTDs (mean age 28.8±10.0; 59.9% female) were randomized (TG n=206; CG n=210, two donors were excluded post-randomization due to non-consent or not being a first donor). In the TG, 90.8% were reachable by telephone. The return rate after 6 months was 10.8% (95%-CI [0.9%; 20.6%]; p=.033) higher in the TG (65.0%) compared to the CG (54.3%). The median time at which 50% of donors have returned was 24.2 weeks in the CG compared to 14.8 weeks in the TG (p=.002). The intervention effect in men (CG 48.8%; TG 70.6%; diff 21.8%) was larger than in women (CG 57.8%; TG 61.2%; diff 3.4%), although the interaction test was not significant (p=.059).

**Conclusion:** Contacting FTDs personally and offering an appointment after their first donation can significantly increase the return rate within 6 months and also motivate FTDs to return significantly sooner for a 2nd donation. Male donors appear to be more receptive to this type of intervention. Whether the effect of the intervention helps to establish increased donor retention in the long term should be the subject of further studies.

**Disclosure Statements:** No conflicts of interests.

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**Fig. 1**

| Condition | All | Control group | Telephone group |
|-----------|-----|---------------|-----------------|
| Return rate ITT, % (n) | 59.6 (248) | 54.3 (114) | 65.0 (124) | 0.003 |
| Male | 58.9 (100) | 48.8 (40) | 70.6 (60) | 0.059 |
| Female | 59.4 (148) | 57.8 (74) | 62.1 (74) | 0.092 |
| Return rate PP, % (n) | 60.6 (241) | 54.3 (114) | 67.6 (127) | 0.010 |
| Restricted mean time to return within 6 months, weeks ± SD | 18.27 ± 7.7 | 19.36 ± 7.61 | 17.16 ± 7.64 | 0.002 |

Table 1: Interaction test comparing the intervention effect in men and women.

**Fig. 2**

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**Prediction of patient individual platelet demand in a large tertiary care hospital**

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**Background:** An increasing shortage of donor blood is expected, considering the demographic change in Germany. In addition, the storage of platelet concentrates is a challenge due to the short shelf life and the high daily fluctuations in consumption. Therefore, the objective was to evaluate multimodal data from multiple source systems in the hospital to form a prediction of platelet transfusions in the next three days on a per-patient level and, if so, how many units a patient needs for the next three days.

**Methods:** Data of 255,107 (51% female) patients between 2017 and 2021 were collected. For each patient, the number of received platelet concentrates, platelet blood count, toxic medications, acute platelet diseases, medications, and procedures, age, gender, and the time span of a patient stay were collected. A two-step model was trained on samples using a sliding window of 7 days input and 3 days target. The first model predicts whether a patient will be transfused at all. The second model predicts if a patient will receive none, one, or two or more platelet concentrates for each of the three days. Both models were trained with excessive hyperparameter search using patient-level repeated 5-fold cross-validation to optimize the average macro F1-score.

**Results:** The trained models were tested on 1,052 unique patients with 11,374 samples. The system has an accuracy of 0.863 and F1-score of [0.93; 0.56; 0.20] for day 1, an accuracy of 0.86 and F1-score of [0.92; 0.39; 0.12] for day 2 and an accuracy of 0.86 and F1-score of [0.92; 0.33; 0.10] for day 3. Due to the high-class imbalance, the model predictions do not generalize well for transfusions of three or more platelet concentrates compared to the training metrics.

**Conclusion:** A patient individual AI-based platelet forecast could improve logistics management and reduce blood product expiry. The number of platelet concentrates to be transfused to a subject was predicted by our model for the next three days. Due to the class imbalance, outliers with two or more transfusions per day underperform. As outliers can be mapped to specific stations, a more granular prediction model should ensure further improvement. Furthermore, a clinic-wide consumption can be derived.

**Disclosure Statements:** The authors declare that there is no conflict of interest.
Sociodemographic characteristics of active blood donors and registered stem cell donors in Germany: Results from a population-representative survey

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Background: The health care system relies heavily on active blood donors and potential stem cell donors to care for seriously ill patients. To target recruitment campaigns, it is important to know the social characteristics of these donors. We, therefore, describe the sociodemographic characteristics of active German blood donors and registered stem cell donors and highlight the differences between both groups.

Methods: We conducted a secondary analysis of data from a population-representative survey of the German Federal Centre for Health Education (BZgA) from 2018. The survey data were provided by Gesis data archive for secondary data analyses. In this survey, 4,001 randomly selected participants provided information about their previous whole blood donations and whether they had registered as stem cell donors. We determined the proportion of active blood donors who have made at least one whole blood donation in the past 12 months and the proportion of registered stem cell donors. Multiple logistic regressions were used to describe the sociodemographic characteristics of active blood donors and registered stem cell donors.

Results: Among the participants of the survey, 10.3% were active blood donors and 22.0% reported that they had registered as potential stem cell donors. The highest proportion of active blood donors was found among younger participants (OR: 2.14), among middle-income participants (OR: 1.74) who are from rural areas (OR: 1.64). There were no significant educational differences. In contrast, registration as a stem cell donor was particularly common among highly educated participants (OR: 1.42) with educational differences. In contrast, registration as a stem cell donor was particularly common among highly educated participants (OR: 1.42) with educational differences. In contrast, registration as a stem cell donor was particularly common among highly educated participants (OR: 1.42) with educational differences. In contrast, registration as a stem cell donor was particularly common among highly educated participants (OR: 1.42) with educational differences. In contrast, registration as a stem cell donor was particularly common among highly educated participants (OR: 1.42) with educational differences.

Conclusion: To increase blood donation as well as stem cell donation, blood services should try to increase donor motivation as well as donor retention among urban dwellers. Studies on rural-urban differences in donor motives and barriers would be helpful in developing successful strategies. In addition, future campaigns to increase registration as a stem cell donor should be carefully revised to reach more people with low education and low income.

Disclosure Statements: No conflict of interests.
Enhanced platelet turnover induced by platelet apheresis and platelet reactivity

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Background: Platelet turnover comprises the processes of platelet generation, consumption and clearance. We recently identified a young platelet subpopulation (RNA; HLA-1- and GPVI-rich) with higher reactivity circulating under steady state platelet generation. We now aimed to investigate, how an increased turnover induced by platelet apheresis affects the proportion of these young platelets and its impact on overall platelet function.

Methods: Blood from healthy platelet apheresis donors (n=26) was drawn before apheresis (day 0) and on days 3 and 10 after apheresis. Young platelets were identified based on residual RNA staining with Cy5-conjugated oligo-dT/oligo-dA and by HLA-1 expression. Platelet reactivity was determined after stimulation with TRAP-6 (thrombin receptor-activating peptide), CRP (collagen-related peptide) and ADP (adenosinediphosphate) and assessment of CD62P-expression (alphagranule release) and PAC-1 binding (integrin αIIbβ3 activation) at each time point.

Results: The proportion of young platelets significantly increased on day 3 after apheresis compared to day 0 (RNA-positive platelets: 35.1% vs 10.5%, p=0.0089; HLA-1-rich platelets: 115% vs 100%, p=0.0004). Concomitantly, platelet reactivity was increased on day 3: Mean fluorescence intensity (MFI) PAC1-binding after stimulation with TRAP-6: 5857 vs 3728, p=0.0071; and CRP: 7956 vs 6115, p=0.0413; MFI CD62P expression after stimulation with TRAP-6: 5825 vs 31735, p=0.0526; and CRP: 61216 vs 35383: p=0.0245. The responso ADP did not change. Reactivity returned to baseline at day 10.

Conclusion: An increased platelet turnover induced by platelet apheresis is accompanied by a transient increase of platelet reactivity in healthy donors due to an increased proportion of circulating young platelets. These findings may have consequences for repeat apheresis donors and for patients with a permanentlyincreased platelet turnover and high thrombotic risk e.g., cardiovascular disease.

Disclosure Statements: No conflicts of interests.

VS-9-2
Anti-HPA-5b antibodies do not cause platelet clearance in vitro

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Background: Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is an immune mediated bleeding condition, in which maternal IgG antibodies (abs) cause Fc-dependent phagocytosis and fetal thrombocytopenia. In Japan, most of FNAIT cases are caused by anti-HPA-4b abs reacted with αIIbβ3 integrin. The second most common abs are anti-HPA-5b reacted with α2β1 integrin. In most FNAIT cases, anti-HPA-5b abs caused mild bleeding tendency. Therefore, the clinical relevance of anti-HPA-5b abs is still questionable.

Methods: Here, we asked the question whether monoclonal antibodies (mAbs) against α2β1 and anti-HPA-5b could induce platelet clearance in vitro. Platelet phagocytosis was performed using pHrodo labelled platelets as described (Takahashi et al., 2017). The phagocytosis index (PI) and the antibody binding were analyzed by flowcytometry. PI>8.5 is defined as positive phagocytosis. Five mAbs (AK7, 16B4, 31H4, G19 and G14) and four anti-HPA-5b sera as well as mAb SZ21 and anti-HPA-4b against αIIbβ3 were analyzed with HPA-4 and HPA-5 phenotyped platelets.

Results: Binding studies by flow cytomtery showed anti-α2β1 mAbs and anti-HPA-5b abs reacted with platelets, but significantly weaker when compared to anti-αIIbβ3 and anti-HPA-4B. Of note, all these abs did not lead to P-Selectin, CD47 and PS expression associated with platelet activation and apoptosis. Analysis of platelets sensitized with mAbs against α2β1 showed extremely low platelets clearance (PI=8.3±8.5) compared with anti-αIIbβ3 sensitized platelets (PI=35.0±8.7). Similarly, all anti-HPA-5b tested so far, did cause significant platelet phagocytosis (PI=8.0±7.3). In the control experiments, mAb against αIIbβ3 as well as anti-HPA-4b antibodies (PI=61.4±38.0) caused significant platelet phagocytosis.

Conclusion: In contrast to anti-HPA-4b, our result demonstrated that anti-HPA-5b did not induce platelet clearance, most probably due to the low copy number of α2β1 compared to αIIbβ3 (around 25 times less). This result is in accordance with our recent experimental and epidemiological studies (Alm et al., 2022). In contrast to αIIbβ3, α2β1 is also found in many other cells including trophoblast. The question whether anti-HPA-5b abs could impair trophoblast function during embryo implantation is intriguing.

Disclosure Statements: None

VS-9-3
Rapid and complete Clearance of HPA-1a mismatched Platelets in a human Model of Fetal and Neonatal Alloimmune Thrombocytopenia by a hyperimmune Plasma derived polyclonal HPA-1a antibody

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Background: Fetal/neonatal alloimmune thrombocytopenia (FNAIT) is a rare and potentially life-threatening bleeding disorder of the fetus/newborn. Antibodies against human platelet antigen 1a (HPA-1a) are associated with the most frequent and severe cases of FNAIT. There are no approved therapies for FNAIT prevention or treatment. RLYB211 is an investigational, plasma-derived, polycional anti-HPA-1a hyperimmune IgG being developed to prevent FNAIT.

Methods: In a randomized, single-blind, placebo-controlled, single-center, phase 1/2 proof-of-concept study (EudraCT Number: 2019-003459-12) we investigated whether a single dose of 1000 IU of RLYB211 could markedly accelerate the elimination of HPA-1ab-mismatched platelets transfused into healthy, HPA-1a–negative participants as compared with placebo. Healthy men (aged 18 to 65 years) who were both HPA-1a- and HLA-A2-negative and had a body mass index (BMI) <35 kg/m² received intravenous RLYB211 (n=6) or placebo (n=2). 1 h after transfusion of HPA-1ab positive platelets. The primary endpoint was the half-life of transfused platelets in circulation after administration of RLYB211 or placebo, determined by flow cytometry.
Results: RLYB211 markedly accelerated the elimination of HPA-1ab platelets in all participants versus placebo (half life of transfused platelets 0.32 hrs. vs. 65.29 hrs. respectively; p value <0.001; Fig. 1). Few treatment-emergent adverse events (TEAEs) were reported; 2 (headache and nausea) were possibly related to treatment, and both were in the RLYB211-treated participants. No participants had developed HPA-1a antibodies at 24 weeks (n=8).

Conclusion: These results establish proof of concept for prophylactic administration of RLYB211 to produce rapid elimination of HPA-1a-mismatched platelets and support its potential use for the prevention of HPA-1a alloimmunization and occurrence of FNAIT.

Disclosure Statements: MKj and JKK are stockholders of Prophylix AS, a Norwegian biotech company, which produced the study drug. MKj and JKK are currently consultants for Rallybio, which is the Sponsor of the study. ZB is employee of Rallybio. The remaining authors declare no competing financial interests.

Flowcytometry-based phagocytosis assay of DAT+ erythrocytes as in-vitro surrogate for immune-mediated hemolysis

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Background: Extravascular immune hemolytic anemia (IHA) triggered by antibodies against red blood cell antigens is caused by tissue macrophages. Monocyte Monolayer Assay (MMA) has been used to assess IHA in vitro, but due to inherent variability and microscopic readout its diagnostic power is limited. Here we propose a flowcytometry-based approach to quantify Fc receptor-mediated phagocytosis by activated human blood monocytes incubated with antibody-coated erythrocytes (ACE).

Methods: CD14+ monocytes from human whole blood were primed with phorbol-12 myristate-13-acetate in order to trigger differentiation into macrophages (MA). Subsequent incubation with ACE, selected by presence of a positive direct antiglobulin test (DAT+), was carried out in 1:6 ratio (MA:ACE) for 1 h at 37°C. We quantified MA-ACE agglutinates by FACS upon staining with fluorophore-conjugated antibodies against CD14 and CD235a (Glycophorin A). Antibody-mediated agglutination was inhibited by co-incubation with IgG (Privigen). We used cytospin preparations to microscopically assess phagocytosis of MA-ACE agglutinates. In vitro hemolysis of ACE was determined by free hemoglobin.

Results: More than 600 experiments were performed with monoclonal and polyclonal antibodies of various blood group epitope specificites (Anti-D, -M, -N, -s, -Jka, -Ge and autoantibodies) to trigger in vitro phagocytosis (Fig. 1). The number of MA-ACE agglutinates proved to depend on many factors. In vitro hemolysis as measured by free hemoglobin is correlated with quantitative occurrence of MA-ACE phagocytosis. Microscopically assessed phagocytosis was also in good and robust concordance with FACS-based assessment of MA-ACE agglutinates. Control of test parameters combined with FACS based assessment of MA-ACE phagocytosis provided reproducible and statistically robust surrogate of in vitro hemolysis.

Conclusion: Flowcytometry-based assessment (Fig. 2) of in vitro interaction between macrophages and antibody-coated erythrocytes allows quantification of cellular immune hemolysis. The power of our approach to also predict in vivo hemolytic potency of anti-red blood cell antibodies and circumvent limitations of MMA is currently being determined.

Disclosure Statements: None
**Abstracts**

**VS-9-5**  
Anti-HPA-5b antibodies and foetal and neonatal alloimmune thrombocytopenia: Incidental association or cause and effect?  

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**Background:** Most cases of foetal and neonatal thrombocytopenia (FNAIT) are caused by maternal anti-human platelet antigen-1a antibodies (anti-HPA-1a). Anti-HPA-5b antibodies are the second most common antibodies in suspected FNAIT cases. Given the high prevalence of anti-HPA-5b antibodies in pregnant women delivering healthy newborns, the association with FNAIT may be coincidental.  

**Methods:** A systematic review of the literature related to FNAIT was conducted according to the PRISMA guidelines. A retrospective analysis of a single-centre cohort of 817 suspected FNAIT cases was included.  

**Results:** The pooled prevalence of anti-HPA-5b antibodies in unselected pregnant women of European descent was 1.92% (n=3111), compared with 3.4% (n=5003) in women with suspected FNAIT. We found weak evidence that a small proportion of pregnant women presenting with anti-HPA-5b antibodies will give birth to a newborn with mild thrombocytopenia. In a cohort of suspected FNAIT cases (n=817) the neonatal platelet counts were not different between cases with and without maternal anti-HPA-5b antibodies. The prevalence of maternal anti-HPA-5b antibodies was not different between neonates with intracranial haemorrhage and healthy controls.  

**Conclusion:** The current experimental and epidemiological evidence does not support the hypothesis that anti-HPA-5b antibodies cause severe thrombocytopenia or bleeding complications in the foetus or newborn.  

**Disclosure Statements:** The authors declare that they have no competing interests.

**VS-9-6**  
Analysis of anti-platelet β3 antibodies profile in immunized β3 knockout mice  

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**Background:** HPA-1a alloantibodies induce variable symptoms in fetuses from thrombocytopenia to severe intracranial bleeding. These antibodies are heterogeneous and target different epitopes on β3 alone or in a complex with a subunit (αIIb or αv). The later antibodies mainly target endothelial-specific antibodies and are known to be involved in the mechanism of FNAIT-mediated bleeding.  

**Methods:** In the current study, immunization of β3 ko (C.129S2-igg3tm1Hyn/J-) female mice with wild-type platelets, led to the production of anti-β3 antibodies. Breeding of immunized mice with wild-type male mice proceeded to pregnancy in β3 pre-immunized mice. Ultrasound analysis of the fetus (E12-E19) indicated normal fetal development up to E15. However, ultrasound analysis identified fetal death after E15. Mice sera before and after pregnancy were absorbed on HEK cells expressing hybrid αIIbβ3 integrin (human/mouse) and reactivity of serum, absorbate and eluate with αIIbβ3 (mouse) or αvβ3 (mouse) expressing HEK cells were examined in flow cytometry. Functional analysis was conducted in angiogenesis using mouse endothelial cell line (3B11).  

**Results:** Investigations indicated a significant enhanced immune response against β3 on platelets (mouse 1: p=0.0043, 2: 0.01476, 3: 0.0081) after pregnancy. Analysis of absorbate using hybrid protein expressing cells identified a reactivity with αIIbβ3 expressing cells which was enhanced after pregnancy. Absorbate from only one mouse (No. 1) after pregnancy was reactive with αvβ3 cells, indicating presence of endothelial-specific β3 antibodies. Functional analysis of all sera revealed a slight angiogenesis blockage in two sera after pregnancy (mouse 2: p=0.0295, mouse 3: p=0.041). Absorbate of the same mice showed no angiogenesis blockage. Both serum and absorbate of mouse 1 strongly inhibited angiogenesis (serum p=0.0166 and absorbate p=0.0066).  

**Conclusion:** This result indicated a) a boosting effect of pregnancy on immune response against β3 in pre-immunized β3 KO mice, b) diverse antibody profiles in immunized mice despite similar immunization protocol, c) presence of endothelial-specific anti-β3 antibodies in one immunized mouse that blocked endothelial angiogenesis, and d) a minor angiogenesis blockage induced by anti-β3 specific antibodies.  

**Disclosure Statements:** NA

**VS-9-7**  
Distinction between anti-D and anti-C from anti-G is essential in pregnant women  

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**Background:** Anti-G antibodies appear like a combination of anti-D and anti-C. The serological distinction between anti-D and anti-C from anti-G is important in women of child-bearing age or in case of pregnancy because patients with Anti-G can be immunized to RhD and therefore require Rh immune globulin (RhIG). A hemolytic disease of the fetus and newborn due to anti-G is usually less severe than one induced by anti-D and anti-C.  

**Methods:** The identification was performed in the indirect antiglobulin test using untreated and papain treated red cells in the gel technique. For differentiation of anti-C and -D from anti-G a sequential elution and absorption was performed, because C- and D- positive red blood cells express the G antigen. The plasma was incubated with RhD +, ccee red cells. The adsorbed plasma was tested for antibodies. An eluate from the adsorbed RhD +, ccee red cells was tested for antibodies and absorbed on RhD −, ccee cells. The absorbed eluate was also tested for the presence of antibodies. To confirm the antibody specificities, the patient sample was sent to a reference laboratory where rare test cells RhD+, ccee, G- and RhD−, ccee, G+ were available.  

**Results:** Anti-C and anti-D antibodies were identified in a 41-year-old woman in her third gestation by an external laboratory. Previous two pregnancies had been uneventful and included the RhIG administration. Serological tests of the maternal plasma confirmed anti-D (32) and anti-C (64). The blood group was AB, RhD −, ccee, K-, the plasma absorbed with RhD +, ccee red cells confirmed anti-C, the eluate revealed an anti-G. The adsorbed eluate did not reveal any antibodies. Anti-C and anti-G were confirmed by an external reference laboratory. After delivery the newborn had no pathological icterus, her blood group was typed A, RhD+, ccee, K−, the direct antiglobulin test was positive (2+). The mother received regular postpartum RhIG.  

**Conclusion:** Serological differentiation of anti-D and anti-C from anti-G is difficult and time-consuming but must always be performed in case of pregnancy. In the absence of anti-D antibodies, the pregnant woman must be administered RhIG to prevent immunization against the RhD antigen.  

**Disclosure Statements:** The authors declare no conflict of interest.

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Screening for Illicit Drugs in Blood Donors Reveals Low but Steady Incidence of THC and Amphetamines in an Urban-predominated Blood Donor Repertoire

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Background: In order to increase blood infection safety as well as to avoid transfusion of toxic substances, the blood donor questionnaire (BDQ) aims to identify and to exclude donors taking illicit drugs. From donor interviews in our center it became evident, that donors at risk exist and that besides tetrahydrocannabinol (THC), also amphetamine and metamphetamine are consumed. Published data indicates that drug abuse may not be reliably reported by donors.

Methods: From 2017-2020, donors or whole blood as well as platelet and plasma apheresis in the age risk group (age 18-30 years) were randomly selected or included upon suspicion (ca. 1.5 % of all donations). All selected donors did not admit illicit drug use in the BDQ and were informed and consented for drug screening. Donors were tested for THC, amphetamine and derivates, cocaine and opiates in blood using high performance liquid chromatography tandem mass spectrometry. Positive results were repeated for confirmation (cutoff 1.0 ng/ml). Blood products were released only after negative results in the drug screening test. Positive donors were informed and excluded permanently (cocaine, opiates, amphetamines) or for one year (THC) from donation.

Results: Of the total 2600 donors tested, 369 (14% of tested donations; ca. 0.22% of all donations) proved positive. In 244 samples (67% of positively tested donors) we detected THC, in 33 (9%) amphetamine or metamphetamine and in 66 (18%) both THC and amphetamine or methamphetamine. Opiates and/or cocaine were found in 17 (5%) of the tested donors. Within our observation period, the rate of the detected positive donations decreased from 25% to 7% of the tested donations, corresponding to a decline from 0.325% to 0.078% of all donations during the observation period (p< 0.00001 using Chi Square Test).

Conclusion: Illicit drugs may be present at a frequency between 0.1 and 1% in blood donors in urban donation centres. The observed decline in the frequency of detecting drugs in donors indicates that it is possible to remove drug-abusing donors from the overall donor repertoire. Further research is needed to elucidate whether the detected drug concentrations may be harmful to transfusion recipients and/or reduce blood-borne infectious risk, and thus could affect patient safety.

Disclosure Statements: No conflicts of interests.

Plasmapheresis in TTP – Can We Give It Up?

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Invited talks abstract/summary: Therapy for immune mediated thrombotic thrombocytopenic purpura (iTTP) is based on three pillars. Immunosuppressive agents, inhibiting the interaction between von Willebrand factor multimers and platelets and the thickest column to date – plasmapheresis. Since the introduction of Plasma Exchange (PEX), the mortality rate has dropped from 90 to 10%. This high efficacy is in contrast to the complexity of this treatment and the complications caused by this procedure. Is it time to abolish this method?

Significant changes have recently been introduced in the treatment of iTTP – the use of anti-CD20 (rituximab) in the early stages of the disease and the administration of Caplacizumab, a von Willebrand factor-directed antibody fragment. Do these changes make it possible to give up plasmapheresis? Can we reduce the burden of TTP therapy at this time or are further changes in treatment approach required before we consider omitting plasmapheresis?

Currently no randomized controlled trials (RCTs) are available to examine these questions. Case reports, registries and data mining from other autoimmune disorders will be presented to stimulate the establishment of a collaborative multinational trial to look at currently available therapies to examine whether it is time to change the treatment paradigm of TTP.

Disclosure Statements: no conflict of interest.
Hämolytische Erkrankung beim Fetus und Neugeborenen

NIPT-RhD Screening: A challenge for the laboratory
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Background: The German Federal Joint Committee determined, that a noninvasive prenatal test for fetal RhD (NIPT-RhD) assay must have a sensitivity of 99 % or higher (lower limit of the 95 % - confidence interval) and a specificity of 96 % or higher (lower limit of the 95 % - confidence interval). In addition, in 2019 a corrigendum of the In-Vitro-Diagnostic Medical Device Regulation (IVDR, 2017/746) was issued by the European Parliament and the Council of the European Union which placed noninvasive prenatal testing of fetal blood groups under the highest risk class D. Furthermore, testing for RhD is challenging because of the high number of genetic RHD variants, which can be present in mother or fetus.

Methods: A literature search is performed and queries are sent to providers of diagnostics in order to determine the availability of commercial assays, which meet the German requirements. German representatives from the Medical Device Coordination Group (MDCG) as the German contact points to the EU commission are interviewed in order to evaluate the chance of changing the current IVDR legislation. In addition, published in-house methods from NIPT-RhD screening laboratories are reviewed.

Results: Results will be presented during the congress.

Conclusion: Providing high quality accurate NIPT-RhD screening results to the vulnerable patient group of RhD-negative pregnant women is the ultimate goal of any medical laboratory offering such a service. Since key players in Germany and other European countries tried to ensure the highest possible accuracy, the possibility that diagnostic companies will withdraw their test kits from the German market must not be ignored.

Increased plasma level of soluble P-selectin in non-hospitalized COVID-19 convalescent donors
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Background: COVID-19 has severe implications on the vascular and coagulation system. A procoagulant platelet phenotype has been reported for the acute disease phase. Soluble P-selectin (sP-sel) in the plasma is a surrogate biomarker of platelet activation. Increased plasma levels of sP-sel have been reported in hospitalized COVID-19 patients associated with disease severity. In a longitudinal study we evaluated the sP-sel plasma concentration in blood donors who previously suffered from moderate COVID-19.

Methods: 154 COVID-19 convalescent and 111 non-infected control donors were recruited for plasma donations and for participation in the CORE research trial. First donations were performed 6 to 56 weeks after COVID-19 diagnosis. Blood samples were taken at up to three plasma donations (T1, T2, T3) within a time period of 8-12 weeks. Baseline characteristics including COVID-19 symptoms of the donors were recorded based on a questionnaire. Platelet function was measured at T1 by flow cytometry and light transmission aggregometry in a subset of 25 COVID-19 convalescent and 28 control donors. The sP-sel plasma concentration was determined in a total of 704 samples by using a commercial ELISA.

Results: In vitro platelet function was comparable in COVID-19 convalescent and control donors at T1 (3-9 months after disease diagnosis). Plasma samples from COVID-19 convalescent donors revealed a significantly higher sP-sel level compared to controls at T1 (1.05 ± 0.42 ng/mL vs. 0.81 ± 0.30 ng/mL; p<0.0001) and at T2 (0.96 ± 0.39 ng/mL vs. 0.83 ±
0.38 ng/mL; p=0.0098). At T3 the sP-sel plasma level was comparable in both study groups. Most of the COVID-19 convalescent donors showed a continuous decrease of sP-sel from T1 to T3.

**Conclusion:** Increased sP-sel plasma concentration as a marker for platelet activation could be demonstrated even weeks after moderate COVID-19, whereas, in vitro platelet function was comparable with non-infected controls. We conclude that COVID-19 and additional individual factors could lead to an increase of the sP-sel plasma level.

**Disclosure Statements:** The authors declare no conflict of interest.

**VS-13-3**

**Long-term Follow up (> 1 year) of Plasma Donors and Patients treated with COVID-19 Convalescent Plasma within the CAPSID trial**

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**Background:** COVID-19 Convalescent Plasma (CCP) showed beneficial effects when given early in the treatment course or when it contains high-titre of neutralizing antibodies. Here we present a long-term follow up of patients of the multicentric national randomized CAPSID trial that investigated the effect of CCP in hospitalized COVID-19 patients. CCP donors were also included in the follow up and severed as a control group of patients with mild to moderate disease.

**Methods:** Patients and donors were invited to participate in the long-term follow up. Data on long-term overall survival (OS) were available for n=52 patients (control group: n=22, high titre CCP: n=16, low-titre CCP: n=14) and n=113 donors. Structured interview and a quality of life (QoL) assessment by questionnaires (FACT fatigue, FACT dyspnea and EQ-ED-5L) were performed. Visits took place online or on site. Laboratory tests included neutralizing antibody testing by PRNT and inflammation markers. Data are given as median with IQR. Medical events during follow up were reported in 27% of donors and 16% of patients (p=0.164) with grade 3 or higher in 9% of donors and ~16% of patients (p=0.164) with grade 3 or higher in 9% of donors and 371–417 days after randomization.

**Results:** Medical events during follow up were reported in 27% of donors and 16% of patients (p=0.164) with grade 3 or higher in 9% of donors and 16% of patients (p=0.164) with grade 3 or higher in 9% of donors and 371–417 days after randomization.

**Conclusion:** COVID-19 convalescent plasma (CCP) remains a potential therapy of COVID-19, e.g. for new variants and for patients with impaired immune response. The trial COVID-19 takes into account lessons learned from previous trials and combines it to a novel approach:

• CCP with very high levels of SARS-CoV-2 antibodies from donors with previous SARS-CoV-2 infection (inf) and vaccination (vax)

**Disclosure Statements:** Funding: the CAPSID trial has been supported by the German Federal Ministry of Health.

Dr. Victor M Corman is named together with Euroimmun on a patent application filed recently regarding the diagnostic of SARS-CoV-2 by antibody testing. The other authors do not declare a conflict of interest related to this trial.

**VS-15-4**

**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 pandemic with a new pathogen: Experience from collection of very high-titre plasma from superimmunized individuals**

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5Dr. Victor M Corman is named together with Euroimmun on a patent application filed recently regarding the diagnostic of SARS-CoV-2 by antibody testing.

