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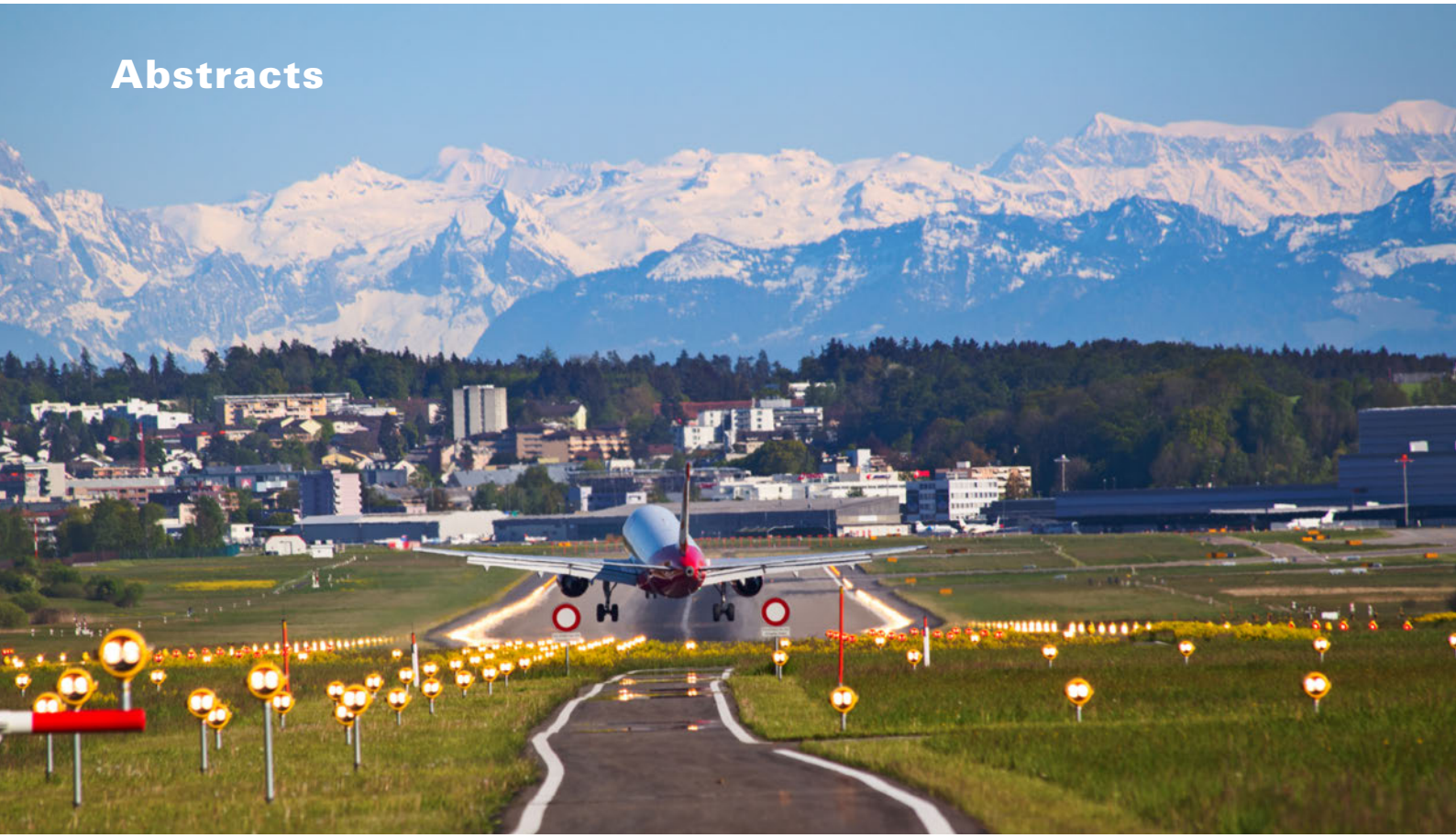
Abstracts



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ZURICH (SWITZERLAND), JUNE 26–28, 2019

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TABLE OF CONTENTS

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SWISS HEMATOLOGY & ONCOLOGY CONGRESS (SOHC)

NOVEMBER 19–21, 2020

RADISSON BLU HOTEL, ZURICH AIRPORT

CLINICAL HEMATO-ONCOLOGY (LYMPHOMA, MYELOMA, LEUKEMIA, TRANSPLANTATION FOR SSH AND SSMO)

993

In children with chemotherapy 39.0°C is a safe fever limit. The randomized controlled multicenter SPOG 2015 FN Definition Study

Koenig C[1], Bodmer N[2], Agyeman P[3], Niggli F[2], Adam C[4], Ansari M[5,6], Eisenreich B[7], Keller N[1], Leibundgut K[1], Nadal D[2], Roessler J[1], Scheinemann K[8,9,10], Simon A[11], Teuffel O[12,13], von der Weid NX[9], Zeller M[14], Zimmermann K[15,16], Ammann RA[1]

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Background: Fever in severe neutropenia (FN) is the most frequent potentially lethal complication of chemotherapy for cancer. In children, FN mortality is <1% thanks to emergency treatment including empirical broad-spectrum antibiotics. The temperature limit defining fever (TLDF) used clinically, varies from 37.5°C to 39.0°C, reflecting the lack of evidence. As previously published, a high versus low TLDF can avoid FN diagnoses in patients spontaneously recovering without therapy. This study aimed to determine if a high TLDF of 39.0°C ear temperature is non-inferior to a low TLDF of 38.5°C regarding safety in pediatric oncology.

Methods: In this randomized controlled non-blinded multicenter study, pediatric patients with chemotherapy for cancer were randomized in monthly clusters to a TLDF of 39.0°C or 38.5°C (NCT02324231). FN diagnosis and therapy below the randomized TLDF was allowed for clinical reasons. The primary outcome was the rate of FN with any safety relevant event (SRE) per chemotherapy exposure time (CET). SREs were death, admission to intensive care unit (ICU), severe sepsis and bacteremia.

Results: Six of 9 sites of the Swiss Paediatric Oncology Group (SPOG) recruited patients of all diagnostic categories from April 2016 to August 2018. After the second of 3 planned interim analyses, the study stopped for success. 269 patients were randomized 2547 times during 195 years of CET. All were treated per protocol. An SRE was diagnosed in 72 (20%) of 360 FN episodes (death, 0; ICU admission, 16; severe sepsis, 22; bacteremia, 56). In 92 CET years randomized to 39.0°C, 151 FN episodes were diagnosed (rate, 1.64/year), including 51 (34%) below 39.0°C and 22 (15%) with SRE (0.24/year). In 103 CET years randomized to 38.5°C, 209 FN episodes were diagnosed (2.03/year), including 28 (13%) below 38.5°C and 50 (24%) with SRE (0.49/year). The mixed Poisson regression rate ratio of FN with SRE in 39.0°C versus 38.5°C was 0.57, with a 95% upper confidence limit of 0.72. The predefined non-inferiority margin was 1.33.

Conclusions: In pediatric patients with chemotherapy for cancer, the use of a high TLDF of 39.0°C versus 38.5°C ear temperature is both efficacious and safe. For Switzerland and comparable settings, 39.0°C can be recommended as new evidence based standard TLDF. The responsible physician will decide to diagnose and treat FN below this TLDF if clinically indicated. In non-comparable settings, confirmatory trials are needed before clinical use.

1002

Time to antibiotics and clinical outcomes in fever and neutropenia during chemotherapy for cancer, a systematic review

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Background: Fever in chemotherapy-induced neutropenia (FN) is the most frequent potentially lethal complication in patients with cancer. Prompt empiric broad-spectrum antibiotic therapy is the standard of care. The association of time to antibiotics (TTA) with clinical outcomes is not clear, however, and recommendations are based mainly on studies involving immunocompetent subjects with sepsis. We systematically reviewed the available data on the association between TTA and clinical outcomes in patients with FN.

Methods/Design: The search covered 7 databases, reference lists were reviewed, forward citations searched and experts contacted. Studies were screened, and data extracted by one researcher and independently checked by a second. Confounding biases and study quality were assessed with the ROBINS-I tool. Safety (composite outcome: death, intensive care unit (ICU) admission, sepsis) and treatment adequacy (relapse of primary infection, persistence or recurrence of fever) were assessed as primary outcomes.

Results: Of 5389 titles and abstracts screened, 142 studies were retrieved and 10 studies, were included. All were observational studies, and all but one with at least moderate risk for bias, mostly due to baseline confounding. Predefined outcomes were reported variably. There was inconsistency among the findings regarding safety. Two studies at lower risk for bias and controlling for triage bias showed a possible association between longer TTA and impaired safety: specifically a lower 28-day mortality was found in patients with TTA ≤30 min (3.0%) vs. 31-60 min (18.1%) (n = 307; HR, 1.18; 95% CI, 1.10 to 1.26); and less adverse events (mortality, ICU admission, fluid resuscitation) in patients with TTA ≤60 min (5.2%) vs. 61-120 min (14.2%) (n = 1628; OR, 2.88; 95% CI, 1.70 to 4.89). Meta-analysis was feasible on 4 studies at moderate risk of bias, with 3 studies each reporting on death (OR 0.78, 95% CI 0.16 to 3.69) and on ICU admission (OR 1.43 95% CI 0.57 to 3.60). No study reported data on treatment adequacy. Triage bias, i.e., faster treatment of patients in reduced general condition or with known risk for poor outcome, was identified as a relevant confounding factor.

Conclusion: There seems to be an association between longer TTA and impaired safety. More precise knowledge about TTA effects on safety are important to optimize treatment guidelines for FN. Controlling for triage bias and other confounders is possible and necessary to gain further evidence.

1007

Integrated Efficacy Results from the Phase II and Phase III Studies with Caplacizumab in Patients with Acquired Thrombotic Thrombocytopenic Purpura

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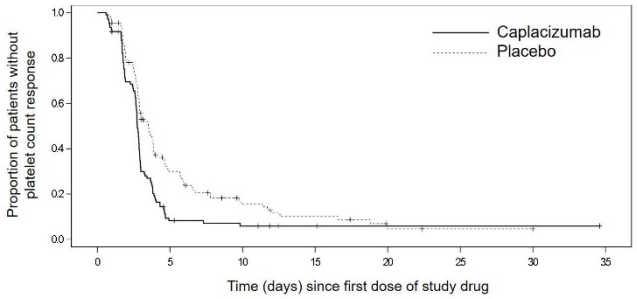
Introduction: Acquired thrombotic thrombocytopenic purpura (aTTP) is a rare, life-threatening thrombotic microangiopathy, characterized by a disseminated formation of von Willebrand Factor-platelet rich microthrombi. The efficacy of caplacizumab in aTTP, in conjunction with plasma exchange (PE) and immunosuppression, was demonstrated in placebo-controlled Phase II and III studies. Here, we present the integrated efficacy results of these studies.

Methods: All randomized subjects in the two studies were included in the analysis. Subjects had a single-blind (SB) or a double-blind (DB) treatment period followed by a 30-day follow-up period. Phase III study subjects could have an open-label caplacizumab treatment (in case of exacerbation during the DB treatment). The primary endpoint was time to platelet count response. Secondary endpoints included mortality rate; the number of PE days; the proportion of subjects with a) TTP-related death, recurrence of TTP or at least one treatment-emergent major

thromboembolic event during treatment (composite endpoint); b) a recurrence of TTP; c) refractory TTP.
Results: 220 subjects were randomized, 108 to caplacizumab and 112 to placebo. Groups were well balanced except for an imbalance regarding TTP history. There was a significant difference in favour of caplacizumab in time to platelet count response (p <0.001). Treatment with caplacizumab resulted in a 72.6% reduction in the composite endpoint during the DB/SB treatment period (p <0.0001). Treatment with caplacizumab reduced recurrences of TTP by 84.0% during the DB/SB treatment period (p <0.0001), and by 49.5% during the overall study period (p <0.005). Zero vs. 7 (6.3%) subjects in the caplacizumab group had refractory TTP (p <0.01). No patients died in the caplacizumab group vs. 4 in the placebo group during the DB/SB treatment period (p <0.05). There was a reduction in the mean number of PE days of 3.9 days in the caplacizumab vs. placebo group (Table 2).



TITAN: Response was defined by a recovery of platelets count ≥150,000/µL. This response had to be confirmed at 48 hours after the initial reporting of platelet count recovery equal to or above 150,000/µL by a de novo measure of platelets count ≥150,000/µL and LDH ≤2 x upper limit of normal range.
HERCULES: Response was defined as initial platelet count ≥150,000/µL with subsequent stop of daily PE within 5 days.

Conclusions: This integrated efficacy analyses confirmed results from Phase II and III studies showing that caplacizumab significantly reduces time to platelet count response, and resulted in clinically meaningful and significant reductions in (i) the proportion of subjects with TTP-related death, a recurrence of TTP, or at least one major thromboembolic event; (ii) the rate of death due to TTP (iii) a recurrence of TTP; (iv) refractory TTP; and (v) the mean number of PE days, during the treatment period.

1019

Comparison between two Reduced Intensity Conditioning regimens in patients with a myeloid malignancy: a single center experience comparing FB2 with FluMel

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Introduction: Hematopoietic Stem cell transplantation remains the only curative option for high-risk myeloid neoplasm. The optimal conditioning regimen is still debated. To address this issue, we conducted a single-centre retrospective analysis to compare two different RIC regimens in adult patients transplanted for myeloid malignancy from 2001 to 2018. A total of 137 patients were analysed, 74 of them treated with Busulfan-based (FB2) and 63 with Melphalan-based conditioning regimen (FluMel). All patients received antithymocyte globulin (ATG). Partial *in vitro* T-cell depletion was performed using alemtuzumab for low risk patients.
Results: Patients' characteristics were well balanced in the two groups with a median follow up of 46 months. The most frequent underling disease in both groups was AML (59.5 and 69.8% for FB2 group and FluMel group, respectively) and the stem cell source was peripheral blood in 94.6 and 96.8% of patients. More patients in the first group had near to significant worst Karnosfky status (<90) at transplant compared to second (35.1 vs 19%, p = 0.057) and more patients received a T-partial depleted graft (54.1 vs 33.3%, p = .028). The 3-year overall survival and disease-free survival were of 43.0 and 36.5%, respectively, after FB2 and 54.9 and 52.0% after FluMel, respectively, without significant difference (p = .41 for OS and .15 for DFS). The cumulative incidence (CI) of grade 2 to 4 acute graft-versus-host disease (aGVHD) was 16.2% after FB2 and 38.3% after FluMel (p <.001). The CI of chronic GVHD at 3 years was 13.9% in FB2 and 22.1% in FluMel group (p = .24). The CI of Non-relapse mortality at 3 years was 18.7% after FB2 and 29.6% after FluMel (p = .11). The CI of relapse at 3 years was 44.8% for the first and 18.4% for the second group (p <.001). No difference in 3-years GVHD-free/relapse-free survival (GRFS) was observed (25.5% for FB2 and 37.6% for FluMel, p = .48).
Conclusion: When comparing two RIC regimens for myeloid neoplasms, we observed a higher incidence of aGVHD after FluMel whereas no statistical difference was noted for the cGVHD occurrence. While the toxicity appears to be higher after FluMel, this result is counterbalanced by a higher proportion of relapse after FB2, accounting for no difference in OS, DFS and GRFS between the two groups. These findings could be partially explained by a larger proportion of patients receiving a partial T-depletion after FB2 RIC, but a larger trial is needed to clarify this issue.

1033

Outcome of patients with chronic myeloid leukemia transplanted with HLA-identical sibling donor and partial T-cell depletion. A single center experience with a very long follow-up

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Background: Transplant-related mortality is considered too high to justify allogeneic hematopoietic stem cell transplantation (alloHSCT) as first-line treatment for chronic myeloid leukemia (CML) patients in chronic phase (CP). AlloHSCT is currently considered for patients failing to at least 2 TKIs or with disease in advanced phase. Nevertheless, the optimal timing for transplant referral is still not well defined.
Methods: We performed a retrospective analysis on 23 consecutive patients with CML in CP receiving first transplants from an HLA-identical sibling donor with partially T-cell depleted grafts from 1998 to 2016 at our center. Partial T-cell depletion (pTD) consisted of *in vitro* alemtuzumab incubation of a part of the graft for infusion at day 0 while the rest, containing 100×10^6 CD3+ cells/kg was given as a T-cell add-back at day 1. Molecular monitoring was performed by 3-month BCR-ABL1 RT-qPCR testing in peripheral blood during at least a 5-year period after HSCT. Thereafter, 3-month testing schedule was maintained where possible, or followed by a 6-month one.
Results: Median age at HSCT was 36 years (range, 18-58). Twelve patients were TKI-naïve at HSCT (1998-2001 period), 4 patients had presented suboptimal response or/and intolerance to imatinib (2002-2004 period), while the last seven patients had presented suboptimal response or/and intolerance to imatinib, dasatinib and nilotinib (2005-2016 period). All patients engrafted. 14 patients presented molecular relapse and one patient hematological relapse with a median interval from transplant to relapse of 9 months (range, 5-70). 17 patients received DLIs (15 for relapse and 2 for mixed chimerism), while 4 patients in relapse also received TKI. Without prior administration of DLI, 3(13%) patients presented grade II aGvHD and 2 patients moderate cGvHD. After DLI, aGvHD occurred in 3 and cGvHD in 3 patients. One patient died of disease progression 3 years after HSCT and one of myocardial infarction 19 years after HSCT. With a median follow-up of 14.4 years (range 2.3-20), 15-year OS and LFS were 95%. At the time of the analysis 21/23 patients were alive and in major molecular response.
Conclusion: These results of excellent long-term survival and no transplant-related mortality suggest that pTCD improves the outcome of CP-CML patients transplanted from an identical sibling donor and they can be useful for deciding risk-adapted strategies.