**Background:** COVID-19 convalescent plasma (CCP) shows potential benefit for COVID-19 patients with mild to moderate disease. The addition of CCP added to standard treatment in severe COVID-19 showed a trend to better OS and QoL. We had previously reported significant better outcomes in the high-titre CCP subgroup (until day 60). This was even more pronounced during the long-term follow up (> 1 year).

**Methods:** We report the initial experience of collection of very high-titre plasma units (defined as ≥4,000 BAU/mL in the QuantiVac ELISA) for this COVIC-19 trial. We recruited 348 potential donors (151 male, 197 female) who had passed initial eligibility check. S-Ab were measured by anti-SARS-CoV-2 QuantiVac ELISA (Euroimmun): mean 4229 BAU/mL (IQR 2.239-5.486 BAU/mL). High S-Ab in the QuantiVac assay correlated with previous SARS-CoV-2 infection (inf) and vaccination (vax). High S-Ab were measured by anti-SARS-CoV-2 QuantiVac ELISA (Euroimmun): mean 4229 BAU/mL (IQR 2.239-5.486 BAU/mL). High S-Ab were measured by anti-SARS-CoV-2 QuantiVac ELISA (Euroimmun): mean 4229 BAU/mL (IQR 2.239-5.486 BAU/mL). High S-Ab in the QuantiVac assay correlated with previous SARS-CoV-2 infection (inf) and vaccination (vax).

**Results:** We analyzed the association between the order of immunizing events and S-Ab. Highest S-Ab were observed among those with a break-through infection after 2 vax, followed by a booster (3rd dose post inf.) (median 5.840 BAU/mL; 76.7% ≥4,000 BAU/mL) or breakthrough infection after 2 vax, followed by a booster (3rd dose post inf.) (median 5.840 BAU/mL; 76.7% ≥4,000 BAU/mL).
Abstracts

3 vax (no further booster; median 3,841 BAU/ml; 47.9% ≥4,000 BAU/ml). S-Ab were lower in those with inf before vax followed by 1 vax (median 1,806 BAU/ml; 18.1% ≥4,000 BAU/ml) or >1 vax (median 2,586 BAU/ml). S-Ab declined rapidly: 42% of donors with S-Ab ≥4,000 BAU/ml had declined below this threshold in the short interval until 1st plasmapheresis and further 6% until 2nd apheresis. Further follow-up will be presented.

Conclusion: Taking into account all eligibility criteria only 8.6% of individuals willing to donate could provide plasma units meeting the criteria of high-titer plasma for COVID-19. 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 immediate start of apheresis to take advantage of the short period of very high mAb.

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Disclosure Statements: None.

VS-13-5

Humoral and cellular immunity after the third vaccination against SARS-CoV-2 in hematopoietic stem cell transplant recipients

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Background: Protecting vulnerable groups from severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) infection is of great importance, as they suffer more often from severe or fatal courses. The immune response after a third vaccination is insufficiently studied in patients after hematopoietic stem cell transplantation (HSCT).

Methods: We analyzed the immune response before and after a third vaccination against SARS-CoV-2 in HSCT patients and healthy controls. This is the first report on neutralizing antibodies against 11 variants of SARS-CoV-2, analyzed by competitive fluorescence assay. Humoral immunity was also measured by semi-/quantitative ELISA and neutralization tests. Cellular immunity was assessed using interferon-gamma (IFN-γ) and interleukin-2 (IL-2) ELISpots.

Results: After the third vaccination, the cellular immune responses were 2.4-fold higher for IFN-γ in healthy controls vs. HSCT patients, and 3.4-fold higher for IL-2. On average, healthy controls vs. HSCT patients had 1.5-fold higher concentrations of neutralizing antibodies against variants and 1.6-fold higher antibody ratios and 4.4-fold higher antibody concentrations against wild-type SARS-CoV-2.

Conclusion: We show that immune responses of HSCT patients are significantly lower vs. healthy controls, but HSCT patients also exhibit neutralizing antibodies to variants of SARS-CoV-2. Our findings may contribute to the adaptation of vaccination strategies and further protective measures for immunocompromised individuals.

Disclosure Statements: No conflicts of interest.

VS-14

Neighbor Day Israel II

Revival of Whole Blood: The Israeli National Blood Program

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Invited talks abstract/summary: Use of LTOWB as the preferred resuscitation fluid for Massive Hemorrhage, greatly simplify resuscitation effort logistics. Transfusing LTOWB to non-group O recipients does not cause immune-related hemolysis. Necessary steps for such decision included: Review international experience using LTOWB. Evaluate transfusion needs of severely bleeding patients in the military pre-hospital system, evacuated by Air or Ground transportation and in the civilian hospitals. Involve Magen David Adom National Blood Services (MDANSB), responsible for collection, processing, testing and supply of blood to all the hospitals nationwide, and the Israeli Defense Forces (IDF). Create a protocol for anti-A, anti-B titration in group O male donors, using automated blood typing equipment. Build a LTOWB donors database and manage the national LTOWB inventory. Automated titration was created and validated in 2018, using PK7300 blood typing machine, for a critical titer of anti-A and B as the equivalent of <50 (saline, 5’RT), yielding 15% of O type donations. In 2021, following an uneventful experience, the titer was changed to <100, yielding 45% of O type donations. Data on LTOWB supply, usage and discard rate was collected from MDABS database and reports from the users (9 hospitals and the IDF). Between 6/2018-6/2022, about 3,835 / 35,920 O type blood donations were identified as LTO. Of them, 1011 (26%) were transfused to massively bleeding patients: 52 units in the military pre-hospital setting and 959 in Emergency/Trauma units in the hospitals. About 51% were transfused as LTOWB and 49% as Packed cells that were separated from the LTOWB after 21 days. 78% of the LTOWB were discarded.

Disclosure Statements: The authors have no conflicts of interest to declare.

VS-13-6

COVID-19 causes a change in the dynamics of hemostasis during extracorporeal membrane oxygenation (ECMO)

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Background: In severe cases of COVID-19 ECMO is needed as a life-saving intervention. COVID-19 as well as ECMO may cause severe coagulopathies manifesting themselves in micro and macro thrombosis. Previous studies established D-dimers as a marker for critical thrombosis of the ECMO system while increased D-dimers on admission are associated with a higher mortality in COVID-19 patients. It is therefore crucial to determine if COVID-19 poses an increased risk of early thrombosis of the vital ECMO system.

Methods: 40 patients who required ECMO support were enrolled in a retrospective analysis and assigned into 2 groups. The COVID group consisted of COVID-19 patients who required ECMO support (n=20), whereas ECMO patients without COVID-19 were assigned to the control group (n=20). D-dimers, fibrinogen, antithrombin III (ATIII), lactate dehydrogenase (LDH) and platelet count were visualized using locally weighted scatterplot smoothing. For model validation MANOVA tests were used to confirm the effect of group membership on the parameters over time.

Results: In COVID-19 patients D-dimers reach their peak 2–3 days earlier than the control group. In COVID-19 fibrinogen is consumed steadily while in the control group fibrinogen levels increase rapidly after 10 days. Both groups experience a rapid increase in ATIII beyond 130 % at the start of ECMO. In the COVID-19 group platelet count decreased from 210 gigi/l to 130 gigi/l after 6 days, while in the same time span in the control group platelets decreased from 180 gigi/l to 100 gigi/l. In both group a prominent increase in LDH beyond 5000 U/l occurs.

Conclusion: The early increase in D-dimers and decrease in fibrinogen suggests that COVID-19 patients bear an increased risk of early thrombosis of the ECMO system compared to other diseases. Additionally, the control group shows signs of severe inflammation 10 days after the start of ECMO which were absent in COVID-19 patients.

Disclosure Statements: The authors have no conflicts of interest to declare.
Implementation of the use of LTOWB started in Israel in 2018, to improve treatment and prevent death from massive hemorrhage. The IDF and 30% of the hospitals nationwide introduced its use. Automated Anti A and B titration enables identification of LTOWB donors, creation of database, inventory and supply of units to military and civilian pre and in-hospital settings. Further efforts are needed to minimizing wastage of the non-used units. A multi-disciplinary expert group, including Transfusion Medicine specialists, should recommend introduction of such changes to the Authorities.

Disclosure Statements: I have no conflict of interest

VS-15
Thrombozyten-Immunhämatologie

VS-15-2
Target number and levels of glycoprotein-specific platelet autoantibodies predict lower platelet counts in thrombocytopenic patients

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Background: Autoantibodies directed against platelet glycoproteins are a major pathophysiologic mechanism in immune thrombocytopenia. The diagnosis of ITP relies on clinical criteria and no gold standard is available. We hypothesized that if an assay identifies clinically relevant G-PA in a patient, antibody level and number of targeted glycoproteins should be associated with thrombocytopenia if the test is diagnostic and the identified G-PA mediates a major pathophysiologic mechanism in ITP.

Methods: We performed a retrospective cohort study using data available in our platelet reference laboratory information system by searching for screened for G-PA. Increased antibody levels predict lower platelet counts.

Results: 15,031 adults, of which 573 (16.2%) and 1,859 (12.4%) had a positive titre of G-PA and G-PA level was available. G-PA were identified using the direct monoclonal antibody-specific immobilization of platelet antigen (MAIPA) assay by immobilizing two major platelet glycoproteins (GP), IIb/IIIa and Ib/IX. Antibody levels were recorded on a semi-quantitative scale.

Conclusion: The presence of autoantibodies against GP IIb/IIIa or GP Ib/IX is associated with lower platelet counts in adults, but not in children. We conclude that detecting G-PA is diagnostic for pathological antibodies and that appropriate assays are useful when evaluating patients suspected of suffering from ITP.

Disclosure Statements: None of the authors have conflicts of interest to declare.

VS-15-3
Procoagulant platelets as a diagnostic tool for the evidence of a heparin-induced thrombocytopenia by flow cytometer

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Background: Heparin-induced thrombocytopenia (HIT) is a prothrombotic, immune-mediated adverse reaction after exposure to unfractionated heparin (UFH) or low molecular heparin (LMWH). The diagnosis of HIT can be confirmed using functional assays such as the Heparin-Induced Platelet Activation assay (HIPA assay). However, functional assays are technically demanding and routinely available only in specialized laboratories. Recently, we showed that anti-PF4/Heparin antibodies induce procoagulant platelets.

Methods: The aim of the current study was to establish a flow cytometer-based method to detect procoagulant platelets in platelet-rich plasma (PRP) for the diagnosis of HIT. Sera samples from patients with HIT (HIT group) were incubated with PRP from healthy donors. Procoagulant platelets were determined by double expression of P-selectin (CD62p) and phosphatidylserine (PS) externalization by flow cytometry.

Results: Sera from HIT-diagnosed patients but not from the control-group induced a significant increase in the procoagulant platelet subpopulation in the presence of 0.2 U/mL heparin (% double positive CD62P/PS: 1.2±1.1 vs 15.8±8.1, p=0.0021). The optimal incubation time was 60 minutes. A donor dependency of the flow cytometric method was not observed. In addition, the use of washed platelets and PRP with HIT-sera led to the same results in the flow cytometric method (34.2±6.3 vs 30.1±2.2, ns).

Conclusion: Our data suggest that PRP-based protocol can be used to detect the ability of HIT antibodies to induce procoagulant platelets by flow cytometry. Ongoing study is currently investigating the clinical implementation of this protocol in the diagnostic work up for HIT.

Disclosure Statements: I have no conflict of interest to declare.

VS-15-4
Platelet clearance caused by monoclonal antibodies against different subunits of GPIb/IX complex

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Background: In patients with immune thrombocytopenia, autoantibodies GPIb/IX could induce platelet clearance viaFc-dependent and -independent mechanism. Recent studies showed that antibodies against LBD (Ligand Binding Domain) of the GPIbα could activate GPIb/IX, induced platelet desialylation and platelet clearance by hepatocytes and/or macrophages. Interestingly, further studies demonstrated that antibodies (mAbs) against GPIb/IX subunit could differentially regulate this process.

Methods: Here, we compared the capability of mAbs against LBD (AP1, AK2), GPIbβ (RAM.1, G10, G27) and GPIbIX (FMC225) to induce platelet phagocytosis in vitro. Mabs G10 and G27 were produced in our laboratory. Mabs were characterized using platelets, wild-type and mutants of GPIb/IX expressed on CHO cells and recombinant GPIb/IX, GPIbE (ectodomain) proteins by flow cytometry, immunoprecipitation and immunoblotting. Platelets clearance was analyzed by Platelet Suspension Phagocytosis Assay (PSPA) using pHrodo-labelled platelets. The phagocytosis rate of monocytes that internalized sensitized platelets and the binding of antibodies onto platelets were analyzed by flow cytometry.

Results: By immunochenical analysis we found that mAb G27 reacted with the intracellular part of GPIbβ. In contrast, mAb G10 recognized unique epitopes on the extracellular GPIbβ subunit. Flow cytometry
analysis showed that except mAb Gi27, all mAbs, reacted with platelets and GPIb/IX transfectants. Sensitization of platelets with anti-LBD and anti-GPIX caused significant platelet phagocytosis (~40%), although lower when compared to anti-αIIbβ3 (AP3) mediated platelet clearance (~55%). Both, anti-αIIbβ3 as well as anti-GPIX/PIX mediated platelet phagocytosis was abrogated by Syk inhibitor (R406). Interestingly, mAb RAM.I, but not mAbs Gi10 and Gi27 (control) against GPIbβ induced platelet clearance. Conclusion: In this study, we found that antibodies against LBD and GPIX could induce platelet phagocytosis in PSMA. This capability is weaker compared to anti-αIIbβ3 antibodies, most probably due to lower copy number of GPIb/IX complex on the platelet surface. The fact that different mAbs (RAM.I and Gi10) against GPIbβ showed different functional effects, may open the use of anti-GPIXβ derivative to inhibit platelet clearance caused by anti-GPIX/PIX autoantibodies.

Disclosure Statements: NONE

VS-16
Immunogenetik

VS-16-2
Higher risk for chronic graft-versus-host disease (GvHD) in HLA-G mismatched transplants following allogeneic hematopoietic stem cell transplantation (allo-HSCT)

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Background: HLA-G has been shown to promote donor tolerogenicity. We hypothesized that in an allo-HSCT setting, HLA-G mismatches may negatively impact the HLA-G mediated tolerogenicity. HLA-G mismatches may contribute to the onset of chronic GvHD, especially in younger patients and should therefore be avoided when possible.

Discussion Statements: The authors declare that they have no conflicts of interest. There are no commercial interests associated with this study. The funding sources did not influence the content of the manuscript or the decision to submit for publication.

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VS-16-3
The alloCELL program: Patient monitoring, donor selection and GMP-compliant manufacturing of virus-specific T cells from stem cell, family and third-party T-cell donors

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Background: Intensive immunosuppression to prevent graft rejection and GvHD leads to impaired T-cell immunity in HSCT and SOT patients. These are at high risk for infection with and reactivation of opportunistic pathogens such as CMV, EBV, HHV6, ADV and BKV, which are associated with significant morbidity and mortality. The inadequacies of conventional therapies have increased interest in T-cell immunotherapy. Here, timely T-cell donor recruitment and rapid production of antiviral T cells are required.

Methods: To improve T-cell donor recruitment, the alloCELL registry was established (www.alloCELL.org), currently recording >3,500 HLA-typed donors with extensively characterized antiviral T-cell repertoire. The registry has been extended by convalescent COVID-19 donors. The alloCELL lab established protocols to consider clinical requirements of patients at high risk or with failed conventional therapy. The manufacturing license for clinical-grade virus-specific T-cell products using Cytokine Capture System and CliniMACS Prodigy was obtained. T-cell donors are considered eligible if ≥0.1% specific interferon-γ’ T cells are detectable. A related haploidentical or ≥5/10 HLA-matched alloCELL donor is recommended if the stem cell donor is not eligible.

Results: As of April 2022, >410 multi-/monovirus-specific T-cell products were generated for clinical applications by using overlapping peptide pools that cover the complete sequence of a viral protein. For patients in need of an unrelated third-party donor, suitable donors were found and clinical grade T-cell products were provided within 1.5 weeks after request with an HLA compatibility ≥5/10. The applied T cells were monitored to determine frequency, chimerism and TCR repertoire. Patients who received antiviral donor T cells did not show severe adverse effects and in 80% of the cases, antiviral T cells were detected in blood after T-cell transfer. Of note, there is evidence that adoptive T-cell transfer induces endogenous T-cell responses.
Conclusion: Success of antiviral T-cell transfer benefits from (i) accurate monitoring of viral load and antiviral T-cell frequencies in patients, and (ii) early and fast selection of suitable T-cell donors. Our data support clinical safety and efficacy of third-party antiviral T cells.

Disclosure Statements: The Authors declare no Conflict of Interest.

Genetically engineered lungs are protected from immune rejection in the absence of immunosuppression

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Abstracts

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The wonderful world of Plasma - an insight into the blood plasma market

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Background: Blood plasma is a wonderful product. It cannot be produced; it can only be donated as a gift from one life to another. The need for blood in our transfusion system is more obvious. Plasma, the liquid part of the blood, is less commonplace. Neither the way it is collected, nor its use and need are elements with which many people are very familiar. Plasma is a source of proteins that are lifesavers and treatment at the same time.

Method: Plasma can be obtained either from a blood or a direct plasma donation by plasmapheresis. Approximately 300 ml of plasma (“Recovered Plasma”) are obtained from a blood donation. In a direct plasma donation (“Source Plasma”), up to 850 ml (1000 ml in the USA) can be donated.

Results: While all recipients of the control group rejected before post-operative day (POD) 100, five of seven recipients of SLA-silenced lungs survive without rejection currently four years after transplantation in the absence of immunosuppression. After removal of immunosuppression at POD 28, frequencies of donor specific T-cells were significantly lower in the SLA-silenced lung transplant group (CD4: 13.4±3.3%; CD8: 21.1±2.1%, p<0.01) and an almost complete SLA class II silencing (p<0.01).

Conclusion: Levels of antibodies increased after transplantation in both groups, but to a much lower extent in the recipients of SLA-silenced grafts (p<0.05). This study shows the power of genetic allograft engineering to reduce their immunogenicity enabling graft survival even in the absence of immunosuppression and opens a fully new horizon in organ transplantation.

Disclosure Statements: Rainer Blasczyk and Constanca Figueiredo are inventors in patents related to this abstract.
Plasmapheresis is safe in donors with low IgG levels: Data from a prospective, controlled multicentre study

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Invited talks abstract/summary: Introduction: Although plasmapheresis is considered safe, data about long-term effects of plasma donation on IgG levels are sparse. The aim of the present investigation was to evaluate if there is a need to permanently defer plasmapheresis donors who donated three times with an IgG level below 6.0 g/L.

Methods: Data of adverse events (AEs) including infections were retrieved from a controlled, prospective multicentre study of healthy plasmapheresis donors, participating in an individualised donation programme stratified by body weight and initial IgG level (individualised arm) or in standard plasmapheresis according to current hemotherapy guidelines (control arm). IgG was monitored at every fifth plasmapheresis, and donors with IgG levels below the threshold were identified and followed up for possible AEs.

Results: 1,491,223 donations in 14,281 donors in the individualised arm and 97,540 donations in 1,462 donors in the control arm were included. Donation-based incidences of at least severe AEs and any infections were observed in either donor arm.

Conclusions: No compromised donor safety in donors with ≥ 3 IgG measurements below the threshold was observed, indicating that plasmapheresis donors, participating in an individualised donation programme stratified by body weight and initial IgG level (individualised arm) or in standard plasmapheresis according to current hemotherapy guidelines (control arm), is considered safe, data about long-term effects of plasma donation on IgG levels are sparse. The aim of the present investigation was to evaluate if there is a need to permanently defer plasmapheresis donors who donated three times with an IgG level below 6.0 g/L.

Disclosure Statements: none

RhD conversion in red blood cell concentrate supply – the strategy of the German Red Cross Blood Transfusion Service Baden-Württemberg – Hessen as supplier of hospitals without blood donation department

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Invited talks abstract/summary: Background: Red blood cell concentrates (RBC) of blood group O RhD negative are compatible with most patients. They are, therefore, preferably transfused in emergency situations when the blood group of the patient is not known yet. Sometimes they are also regarded as a kind of universal RBC. However, O RhD negative RBC are a scarce commodity due to the fact that only 6% of the German population and therefore, of the blood donor community, are O RhD-negative.

Methods: Different approaches are pursued in order to keep requirement and supply in workable order. First, donor management tries to approach donors who are RhD-negative in a prioritised manner and organises RhD focused blood drives. Second, our education programmes in the hospitals clearly show, that O RhD-negative are not universal RBC that “fit all in all cases”, e.g. 17% of patients are RhCC and should receive Rhcc RBC only in emergency situations. Third, our online network enables physicians on duty as well as supply managers to balance requirement and supply in a timely manner. Fourth, a slightly higher price for O RhD-negative RBC and a “sale or return” policy for AB RBC enables hospitals to actively control their use of scarce blood group RBC.

Results: By using the four abovementioned approaches, our blood transfusion service (BTS) is able to supply most of the RhD-negative RBC requirements. In accordance with the German guidelines, RhD-negative females of childbearing potential can be supplied in virtually all cases with RhD-negative RBC, as well as RhD-negative children. We also supply RhD-negative patients with long lasting RBC support requirement, e.g. hematology/oncology patients, with RhD-negative RBC. In this last patient group, we cannot guarantee a 100% success rate. RhD-negative females older than 50 years of age and RhD-negative males, both without long lasting RBC requirement, have to be switched to RhD-positive RBC at times. In such cases, our physicians counsel their clinical counterparts in every detail in order to make this switch safe for the patient.

Conclusions: Only in close cooperation between our BTS and the supplied hospitals, our strategy of RhD conversion in RBC supply is able to work well. Therefore, repeat counselling, education and mutual trust are the cornerstones of this strategy.

Disclosure Statements: Authors are employees of the German Red Cross Blood Transfusion Service Baden-Württemberg - Hessen.
New ultraviolet C light-based method for pathogen inactivation of red blood cell units

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Background: Pathogen inactivation (PI) technologies for platelet concentrates and plasma are steadily becoming more established, but new PI treatment options for red blood cells (RBCs), the most commonly used blood component, still need to be developed. We recently established a novel approach to reduce pathogens in RBC units employing ultraviolet C (UVC) light. Here we present detailed information about the procedure and additional results on product quality.

Methods: The UVC-based PI procedure for RBCs consisted of four steps:
Step 1: RBCs in blood bags were diluted with 300 mL of the additive solution PAGGS-C (bct about 30%).
Step 2: Diluted RBCs (about 600 mL) were transferred to illumination bags (THERAPLEX UV disposable kit, Macopharma).
Step 3: The illumination bags were loaded onto a MacoTronic illumination machine and UVC-treated under vigorous agitation (300 rpm).
Step 4: The UVC-treated RBCs were centrifuged and the supernatant was removed to obtain reconcentrated RBCs with a final hematocrit of about 60%.

Whole blood-derived leukoreduced, UVC-treated RBCs suspended in the newly developed third generation additive solution PAGGS-C served as test samples and RBCs in SAG-M as untreated controls.

Results: In vitro analyses were performed weekly to measure RBC quality, metabolic and functional parameters of UVC-treated versus untreated RBCs during 36-day storage. UVC treatment of RBCs in PAGGS-C significantly influenced some in vitro parameters. Compared to controls, hemolysis was higher in UVC-treated RBC units, but was still below 0.8% after 36 days of storage. Extracellular potassium increased early after PI treatment and reached about 70 mmol/l by the end of storage. UVC-treated RBC units had higher glucose (p<0.05, all days tested). The 2,3-DPG concentrations were significantly prolonged and higher in UVC-treated RBCs than in control RBCs during storage. RBC concentrations remained unchanged until the end of storage.

Conclusion: UVC treatment is a simple procedure that may facilitate the implementation of PI of RBCs in blood banking processes. The data suggest that the biochemical properties of RBCs can be well maintained during storage after UVC treatment.

Disclosure Statements: Project grants for the development of UVC-based PI technology for platelets were received from Research Foundation of the German Red Cross Blood Services.
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Joint patent application was filed for the Technology by Axel Seltsam, Ute Gravemann and Wiebke Handke.

Influence of purification and anticoagulant on analysis of plasma-derived EVs by imaging flow cytometry

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Background: Extracellular vesicles (EVs) are secreted by virtually all cells. Depending on their origin they appear as promising biomarkers in health and disease, especially if they can be recovered in plasma samples. However, there are many parameters that affect the detectability of EVs in given samples. Amongst others the anticoagulants being used as well as required processing steps in obtaining and preparing plasma for the EV analysis influence the diagnostic usability of EVs essentially.

Methods: Recently, we have qualified imaging flow cytometry (IFCM) as a valid method for the phenotypic characterization of antibody labelled EVs at the single object level. In contrast to many other novel single EV characterization methods, IFCM does not require any major pre- and post EV labelling processes. Antibodies can be simply added to EV containing biofluids and analyzed. Using a panel of 20 different antibodies and applying our optimized IFCM analysis strategy, we now have investigated impacts of conventionally applied processing steps, i.e. the centrifugation step as well as that of the eight most frequently used anticoagulants on the detectability and the phenotypic appearance of EVs within the plasma of healthy donors.

Results: Our results demonstrate that the speed of the clearance centrifugation step (2,000, 2,500, 3,000 x g centrifugation, or following Ficoll gradient centrifugation) do not recognizably affect the composition of detected EV populations. In contrast, the choice of the anticoagulant significantly impacted the composition of the different EV populations. While we hardly observed any impact on myeloid cell derived EVs, e.g. CD16+ or CD71+ EVs, the abundance of CD9+, CD41+ and CD61+ EVs, assumedly platelet-derived EVs, was significantly affected. Most CD41+ or CD61+ EVs were recovered in serum or, when Heparin was used as anticoagulant, the lowest amount was detected in EDTA containing plasma.

Conclusion: This, our analyses demonstrate significant impacts of anticoagulants on the detectability of certain EV types, especially on PL-derived EVs.

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GMP conform production of isoagglutinin depleted human plasma for blood group independent transfusion

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Background: Plasma is an essential blood product. The anti-A and anti-B antibodies contained in the plasma demand 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 for blood group independent transfusion. The principle of the method is based on isoagglutinin adsorption onto added red cell from red cell concentrates (RCC).

Methods: The before identified RCC volume required for the reduction of the isoagglutinin titer was aliquoted and stored until use at 2-6 °C. 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 (Macopharma, France). 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 separated using an automatic Macopress Smart (Macopharma) before isoagglutinin depleted FFP units were deep frozen again. Plasma quality was ensured by measuring isoagglutinin titers, cell free hemoglobin, coagulation factors V, VIII, XI, and sterility.

**Results:** The volume of RCC blood group B, A, and AB, respectively, required to decrease the isoagglutinin titer < 1:2 was 13.5±1.6 mL for one FFP unit group A, 21.4±2.4 mL for B FFP, and 37.5±8.7 mL for O FFP. All quality parameters are well preserved except for cell free hemoglobin of group A FFP, which increased slightly after isoagglutinine adsorption (Table 1), however, values were far below the ones accepted in red cell concentrates (up to 80 µM). All plasma units are sterile.

**Conclusion:** We present an automatic GMP conform procedure for the production of isoagglutinin depleted plasma from group A, B, and O plasma. The procedure uses standard equipment and is applicable to any blood bank laboratory.

**Disclosure Statements:** none

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**Table 1. Quality parameters before and after isoagglutinin depletion treatment (mean±standard deviation)**

| Plasma group | A (n=12) | B (n=9) | O (n=9) |
|--------------|----------|--------|--------|
| Before       | After    | Before | After  | Before | After  |
| treatment    | treatment| treatment| treatment| treatment| treatment|
| Cell free hemoglobin (µM) | 26.0±15.0 | 53.7±4.7 | 26.0±15.0 | 23.8±10.9 | 22.2±7.3 | 26.6±10.3 |
| Factor V (%) | 97.8±7.3 | 96.3±7.8 | 97.8±7.3 | 96.3±7.8 | 101.2±10.3 | 96.2±12.1 |
| Factor VIII (%) | 107.3±15.3 | 102.8±16.6 | 107.3±15.3 | 102.8±16.6 | 77.4±10.3 | 75.5±11.0 |
| Factor XI (%) | 95.7±20.1 | 94.8±19.6 | 95.7±20.1 | 94.8±19.6 | 89.9±13.2 | 85.1±11.7 |

Tab. 1

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**Conclusion:** Transfusion of 2 FFP / week from “elite donors” to SCTx patients is a safe method which seems to protect from CMV reactivation encouraging to perform a phase II clinical trial

**Disclosure Statements:** IKT Ulm offers plasmapheresis products

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**VS-19-6**

**Apoptosis inhibition during cold storage of platelet concentrates better maintains both platelet functionality and platelet half-life**

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**Background:** Apheresis-derived platelet concentrates (APCs) is an essential medical treatment. However, the storage at room temperature enhances the risk of bacterial infection post-transfusion. Recently, we showed that cold storage of APCs better conserves platelet functions but it reduced cell half-life due to the activation of the apoptosis. The aim of this study was to investigated the impact of apoptosis inhibition on platelet functions and half-life during cold storage.

**Methods:** APCs were stored for 1, 4, 7 and 10 days at 4°C with or without the apoptosis inhibitor G04 (RhoA-GTPase inhibitor). The activation and aggregation abilities were assessed by flow cytometer (CD62 and CD63, upon TRAP incubation) and aggregometry (after TRAP and ristocetin stimulation), respectively. Thrombin generation was detected using a thrombography and thrombus formation was assessed by thromboelastography. The platelet responsiveness to the extracellular matrix protein fibrinogen was determined performing the adhesion assay. Platelet survival was investigated using the NOD/SCID mouse model.

**Results:** During cold storage of APCs the presence of the inhibitor better maintained CD63 expression (Fold increase: CD63, p=0.035, day 7) while comparable expression of CD62 was observed with or without inhibitor. Furthermore, the aggregation ability was not impaired in the presence of the inhibitor G04, as well as the thrombin generation and thrombus formation at each storage point. Next, the presence of the inhibitor better conserved the ability of platelets to adhere to fibrinogen compared to cells stored without G04 (% adherent cells/field: p=0.043, day 7). Importantly, a higher percentage of circulating platelets was detected upon incubation with G04 for 7 days compared to untreated cells (% human platelets, 5h post injection: p=0.046).

**Conclusion:** Our findings show that the inhibition of the cold-induced platelet apoptosis significantly reduces the clearance of cold-stored platelets without affecting cell functionality including activation, aggregation, thrombin generation, thrombus formation and adhesion. Therefore, the use of apoptosis inhibitors may be a promising strategy to prolong the storage time and reduce the risk of bacterial infection post transfusion without impairing platelets functionality and improving cell half-life.

**Disclosure Statements:** NONE
Novel blood group gene variants identified by massively parallel sequencing

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Background: Massively parallel sequencing (MPS) for all genes encoding the known blood group antigens as well as platelet (HPA) and neutrophil antigens (HNA) has been established for the workup of complex cases in immunohematology. Here, we applied the gene panel sequencing that included all exons of 59 genes encoding the blood group systems (ISBT 2016) as well as platelet (HPA) and neutrophil antigens (HNA). MPS was applied to 10 samples with suspected alloantibodies against HFA in the Knops (KN) system (6 cases) and the John-Milton-Hagen (JMH) system (4 cases) was analyzed. Sequencing data were evaluated by using the Variant Interpreter software tool (Illunina). PCR-SSP methods were established for new variants in order to confirm sequencing results.

Results: Known ABO gene variants (*AW09, *AW31, *AW45) were identified in 3 cases with weak A antigens. New ABO alleles were identified in a case with weak A antigens (c.664G>A; p.V222M) and in a case with O phenotype (c.6099A>T; p.E2033D). One patient was homozygous for the KEL*02.36 allele known for the KETI-negative phenotype.

Discussion: The authors have no conflict of interest.
Conclusion: We identified rare or novel gene variants by using MPS in cases with aberrant blood group phenotypes or serologically not clearly identified allotypes against HFA. Homozygous or two heterozygous (compound heterozygosity) gene variants could confirm the suspected blood group system and explain the antibody specificity.

Disclosure Statements: The authors declare no conflict of interest.

VS-21-4
RHD Donor screening in Switzerland: Resolving novel alleles by nanopore-sequencing
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Background: Multiple RH1 (RhD) variants cause very weak RH1 expression and may be missed even by phenotyping methods including indirect antiglobulin test. In 2012, molecular routine screening for the presence of RHD was therefore implemented in Switzerland for all serologically RH1 negative first-time donors, according to the respective guidelines of the Swiss Red Cross. This screening strategy revealed previously unknown RHD alleles, which we resolved by Sanger and third-generation nanopore sequencing.

Methods: Here, we present results from the RHD screening since 2017. Screening of all RH1-negative donors was performed with the RCC-FluoGene D-Screen kit including primers for RHD exons 3, 5 and 10 (inno-train, Germany). In case of positivity, Rhesus genotypes/phenotypes were reassessed by commercially available SSP-PCR kits as well as standard and extended serological techniques. Sanger-sequencing and newest long-read sequencing strategy provides evidence for ~33% of the donors. One heterozygous blood group alleles as complete gene haplotypes could become the emerging standard.

Results: More than 10,000 serologically RH1 negative samples have been screened at the Blood Transfusion Service Zurich in the last 5 years. Overall, 0.57% (n = 58) were genetically positive for at least one of the three typed RHD exons. Strikingly, our combined sequencing strategy elucidated three novel RHD alleles. All were caused by frameshift mutations and serologically defined as null-alleles, also by adsorption/elution techniques, when applicable: one sample had a small duplication in exon 3 (c.395_396delp.K133Gfs*10), one sample had a single basepair deletion in exon 2 (c.245delT, p.F82Sfs*17) and the third donor carried an allele with a 4-bp deletion (c.1199_1202del, p.K400Ifs*48) in exon 9 in addition to the DAU-specific SNV c.1136C>T.

Conclusion: Molecular RHD screening of RH1 negative donors represents an efficient strategy to detect RH1 variants of very low expression, hence reducing the potential risk of alloimmunization in patients. Here we describe three previously unknown RHD variants all defined as null alleles based on genetic and phenotypic data. Remarkably, confirmation of all novel alleles by our RHD long-read sequencing strategy provides evidence that ONT is a reliable and emerging tool for routine diagnostics.

Disclosure Statements: None of the authors report conflict of interest.

VS-21-5
Case example of Nanopore sequencing for resolving genotype-phenotype discrepancies in the Duffy blood group at haplotype scale: discovery of a novel null allele in a FY*A/FY*B heterozygous individual
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Background: The Duffy (Fy) blood group is encoded by ACKR1. The FY*A/FY*B alleles are defined by the SNV c.125G>A. Weak alleles are mainly caused by c.265C>T and null alleles often involve c.-67T>C in the promoter, both linked to FY*B. Genotyping these SNVs usually confirms serological results. However, rare unexplained phenotype-genotype discrepancies may occur. We resolve such a discrepant case using nanopore sequencing, which, unlike Sanger sequencing, allows haplotype generation along the whole gene.

Methods: Phenotyping was performed using standard serological techniques. Genotyping of the three aforementioned SNVs in ACKR1 was carried out with MALDI-TOF mass spectrometry, a high-throughput platform that we have been using for routine blood donor genotyping of 46 selected blood group antigens. In case of phenotype-genotype discrepancy unexplained by the routinely typed SNVs, genotyping was reconfirmed using commercial PCR-SSP kits (inno-train, Germany). To identify the genetic variation causing unexplained discrepancy in one donor, ACKR1 including flanking regions (~2.1 kb) was amplified and sequenced as haplotype by nanopore sequencing by Oxford Nanopore Technologies (ONT). Sanger sequencing of the two ACKR1 exons was used to confirm the results.

Results: Since 2015 more than 40,000 donors have been screened for FY genotype at Blood Transfusion Service Zurich. Phenotypes were available for ~33% of the donors. One heterozygous FY*A/FY*B carrier with FY(a-b+) phenotype was identified, pointing to a FY*01N allele in the absence of c.265T or c.-67C. Indeed, we found a 1 bp deletion (c.655delG) accompanying by a SNV c.657C>G (rs748896745) in the second ACKR1 exon, both discovered by ONT on reads of the FY*A allele. The frameshift mutation is yet undescribed and was confirmed by Sanger sequencing.

Conclusion: Nanopore sequencing proved well-suited and accurate to resolve unclear discrepancy between Duffy blood group genotype and phenotype. In particular, it provided clinical utility by directly phasing a novel frameshift mutation to the respective FY*A/FY*B allelic background. As case example, this work demonstrated that sequencing new blood group alleles as complete gene haplotypes could become the emerging standard.

Disclosure Statements: None of the authors report conflict of interest.

VS-21-6
Comparison of different methods to optimize immunohematological diagnostics in case of interference due to monoclonal antibodies against CD38
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Background: Daratumumab (DARA) is a human monoclonal antibody directed against the surface protein CD38 and has been approved for the treatment of multiple myeloma. CD38 is found on the cell surface of many immune cells and in smaller numbers on RBCs. For this reason,
antibodies against CD38 cause panagglutination in indirect Coombs-Test (IAT). As a result, clinically relevant antibodies may be masked and therefore not detected. Aim of this study is to optimize diagnostic for patients receiving anti-CD38.

Methods: For this Study, treatment of test RBCs with 0.2 M DTT, production and use of Fab fragments and direct use of For 0.04 M DTT were compared.

In an initial test to assess the reaction strength of anti-CD38, DARA was added to plasma and titration was performed in the IAT using gel cards. All three methods were evaluated with plasma spiked with antibodies and DARA and 20 patient samples for avoidance of interference by anti-CD38 and detection of clinically relevant antibodies in the IAT. In addition, time and cost of each method were recorded and compared to evaluate their use in routine diagnostics.

Results: The use of 0.2 M DTT-treated RBCs and Fab fragments suppressed anti-CD38 interference in patient plasma in 90% and 95% of cases. In contrast, 0.04 M DTT only achieved negative results in 45% of the samples. The 0.2 M DTT method correctly identified 4 out of 6 antibodies, whereas the Fab-method and 0.04 M DTT direct method correctly identified 6 of 6 antibodies – however, there is a dilution effect with both methods (3:5 and 1:2), so weak antibodies might be missed. The 0.04 M DTT direct method is unable to detect IgM antibodies. The cost and time required depends on the number of samples per month, with the 0.04 M DTT direct method being the cheapest, whereas the 0.2 M DTT method achieves the fastest results in the antibody screening test.

Conclusion: Both the treatment of RBCs with 0.2 M DTT and the use of Fab fragments are reliable methods to prevent the interference of anti-CD38. The main advantage of the 0.04 M DTT direct method is its low cost. Fab fragments achieved the best results in terms of avoiding the interference caused by anti-CD38 and the detection of both IgG and IgM antibodies. 0.2 M DTT treated RBCs could not detect antibodies of the KEL-System. This can be resolved by providing K- and Kp-compatible RBC concentrates.

Disclosure Statements: There is no conflict of interest.

VS-21-7
Third-generation sequencing detects a novel variant in the regulatory RUNX1 motif of the ABO gene causing mixed-field agglutination in an AB individual

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Background: Mixed-field agglutination in ABO phenotyping (A/B) has been linked to rare variants in ABO exon 7 and regulatory regions. Complete knowledge about regulatory regions, however, hinders the discovery of genetic causes of unexplained ABO phenotypes. Long-read sequencing has great potential in this regard by enabling complete gene haplotype sequencing. As proof of concept, we used nanopore sequencing to resolve a case of mixed-field agglutination in ABO forward typing in a blood donor.

Methods: The ABO phenotype was determined by standard serological methods, including anti-A1 and anti-H specific agglutination. Commercially available PCR-SSP kits (inno-train, Germany) were used for ABO genotyping. Expression of A-, B-, and H-antigen was measured by flow cytometry. We excluded potential presence of chimerism by digital PCR (STILLA, France). The entire ABO gene was amplified by two overlapping long-range PCR fragments (~13 kb each). PCR-products were sequenced with newest sequencing technology of Oxford Nanopore Technologies (ONT). Results were confirmed using Sanger sequencing. In addition to the blood donor, we also analysed her mother and brother.

Results: We observed a mixed-field reaction with anti-A in a blood donor genotype as AB. Agglutination with anti-H was weak and absent with anti-A1. In concordance, we found ~20% erythrocytes expressing A and B while ~80% had only B antigen. Nanopore sequencing revealed a novel heterozygous g.10924C>A variant on the A-allele in a known transcription factor binding site for RUNX1 in intron 1 (~5.8 kb site). Inheritance of the novel SNV was proven by the donor's mother, who was genotyped AO, and shared the anti-A-specific mixed-field agglutination (~35% of A-expressing cells). As expected, the novel variant was absent in the donor's brother, who had phenotype O and genotype O,O.

Conclusion: We discovered an unknown SNV causing an A3 phenotype. The SNV falls into the 8-bp RUNX1 motif located in the large intron 1, which is rarely sequenced. Our finding extends current knowledge of four other variants affecting this motif, all leading to A/B or A/A/AB phenotypes. Our strategy of ONT-sequencing of long-range PCRs allowed haplotype generation of the entire ABO gene. This simplifies the crucial assessment of known and unknown regulatory regions in cases of complex antigen expression.

Disclosure Statements: None of the authors report conflict of interest.

VS-21-8
Mono-allelic partial cd177 expression results in reduced protein expression on neutrophils

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Background: About 95–97% of the Caucasian population express CD177 on the surface of their neutrophils whereas the remaining 5–3% do not express the protein. Gene mutations have been linked to null-phenotype and a decreased expression of CD177 on the surface of neutrophils. Copy number variations have not been linked to variable CD177 expression. The aim of this study was to analyse CD177-gene copy number variations in a cohort of German blood donors.
Methods: Quantitative PCR for CD177 was achieved by amplifying CD177 exon 1 in parallel to the JK gene and calculating the differences of the ct values generated by the specific hydrolysis probes for CD177 exon 1 and for JK. A cohort of 144 German blood donors was analysed. Expression of CD177 on the surface of granulocytes was determined by FlowGIFT/WIFT and SASGA using known HNA-2 antisera and a monoclonal anti-CD177 antibody (MEM166 FlowGIFT/WIFT).

Results: Among 144 German blood donors we did not find any subject carrying three copies of the CD177 gene. In contrast, we identified one individual expressing only one allele of CD177. The surface expression of CD177 on neutrophils of this proband was further characterised by FlowGIFT/WIFT and SASGA and compared to probands with either normal CD177 expression (wt) or probands who were heterozygous for the c.787 A>T or c.1291G>A mutation. The CD177 expression was significantly below the expression of neutrophils with CD177 wt, with heterozygous c.787 A>T mutation and with heterozygous c.1291G>A mutation. The expression was within the range of neutrophils with of compound heterozygotes for both the c.787 A>T and c.1291G>A mutations.

Conclusion: In our cohort of German blood donors, we could not confirm the frequent presence of three copies of CD177 described in a US-American donor population. However, we could demonstrate a partial mono-allelic gene expression of CD177. This partial mono-allelic expression resulted in a significantly reduced but distinct expression of CD177 on the surface of neutrophils.

Disclosure Statements: No conflict of interest to declare.

VS-21-9
Uptregulation of check-point ligand PD-L1 in patients with PNH explained by proximal complement activation
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Background: Inhibitory immune checkpoints are pivotal for sustaining self-tolerance, but exaggerated expression of checkpoint ligands may aid immune escape in various types of cancer. It is still insufficiently understood how different branches of the human immune system affect the expression of checkpoint ligands.

Methods: We reveal how the activation of the innate defense complement cascade stimulates the upregulation of the important checkpoint ligand PD-L1. In addition to the investigation of PNH cells, we used various in vitro models of alternative and classical pathway activation to unravel the relationship between complement activation and PD-L1 upregulation. In these models, we also used inhibitors of the complement system that act at different levels, for example, at the C3 or C5 level.

Results: Mechanistically, we demonstrate that complement activation leading to the decoration of particles/cells with C3- and/or C4-opsonins increased PD-L1 expression on neutrophils and monocytes as shown for different in vitro models of classical or alternative pathway activation. We further showed that in vitro complement inhibition at the level of C3, but not C5, inhibits the alternative pathway-mediated upregulation of PD-L1. We demonstrate by means of soluble PD-L1 that this observation translates into the clinical situation when PNH patients are treated with either C3 or C5 inhibitors.

Conclusion: In summary, we establish a link between complement activation and the expression of the checkpoint ligand PD-L1 in vitro and in vivo[cc1]. This crosstalk has considerable clinical implications since complement attack may be accompanied by immunosuppression as a counter-regulatory process. Together, the presented data show that the checkpoint ligand PD-L1 is increased in PNH patients, which correlates with proximal complement activation.

Disclosure Statements: None
VS-22-4
Establishing Haemovigilance in Africa – A Regulatory Systems Strengthening (RSS) Example
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Background: Haemovigilance (HV) is not yet well established in the majority of African countries. An effective HV system, however, can increase the safety of blood donors and recipients tremendously. Therefore, the BloodTrain (BT) project selected HV as the first regulatory function to be strengthened through an extensive technical support. BT aims to increase availability, safety and quality of blood products through RSS, focusing on the partner countries (PC) Ghana, Tanzania, Zambia and Zimbabwe.

Methods: By conducting training workshops BT raised awareness and build capacity in HV. In order to establish HV systems, HV Task Teams (HTTs) were formed in each of the PCs, composed of representatives from both the National Blood Transfusion Service and National Regulatory Authority. The HTTs were tasked to lead development of key HV system documents with the support of BT. BT organized and hosted the meetings (both in-person and on-site) and supported the development/elaboration of documents through facilitation and providing expertise. During stakeholder meetings that included representatives from relevant institutions, i. a. hospitals, the proposed system and developed documents were reviewed, adapted and finalized.

Results: Key documents that have been developed in the PCs in the course of the HV support are national HV frameworks, guidelines and forms that bring together each of the HV systems, defining responsibilities, the scope of reporting and overall purpose. BT provided initial drafts, modified to fit each PC based on system specific details from previous comprehensive system analyses and benchmarking exercises. These drafts were reviewed and further customized in the HTTs and later in large stakeholder meetings, accommodating the perspectives of all relevant stakeholders. Two out of four PCs are already organizing the official launch of the national HV system and work is ongoing to fully/legally implement the agreed-upon HV system.

Conclusion: The national launch of the HV system in the PCs mark the starting point of the implementation phase during which the training will focus on completion and assessment of HV reports and potentially arising challenges. HV is only one of many regulatory functions within the field of blood regulation. The successful establishment of the HV systems promotes BT’s approach of capacity building paired with technical support. Support of the other regulatory functions will follow the same setup.

Disclosure Statements: The authors do not have any conflict of interest.

VS-22-5
Neural Network Based Flow Morphometry for Assessment of Red Blood Cell Storage Lesion
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Background: During storage, red blood cells (RBCs) undergo progressive biochemical and morphological changes referred to as storage lesion. RBC concentrates (RCCs) quality is usually assessed by quantifying hemolysis prior to transfusion. Hemolysis, however, reflects only already lysed RBCs, but not the number of aged cells known to compromise post-transfusion survival. We hypothesize, that morphological analysis is better suited to predict actual post-transfusion survival, increasing the safety of RCCs.

Methods: As an alternative to the standard blood smear, microfluidic systems with suspended RBCs in laminar flow can provide automated discrimination of RBCs morphologies using image analysis with large cell statistics. We have combined statistical power of flow morphometry using an in-house developed microfluidic system with high image classification power of a convolutional neural network (CNN) to assess changes in RBCs morphology caused by storage lesion. Five RCCs were stored under blood bank conditions for 12 weeks to represent advanced morphological stages. Samples were taken at week 2, 5, 6, 8, 10, 12 and analyzed by flow morphometry. In addition, hemolysis was measured in all RCC samples to verify correlation with morphological measurements.

Results: CNN-based image classification successfully achieved morphological resolution corresponding to nine RBC classes, with an overall classification accuracy of 92% compared to manual classification by trained personnel. We use the term lesion index to refer to the percentage of irreversible spherical morphologies correlated to post-transfusion survival. Furthermore, our results provide strong evidence that the lesion index can predict the degree of hemolysis in RCCs during storage. We were able to establish a preliminary threshold of 11.1% of the lesion index, which is equivalent to the 0.8% threshold for hemolysis. Consequently, the lesion index can be used to determine sufficient quality of an RCC according to regulatory guidelines.

Conclusion: The CNN-based flow morphometry and the calculated lesion index allow a reliable evaluation of RCC quality. The method also reduces the need for complex laboratory procedures. Therefore, it is strongly advisable to include the lesion index as an additional marker of storage lesion in clinical routine. In contrast to hemolysis, lesion index may serve as a good indicator of post-transfusion survival. Thus, both measurements together could provide increased safety and efficacy of stored RCCs.

Disclosure Statements: No conflicts of interests.

VS-22-6
Rapid increase of platelet reactivity by short-term cold storage
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Background: In comparison to room temperature (RT), storage of platelet concentrates (PC) at cold temperature (CT) has been proposed as an approach to increase platelet reactivity. In this context, we could recently show that cold-induced attenuation of inhibitory signaling is an important underlying mechanism. For practical reasons in blood banking, it is essential to define required time periods of cold storage until increased platelet responsiveness is achieved, which we addressed in this study.

Methods: Platelet-rich-plasma (PRP) was prepared from freshly obtained citrated whole blood samples. PRP was stored for 1 h at RT (22±2 °C) or at CT (4±2 °C), followed by preparation of platelets for subsequent comparative analysis. The investigations comprised light transmission aggregation using threshold agonist concentrations of adenosine-diphosphate (ADP) and collagen. Inhibitory signaling was explored by Western Blot analysis and by flow cytometric measurement of basal or induced vasodilator-stimulated phosphoprotein (VASP) phosphorylation. Adhesion studies were performed on collagen-coated slides under flow conditions using a live cell imaging system.

Results: Compared to RT, basal VASP phosphorylation levels decreased under CT by approximately 20% within 1 h of refrigeration. Prostaglandin E1- or nitric oxide donor-induced levels were similarly reduced. ADP- and collagen-induced threshold aggregation values were enhanced by up to 30-40%. In addition, platelet-covered areas on collagen-coated slides were increased after storage at CT.

Conclusion: Under cold storage, inhibitory signaling is rapidly attenuated within 1 h in PRP, as a milieu similar to PC, accompanied by an enhancement of aggregation responses and adhesion on coated surfaces. Therefore, short-term refrigeration may reflect a rational approach to obtain platelets with higher reactivity for therapy of acute hemorrhage.

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Tab. 1. Summary of detected PLG standard contents [nmol/L] of unknown samples through different detection systems, including standard deviation

| Sample | STA*-Stachrom® Plasminogen [nmol/L] | FIDAm | Glu-PLG ELISA [nmol/L] | Total PLG ELISA [nmol/L] |
|--------|-------------------------------------|-------|-------------------------|---------------------------|
| V1     | 1,782.61                            | 1,245.22 ± 24.35 | 2,809.66 ± 487.62        | 3,263.59 ± 43.48          |
| V2     | 2,260.87                            | 1,719.32 ± 1.20 | 3,387.19 ± 482.69        | 4,113.11 ± 286.35         |
| V3     | 1,543.48                            | 1,933.19 ± 8.91 | 2,490.40 ± 163.62        | 3,187.16 ± 395.72         |
| V4     | 2,130.43                            | 2,274.82 ± 26.09 | 3,907.30 ± 654.18        | 4,679.11 ± 319.55         |
| V5     | 2,869.57                            | 3,598.17 ± 22.12 | 5,179.96 ± 737.69        | 6,679.29 ± 1,529.97       |

STA* analyzer read out gives no information about standard deviation
Liver disease and hemostasis

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Invited talks abstract/summary: Patients with liver diseases can present with various abnormalities in routine laboratory tests of hemostasis, that commonly indicate an increased risk of bleeding, including thrombocytopenia or prolonged clotting times. However, as the complex changes in the hemostatic system of patients with liver disease not only affect procoagulant but also anticoagulant pathways as well as the fibrinolytic system, the risk of thrombosis is also increased. As the alterations develop concurrently, the hemostatic system remains balanced but can be disturbed more easily, making prevention and treatment of bleeding and thrombosis in patients with liver disease a challenging task. This talk will provide an overview of the hemostatic changes, the interpretation of laboratory tests of hemostasis, and therapeutic options to counteract coagulopathy in liver disease.

Disclosure Statements: None to declare.

Inherited von Willebrand disease and acquired von Willebrand syndrome: mechanisms, diagnostics and treatment

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Invited talks abstract/summary: The multimeric plasma glycoprotein von Willebrand factor (VWF) mediates platelet adhesion to the injured vessel wall and stabilizes circulating coagulation factor VIII (FVIII). Quantitative and/or qualitative VWF abnormalities thus lead to impaired primary and/or secondary hemostasis. Von Willebrand disease (VWD) is the most common inherited bleeding disorder with a prevalence of up to 1%. VWD is caused by autosomal dominant or recessive mutations in the VWF gene located at the short arm of chromosome 12, thus affecting both females and males (so-called pseudohemophilia). Diagnosis of VWD is based on abnormal bleeding symptoms, as assessed, for example, by the MCMDM-1 VWD bleeding questionnaire or other validated bleeding assessment tools, a positive family history, and pathological VWF/FVIII laboratory test results. While VWD type 1 and 2 are characterized by quantitative and qualitative VWF abnormalities, respectively, plasma VWF is virtually absent in patients with VWD type 3, who may, in addition to mucocutaneous hemorrhages, experience spontaneous or traumatic joint bleeds due to significant FVIII deficiency, resulting in debilitating arthropathy as observed in patients with hemophilia A. Antifibrinolytics such as tranexamic acid, the synthetic vasopressin analog desmopressin (DDAVP), which induces V2 receptor-mediated secretion of VWF/FVIII from endothelial Weibel-Palade bodies, and either plasma-derived or recombinant VWF concentrates are used for bleeding prevention and treatment. Acquired von Willebrand syndrome (AVWS) is caused by various pathophysiological mechanisms. These include, but are not limited to enhanced shear-dependent, ADAMTS13-mediated VWF proteolysis in patients with severe aortic valve stenosis, aberrant degradation of VWF by other enzymes such as plasmin in patients with hyperfibrinolysis, functional inhibition or accelerated immune-mediated clearance of VWF by autoantibodies in patients with systemic lupus erythematoses or monoclonal gammapathy, and increased VWF adsorption to platelet GPIb in patients with essential thrombocythemia. Identifying the underlying pathophysiology of AVWS has significant implications for diagnosis and treatment of this potentially life-threatening and still under-recognized bleeding disorder.

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Applying machine-learning algorithms for prediction of fainting during blood donations

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Background: Fainting is a well-known side effect of blood donation. Such negative experiences can diminish the return rate for further blood donations. Identifying factors associated with fainting could help to prevent adverse incidents during blood donation. Machine-learning algorithms are able to identify factors contributing towards these events.

Methods: Data of 85,040 blood donations from whole blood and apheresis donors within two consecutive years were included in this retrospective study. Demographic characteristics (age, sex, blood type), donor specific information (blood pressure, pulse, medical questionnaire) and donation specific data (donation procedure, donation site, association type) were used as features for two different machine-learning algorithms (random forest, potential support vector machine). These data originated from standard procedures during the time course of blood donation. The created models were tested by AUC-ROC analysis and determination of balanced accuracy.

Results: 925 mild, moderate or severe fainting reactions were observed over a study period of 24 months. 44.5% of vasovagal reactions that occurred during blood donation involved first-time donors, who represented only 12.2% of the donor population overall. Weight, systole, age, diastole, height and the number of previous donations were top ranked with random forest (RF) classification. Mean accuracy of the RF classifier was 87.95% whereas balanced accuracy had a mean of 65.82%. The most important features predicting fainting during blood donation were weight, systolic blood pressure, age and height, whereas gender was not identified as an important prediction factor for fainting although highly significant differences were found (p<0.01).

Conclusion: Machine-learning algorithms can be used to establish prediction models of fainting in blood donors. Features identified by RF are more interesting whereas PSVM mainly focused on first-time donors. These new tools can contribute to reduce adverse reactions and therefore to improve donor safety as well as minimize negative associations relating to blood donation. Further research such as incorporating weather data into the new computational algorithms is planned to improve the donor experience.

Disclosure Statements: The authors declare that there is no conflict of interest.

Changing blood donation through the use of a donor app

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Background: One year ago, we introduced our mobile app for blood donation: donors have received it exceptionally well and it has definitely improved the process of our blood donations. The app allows users to prepare for the donation ahead of time and to receive the medical report upon completion and validation in the lab. The net effect is that both the donor and the medical personnel are the beneficiaries of tangible efficiency gains, exemplifying what digital transformation is all about.

Methods: The app uses two-factor authentication and distinguishes between two user types. Registered users can explore the basic features of the app until they are verified during a blood drive: now they are allowed to access their medical data as well as medical history from past donations. Once the donation questionnaire is completed the donor receives a message indicating in advance whether admission or deferral is more likely. Moreover, verified users see their next donation permission directly from the system of the blood center.

With notifications, donors are informed of a new available medical report. Invitations to blood drives are targeted, convenient and low-cost.

Results: After one year there are more than 35,000 users that use the app: more than 16,000 of them can access their medical data, being verified users. In total 21,500 donations were processed with the digital questionnaire; more than 175,000 medical reports can be digitally accessed via the app.

The number of donors using the digital questionnaire is constantly and steadily increasing and currently at a 42% average. Overall, there is no difference in age or gender concerning the usage of digital vs. paper questionnaires, although regional differences do exist. Deferrals have decreased by 30% among app users but there is no difference in the causes for the remaining deferrals.

Conclusion: We are able to streamline our workflow because digital questionnaires do not require further processing. All the data is integrated into the blood bank system automatically. The donor experience is improved across the entire process, where one advantage is the estimation of admission in advance. This saves them a trip to the blood drive and there are fewer unnecessary deferrals overall. For verified users, the secure access to their medical data and history is well established and highly used.

Disclosure Statements: The authors declare that there is no conflict of interest.
Establishment of a mobile plasma donation bus in Germany

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Background: The shortage of plasma products and the dependence on oversea supply is a known problem in Europe and has even aggravated during the COVID-19 pandemic. New ways and initiatives for plasma donations are strongly required. Thus, we established the first truly mobile plasma donation site in Germany, the “Plasma Bus”.

Methods: We formed a “task force” for the bus-project including technicians, IT specialists, PR employees, the quality management department, a construction company, plasma machine operators and physicians. This team designed all of the necessary equipment and procedures inside and around the bus and supervised the corresponding implementation steps. Suitable plasma machines and custom made storage for medical equipment and plasma products were selected and installed in the bus. All Requirements for a donation procedure following national guidelines were ensured. Instructions and SOPs were put in place for plasma donations in the bus.

Results: Three “Aurora” plasmapheresis machines (Fresenius Kabi) were installed and validated in the plasma bus. All further requirements for donor management, plasma donation, storage and transportation were established. A VPN-tunnel based connection to the medical IT-system was implemented. The workflow for plasma donations in the bus was delineated in detail. Extensive staff-training for “in bus” donations took place.

Conclusion: Mobile plasma donations based on the concept of a “plasma bus” are possible. They could be a helpful tool regarding the dramatic shortage of plasma. In addition, collection of plasma products with special requirements such as convalescent plasma may be expanded to rural areas. We compiled a model for this type of donation sites for identifying and overcoming potential pitfalls. Thereby we propose a blueprint for the implementation of a mobile plasma donation site.