1034

Gilteritinib prolongs survival in patients with FLT3-mutated relapsed/refractory AML: phase 3 ADMIRAL trial results

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Introduction: Gilteritinib is a potent/selective oral FLT3 inhibitor. Based upon interim analysis response rates from the ADMIRAL phase 3 study

of gilteritinib vs salvage chemotherapy (SC) in patients (pts) with relapsed/refractory (R/R) *FLT3*-mutated (*FLT3*$^{mut+}$) AML (NCT02421939), gilteritinib was approved as single-agent therapy in this population. We present the final results of this pivotal trial.

Methods: Adults with confirmed *FLT3*$^{mut+}$ AML (*FLT3*-ITD or *FLT3*-TKD D835/I836 mutations) refractory to induction chemotherapy, or in untreated first relapse, were randomized (2:1) to receive continuous 28-day cycles of 120-mg/day gilteritinib or prerandomization-selected SC: low-dose cytarabine (LoDAC), azacitidine (AZA), mitoxantrone/etoposide/cytarabine (MEC), or fludarabine/cytarabine/granulocyte colony-stimulating factor/idarubicin (FLAG-IDA). Prior FLT3 inhibitor use, other than midostaurin or sorafenib, was excluded. Overall survival (OS) and the combined rate of complete remission/complete remission with partial hematologic recovery (CR/CRh) were co-primary endpoints. Safety/tolerability was also examined.

Results: A total of 371 pts were randomized: 247 to gilteritinib and 124 to SC (MEC, 25.7%; FLAG-IDA, 36.7%; LoDAC, 14.7%; AZA, 22.9%). Median age was 62 years (range, 19-85). Baseline *FLT3* mutations were: *FLT3*-ITD, 88.4%; *FLT3*-TKD, 8.4%; both *FLT3*-ITD and *FLT3*-TKD, 1.9%; unconfirmed, 1.3%. Overall, 39.4% of pts had refractory AML and 60.6% had relapsed AML. Patients assigned to gilteritinib had significantly longer OS (9.3 months) than SC (5.6 months; hazard ratio for death = 0.637; $P = 0.0007$); 1-year survival rates were 37.1% and 16.7%, respectively. The CR/CRh rates for gilteritinib and SC were 34.0% and 15.3%, respectively (nominal $P = 0.0001$); CR rates were 21.1% and 10.5%. Common adverse events (AEs) in all randomized pts were febrile neutropenia (43.7%), anemia (43.4%), and pyrexia (38.6%). Common grade ≥3 AEs related to gilteritinib were anemia (19.5%), febrile neutropenia (15.4%), thrombocytopenia (12.2%), and decreased platelet count (12.2%). Exposure-adjusted serious treatment-emergent AEs were less common with gilteritinib (7.1/patient-year) than SC (9.2/patient-year).

Conclusions: In pts with R/R *FLT3*$^{mut+}$ AML, gilteritinib demonstrated superior efficacy compared with SC and had a favorable safety profile. These results change the treatment paradigm for R/R *FLT3*$^{mut+}$ AML and establish gilteritinib as the new standard of care.

1035

A simple acute phase protein score to predict long-term survival in patients with acute myeloid leukemia

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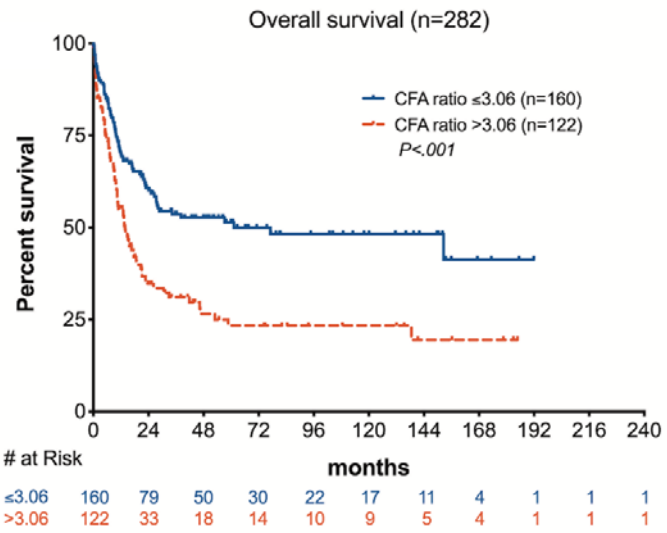
Introduction: High levels of acute phase reactants can be associated with adverse outcome in patients with various solid tumor types. For patients with acute myeloid leukemia (AML), this correlation is unknown.

Methods: We retrospectively investigated the prognostic value of pre-treatment acute phase protein levels in 282 consecutive newly diagnosed AML patients undergoing at least one cycle of intensive induction chemotherapy between 2000-2018. We developed a new score integrating pre-treatment C-reactive protein (CRP), fibrinogen, and albumin termed CFA ratio. Moreover, we assessed the modified Glasgow prognostic score (mGPS), which comprises elevated CRP and decreased albumin levels to predict outcome.

Results: Patients were stratified into two groups: Patients with a CFA ratio below 3.06 had decisively better progression free (26.2 vs. 7.7 months; P <.001), disease free (56.4 vs. 8.7 months, P <.001) and overall survival (61.2 vs. 13.8 months; P <.001; Figure 1). Results remained significant for PFS and OS when adjusting for confounders including ELN risk group. Early mortality also tended to be lower in the low CFA ratio group. Finally, patients with lower modified Glasgow prognostic score (mGPS) had better outcome.



Conclusion: Our data suggest that an elevated CFA ratio as well as a high mGPS are associated with adverse outcome in patients with newly diagnosed AML undergoing intensive induction. These parameters should be prospectively evaluated for their contribution to risk profiling in AML patients as they may provide an additional, rapidly available assessment of prognosis at diagnosis.

1037

High dose bendamustine and melphalan (BenMel) – a novel conditioning approach before second autologous transplantation in myeloma patients

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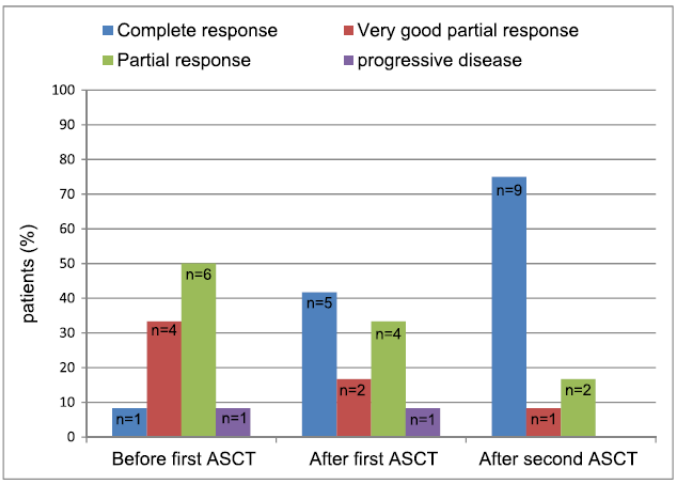
Introduction: Consolidation in myeloma patients with high-dose melphalan chemotherapy (Mel HDCT) and autologous transplantation (ASCT) is standard of care. However, definite cure remains exceptional despite intensive treatment, and improving effectiveness of HDCT remains an unmet clinical need. Combining intensified bendamustine with melphalan (BenMel) for HDCT together with ASCT may represent a novel conditioning option.

Methods: In this single-center prospective study, we analyzed safety and efficacy of combining dose-intensified bendamustine (200 mg/m^2 on days -4/-3) with high-dose melphalan (100 mg/m^2 on days -2/-1) before a second (tandem) ASCT in 12 myeloma patients, who have received Mel HDCT/ASCT1 for consolidation of first-line remission. We compared toxicities, engraftment and duration of hospitalization between melphalan conditioning (ASCT1) and BenMel HDCT/ASCT2.

Results: Twelve patients received BenMel HDCT before ASCT2 because of high-risk cytogenetics and/or failure to achieve complete remission (CR) after Mel HDCT/ASCT1. Comparing Mel HDCT/ASCT1 and BenMel HDCT/ASCT2, we observed no differences in hematologic recovery and tolerance. Acute renal injury after BenMel conditioning occurred in three (25%) patients, but was reversible in all cases, and there were no treatment related deaths. Cardiac toxicities were observed in two patients (17%) after Mel HDCT/ASCT1, but were not observed following BenMel HDCT/ASCT2. Neutrophil recovery was similar after BenMel HDCT/ASCT2 as compared to Mel HDCT/ASCT1 (day +11 versus day +12), and all patients had complete neutrophil and platelet recovery after BenMel HDCT/ASCT2. Duration of hospitalization was not different after Mel HDCT/ASCT1 compared to BenMel HDCT/ASCT2 (17 days and 18 days, respectively). The CR rates were increasing from 42% after Mel/ASCT1 to 75% after BenMel/ASCT2 (Figure 1). We identified a PFS one year after ASCT2 was 67% and OS was 83%, and the median PFS was 18 months, while the median OS was not reached.



Conclusions: These data suggest that dose-intensified bendamustine with melphalan HDCT is safe and effective and warrants a prospective randomized comparison to standard melphalan HDCT in myeloma patients.

1039

Renal toxicity of dose-intensified bendamustine-based high-dose chemotherapy in lymphoma and myeloma patients

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Background: Relapse after BEAM high-dose chemotherapy (HDCT) and autologous stem cell transplantation (ASCT) remains the major cause of death in patients with lymphomas and multiple myeloma (MM) after HDCT. Introducing dose-intensified bendamustine by replacing carmustine in the BEAM regimen (BeEAM) or by combining it with melphalan (BenMel) is a promising strategy to lower the relapse rates, but renal toxicity emerges as a major concern.
Methods: We investigated renal toxicity in a series of consecutive lymphoma patients treated with BeEAM and MM patients treated with the same dose of 400 mg/m^2 bendamustine together with full-dosed (200 mg/m^2) melphalan.
Results: A total of 122 consecutive patients were analyzed. Acute kidney injury related to bendamustine (rAKI) occurred in 51 patients (41.8%) and was completely reversible in n = 50/51 (98%). rAKI was mild to moderate in 90% of affected patients and did not increase treatment-related mortality after ASCT. 3 of 51 patients (6%) with rAKI required transient renal dialysis to enable recovery from renal damage. Occurrence of rAKI correlated ($p < 0.05$) with age >60 years, previous AKI, cardiovascular comorbidities and concomitant nephrotoxic drugs. In addition, rAKI correlated ($p = 0.004$) with the development of cardiovascular complications during hospitalization. No differences in the incidence of rAKI were observed in both MM and lymphoma patients.
Conclusion: Our data suggest that treatment-related acute renal toxicity is a common event in lymphoma and MM patients receiving dose-intensified bendamustine HDCT before ASCT. However, renal impairment is reversible and manageable. Importantly, our data identify a subgroup of patients at increased risk for the development of renal damage following bendamustine-based HDCT. Such patients should be strictly monitored during hospitalization, and a generous hydration strategy before, during and after administration of bendamustine is recommended. Moreover, assessing the pre-transplant renal risk profile may help to identify those patients, which may not be candidates for bendamustine-based HDCT thereby avoiding prolonged hospitalization due to rAKI and eventually dialysis. Thus, our results may contribute to design appropriate selection criteria for dose-intensified bendamustine as part of the conditioning regimens preceding HDCT/ASCT in lymphoma and MM patients.

1040

Autologous stem cell transfusions on multiple days in patients with multiple myeloma – does it matter?

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Background: Autologous stem cell transplantation (ASCT) requires at least 2.0´10^6 CD34+ cells/kg b.w. In patients with poor CD34+ mobilization at the first apheresis day, additional apheresis procedures are needed together with additional DMSO doses, increasing the risk of DMSO associated toxicities. At our center, maximum transplant volume is 300 mL per day with DMSO concentration of 5% with a maximum of 60 g DMSO per day. For patients with transfusion volumes above these limits, transplant procedure is split over several days.
Methods: We evaluated the effect of ASCT split over multiple days compared to single day procedure in 271 consecutive myeloma patients undergoing first standard 200 mg/m^2 melphalan conditioning with ASCT at our center between 2006 and 2014.
Results: 244 pts (90%) received ASCT in 1 day (Tx_1 group). The $Tx_{2\text{-}3}$ group comprised 23 patients receiving stem cells on two days and four patients on three days. The Tx_1 and $Tx_{2\text{-}3}$ groups did not differ in clinical characteristics, induction treatment, and remission status. We observed that plerixafor tended to be needed more frequently in $Tx_{2\text{-}3}$ ($p = 0.0715$). At collection day, peripheral CD34+ counts were lower in $Tx_{2\text{-}3}$. The final transplanted autograft volume was higher in $Tx_{2\text{-}3}$. Transplanted CD34+ cells/kg b.w. were lower in $Tx_{2\text{-}3}$, suggesting higher dilution of CD34+ cells ($p < 0.0001$). Median neutrophil recovery was 18 days for $Tx_{2\text{-}3}$ vs 12 days for Tx_1 ($p = 0.0048$), for platelets 18 vs 14 days ($p = 0.0004$). $Tx_{2\text{-}3}$ had longer median hospitalization (23 vs 19 days; $p = 0.0006$), and fever was more frequent in $Tx_{2\text{-}3}$ than Tx_1 (96% vs 76%; $p = 0.0131$). Finally, $Tx_{2\text{-}3}$ had shorter median relapse-free survival (21 vs 40 months; $p = 0.0245$), and shorter median OS (55 vs 93 months; $p = 0.0134$).
Conclusion: Multiple day transplantation is associated with poor CD34+ mobilization and was observed in ~10% of myeloma pts. Pts with multiple day transplant procedures had later neutrophil/platelet engraftment, longer hospitalization, more febrile episodes, and inferior OS/RFS. Multiple day transplant procedures assign myeloma pts to a high-risk group, deserving careful monitoring. These pts may benefit from prolonged maintenance treatment, but there may be poor tolerance due to a reduced stem cell pool. Alternatives to multiple day transplant procedures, e.g. reduced DSMO dose in the autografts in pts with high transplant volume, should be evaluated.

1041

MRD negativity and poor stem cell mobilization predict excellent outcome after autologous transplant in NPM1 mutant AML

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Introduction: *NPM1* mutations (*NPM1*mut) are associated with favorable prognosis in acute myeloid leukemia (AML) patients, but outcomes after autologous transplantation (ASCT) vary widely when only considering pre-treatment characteristics. Therefore, the inclusion of post-treatment characteristics into therapeutic algorithms may provide important helpful information.
Methods: We assessed the value of combining two post-treatment parameters in *NPM1*mut AML patients to predict outcome after ASCT in first remission (CR1). Accordingly, achievement of MRD negativity in this study was defined by *NPM1*mut levels $<10^{-5}$; and low versus high mobilization of peripheral CD34+ cells at peripheral stem cell collection was compared.
Results: Among 42 *NPM1*mut AML patients, 48% achieved *NPM1*mut MRD-negativity (MRD^{neg}) in the bone marrow after two induction cycles. Median level of circulating CD34+ cells at the day of peripheral stem cell collection was 45/µL, thereby separating CD34+^{low} and CD34+^{high} patients. Overall survival (OS) was better for MRD^{neg} versus MRD^{pos} patients (median not reached versus 18 months; P <.0001) and for CD34+^{low} versus CD34+^{high} mobilizers (not reached versus 34 months; P = .011). The number of deaths was higher for CD34+^{high} versus CD34+^{low} mobilizers (62% versus 19%, P = .01). Thus, higher levels of mobilized CD34+ cells at the day of peripheral stem cell collection and MRD-positivity in the bone marrow after two cycles of induction treatment are associated with adverse outcome. By combining these two parameters, the OS of the subgroup of MRD^{neg}/CD34+^{low} patients was excellent (no deaths), but was dismal in MRD^{pos}/CD34+^{high} (median 15 months) suggesting that MRD status confers stronger prognostic information than the stem cell mobilization potential in *NPM1*mut patients. Multivariate analysis confirmed the independent positive predictive value of MRD-negativity and low stem cell mobilization for PFS and OS.

Conclusion: MRD^{neg}/CD34^{low} *NPM1*mut AML patients have an excellent outcome after autologous transplant consolidation. In contrast, MRD^{pos}/CD34+^{high} *NPM1*mut AML patients have a 100% probability for treatment failure despite achieving cytomorphologic complete remission. These results may contribute to the selection of patients with *NPM1*mut AML, who will benefit most from ASCT consolidation and, ultimately, may facilitate the decision process between allogeneic and autologous stem cell transplantation in this genetic subgroup.