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Machine learning modelling to precisely predict hemoglobin and iron content in individual packed red blood cell units

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Background: Despite substantially varying, the concentration neither of the pharmaceutically active agent Hb, nor of the potential noxa heme iron is known for individual pRBC units and cannot be considered for transfusions. Thus, rules of thumb are applied for pRBC transfusions worldwide. Transfusion-associated iron overload, PBMs and changing demographics demand more rational, evidence-based use of pRBCs, and accurately determining individual Hb and iron loss could improve blood donors’ safety.

Methods: Invasive quality control revealed that Hb (38.5 g to 79.9 g) and heme iron (129.41 mg to 268.56 mg) vary substantially between pRBCs (n=6,058). Without compromising their integrity we sought to predict Hb and iron content of any given pRBC unit applying eight different machine learning models in a first cohort of 6,058 pRBCs. Based on all routinely collected features during blood donation, production and quality control testing, we identified the model with the best trade-off between performance and complexity. This model was then assessed in a second, independent cohort of 2,637 pRBCs. Facilitating clinical access we developed a web application to predict single pRBC units and entire data sets.

Results: We predicted individual Hb and iron content of pRBCs in this cohort with a mean absolute prediction error of ±1.43 g Hb/4.80 mg iron and a corresponding standard deviation of ±1.13 g Hb/3.81 mg iron, and we identified for the first time relevant factors determining the Hb and iron content of pRBCs. We further showed that applying this algorithm could lead to substantial dose and cost reduction of iron chelation therapy.
Conclusion: Our concept unlocks tailored application of pRBCs with unprecedented precise prediction of their respective Hb and iron content. Enabling reliable pRBC dosing per pharmaceutically active agent, monitoring iron uptake in chronically transfused patients and individual iron loss in donors is paving the way toward individualized patient and donor blood management.

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PS-1-7

Function of Pathogen Reduced Cryoprecipitate stored for 36 Months and Thawed for 120 Hours at Ambient Temperature

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Background: Cryoprecipitate (Cryo) is a pooled-plasma-derived blood product, enriched for Fibrinogen (Fb), Factor VIII, and FXIII. Pathogen reduction (PR) of plasma makes blood centre Cryo production an attractive source of fibrinogen concentrate. We evaluated the feasibility and efficacy of the production of Cryo from PR-plasma. The main objective is compliance of PR-Cryo with EDQM guidelines. We report the results of a validation study over a storage time of 3 years and after 5-day storage post-thawing.

Methods: Two ABO-identical units of concurrent apheresis plasma (325 mL each), were pooled, treated with INTERCEPT® Blood System for plasma (amotosalen-UV-A), transferred into a single bag (650 mL) and frozen to < -25°C within 45 minutes using a shock freezer. After one week of storage, plasma was thawed slowly at 2-6°C overnight and Cryo was separated by centrifugation at 4°C. The PR-Cryo units were frozen and stored at < -25°C. Six PR-Cryo units were thawed after 1, 3, 12, 24 and 36 months respectively, and stored at room temperature (20-24°C) over 5 days. Samples were taken 1h, 24h, 72h and 120h after thawing to evaluate the stability of Fb, FVIII, vWF and FXIII.

Results: The volume (mean ± SD) of the PR-Cryo units was 90±5 mL. All measured clotting factor activities remained stable with minor variations over 36-month storage at < -25°C. At 36 month and after 5 days post-thawing at RT (n=6) Fb content was 539±155 mg/unit, FVIII, vWF and FXIII contents were 156±11, 301±32 and 189±49 IU/unit, respectively. These factors exceeded the EDQM minimum values on average 3.9-fold for Fb, 3.1-fold for FVIII and 3.0-fold for vWF, respectively versus an untreated control. K+ concentrations never exceeded 25mM until day 8. Yet our data show that K+ differs to all other parameters, increases rapidly after irradiation. We show that washing is a potent tool to lower K+ levels in RCCs for neonatal transfusion, which could be used to extend the maximum storage period of neonatal RCCs for up to three days.

Conclusion: K+ values and further metabolic parameters can be reliably validated in routine RCCs and show minimal variations between individual donations. K+ concentration never exceeded 25mM until day 8. Yet our data show that K+ differs to all other parameters, increases rapidly after irradiation. We show that washing is a potent tool to lower K+ levels in RCCs for neonatal transfusion, which could be used to extend the maximum storage period of neonatal RCCs for up to three days.

Disclosure Statements: The authors have no conflicts of interest to declare.

PS-1-8

Neonatal red blood concentrates contain highly predictable extracellular K+ levels, which can be controlled by washing and may allow extended shelf life

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Background: Transfusion of red blood cells in neonates requires tight control of K+ levels. Transfusion guidelines recommend use of RCCCs aged 7 days or less. Refined data and recommendations for handling of neonatal RCCCs are missing. We aimed to (i) assess K+ metabolic parameters and hemolysis during storage of RCCCs and (ii) investigate the influence of irradiation and a washing step, in order to optimize the product quality and possibly extend the shelf life of neonatal RCCCs.

Methods: A total of 26 RCCCs were prepared in three series from whole blood donations by established centrifugation, buffy coat separation and red cell inline leucocyte filtration. RCCCs were stored in SAGM (saline-adeno-glucose-mannitol) solution at 4±2°C over 42 days and analyzed at different time points. The products were washed at different time points: on day 1, 8 and after irradiation on day 8. Biochemical parameters (glucose, LDH, lactate, ATP, free hemoglobin, hemolysis) were determined using established methodologies. K+ determinations were performed using colorimetry and were validated using flame photometric determination. RCCCs were gamma-irradiated using a 137Cs source. We report the results of a validation study over a storage time of 3 years and after 5-day storage post-thawing.

Results: RCCCs washed on day 1 showed 1.31-fold glucose levels compared with controls on day 1. Also, minimal but significant improvement in K+ and lactate levels was observed. In contrast, RCCCs washed on day 8, showed 1.52-fold glucose compared with controls, and significantly lower concentrations of lactate, LDH and K+.

RCCs washed directly after irradiation on day 8 showed significant improvement in glucose, lactate, LDH, free hemoglobin during further storage, less hemolysis (except day 42) and higher ATP from day 32 on. Directly after washing, irradiated RCCs K+ concentrations were reduced to 1.12mM ±0.05 vs. 21.20mM ±2.78 in unwashed units (means±SD). On day 10, K+ levels were 16.8mM ±0.85 in washed units vs. 36.80mM ±2.13 in unwashed units.

Conclusion: K+ values and further metabolic parameters can be reliably validated in routine RCCCs and show minimal variations between individual donations. K+ concentration never exceeded 25mM until day 8. Yet our data show that K+ differs to all other parameters, increases rapidly after irradiation. We show that washing is a potent tool to lower K+ levels in RCCCs for neonatal transfusion, which could be used to extend the maximum storage period of neonatal RCCCs for up to three days.

Disclosure Statements: The authors have no conflicts of interest to declare.

PS-1-9

Increase of whole blood donation volume from 450 to 500 ml results in quantitative and qualitative improvements in all three derived products

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Background: Whole blood donation volume is generally limited to a range of 450 ml to 500 ml to yield sufficient quantity of transfusion doses for patients, as well as for the sake of donor safety. We aimed to determine the product quality increase by increasing routine donation volume of 450 to 500 ml, and asked for the improvements in product quantity and quality.

Methods: Whole blood donations were obtained from routine voluntary donors. Donations were collected into quadruple bag systems with integrated red cell inline filters (LQE) prefilled with 63ml (450 ml donations) or 70 ml (500 ml donations) Citrate-Phosphate-Dextrose (CPD) solution

Disclosure Statements: n.a.
using automated mixing balances (MacoPharma). Whole blood was stored for maximally 23h before centrifugation at 3931 x g for 17min. Separation was performed within 1 h after end of centrifugation on semiautomated processors (MacoPress; MacoPharma). Whole blood was stored for maximally 23h before centrifugation at 3931 x g for 17min. Separation was performed within 1 h after end of centrifugation on semiautomated processors (MacoPress; MacoPharma). SAG-M red cell storage solution (450 ml donations) was replaced by PAGGS-M (500 ml donations). Cellular and biochemical parameters were performed using established methods.

**Results:** In red cell concentrates (RCC), total hemoglobin increased by 11.7 % from 47.8 ± 4.8 g (450 ml) to 53.4 g ± 6.1 g (500ml; means ± SD), whereas hemolysis remained unchanged at a mean of 0.19% in 450 ml donations after day 42 and 500 ml donations after day 49. RCC could be irradiated until day 14 and used thereafter until another 14 d. Pooled platelet concentrates (PC) showed 8 % increased platelet content (2.7 ± 0.3 x 10e11/unit from 4 buffy coats). Plasma (FFP) volume increased by 9.5 % from 284 ± 4 ml to 311 ± 4 ml. Coagulation factor VIII quality was preserved with 90.8 ± 11.3 % after 1 month storage at < -30°C with an increased whole blood holding time of 23h, compared to FFP from 450 ml donations which had a maximum hold time of 18 h.

**Conclusion:** It was possible to adapt 5 blood product licenses by the Paul-Ehrlich-Institute in one step, thereby increasing hemoglobin in RCCs and PLT yield in PCs, improving quality of irradiated RCC and preserving it in irradiated PC. As well, plasma volume increased and FVIII was preserved in FFPs after extending the pre-centrifugation hold time from 18 to 23h. The modifications resulted in more flexible workup and handling during component production as well as for clinical application.

**Disclosure Statements:** No conflicts of interests.

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**Reduced platelet wastage following conversion from 5-day conventional platelets to 7-day pathogen reduced platelets**

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**Background:** In Europe, pathogen reduced platelet concentrates (PR-PCs) may be stored up to 7 days post collection, which is routine practice use in many countries. We assessed changes in wastage rates and the distribution of PCs by day-since-collection at an academic medical center in Austria before and after the conversion from 5- to 7-day storage, and the introduction of PR.

**Methods:** Data on PC collections, PC age at transfusion (in days since collection), and reason for discard were retrospectively analysed for two discrete 3-year time periods before and after 2016 when the conversion to PR-PC prepared with the INTERCEPT® Blood System for Platelets occurred. During period 1 ([P1] 2013–15) conventional PCs were stored for a maximum of 5-days with bacterial culture and irradiation. Period 2 ([P2] 2017–19) included PR-PCs stored for up to 7-days without irradiation or bacterial culture. Data were stratified by age (day since collection) and collection type; discarded units were stratified by reason for discard. Summary statistics were calculated. T-tests were used to compare differences in independent proportions.

**Results:** Overall PC usage increased by 5.7% in the course of the study, from 18,581 (P1) to 19,640 PCs (P2). Whole blood-derived PCs (WB-PCs) accounted for 80.8% of PCs in P1 and 74.8% in P2. Apheresis PC (A-PC) use increased by 39.2% in P2 and accounted for 100% of all PCs transfused on Day 1 in P2 (Fig. 1). Overall wastage declined significantly from 19.1% of PCs in P1 (range: 22.6%-13.4%) to 7% in P2 (range: 5.9%-9%) (p<0.0002). Overall wastage was more prominent for WB-PCs, (from 18.1% in P1 to 7.2% in P2). Out-dating accounted for >90% of waste in P1 (range: 81.3%-99%) and 63.2% in P2 (range: 28.1%-87%). A-PCs were less likely to expire by outdate than WB-PCs in both periods. Daily PC usage rates stabilized in P2 (Fig. 1).

**Conclusion:** Platelet waste declined significantly and stock management was harmonized following the conversion from irradiated PCs with 5-day storage to PR-PC with 7-day storage. In addition, the introduction of pathogen reduction helped to simplify overall logistics as gamma irradiation and bacterial screening became obsolete.

**Disclosure Statements:** The Graz Blood Center performs company-funded trials for Cerus and is under contract to perform routine PR for platelets and plasma. PS has received honorarium, PS and KR have received reimbursement for travel expenses in the past 5 years. WH declare no conflicts of interest.
Simultaneous heart surgery and red blood cell exchange by apheresis system connected to a heart-lung-machine. 3-year-old child with homozygous sickle cell disease: A case report

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Background: A case report of interdisciplinary treatment of a 3-year-old child with homozygous sickle cell disease (HbSS with persisting HbF) with cardiac insufficiency based on bi-atrial insufficiency (TI/MI) and restrictive cardiomyopathy of unknown origin. An emergency treatment for reconstruction of the AV valves by cardiopulmonary bypass was recommended. Together we decided to perform the exchange of red blood cells perioperatively by an apheresis system connected to the heart lung machine (HLM).

Methods: According to the current guidelines urgent heart surgery should be done with decreased HbS-levels (< 30% pre-, <15% perioperatively) to avoid risk of sickle complication like hypoxemia, hypothermia, acidosis and sickling crisis. Patient data: body weight 10kg, height 90cm, total blood volume estimated 750ml. Hb 8.5 g/dl, Hct 26%. Priming of the apheresis machine with RBC (reconstitution with plasma to hematocrit level of 50%) and connection with the heart-lung-machine (HLM). Simultaneous to the open heart reconstruction “de Vega/ Paneth plastic” we performed an automated exchange transfusion (aRBCX) with 3 RBC. Apheresis data: MNZ-Set, Spectra Optia, 35 min, start hct 25%, estimated hct 35%, hct of RBC 62%, 805ml RBC, FVZ 12%.

Results: Regardless of the lack of experience and literature in high risk paediatric patients with homozygous sickle cell disease in need of open heart operation, we could perform a safe and effective procedure of red blood exchange. HbS-level decreased to 12% as we expected corresponding to the fraction of residual cells (FVZ). Hb at the end 11.6 g/dl, hct 36%. Postoperative no complications, discharge after two weeks. A control in outpatient clinic of paediatric haematology one month later showed a stable heart situation with less signs of heart insufficiency and gain of weight. Medication with Hydroxycarbamid was well tolerated.

Conclusion: With an individualized concept of combining open heart operation with simultaneous aRBCX by connecting an apheresis system to a heart lung machine, we saved the patient from another procedure with additional vane access and anaesthesia. Contrary to an exchange only to HLM there was no need of complicated plasma/coagulation management.

We are convinced that this case will help to handle similar cases of paediatric sickle cell disease patients in need of cardiac intervention.

Disclosure Statements: No conflicts of interests.
Exchange transfusion for hyperleukocytosis in childhood leukemia – A case report

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Background: Initial hyperleukocytosis is a life-threatening complication in childhood leukemia. Immediate leukocyte reduction by automated leukapheresis (LA) or exchange transfusion (ET) may be required in context of tumor lysis syndrome, disseminated intravascular coagulopathy or leukostasis syndrome. Little data is available regarding safety, efficacy and overall clinical benefit in children, and practical experience in the use of LA or ET is often limited.

Methods: A previously healthy, 6-year-old, male (124 cm, 24kg) who presented in an emergency situation with petechiae, extensive cervical lymphadenopathy, pallor and hepatosplenomegaly with WBC 302.4 x10^9/L (84 % blasts), Hb 11.8 g/dl, PLT 67 x 10^9/L, LDH >30 µkat/L, and uric acid 518 µmol/L. Due to increasing apathy and cutaneous hemorrhage we decided for rapid reduction of the intravascular leukemic cellular burden before a definite diagnosis of the leukemic subtype was available. ET (100-150ml/kg body weight fractionated into 10-50 mL single portions) was manually performed by repeated cycles of aspiration and subsequent substitution of red packed cells and fresh frozen plasma in a ratio of 2(-3):1, via venous or arterial access.

Results: After an 8 hour procedure WBC count decreased to 145.8 x 10^9/L, with a Hb level of 9.8 g/dl and a PLT count of 59 x 10^9/L. ET was well tolerated without any immediate complications. Subsequently, diagnosis of cortical T-ALL was made and cytoreductive treatment according to the current AIEOP-BFM ALL protocol was started. Adequate response was documented on d15, contributing to more favorable prognosis.

Conclusion: ET provided an effective and safe leukocyte reduction thus preventing possible life-threatening events. In emergency situations, however, available resources and experience of staff may dictate definite treatment decisions. Further and more systematic analysis of the value of ET and LA in hyperleukocytosis is warranted to optimize emergency treatment situation for this cohort of very sick children.

Disclosure Statements: No conflicts of interests.
Autologous and Allogenic Serum Eye Drops – Validation Strategy Update

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Background: Serum Eye Drops (SED) are vital for treating a variety of ocular diseases and disorders. Establishing high quality SED is therefore an important step in alleviating patient symptoms. This report describes the accompanying validation of our manufacturing and storage process for autologous (au) and allogenic (al) SED. The aim of the study was: producing SED containing 100% serum, with a shelf life of 6 months when stored at -20°C and transport directly to patient homes using cooled transport bag.

Methods: Whole blood was collected with TF304 sterile blood collection system (Meise) and Donoc (Müller Medical) blood collection mixer. After centrifugation (2x 15min 3500rpm), the resulting serum was packaged in sterile ATS 36 (Meise) applicator sets. The validation plan included exposing the transport bag (PYB2, Versapak Europe) to temperatures of 20-24°C and 30-34°C (8 h respectively) and monitoring internal bag temperature. SED quality control parameters (min. 6 each from healthy donors and patients) were visual control, sterility, leucocyte concentration, pH, TGF-ß, VEGF and vitamin A. Samples were obtained from freshly manufactured product (A), after 24 h freezing (B) and after 6 months frozen storage (C). Data are presented as median values.

Results: The transport bag maintained an internal temperature below 0°C for a minimum of 8 h at both temperatures ranges (n=6). The results are based on data of au (n=8) and al n=8). Regarding sterility, visual control and leucocyte counts, there were no discrepancies between A, B and C. pH and Vitamin A were comparable between A and B: pH 7.61 vs pH 7.64 and 0.62 µg/ml and 0.61 µg/ml, respectively. TGF-ß and VEGF showed non-significant differences between A and B: 26.3 ng/ml vs 21.6 ng/ml (p=0.15) and 273.5 pg/ml vs 375.5 pg/ml (p=0.50), respectively. After 6 months storage, SED pH increased to 8.0 vs a pH 7.7 for 24 h frozen material (au and al n=13). Concerning vitamin A, TGF-ß and VEGF there was no relevant loss of quality after storage.

Conclusion: Quality data of autologous and allogenic SED appear consistent between freshly manufactured SED and SED frozen for 24 h. Validation of the storage process showed the suitability of storage at -20°C for 6 months without loss of quality. The PYB2 transport bag maintained an adequate internal temperature below 0°C for 8 h at 20-24°C and 30-34°C and is therefore appropriate for use in delivery of frozen SED. The validation for autologous and allogenic SED products was completed successfully.

Disclosure Statements: None
PS-1-17
Serum Eye drop experience in manufacturing: 10 years autologous – 5 years allogeneic

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Background: Serum eye drops are an experimental therapy for severe forms of dry eyes, increasingly in use worldwide. Until now only few prospective studies were published, several retrospective evaluations reported.

Methods: At our blood center since 2012 autologous serum eye drops (ASAT, ASED) are manufactured, since 2017 allogeneous serum eye drops (FSAT, FSED). Data of donation, manufacturing, and if possible, clinical result were evaluated.

Results: Since 2012 more than 2000 serum eye drop preparations were manufactured. Between 2017 and 2021 399 allogeneous preparations were made for 127 patients in the setting of individual healing attempts. Even in a comparatively large University Blood center (30,000 whole blood donations, 60-150 donors daily) the search for the suitable donor with respect to the eligibility criteria can be challenging, particularly as manufacturing is timed for each individual patient. Due to quarantine until negative microbiological result, products can be issued up to 2 weeks after request only. This may be very long for urgent indications.

Conclusion: Permission for general manufacturing and delivery from stock would be desirable. For this to achieve an international recommendation for standardization of donor eligibility and manufacturing in general would be very helpful.

Disclosure Statements: No conflicts of interests.

PS-1-18
Coagulation factor activity in 4°C stored universal plasma manufactured with UBP Glycosorb® columns

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Background: Therapeutic plasma is widely used in coagulation factor (CF) deficiency and frozen storage is favorable, but compatible plasma may be lacking under certain circumstances due to increasing global needs. Glycosorb® UBP columns significantly eliminate isoagglutinin from donor plasma to gain fresh universally applicable plasma. Here, we analyzed the impact of different plasma flow rates on the activity of multiple CF and measured the remaining activity after storage at 4°C for up to 6d.

Methods: A pool of freshly produced donor plasma (957 mL, blood group B) was used with three UBP columns, which contain 4 mL of a specific gel agent for the adsorption of isoagglutinins. A different flow rate (180 mL/h, 240 mL/h, and 360 mL/h) was used for each column to determine if contact time was a relevant factor in the potential loss of CF. 325 mL plasma were passed twice over each column. Samples were taken before (0 h) and after each treatment (650 mL) and from the 4°C-stored plasma after 24 h, 72 h, and 144 h. All samples were snap-frozen in liquid nitrogen and stored at -80°C until analysis: isoagglutinins were titrated (Ortho Vision® Max, OCD) and a large panel of CF was measured (BCS XP® System, Siemens Healtheneers).

Results: Initial Anti-A1 isoagglutinin titers (IgG 1:64, IgM 1:16) were reduced to (IgG 1:4, IgM 1:2) after 325 mL and remained unchanged after 650 mL, with no significant effect of the flow rate used. Moreover, different plasma flow rates had an only minor impact on CF activity when comparing treated and untreated plasma. Storage at 4°C resulted in some decrease in CF activity compared to freshly treated plasma, but no relevant reduction in CF activity was observed between time points 0 and 144 hours (table 1). Meaning, the activity did not drop below 70% in any CF - e.g. (in brackets: column number and result): FV 120.4% (C1:93.3%, C2:77.6%, C3:88.0%), FVII 100.9% (C1:79.1%, C2:90.5%, C3:79.6%) and FVIII 153.3% (C1:81.1%, C2:70.6%, C3:76.8%).

Conclusion: Universal plasma with low isoagglutinin titers was produced with three UBP columns applying plasma at low (180 mL/h), medium (270 mL/h), and high (360 mL/h) flow rates. Taken together, none of these flow rate settings significantly affected measured CF activity in the treated plasma. Furthermore, after storage at 4°C for 144 hours, an acceptable CF activity of more than 70% was observed, which is an important goal in plasma production.

Disclosure Statements: We declare no conflicts of interest considering this abstract. UBP columns were provided by Glycorex.

| plasma | UBP column 1 | storage at 4°C | UBP column 2 | storage at 4°C | UBP column 3 | storage at 4°C |
|--------|--------------|----------------|--------------|----------------|--------------|----------------|
| Anti-A1 IgG titer | 1.24 | 1.4 | 1.4 | 1.4 | 1.4 | 1.4 |
| Anti-A1 IgM titer | 1.16 | 1.2 | 1.2 | n/a | n/a | n/a |
| PII [%] | 104.8 | n/a | 97.9 | 102.6 | 103.4 | 100.3 |
| Pll [%] | 120.4 | n/a | 111.2 | 100.1 | 95.7 | 93.3 |
| FV [%] | 100.9 | n/a | 102.3 | 94.8 | 83.0 | 79.1 |
| FVII [%] | 153.3 | n/a | 147.7 | 101.1 | 79.8 | 81.3 |
| FX [%] | 88.7 | n/a | 85.2 | 78.0 | 76.6 | 76.9 |
| FIX [%] | 112.8 | n/a | 109.9 | 108.1 | 104.2 | 101.1 |
| FII [%] | 69.6 | n/a | 97.4 | 90.7 | 91.2 | 91.9 |
| FIII [%] | 69.7 | n/a | 85.6 | 81.9 | 81.3 | 85.0 |
| Protein C [%] | 110.9 | n/a | 110.9 | 108.9 | 106.7 | 107.9 |
| Protein S [%] antigen | 113.1 | n/a | 109.8 | 104.2 | 101.0 | 101.0 |
| Factor V activity | 119.3 | n/a | 126.1 | 101.5 | 95.0 | 79.6 |
| vWF activity | 125.2 | n/a | 135.4 | 125.4 | 125.9 | 117.9 |
| vWF antigen | 124.5 | n/a | 128.0 | 120.7 | 120.0 | 120.0 |
| ATIII [%] | 103.2 | n/a | 95.8 | 98.2 | 94.3 | 98.0 |
| Fibrinogen (mg/dL) | 250.0 | n/a | 247.0 | 245.0 | 246.0 | 243.0 |

Table 1: Isoagglutinin titers and coagulation factor activity in plasma at different time points
PS-1.19

Uncertainty: Are Serum Eye-drops Blood Products? Patient Care Suffers from Regulatory Stumbling Blocks, Deficiency of Transparency and Acceptance

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Background: Serum eye-drops (SED) prepared from autologous or allogenic serum are a treatment option for otherwise therapy-resistant severe cornea diseases (dry eyes). They are highly effective, well-tolerated and promote healthy growth and healing of the cornea. SED is usually manufactured in accordance with drug law regulations for blood components. But what are SED? Are they blood products or plasma derivatives? Do we have a clear regulatory framework for the use of these drugs today?

Methods: Recently, our group conducted a detailed validation of the overall concept of patient care with SED. Briefly, we examined patient selection, request and approval procedures, suitability examination, whole blood collection, serum processing, quality and safety examinations, deep-frozen interim storage, temperature-controlled transport, dispensing by pharmacy, storage and reimbursement by health insurance companies. Frauke Dormann et al will present the results of the validation in a separate abstract at (DGTI Congress 2022). The multifaceted experiences gained outside of the validation results will be shown in detail and critically commented on.

Results: Although SED originate from autologous or allogeneic blood, they are outside the scope of the German Hemotherapy Guideline and are therefore not a defined blood component. Neither the European nor the German Pharmacopoeia list serum eye drops. As a result, there is a great deal of uncertainty regarding SED status and regulation of manufacturing, testing, storage, transport and delivery. Facilities currently producing SED use different technologies, assorted quality, and safety criteria like other blood products. A critical obstacle is the restrictive attitude of the health insurance companies, which often refuse to reimburse SED. Consequently, many patients are not adequately treated and risk the deterioration of vision up to blindness.

Conclusion: In the production and sales practice of SED we are confronted with many ambiguities and difficulties as well numerous obstacles and deficits due to insufficient regulations. It is therefore imperative that the hemotherapy community engage in a dialogue in order to develop proposals and possible solutions that could serve as recommendations for the responsible scientific advisory board of the BÄK as framework for an adequate future sub-chapter of the German Hemotherapy Guideline.

Disclosure Statements: There is no conflict of interest.

PS-2

Immunhämatologie und immunologische Grundlagen I. Fetomaternale Inkompatibilität

PS-2-1

Evaluation of single nucleotide variations in intron 1 of the ABO gene as specific markers for the A1 allele

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Background: A specific marker for the ABO*A1 allele is still missing. Thus, the molecular diagnosis of the A1 allele is based on the exclusion of gene variants leading to B or O alleles. In a previous study performed at the Swiss Red Cross Blood Transfusion Service Zurich four candidate single nucleotide variations (SNVs) in the ABO intron 1 were identified. We evaluated the diagnostic sensitivity and specificity of the four SNVs in 1,340 blood donors with known ABO phenotype and genotype.

Methods: ABO forward and reverse phenotyping of the blood donors was performed as routine analysis. The ABO genotype was determined by using a low resolution PCR-SSP method for detection of the O1, O2, B, and A2 alleles. For genotyping of the four SNVs (rs1554760445, rs507666, rs532436) we applied TaqMan assays with endpoint detection of fluorescence according to a standard protocol. Genotypes of the SNVs were compared with the ABO phenotype and the low resolution genotype.

Results: The ABO phenotype and the low resolution ABO genotypes indicated absence or presence of 1 or 2 A1 alleles. The SNVs showed false positive and false negative results in 15 samples. The rs1554760445 revealed A1 specificity of 99,85% (4/2678 false positive alleles) and sensitivity of 99,78% (6/2678 false negative alleles). Very similar results were obtained for rs2519093 (99,78%; 99,85%), for rs507666 (99,81%; 99,81 %) and for rs532436 (99,85%; 99,74 %).

Conclusion: The ABO intron 1 SNVs had a strong correlation with the A phenotype and the proposed A1 allele. However, none of the SNVs showed a complete genotype-phenotype correlation demanded for a reliable diagnostic marker. Additional SNVs or combination of SNVs should be evaluated as diagnostic Markers for the A1 blood group.

Disclosure Statements: The authors declare no conflict of interest.

PS-2-2

Antigen Masking Indirect Antiglobulin Test: A New Reagent with Improved Protocols

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Background: Anti-CD38 antibodies are increasingly used in the clinic. CD38 is a surface molecule expressed on a number of cells and tissues, including erythrocytes. After administration of anti-CD38, interference regularly occurs in indirect antiglobulin tests (IAT). A strategy for mitigation of this interference is the antigen masking IAT (AMIAT), where the antigens on the erythrocyte are masked by an antibody fragment. The new AMIAT reagent DaraEx plus contains the Fab fragment of an anti-CD38 antibody.

Methods: Incubation protocols were tested for DaraEx plus. We analyzed whether the sample material has an influence on the AMIAT and tested whether worst case concentrations of anti-CD38 antibodies can be effectively inhibited. Finally, 21 plasma samples of patients treated with Daratumumab that were otherwise negative for irregular antibodies were tested in an AMIAT with DaraEx plus. All tests were performed in Bio-Rad I-D System gel cards.

Results: We developed a quick protocol that inhibited 15 of the 21 samples and a more material intensive protocol that worked for 5 of the 6 samples that had not been inhibited by the quick protocol. 1 mg/ml Daratumumab could be inhibited and the sample material had no influence on the inhibition efficacy. With DaraEx plus, a pre-incubation step was not necessary.

Conclusion: These data suggest that DaraEx plus treatment is a simple and effective way to mitigate the interference of anti-CD38 antibodies.

Disclosure Statements: CPH, SA, and CS are employees of imusyn GmbH & Co. KG. CS is a designated inventor of the technology presented here.
PS-2-3

Anti-HNA-1d specificity is frequently observed in anti-HNA-1b alloantisera

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Background: Four amino acids are involved in epitope formation of HNA-1 alleles, located at positions 36, 65, 78, and 82. HNA-1a and HNA-1b alloantibody epitopes were recently characterized. Anti-HNA-1a binds to p.65N exclusively, whereas anti-HNA-1b binds to either p.36S, or p.82N, or both. However, the HNA-1b allele also carries the HNA-1d epitope p.78A p.82N. The current study aimed to identify compound antibody specificities in HNA-1b alloantisera, especially the presence of Anti-HNA-1d.