1042

Introducing CAR-T therapy in Switzerland – a clinical and diagnostic perspective

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Background: In 10/2018, the first CAR-T treatment using Kymriah® (Novartis) was approved by Swissmedics for commercial application in patients below 26 years with rel/ref B-ALL and for adults with DLBCL after ≥2 lines. Agreements between insurance companies, cantonal authorities and the company are required for individual patients in this initial era.
Methods: Our center initiated an accompanying laboratory program to monitor the risk for CRS development in CAR-T cell recipients. We established IL-6 measurement (ELISA) and a ddPCR assay for CAR-T cell specific TCR measurement using peripheral blood (PB). We elucidated the immunologic environment by repetitive assessments of lymphocyte subpopulations.
Results: A 25-yr old female with *BCR-ABL1*+ B-ALL relapsed 5 years post-transplant. Ponatinib and salvage chemotherapy induced molecular MRD-negative CR2 before admission for CAR-T therapy. Lymphocyte apheresis in 11/2018 yielded sufficient CD3+ cells (11.68 x 10(9) cells) allowing generation of CAR-T cells (Novartis, USA) within specification limits. In 01/2019, cyclophosphamide/fludarabine was given for lymphocyte depletion (3 days) followed by Kymriah® infusion. Peripheral lymphocytes declined to 0.16 G/L, with complete B-cell aplasia. The patient developed no CRS or CRS associated encephalopathy. CRP and IL-6 levels remained near-normal, and maximum ferritin peaked at 2'805 ng/mL. Tregs (FoxP3+/FoxP3Δ2) declined from 12.8% to 8.7% of CD4+/CD3+ cells. By ddPCR, Kymriah®-specific TCR in PB became detectable at day +8 following Kymriah® infusion, with a maximum of 15'330 copies of CTL019/µg DNA at day +14 and rapid decrease thereafter to undetectable levels from day +23. Further follow-up was uneventful apart from HSV and RSV infections in weeks 3-5, and ongoing immunoglobulin deficiency requiring substitution.
Conclusion: CAR-T therapy entered hemato-oncologic practice in Switzerland. A first patient with MRD-negative B-ALL experienced an uneventful post-infusion course corresponding to near-normal IL-6 and CRP levels, with mild ferritin elevation. Using a newly developed ddPCR assay, we could monitor CAR-T cell levels in the PB. Compared to assays used in trials, a moderate increase of CAR-T cells in PB was observed peaking at day +14 followed by a rapid decline (Fig. 1). We intend to expand this accompanying laboratory program in subsequent CAR-T cell treatments aiming to monitor CRS or CRS associated encephalopathy during both in- and outpatient periods.

1047

Excess rebound thrombocytosis after induction chemotherapy is a strong biomarker for favorable outcome in AML patients

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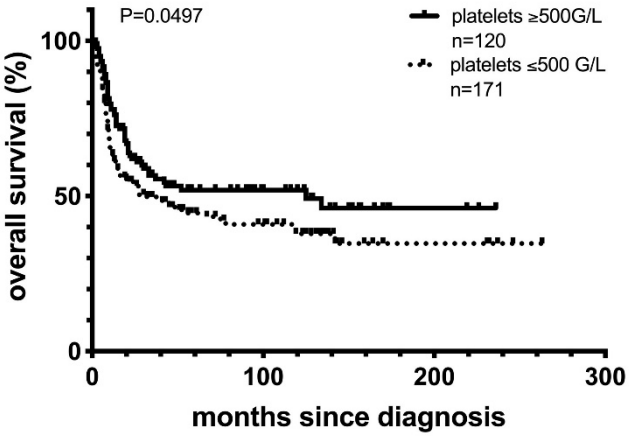
Introduction: Whereas the molecular background of AML is being increasingly clarified, the kinetics of hematologic recovery following induction chemotherapy remain obscure. In particular, platelet recovery may vary between incomplete and excess recovery in AML patients achieving CR.
Methods: We analyzed platelet recovery after a first induction cycle in 291 consecutive AML pts undergoing intensive therapy. We defined excess platelet rebound (EPR) as platelet increase exceeding 500 G/L.
Results: We observed the phenomenon of EPR following induction treatment in 120 (41.2%) pts (termed "EPR+" patients). Platelet levels reached their maximum value after a median of 32 days. EPR+ patients had lower platelets at diagnosis, higher marrow infiltration, and more frequently *NPM1* mutations than EPR negative ("EPR-") pts, and they were associated more often with ELN favorable risk. Absence of EPR correlated with complex karyotypes, ELN intermediate-I or adverse risk, and therapy-related AML. We found no correlation between inflammation parameters such as CRP and EPR. Importantly, OS was better in EPR+ patients (125 versus 41 months; P = .04), as was DFS. By multivariate analysis, EPR+ was an independent parameter associated with favorable outcome. Finally, high thrombopoietin (TPO) levels at diagnosis indicated EPR+ (p <0.0001), while *GATA-1*, *GATA-2* and *MPL* mRNA expression did not differ between EPR+ and EPR- patients. Transcription factors blocking early megakaryopoiesis were upregulated in EPR-, while *NFE2* involved in late megakaryocyte differentiation was increased in EPR+ patients.



Conclusion: Achieving EPR (platelets ≥500 G/L) following first induction cycle is associated with favorable outcome and good-risk genetic subgroups of AML. Our study provides novel insights into the kinetics of platelet recovery following induction chemotherapy in AML patients.

1049

Marked peripheral blood plasmacytosis as epiphenomenon of angioimmunoblastic T-cell lymphoma mimicking plasma cell leukemia

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Background: Marked plasmacytosis in peripheral blood with simultaneously increased serum protein raises the suspicion of plasma cell leukemia (PCL). However, the confirmation of the clonal origin of these plasma cells is crucial as there are various diseases associated with polyclonal plasma cell proliferation. We report a very rare case with marked plasmacytosis mimicking PCL as an epiphenomenon prior to the definitive diagnosis of an angioimmunoblastic T-cell lymphoma (AITL).
Results: In April 2019, a 70-year-old female patient was referred to our hospital for evaluation of plasmapheresis because of suspected PCL with signs of hyperviscosity syndrome as she presented with a rapid onset of dyspnea NYHA grade III. Blood values showed a moderate to severe anemia with a hemoglobin of 60 g/l, normal thrombocyte count and leukocytosis of 13.85 G/l due to an elevated plasma cell count of 29%. Additionally, elevated serum protein of 106 g/l with a low albumin fraction of 28 g/l was measured, but normal calcium and creatinine values were present. Strikingly, flow cytometric immunophenotyping of the peripheral blood revealed a polyclonal plasma cell population with a physiological marker profile and a very small clonal CD4+CD3dimPD-1+CD10+ T-lymphocyte population. Further diagnostic workup confirmed the diagnosis of an AITL Ann-Arbor stage IV with reactive plasmacytosis and consecutive hyperglobulinemia (IgG 35.5 g/l, IgA 11.65 g/l, IgM 31.7 g/l). Respiratory symptoms were attributed to the hyperviscosity syndrome and a coexisting respiratory syncytial virus infection. The patient was treated immediately with high dose intravenous corticosteroids, which rapidly led to the disappearance of the plasma cells in the peripheral blood and the decrease of serum protein as well as to clinical improvement.
Conclusion: Marked polyclonal plasmacytosis with consecutive hyperglobulinemia is a very rare first manifestation of AITL. Initial findings can lead to the suspected diagnosis of PCL and special attention should be devoted not to miss an underlying small AITL clone in flowcytometric analysis of peripheral blood samples of these patients.

1051

Pegylated Interferon in Myelodysplastic/Myeloproliferative Syndrome with Ringsideroblasts and Thrombocytosis

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Background: In the WHO 2016 the previously provisional entity refractory anemia with ring sideroblasts and thrombocytosis was recognized as a distinct entity and renamed as myelodydplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T). Although the diagnostic criteria are well defined and there are several studies of the molecular landscape of the disease, there is no approved treatment regimen, due to the lack of clinical trials. Therapeutic options are chosen either on a MDS- or MPN-based. Prevention of thromboembolic events is reasonable, especially in the presence of JAK2V617F mutation. However, the use of platelet-lowering drugs is often limited by anemia and hydroxyurea harbors a potential risk for secondary malignancies. We present 3 cases successfully treated with pegylated interferon (pIFN) and provide insight in the patients JAK2-allele burden measurement and additional mutations obtained with next generation sequencing (NGS).
Results: Three patients with diagnosis of MDS/MPN-RS-T were treated with a dose of 135µg pIFN during an observation period between 4 and 29 months. Thrombocyte values were rapidly decreased after treatment initiation. During observation period, hemoglobin levels remained stable. In one case an improvement in hemoglobin levels even was achieved. Side effects of the treatment was only minimal and well tolerated of all patients and no thromboembolic event occurred under additional acetylsalicylic acid medication. All 3 patients had a JAK2V617F mutation and the morphological finding of >15% ring sideroblast, as well as thrombocyte count above 600G/l. In the additional NGS analysis, two patients showed a mutation in the SF3B1 gene, one with an additional DNMT3A mutation. In the third case, NGS results revealed a mutation in the splicing factor U2AF1. JAK2-allelel burden was obtained at the beginning and showed a decrease of a mean value of 38% as measured by digital droplet PCR.
Conclusion: In summary we show that in patients with MDS/MPN-RS-T treated with 135µg of pIFN achieved rapid decrease of thrombocyte values without lowering hemoglobin levels. JAK2-allele burden reduction and a low side effect profile further demonstrate a valuable treatment option for these patients. However, larger studies are needed to confirm the benefit of the therapy and to fully evaluated clinical long term outcome.

1055

Impact of T-cell depletion on outcome in patients undergoing allogeneic hematopoietic stem cell transplantation for acute myeloid leukemia

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Background: Allogeneic stem cell transplantation (HSCT) remains the only curative option for most patients with acute myeloid leukemia. At the Geneva University Hospital, we use *in vitro* partially T-cell depleted grafts in selected patients with <0.1% Mesurable Residual Disease (MRD) at the time of transplantation.
Methods: We conducted a single-center retrospective analysis to compare outcomes in adult patients who were transplanted with T-cell replete or *in vitro*partially T-cell depleted grafts (TDEP) for AML in first complete remission between 1998 and 2018, with either HLA-identical or 10/10 matched unrelated donors. *In vitro* partial T-cell depletion was performed using alemtuzumab.
Results: A total of 145 patients were analyzed. 89 (61%) and 56 (39%) received partially TDEP and T-cell replete grafts, respectively. Patients in the partially TDEP group were significantly younger (median age 50 vs 58.5 years (p <0.05)). Their EBMT score was significantly lower (EBMT score 1: 11.2% vs 1.8%; score 2: 46.1% vs 25%; score 3: 34.8% vs 60.7%; score 4: 7.9% vs 7.1%; score 5: 0% vs 5.4%; p <0.05) and their Disease Risk Index was significantly lower (low: 3.4% vs 0%; intermediate: 83.1% vs 69.6%; high: 10.1% vs 30.4%; p <0.05). RIC regimen was used in 31.5% in the TDEP group in 66.1% in the T-cell replete group (p <0.05). Stem cell source was PBSC in 98.9% in the partially TDEP and 95.6% in the T cell replete group, respectively. The 3-year overall survival (OS) and progression-free survival (PFS) were 55.9% and 49.2% in the patially TDEP group and 42.2% and 41.6% in the T-cell replete group (p = 0.94 for OS and 0.53 for PFS). The 3 year cumulative incidence (CI) of grade II-IV aGVHD was 22% in the TDEP group and 61.7%n the T-cell replete group (p <0.05) and for cGVHD it was 11.9% and 40.1%, respectively (p <0.05). The CI of non-relapse mortality at 3 years was 27% in the T-cell replete group and 15.1% in the TDEP group (p = 0.124) and the CI of relapse at 3 years was 35.8% and 31.5% respectively (p = 0.53). Importantly, partial T-cell depletion had no impact on OS (HR 0.99%, p = 0.98) in a multivariate analysis.
Conclusion: Partial T-cell depletion significantly reduced the CI of grade II-IV aGVHD and cGVHD in patients with AML with no significant impact on OS and RI.

1058

Trends of Incidence, Mortality, and Survival of Chronic lymphocytic leukaemia/ Small lymphocytic lymphoma in Switzerland between 1995 and 2014

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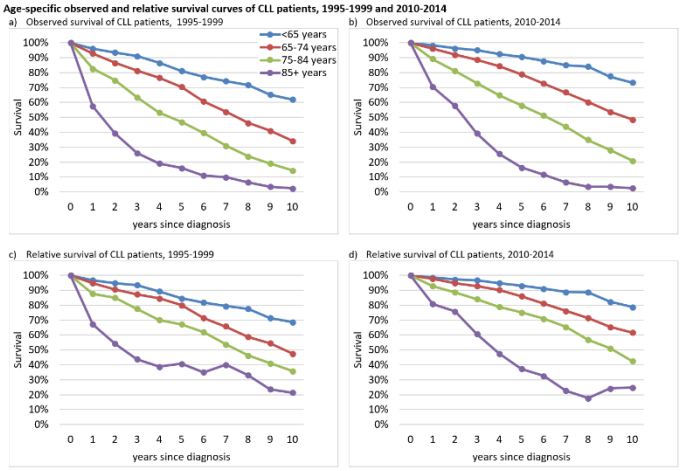
Background: Chronic lymphocytic leukaemia (CLL)/ small lymphocytic lymphoma (SLL) is the most prevalent leukaemia and the second most common B cell neoplasm in Western countries typically diagnosed in elderly people. During the last 20 years, CLL/SLL treatment has undergone considerable changes that have been justified by improved clinical outcomes in randomised controlled trials. Currently, no data have been published from Switzerland to assess effectiveness of recent health-care advances in CLL/SLL on a population-based level.
Methods: We retrospectively studied registry data from the National Institute for Cancer Epidemiology and Registration (NICER) database in Switzerland from 1995 to 2014. We investigated trends in incidence, mortality and survival for consecutive 5-year periods.
Results: We obtained 5612 cases with chronic lymphocytic leukaemia or small lymphocytic lymphoma. Median age at diagnosis was 72 years. Age-adjusted incidence rate shows a slight non-significant increasing trend from 4.4 per 100'000 person-years from 1995 until 2009 to 4.8 per 100'000 person-years from 2005-2009, followed by a sharp decline to 3.8 from 2010-2014 due to reporting delay . Five- and 10-year age-specific RS increased from 76.8% (95%CI 72.9-80.2) and 53.2% (95%CI 48.3-57.9) in 1995-1999 to 83.8% (95%CI 81.0-86.3, p <0.01) and 63.2 (95%CI 59.1-66.9, p <0.01) in 2010-2014. OS and RS is better in younger patients. Incidence and mortality were significant higher in males and females had better relative survival.



Incidence and mortality of CLL/ SLL in Switzerland, 1995-2014

	1995-1999			2000-2004			2005-2009			2010-2014		
	N°	Rate[b]		N°	Rate[b]		N°	Rate[b]		N°	Rate[b]	
	per year	Crude	adjusted	per year	Crude	adjusted	per year	Crude	adjusted	per year	Crude	adjusted
Incidence												
Overall	392	5.6	4.4	455	6.3	4.6	500	6.6	4.8	440	5.4	3.8
Sex												
Males	228	6.7	6.1	255	7.2	6.2	295	7.9	6.4	264	6.6	5.1
Females	164	4.6	3.1	200	5.5	3.5	205	5.3	3.4	176	4.3	2.7
Age												
<65 years	118	2.0	1.9	126	2.0	1.9	142	2.2	1.9	123	1.9	1.6
65-74 years	119	20.7	20.5	130	21.8	21.6	160	25.1	24.8	140	19.1	19.1
75-84 years	113	31.8	31.2	148	36.2	35.1	139	32.3	32.2	122	26.5	26.2
85+ years	41	31.0	31.0	55	37.8	37.8	58	35.0	35.0	55	29.4	29.4
Mortality												
Overall	133	1.9	1.2	151	2.1	1.3	161	2.1	1.2	154	1.9	1.0
Sex												
Males	78	2.3	2.0	82	2.3	1.9	89	2.4	1.8	87	2.2	1.5
Females	54	1.5	0.8	69	1.9	1.0	72	1.9	0.8	68	1.7	0.7
Age												
<65 years	14	0.2	0.2	16	0.3	0.2	14	0.2	0.2	9	0.1	0.1
65-74 years	27	4.6	4.5	34	5.7	5.6	29	4.5	4.4	31	4.2	4.1
75-84 years	49	13.8	12.9	56	14.2	13.5	61	14.3	13.3	56	12.2	11.5
85+ years	42	32.2	32.2	45	30.9	30.9	57	34.4	34.4	59	31.2	31.2

[a] Incidence: mean annual case frequency extrapolated to the whole Swiss population from cases observed in the cancer registries.
Mortality: mean annual case frequency derived from nationwide cause of death statistics.
[b] per 100,000 person-years.

Conclusion: Age-adjusted incidence rates are similar over time and compared to other countries, but timely reporting of CLL diagnosis remains a challenge. Survival has improved over time across all age groups.

1093

Evaluation of a clinical tool for early detection of infection and sepsis in patients after high-dose chemotherapy

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Introduction: Patients in aplasia after high-dose chemotherapy and autologous haematopoietic stem cell transplantation (HD-aHSCT) are at high risk for infection and sepsis. Timely initiation of empiric antibiotic treatment is crucial but the best point of time is unclear. A clinical tool for early sepsis recognition by ward nurses is associated with improved clinical outcome parameters in patients with sepsis (Torsvik 2016). We evaluated the utility and effectiveness of a clinical tool for early detection and initiation (EDI) of empiric antibiotic treatment in patients after HD-aHSCT. This EDI-tool was systematically applied by trained ward nurses and consists of a combination of vital parameter changes (heart rate, hypotension, respiration rate, level of consciousness, O_2-saturation, temperature) with clinical signs and symptoms frequently associated with infection and sepsis after HD-aHSCT. The clinical utility and effectiveness of this tool was evaluated.

Methods: Prospective cohort study and historical comparison with a cohort of HD-aHSCT patients from 01/2012 to 08/2016 when empiric antibiotic treatment was triggered by conventional criteria for febrile neutropenia (FN) or physicians¢ discretion.

Results: 174 patients (median age 58.3 years) treated with HD-aHSCT from 2012 to 2018 at a single institution with a total of 186 periods of aplasia were included. EDI was systematically applied in 86 periods of aplasia after HD-aHSCT from 09/2016 to 12/2018: Treatment with empiric antibiotics was initiated in 67.5% (56/83) of cases due to EDI-criteria, in 22.8% (19/83) because of conventional criteria for FN. A clinical diagnosis of infection and specific treatment occurred in 4.8% (4/83) independent of EDI- or FN-criteria; no antibiotics were administered in absence of FN and EDI-criteria in 4.8% (4/83), respectively. Table 1 for further data.

Table 1: Demographic and clinical data, Median (95% Confidence interval)

	Before EDI 01/2012 - 08/2016 N=103	After start of EDI 09/2016 – 12/2018 N=83	P-value
Age (years)	**58.6** (54.8 – 58.5)	**56.4** (50.4 – 56.8)	**0.10**
Type of HD-Treatment	**Melphalan: 78.6%** (81/103) **BEAM: 16.5%** (17/103) **Others (GC): 4.8%** (5/103)	**Melphalan: 66.3%** (55/83) **BEAM: 16.7%** (14/83) **Others (AML,GC): 16.7%** (14/83)	**0.068** **1.0** **0.013**
Duration of aplasia (days)	**7.0** (6.6 – 7.4)	**7.0** (6.5 – 7.2)	**0.57**
Blood cultures positive	**43.7%** (45/103)	**30.1%** (25/86)	**0.068**
No febrile neutropenia, No antibiotic treatment	**4.8%** (5/103)	**4.8%** (4/83)	**1.0**
Duration of i.v. antibiotic treatment (days)	**9** (8.6 – 10.7)	**7** (6.9 – 9.0)	**0.025**
30-day mortality	**2.7%** (3/103)	**0%** (0/83)	**0.25**
Rate of ICU-admission	**6.8%** (7/103)	**2.4%** (2/83)	**0.2**
Duration of ICU stay per aplasia (days)	**0.48** (0.09 – 0.89)	**0.17** (-0.07 – 0.41)	**0.17**
Duration of hospitalisation (days)	**21.1** (19.7 - 22.4)	**20.0** (19.0 – 21.0)	**0.22**

Legend table 1: AML – acute myeloid leukemia, BEAM - carmustine, etoposide, cytarabine and melphalan, EDI - tool for early detection and initiation, GC – germ cell tumour, HD – high dose, ICU – intensive care unit.

Conclusions: A clinical tool for early detection of infection and sepsis, systematically applied by trained ward nurses, is useful for triggering treatment with empiric antibiotics in patients in aplasia after HD-aHSCT and does not lead to longer administration of i.v.-antibiotics. Associated clinical outcomes seem to trend favourably but should be interpreted with caution due to small numbers.

Reference: Torsvik M et al. [2016]. Early identification of sepsis in hospital inpatients by ward nurses increases 30-day Survival. Critical Care (2016) 20:244 DOI 10.1186/s13054-016-1423-1

1095

Lymphocyte apheresis before CAR-T therapy – a first series of eight patients at the University Hospital Bern

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Background: Since October 2018 CAR-T therapy with Kymriah® (Novartis) is available in Switzerland for treatment of relapsed or refractory B-ALL patients below the age of 26 years or patients with diffuse large B-lymphoma (DLBCL) relapsed or refractory after at least two lines of treatment. Until April 2019, eight patients (one pt with B-ALL and seven pts. with DLBCL) underwent lymphocyte apheresis before CAR-T therapy at the University Hospital Bern.

Methods: Each of the lymphocyte apheresis procedures was performed according to the Novartis handbook for production of Kymriah®. The pre-collection requirements were fulfilled and the collection recommendations were applied in all patients. We analyzed the collection characteristics in our set of patients.

Results: Nine uneventful lymphocyte collections have been conducted in eight patients with a median age of 70 years (range 25 – 81 years). One patient underwent two apheresis procedures since the final product from the first apheresis did not fulfill the quality requirements after manufacturing by Novartis. The median peripheral leukocyte and lymphocyte levels by multiparameter flow cytometry (BD FACSCanto II) at start of apheresis were 7.51×10^9/l (range 5.15×10^9/l- 18.10×10^9/l) and 1.51×10^9/l (range 0.28×10^9/l – 2.9×10^9/l), respectively. The median level of CD3+ cells at the start of the apheresis was 1.32×10^9/l (range 0.11×10^9/l – 2.66×10^9/l). The median collection duration was 173 minutes (range 114 – 273 minutes) and the median ratio of volume processed to WBV (patient whole blood volume) was 2.45 (range 1.9 – 6.7). There was a significant negative correlation between CD3+ levels and volume processed/WBV (Spearman rank correlation, r: -0.810, p = 0.022). The median end value of collected CD3+ cells was 6.01×10^9 (range 2.41 $x10^9$ - 11.9 $x10^9$) fulfilling the requirement of the minimum of 1.0×10^9 CD3+ cells for all patients as requested for Kymriah® production. Finally, the CD4/CD8 ratio at start and end of the apheresis procedure were comparable (median levels (range): 0.77 (0.36-1.71) vs 0.80 (0.30-1.60), respectively; p = 0.34; Wilcoxon matched-pairs signed rank test).

Conclusion: Our single-center experience of lymphocyte collection for CAR-T therapy suggests that uneventful, efficient and successful CD3+ cell collection can be equally performed in older patients as well in patients with very low peripheral CD3+ values without changes in the CD4/CD8 ratio.

1097

Acquired hemophilia A associated with and complicating multiple myeloma

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Acquired hemophilia A (AHA) is a rare autoimmune disease caused by circulating autoantibodies inhibiting coagulation factor VIII (FVIII), leading to a clinically significant bleeding diathesis. In half of AHA cases no underlying cause is identified, while in the other half AHA is associated with autoimmune disease, cancer, use of certain drugs, pregnancy or the post-partum period. An association of AHA and multiple myeloma is extremely rare.

We present the case of a 77-year-old man admitted to a peripheral hospital due to a compartment syndrome of the left calf following a minor trauma. The patient was on apixaban for atrial fibrillation. Two months earlier, he had undergone a colonoscopy with polyp removal because of gastrointestinal bleeding; no coagulation tests were available from that time. Despite two surgical interventions, the calf bleeding persisted. Laboratory tests revealed a prolonged aPTT (119s) and a FVIII:C of 2%. AHA was suspected and the patient was transferred to our hospital. A high titer FVIII inhibitor of 102 BU/ml confirmed the diagnosis. Treatment with steroids (1 mg/kg body weight) and cyclophosphamide 150 mg/d was started. In addition, we performed 7 cycles of immunoadsorption to rapidly reduce the FVIII inhibitor. Within 20 days, aPTT and FVIII:C had normalized.

In search of an underlying cause, further diagnostics revealed an IgG kappa paraprotein of 9.2 g/l. Bone marrow biopsy showed clonal plasma cell infiltration of 15%, and the skeletal survey multiple hypo-dense lesions in the vertebras. No other cause of AHA was identified. Based on these findings, we diagnosed multiple myeloma and initiated VRD (bortezomib/lenalidomide/dexamethasone) therapy. On day 11 of the 2nd VRD cycle (58 days after diagnosis), a laboratory control demonstrated a low-titer FVIII inhibitor of 0.31 BU/ml, a normal FVIII:C and aPTT. Clinical evaluation was favorable as well, as no further bleedings occurred.

Treatment of AHA is based on four pillars: control of bleeding, avoidance of procedures that may induced bleedings, inhibitor eradication and treatment of the underlying disease. This case, together with others described in the literature, emphasizes the possibility of plasma cell diseases as a underlying cause of AHA. Accordingly, protein-electrophoresis, immune-fixation and free light chains should be assessed in all cases of AHA. Early intervention with immunoadsorption can be lifesaving in acute cases with high FVIII inhibitor titers.

1098

Management of Erroneous Subcutanous Injection of Vincristine

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The extravasation of chemotherapeutic drugs is a potentially severe complication that has to be considered an oncological emergency. Consequences depend on a variety of factors, especially whether the nature of the agent involved is vesicant or irritant and the amount of the extravasated substance.

Vincristine is part of the group of vesicant chemotherapeutic drugs and has to be given strictly intravenously. In case of extravasation, chemical cellulitis with symptoms ranging from erythema to cutaneous necrosis is the consequence, with the risk of one third of ulceration and necrosis. A previous study on 7 patients demonstrated the save administration of hyaluronidase as an antidote to extravasation of vinca alkaloids, including one patient with extravasation of vincristine.

We report the case of a 49-year-old non-obese woman with pre-B cell acute lymphoblastic leukaemia (ALL) who was under induction therapy and was enrolled in the GRAALL-2014 study, a multicenter trial for the treatment of ALL. On the 8th day of induction treatment, 2 ml containing 2mg of vincristine were administered subcutaneously in the abdominal wall by mistake. The error was discovered only 4 days later, when the patient suffered from increasing pain at the injection site. As the injection was done 4 days before, no emergency measurement as liposuction or hyaluronidase administration was undertaken. Our patient was treated with topical dimethyl sulfoxide (DMSO) plus vitamin E in the form of wet compresses that were applied during 1 hour every 6 hours for 13 days (day 4-17). Subsequent doses of vincristine IV were administered without provoking a so-called flare reaction. Over two years later, and after completion of the first year of maintenance therapy, a small hyperpigmented area in the injection site remains the only residue of the accident. In our case the extravasation has not been detected quickly and treatment was delayed for several days. Our case demonstrated that an extravasation of vincristine is not an absolute contraindication to continue the treatment with this drug if this treatment is considered crucial for the patient. The benefits of the treatment and the potential risk of a flare reaction have to be taken into consideration.

1102

Clinical follow-up of patients with idiopathic erythrocytosis

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Background: Erythrocytosis corresponds to an increase in the red blood count, hemoglobin (Hb) concentration and hematocrit (Htc) above the reference range adjusted to age, sex and living altitude. Clinical data on patients suffering from primary erythrocytosis are sparse. Due to its rare incidence, primary erythrocytosis frequently represents a challenge for the diagnosis. The management of such patients is demanding as no clear standards of treatment have been defined. We aim to analyze retrospectively the clinical and laboratory features and to describe the management of this entity at our clinic.

Methods: From 642 patients that were encoded as polycythemia in our internal registry, we identified 18 patients (2.8%) that did not fulfill the criteria for polycythemia vera, nor had a secondary underlying cause of polycythemia and were diagnosed with idiopathic erythrocytosis. They were followed in our clinic between 2006 and 2019.

Results: 18 patients with a median age of 32 years (r 20-67) were identified, 79% (14/18) were males. 83% of all patients (15/18) had a median follow-up of 27 months (r 3-132). Four presented initially with symptoms, probably, but not undoubtedly related to hyperviscosity (skin itching, erythromelalgia, headache). The median Hb-value at the presentation was 171 g/l (r 142-195) and Htc 49% (r 43-57%). The median Hb-value at the last consultation was 172 g/l (r 137-187 g/l) and Htc 48% (r 40-52%). No thromboembolic complications occurred in any of the patients either before or after diagnosis. There was no evolution to leukemia nor to any other myeloproliferative disorder. Twelve patients (67%) were initially treated with venesection which was interrupted during the follow-up, except in two cases. The median follow up since the interruption of venesection was 23 months (r 12-106). Symptomatic iron deficiency, as a consequence of venesection, was observed in 3 patients. Initially five patients received antiplatelet drugs which were withdrawn during the follow-up, except in those with other indications.

Conclusion: This population of patients with idiopathic erythrocytosis, predominantly constituting by young men, neither presented with thrombotic complication before or after the diagnosis, nor with other hematological transformation. The management observed is in line with the data emerging during the last few years that show, that primary erythrocytosis patients do not require a polycythemia vera-like management.

1120

Lymphocytopenia at diagnosis is an independent risk-factor in patients with IPSSR-low-risk MDS

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Background: Lymphopenia is associated with an increased mortality in several medical conditions, including solid tumours and lymphoma. Its impact in MDS is less well studied.

Methods: The Düsseldorf MDS-registry was searched for patients sufficiently characterized for IPSS-R-calculation and with information about the absolute lymphocyte count (ALC) at diagnosis. Cases with an ALC >5000/mcl were excluded. The influence of the ALC on overall survival (OS) was determined by Kaplan-Meier analysis and by multivariable Cox regression models.

Results: 1027 patients with a median follow-up of 28 months (mo) were identified, with a median ALC of 1215/mcl (SD of the mean 761; Range 0-4930/ mcl). Age <or> 65a was not associated with differences regarding the ALC, as were the different categories of the MDS-comorbidity index.
A higher ALC was seen in MDS-SLD/MLD-RS compared to MDS-SLD/MLD (median 1410 vs. 1090/mcl, p <0.001), primary vs. secondary MDS (median 1250 vs. 1040/mcl, p = 0.004), lower (IPSS-R [very]-low risk vs. higher (IPSS-R intermediate and [very-]high risk) MDS (1280 vs. 1170/mcl, p = 0.011) and in case of transfusion-independency (median 1260 vs. 1150/mcl p = 0.012). MDS with/without excess blasts or bone marrow fibrosis did not differ significantly. An ALC <1200/mcl was associated with lower peripheral blood values (median absolute neutrophil count 1350 vs. 1920/mcl [p <0.001]; median platelet count 100 vs. 138 G/l [p <0.001]; median haemoglobin 97 vs. 93 g/l, [p = 0.047]).
Survival analyses of cases with primary MDS not having received stem-cell transplantation or induction chemotherapy (n = 724) showed a significant lower OS with an ALC <1200/mcl (median 36 vs. 56 mo, p <0.002). The additional prognostic value was restricted to the IPSS-R low-risk group, but retained its independent prognostic value after inclusion into a multivariable Cox regression model together with age <or> 65a and LDH <or> normal value (240 U/l) (p = 0.023).
Conclusion: A low ALC is an independent prognostic marker for patients with IPSS-R low-risk MDS . Its association with a higher degree of cytopenias and several morphological and clinical features implies a causal relationship with the underlying pathobiology. This hypothesis is supported by the independency of the ALC from age and comorbidities. Further studies are necessary to define the ALC most suitable for prognostication and to identify the biology responsible for the impairment of lymphoid homoiostasis in MDS.

1121

Successful high dose chemotherapy and autologous stem cell transplantation in a patient with AIDS-related primary CNS lymphoma

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Background: In the HIV population, occurrence of primary CNS lymphoma (PCNSL) has declined over time with effective treatment using highly active antiretroviral therapy (ART), as its occurrence strongly correlates with a lower CD4 count. As a consequence, the incidence of PCNSL in AIDS patients (pts) appears to be extremely low. Current standard therapy for PCNSL in immunocompetent pts bases on high-dose methotrexate (MTX), cytarabine and thiotepa followed by autologous stem cell transplantation (ASCT). In pts with AIDS with a low CD4 cell count high dose chemotherapy with ASCT is not recommended because of the unfavourable prognosis.
Case presentation: A 37-year-old, Turkish pt had been diagnosed with HIV during pregnancy 12 years ago. As a result of lack of adherence to ART due to a difficult social situation, she experienced repetitive virologic failure and multiple resistances and suffered from various AIDS-defining infections. The patient was admitted to the emergency department with generalized epileptic seizure. CD4 cell count 7/µl, the MRI showed a large left-sided occipital lesion. Biopsy showed an EBV-associated PCNSL. Antiretroviral and corticoid therapy were initiated. The patient was treated with 4 cycles of high-dosed MTX, 2 cycles with cytarabine and thiotepa and rituximab. Stem cell collection could be performed with 9.55 million CD 34 pos./kg BW after 5th cycle. High dose CT with BCNU and thiotepa was started at CD4 lymphocyte count of 59/yl and engraftment was on day 11 after ASCT. Beside oral candidiasis and neutropenic fever, no severe infection occurred in the posttransplant episode.
Discussion: Patients with AIDS have a poorer prognosis due to the increased toxicity and limited intensity of CT, with median survival of 13.5 months, even when treated with multimodal therapy. Evidence for standard therapeutic approaches in these patients is lacking. We chose a curative approach considering the young age of our patient and absent comorbidities apart from the AIDS disease even if cases in literature are not reported.
Conclusion: Our case demonstrates the feasibility of high dose CT with ASCT in selected patients with AIDS-related PCNSL. The challenge of the infection-related complications is controllable with interdisciplinary collaboration of infectious disease specialists and haematologists/oncologists.

1122

Fulminant Hemophagocytic Lymphohistiocytosis in a patient with previously untreated Chronic Lymphocytic Leukemia

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Background: Hemophagocytic Lymphohistiocytosis (HLH) is a life-threatening condition with activation of macrophages, ineffective T- and NK-cell activity and uncontrolled cytokine-release. It seems to be more common in T-cell than in B-cell disorders such as Chronic Lymphocytic Leukemia (CLL).
Case Report: We report a case of a 69-year-old male who was diagnosed with CLL in March 2018 with splenomegaly and documented *TP53* mutation. In August 2018 he presented with malaise, fever and palpitations. His blood work showed pancytopenia, high levels of ferritin, sIL-2R and triglycerides. Six out of eight diagnostic criteria of the HLH-2004 guidelines were fulfilled. We started treatment with ibrutinib (420 mg/d) as the most effective treatment of CLL with *TP53* mutation, glucocorticoids and antibiotics. Rituximab (375 mg/m^2) was given when EBV-reactivation was documented (viral load: 69'096 IU/ml). Bone marrow biopsy revealed hypercellularity and diffuse infiltration of B-CLL without evidence of HLH. After initial stabilization, the patient developed lactate-acidosis, kidney and liver failure and was treated in the intensive care unit. Ibrutinib was paused and hemofiltration and mechanical ventilation were started without improvement of his condition. He developed massive lymphocytosis and died despite maximum intensive care support. A post-mortem liver biopsy revealed extensive hemophagocytosis.
Conclusion: HLH is a life-threatening condition with the need of prompt treatment. Patients should be cared for interdisciplinary by hematology, immunology and intensive care medicine. Response may be monitored by Ferritin, Fibrinogen and sIL-2R/IL-6. HLH is known to be triggered by infections, medication and cancer such as lymphoproliferative disorders. An association between ibrutinib and HLH has been described (Poole, Girard et al. 2017) but our patient had fulfilled criteria of HLH before treatment was initiated and ibrutinib has been reported to inhibit macrophage function (Lionakis, Dunleavy et al. 2017). It is unknown if further alternative treatments of HLH including etoposid and cytokine-targeted therapies such as Emapalumab (Interferon-Gamma-Antagonist) and Tocilizumab (IL-6-Antagonist) may have altered the fulminant course.