Methods: For investigation of binding epitopes for HNA-1b alloantibodies, transfected HEK293-F cells stably expressing different HNA-1 allele variants in five amino acid positions p.36, p.65, p.78, p.82, and p.106 were generated and tested against previously well-characterized HNA-1b antisera (n=11) in an antigen capture assay. For further differentiation of compound antibody specificities, in particular regarding the presence of Anti-HNA-1d, randomly selected sera with p.82N specificity or compound p.36S and p.82N specificity were additionally analyzed by using adsorption and elution methods.

Results: Three amino acids, p.36S, p.78A and p.82N are involved in epitope formation of HNA-1b. The following specificities were identified in eleven HNA-1b alloantisera: p.36S (6/11), p.82N (9/11) and p.78A p.82N (8/11) of which p.36S was identified as sole entity in 2/11, whereas 9/11 antisera contained a polyspecific mixture of anti-p.36S, p.82N (1/11) and anti-p.78A p.82N in combination with anti-p.82N (5/11) or compound specificities of anti-p.36S, p.82N and p.78A p.82N (3/11). In seven out of eight antisera with p.82N specificity, anti-p.78A p.82N was detected.

Conclusion: Analysis of HNA-1b antisera indicates compound specificities for HNA-1b alloantibodies with a high variation between HNA-1b immunized individuals. Amino acids p.36S, p.82N and p.78A p.82N are necessary for HNA-1b epitope formation. The HNA-1d epitope is recognized by 73% (8/11) of HNA-1b immunized individuals.

Disclosure Statements: No conflicts of interests.

PS-2-4

The burden of false RhD phenotyping in patients with partial D – RhD typing in a case of RHD*10 DAU

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Background: Serologic RhD typing may be unreliable for purposes in blood transfusion and pregnancy surveillance as monoclonal anti D reagents can react in full strength with weak and partial D types. We were close on false serologic RhD typing in things like that because agglutination tests reacted with high strength in different assays of a pregnant patient with partial D. It was rather by chance that the same specimen was submitted to genotyping whereby a variant RHD*10 (DAU) was detected.

Methods: 3055 samples of a multi-ethnic cohort of pregnant women from a 1-year collection period were taken for blood group serology according to legal guidelines.

AB0 and RhD serology. Methods were tube agglutination, column agglutination using gel cards, analyzer-based microplate agglutination. All conspicuous specimens and those from non-European descent were submitted to RHD genotyping to detect D variants.

Other assays. Alloantibody screening was done by indirect antiglobulin tests in LISS-Coombs at 37 ºC. Direct Coombs tests served to detect erythrocyte sensitization as well as to control red blood cell sensitization by monoclonal anti-D and to detect their agglutinability with check cells.

RHD analysis. Genotyping was done by SSP-PCR.

Results: RhD serology was not straightforward in 21 cases. Subsequent analyses by RHD genotyping revealed 18 weak D and 4 partial D types (Table 1). The specimen from a pregnant African woman yielded the blood group 0 RhD positive (ccDec) by serology and an obviously normal RhD antigen (Fig. 1). Also, other tests with different RhD reagents (monoclonal IgM and IgG antibodies TH28, MS26, D175-2, MS24, MS201, MS80, MS26, LHMS50/3, TH 28, RUM 1, LHMS59/20, 175-2, D7B8) were not suspect of partial D. In contrast to serology, a partial D (DAU cluster, RHD*10, RHD*DAU0) was detected by RHD genotyping. This D variant may cause alloimmunization and should be classified RhD positive as blood donor and RhD negative as blood recipient in transfusion.

Conclusion: False RhD phenotyping by monoclonal antibodies is mainly based on the diversity of D antigens and due to the heterogeneity of agglutination reactions inherent to serological methods. They are liable to fall in...
the detection of relevant D variants with potential risks for alloimmunization in transfusion. RHD genotyping is the method of choice to secure the RhD status. The diversity of RHD alleles and ethnicity are comprehensive variables that have an impact on diagnostic strategies.

Disclosure Statements: Disclosure Statements: Laboratory tests were partially delegated to the DRK Blood Donor Service Bad Kreuznach, Germany.

Abstracts

PS-2-5
Is molecular prediction of a Del phenotype of a previously unknown RHD allele feasible?

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Background: While the number of known RHD alleles is steadily increasing, the serologic characterization of the expressed phenotype is often insufficient. Increasingly, online accessible computational tools are used as substitute to predict the putative phenotype. A systematic evaluation of the validity of such results was lacking. Therefore, the utility of such tools to discriminate Del from RhD negative and weak D samples was evaluated.

Methods: The impact of missense mutations was evaluated for 17 Del, 6 Del-like and 23 weak D alleles using Provean, Sift and Polyphen-2. Alleles with single nucleotide variations (SNV) near splice-sites were collated from Human RhesusBase and Rheference. The impact of the SNV was evaluated using MaxEntScan based on the differences from wild type scores. The obtained results were compared using median, quantiles and range and evaluated using Mann-Whitney U-test.

Results: Median SIFT and Polyphen-2 scores of Del, Del-like and weak D alleles with missense modifications indicated a near maximal impact. For Provean, the median Del-score was -5.404 (range -10.563 to -2.031; Del-like -4.704 (-7.991 to -3.121), weak D -4.381 (-12.962 to 1.547). Of 72 alleles with SNV near splice-sites, 14 alleles were Del (16 RhD neg, 6 RhD neg or Del, 26 weak, 4 D pos or partial D and 6 no data). Median MaxEntScan impact score was -8.065 for Del (range -8.97 to -1.24; RhD neg -8.18 (-11.02 to -1.29), weak -1.845 (-8.51 to 4.13). Due to the large overlap, no significant differences were detected.

Conclusion: Computational tools usually indicate relevant impact of mutations altering the phenotype expressed by an allele and are useful to explain the impact of the mutation. There is considerable overlap of the results obtained with alleles expressing a RhD negative, Del or weak D positive phenotype. Currently, it is not possible to discriminate these phenotypes by computational tools. A serologic investigation of a sample with unknown phenotype remains the gold standard and should be documented.

Disclosure Statements: There are no conflicts relevant for this abstract.

PS-2-6
Reduced Expression of Kell Blood Group Antigens Including Jsa and Jsb Induced by Compound Heterozygosity for KEL Allele Variants

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Background: A Lebanese blood donor with compound heterozygosity for KEL allele variants was identified by NGS blood group screening (BluStar,NRW) in non-middle European donors. Detected variants were assigned in comparison to the reference sequence or the most probable known allele and predicted a Jsa(a+b-) phenotype. Extended molecular typing as well as serological tests should confirm the allocation of variant nucleotides to both alleles and the corresponding phenotype.

Methods: NGS of 22 blood group systems including KEL was performed by an in-house exon-based strategy on a MiSeq sequencing device (llumina Inc., San Diego, USA). Additionally, single nucleotide polymorphism (SNP) specific PCR SSP reactions and Sanger sequencing with SNP specific amplification and sequencing primers were applied to assign the detected variant nucleotides to both alleles. Indirect antiglobulin test was used for phenotyping of the donor’s red blood cells for Jsa, Jsb, K, k, Kp^a and Ku by applying two sources of polyclonal human sera (with exception of Ku; Antitoxin GmbH, Germany and SCARF) in the gel technique (BioRad, CH).

Results: NGS determined the genotype KEL*02N.05/KEL*02.06. A580V, 1899A>G resulting in a silenced KEL due to a rare KEL*02N.05/A exchange (K, Kp^a and Jsa not expressed) and a second variant KEL*02 with a novel KEL*02.1790T->C(T(A580V), c.1790T>C(L597P), c.1899A>G (table). Due to the KEL*02.1790T->C variation, the phenotype of the donor was predicted as Js(a+b-). Contrary, Sanger sequencing and SNP specific
SNPs to both haplotypes. The effect of new variants should always be variation in both blood group alleles is critical for the assignment of single the expression of the whole Kell protein, too, showing that complex vari-

ified by serology.

significant weakened Jsb

notype. Surprisingly, serological tests showed a Js(a+b+) phenotype with

Conclusion: The KEL*2027G>A variation in one haplotype with c.1790T>C did not silence the Js expression while the novel KEL*c.1739C>T in one haplotype with the reference KEL*c.1790T signific-

variation in one haplotype

weak, very

weak antigen expression

**Expression of allele 1 (Jsb)** may be modified due to KEL*2027G>A

***Expression of allele 1 (Jsb)** may be modified due to KEL*2027G>A

(A580V) together with the Js b with

KEL*c.1739C>T

KEL*c.2027G>A

was located

was 1024 in elution. No further transfusion was indicated. A control after 4 days did not show an indication for transfusion and the newborn was discharged in a good physical condition for regular check-ups by the resident pediatrician.

Conclusion: Implementation of Rh D antibody prophylaxis in Rh D-negative non-immunized women has led to a marked reduction of the incidence of Rh D alloimmunization and eventually HDFN. However, maternal Rh-D antibodies can induce delayed HDFN, even without signs of hemolysis at birth. Frequent pediatric controls of newborns with maternal alloantibodies in the first weeks after birth are of great importance to avoid complications of a delayed HDFN.

PS-2-7

Maternal anti-D antibody inducing delayed hemolytic disease of newborn

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Background: Hemolytic disease of fetus and newborn (HDFN) is caused by maternal antibodies that react to erythrocyte antigens of the fetus. HDFN is associated with perinatal mortality and long term neurological deficit in severe cases. Autoantibodies will lead to hemolysis of fetal erythrocyte cells and cause fetal and neonatal anemia. The Rhesus D (Rh D) antigen is a frequent cause of HDFN. In most cases, the maternal alloantibody transported through placenta leads to hemolysis already in utero.

Methods: Only few cases of delayed-onset hemolysis of the newborn are reported yet.

Case Report: We present a case with high titer of maternal anti-D at birth with normal hemoglobin level. The newborn’s direct antiglobulin test (DAT) was positive for immunoglobulin G, with an anti-D titer of 4096 identified by red cell elution studies. Hemoglobin was 15.6 g/dl, total bilirubin was 7.9 mg/dl (normal < 4 mg/dl) and lactate dehydrogenase (LDH) was 1319 U/l (normal < 799 U/l). After phototherapy total bilirubin decreased to 10 mg/dl and the newborn was discharged with a hemoglobin of 17.4 g/dl and without any complications.

Results: Twenty days after birth the newborn was admitted to the child-

Case Report assigned the KEL*c.1739C>T (A580V) together with the Js encoding KEL*c.1790T to one haplotype, while the KEL*c.2027G>A was located on the Js encoding allele (KEL*c.1790T>C). This implies a Js(a+b+) pheno-

type. Surprisingly, serological tests showed a Js(a+b+) phenotype with significantly weakened Js and moderately weakened Js', Ku, Kp and k.

Conclusion: The KEL*2027G>A variation in one haplotype with c.1790T>C did not silence the Js expression while the novel KEL*c.1739C>T in one haplotype with the reference KEL*c.1790T signific-

variation in one haplotype

weak, very weak antigen expression

**Expression of allele 1 (Jsb)** may be modified due to KEL*2027G>A

***Expression of allele 1 (Jsb)** may be modified due to KEL*2027G>A

PS-2-8

Genotyping of 413 serologically inconspicuous routine samples reveals many RHCE and RHD variant alleles

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Background: Since years, blood group genotyping has been used as a reasonable addition in immunohematology labs in order to aid with question-

able results. The aim of this study was to genotype random samples from the German Red Cross with a real-time RHCE/RHD assay in order to identify the percentage of variant alleles that have not been noticeable by routine serological testing.

Methods: 413 samples with pre-existent RhD and RhCE serotypes (obtained by standard techniques) were genotyped for the RHD and RHCE genes with a TagMan Probe 48-well assay (RBC-FluGene CDE eXtend, inno-train Diagnostik GmbH). The kit detects RHCE*’s, ‘C, ‘E alleles plus variants like RHCE*CeCw and others by detecting the RHCE SNPs 48G/G>C, 122G>A, 733C>G and 1006G>T. For analyzing the RHD locus 43 different primer-probe-mixes identify RHD*01, RHD*01N.01 (and other null-alleles), as well as RHD variants, DELs, D weak and partial Ds. The samples were analyzed by two methods: end point fluorescence and real-time PCR. The results were automatically evaluated by software.

Results: With respect to RHCE, the analyzed samples revealed 96.6% concordance between serological and molecular typing. In 13 out of 413 samples genotyping detected the presence of the RHCE 122A>G SNP that codes for the Cw phenotype. One sample was serologically typed c+, while genotyped as c+. This discrepancy is currently under further investigation. Genotyping of RHD locus revealed 9 variant alleles: namely 5x D
weak types, 3x D partial and 1x DEL (see table 1 for detected alleles). Both molecular methods, end point PCR and real-time PCR, agreed to 100%.

| RHCE/RHD alleles detected: | n | % |
|---------------------------|---|---|
| RHCE*CeCW                 | 13| 3.15|
| RHCE*c+ (genotype) vs. c- (serology) | 1 |
| RHD*01W.1                 | 1 | 1.21 |
| RHD*01W.2                 | 2 |
| RHD*01W.3                 | 1 |
| RHD*01W41/45/75*          | 1 |
| RHD*07.01                 | 2 | 0.73 |
| RHD*25                    | 1 |
| RHD*01EL.08               | 1 | 0.24 |

**Conclusion:** The study shows that molecular RBC typing methods represent a reasonable addition or even a substitution to serology. Low manual hands-on-time and fully automated result evaluation with assay time of 70 minutes provide a beneficial opportunity for molecular blood group testing. The used genotyping assay facilitates to solve unclear results or even reveal allelic variants that were overseen by serological testing, as shown in 5 % of the samples (23/413) tested in this study.

**Disclosure Statements:** The author is employed at inno-train Diagnostik GmbH.

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### PS-2-9

**Detection of a novel RHD gene mutation in red blood cells**

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**Background:** Beside ABO, the Rh blood group system is the second most significant one. It is the most polymorphic blood group, with variations due to deletions, gene conversions, and missense mutations. The Rh blood group includes the D gene, which encodes the RhD protein, and a second gene that encodes the RhC and RhE antigens. The two genes are found in a cluster on chromosome 1. Sequencing of RHD coding regions can enable compatible and safe blood transfusion.

**Methods:** Automated Blood group-testing was performed with NEO Iris, Immucor and IH-1000-ID-Cards System, BioRad. The genotyping was performed using the SSP Inno-Train kits (CDE SCP0520, Dweak SWP0421, DAddOn SAD0221, ZygoFast SZF0320). RHD exons and flanking introns were sequenced using ABI kit (BigDye Terminator 00886303). DNA-protein mutation analysis was performed with DNA Star software.

**Results:** In the serological blood group-testing, the patient was blood typed AB and Rhesus Ccee with weakened D. The initial SSP PCR genotyping detected a normal RHD*01.01N.01 = Dd. However, this result could not explain the serological results. Therefore genomic DNA sequencing (Sanger) was performed and resulted in an Exon 1 c.23C>T mutation. The new base triplet in the N-terminal part of RHD codes for a phenylalanine residue at amino acid position 8 (p.Ser8Phe).

**Conclusion:** This mutation is not listed in the ISBT RHD allele database (v6.0 30-NOV-2021) or the RhesusBase established by Wagner and Flegel. We consider the mutation until now unknown. It likely affects expression and/or membrane integration. It is uncertain if carriers of this mutation can be immunized with RhD positive blood. We transfused the patient only with RhD negative blood. This case shows that SSP genotyping may not be sufficient to identify rare patients with potential allo-immunization risk.

**Disclosure Statements:** None
PS-2-10
The solid phase assay detects clinically relevant anti-Jk antibodies with higher sensitivity than column agglutination

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Background: After introducing solid phase antibody screen in our laboratory, we observed an increased frequency in the detection of anti-Jk antibodies. The solid phase has been reported to be more sensitive for Jk than column agglutination. We were interested to learn whether Jk antibodies detected in solid phase differ from those detectable in column agglutination.

Methods: Anti-Jk identified in a solid phase assay (Capture-R, NEO Iris; Immucor, Dreieich, Germany) were re-tested by column agglutination (CA; indirect Coomb’s test on gel cards (BioRad, Dreieich, Germany). All antibodies were titrated in solid phase. IgG subclass composition was determined using Gamma Eli Kit II (Immucor) and the Human IgG Subclass Profile (Innogen, Carlsbad, USA) for 14/27 samples.

Results: Out of 27 anti-Jk antibodies, 16 were only reactive in the solid phase assay, whereas 11 antibodies were also detectable by CA. Antibody titers for solid phase anti-Jk were in the range of 1 to 4 (mean: standard deviation = 2.25 ± 0.39), and antibody titers for anti-Jk detected by both methods were in the range of 1-16 (7.54 ± 1.75; p=0.013). The composition of IgG subclasses was different between the two groups of anti-Jk.

Conclusion: The solid phase system detects anti-Jk antibodies with significantly lower titers in comparison to CA. The IgG subclass composition is not different between solid phase only antibodies and those detectable by both methods, indicating equal clinical significance. We conclude that compared to CA, the solid phase system detects clinically relevant anti-Jk antibodies with higher sensitivity.

Disclosure Statements: none

PS-2-11
The effect of mabs against ß3 integrin on endothelial angiogenesis

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Background: Exposure of a HPA-1a negative mother to HPA-1a positive platelets originating from the fetus induces alloimmunization against HPA-1a, responsible for up to 80% of FNAIT cases in Caucasians. Endothelial-specific anti-HPA-1a alloantibodies have been documented as pathological antibodies involved in the mechanism of FNAIT-mediated bleeding. Blockage of angiogenesis and sprouting during fetal brain vascularization have been considered to be involved in FNAIT-mediated intracranial bleeding (ICH). Antibodies with higher sensitivity.

Methods: In the current study HUVECs angiogenesis on matrigel in presence of mab against αvβ3 (clone LM609) or anti-β3 (clone AP3, SZ21 and 26.4) or anti-αv (clone P2W) or anti-HLA class I (W6/32) or anti-HPA-1a sera was evaluated in real-time using a Leica DMi8 Thunder Imager Live-cell microscopy system.

Results: Angiogenesis analysis indicated a maximum tube formation (evaluated by tube length) four hours after cell culture that declined after six hours. Presence of mab against αvβ3 significantly inhibited tube formation (up to 30%), number of branching points (up to 40%), and total loops (up to 60%). No blocking effects were observed with anti-β3 or anti-αv (or anti-HLA class I mabs. Further blockage of Fc gamma receptors (FCR) using specific mabs reveals a partial FC-dependent effect of anti-αvβ3, via FCGRII. Endothelial treatment with anti-HPA-1a sera, demonstrated an angiogenesis blocking effect only for ICH positive but not ICH negative sera. This effect was persistent even after absorption on chimer β3 (cβ3 protein).

Conclusion: These results confirmed the blocking effects of antibodies against αvβ3 but not β3 or αv on endothelial angiogenesis. Similarly, serum from ICH positive cases inhibited angiogenesis that was not hindered even after absorption of anti-β3 antibodies.

Disclosure Statements: NA

PS-2-12
Development of a test cell-panel that provides the framework for fast, sensitive and reliable FNAIT-causing HPA antibody detection

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Background: To date, antibodies directed against human platelet antigens (HPAs) are not routinely investigated because they are less common compared to erythrocytic antibodies. However, if pregnant women develop allo-antibodies to paternally inherited HPAs, fetal/neonatal allo-immune thrombocytopenia (FNAIT) may occur, which can lead to cerebral hemorrhage with severe residual syndrome. Therefore, a test system for early detection of the main causing antibodies for FNAIT may be of clinical interest.

Methods: Clinically relevant for FNAIT are antibodies directed against HPA-1a or HPA-5b, as well as the recent suggested cross-reactivity of anti-HPA-1a antibodies with the endothelial αv/β3 integrin heterodimer. In order to develop a test cell-panel that can identify these HPA antibodies, we generated xenogeneic cells carrying either the integrin αllb/β3 (HPA-1a) heterodimer, the α2β1 (HPA-5b) or the endothelial αv/β3 (HPA-1a) integrin complex. Binding of HPA-specific human IgG antibodies to the test cells is detected by flow cytometry using an APC-coupled anti-human IgG.

Results: Using patient plasma with pre-described HPA antibodies, we could show that the generated test cells effectively present the antigen and are able to detect and discriminate anti-HPA-1a and -5b antibodies. With this HPA test cell panel, all available patient plasmas with presumed anti-HPA antibodies according to conventional diagnostics were analyzed, of which the results from 16 out of 19 samples could be confirmed. These included 10 samples collected for a study, in which plasma was obtained from women immunized against HPA-1a in a previous pregnancy. Flow cytometric analysis confirmed the presence of anti-HPA-1a antibodies in all plasma samples. The analysis also revealed that 6 of the 10 plasmas were also reactive with the αv/β3 complex.

Conclusion: According to the results generated so far, we posit that our system is capable of detecting anti-HPA antibodies with high sensitivity and specificity. Our novel test system could enhance HPA antibody diagnostics and support pregnancy screening for anti-HPA antibodies. We are additionally working on the feasibility of further developing the assay into an easy-to-use test system that does not rely on time-consuming analysis by flow cytometry.

Disclosure Statements: CG and HB have invented the technology at the base of the presented work which is registered world-wide (EP3062109A1 / JP2017545404A / CA2977436A1 / US5/552,657 / PCT/EP2016/053989 / AU2016223431A). ES is the CMO of the company which owns the IP according to the German Law on Employees’ Inventions.
Analysis of different anti-HPA-1a mediated platelet clearance and their inhibitors in whole blood by platelet suspension phagocytosis assay

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Background: Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is an immune mediated bleeding condition, in which maternal anti-HPA-1a (mostly) IgG antibodies (abs) pass through the placenta, leading to Fe-dependent phagocytosis of opsonized HPA-1a (+) fetal platelets and thrombocytopenia. Here, we established a fluorescence-based platelet phagocytosis assay (PSPA) in whole blood to measure the capability of different anti-HPA-1a abs.

Methods: Platelets were labelled with pH-sensitive fluorescence dye (pHrodo), sensitized with anti-HPA-1a abs and incubated with whole blood. The phagocytosis rate of monocytes that internalized platelets and the binding of abs onto platelets were analyzed by flow cytometry. Sera from FNAIT cases (n=15) with and without anti-HLA class I antibodies were tested. Standard anti-HPA-1a abs derived from FNAIT cases with intracranial bleedings containing anti-HPA-1a reacted with αvβ3 complex. In some experiments, inhibitors (deglycosylated type I and type II monoclonal antibodies against HPA-1a or against FcγRII) were added prior to phagocytosis.

Results: When anti-HPA-1a sera from FNAIT cases were tested, 13/15 sera containing induced clearance of HPA-1a (+), but not HPA-1a (-) platelets. Significant correlation was found between antibody binding and phagocytosis rate (r=0.92, P<0.0001). Both type I and type II anti-HPA-1a abs reacting with β3 integrin induced platelet clearance. In contrast, type III anti-HPA-1a abs reacting with αvβ3 complex did not trigger platelet phagocytosis. Blockage experiments showed that deglycosylated mAbs against HPA-1a reduced anti-HPA-1a mediated platelet phagocytosis. However, these mAbs did not achieve complete inhibition. Interestingly, not only anti-FcγRI and anti-FcγRII, but also anti-FcγRIII inhibited anti-HPA-1a mediated phagocytosis in our assay.

Conclusion: PS-PA allows in vitro analysis of platelet clearance due to different types of anti-HPA-1a abs. This method may not only improve platelet antibody diagnostic to predict the severity of FNAIT, but also provide the possibility to test different inhibitors.

Disclosure Statements: none

Improvement of accuracy in noninvasive fetal genotyping of fetal KEL and ITGB3 (HPA-1a) using single molecule consensus sequencing

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Background: Hemolytic disease of the fetus and newborn and fetal/neonatal alloimmune thrombocytopenia are caused by maternal antibodies. 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. However, previous NGS protocols for noninvasive prenatal diagnostic revealed limited sensitivity.

Methods: A primer panel was designed to target sequences flanking single-nucleotide polymorphisms (SNPs)/exonic regions of red blood cell antigens and human platelet antigens. Single molecule consensus sequencing was performed by adding molecular barcodes (unique molecular identifier, UMI) to the initial cffDNA during library preparation. Limit of detection and false-positive antigenic alleles, linearity and inter-assay variation were estimated for detection of ITGB3*176T (HPA-1a) and KEL*01 (K) alleles in cell free plasma samples with an improved NGS protocol employing single molecule consensus sequencing. A side-by-side comparison with a standard NGS protocol was performed in plasma samples of 21 pregnant women at risk.

Results: The limit of detection for ITGB3*176T was 0.2058 % (mean+3SD) or 0.6298 % (mean+10SD) respectively. For KEL*01 the limit of detection was 0.1706 % (mean+3SD) or 0.5314 (mean+10SD). The % of the false positive anti-HPA-1a reduced anti-HPA-1a mediated platelet phagocytosis. Blocking experiments showed that deglycosylated mAbs reacting with β3 integrin induced platelet clearance. In contrast, type III anti-HPA-1a abs did not trigger platelet phagocytosis.

Conclusion: Next generation sequencing with short amplicons and single molecule consensus sequencing is a sensitive and specific tool for noninvasive prenatal diagnosis of fetal blood groups.

Disclosure Statements: Conflict of interest

PS-2-13

PS-2-14

PS-2-15

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Background: Neonatal alloimmune thrombocytopenia is a disorder of the newborn due to fetomaternal incompatibility of platelet antigens. Polymorphisms associated with platelet alloimmunisation are primarily located in ITGB3 & ITGA2B, though, five single nucleotide variants (SNV) are described for ITGA2 as well. This gene encodes for the a chain of Gp IIa/IIIa. The rare variant rs780441074 of ITGA2 (estimated frequency <10⁻⁵) leads to p.Asn57Lys. So far, no alloimmunisation is described for this SNV.

Methods: Here, we present a case of a female newborn with a suspected NAIT (initial platelet count: 84 Gpi/l, nadir: 52 Gpi/l) and a presumably new alloantibody. Blood samples of all direct family members and of several general relatives were analysed. Antibody detection was performed by indirect MAIPA. Serological analysis included a crossmatch between paternal platelets and maternal serum. Initial genotyping was performed by commercial SSP-PCR. For sequencing of ITGA2 a direct taq-cycle method was chosen.

Results: No maternal platelet specific alloantibody could be detected by indirect MAIPA. For the most relevant HPA systems, no incompatibility between mother and newborn was found. However, a crossmatch between maternal serum and paternal platelets was strongly positive for Gp IIa/IIIa. Thus, the existence of an antibody to a private antigen was assumed and sequencing of both eligible genes (ITGA2 and ITGB1) was initiated. The rare SNP rs780441074 was found in the father and the newborn, but not in the mother. At GnomAD, an allele frequency of 2.39x10⁻⁵ is given, whereby no homoyzogte sample was found yet. Figure 1 shows a pedigree of the family. No bleeding complications were reported in any relative assuming a benign behaviour of this type.

Conclusion: It is intriguing to speculate that this case of a neonatal thrombocytopenia is caused by an alloantibody directed against a rare polymorphism in ITGA2. Actually, the impact of Gp-IIa/Iia antibodies on NAIT is discussed controversially. In addition, further serological investigations are needed to prove this alloantibody specificity. However, this case shows strikingly that a serological crossmatch is essential for detecting antibodies against new antigens.

Disclosure Statements: None of conflict of interest
PS-2-16
Maternal human leukocyte antigen (HLA) class II genotype is not a predictor of immunization against HLA class I in neonates with suspected fetal/neonatal alloimmune thrombocytopenia (FNAIT)

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Background: Antibodies against HPA-1a, formed by the maternal immune system, are causing most cases of FNAIT in white Europeans. Anti-HPA-1a is detected in less than 0.2% of all pregnancies. The maternal HLA-DRB3*01:01 is strongly associated with the propensity for mounting the maternal immune response. In contrast, up to 50% of all mothers form anti-HLA class I antibodies during pregnancies, but it is unclear whether or not a minority of these antibodies may be capable of causing FNAIT.

Methods: We included 95 cases of suspected FNAIT (newborn platelet count < 100 G/l, no other obvious reason for thrombocytopenia) with no anti-HPA antibodies detectable. Maternal serum was analysed for the presence of anti-HLA antibodies by lymphocytotoxicity testing, and maternal DNA was genotyped for HLA class I and II by SSO. Fetal DNA was genotyped for HLA class I by SSO.

Results: A total of 57 mother/child pairs with anti-HLA antibodies (cases) and 38 mother/child pairs without anti-HLA antibodies (controls) were identified. There were no differences in maternal HLA class II frequencies between the two groups. Also, fetal HLA class I frequencies did not differ between cases and controls. When the analysis was restricted to pairs where anti-paternal anti-HLA specificities were present in maternal serum (41 cases, 38 controls), HLA-DQB1*02 was more frequent in cases (0.27) than in controls (0.13; p=0.0326, chi-square), but the allele frequencies for both groups did not differ significantly from published population data for Germany (DKMS: 0.1959; p>0.05, chi-square).

Conclusion: The causative role of maternal anti-HLA class I antibodies in FNAIT remains controversial. This study was conducted to identify a potential genetic predictor of immunization against HLA class I. However, we were unable to identify a clear association between maternal HLA class II alleles and the propensity for mounting an anti-HLA class I immune response in a cohort of thrombocytopenic newborns.

Disclosure Statements: None

PS-2-17
Successful use of erythropoietin in a case of late anemia following intrauterine transfusions

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Background: Most severe courses of hemolytic disease of the fetus and newborn (HDFN) are caused by antibodies against the RhD antigen. However immunization against other rhesus antigens may also lead to a severe course of HDFN. Intrauterine transfusions (IUT) have significantly improved chances of survival of affected fetuses and perinatal outcome.
A possible effect of IUT is a prolonged anemia of the newborn, sometimes lasting for several months, with a significantly increased need for transfusions.