1143

Presence of Reed-Sternberg cells in bone marrow cytology

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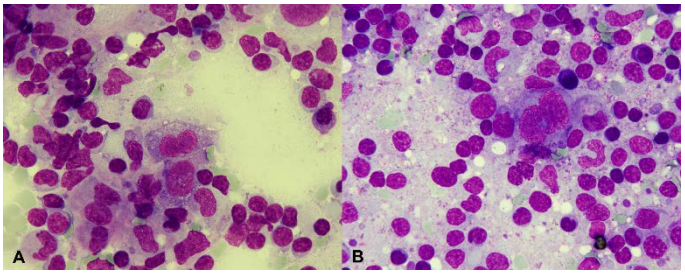
Case description: A 76-year old female with a diagnosis of T-LGL leukemia with occasional neutropenias and rare agranulocytosis recently presented at our institution with fever, diarrhea and denutrition, as well as palpable splenomegaly. Blood count showed bicytopenia (anemia and thrombocytopenia) with normal neutrophil count. A CT-scan confirmed the splenomegaly associated with multiple enlarged lymph nodes (mediastinal, retroperitoneal and iliac). A follow-up PET-CT-scan revealed hypercaptation of these adenopathies, but also spleen, liver and a diffuse bone marrow hypercaptation. A bone marrow aspiration and biopsy was performed; cytology revealed hypercellularity with presence of numerous large sized, basophilic, often vacuolated and nucleated cells (panels A and B).



The fact that these cells were mostly binucleated raised the suspicion of Hodgkin's lymphoma. Diagnosis was confirmed by the histological analysis, which also demonstrated the presence of LGL lymphocytes.
The visualisation of Reed-Sternberg cells in bone marrow cytology is extremely rare, even in cases with bone marrow involvement, but the presence of these typical cells should always raise the suspicion of Hodgkin's disease.

1152

An unusual case of pure red cell aplasia: results of a Daratumumab treatment

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Introduction: Daratumumab, a monoclonal antibody against CD38, has been studied in the setting of red cell aplasia following allogenic transplantation. The persistence of antibodies direct against red blood cells due to the ABO major incompatibility between donor and recipient, can induce an acquired pure red cell aplasia. The use of Daratumumab has shown promising results with normalisation of red cell count.
Case report: A 55-year-old man with no notable medical history presented for evaluation of a symptomatic aregenerative normochromic normocytic anemia with a hemoglobin of 82 g/l, reticulocyte count of 20 G/l, normal haptoglobin and elevated erythropoietin. Bone marrow biopsy revealed a hypercellular aspirate, with erythroid precursors majorly represented by basophilic erythroblasts. Cytogenetic studies showed a normal karyotype. Viral, autoimmune and toxic screening were negative. The CT imaging did not show a neoplastic lesion. In vitro culture growth of erythroid progenitors confirmed a maturation block of the erythrocyte, and the measurement of the telomers length was normal. In light of these results, we supposed the patient to have an atypical myelodysplastic syndrome, or an immune aetiology. We started an immunosuppressor treatment of prednisone and ciclosporin, with a transitory amelioration of the anemia. One year later the patient became progressively transfusion dependent. The attempts with high doses of erythropoietin and valproate were unsuccessful. The bone marrow biopsy 4 years later showed no molecular abnormalities at NGS analysis and an evolution towards pure red cell aplasia in the absence of erythroid precursors. We started a Rituximab treatment and then Sirolimus, both of them unsuccessful. Due to the clinical stability and moderate transfusion needs we did not perform an allogenic transplantation.
Basing on recent publications of Daratumumab treatment in the setting of red cell aplasia following allogenic transplantation, we started the anti-CD38 monoclonal antibody. The patient received a total of 4 doses of 16 mg/Kg per week.
Conclusion: In the literature, the initial response to the treatment is seen 1 to 4 weeks after the first treatment. We do not observe a rise in the hemoglobin nor in the reticulocyte count 5 weeks after the first dose, and the patient is still transfusion dependant. The absence of response to daratumumab suggests that the pathogenic mechanism of the disease is complex and needs further investigations.

CLINICAL SOLID TUMOR ONCOLOGY

972

Treatment landscape of GEP-NET's in correlation to clinical outcome at ENETS CoE in Switzerland

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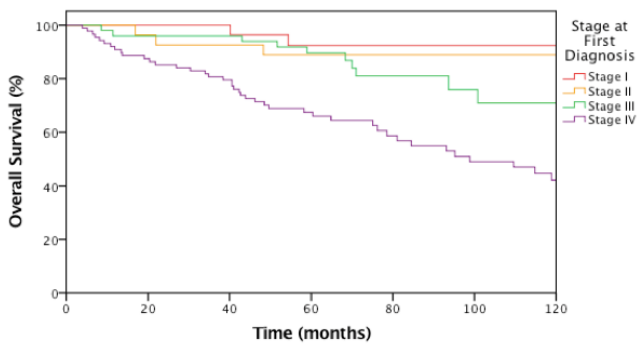
Introduction: Gastroenteropancreatic neuroendocrine tumors (GEP-NET) are heterogeneous with respect to biological behaviour and prognosis. The therapeutic landscape of GEP-NET's has evolved rapidly in recent years. Current and emerging treatment options include somatostatin analogues, radiolabeled somatostatin analogues, mTOR or tyrosine kinase inhibitor as well for neuroendocrine carcinoma (NEC) chemotherapy combinations. Limited data exist for treatment sequences although this is of upmost clinical interest.
Aim(s): The aim of this project was to analyse outcome according to treatment sequences of GEP-NET in a real life cohort.
Materials and methods: Between 2012-2016 we screened 328 NET patients at ENETS Center of Excellence, Zurich, Switzerland retrospectively. Patients' and disease characteristics such as tumor type, histologic grading, treatment lines and dates of follow up were documented by chart review. We calculated PFS and OS according to each therapy line.
Results: A total of 256 GEP-NET patients were included for final analysis. Subgroup analyses showed 92 (36%) of small intestine, 88 (35%) pancreatic, 21 (8%) of unknown primary site, 19 (7.5%) were NET of the appendix, 16 (6%) gastric NET, 11 (4%) rectal, 7 (3%) of colon or cecum and 1 (0.5%) NET of the gallbladder. 25 (10%) suffered of a neuroendocrine carcinomas (NEC) or NET-G3, 53% were male with a median age 57 (range 13-86). We observed a significant correlation in longer progression free survival rate between surgical interventions, treatment with SSA (Cox regression, chi-square, $p = 0.03$), SIRT (Cox regression, chi-square, $p = 0.005$) and cytotoxic chemotherapy (Cox regression, chi-square, $p = 0.009$). Median OS was 71 months. Higher grading (G2 and G3) and advanced disease (Stage III and IV) predicted shorter overall survival (OS) on Kaplan Meier Analysis (both log-rank, $p < 0.001$).



Conclusion: We identified a unique real life population of GEP-NET at our ENETS center in Zurich. We could illustrate different PFS and OS depending on implemented therapies, which were adjusted for tumor grading (G1-G3). According to these conclusive findings in our study we could perform a suggested treatment algorithm for advanced GEP-NET and we expect to build foundation to future prospective and randomized studies of treatment sequences in GEP-NET patients.
Keywords: GEP-NET, treatment sequences, SSA, real-life population, survival analysis

1006

Incidence, mortality, and survival trends of soft tissue and bone sarcoma in Switzerland between 1996 and 2015

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Background: Research on soft tissue (STS) and bone sarcoma (BS) is increasingly in the focus of physicians and pharmaceutical companies. Expanding knowledge has improved the management of sarcoma and potentially survival. Here we provide the first population-based data on time trends of STS and BS incidence, mortality, and survival in Switzerland diagnosed between 1996 and 2015.
Methods: We performed a retrospective registry study with data from the National Institute for Cancer Epidemiology and Registration (NICER) database in Switzerland from 1996 to 2015.
Results: We identified 5500 STS and 876 BS patients, respectively. The three most common STS subtypes were undifferentiated/unclassified sarcoma (23.1%), liposarcoma (20.2%) and leiomyosarcoma (20.0%). Chondrosarcoma, osteosarcoma and Ewing sarcoma represented 43.8%, 31.6% and 20.8% of the BS group, respectively. The age-standardized incidence and mortality rates in 2011-2015 were 4.47 and 1.42 per 100,000 person-years for STS, and 0.93 and 0.42 for BS. Age-standardized incidence of STS in males was significantly higher 1996 – 2000 compared to 2001 – 2015, however mortality rates did not change significantly over time. Five-year relative survival (RS) for STS improved significantly from 56.6% [95%CI 53.1%-59.9%] (1996-2001) to 62% [95%CI 59.0-64.8] (2011-2015) (p = 0.017). No improvement of 5-year RS for BS could be observed (RS 1996-2000: 67.3 [95%CI 58.0-74.8]; RS 2011-2015: 71.1% [95%CI 64.3-76.8]; p = 0.728).
Conclusion: Incidence rates of STS and BS have been stable since 2001. The longer relative survival in STS can be attributed to advancements in sarcoma patient management.

1025

Expression signature of gastroenteropancreatic neuroendocrine tumors in correlation to clinical outcome at ENETS centre of excellence Zurich

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Background: Gastroenteropancreatic neuroendocrine tumors (GEP-NET) are a heterogeneous tumor entity with respect to biological behaviour and prognosis. Currently there is not much knowledge about immunohistochemical expression profiles of different tumor subtypes, indicating a less favourable clinical course or faster progression. Hence definition of new predictive and prognostic biomarkers is of upmost clinical interest.
Methods: Tissue microarray (TMA) blocks were constructed with 147 tissue samples of tumor diagnosed from 2000 to 2017, including primary tumors and metastasis. Microarray sections were immunostained for SOX-9, SOX-10, Ki-67, SSTR-2 +, TTF1, PD-L1, ER-α and β, PR and AR as well as ChromograninA and Synaptophysin. Furthermore BRAF analysis was made for each sample. Biomarker expression will be correlated with clinico-pathological variables and tested for survival prediction using Kaplan-Meier and Cox regression methods.
Results: 270 patients were screened between 2000 – 2017 and 93 patient's material was sufficient for TMA analysis. Subgroups analysis showed 32 pancreatic, 37 ileum, 10 duodenum, 7 appendix, 3 colorectal, 3 gastric and 1 NET of gallbladder. 50% were male, the median age 63.5 years (range 19-88y). Expression of AR, ER-β, TTF1 and PD-L1 was only present in a small number of pancreatic NETs. 27.2% of all samples were ER-α positive, 30% PR positive and 67.4% had SOX-9 expression, SOX-10 was positive in 6.8%, but with very weak expression.
Higher grading, advanced stage and lack of SSTR-2 positivity, predicted for shorter overall survival (OS) on univariate analysis. Other expression markers as well as BRAF status are planned for multivariate analysis now and will be presented at time of presentation
Conclusion: We have identified a frequent expression of new markers in a broad cohort of GEP-NETs. This finding might be used for patient stratification and to optimize treatment decisions in GEP-NETs independently from stage and grading.

1056

Prospective, observational study of perceived effects of skin care and management of cetuximab related skin reactions – PROSKIN study

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Introduction: Cetuximab is a monoclonal antibody against the epidermal growth factor receptor (EGFR) and is approved for the treatment of metastatic colorectal cancer (mCRC) and recurrent/metastatic squamous cell cancer of the head and neck (r/m SCCHN). Skin toxicities, most often acne-like rash are seen in the majority of patients (pts).
Methods: This prospective observational study has a non-experimental cohort design to assess management strategies of cetuximab-related skin reactions and their perceived effect in pts with mCRC and r/m SCCHN. Pts with mCRC or r/m SCCHN who received at least one dose of cetuximab were eligible. Current radiotherapy was an exclusion criterion. The primary endpoint was physician's perceived effectiveness of skin products.
Results: Between October 2012 and April 2016, 147 pts were screened and 125 pts were included. 73.6% were men. The mean age was 63.3 years (range, 29-84). 91 pts (72.8%) were diagnosed with mCRC and 34 pts (27.2%) with r/m SCCHN. The most frequently used skin products were moisturizing (77.6%), lipid regenerating (56.8%) or urea containing products (52%), systemic antibiotics (49.6%), and vitamin K1 cream (43.2%). 21.6% of pts received systemic antibiotics prophylactically from beginning. The frequency of acneiform rash grade ≥2 increased from 12.6% at week 2 to 21.7% at week 16. Moderate to very strong effectiveness was perceived by a majority of physicians for pts who received systemic antibiotics at week 6 (62.2%) and at week 16 (60.7%). Similar rates were seen for vitamin K1 cream (week 6: 68.3%; week 16: 68.8%). For pts receiving moisturizing products, moderate to very strong effectiveness was perceived in only 43.8% at week 6 and 52.8% at week 16. Similar effectiveness was observed for lipid regenerating (41.5% and 52.6%) and urea containing products (37.3% and 45.9%). At week 2, 24.4% pts reported an impact of skin reactions on daily life. Thereafter this proportion increased to 47.1% at week 6 and 56.7% at week 16. A majority of pts reported no impact of skin care on skin reactions at week 2 (62.2%). Thereafter, the proportion decreased to 30.4% at week 6 and 23.3% at week 16.
Conclusions: A large number of relatively low-cost general skin care products are most commonly used. There is high physician's perceived overall efficacy for systemic antibiotics and vitamin K1 cream. The overall efficacy of other products was considered as weak.
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1062

Primary results from the SAUL study: atezolizumab for locally advanced or metastatic urothelial carcinoma (UC) or non-UC

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Background: Atezolizumab (atezo), a monoclonal antibody that targets PD-L1, is an approved therapy for locally advanced/metastatic UC based on results of phase II and III clinical trials. SAUL (NCT02928406) evaluated safety and efficacy of atezo in a broader patient (pt) population, including pts ineligible for the IMvigor211 phase III trial.
Methods: Pts with locally advanced (T4bNany or TanyN2–3) or metastatic (M1) UC or non-UC of the urinary tract received atezo 1200 mg every 3 weeks until disease progression or unacceptable toxicity. IMvigor211-like pts and pts with renal impairment, ECOG PS 2, treated asymptomatic CNS metastases or stable controlled autoimmune disease were eligible. The protocol was approved by Institutional Review Boards/Ethics Committees. The primary endpoint was safety; efficacy endpoints included overall survival (OS), progression-free survival (PFS), overall response rate (ORR) and duration of response (DoR).
Results: Between Nov 2016 and Mar 2018, 1004 pts were enrolled; 997 received atezo. Median age was 68 years, 10% had ECOG PS 2, 5% had non-UC histology, 77% were male and 98% were platinum pretreated ([neo]adjuvant or advanced setting). Immune cell PD-L1 status (VENTANA SP142) was low (IC0/1) in 66% and high (IC2/3) in 27% (unknown in 7%). By 16 Sep 2018, median duration of follow-up was 12.7 mo. Median number of atezo cycles was 5 (range 1–28); 220 pts (22%) remained on atezo and 555 (55%) had died. Treatment-related grade (G) ≥3 adverse events (AEs) occurred in 13% (table), most commonly fatigue, asthenia, colitis and hypertension (each in 1%). Median OS was 8.7 (95% CI 7.8–9.9) mo, 6-mo OS rate 60% (95% CI 57–63%), median PFS 2.2 (95% CI 2.1–2.4) mo and ORR 13% (95% CI 11–16%), including

complete responses in 3%. Median DoR is immature (95% CI 13.2 mo–not estimable). In the IMvigor211-like subgroup (ie excluding pts with ECOG PS 2 and other IMvigor211 exclusion criteria), median OS was 10.0 (95% CI 8.8–11.9) mo, 6-mo OS rate 65% (61–69%), median PFS 2.3 (2.2–2.6) mo and ORR 14% (11–17%).

Conclusions: SAUL confirms the tolerability of atezo in a 'real-world' UC and non-UC population. Efficacy in both the IMvigor211-like subgroup and the broader unselected population is consistent with previous anti-PD-L1/PD-1 pivotal UC trials. These results support use of atezo in UC or non-UC, including pts with limited available treatment options.

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1063

CUPISCO study: Molecularly guided therapy versus standard chemotherapy in patients with carcinoma of unknown primary

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Introduction: Carcinomas of unknown primary (CUPs) are heterogeneous tumours of diverse origins that often have poor prognoses and high unmet clinical need. Their heterogeneity makes the conduct of clinical trials difficult. The CUPISCO study aims to compare the overall efficacy and safety of molecularly guided therapy (MGT) with standard platinum-based chemotherapy for patients (pts) with CUP.