**Methods:** Clinical course: A 35-year-old female patient with migration background presented to our gynecology outpatient clinic in her 5th pregnancy at 20 weeks of gestation. The patient had a history of 2 normal pregnancies and 2 infants who died from HDFN after birth. During the current pregnancy, two IUT were given. The infant was delivered in 28th week of gestation. Exchange transfusion was not required, but two transfusions within the following 4 weeks. Standard serologic tests for detection and titration of red blood cell antibodies were applied using the gel card technique (antibody screening, indirect antiglobulin test (IAT) and direct antiglobulin test (DAT)).

**Results:** Maternal blood group was typed 0 RhD+, CCee, K-, with Anti-E (256), Anti-c (4) and Anti-K (64). Before receiving IUT, a fetal blood sample was typed 0 RhD+, CCee, K-, DAT was strongly positive (++) and acid elution showed anti-E and anti-c. The newborn’s blood group was 0 RhD+, CCee, K-, corresponding to IUT. DAT was negative, IAT confirmed anti-E, anti-c and anti-K. Autologous red blood cells were not detectable for two months, the reticulocyte counts were low. Treatment with erythropoetin (EPO) was initiated at 4 weeks of postnatal age. Two weeks after EPO initiation, the infant received the last unit of red blood cells, 1 week later the reticulocyte count increased accompanied by a slightly positive DAT indicating successful erythropoiesis.

**Conclusion:** EPO might be an useful option in the treatment of late anemia after IUT in neonates with HDFN and might reduce the need of transfusions.

**Disclosure Statements:** The authors declare no conflict of interest.

**PS-2-18**

**Non-invasive prenatal test for fetal RhD (NIPT-RhD): Interim analysis of an ongoing prospective confirmatory cohort-study**

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**Background:** According to current German maternity guidelines a non-invasive prenatal test for fetal RhD (NIPT-RhD) has to be offered to all RhD-negative pregnant women between week 11+0 and 29+6 of gestation aiming to allow targeted antenatal RhD-prophylaxis in case of a RhD-positive fetus. However, any NIPT-RhD offered requires careful validation. Therefore, we perform an ongoing prospective study in order to determine sensitivity and specificity of our NIPT-RhD setup.

**Methods:** The FetoGnost Kit RhD (Ingenetix, Vienna) has been already validated in the university hospital of Vienna (Legler et al. Arch Gynecol Obstet. 2021;304:1191). We evaluated our deviation from the original protocol: Cell-free DNA was extracted from plasma with the QIA symphony SP instrument (Qiagen, Hilden). Real-time PCR was performed with QuantStudio 5 (Thermo Fisher Scientific, Darmstadt). In the first part of a prospective confirmatory study we offered two NIPT-RhD tests to RhD-negative pregnant women. The second part of this study consists of an ongoing survey, where NIPT-RhD is performed as a clinical service and women are contacted about four weeks after delivery in order to query newborns’ serological RhD-status.

**Results:** In the first part of this study 205 samples were received. Four samples were excluded because the inclusion criteria were not met. In four cases the mother carried an RHD gene and the fetal RhD status could not be determined. The remaining samples (n=197) were tested by two blinded operators with the NIPT-RhD method described above and an in-house reference assay (Müller et al. Transfusion 2008;48:2292). Complete concordance was obtained in 130 RHD-positive and 67 RHD-negative cases. Of the 969 patients tested with the new NIPT-RhD setup until April 2022, 513 women were invited to participate in our survey and 200 responded. NIPT-RhD results were confirmed in 122 RHD-positive and in 78 RhD-negative cases, no discrepancies were observed.

**Conclusion:** According to this interim analysis the combination of the QIA symphony SP instrument with QuantStudio 5 yields reliable NIPT-RhD results when the FetoGnost Kit RhD is used. Because it is not uncommon that an RhD-negative mother carries a RHD gene, more research is required to allow correct determination of the fetal RhD status also in those more difficult cases.

**Disclosure Statements:** TJL has received lecture fees, consultancy fees and travel reimbursements. Ingenetix, the University Medical Center Göttingen and LADR Zentrallabor Dr. Kramer & Kollegen finance the study.

**PS-3**

Hämovigilanz | Immunhämatologie und immunologische Grundlagen II. | Hämostaseologie – Koagulopathien, Plasmaderivate und Hämophilie | Pädiatrische Transfusionsmedizin

**PS-3-1**

**Systematic analysis of adverse transfusion reactions reveals both, transfusion adverse events and handling errors**

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**Background:** Regular surveillance of transfusion-related adverse events is good practice in clinical hemovigilance. Their workup is regulated by the Cross-sectional Hemotherapy Guidelines. Reports are however triggered by personnel and underreporting may occur. We asked whether the evaluation of the incidence, quality and severity of transfusion reactions can also yield information on logistical quality of transfusion practice.

**Methods:** In a center with annual transfusion of around 16.000 red cell concentrates, 5000 platelet concentrates and 5000 units of plasma, we evaluated both, transfusion-associated adverse events and adherence to transfusion practice guidelines. A total of 322 transfusion cases were evaluated through analysis of transfusion medical records over the past 9 years and in addition, when death was associated with transfusion, also digitized sections and court records from 1990 to 2019.

**Results:** Adverse events were reported at an incidence of 6/10.000 transfusions, corresponding to a mean of 35 cases per year (range, 23-42). Allergic reactions were most frequent (55.9%). We show that only 80.1% of physicians complied with the workup rules for transfusion reactions in the national guidelines. 9 % of transfusion reactions were associated with staff errors. Of these, the most common were incorrectly performed or omitted bedside test (3.7%), omitted identity check (1.6%), wrong component issued (1.6%). Transfusions were prescribed without indication at 1.2%. Statistically significant associations were found for allergic reactions and red cell concentrates (< p = .001) and for plasma transfusion and reduced clinical symptoms (p = .002).

**Conclusion:** Our analysis of 322 individual cases showed expectable distribution, quality and incidence of adverse transfusion reactions. In addition,
it revealed an unexpectedly high incidence of staff handling errors. Thus, guideline-conform workup of transfusion reactions can be utilized for further improvement of clinical transfusion quality, to sensitize transfusion staff in the handling of hemotherapeutics, and to enhance patient safety.

Disclosure Statements: No conflicts of interests.

PS-3-2
In vitro data for depletion of murine granulocytes via CAR-NK92 cells

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Methods: To target neutrophil granulocytes, a second-generation CAR with a 4-1BB co-stimulatory domain was designed against Ly6G (lymphocyte antigen 6 complex locus) and expressed on NK92 cells by lentiviral transduction. The scFv is derived from the Gr1 antibody clone, which in addition to Ly6G also binds Ly6C, but with a markedly lower affinity. The naturally inherently Ly6G/C-negative human cell line MDA-MB468 was modified to express either Ly6G or Ly6C at various expression levels. Functionality of the CAR constructs was investigated by Europium–release (EU) assays and live-cell imaging. In addition to the Ly6G/C positive cells, primary granulocytes and monocytes from murine bone marrow were used as targets; appropriate controls were included.

Results: CAR-NK92 cells showed specific killing of Ly6G expressing target cells independent of antigen dose, implying high affinity of the scFv. By contrast, Gr1-CAR-NK92 cell do not kill Ly6C expressing target cells. For primary murine granulocytes, time-sensitive specific killing was shown, whereas no killing was shown for murine monocytes.

Conclusion: We have generated a highly functional CAR-construct that specifically kills Ly6G expressing cells. Gr1 dependent cross-reactivity with Ly6C was excluded. Using anti-Ly6G targeted CAR-effector cells we can now establish a model of long-term immune neutropenia. Besides providing answers to the role of neutrophils for immature hematopoiesis, the model should investigate CAR-T-longevity, anergy and exhaustion, as well as investigating drugs in neutropenic infection models.

Disclosure Statements: The authors declare no conflict of interest.

PS-3-3
Case report of a patient with a newly detected Bombay phenotype

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Background: The so called Bombay blood group is a rare phenotype characterized by missing H antigen on red cells. This is typically caused by null alleles of FUT1 and FUT2, two genes encoding for fucosyltransferases. Whereas inactivating variants in the latter are quite common, they are very rare for FUT1. Generally, a potent anti-H can be found in the individual’s plasma. For patients with rare blood groups or with antibodies against high frequency antigens, supply of blood products is often challenging.

Methods: Here, we present a case of a 41 year old female patient admitted due to an elective uterine fibroid surgery. Due to a possible need of packed red blood cells (pRBC), blood group typing and antibody screening was initiated. Standard serological testing was performed by haemagglutination with a column technique (Ortho Biovue). In addition to standard antibody screening, several in-house assays and rare red blood cells were used. Serological typing of H antigen was performed with commercial lectins (Optima; CE Immunodiagnostika) and additionally, with an anti-H containing plasma of a likewise “Bombay” typed patient. Sequencing of FUT1 and FUT2 was performed using a direct taq-cycle method.

Results: In IAT, a panagglutinating antibody was detected (titre: 4). Reaction strength was not decreased by ficin or DTT treated cells. In tube technique, the antibody was active in saline at 4° C (titre: 8) and at room temperature. At 37° C, only weak agglutinations were observed. In IAT, patient’s plasma did not react with two “Bombay” cells. H-deficiency of the patient’s red cells was confirmed by lectins and a human derived anti-H. Sequencing revealed null alleles for both corresponding genes: FUT1*0IN.12 and FUT2*0IN.02, respectively. However, additional allo-antibodies could not be completely excluded and no compatible units of pRBC were available. Thus, the patient was discharged and encouraged to undergo an autologous transfusion programme.

Conclusion: Our case shows that anti-H in patients with Bombay phenotype can readily be misinterpreted as an additional non relevant cold reacting antibody. Though lectins are available for detection of H antigen, it is recommended to confirm an H-deficiency in these cases by a human anti-H. Indications for preoperative autologous blood donation are warranted due to improved blood safety. However, this form of blood supply still has a high value in cases with relevant antibodies against high frequency antigens.

Disclosure Statements: No conflict of interest.

PS-3-4
An unusual case of diclofenac-induced immunohemolytic anemia: importance of immunohematologic studies in confirming the diagnosis

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Background: Diclofenac-induced immune hemolytic anemia (DIIHA) is caused by drug-dependent antibodies (ddab) and is often accompanied by drug-induced autoantibodies (aab). Ddab usually disappear immediately when the drug is discontinued, while aab usually take weeks or months to resolve. We describe for the first time that diclofenac-induced aab disappeared rapidly after discontinuation of diclofenac, which ultimately led to confirmation of the diagnosis.

Methods: Serological studies were performed with standard techniques using gel cards and tube testing. Detection of drug-dependent antibodies were performed in the presence and absence of diclofenac and its ex vivo antigens (urine of patients taking diclofenac).

Case Report: A 42 year old male with no significant previous medical history presented with acute icterus (bilirubin 27 mg/dl), anemia (Hb 8,7 g/dl), thrombocytopenia (PLT 10/ul) and anuria for 24 h. Further laboratory investigation showed LDH of 771 U/l, creatinine of 3,8 mg/dl and moderate proteinuria. Extensive diagnostic workup was initiated to clarify bicitopenia and AFE.

Results: Immunohematologic investigation revealed the classic serologic picture of warm-reactive autoantibodies (DAT IgG4+, panagglutinating antibodies in eluate and plasma). AIHA was diagnosed and therapy with high-dose steroids was initiated. However, a few days later, a marked change in the serological picture was noted: DAT IgG +/−, negative eluate and antibody screening in IAT. After sample mix-up was ruled out, drug-induced hemolytic anemia was suspected. Re-questioning of the patient revealed that he had taken diclofenac frequently in the weeks prior to hospitalization. Drug testing confirmed antibodies to RBCs reacting
only in the presence of different diclofenac ex vivo antigens. Four months after discharge, the patient was fully recovered.

**Conclusion:** A rapidly changing serological picture, especially when auto-antibodies to RBCs are detected, should always suggest a drug etiology. Our case highlights the importance of a thorough history and, in particular, the role of immunohematologic laboratory testing in confirming the diagnosis of DIIHA.

**Disclosure Statements:** The authors declare no conflict of interest

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**PS-3-5**

**Antibody-induced procoagulant platelets cause increased thrombus formation in heparin-induced thrombocytopenia**

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**Background:** Thromboembolic events are life-threatening complications frequently observed in patients with heparin-induced thrombocytopenia (HIT). The interactions of platelets (PLTs) with different actors of the adaptive and innate immune system are increasingly identified to cause prothrombotic conditions in HIT. However, the impact of antibody (Ab)-induced procoagulant PLTs and their potential regarding thrombus formation remain elusive.

**Methods:** PLTs from healthy individuals were incubated with IgG fractions from HIT patients or healthy controls (HCs) in the presence of buffer, low- (0.2 IU/s) or high (100 IU/s) dose heparin. For the detection of changes in the expression level of PLT CD62p (P-selectin) and procoagulant phosphatidylserine (PS), a flow-cytometric procoagulant PLT-assay was utilized. To investigate whether Ab-induced procoagulant PLTs might promote prothrombotic conditions and increased clot formation, calibrated automated thrombogram (CAT) analysis and an ex vivo model of thrombosis were used, respectively.

**Results:** HIT IgGs induced significant increase in procoagulant PLT formation whereas these changes were not detected in buffer as well as in the presence of high dose heparin (mean ±SEM: 33.71±3.88 vs. 4.30±1.13, p value 0.0039; and vs. 4.92±1.99, p value 0.0039, respectively). Remarkably, CAT analysis revealed that procoagulant PLTs changes harbored an increased prothrombotic potential as significant higher thrombin was found in the presence of low dose heparin but not in buffer (thrombin (nM)±SEM: 452.70±15.67 vs. 312.00±10.76, p value 0.0001). Importantly, Ab-induced procoagulant PLTs that were perfused through collagen-coated microfluidic channels induced dramatic increases in thrombus formation.

**Conclusion:** Findings of our study indicate that HIT Abs harbour the ability to induce a procoagulant PLT phenotype in a heparin-dependent pathway. The observation that Ab-induced procoagulant PLTs were associated with increased thrombin and thrombus formation directly towards a potential relevance of Ab-induced procoagulant PLTs in this complex coagulation disease.

**Disclosure Statements:** There are no potential conflicts of interest

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**PS-3-6**

**The challenge of finding compatible blood units for a sickle cell disease patient with multiple alloantibodies**

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**Background:** A 53-year-old patient was admitted due to a sickle cell crisis. The hemolytic markers were significantly elevated and hematuria was present. Her general practitioner had transfused her with two blood units 11 days prior. She was transferred to the ICU for further treatment. On day two, the antibody screening was positive, DAT was negative and cross matching was negative, thus she was able to receive two blood units. However, there was no hemoglobin increase and the hemolytic markers increased.

**Methods:** Automated blood group-testing was performed with NEO Iris®, Immucor and HI-1000-ID-Cards® System, BioRad. Detection and differentiation of red blood cell antibodies was done with Capture-R Ready-Screen®, Capture-R-Ready-ID Extend I, Immucor, with LISS/Coombs ID-Cards, IF-DiaPanel, BioRad and Data-Cyte® Plus 0.8% Reagent Red Blood Cells, Grifols. DAT was performed using Immucor-NEO®, BioRad HI-1000®, DC Screening I, DAT IgG Dilution and IgG1/G3 BioRad cards. The genotyping was performed using SSP Inno-Train® kits (KKD SKP0121, MNS SNM1120, Rare ID SSP0620).

**Results:** The cross matching for the following blood units along with DAT and antibody screening was positive. There was a strong coating of the red blood cells with IgG, C3d, IgG1 and IgG3. The antibody panels were positive, however a specific antibody could not be identified. Anti-Jk(b) and Anti-M were suspected. The genotyping results from the following blood groups were Kidd: Jk*(a+)1, Jk*(a+), Duffy: FY*(a+), FY*(a+), Fy (Fya-b+), MNSs: GYPA*02, (N+), GYPB*03, 04 (S+ s+), and Lutheran: Lu*02, Lu(b+). In cooperation with the BRK Immunohematology Laboratory Munich, anti-Jk(b) along with anti-M was confirmed. Anti-Fy3 was also suspected.

**Conclusion:** This case emphasizes that prophylactic antigen matching of RBCs could decrease the risk of immunization in sickle cell disease patients. With the help of genotyping, alloantibodies from other blood groups could be excluded and anti-Jk(b), anti-M could be confirmed. The transfusion of one antigen matched blood unit improved the hemoglobin level and decreased the hemolytic markers. Her condition stabilized and she was discharged.

**Disclosure Statements:** None

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**PS-3-7**

**Resolving sanger sequencing ambiguities with nanopore technology**

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**Background:** Nanopore sequencing measures the change of an ionic current while molecules pass the nanopenes. The change identifies the passing nucleotides from DNA or RNA molecules. We tested the feasibility of this technology for examination of HNA1 and HNA2 in samples with sanger sequencing ambiguities using the benefit of the nanopore method to generate long reads and to avoid the need to perform individual haplotype-specific sanger sequencing.

**Methods:** Commercial kits from Oxford Technologies for a) rapid 1D sequencing and b) 1D amplicon sequencing with and without barcoding were used. Long-range PCR generated amplicons from 5” to 3” end of the HNA1 and HNA2 gene, respectively. Identification of sequences was done with Geneious (Biomatters Ltd) and MacVector (MacVector Inc.)
Results: Both sequencing kits (rapid 1D, 1D amplicon) were suitable to examine samples with ambiguities. With real-time base-calling and internal quality control, the rapid 1D sequencing kit generated > 100 000 reads in a run-time of 6h and > 200 000 reads in a run-time of 18h runs. The resulting coverage for the gene of interest was > 20 000x and > 50 000x, respectively. By using the 1D amplicon kit and introducing barcodes for the examination of six samples in parallel the mean number of usable reads were 4500 for each sample and the resulting coverage about 2500x. More than 50% of reads generated by both sequencing kits showed a read length > 7100 basespairs and thus gave information about the combination of polymorphisms for each haplotype. 

Conclusion: Nanopore sequencing introduces an attractive alternative to resolve sequencing ambiguities detected by the sanger method. While the library preparation for rapid 1D sequencing uses only 10 minutes hands-on-time, the 1D amplicon kit requires two hours. The opportunity for bar-coding of 2 - 96 samples makes the 1-D amplicon sequencing kit more cost-effective for analyzing haplotype-specific polymorphisms. 

Disclosure Statements: No conflicts of interests.

PS-3-8
Alloimmunisation in patients treated with anti-CD38 (Daratumumab)
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Background: It is well known that plasma from patients treated with human anti-CD38 monoclonal antibodies shows a panreactivity in the indirect antiglobulin test that may mask the detection of allo-antibodies. To overcome this interference, strategies such as treatment of the test cells with dithiothreitol (DTT) and trypsin have been developed. To reduce the allo-immunisation risk, it is recommended to transfuse Rh, K and Kp(a) compatible blood units to patients undergoing a treatment with anti-CD38.

Methods: To assess the red cell alloimmunisation in patients treated with anti-CD38 monoclonal antibodies shows a panreactivity in the indirect antiglobulin test that may mask the detection of allo-antibodies. To overcome this interference, strategies such as treatment of the test cells with dithiothreitol (DTT) and trypsin have been developed. To reduce the allo-immunisation risk, it is recommended to transfuse Rh, K and Kp(a) compatible blood units to patients undergoing a treatment with anti-CD38.

Results: Over a period of more than 5 years, 732 pre-transfusion work-ups were done for 128 patients treated with anti-CD38. A large number of these patients received blood products prepared by the hospital in charge of the patient. However, in 165 transfusion episodes, 284 blood units were prepared for administration in our laboratory. In seven patients, alloantibodies were identified of which only one, an anti-E reacting in papain, was not detected before the first administration of anti-CD38. 

Conclusion: The alloimmunisation rate in these patients is low, even lower compared with other transfused patients (2-5%) (Tormey et al., 2015). As negative controls, a random AB serum instead of the patient's serum and saline instead of the drug were used. Tests were repeated 16 and 39 days after discontinuation of the drug. Hb and hemolytic laboratory markers (e.g. bilirubin, LDH and haptoglobin) were monitored.

Results: Hb dropped from 82 to 27 (d23) and from 96 to 39 g/l (d24) following IV Ceftriaxone. Three packed RBCs were administered on d23/d24. LDH and bilirubin were slightly elevated; contrary to expectations, the results of haptoglobin and free hemoglobin were normal. Serological investigations revealed a strong C3d-positive DAT. The serum ab screening test was negative in IAT and the eluate was non-reactive. Serum drawn at the time of the hemolytic reaction was strongly reactive when incubating with RBCs in the presence of the drug (3+) and its metabolite (2+), while controls remained negative, clearly demonstrating presence of Ceftriaxone ab. Repeated testing d16 after drug cessation showed a similar result. On d39 no Ceftriaxone ab were detected.

Conclusion: Here we present a pediatric case with acute, life-threatening Ceftriaxone-dependent hemolysis and hemodynamic instability. After discontinuation of Ceftriaxone and administration of Methylprednisolone, Clemastine plus transfusion, hemolitic remission was rapid. Notably, hemolytic markers remained proportionally unaffected. DIHA needs to be considered in individuals with acute, C3d-positive hemolysis coinciding with new drug treatment and the relevant medication has to be stopped at once.

Disclosure Statements: None

PS-3-10
Genotyping assistance in blood typing and in antibody screening in a patient with steroid refractory autoimmune hemolytic anemia and B-cell lymphoma
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Background: The patient was admitted in the LMU hospital due to his deteriorating condition caused by a B-cell lymphoma. Due to the patient's anemia, blood transfusions were required daily. Blood typing was performed and the isosagguitin 0 was positive, making blood typing invalid. Furthermore, DAT was positive with strong IgG and C3d coating. Warm and cold autoantibodies were detected. Antibody screening along with the antibody differentiation panels and cross matching were positive.

Methods: Blood typing was performed using NEO Iris®, Galileo®, Immucor, and IH-1000-ID-Cards” System, BioRad. Detection and software. Additional tools for interpreting the nanopore sequencing data were the Galaxy nanopore workflow (usegalaxy.org; v22.01.1) and the EPZ2ME-labs-launcher (ONT).
antibody differentiation was done with Capture-R Ready-Screen®, Capture-R-Ready-ID EXT I, Immucon, with LISS/Coombs ID-Cards, ID-DiaCell I-III, EXT ID-DiaPanel, BioRad and Data-Cyte® Plus 0.8% Reagent Red Blood Cells, Grifols. DAT was performed using Immucor-NEO®, BioRad IH-1000®, DC Screening I, DAT IgG Dilution and IgG1/ G3 BioRad cards. The genotyping was performed using BAG: AB0-TYPE variant 113AV1, Inno-Train® kits (AB0-Subtype SST0421, KKD SKP0121, MNS SMN1120, Rare ID SSP0620).

**Results:** Despite daily transfusions, the hemoglobin levels did not increase and there was an increase in the hemolytic markers. Cyclophosphamide and dexamethasone were given. In the following days, a plasmapheresis was performed, after which hemolytic parameters decreased and the patient's condition improved temporarily. However, the patient's condition deteriorated again and a splenectomy was performed. From a transfusion medicine perspective, underlying alloantibodies had to be excluded. The patient was AB0*O.01.02, A1.01 corresponding to the phenotype A1. The results in following blood groups were Kidd: Jk*01, Jk(a+), Duffy: FY*01,02, Fy(a+) (b+), MNs: GYP*A*01, (M+), GYPB*03, 04 (S+ s+), and Lutheran: LU*02, b+. (b+).

**Conclusion:** After splenectomy, the patient developed sepsis and was treated with meropecten. Fortunately, the patient's condition improved to the point where blood transfusion was no longer frequently required. Using genotyping of the patient, we were able to safely transfuse the patient with A RBCS and exclude alloantibodies in the Kidd, Duffy and MNs System. For invalid serological blood typing, genotyping can be used for the selection of antigen matched blood units.

**Disclosure Statements:** None.

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**PS-3-11**

**Rivaroxaban levels in plasma show greater inter-individual variation than apixaban levels in treatment of venous thromboembolism**

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**Background:** Direct oral anticoagulants (DOACs) have become the standard of care in the treatment of venous thromboembolism (VTE). While routine monitoring is not required, measurement of DOAC levels in plasma is advised in a growing number of special situations. In default of therapeutic ranges, guidelines recommend reporting DOAC levels together with “expected” ranges from the literature. In this study we assessed DOAC level variation and agreement with expected levels in a large VTE patient population.

**Methods:** Citrated plasma samples from patients receiving DOACs for treatment of VTE were collected (rivaroxaban, n=1471; apixaban, n=725). Drug concentrations were determined by measuring the anti-Xa activity using drug-specific calibrators. Expected (5th-95th percentile) peak/trough level ranges were 189-419/6-87 ng/mL for rivaroxaban 20 mg OD (Mueck et al. 2011), 91-196/1-38 ng/mL for rivaroxaban 10 mg OD (Mueck et al. 2008), 59-302/22-177 for apixaban 5 mg BD, and 30-153/1-90 for apixaban 2.5 mg BD (Summary of Product Characteristics). Correlations of drug levels with the patients’ sex, age, body weight, body mass index and d-dimer levels were analyzed.

**Results:** In patients on rivaroxaban, observed peak level ranges were lower than expected, with peak/trough levels of 98-367/8-55 ng/mL for 20 mg OD, and 51-211/5-27 ng/mL for 10 mg OD. At peak, 43.5% (rivaroxaban 20 mg OD) and 32.5% (rivaroxaban 10 mg OD) of samples were not in the expected range, at trough 7.7%. Under apixaban, agreement of observed and expected peak/trough levels was better, with 63-299/13-114 ng/mL for 5 mg BD and 37-161/7-68 ng/mL for 2.5 mg BD, 7.5-13.0% of peak samples and 6.9% of trough samples were not in the expected range. Repeatedly measured drug levels in subgroups of patients showed a strong correlation (r≥0.77). Age and drug levels correlated weakly (r≤0.33).

**Conclusion:** The observed real-world variation of DOAC levels showed good agreement with reference data only at trough post-dose intervals. The obtained data strongly suggest that DOAC levels, especially in patients on rivaroxaban, should be measured at trough and not at peak post-dose intervals. Further studies are warranted to determine clinically relevant thresholds of DOAC levels.

**Disclosure Statements:** None to declare.

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**PS-3-12**

**Procoagulant platelet activity in VITT induced increased thrombus formation**

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**Background:** Vaccines against SARS-CoV-2 virus significantly reduce morbidity and mortality of the pandemic. But with millions of people vaccinated in a short period of time, even very rare side effects like the clotting disorder vaccine-induced thrombotic thrombocytopenia (VITT) became apparent. We recently identified an increase in procoagulant platelets in these patients, which is even higher than in a previously reported cohort of COVID-19 patients.

**Methods:** 8 patients (4 female and 4 male) who were hospitalized with suspected thrombotic complications 5 to 16 days after ChAdOxi nCoV-19 vaccination were included in this study. The median age was 38 years. All patients had thrombocytopenia at admission. Three had a fatal outcome and five were successfully treated. The blood samples were analyzed by using enzyme immune assays, flow cytometry, ex vivo thrombus formation assay and heparin-induced platelet aggregation assay.

**Results:** All sera from VITT patients contained antibodies against PF4 [OD 3.0±0.68] with the ability to activate platelets (8/8). Sera induced significant increase in procoagulant markers (CD62P and phosphatidylserine externalization) [CD62P/PS positive PLTs: 40.8±27.0%] compared to COVID-19 patients [FI CD62P/PS positive PLTs:71±17.7%]; p=0.8977. The formation of procoagulant platelets could be significantly reduced by use of the monoclonal IV3 antibody as well as IVIG [FI CD62P/PS positive PLTs:1.01±0.36]; p=0.0001. In thrombus formation model, IgGs from VITT patients induced increased platelet surface area (8.64±0.53, SAC±SEM) compared to control (0.72±0.00, SAC±SEM); p=0.001, which was inhibited by IVIG (4.07±0.51, p=0.001).

**Conclusion:** Our ex vivo microfluidic thrombus formation model supports the significance of procoagulant platelet in the pathogenesis of VITT. It may offer significant clinical implications and therapeutic options like evaluation of IVIG as a recommended therapy or other drugs for treatment of clinical picture of VITT.

**Disclosure Statements:** no disclosures.
IL-6 as a Biomarker for Bleeding Events in Hemophilia

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Background: Monocytes of hemophilia patients reside in a state of heightened activation. To identify recurrent inflammation in hemophilia, we assessed the acute-phase response in the blood of patients with hemophilia A and B.

Methods: To assess the acute-phase response in hemophilia patients, we collected blood from 59 adult patients with mostly severe hemophilia A and B compared to 54 healthy controls. To analyze inflammation in the context of bleeding, we collected blood from hemophilia patients with acute bleeding events and induced a needle puncture injury in the knee joint of transgenic hemophilia mice. Results: Compared to healthy controls, blood levels of IL-6, CRP and LBP were significantly elevated in the entire cohort of hemophilia patients but exhibited a particularly pronounced increase in obese hemophilia patients (BMI ≥ 30). Subgroup analysis of the non-obese hemophilia patients (BMI 18-29.9) revealed a significant spike of IL-6, CRP and LBP in connection with an increase of sIL-6Ra in patients with bleeding events within the last month. Non-obese hemophilia patients without recent bleeding had IL-6, CRP and sIL-6Ra blood levels similar to healthy controls. The role of IL-6 as a marker of bleeding in hemophilia was confirmed in hemophilia A and B.

Conclusion: These findings indicate that acute-phase reactants in combination with sIL-6Ra could be sensitive biomarkers for the detection of acute and recent bleeding events in hemophilia.

Disclosure Statements: None.