Methods (Study Design): Eligible pts have a histological diagnosis of adeno- or poorly differentiated carcinoma without detectable primary tumour according to ESMO diagnostic guidelines (nonspecific CUP subset per ESMO definition only); Eastern Cooperative Oncology Group performance status 0–1; ≥1 measurable lesion; and are naive to systemic therapy. All pts receive hybrid capture-based comprehensive genomic profiling (FoundationOne®, FoundationACT®) to assess tumour genomic alterations, microsatellite instability and tumour mutational burden. Pts who achieve complete response [CR], partial response [PR] or stable disease [SD] after 3 induction chemotherapy cycles of carboplatin/paclitaxel, carboplatin/gemcitabine or cisplatin/gemcitabine are randomised (3:1) to investigator's choice (IC) from 9 MGT regimens (7 targeted therapy regimens, 2 immunotherapy regimens) or 3-6 further chemotherapy cycles. Randomisation is stratified by gender and response during the induction period (CR + PR vs SD). A key element of the trial design is a 'Molecular Tumour Board (MTB)', comprising the investigator, reference pathologist, reference oncologist and cancer genomics consultant (when needed), who advise investigators on MGT choice based on tumour genomic profiles. Pts with progressive disease during the induction period will be assigned to IC of MGT regimens with advice from the MTB. The primary efficacy endpoint is investigator-assessed progression-free survival (time from randomisation to first occurrence of disease progression per Response Evaluation Criteria in Solid Tumors v1.1, or death from any cause). Enrolment of 790 pts is planned across 26 countries and ~116 sites. Recruitment is ongoing; 167 pts have been screened to date (NCT03498521).

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1064

KATHERINE: Trastuzumab emtansine vs trastuzumab as adjuvant therapy in patients with HER2-positive early breast cancer

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Introduction: Patients (pts) with HER2-positive early breast cancer (BC) with residual invasive disease after neoadjuvant chemotherapy plus HER2-targeted therapy have a high risk of recurrence and death. The current standard of care is continuation of the same HER2-targeted therapy for one year. Trastuzumab emtansine (T-DM1) has shown activity and a favorable benefit-risk profile in metastatic patients with disease progression after prior chemotherapy plus HER2-targeted therapy. Thus, T-DM1 may also be active in pts with residual invasive disease after neoadjuvant HER2-targeted therapy.

Methods: In the open-label, global phase III study KATHERINE (NCT01772472), pts with centrally confirmed HER2-positive (IHC3+ or ISH+) primary BC (T1–4, N0–3, M0) who received neoadjuvant chemotherapy plus HER2-targeted therapy (including a taxane and trastuzumab), with residual invasive disease after surgery were randomized 1:1 to T-DM1 (3.6 mg/kg IV q3w) or trastuzumab (6 mg/kg IV q3w) for 14 cycles and stratified by clinical stage at presentation, hormone receptor status, single vs dual neoadjuvant HER2-targeted therapy, and pathological nodal status after neoadjuvant therapy. Pts received radiotherapy and/or endocrine therapy per local standards. Primary endpoint: invasive disease-free survival (IDFS). Secondary endpoints: IDFS including second primary non-BC, DFS, distant recurrence-free interval, overall survival (OS), and safety.

Results: Based on a pre-specified interim analysis (after 67% of required IDFS events, efficacy stopping boundary HR≤0.732 or p <0.0124), the IDMC recommended full analysis. With 256 IDFS events reported, administration of T-DM1 significantly improved IDFS compared with trastuzumab (unstratified HR = 0.50; 95% CI: 0.39 to 0.64; p <0.0001). IDFS events occurred in 91 pts (12.2%) in the T-DM1 arm vs. 165 (22.2%) in the trastuzumab arm. T-DM1 treatment increased estimated three-year IDFS rates (88.3% vs 77.0% with trastuzumab). A consistent benefit was shown across all stratification subgroups. With 98 deaths reported, the OS analysis is immature (HR = 0.70; 95% CI: 0.47 to 1.05; p = 0.085). Safety data were consistent with the known safety profile of T-DM1, with expected increases in AEs associated with T-DM1 vs trastuzumab alone. One grade 5 AE (0.1%) occurred in each arm.

Conclusions: Adjuvant T-DM1 substantially improved IDFS in pts with HER2-positive early BC with residual disease after completion of neoadjuvant therapy.

Geyer CE et al., SABCS 2018.

1070

IMpassion130: Efficacy in immune biomarker subgroups of atezolizumab + nab-paclitaxel in patients with triple-negative BC

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Introduction: The Phase III IMpassion130 study (NCT02425891) evaluated atezolizumab (anti–PD-L1) + *nab*-paclitaxel (nabPx) vs placebo + nabPx as first-line treatment for pts with metastatic triple-negative breast cancer (TNBC). The study met its co-primary PFS endpoint in ITT pts and in pts with PD-L1 ≥1% on tumor-infiltrating immune cells (IC+). Clinically meaningful OS benefit was seen at interim OS analysis, notably in pts with PD-L1 IC+ tumors (Table). Here we report exploratory efficacy data in immunologically and clinically relevant, biomarker-defined subgroups.
Methods: Pts had histologically documented metastatic or unresectable locally advanced TNBC (evaluated locally per ASCO-CAP). Pts were randomized 1:1 to nabPx 100 mg/m^2 IV (d1, 8 and 15 of a 28-d cycle) + atezolizumab 840 mg IV q2w or placebo (A-nabPx or P-nabPx) until progression or toxicity. Exploratory biomarkers were centrally analyzed: PD-L1 on tumor cells (TC) by VENTANA SP142 IHC assay, intratumoral CD8 by IHC, stromal tumor-infiltrating lymphocytes (sTILs), *BRCA1/2* mutational status and ER/PR/HER2 status.
Results: PD-L1 IC was highly predictive of A-nabPx efficacy (Table). The majority of PD-L1 TC+ tumors were also PD-L1 IC+. Intratumoral CD8, but not sTILs, were well correlated with PD-L1 IC. Consequently, CD8 was predictive of A-nabPx efficacy for PFS/OS, while sTILs only predicted PFS benefit. Local vs central TNBC assessment was concordant in most pts. Local vs central lab–defined TNBC populations derived similar benefit from A-nabPx. Efficacy by *BRCA* status will be presented to evaluate the benefits of immunotherapy for this subgroup.

Population	A-nabPx	P-nabPx
Primary data, stratified		
ITT, n	451	451
mPFS (95% CI), mo	7.2 (5.6-7.5)	5.5 (5.3-5.6)
PFS HR (95% CI)	0.80 (0.69-0.92); *P*=0.0025	
mOS (95% CI), mo	21.3 (17.3-23.4)	17.6 (15.9-20.0)
OS HR (95% CI)	0.84 (0.69-1.02); *P*=0.0840	
PD-L1 IC+, n (%)	185 (41%)	184 (41%)
mPFS (95% CI), mo	7.5 (6.7-9.2)	5.0 (3.8-5.6)
PFS HR (95% CI)	0.62 (0.49-0.78); *P*<0.0001	
mOS (95% CI) mo	25.0 (22.6-NE)	15.5 (13.1-19.4)
OS HR (95% CI)	0.62 (0.45-0.86)[a]	
Exploratory/biomarker data, unstratified		
PD-L1 TC evaluable, n	449	451
PD-L1 TC+, n (%)	38 (8%)	40 (9%)
PFS HR (95% CI)	0.51 (0.31-0.84)	
OS HR (95% CI)	0.63 (0.33-1.21)	
CD8 evaluable, n	371	349
CD8 ≥0.5%, n (%)	261 (70%)	239 (68%)
PFS HR (95% CI)	0.74 (0.61-0.91)	
OS HR (95% CI)	0.66 (0.50-0.88)	
sTIL evaluable, n	448	444
sTIL ≥10%, n (%)	147 (33%)	137 (31%)
PFS HR (95% CI)	0.66 (0.50-0.86)	
OS HR (95% CI)	0.75 (0.51-1.10)	
cTNBC evaluable, n	420	412
cTNBC ITT, n (%)	307 (73%)	317 (77%)
PFS HR (95% CI)	0.81 (0.68-0.98)	
OS HR (95% CI)	0.85 (0.67-1.08)	
cTNBC PD-L1 IC+, n (%)	133 (43%)	134 (42%)
PFS HR (95% CI)	0.67 (0.51-0.88)	
OS HR (95% CI)	0.69 (0.47-1.00)	

Data cutoff: 17 April 2018 (12.9-mo median follow up).
cTNBC, centrally confirmed TNBC
TC/IC+, PD-L1 ≥1% (VENTANA SP142 assay)
[a] Not formally tested per hierarchical study design.

Conclusions: Exploratory efficacy analyses from IMpassion130 suggest consistency between local and central ER/PR/HER2 testing and that PD-L1 IC is the most robust predictive biomarker for selecting untreated mTNBC pts who benefit from A-nabPx.
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1072

IMpower150: an exploratory analysis of efficacy outcomes in patients with EGFR mutations

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Introduction: Atezolizumab (atezo; anti–PD-L1) inhibits PD-L1 to restore anticancer immunity; bevacizumab (bev) may enhance atezo efficacy by inhibiting VEGF immunosuppression and promoting T-cell tumour infiltration. Atezo + bev + chemotherapy (chemo) prolonged progression-free survival (PFS) and overall survival (OS) vs bev + CP in patients (pts) with first-line nonsquamous NSCLC in the randomised Ph III IMpower150 study, including pts with EGFR or ALK genomic alterations. Here, we further analyse the efficacy of atezo and/or bev with chemo in pts with EGFR mutations (EGFR-mt).
Methods: The 1202 enrolled pts received atezo (A) 1200 mg + bev (B) 15 mg/kg + carboplatin (C) AUC 6 + paclitaxel (P) 200 mg/m^2 (ABCP) or A + C + P (ACP) or B + C + P (BCP) by IV q3w for 4 or 6 cycles per investigator (INV) decision, then q3w maintenance with atezo + bev, atezo or bev, respectively. Co-primary endpoints were OS and INV-assessed PFS in the ITT–wild-type population (excluded pts with *EGFR* or *ALK* genomic alterations). Exploratory analyses included OS and INV-assessed PFS in pts with *EGFR*-mt disease, pts with sensitising *EGFR* mutations and pts with *EGFR*-mt disease who had prior TKI therapy.
Results: These data represent ≥ 20-mo follow-up (data cutoff: 22 Jan 2018) in the ITT population. 124 pts were *EGFR*-mt, including 91 with a sensitising mutation. Baseline characteristics of *EGFR*-mt pts across the treatment arms were generally comparable to the ITT population. OS was improved with ABCP vs BCP in *EGFR*-mt pts, especially in pts with sensitising *EGFR* mutations (HR, 0.31 [95% CI: 0.11, 0.83]). This benefit extended to PFS (HR, 0.41 [95% CI: 0.23, 0.75]). See table for full efficacy results. Safety was similar between the *EGFR*-mt subgroup and the ITT population.

	mOS, mo			HR (95% CI)	
	ABCP	ACP	BCP	ABCP vs BCP	ACP vs BCP
EGFR-mt	NE n = 34	21.4 n = 45	18.7 n = 45	0.61 (0.29, 1.28)	0.93 (0.51, .68)
Sensitising *EGFR* mutation[a]	NE n = 26	21.2 n = 33	17.5 n = 32	0.31 (0.11, 0.83)	0.90 (0.47, 1.74)
Received prior TKI therapy	NE n = 22	18.4 n = 27	17.5 n = 28	0.39 (0.14, 1.07)	0.39 (0.14, 1.07)
	mPFS, mo			HR (95% CI)	
EGFR-mt	10.2 n = 34	6.9 n = 45	6.9 n = 45	0.61 (0.36, 1.03)	1.14 (0.73, 1.78)
Sensitising *EGFR* mutation[a]	10.3 n = 26	6.0 n = 33	6.1 n = 32	0.41 (0.23, 0.75)	1.01 (0.61, 1.70)
Received prior TKI therapy	9.7 n = 22	5.7 n = 27	6.1 n = 28	0.42 (0.22, 0.80)	1.20 (0.69, 2.09)

[a] Sensitising *EGFR* mutations are defined as exon 19 deletions or L858R mutations.
NE, not estimable.

Conclusions: IMpower150 is the first randomised Ph III trial of a checkpoint inhibitor to show a benefit in pretreated *EGFR*-mt pts. Adding atezo to standard-of-care bev and chemo provided survival benefit in *EGFR*-mt pts who have failed TKIs, for whom this regimen may represent a new treatment option.
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1089

Bazedoxifene as a novel strategy for treatment of pancreatic and gastric adenocarcinoma

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Experimental studies have shown that the IL6/GP130/STAT3 pathway is involved in pancreatic cancer tumorigenesis and progression as well as in the development of other tumors. Bazedoxifene, a selective estrogene receptor modulator clinically available for the treatment of osteoporosis, has been shown to be an effective GP130/STAT3 signaling inhibitor through in vitro and small animal studies. Our aim was to investigate the effect of bazedoxifene on tumor progression in patients with advanced pancreatic and gastric tumors.

We analyzed the data of 12 patients (8 suffering from pancreatic and 3 from gastric adenocarcinoma, 1 from cholangiocarcinoma), with locally advanced and/or metastatic disease, median age 70 years old (range 49 – 87 years). Bazedoxifene was given orally at a dose of 20 mg per day for a median duration of 13 months (range 6 – 25 months). 3 patients received bazedoxifene as monotherapy, 8 patients were under concomitant chemotherapy (Gemcitabine and Nab-Paclitaxel weekly).

Results showed tumor marker reduction in 5 patients and metabolic regression on PET-CT in 6 patients. Weight was gained in 5 patients. Two patients developed deep vein thrombosis and one pulmonary embolism, the treatment was otherwise well tolerated. Five patients died after a median of 13 months under Bazedoxifene, out of which two deaths for non-oncological reasons. An immunohistochemical study of pSTAT3 was performed in 6 patients, out of which 3 were positive.

Our preliminary data indicates that bazedoxifene is a potential new therapeutic option for pancreatic and gastric cancer therapy, through inhibition of GP130 signaling. It is already clinically available, safe to use and represents a low cost. It might be administrated at an early stage with current strategies. Based on these preliminary results, we will initiate a prospective clinical study.

1105

Sequencing solid tumors with FoundationOne test: does it play a role?

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Introduction: Molecular profiling assays are becoming widely available and provide valuable information on genomic tumor characteristics, which can identify targeted therapies or immunotherapies for cancer patients. However, the clinical impact of such tests remains unclear. Within our institution, we first investigated, which patients undergo such test and second we analyzed the impact of FoundationOne® Comprehensive Genome Profiling Test (FOne) on clinical decisions.

Methods: We conducted a retrospective cohort review (2017 – 2018) of patients with solid tumors under standard diagnostic care who received FOne testing. We reviewed the therapies that were proposed by FOne and studied whether they led to a therapeutic alteration

Results: 71 patients were identified, of which the majority presented with progressive disease (80%). Among the cancer types most frequently tested were adenocarcinoma of the colon (14%), prostate (8%), lung (4%), intrahepatic cholangiocarcinoma (8%) and breast invasive ductal carcinoma (4%). In 16 patients (22%), therapies suggested by FOne were approved in patient's tumor type while in 30 cases (42%) therapies were approved in another tumor type. For an additional 13 patients (18%) only therapies tested in clinical trials were reported. Four patients (6%) received a new therapy based on the FOne result: cancer of unknown primary (Everolimus due to a TSC1 mutation), cutaneous angiosarcoma of the scalp (Pembrolizumab due to a high tumor mutational burden [TMB]), gastrointestinal neuroendocrine carcinoma (Ipilimumab and Nivolumab due to an intermediate TMB) and mucinous adenocarcinoma of the appendix (Talazoparib due to an ATM mutation). For 11 patients (15%), a new therapy option was identified by FOne, which due to the current treatment plan might be considered for later use. 3 cases (4%) were evaluated for potential clinical trial enrollment. For an additional 6 patients (8%), the therapies proposed by FOne were already established on the basis of previous testing (e.g. smaller genomic panels, IHC, FISH).

Conclusion: Overall, 18 (25%) patients received a new therapy option by FOne after standard of care diagnostics. Therapeutic alterations were observed particularly in patients with a rare or unknown tumor type.

1106

Androgen deprivation therapy with enzalutamide or placebo in metastatic hormone-sensitive prostate cancer: ARCHES

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Background: Enzalutamide (ENZA), a potent androgen receptor inhibitor, has demonstrated benefit in men with metastatic and nonmetastatic castration-resistant prostate cancer (CRPC).

Methods: ARCHES is a multinational, double-blind, Phase 3 study (NCT02677896). Patients with metastatic hormone-sensitive prostate cancer (mHSPC) were randomized 1:1 to ENZA (160 mg/day) + ADT or placebo (PBO) + ADT, stratified by disease volume (CHAARTED criteria) and prior docetaxel therapy. Primary endpoint was radiographic progression-free survival (rPFS), assessed centrally, or death within 24 weeks of treatment discontinuation. Secondary endpoints included time to prostate-specific antigen (PSA) progression, PSA and radiographic responses, and overall survival (OS). Treatment continued until disease progression or unacceptable toxicity.

Results: 1150 men were randomized to ENZA (n = 574) or PBO (n = 576); baseline characteristics were balanced between groups. Overall, 67% had distant metastasis at initial diagnosis; 63% had high volume disease; 18% had prior docetaxel. Median follow-up was 14.4 months. ENZA + ADT significantly improved rPFS (**Table**); similar significant improvements in rPFS were reported in prespecified subgroups of disease volume, pattern of spread, region, and prior docetaxel (hazard ratios [HRs] 0.24–0.53). Secondary endpoints improved with ENZA + ADT (**Table**); overall survival data are immature. Grade 3–4 adverse events (AEs) were reported in 23.6% of ENZA patients versus 24.7% of PBO patients, with no unexpected AEs.

Endpoint	ENZA+ADT (n=574)	PBO+ADT (n=576)
Primary: rPFS, HR (95% CI)	0.39* (0.30, 0.50)	
Median (months)	NR	19.4
Key secondary		
Time to PSA progression, HR (95% CI)	0.19* (0.13, 0.26)	
Time to initiation of new antineoplastic therapy, HR (95% CI)	0.28* (0.20, 0.40)	
PSA undetectable (<0.2 ng/mL) rate,[a] %	68.1*	17.6
Objective response rate,[a] %	83.1*	63.7

CI=confidence interval; NR=not reached; *p<0.0001; [a]Of those with detectable PSA or measurable disease at baseline, respectively

Conclusions: ENZA + ADT significantly improved rPFS and other efficacy endpoints versus PBO + ADT in men with mHSPC, with a preliminary safety analysis that appears consistent with the safety profile of ENZA in previous CRPC clinical trials.

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1110

Prostate-specific antigen response with enzalutamide in nonmetastatic castration-resistant prostate cancer: PROSPER

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Background: Patients (pts) with nonmetastatic (nm) castration-resistant prostate cancer (CRPC) and rapidly rising prostate-specific antigen (PSA) are at high risk of developing metastatic (m) CRPC. Enzalutamide (ENZA) improves overall survival (OS) and radiographic progression-free survival in pts with mCRPC.
Methods: PROSPER is a randomized, double-blind, placebo (PBO)-controlled, multinational Phase 3 study (NCT02003924) in pts with asymptomatic nmCRPC, PSA doubling time ≤10 months and PSA ≥2 ng/mL at screening. All pts continued androgen deprivation therapy (medical or surgical castration) and were randomized 2:1 to ENZA 160 mg or PBO. Primary endpoint: metastasis-free survival (MFS). Secondary endpoints included time to first use of new antineoplastic therapy, PSA progression, PSA response, OS, and safety.
Results: 1401 pts were enrolled. Baseline characteristics were balanced between groups (**Table**). Compared to PBO, ENZA significantly (p <0.0001) reduced risk of MFS (hazard ratio [HR] 0.29; 95% confidence interval [CI] 0.24, 0.35), time to first use of new antineoplastic therapy (HR 0.21; 95% CI 0.17, 0.26), and time to PSA progression (HR 0.07; 95% CI 0.05, 0.08; **Table**). A significantly greater proportion of pts had confirmed PSA responses with ENZA vs PBO, respectively (**Table**). Median duration of treatment was 18.4 months vs 11.1 months for ENZA vs PBO. In the first interim analysis of OS there was a trend in favor of ENZA (HR 0.80; 95% CI 0.58, 1.09; p = 0.1519). Adverse events (AEs) were higher with ENZA vs PBO (any grade: 87% vs 77%; grade ≥3: 31% vs 23%; serious: 24% vs 18%); 10% of ENZA pts vs 8% of PBO pts discontinued treatment due to AEs.

Baseline characteristic	ENZA+ADT (n=933)	PBO+ADT (n=468)
Age, median, y	74	73
PSA doubling time <6 months, n (%)	715 (76.6)	361 (77.1)
Serum PSA, median, ng/mL	11.1	10.2
Endpoint		
Patients with baseline PSA values, n (%)	933 (100)	467 (99.8)
Patients with ≥1 post-baseline assessment, n (%)	887 (95.1)	439 (93.8)
Confirmed PSA response ≥50%, n (%)	712 (76.3)	11 (2.4)
p value	<0.0001	
Confirmed PSA response ≥90%, n (%)	522 (55.9)	2 (0.4)
p value	<0.0001	
Confirmed PSA response to undetectable level, n (%)	90 (9.6)	0 (0)
p value	<0.0001	

Conclusions: In pts with nmCRPC and rapidly rising PSA, ENZA treatment resulted in a clinically meaningful and statistically significant reduction in the risk of developing mCRPC and increased the median time to PSA progression. PSA responses were significantly greater with ENZA vs PBO. AEs were consistent with the established safety profile of ENZA.
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1118

All predictive makers for first line treatment of advanced Non Small Cell Lung Cancer in 3 working days: the USZ Algorithm

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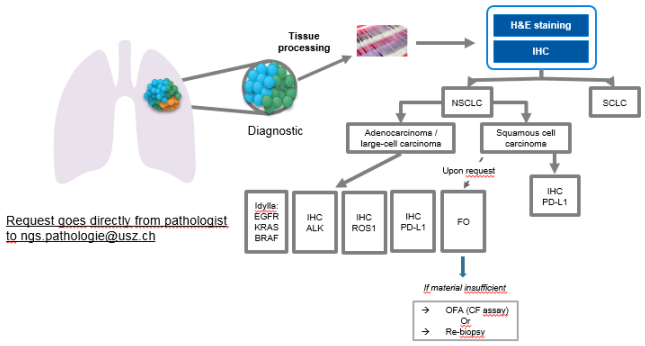
Background: molecular analysis of non small cell lung cancer (NSCLC) have been included as part of routine over the last ten years. Most of such analyses are performed upon requests and might differ from clinician to clinician. Moreover, clinician as well as pathologist are confronted with the development of multiple technologies growing at incredible pace and the lack of material to be analysed when at different timepoints. Based on these premises, we established a diagnostic algorithm to facilitate completeness, accelerate results and enable to offer to all patients the most accurate care. Such algorithm can be implemented to several institutions.
Methods: The algorithm applies to the molecular diagnostic of squamous (SqC) and adenocarcinoma (AdC) of the lung. As soon as the histological diagnosis is confirmed, for SqC an immunohistochemistry (IHC) for PD-L1 is performed; for AdC a parallel IHC for PD-L1, ROS1 and ALK is performed, as well as a molecular analysis of EGFR, KRAS and BRAF via Next generation sequencing using the Idylla platform. This leads to a broad diagnosis containing all therapy-relevant information for the first line treatment within 3 workdays.
At a second time-point, a complete molecular tumor profiling using the FOneCDx System is performed. In the case of insufficient DNA-Material to perform a FOneCDx analysis we adopt the Oncomine Focus Assay. A liquid biopsy can be performed at any timepoint and recommended only at treatment-resistance. All results are discussed in our weekly interdisciplinary molecular tumorboard.
Results: This new standardized diagnostic algorithm leads to a fast diagnosis including all therapy determining information for the treatment of advanced NSCLC. The exhaustive analysis can provide important information for further line treatment, eventually the possibility to include the patient in clinical trials or early access programs and is an important step further in the direction of precision medicine.



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1123

When SUV matters: FDG PET/CT at baseline correlates with survival in soft tissue and Ewing sarcoma

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Introduction: The role of PET/CT scans in the management of sarcomas and as a prognostic tool has been widely studied. However, it remains unclear, which metric is the most useful. We aimed to investigate if volume-based PET-metrics are superior to maximal standardized uptake value (SUV_{max}) and other metrics in predicting survival of patients with soft tissue sarcoma, bone sarcoma and Ewing sarcoma family tumors.
Methods: In this single-center, retrospective cohort study we identified a total of n = 88 patients who were diagnosed with soft tissue or bone sarcoma and had a staging scan at our institution before initial therapy. We used a Wilcoxon signed-rank test for significance analysis to assess,

which PET/CT-metric correlated with survival in different patient subgroups. Receiver-operating-characteristic analysis were used to calculate optimal cut-off values using the Youden Index.
Results: In our cohort, the PET-metric that correlated most significantly with short survival time in soft tissue sarcoma and Ewing sarcoma family tumors was a high SUV_{max} ($p = 0.001$ and $p = 0.017$, respectively), with cut off values of 5.5 and 8.5 mg/dl, respectively. However, no PET-metric but only tumor-volume correlated significantly ($p = 0.035$) with survival in primary bone sarcomas. Total lesion glycolysis was significantly associated with long survival only in Ewing sarcoma ($p = 0.03$).
Conclusion: Our analysis shows that the outcome of soft tissue, bone and Ewing sarcoma is associated with different PET/CT-metrics. We could not confirm previously described superiority of volume-based metrics in soft tissue sarcomas, for which we found SUV_{max} to be the best prognostic factor. However, bone sarcomas should probably be evaluated with tumor volume rather than FDG PET activity.

1126

Dependency on the adenosine deaminase ADAR1 in cancer cells

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Systematic exploration of cancer cell vulnerabilities can inform the development of novel cancer therapeutics. Here, through analysis of genome-scale loss-of-function datasets, we identify adenosine deaminase acting on RNA (ADAR or ADAR1) as an essential gene for the survival of a subset of cancer cell lines. ADAR1-dependent cell lines display increased expression of interferon-stimulated genes. Activation of type I interferon signaling in the context of ADAR1 deficiency can induce cell lethality in non-ADAR1-dependent cell lines. ADAR deletion causes activation of the double-stranded RNA sensor, protein kinase R (PKR). Disruption of PKR signaling, through inactivation of PKR or overexpression of either a wildtype or catalytically inactive mutant version of the p150 isoform of ADAR1, partially rescues cell lethality after ADAR1 loss, suggesting that both catalytic and non-enzymatic functions of ADAR1 may contribute to preventing PKR-mediated cell lethality. Together, these data nominate ADAR1 as a potential therapeutic target in a subset of cancers.

EXPERIMENTAL HEMATOLOGY / ONCOLOGY

975

Reactive oxygen/nitrogen species contribute substantially to the antileukemia effect of APO866, a NAD lowering agent

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Introduction: APO866 is a small molecule drug that specifically inhibits nicotinamide phosphoribosyltransferase (NAMPT), a key enzyme involved in nicotinamide adenine dinucleotide (NAD) biosynthesis from the natural precursor nicotinamide. Although, the antitumor activity of APO866 on various types of cancer models has been reported, information regarding mechanisms by which APO866 exerts its cytotoxic effects is not well defined. We investigated here the effect of APO866 treatment on reactive oxygen/nitrogen species (ROS/RNS) release in hematopoietic malignant cells and explored, using pharmacological or genetic inhibition, the contribution of ROS/RNS production to the anti-leukemia activity of APO866.
Methods: Malignant cells from different haematological malignancies were exposed to APO866 and generation of various types of intracellular ROS/RNS was measured using specific ROS/RNS probes and flow cytometry. Pharmacological inhibition tests and genetic approaches were used to delineate the contribution of ROS/RNS production to APO866-induced cell death.
Results: APO866 induces a strong, time-dependent increase in highly reactive ROS, nitric oxide, cytosolic/mitochondrial superoxide anions and hydrogen peroxide. We provide evidence that APO866-mediated ROS production triggers cell death through PARP1 activation and mitochondria depolarization. Inhibition of PARP1 activation prevented cytosolic superoxide anion accumulation, caspase activation, mitochondria depolarization and abrogates APO866-induced cell death, suggesting that the integrity of PARP1 status is required for cell death. Conversely, PARP1 activating drugs enhanced the anti-leukemia activity of APO866
Conclusion: Our data show that APO866 induces ROS/RNS productions, which mediate its antileukemia effect. These results support testing new combinatorial strategies to enhance the antitumor activities of APO866.

994

Comprehensive characterization of vulnerable and bypassing oncogenic signaling kinases/pathways by phosphoproteomics in myeloid cell lines treated with targeting compounds

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Background: Myeloid Neoplasms are heterogeneous disorders caused by sequential accumulation of mutations in haematopoietic stem cells (HSC). They are characterized by a propensity to evolve towards secondary acute myeloid leukemia (sAML). Response to targeted treatment as well as clonal evolution is currently not predictable based on genetic approaches. Therefore, identification of more reliable biomarkers for response is a relevant and unmet need for "precision medicine".
Aim: The aim of this project is to construct a pipeline for the analysis of phosphoproteomic (PP) data to i) identify differential phosphosites, ii) infer targetable kinases and iii) infer overactive oncogenic pathways. Here, we present the validation phase in myeloid cell lines perturbed with kinase inhibitors.

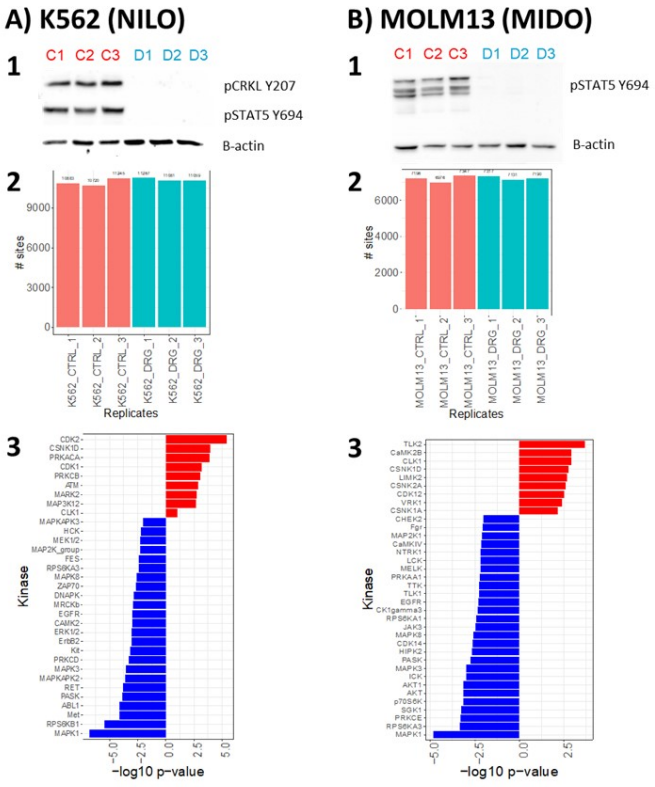

Figure 1A/1B: K562 and MOLM13 perturbed with Nilotinib (NILO) and Midostaurin (MIDO), respectively. 1) Western blot of K562/MOLM13 showing three replicates in control (C1-C3) and NILO/MILO perturbed conditions, respectively (D1-D3). Dephosphorylation of down-stream targets after drug exposure (BCR-ABL1: pCRKL Y207/pSTAT5 Y694; FLT3: pSTAT5 Y694). 2) Number of PPs identified in the three replicates of every condition (control vs drug). 3) KSEA waterfall plot treated compared to controls with overactive (red) and underactive (blue) kinases.

Methods: Two myeloid cell lines K562 and MOLM13, mainly driven by *BCR-ABL1* and *FLT3* kinases, respectively, were used. They were inhibited with *Nilotinib* (NILO) and *Midostaurin* (MIDO), respectively. PPs were enriched with titanium-dioxide and analyzed by mass spectrometry (nanoLC-MS^2). A *Kinase Substrate Enrichment Analysis* (KSEA) pipeline was developed in R. This pipeline is based on the *SetRank* enrichment algorithm integrating substrate-kinase datasets from five experimentally validated databases complemented by *NetworKIN*, an in-silico kinase motif recognition tool. The pipeline is supported by a shiny web-app that allows visualization and interactive interrogation of data on differential phosphosites, kinases and oncogenic signaling pathways.
Results: NILO in K562 showed expected inhibition of ABL1, KIT and down-stream effectors of MAPKs as well as additional vulnerable kinases such as RPS6K, MET and RET (Figure 1A). MIDO in MOLM13 showed expected inhibition of PRKC and downstream kinases of FLT3 (AKT1, JAK and MAPK) as well as additional vulnerable kinases such as RPS6K and SGK1 (Figure 1B). At the same time, overactive kinases emerged such as CDK1/2, CSNK1D and PRKs in K562 and TLK2, CLK1 and Casein-kinases in MOLM13.
Conclusion: Our data validated the utility of our pipeline. It identified bypassing kinases that evade killing of cancer cells and could represent candidates for combinatorial treatment. We provide a novel analysis pipeline for unsupervised characterization of kinase/pathway activities that allow characterization of response/resistance and identification of targets for combinatorial treatment. We are currently validating our pipeline in primary AML samples.

1001

Blood cell production is not influenced by bone health status in homoeostasis – the CoLaus/OsteoLaus cohort

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Background: The hematopoietic stem cell (HSC) niche constitutes a complex bone marrow (BM) microenvironment critical to tightly control HSC proliferation. Osteoporosis is characterized by both reduced bone mineral density (BMD) and microarchitectural deterioration, which constitute the most frequent alteration of the BM microenvironment. It still remains unclear to which extent modification of the BM microenvironment, including in the specific context of osteoporosis, influences blood cell production.
Aims: To describe the association between lumbar spine BMD and microarchitecture (measured by the trabecular bone score – TBS), and complete blood counts.
Methods: We analyzed the complete peripheral blood counts and bone parameters of the postmenopausal women taking part of the CoLaus/OsteoLaus cohort, a Caucasian population-based sample in Lausanne, Switzerland. BMD and TBS were measured at 5-year interval. For this study, we focused on patients with homeostatic hematopoiesis. Exclusion criteria were any active treatment or disease that could influence haematopoiesis and any active treatment for osteoporosis. Bivariate and multivariate associations between each peripheral blood cell count and BMD or TBS were performed.
Results: Our study included 803 and 901 women, respectively for the two timepoints. Significant associations between blood counts and BMD or TBS were found for certain blood markers within the cohort, but none was consistent across bone markers or timepoints.
In order to investigate an effect of extreme values reflecting the highest and the lowest bone quality, we compared participants in the highest BMD and TBS tertiles and participants in the lowest BMD and TBS tertiles. Neutrophils were significantly different in the lowest BMD and TBS tertile (3.18±0.09 vs. 3.47±0.08 G/l, p = 0.028) at the first assessment. At the second assessment, significant differences were found for leucocytes (5.90±0.11 vs. 5.56±0.10 G/l, p = 0.033), lymphocytes (1.87±0.04 vs. 1.72±0.04 G/l p = 0.033) and monocytes (0.49±0.01 vs. 0.46±0.1 G/l, p = 0.033). No significant association was therefore reproducible between timepoints.
Conclusion: In this cohort of postmenopausal women, BM microenvironment changes related to bone condition did not have a reproductible impact on blood cell production in homeostasis. This study underlines the complexity of understanding the clinical relevance of changes in the BM and prompt further research in stress hematopoiesis.