Visualization of Gliomas with Clot-binding Peptides

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Background: Glioblastoma (GBM) is a highly aggressive brain tumor characterized by necrosis, hemorrhage and thrombosis. The outcome of GBM correlates with the extent of surgical tumor removal. We wanted to test if the formation of a fibrin-rich extracellular matrix provides binding sites for clot-binding peptides that support fluorescent-guided recognition and surgical removal of astrocytomas.

Methods: We analyzed fibrin formation in astrocytoma samples from patients using immunohistochemistry. To assess the uptake of clot-binding peptides into brain tumors, we injected fluorescein-labeled peptides intravenously in GBM-bearing mice. Two-four hours later the brain and control organs were isolated and tissue sections were evaluated by fluorescence microscopy. In addition, we assessed peptide uptake into intracranial brain tumors in intact mice using a fluorescence endoscope in situ. The role of clotting was tested in GL-261 glioblastomas grown in transgenic hemophilia A mice.

Results: We demonstrated a marked upregulation of clot formation in the interstitial spaces of astrocytoma patients while healthy brain is fibrin-free. Five peptides with affinity for clot or brain injury (CLT1, CLT1-IK, CLT2, CREKA, CAQK) were injected into GBM-bearing mice to visualize fibrin accumulation. Subsequent analysis showed the strongest fluorescence after injection of CLT1 while unspecific control peptides did not generate any fluorescence. The fluorescent label of CLT1 was specific for clot in brain tumor tissue whereas no fluorescence was detectable in tumor tissue in absence of clotting activity or in normal brain. Fluorescence endoscopy showed specific fluorescence by the clot-binding peptide in brain tumors of intact mice in situ.

Conclusion: Our data demonstrate stage-dependent fibrin deposition in astrocytomas that can be visualized with the clot-binding peptide CLT1 in vivo. Moreover, our data suggest that CLT1 can improve fluorescent-guided detection and removal of astrocytoma in situ.

Disclosure Statements: None
Whole genome expression analysis resolves the correlation between genetic knockout of single genes and the obtained phenotype in relation to FVIII secretion

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Background: We have shown that GABARAPs proteins modulate FVIII secretion; however, GABARAP gene KO decreased FVIII secretion by 40%, whereas GABARAP1L and GABARAP2L gene KOs increased FVIII secretion by 70 and 100%, respectively. To identify the underlying reason, we performed gene rescue experiments and 3’RNAseq to identify DEGs. Our overall aim is to identify the link between the individual gene knockouts and FVIII secretion.

Methods: We rescued each KO by transient transfection of a cDNA vector expressing the corresponding KO gene; the highly expressed genes (CANX, CALR, LMAN1, and MCFD2) were under CMV promoter while the relatively lower expressed genes (all GABARAPs) were under SV40 promoter. The FVIII activities in the medium were measured using chromogenic assay. To further study the intracellular pathways underlying the change in FVIII secretion, we performed 3’RNA sequencing of wild type HEK293 cells and the eight single knockouts. We used bioinformatics tools to determine a list of differentially expressed genes, the affected pathways (if any) and its activation scores.

Results: The reintroduction of proteins of the conventional secretion pathway (CANX, CALR, LMAN1 and MCFD2) returned the FVIII activity in the medium to the direction of wild type values; in contrast, GABARAP1 and GABARAP2 did not show a significant rescue. Pairwise comparison between knockouts and HEK293 at 5% FDR showed 68,1087 and 199 differentially expressed genes in CALR, GABARAP1 and GABARAP2 knockout respectively; all other KOs showed less than 15 genes to be differentially expressed at 5% of FDR. IPA analysis of the DEG showed significant enrichment for pathways related to energy metabolism, among which ATP production was predicted high and low for increased and decreased FVIII secretion knockout respectively.

Conclusion: The rescue process was successful for transporter molecules returning the FVIII secretion values to normal, while the others did not reach normal values. The RNA-seq analyses showed that the latter are involved in the metabolism of the cells requiring more suitable conditions for rescue. 3’RNA sequencing results underscore the need for high ATP for the release of FVIII from the ER, these knockouts are considerably showing the change in ATP production correlated with change in FVIII secretion.

Disclosure Statements: no conflict of interest.

Diagnostics and blood management of an infant with McLeod contiguous gene deletion syndrome

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Background: X-linked chronic granulomatous disease (XL-CGD) is caused by single mutations or larger deletions of CYBB. Deletion involving XK is associated with reduced Kell blood group antigens expression on RBCs and absence/truncation of XK protein with absence of Kx antigen (McLeod phenotype). RBCs of healthy donors carrying XK protein and Kx antigen can induce anti-Kx and anti-Km in patients with McLeod phenotype. Thus, blood management of Kx- patients is a challenge, particularly in the context of HSCT.

Methods: A four-month-old infant with XL-CGD and Duchenne muscle dystrophy was referred for HSCT evaluation. An additional McLeod phenotype was identified. In an interdisciplinary multi-center effort the blood management including procurement of Kx- packed (p) RBCs was...
orchestrated, and a sophisticated schedule balancing donations, storage times, separation of satellite pRBC bags and irradiation was developed. **Results:** McLeod phenotype was confirmed by serology (K-kw,Kp(a–b–), Jk(b–),Kx–), flow cytometry (absence of Kell antigens) and molecular blood group typing (K–1.1–2.3, –4.6–7). Further molecular genetics revealed a large (>5.8 Mb), previously unknown, deletion involving DMD, RPGR, LANCL3, XK, CYBB and DYNLT3. No Kx- and RhD compatible donors were identified by International Rare Donor Programs, but a compatible donor was known to the Swiss Red Cross Zurich, where a first blood collection was performed, followed by a second donation in Freiburg. Our concept enabled blood supply over the entire peritransplant phase. The patient was discharged with complete chimerism at d68, and is well and independent of transfusions at d150 without signs of GVHD. **Conclusion:** McLeod phenotype poses a substantial challenge for blood management, especially in the context of HSCT, where timing of complex procedures, availability of compatible stem cell and blood donors, as well as logistics and the storage life of irradiated pRBCs must be considered.

Close international collaborations with three transfusion medicine institutions and the clinic ensured the successful HSCT treatment of the young patient.

**Disclosure Statements:** n.a.

**PS-4**

Hämatoptetische Stammzellen – Grundlagenforschung und Transplantation | Zelltherapie und Gentherapie | Neue Blutprodukte – Neue Anwendungen | Rechtliche Grundlagen, Qualitätssicherung und regulatorische Aspekte | Zukunftsgebiete der Transfusionsmedizin

**PS-4-1**

Quality and shelf-life of cryopreserved allogenic stem cell transplants processed under GMP-conditions

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**Background:** Providing a stem cell product for a patient is a complex and time-consuming process with numerous challenges like donor non-availability or pandemic-related travel restrictions. The establishment of a stem cell bank for cryopreserved peripheral blood stem cells aims to accelerate the overall process and make ready-to-use products quickly available to patients. We use a routine process under GMP-conditions to provide durable products with high quality and low cell loss after freeze-thaw cycle.

**Methods:** Allogenic stem cell apheresis products were processed at our GMP-clean room facility. Each product was split in dedicated cryobags with a final volume of 2 × 100 ml and cryopreserved using 5% DMSO and a controlled rate freezer. Products were stored in the vapour phase of liquid nitrogen. For quality and shelf-life investigations, bags and/or retain samples were thawed for analysis. Assays for cell counts (CD34+, CD45+), and viability (assessed by 7AAD) were performed using standard flow cytometry methods (BD FACSCyto flow cytometer, antibodies from BD; ISHAGE protocol). Colony forming unit assays were performed to assess potency of stem cells. Recovery data refer to pre cryopreservation results. All results were expressed as median.

**Results:** Quality investigations on thawed products were performed to verify the influence of cryopreservation. Post-thaw viability of CD34+ and CD45+ cells was >80% and >60%, respectively. A moderate cell loss was seen compared to viability before cryopreservation (>95%/>90%). Recovery of viable CD34+ cells after thawing was >70%. To study the influence of long-term storage, shelf-life investigations on thawed products were done after 3 and 6 months. Shelf-life investigations showed similar results on post-thaw viability of CD34+ and CD45+ cells and on recovery of viable CD34+ cells compared to quality investigations shortly after cryopreservation. Proliferation was detected at any time. Thus, there are no negative trends caused by long-term storage.

**Conclusion:** Our process of cryopreservation results in stem cell transplants with high quality and moderate cell loss after freeze-thaw cycle. Data on loss of quality or cell numbers are comparable to published results of cryopreserved products. Moreover, no negative trends on recovery of leucocytes and viable CD34+ cells were caused by long-term storage. Until now, shelf-life of our cryopreserved peripheral blood stem cell products was proved for 6 months. Shelf-life is constantly tested for extension.

**Disclosure Statements:** No conflicts of interests.

**PS-4-2**

Transcriptional regulation of the ABO blood group antigen expression and its impact on erythropoiesis

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**Background:** The hematopoietic development is orchestrated by gene regulatory networks that progressively induce lineage-specific transcriptional programs. To guarantee the appropriate level of complexity, flexibility, and robustness, these networks rely on transcriptional and post-transcriptional circuits involving both transcription factors (TFs) and microRNAs (miRNAs). Previously, we found that miRNAs play a critical role in the regulation of the ABO blood group (BG) system by simultaneous targeting TFs and the glycosyltransferase. In addition, numerous miRNAs present in red blood cells (RBCs) show a partially differential expression pattern depending on the BG genotype and comprise potential binding sites in the 3’UTR of the mRNA of TFs involved in the regulation of erythropoiesis. Therefore, we aimed to investigate if the ABO BG type has an impact on the erythropoiesis.

**Methods:** Peripheral hematopoietic stem cells (HSCs) of individuals with different ABO BG were subjected to in vitro differentiation in a colony-forming unit assay and analyzed with regard to the capacity to form burst forming unit erythrocytes. In addition, the expression of differentiation markers, miRNAs, and different TFs were analyzed at different time points.

**Result:** HSCs from BG B individuals (N = 14) showed a significant accelerated erythropoiesis compared to HSCs from donors of BG A (A: 22.5±2.0%, B: 31.3±2.2% proerythroblast at day 5 of differentiation) as differentiation markers, such as CD71, CD36 and CD235a in average appeared 2 days earlier in carriers of BG B, as compared to BG A. In addition, HSC from BG B (53.7±2.5) yielded more and larger BFU-E per CD34 cell, compared to HSCs from BG O and A, respectively (O: 41.9±4.0, A: 43.1±3.1). Further, we found decreased mRNA and protein expression levels of TFs (RUNX1, HES-1 and SP1) and corresponding miRNAs (miR-215-5p, -182-5p, -331-3p) in erythroid precursor cells of BG B donors compared to BG A.

**Conclusion:** Our study for the first time reveal a correlation between ABO BG and the velocity and capacity of RBC differentiation in vitro. We hypothesize that miRNAs differentially present in HSCs and RBCs depending on ABO BGs interact with erythroid lineage specific TFs and thus account for differences in hematopoietic differentiation. A deeper understanding of the gene regulatory networks of erythropoiesis could therefore open the door for therapeutic interventions in diseases where BG receptors promote disease pathology.

**Disclosure Statements:** We have no conflict of interest.
**Abstracts**

**Disclosure Statements:** TT and WSW are named as inventors on patents in the field of cancer immunotherapy owned by their respective institutions. Other authors declare that they have no competing interests.

**Background:** NK cells represent attractive effectors for cancer immunotherapy due to their ability to rapidly eliminate cancer cells. However, many ovarian and breast carcinomas are resistant to NK cell killing, resulting in poor outcome of such therapies. PARP inhibitors (PARPi) were recently shown to render leukemic stem cells sensitive towards NK cell killing by upregulation of NK cell activating ligands. Whether this strategy can also be applied for ovarian or breast carcinoma cells is not clear.

**Methods:** Here, we used a high throughput live-cell imaging system to investigate the impact of the PARPi Olaparib, AG-14361 or Talazoparib on PARP-mediated cytotoxicity of NK cells. The HER2 (ErbB2)-positive ovarian and breast cancer carcinoma cell lines SKOV-3 and MDA-MB-453, which are both resistant to clinically applicable NK-92 cells, were exposed to PARPi, and their effect on specific lysis by parental NK-92 cells or the HER2-CAR engineered derivative NK-92/5.28.z was examined. CAR retargeting on NK cells may offer an attractive approach to better harness a patient’s endogenous NK-cell potency towards otherwise NK-resistant ovarian and breast carcinoma cells. Whether this strategy can also be applied for ovarian or breast carcinoma cells is not clear.

**Results:** We found that co-incubation with PARPi improved natural cytotoxicity of NK-92 towards SKOV-3 and MDA-MB-453 cancer cells. This was also confirmed in 3D spheroid culture systems. Interestingly, both cell lines are highly positive for NKG2D and DNAM-1 ligands, and incubation with PARPi resulted only in limited further upregulation of ligand expression. In agreement with this observation, blocking experiments showed that neither NKG2D ligand nor DNAM-1 ligand upregulation were responsible for sensitization towards NK-92. CAR retargeting on its own already markedly improved killing of HER2-positive cancer cells, with combination with PARPi only leading to a modest further increase in NK cell cytotoxicity.

**Conclusion:** Taken together, our data demonstrate that PARPi can directly improve NK-cell potency towards otherwise NK-resistant ovarian and breast carcinoma cells which is independent from NKG2D ligands. This may offer an attractive approach to better harness a patient’s endogenous NK cell response and/or enhance adoptive NK cell therapies.

**Antiviral T-cell frequencies in a healthy population:** Reference values for evaluating antiviral immune cell profiles in immunocompromised patients and their application during the SARS-CoV-2 pandemic

**Disclosure Statements:** The Authors declare no Conflict of Interest.
Background: The interplay between renal proximal tubular epithelial cells (PTEC) and macrophages plays an important role in the progression of acute kidney injury (AKI), caused by the chemotherapeutic agent cis-platin. Because of its high proregenerative capacity, MSC secretome can rescue damaged kidney directly by acting on PTEC and indirectly by modulating macrophage responses. We hypothesize that the MSC secretome is able to modulate the crosstalk between PTEC and macrophages in cisplatin injury setting.

Methods: To test this hypothesis, we first investigated the effect of MSC-derived conditioned medium on ciPTEC and macrophages upon cisplatin injury. We assessed ciPTEC metabolic activity, migration and apoptosis in control and cisplatin-treatment, with and without MSC-CM addition. In a similar experimental setup, we analyzed macrophage polarization based on phagocytosis and marker expression.

Results: Our preliminary study shows that cisplatin treatment on ciPTECs leads to reduced metabolic activity, compromised migratory capacity and increased apoptosis, which are attenuated by MSC-CM. MSC-CM also ameliorates cisplatin effect on ciPTEC gene expression related to DNA damage, apoptosis, oxidative stress and inflammation. MSC-CM promotes macrophages phagocytosis and the expression of M2-associated surface markers, these effects are slightly compromised in cisplatin-treated macrophages.

Conclusion: Our data clearly indicate that MSC-CM ameliorates the injury effects of cisplatin on ciPTECs. Furthermore, it promotes macrophage polarization towards a rather proregenerative function. Whether MSC CM acts synergistically by promoting proregenerative ciPTEC-macrophage interaction will be assessed by measuring the chemokines and cytokines in co-culture system.

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Evaluation of a predictive algorithm for collection of starting material for CAR T cell production

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Background: The starting material for all FDA/EMA approved CAR T cell products are leukapheresis collected autologous peripheral blood leukocyte concentrates (PBLCs) that are shipped to the pharmaceutical manufacturer for expansion, separation and CAR-receptor transduction. An already existing prediction algorithm for CD34+ cell counts (Humpe et al., 2000) was tested for its capability to predict the CD3+ T cell count in PBLC products.

Methods: The prediction accuracy of the algorithm was retrospectively tested by comparing the harvested yield after CD3+ T cell count by fluorescence-activated cell sorting with the predicted cell count of the algorithm based on apheresis characteristics. The numbers were analyzed by a regression analysis.

Results: The linear regression analysis showed R2 = 0.7312 with a P value < 0.0001. The regression is described by Y = 1.267*X + 734.4. The data were collected from leukapheresis products for CAR T cell production from 2018-2021 (n=60). In only two (3.3%) of the 60 cases the predictive algorithm underestimated the harvested yield.

Conclusion: The previously established algorithm for predicting CD34+ cells showed a good determination capability (R2) for predicting CD3+ cells with a high statistically significance. Therefore, it is a useful tool for tailoring the duration of the leukapheresis time according to the therapeutic needs of the patient, especially important for critically ill patients with a higher probability of side effects because of prolonged leukapheresis.

Disclosure Statements: No conflicts.
miRNA profiling to assess protective effects of MSC-conditioned medium on cisplatin-induced injury in proximal tubule epithelial cells

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Background: Kidney disease is a major challenge for health systems, with few treatments available and associated with significant side effects. Aberrant expression of microRNAs appears to be associated with the progression of kidney disease. Several studies have assessed the therapeutic potential of Mesenchymal Stromal Cells in renal disorders that, through the release of cytokines and growth factors, attenuate the renal tubular damage.

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 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, miRNAs 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 compared to untreated ciPTECs. ASC-CM ameliorated the cisplatin toxicity. This effect was most pronounced when assessing ciPTECs migratory activity: while cisplatin significantly reduced the migration in the scratch wound assay, CM leveled it up even exceeding control levels. Small-RNASeq analysis identified 31 differentially expressed miRNAs in the cisplatin-ASC-CM group as compared to ASC-CM group, among them 13 were upregulated and 18 were downregulated. Some of these miRNAs are known to regulate apoptosis and cellular fitness, while others have not been described in this context yet.

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.

Disclosure Statements: We speculate that MSC-conditioned medium exerts a protective effect, amongst others, by modulating miRNA expression in injured renal cells and by this post-transcriptional gene regulation.

PS-4-10

GMP Manufacturing of Cryopreserved peripheral Blood Stem Cell Products

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Background: Hematopoietic stem cell preparations are pharmaceuticals that are typically transfused as fresh products. Due to the travel restrictions caused by the Covid-19 pandemic since March 2020, the supply of fresh stem cell preparations to patients became significantly challenging. To maintain global patient supply, a GMP-compliant manufacturing process for cryopreservation of allogeneic stem cell donations was established and validated.

Methods: The donor leukapheresis is processed from the collection center to the manufacturing site at 2-6°C using qualified containers. In class D clean rooms, this material is further processed in a completely closed process. If necessary, volume reduction is performed by centrifugation. If the suspension is mixed with the cryoprotectant Cryostore CS10 to achieve a final DMSO concentration of 5%. The formulated product is filled into cryobags and cryopreserved under controlled freezing conditions.

The frozen products are stored and transported at ≤ 140 °C. The cell counter NC-200 is used to determine the cell count and viability of the product. 97 products in 2020 and 127 products in 2021 were successfully cryopreserved and transported worldwide.

Results: The mean values of viability of 215 products are 99.3 % (± 1.1 % SD) for fresh apheresis products and 93.1 % (± 6.2 % SD) for the corresponding cryopreserved final product after thawing. For a good recovery after thawing, the age of the apheresis as well as the DMSO contact time are generally considered to be critical factors. The analysis of the viability of 222 cryopreserved end products after thawing shows a correlation in relation to the age of the apheresis (Fig. 1). As the age of the apheresis increases, the viability decreases. Therefore, care should be taken about the age of the apheresis. A correlation between the DMSO contact time and the viability of the end products after thawing was not observed (Fig. 2).

Conclusion: GMP-compliant closed system manufacturing of cryopreserved allogeneic stem cell products provides safe and high quality drugs that can be used for transplantation. We have shown that with our manufacturing process, DMSO, which is potentially toxic to cells, has no effect on cell recovery after thawing. Cryopreservation is therefore a suitable and safe method to provide patients with essential therapy worldwide.

Disclosure Statements: No conflicts of interests.

PS-4-12

Interim 52-Week Analysis of Immunogenicity to the Vector Capsid and Transgene-Expressed Human FVIII in GENEr8-1, a Phase 3 Clinical Study of Valoctocogene Roxaparvovec, an AAV5-Mediated Gene Therapy for Hemophilia A

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Background: AAV-mediated gene therapy vectors represent a complex drug design with multiple components that may impact immunogenicity. Valoctocogene roxaparvovec is an AAV5-mediated gene therapy under investigation for treatment of hemophilia A and encodes a
B-domain deleted human FVIII protein under control of a liver-selective promoter. This report describes immunogenicity data from up to 1 year of follow up from GENER8-1, an ongoing, Phase 3 single-arm study in 134 males with severe hemophilia A.

**Methods:** Patients were required to test negative for AAV5 antibody utilizing a bridging ECLA screening assay and to have had greater than 150 exposure days to FVIII replacement without FVIII inhibitors. Following dose administration, patient plasma was analyzed for AAV5 antibody, and a cell-based AAV5 transduction inhibition assay was used to further characterize the neutralizing potential of AAV5 antibodies. Development of FVIII inhibitors was monitored using the Nijmegen-modified Bethesda assay, and FVIII-specific binding antibody was assessed by bridging ECLA. Peripheral blood mononuclear cells were collected for analysis in a validated IFN-g ELISPOT assay for detection of capsid-specific and hFVIII-SQ-specific cellular immune responses.

**Results:** Immune responses to treatment were predominantly directed toward AAV5 capsid, characterized by the production of anti-AAV5 binding and neutralizing antibodies as well as transient AAV5 capsid-specific cellular immune responses. In general, there was a minor association between AAV5 capsid-specific IFNg ELISPOT responses and plasma levels of ALT at a population level, yet these responses may be a contributing factor to transient elevations in ALT in some participants. There was no significant association between AAV5-specific cellular immune responses and FVIII activity, and no association between FVIII-specific cellular immune response and ALT or FVIII activity.

**Conclusion:** There was no clinical evidence of inhibitor formation after gene transfer.

**Disclosure Statements:** BL, TR, JD, HY, KL, KP, GH, JH, SA, CV, SG: BioMarin employees.

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**PS-4-13**

**Transfer of Human Therapeutic T cells into Expansion Cultures Used for Generation of CAR T Cells is Associated with Discordant Regulation of Adhesion Receptor and Cytoskeletal Genes**

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**Background:** The transfer of patient or donor-derived T lymphocytes into culture medium is a routine step in the production of immune cell therapeutics, including Chimeric Antigen Receptor (CAR) T cells. To better understand changes observed in cultured T cells such as loss of migration potential, we investigated the changes in T cell transcriptome upon exposure to culture conditions as used for therapeutic clinical CAR T cell production.

**Methods:** Human blood T cells were isolated from 8 voluntary donations from male donors aged 20-29 between 10 and 11 AM, using CD3 Fab-Traceless Affinity Cell Selection (TACS) Agarose Columns. Cells were cultured for 7 days in X-VIVO15, 5% HAB Serum, 50U IL-2 and were activated using 10μl/ml of anti-CD3/CD28 for the initial 3 days. Total RNA was isolated from fresh T cells and from T cells cultured for 4h, 12h, 24h, 3 and 7 days. Total RNA libraries were generated using the TruSeq Stranded Total RNA Kit, spiked with RNA Spike-In controls, and sequenced on Illumina NovaSeq. Differential gene expression was analyzed using DESeq2 and was focused on adhesion, migration and cytoskeletal genes.

**Results:** Total RNA per sample correlated with cell numbers. Principal component analysis showed close concordance between individual samples and clear separation of the different time points. Several T cell specific genes including ICOS and TIGIT were upregulated after 4h, but returned to pre-culture values within 3 to 7 days. Of adhesion molecules, integrins alpha 4, 5, and 6 (ITG4A, ITG5A ITG5A6) were downregulated by > 1 log within 4h, whereas integrins ITG2A, ITGAs and ITGAL remained constant or increased. Most chemokine receptors showed minor regulation, however CX3CR1 fell > 1 log at 4h and further declined during all time points. We also found discordant regulation of several actin-interacting genes, miRNAs and IncRNAs.

**Conclusion:** Transcriptome analysis of T cells that were generated under a current manufacturing protocol for clinical CAR T cells showed downregulation of key genes associated with cell cytoskeleton, cell migration and adhesion and migration, in line with observations of disturbed migration behavior of ex vivo expanded T lymphocytes. These results may help to better understand alterations in T cells function during ex vivo expansion and their impact on efficacy of the CAR T cell therapy upon infusion.

**Disclosure Statements:** There is no conflict of interest.

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**PS-4-14**

**Ex-vivo expansion of autologous bone marrow-derived mesenchymal stromal cells for treatment of bone defects:**

**Impact of donors characteristics and quality of starting material of efficacy of expansion**

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**Background:** Mesenchymal stromal cells (MSC) are promising candidates for regenerative and immunomodulatory treatment. We expanded autologous MSC from bone marrow (BM) aspirate for several clinical trials for bone repair. The target dose of the clinical trials ranged from 1 to 2 x10^8 MSC. We observed a substantial heterogeneity of ex-vivo expansion of these autologous MSC. Here we analyzed factors which predict the efficacy of expansion of BM-MSC for clinical trials in our manufacturing center.

**Methods:** We analyzed 56 ex-vivo expansion of BM-derived MSC from autologous donors. Expansion was performed in CellStack cultures in alpha-MEM with platelet lysate as growth factor. BM cells were seeded at a density of 5x10^4 WBC/cm^2 and harvested after 10 or 14 days (p0). MSC harvested from p0 were seeded at a density of 4x10^3 MSC/cm^2 for 5 or 7 days (passage 1, p1). We compare a “short protocol” (5+10 days; target 1x10^8 MSC) and a “long protocol” (14+7 days; target 2x10^8 MSC). Since overall harvest depends on the number of seeded CellStacks and the target dose we used the MSC density of p0 harvest as endpoint which is independent of these parameters, yet critical for the culture area that can be seeded in p1 and therefore the overall harvest.

**Results:** We have analyzed the impact of many donor (=patient) and process related parameters. Age and gender had no significant influence on the MSC density at harvest of p0. This endpoint was significantly lower with the short protocol (1.69x10^4 vs. 2.87x10^3; p<0.0001). The cumulative number of population doublings (p0+p1) was 14.1 vs. 16.7, p<0.0001. There was a positive correlation of MSC density with a short interval between BM aspirate and culture (p<0.001), high concentrations of leukocytes (WBC)(p<0.001), CFU-F (p<0.0103) and high proportion of mononuclear cells (p=0.002) in the BM aspirate, but not the concentration of CD34+ cells (p=n.s.).

**Conclusion:** Ex-vivo MSC expansion is negatively affected by a long interval between BM aspiration and start of MSC culture, poor BM aspirate with low WBC and low CFU-F count. Poor starting material impairs in particular the expansion by the short protocol. Ex-vivo-expansion of autologous BM-MSC can easily achieve target doses >2x10^8 MSC even with poor starting material due to the 7 day longer culture period and higher number of population doublings which can compensate for poor starting material.

**Disclosure Statements:** None.
Impact of miRNA-30a-5p gene knock out on the proliferation and differentiation capacity of erythroblast cell line imBMEP-TUD-BGO (imBMEP).

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Background: Ex vivo production of red blood cells (RBCs) represents a promising alternative for transfusion medicine. Several strategies were described to generate erythroid cell lines from different sources, including embryonic, induced pluripotent and hematopoietic stem cells (HSCs). All these approaches have in common that they need elaborate differentiation cultures while the yield of enucleated RBCs is inefficient. To reduce culture time and increase enucleation rate, we have further engineered an erythroblast line previously established in our lab (imBMEP), by knockout (K.O.) of miR-30a-5p, which has been suggested as a key inhibitor of enucleation.

Methods: imBMEP cells originate from bone marrow derived CD71+ erythroid progenitors. Lentiviral transduction of a c-myc-p2A-BCL-XL antibiotic-inducible vector led to a stable culture (>3 years) under supplementation with 1 µg/ml doxcycline (Dox). Since the cells showed impaired terminal erythropoiesis, we aimed to enhance enucleation rate by electroporation-based CRISPR/Cas9 K.O. of the miR-30a-5p gene locus whose complete deletion was confirmed by sequencing. A stable adult hemoglobin producing cell line was established by single cell cloning. Induction of differentiation via addition of 3U/ml EPO in absence of Dox was monitored over 10 days. Peak of enucleation occurred on day 7, as assessed by flow cytometry. Deformability of reticulocytes was examined using real-time deformability cytometry.

Results: miR-30a-5p K.O. did neither affect morphology, nor erythroid phenotype, but accelerated proliferation with a ~20% reduction in doubling time to 37.6±4.3h. Furthermore, miR-30a-5p K.O. resulted in a significant increase in terminal erythropoiesis capacity with ~75% orthochromat erythroblasts after 10 days of differentiation culture. However, the effect of the K.O. on enucleation was limited with a final rate of 5%. Interestingly, deformability of enucleated imBMEP miR-30a-5p K.O. cells is comparable to that of HSCs derived reticulocytes.

Conclusions: miR-30a-5p K.O. in imBMEP cells resulted in promotion of terminal erythropoiesis in terms of quicker doubling time and increased yield of orthochromatic erythroblasts. However, miR-30a-5p deletion alone seems not to be sufficient to enhance enucleation rate in erythroblast lines, such as imBMEP. To address the impaired enucleation in immortalized cell lines, we are currently employing a systematic shotgun approach, to identify modulators of enucleation.

Disclosure Statements: We have no conflict of interest. The project was in part funded by the DGTI-Stiftung.
Human breast milk bank – Frankfurt experience after > 3500 bottles of delivered donor human breast milk

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Background: If their own mother's milk is not available, the World Health Organization and UNICEF recommend quality-controlled donor milk as the best alternative nutrition for infants, especially preterm and ill infants. To provide the supply of such vulnerable patients in the Rhine-Main region with quality-controlled donor milk, we established the human Milk Bank Frankfurt. Here we present our experience after the delivering of more than 3500 bottles of donor human breast milk.

Methods: The production of our quality-controlled donor milk starts by screening the potential donor. After the donor screening process is completed, the raw milk of each donor is pooled by a single donor concept. We then aliquot the raw milk pool into small volume (50mL) or large volume (100mL) servings and treat them via Holder Pasteurization. Immediately after pasteurization, we freeze the pasteurized milk. One bottle of each batch is analyzed for bacterial contamination, freezing point and its macro nutritional composition of fat, protein and lactose and additionally one bottle is stored as retain sample. If all results are within specification, the batch is released and fed to infants at participating perinatal centers.

Results: The Breast Milk Bank Frankfurt contributed 3515 bottles of human breast milk to the nourishment of more than 65 preterm infants. We received 425 liters of human milk from 44 healthy voluntary donors, out of which we produced 255 batches of pasteurized breast milk. Due to our high quality control parameters not all batched were within specification, which led to a discard rate of 13.6% of the total volume of donated breast milk. All fed batches showed a medium pH value of 6.99, a mean freezing point at –0.54°C and were free of bacterial contamination. The Macro nutrient composition displayed a mean fat content of 3.61%, a protein content of 1.26% and a lactose value of 6.86%.

Conclusion: After two years of supplying milk to the University Hospital Frankfurt, one more hospital (Sana Hospital Offenbach) was included to the Breast Milk Bank Frankfurt. This is a first step towards our goal to make human milk even more available. The excluding rate of 13.6% highlights the biological variability in the composition of donor milk and displays the effectiveness of our process to detect even small numbers of remaining bacteria despite high hygiene requirements of the donation process.

Disclosure Statements: All authors declare that they have no conflicts of interest.

PS-5 Gewebezubereitungen | IT und Automatisierung in der Transfusionsmedizin | COVID-19 | Versorgungsforschung

PS-5-1 Allografts: Expanding the clinician's armamentarium

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Background: In Germany, bone allografts are widely used and have become an attractive alternative to autografts, as a result of improved allograft processing methods that have increased their safety and availability. In orthopaedic surgery bone allograft use has increased by 74.1% between 2008 and 2018. In turn, autograft use has decreased by 14.3% over the same period (Rupp et al., 2021).

Methods: Allografts are obtained from various tissues such as bone, tendon, amniotic membrane, meniscus and skin. Compared to autografts, the allograft spectrum offers a wide range of allograft forms that provide surgeons with an abundant supply during surgical procedures. These allogenic grafts aid in reducing patient morbidity associated with no harvesting comorbidity and pain, shorter hospital stays and decreased costs (Vardanian et al., 2009). Nevertheless, many surgeons still use autografts to this day.

Results: Allografts have a wide applicability that is of great benefit to surgeons. They have access to various allograft forms such as mineralized, demineralized, freeze-dried, paste, powder, chips, strips and putty that allows surgeons to use suitable allografts in indications they deem fit. A common allograft is demineralised bone matrix that is present in many forms and can be used in various indications ranging from spinal fusion, cranio-maxillofacial, non-unions and tumour surgery. Thus, the vast options of allografts and their applicability allows surgeons to use allografts in indications they consider appropriate.

Conclusion: The multi-usability of allografts displays how they act as a secondary scalpel for the surgeon. These types of allografts help equip clinicians with a natural toolbox for the treatment of patients. Allograft use in different settings exhibits the importance of such grafts in human health and recovery.

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.

PS-5-2 Requirement for the acceptance of cryopreserved tendons without inactivation treatment

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Background: The number of allografts compared to the number of autografts used for reconstruction of the ACL has been growing especially concerning revision surgery. Recent studies suggest that fresh frozen tendon grafts (FFT) are superior to grafts undergoing irradiation, chemical processing, freeze-drying or cryopreservation in regard of biomechanical properties and graft failure. At present according to the PEI there is no tissue establishment that has FFT as an authorized tissue preparation in Germany.

Methods: A concept for the sterile harvest of allograft tendon from cadaveric donor was established, following the guidelines for heart valve explanation of the University Tissue Bank of the Charité - Universitätsmedizin Berlin. After the explantation a sample inoculation and microbial testing of rinsing solution was performed.
Results: A procedure for sterile explantation of tendon grafts from cadaveric donors could be successfully established. Requirements for microbial testing on the tissue were laid out and performed. For multiple grafts negative results in all microbial tests were achieved.

Conclusion: A first concept for the establishment of fresh frozen tendon allograft is demonstrated. The procedure could successfully be derived from currently published literature and our many years of experience with other tissue preparations. Further validation studies demonstrating the safety and the equality or superiority of fresh frozen tendon grafts compared to established tissue preparations should be performed in the future.

Disclosure Statements: none

PS-5-3
Platelet lysate as well as human serum can substitute for fetal bovine serum for culturing corneal endothelial cells

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Background: Standard media for culturing human cornea transplants contains FCS bearing the risk of zoonosis and immunological complications besides ethical issues associated with the procedure to obtain FCS. To establish and assess the suitability of alternative culturing media an in vitro method is needed. Besides BHK-21, we tested a human corneal endothelial cell line in media with different formulations substituting for FCS with human serum (HS), platelet lysate (PL) and fresh frozen plasma (FFP)

Methods: BHK-21 cell line from hamster kidney, and HCEC-12 (human corneal endothelial cell line) were cultured with MEM, Human-Endothelial-SFM+FGF (10ng/ml), HAM’s F12, M199 each in the presence or absence of FCS, HS, PL, or FFP. Cell proliferation and vitality was assessed after 2, 3, and 4 days by foto documentation, CyQuant and WST-1 assay, respectively

Results: For BHK-21 and HCEC-12 cells, HS (2.5%) shows higher and PL (2.5%) equal activity compared to 5% FCS. For FFP we obtained inconsistent results.

Conclusion: FCS can easily be replaced with HS or PL while PB should be rather preferred because it is available in much higher amounts than HS, is cheaper and doesn’t compete with patient blood supply. FFP may be pooled before testing in order to address individual differences possibly leading to our inconsistent results. The suitability of PL as media supplement for cornea transplants seems likely and should be assessed.

Disclosure Statements: None

PS-5-4
Algorithm for acceptability and testing of post-mortem blood samples from tissue donors

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Background: The acceptability of postmortem blood samples continues to be regulatory limited to a maximum postmortem time of 24 hours. Previously published studies investigating factors influencing the suitability of postmortem blood samples for infectious serology and nucleic acid amplification testing (NAT) found no or only a questionable correlation to postmortem time. In our opinion, the most crucial aspect of using post-mortem blood samples is the avoidance of false-negative test results.

Methods: Based on the published and validation studies on postmortem blood samples and own investigations, also on the total IgG concentration, a test algorithm was developed. According to this algorithm, a post-mortem blood sample, regardless of its postmortem time, can be used for infectious serological testing with a validated test method if the total IgG concentration is at least 4 g/l (lower limit of our test method is 6 g/l). After testing the mandatory parameters for hepatitis B and C, human immunodeficiency virus and syphilis, appropriate NAT testing, which has to contain an internal positive control, is performed. If all parameters are negative/unremarkable there is a suitability of the tissue donor.

Results: In our preliminary results from 7 tissue donors, the total IgG concentration of the postmortem blood sample was with 7.1 g/l on average above the established limit of 4 g/l in all samples. In all of them, the infectious serological and NAT examinations were unremarkable. The pre-mortem samples that were also tested showed also negative test results.

Conclusion: The test algorithm proposed by us for postmortem blood samples based on a measurement of the total IgG concentration seems to be feasible and sufficiently safe with regard to false negative test results due to the use of a validated NAT method.

Disclosure Statements: No conflicts of interests.

PS-5-5
Meeting the demands for bone allografts: challenges during COVID-19 pandemic

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Background: Bone allografts have been an integral part of orthopedic procedures such as revision hip surgery or reconstruction of large bone defects. Femoral heads and necks were harvested from living donors undergoing total hip arthroplasty (THA). They were stored fresh frozen or processed into bone chips by the Innsbruck Bone Bank. The challenge was to meet the demand for bone grafts through in-house manufacturing even during Covid-19 pandemic restrictions.

Methods: Key data from the bone bank’s registry and the hospital administration between January 2019 and March 2022 were analysed with regard to collection of fresh frozen allogenic femoral heads /necks and the manufacture of bone chips and their discard. Donor suitability and release criteria were assessed in accordance with EU and national legislation.

Results: In 1,249 primary THA surgeries 640 femoral heads and necks were harvested. In the mean 51.4% of these yielded the bulk material for bone chips manufacture. Due to Covid-19 pandemic restrictions the number of grafts retrieved was lowest in Q1 2020 and Q4 2021. The proportion of unreleased transplants remained fairly consistent at 14%, but the reasons for discard varied: technical issues were eliminated, but the increase for discard to >30% due to reasons in donor history required the revision of the questionnaire and the collaboration with the team responsible for listing diagnoses in the medical records. By 2020, the bone chip discard rate had increased to 8%. Hence modification of cleaning and gowning brought it back down to 2%.

Conclusion: Providing high quality bone allografts is a complex task. In this context, it is important to regularly evaluate the registry data and to modify the process accordingly, thus ensuring adequate supply of allografts even in times of significant restrictions.

Disclosure Statements: The authors have no conflict of interests to declare.
AutoPiLoT – Automated guideline-compliant patient-specific blood product allocation and innovative logistics management in transfusion medicine

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Background: Coordinating the need for blood and the availability of blood products will play an increasingly important role, especially considering the demographic change. Hospitals in Germany face an increase in elderly patients dependent on blood transfusions. At the same time, the number of potential blood donors is constantly decreasing. Setting up a blood product monitor with real-time visibility of in-stock numbers can help to optimize the management of blood products.

Methods: The newly established AutoPiLoT monitor enables supervised stored blood products in real-time with less than one-minute latency. Based on a continuous ETL (extract-transform-load) pipeline into multiple hospital information systems, the AutoPiLoT monitor is connected via a central FHIR repository to the required data. The monitor is developed as a web-based application in TypeScript using the React framework. Backend communication with the central FHIR repository is performed using RESTful FHIR APIs. Visualization enables an easy and simple overview.

Results: The AutoPiLoT monitor enables early awareness of a critical blood product shortage. An easy graphical display gives an overview of available and patient-specific products. The AutoPiLoT monitor reduces the time-consuming search in the laboratory information system for in-stock blood products and provides an essential overview of needed blood products well in advance to mobilize donors for unique products required. Discrimination between (free) available and crossmatched products is displayed for red blood cell concentrates. With a short shelf life of four days, blood products will play an increasingly important role, especially in times of crises. It is important that blood establishments can rely on adequate and timely information, as well as on emergency plans.

Conclusion: Using the AutoPiLoT monitor, we achieved efficient and straightforward monitoring of available blood products. The quick and easy display of in-stock and expiring blood products can lead to optimized handling of this valuable resource. The optimized control improves the supply and reduces the decay of blood products at the University Hospital Essen. This represents significant progress in transfusion medicine, both in medical and ethical, and financial terms.

Disclosure Statements: The authors declare that there is no conflict of interest.

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Detection of SARS-CoV-2 Spike-Protein-Specific Antibodies after Vaccination via Nasal and Buccal Swabs: Prospects for Mass Scale Immunity-Screening in Large Populations

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Background: Humoral immunity after SARS-CoV-2 vaccination has been extensively investigated in blood. Far less is known, however, about the potentially induced mucosal immunity. Aim of this study was to develop an ELISA method in order to determine the prevalence of IgG and IgA potentially induced mucosal immunity. Measurements.

Methods: To this end, we prospectively analyzed 69 adult individuals after signed written consent who received their first vaccine dose between February and July 2021. Detection of IgG and IgA Abs was performed using the semi-quantitative ELISA assay from EUROIMMUN for both blood and swab samples after protocol modification for the latter. The eluates of the nasal and buccal swabs were analyzed undiluted while the cut-off was set as for the serum assay. The samples were taken at regular intervals to ensure systematic recording of Ab course before and after each vaccination dose.

Results: After second dose all study subjects developed IgG anti spike Abs in serum, yet 5.9% of them remained negative for IgA. In buccal swabs, positivity rates were 81.2% and 53.1% and in nasal swabs 84.1% and 90.6% for IgG and IgA, respectively. The IgG Abs in buccal swabs correlated more consistently with the respective measurements in blood with a correlation coefficient of r=0.74 while the IgA assay gave less concordant results. It is of note that IgA Abs appeared to be significantly more prevalent in the nasal compared to the buccal mucosa. Adjustment of the assay cut-off as for IgG antibody detection in buccal swabs from 1.1 to 0.2 conferred a sensitivity of 91.8% and a specificity of 100% in a total of 520 comparison measurements.

Conclusion: In conclusion, our findings confirm a weaker yet clear prevalence of Abs in mucosal surfaces after full vaccination against SARS-CoV-2 with IgA anti spike Abs being significantly more prevalent in the nasal cavity. Our method for IgG Ab detection in buccal swabs could be expanded to other pathogens of interest and serve as a reliable alternative to standard serum assays especially in the context of immunity screening of large populations.

Disclosure Statements: No conflict of interest to declare.

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

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Background: Treatments are being developed against severe Covid-19 symptoms, among them the use of convalescent plasma. Two drawbacks are, the large volumes of plasma needed for treatment can lead tocirculatory overload, and the plasma contains unnecessary components that can lead to unwanted side-effects. We investigated the use of immunoadsorption followed by tangential flow filtration as a method to obtain highly concentrated Covid-19 antibody concentrates free of additional plasma components.

Methods: Five convalescent plasma donors (3 men and 2 women) have participated in this study. Two donors donated twice with 1 year gap between the donations. Immunoadsorption was performed with an affinity column adsorber pair (Millenyi Biotec, Bergisch-Gladbach, Germany). The resulting elute contained antibodies dissolved in a glycerine buffer with a total volume of 1100–1500mL. Tangential Flow Filtration System was used to concentrate all eluates 15–21.3-fold using an Omega 30 kD membrane (Pall Corp, Dreieich, Germany) and to exchange the glycerine buffer with 0.9% NaCl. Eluates were then filtered through a sterile filter, before storage at 4°C and -80°C.

Results: All donors have tolerated the immunoadsorption very well with no side effects. The final product contained between 5mg and 194mg of CoV-2 antibodies per donation at a median end-volume of 61±20ml leading to probably eight times more COVID-19 antibodies than in one plasma unit while preserving or even increasing their neutralization capacity up to ten-fold. Glycine levels were reduced to non-hazardous 12.6±20 µmol (see table 1). The product was sterile and remained stable for 0.5 to 1 year in storage. In two cases the concentration of SARS-COV2 antibodies even increased during storage.

Conclusion: Immunoadsorption followed by tangential flow filtration produces CoV2 antibody concentrates of high concentrations without simultaneous removal of unnecessary plasma components. The procedure can be done within one day, including the donation, without compromising the donor’s immune system. Whether these donated antibodies can be used as passive immunization in acutely infected patients remains to be elucidated.

Disclosure Statements: I am employed at the Pall Corporation.
PS-5-11

Increase in SARS-CoV-2 antibody titres in a normal human immunoglobulin preparation for intravenous use

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Background: Human intravenous immunoglobulin preparations (IVIG) contain a consistent level of antibodies by using large plasma pools from healthy donors for production. Thus, they contain antibodies against a broad spectrum of pathogens and are used to protect against serious infections in patients with primary and secondary immunodeficiency. We have investigated the occurrence and quality of antibodies against a novel pathogen, SARS-CoV-2, in an IVIG preparation.

Methods: Consecutive batches of an IVIG preparation (Intratect™) and experimental IgG preparations from convalescent or vaccinated donors are analysed. Modified commercial and in-house ELISA systems were used to measure the binding activities against different pathogens. Anti-SARS-CoV-2 IgG activity was determined with a quantitative in-house ELISA based on the spike protein (Wuhan strain). A Meso Scale Diagnostic Antibodies kit system was employed for the analysis of antibodies against SARS-CoV-2 variants or various other respiratory pathogens.

Results: All IVIG batches tested contained consistent levels of antibodies against common bacteria, viruses and fungi. IVIG batches from before the pandemic showed no detectable anti-SARS-CoV-2 activity. In more recent batches, this activity increased to levels comparable to hyperimmunoglobulin produced from convalescent plasma or vaccinated donors. In parallel, reactivity increased with MERS and anti-SARS-CoV-1, but not with other corona or influenza viruses. The reactivity of the present antibodies against other SARS-CoV-2 variants (alpha, beta, gamma, delta) was similar. They also bound the omicron variant, albeit more weakly.

Conclusion: SARS-CoV-2 antibody levels in an IVIG preparation reached a high level within two years of the pandemic outbreak. As antibody titres correlate with virus neutralisation, this may be beneficial for patients with primary and secondary immunodeficiencies. As clinical trials with individual hyperimmune plasma donations have shown successful prophylaxis and therapy of mild diseases, an IVIG preparation could be beneficial for the treatment of certain patient groups.

Disclosure Statements: All authors are employees of Biotest AG.

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PS-5-12

Hematopoietic stem cells from SARS-CoV-2 infected donors. Is there a risk for transmission?

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Background: Since the global COVID-19 pandemic, the supply of patients with hematopoietic stem cells is challenging. On day of collection, donor for PBSC is completely mobilized and the patient in complete aplasia. In this situation, a cancellation or deferral of collection has life-threatening impact on the recipient. In this case series we investigated the feasibility and the outcome of donors, which were tested positive for SARS-CoV-2 around the day of collection.

Methods: Since the availability of SARS-CoV-2 antigen rapid test systems, all stem cell donors in our institution get tested on the day of collection before entering the facility. For bone marrow donors an additional SARS-CoV-2 PCR test is required. Furthermore, donors are instructed to perform ag-test in case of symptoms of an infection before and after collection. In case of a positive test result before collection, donor registry, donor centre and at least the donor was consulted how to proceed. For bone marrow harvest, the collection was cancelled.

Results: Between November 2021 and April 2022 we performed 571 PBSC-donations and 54 bone marrow harvests. Of them, five donors were enrolled into this observational trial, 4 PBSCs and 1 bone marrow harvest who was tested positive after the donation. In all cases, we collected sufficient amounts of stem cells for transplantation. Donor 2 developed circulation problems and fever during donation, donor 4 after the donation. All symptoms resolved after a short period. All products have been administered to the patient, up to date there are no reports concerning transmission of SARS-CoV-2 so far (Table 1).

Conclusion: SARS-CoV-2 is detectable in blood stream of hospitalized patients but little is known about viremia in immunocompetent donors. Transmission via transfusions has not been described, yet, but the impact of G-CSF during infection and the cellularity of the source opens up questions concerning the safety of stem cell products. In our case series, we observed no serious side effects, neither from donation or infection. Clinically, there was no transmission reported after administration.

Disclosure Statements: Nothing to disclose.

Tab. 1
PS-5-13
Patient supply with convalescent plasma at a university hospital during the first two pandemic waves

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Background: The blood bank of the Department Transfusion Medicine in a University Hospital supplied between April 6th, 2020, and June 9st, 2021, convalescent plasma (CP) to 133 patients and 38 patients in other Bavarian hospitals. We evaluated the data regarding the therapy of our own patients.

Methods: We used the matrix initiated by COVIM - one of 13 joint projects within the network of university medicine and is funded by the Federal Ministry of Education and Research - to examine our own data regarding therapy with CP. We collected following data: patient identification number, age, sex, blood group, date of hospitalization, date of transfusions batch-number (Konservennummer) of applied CP, volume of CP units and total volume, titer of neutralizing antibodies against SAR-CoV-2 in each unit, the total volume of CP, documented comorbidities, date and transfer to ICU, duration of stay in hospital. We also regarded and analyzed the neutralizing antibody titers and the mortality during the hospitalization.

Results: The median age of the patients was 60.9 years (23 to 89). Men were overrepresented at 69.2%. The blood group distribution was: 40.6% (O), 44.4% (A), 8.3 (B) and 6.7% (AB). A total of 347 CPs (volume: 196 ml) were used, a mean of 2.6 units (481.7 ml) per patient. Figure 1 shows the distribution of the neutralizing antibody titers. The need for CP was variable and well correlated with the course of the first two pandemic waves. 38 of 133 patients (28.6%) died. The first CP transfusion was performed on average on day 6 after hospital admission in the deceased and on day 4 after admission in the non-deceased patients. The mortality was slightly lower (23.4%) in the group of 64 patients treated within 48 hours after admission in the hospital.

Conclusion: In the first two waves of the corona pandemics, there were still no reliable study results regarding the dosage of CP. Our results correlate with international experience (Piechotta et al, 2021; Rijnders BJA et al, 2022) and the recommendations of “S3-Leitlinie: Empfehlungen zur stationären Therapie von Patienten mit COVID-19 (28.02.2022)”. In summary: the CP were applied mostly too late and at too low a dose. This may also be related to the low antibody titer in the available CP.

Disclosure Statements: The authors declare that there are no conflicts of interest.

Fig. 1

PS-5-14
Correlation between infection severity and antibody levels in COVID-19 convalescent plasma donors

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Background: COVID-19 convalescent plasma (CCP) has been suggested to be beneficial to prevent disease progression in COVID-19. The effect of the CCP depends on the amount of neutralizing antibodies in the product. There are no criteria for the selection of donors with high antibodies. In this study, we investigated the relationship between infection severity and anti-SARS-CoV-2 antibodies in CCP donors.

Methods: Convalescent plasma donors with a history of COVID-19 infection were included into the study. The infection severity was determined by a self-reported questionnaire. We measured the level of anti-SARS-CoV-2 antibodies (Spike Trimer, Receptor Binding Domain (RBD), S1, S2 and nucleocapsid (NC) protein) in CCP donors using a bead based method (MULTI COV AB, NMI Reutlingen, Germany). We used Spearman’s correlation analysis and Mann-Whitney U test for statistical analyses. Statistical significance was set at p<0.05.

Results: The results from forty-seven CCP donors [22 Male, 25 Female; and mean age (±SD) 41.4±13.7 years] were analyzed. Median duration after acute COVID-19 infection was 97 days (range, 34-401) and median duration of infection was 7 days (range, 0-27). Symptom duration was correlated with IgG Anti-Spike-Trimer (r=0.34; p=0.02), IgG anti-RBD (r=0.29; p=0.04) and IgG anti-NC antibody levels (r=0.42; p<0.01). Duration after infection was negatively correlated with IgG anti-NC antibody level (r=-0.57; p<0.01). The symptom score was correlated with IgG anti-NC antibody level (r=0.42; p<0.01). None of the infection parameters were correlated with the neutralization capacity of IgG anti-RBD antibodies against wild-type SARS-CoV-2 virus.

Conclusion: The duration of the infection correlates with the amount of antibodies in CCP donors. Further studies are needed to develop a clinical prediction model for the selection of donors with high antibody titers.

Disclosure Statements: none

PS-5-15
Vaccine side effects after first, second and booster vaccination against SARS-CoV-2: Data from TüSeRe:exact study

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Background: COVID-2019 caused by SARS-CoV-2 has become a pandemic that has claimed more than 6.2 million lives so far. Vaccination is the most effective tool in the fight against infectious diseases. However, low vaccination coverage is hampering our efforts to contain the COVID-19 pandemic. Vaccine side effects are one of the reasons for reluctance to vaccinate. In this study, we systematically investigated self-reported vaccine side effects (SE) after the first, second, and booster vaccinations.

Methods: The data was collected during the TüSeRe:exact study (Tübingen Monitoring Studie zur exakten Analyse der Immunantwort nach Vakzinierung). The coworkers from University Hospital Tübingen, Center for Clinical Transfusion Medicine and Natural and Medical Sciences Institute were invited to participate in the study. Study participants were asked to fill an online questionnaire and report SE after first, second and booster vaccinations.

Results: A total of 889 participants (mean age: 43 ± 12.7 years; female: n= 664 (77%), male: n= 205 (23%)) were included in the analysis. Pain on injection site was the most common local SE after first, second and booster
vaccination (68%, 70%, 57%, respectively). Fatigue was the most common systemic SE after first, second and booster vaccination (61%, 64%, 46%, respectively). Local and systemic SE were highest after vector based vaccine ChAdOx1 nCoV-19 in the 1. vaccination. However, local and systemic SE were more common after mRNA vaccines (BNT162b2, mRNA-1273) in the 2. vaccination. Compared to BNT162b2 vaccine, more SE have been observed after mRNA-1273 vaccine in the booster vaccination.

Conclusion: Local and systemic SE are common after SARS-CoV-2 vaccines. The frequency of self-reported local and systemic SE are significantly different between mRNA and vector vaccines. SE are more common after mRNA-1273 vaccine than BNT162b2 vaccine.

Disclosure Statements: none

PS-5-16
Results of nanopore sequencing of SARS-CoV-2 isolates of over a year

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Background: Starting in January 2021 we established fast genetic sequencing of the SARS-CoV2 virus genome using the Nanopore sequencing technology. Up to now we analyzed more than 400 virus samples including all of the so called different Variants of Concern (VOC) emerging in Germany.

Methods: We established a method to sequence the whole SARS-CoV-2 genome by Nanopore sequencing using previously taken nasopharyngeal swabs (already used for SARS-CoV-2 PCR testing) as viral RNA source. The whole analysis pipeline from RNA-isolation to virus variant classification and result report can be conducted within 2-3 working days. The method is highly scalable.

Results: We analyzed more than 400 SARS-CoV2 viral isolates from 2021 to the present day. We observed, that most Variants of Concern (VOC) (Alpha, Delta, or Omicron) locally take over all infection events within only one week. While 50-70% of the Alpha and Delta variant isolates show besides the variant defining mutations additional mutations in the S gene, additional mutations in the S gene in Omicron were seen rarely. Also we could see that the Omicron variants nearly had no mutations in the ORF3, and ORF6-8 genes, while mutations in Alpha and Delta were frequent.

Conclusion: We are able to accurately identify different SARS-CoV-2 variants and detect potential new ones in a cost-efficient and fast way even for low sample numbers. Mutational analysis of the different virus isolates show differences in mutation frequency and localization maybe indicating correlations between mutation localization and disease process.

Disclosure Statements: No conflicts of interests.

PS-5-17
BNT162b2 booster vaccination elicits cross-reactive immunity against SARS-CoV-2 variants B.1.1.529 and B.1.617.2 in convalescents of all ages

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Background: Among the greatest challenges of the current pandemic with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is its ongoing evolution, resulting in variants of interest (V0I) and variants of concern (VOC) with an enhanced capacity for immune escape. The likelihood for the emergence of such variants exponentially rises with the time a host is infected with the virus and the virus is reproduced without being properly controlled by the immune system.

Methods: In this prospective observational cohort study we analyzed cellular and serological immune response parameters against SARS-CoV-2 and current variants of concern (VOC) in 147 COVID-19-convalescent and 39 COVID-19-naive individuals before and after BNT162b2 booster vaccination. End points included anti-SARS-CoV-2 IgG and IgA titers, neutralization capacities against wild type SARS-CoV-2 and the VOCs B.1.1.529 and B.1.617.2 as well as SARS-CoV-2-specific T cell IFN-gamma responses.

Results: No significant differences regarding immunological response parameters were observed between younger and older individuals. Booster vaccination induced full recovery of both cellular and serological response parameters including IFN-g secretion and anti-spike antibody titers with strong neutralization capacities against wild type SARS-COV-2 and Delta. Surprisingly, even serological neutralization capacity against Omicron was detectable one month after second vaccination and four months before it had been first observed in South Africa. As a result, more than 90% of convalescent individuals exhibited detectable and 75% strong Omicron neutralization capacity after booster vaccination, compared with 72% and 46% of COVID-19-naive individuals.

Conclusion: Broad and cross-reactive immune memory against SARS-CoV-2 including currently known VOCs can be established by booster vaccination with spike-based mRNA vaccines like BNT162b2, particularly in COVID-19-convalescent individuals of all ages. Nevertheless, especially in COVID-19-naive individuals future variants escaping the memory immune response may require vaccine approaches such as inactivated whole virus vaccines, which include all antigenic components of the virus.

Disclosure Statements: All authors declare no conflict of interest.

PS-5-18
Effects of the COVID-19 pandemic on blood product management in a University Hospital blood bank

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Background: Since the beginning of the COVID-19 pandemic in April 2020 the supply with blood products was largely stable and the demand for red blood cell concentrates (RBCC) could be covered. From June 2020 the supply with blood products was largely stable and the demand for red blood cell concentrates (RBCC) could be covered. From June 2020 the supply with blood products was largely stable and the demand for red blood cell concentrates (RBCC) could be covered.

Methods: Data was analyzed to show the influence of the COVID-19 pandemic on the RBCC distribution management in the blood bank.
Therefore, RBCC cross-matches, delivery, return and transfusions of RBCC were analyzed, so the crossmatch-to-transfusion ratio (CTR: ratio of cross-matched to transfused RBCC) as an efficiency marker could be calculated. In consideration of the CTR the consequences of minimizing the number of RBCC provided for elective surgeries in direct consultation with clinical decision makers on blood bank management efficiency were statistically described in the context of inpatient admissions and operations.

**Results:** The availability on demand and consumption of RBCC were stable from April 2020 to May 2021 (mean value per month, fig. 1). However, the significant drop in RBCC supply from June 2021 was intercepted by a change of the RBCC management strategy as the number of transfusions remained unchanged (fig. 1): for elective operations the amount of RBCC provided was drastically reduced (from 48% to 23%). As a result, the overall CTR decreased from 3.0 on annual average (January 2019 to June 2021) to 1.7 from July 2021 to December 2021 (fig. 2). The number of operations was stable on a monthly average, as well as the inpatient admissions per month from 2019 to 2021 (2512) were only interrupted temporarily in April 2020 (1689).

**Conclusion:** Regarding constant inpatient admissions and performed surgeries, the amount of transfused RBCC was stable. With drastically decreasing the number of RBCC provided for elective interventions a more efficient blood bank management led to a uncompromised patient care and even the target CTR of 1.7 in the surgical field (Gombotz, Patient Blood Management, Anesthesiologist 2013, 62:519-527) could be reached. Therefore, we consider this proceeding worth maintaining for the future.

**Disclosure Statements:** No conflicts of interests.

![Fig. 1](image1.png)

![Fig. 2](image2.png)
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