1021

Progressive long-term avidity decline of CMV- but not EBV-specific memory CD8 T cell clonotype repertoires

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Objectives: Efficient T cell responses rely on the T-cell receptor-peptide-MHC (TCR-pMHC) binding avidity that controls all essential T cell functions. However, it still remains unknown whether the TCR-ligand avidity is a determining factor for the clonal selection and evolution of virus antigen-specific T cells over time.
Methods: We studied the CD8 TCRαβ repertoire composition and selection over a period of 15 years in six healthy humans. In large panels of CMV- and EBV-specific CD8 T cell clones, we determined the TCR-pMHC-CD8 binding avidity and various T cell functions, and performed RNA-Seq analyses on the dominant CMV-specific T cell clonotypes identified at the first time-point and 15 years later.
Results: Within the CMV-specific TCRαβ clonotype repertoires, we observed the preferential selection and expansion over time of clonotypes of lower TCR-pMHC avidity and higher CD8 binding dependency, correlating with reduced functional capacities. Importantly, we identified a distinct molecular signature preferentially expressed by the high avidity T cell clonotypes, including a checkpoint regulator controlling the expansion potential of virus-specific T cells. In contrast, the clonal evolution of the EBV-specific clonotype repertoires was highly preserved, with the presence of the same clonotype distribution (i.e. dominant versus subdominant, low versus high TCR avidity, CD8 binding-independent versus -dependent) during the observation period of 15 years.
Conclusions: Our results indicate that the TCR-pMHC-CD8 binding avidity represents a major determinant of clonal selection and evolution in long-lasting CMV-specific T cells, consistent with the current concept of clonal senescence of high avidity T cells with ageing. However, this is not the case for EBV-specific CD8 T cell repertoires, in which the clonal composition and distribution once established is kept highly constant for at least 15 years. These findings indicate distinct mechanisms regulating the long-term outcome of CMV- versus EBV-specific memory CD8 T cell responses in humans.

1022

Chronic TCR-MHC (self)-interactions limit the functional potential of TCR affinity-increased CD8 T lymphocytes

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Objectives: Affinity-optimized T cell receptor (TCR)-engineered lymphocytes targeting tumor antigens can mediate potent antitumor responses in cancer patients, but also bear substantial risks for off-target toxicities. Identifying adverse events in relation to enhanced TCR-pMHC affinity is therefore becoming essential to improve and ensure the safety of candidate TCRs for clinical trials. Here, we addressed the question whether chronic TCR-A2 (self)-interactions can directly modulate T cell activation, signaling and functional status in the absence of cognate peptide antigen.
Methods: We developed two complementary human tumor-redirected CD8 T cell models engineered with incremental affinity TCRs to the HLA-A2/NY-ESO-1 tumor antigen. The impact of HLA-A2 expression according to TCR affinity was assessed (i) on basal levels of the TCR/CD3 complex and on negative TCR tuning molecules (i.e. CD5 and c-Cbl) as well as (ii) on the quality of T cell responses, independently of the specific antigenic peptide (i.e. basal proliferation, cytokine production by multiplex cytokine profiling and killing assays by IncuCyte).
Results: We describe a central role of HLA-A2 expression *per se* in predisposing redirected HLA-A2/NY-ESO-1-specific CD8 T cells to chronic activation followed by T cell hyporesponsiveness, depending on TCR affinity. HLA-A2^{pos} but not HLA-A2^{neg} CD8 T cells engineered with increased-affinity TCRs displayed TCR/CD3 downmodulation and impaired TCR signaling, even in the absence of cognate antigen. The observed strong T cell activation was associated to enhanced upregulation of c-CBL and multiple inhibitory receptors, and preceded TCR affinity-mediated functional hyporesponsiveness. This stepwise activation-to-hyporesponsive state did not involve the PD-1/PDL-1 regulatory axis, but was recapitulated once affinity-increased HLA-A2^{neg} T cells were chronically exposed to HLA-A2^{pos}-expressing target cells.
Conclusions: Our observations indicate that sustained interactions between affinity-increased TCRs and self-MHC molecules directly adjust

the functional potential of T cells, independently of specific peptide. Moreover, this TCR affinity-mediated hyporesponsive state is novel and has potential implications for the design of affinity-improved TCRs for adoptive T-cell based therapies.

1023

Identification of key stromal niche cell types supporting developmental extramedullary hematopoiesis

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Homeostasis of the entire adult blood system is directly dependent on the self-renewal and differentiation of a rare and uniquely clinically relevant cell subset; hematopoietic stem cells (HSCs). Throughout embryonic development, only few HSCs are generated within large blood vessels during a short timeframe. Thereafter the stem cells colonize the fetal liver (FL), where they undergo a massive proliferative expansion before homing to the bone marrow shortly before birth. We aim to dissect the composition, spatial distribution and molecular characteristics of the FL stromal microenvironment that allows HSCs to favor self-renewal and thus permitting such a formidable expansion of the stem cell pool.
Using knock-in reporter mice, we determined the cellular subsets, which express two crucial HSC support factors: Cxcl12 and SCF. The expression of both is contained to the stromal, non-hematopoietic compartment. Developing DLK1-positive hepatoblasts and CD140b-positive mesenchymal cells were identified to express high levels of the two cytokines. Whole-organ 3D imaging revealed that both cell types densely populate the entire tissue, restricting available niche space, and closely associate with HSCs. Of special interest, gene expression analysis uncovered a strong down-regulation of SCF with progressive developmental time that temporally coincides with declining liver hematopoiesis.
In summary, we describe two putative stromal HSC niche populations that enable a rapidly expanding hematopoietic system in the FL. An overall loss in stromal cell numbers accompanied by a changing cytokine milieu might induce a closing HSC niche and force hematopoiesis out of the FL to the developing bone marrow.

1026

Dissecting mechanisms that drive aging hematopoietic stem cells to quiescence

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Introduction: Hematopoietic Stem Cells (HSC) are multipotent cells that sustain lifelong blood production. Despite their immense turnover, most HSC are quiescent (>90% in steady state). Notably, aged HSCs are more quiescent than young ones, possibly due to altered epigenetic patterns associated with self-renewal and cell differentiation. We hypothesize that increased proliferative history activates an intrinsic program that drives HSC towards quiescence. In this scenario, HSC can re-enter the dormant state upon accumulating divisions to ensure a homogeneous divisional history of the HSC pool throughout life.
Methods: We used a combination of CFSE in vivo labelling and Ki-67 proliferation assay to assess HSC cell cycle status after each division (i.e. from 0- to >5-divided cells). We isolated HSC from young or old mice by FACS sorting, labelled them ex vivo with CFSE and transplanted them into young, non-irradiated recipients. After 1-8 weeks, we isolated HSC from recipient mice and analysed their proliferative history and cell cycle status by FACS. In addition, we established transplantation of CFSE-labelled human HSPC into NSG and MITRG-SKI mice in order to study the divisional behaviour in the context of human hematopoiesis.
Results: Our data show that after each cell division a small subset of HSC goes into quiescence while the rest immediately re-enters cell cycle. In aged HSC the fraction of non-divided cells, compared to young ones. In addition, the amount of spontaneously quiescent HSC after each division increases with age. External stimuli that mimic viral infections, such as PolyI:C treatment, completely abolish this phenotype, suggesting that the intrinsic drive to quiescence can be suppressed upon need. Furthermore, to gain insight in human HSC cycling behavior, we transplanted CFSE-labeled human CD34+ cells from cord blood and mobilized HSC from adult donors into humanized mice and tracked their cycling activity. Results confirm findings in mice that aging is associated with accumulation of quiescent HSC.
Conclusions: Our results seem to confirm our initial hypothesis that increased proliferative history with aging or inflammation drive HSC towards quiescence. This might ensure that the HSC populaiton as a whole goes through a similar overall-turnover at the end of life and therefore might prevent HSC exhaustion and reduce the risk to develop HSC malignancies.

1043

Night transplantation accelerates in vivo leukemia development by enhancing bone marrow homing capacity in leukemic cells

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Xenografts in immune suppressed mice are routinely used for *in vivo* studies on human acute myeloid leukemia (AML). However, a significant proportion of primary AML samples are not able to repopulate mice or require a long time to show detectable engraftment in such models. We report that changing the transplantation time-point from day (5:00 pm) to night (4:00 am) strongly improved and accelerated the engraftment of primary human AML cells in NOD/SCID/IL2Rgnull (NSG) mice. We transplanted sublethally irradiated mice at day or night with equal numbers of freshly thawed AML cells from the same patient. Transplanted mice were monitored for engraftment using flow cytometry and immunohistochemistry. Night transplantation lead to enhanced engraftment compared to day-time for all analyzed samples. In 2 out of 5 samples, engraftment was only observed in the night condition; in the other n = 3 samples, next generation sequencing detected similar distribution of AML related mutations in cells retrieved from day vs. night transplanted mice. Limiting dilution experiments confirmed these findings and indicated that lower numbers of AML cells were required for leukemia initiation when mice were transplanted at night vs. day. Moreover, homing assays performed with labeled human AML cells injected via tail vein and analyzed 12 hours later showed higher BM colonization by leukemic cells when these were injected at night vs. day times. These results were confirmed in a syngeneic MLL-PTD/FLT3-ITD mouse AML model, where leukemia was induced more rapidly when transplanted at night vs. day. First mechanistic explorations indicate that the improved homing during night is independent of the SDF1-CXCR4 axis. Intriguingly, first investigations with healthy human cord-blood derived hematopoietic stem and progenitor cells (HSPC) and mouse bone marrow derived lineage-negative cells) revealed no change in the hematopoietic reconstitution in night vs. day transplantations, suggesting differential regulation in healthy vs. leukemic counterparts.
In conclusion, our results show that night transplantation can be used to accelerate *in vivo* leukemogenesis studies in human xenografts as well as syngeneic mouse models. Transplantation time-points are crucial, and if not kept homogenously within a study they might severely bias results. These results indicate a selective and CXCR4-independent mechanism enhancing homing of leukemic but not healthy HSCPs at night; further explorations are underway.

1044

Chaperone-mediated autophagy and its role in acute myeloid leukemia biology

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Chaperone-mediated autophagy (CMA) is a selective autophagy pathway that mediates lysosomal turnover of soluble cytosolic proteins. It is carried out by the molecular chaperone HSC70/HSPA8 binding to proteins containing the amino acid signature, KFERQ, and targeting them to the lysosome via LAMP2A, a lysosomal membrane-spanning protein. LAMP2A is further stabilized on lysosomal membranes by the co-chaperone HSP90. CMA and associated proteins are frequently upregulated in solid tumors. However, their role in hematological malignancy such as acute myeloid leukemia (AML) is not yet established. AML is a highly heterogeneous form of blood cancer characterized by the clonal expansion of abnormally or poorly differentiated cells of hematopoietic origin. In this project, we aim at understanding the role of CMA in AML differentiation.
Online accessible data from the blood spot server revealed that key proteins involved in CMA such as, *HSC70* and *HSP90*, are highly expressed in immature hematopoietic and AML cells in comparison to mature

healthy granulocytes. These data were further confirmed in a gene expression data set from murine hematopoietic subpopulations (GDS3997). In addition, several AML cell lines (NB4, HT93, HL60, KG-1, MOLM-13, and OCI-AML2) demonstrated high levels of CMA activity. LAMP2A/HSC70 co-localization by immunofluorescence microscopy was used as the standard technique for CMA measurements in all experiments.
Using the well-established NB4 and HT93 acute promyelocytic leukemia (APL) differentiation models, we showed that inducing granulocytic differentiation using all-*trans* retinoic acid (ATRA) leads to a significant decrease in CMA activity. Furthermore, knocking down *HSP90* or *LAMP2A* led to an accelerated while overexpression of LAMP2A led to reduced granulocytic differentiation based on CD11b FACS analysis. Next, we found decreased cellular proliferation in LAMP2A overexpressing cells labeled with CFSE at steady state. This suggests that high CMA activity may contribute to the maintenance of quiescence in AML cells.
Overall, our data suggest that increased CMA activity leads to a block in differentiation and a reduced proliferation rate in AML cells. Therefore, CMA function may contribute to the immature phenotype found in AML cells and potentially to the development of slowly proliferative chemotherapy-resistant cells.

1050

Prognostic significance of a CD33 splicing variant in AML patients undergoing intensive treatment

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Introduction: Given its ubiquitous expression in myeloid leukemic cells, CD33 is an obvious target for intervention. Gemtuzumab ozogamicin (GO) is a CD33 targeting immunoconjugate developed to improve specificity of standard treatment options for AML patients. Trials investigating GO combined with standard induction treatment reported conflicting results. Intriguingly, a single-nucleotide polymorphism (SNP) affecting splicing of the CD33 gene was reported in pediatric AML patients to predict response to GO treatment, whereas such results await confirmation in adult AML patients.
Methods: We analyzed the CD33 genotype by Sanger sequencing in consecutive patients in which DNA was available from leukemic cells at diagnosis of AML at our academic center between 02/2006 and 07/2017. All patients received intensive standard induction chemotherapy without GO.
Results: This study included 144 AML patients at first diagnosis, in which we determined the SNP genotype affecting CD33 splicing. We found that 67 patients (46.5%) had the CC-genotype (wildtype), 63 patients (43.8%) the heterozygote CT-genotype, and the TT-genotype was identified in 14 patients (9.7%). By multiparameter flow cytometry, CD33 expression intensity was comparable between AML patients with CT- and CC-genotypes, whereas patients with the TT-genotype had lower CD33 expression compared to the other SNPs (TT<CC, $p = 0.016$; and TT<CT, $p = 0.037$). The median OS of the entire cohort was 18 months and the median PFS was 12 months. CT-genotype patients tended to have longer survival (median OS; 25 months) compared to the CC-genotype (13 months) and TT-genotype (11 months), but significance was not reached. In contrast, we observed no significant differences between the CD33 genotypes regarding clinical characteristics at diagnosis, mutation profiles, remission to treatment, and relapse rate.
Conclusion: Our data propose that a SNP affecting CD33 splicing determined CD33 expression levels assessed by flow cytometry in AML patients. The different genotypes affecting CD33 splicing seemed to be relevant for survival outcomes in this cohort of AML patients undergoing standard induction treatment in the absence of GO. Therefore, our analysis suggests to thoughtfully investigate a possible impact of the CD33 genotypes on the response to CD33 targeting therapy in combination with standard chemotherapy in patients with AML.

1052

Genetic targeting of ERK1/ERK2 kinases impairs the fitness of the myeloproliferative neoplasm clone

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Background: Myeloproliferative neoplasms (MPN) are hematopoietic stem cell disorders with dysregulated JAK2 signaling. The limited effects of JAK inhibitors relate to compensatory MAPK activation. We hypothesized that ERK1/2 could be a favorable target given the distal position in the MAPK pathway and the essential role for hematopoiesis.
Methods: We genetically targeted ERK1/2 in MPN by combining Jak2V617F with ERK1 and ERK2 knockout alleles and hematopoiesis-specific Mx-Cre.To assess engraftment dynamics and competitive performance of the MPN clone, CD45.2 Jak2V617F bone marrow (BM) +/- ERK1/2 double KO (dKO) was competitively transplanted 1:1 with CD45.1 Jak2 WT BM into WT recipients.
Results: Loss of ERK1/2 in Jak2V617F mice reduced splenomegaly at 3 months. Excessive erythropoiesis was moderated with decreased red cell and reticulocyte counts in peripheral blood and erythroid progenitors in BM. Red cell parameters remained slightly above normal over time without induction of anemia. The microcytic hypochromic red cell features of Jak2V617F mice normalized by ERK1/2 dKO and emergence of leukocytosis and neutrophilia was prevented. Platelets were normal in Jak2V617F mice, while ERK1/2 ablation moderated thrombopoiesis with reduced megakaryocyte progenitors and thrombocytopenia. $Lin^{-}Sca^{+}Kit^{+}$ (LSK) hematopoietic stem/progenitor cells were decreased in ERK deficient Jak2V617F mice suggesting a reduced disease-initiating population. In competitive transplants, all recipients of Jak2V617F ERK1/2 dKO BM engrafted. The Jak2V617F clone as shown by CD45.2 chimerism, was reduced to 19% in mice with deficient vs. intact ERK1/2. The Jak2V617F clone was most prominent in neutrophils and gradually expanded, but remained low and stable in ERK deficient mice.

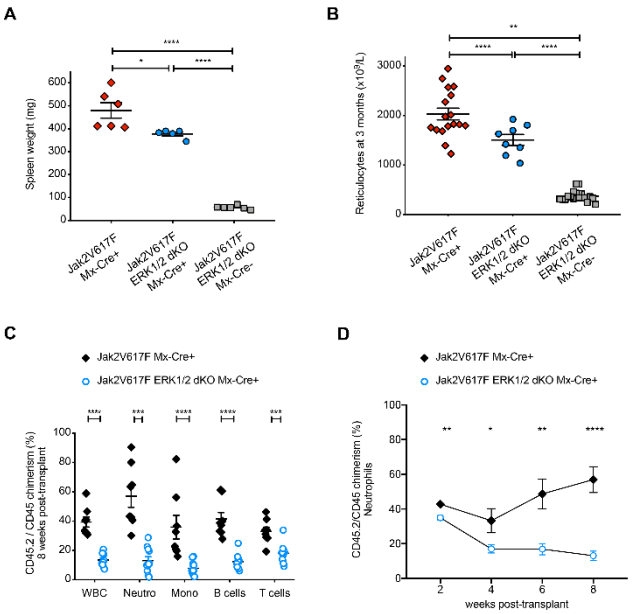

Fig. 1. Genetic targeting of ERK1 and ERK2 kinases impairs the fitness of the myeloproliferative neoplasm (MPN) clone. A. Ablation of ERK1 and ERK2 in Jak2V617F mutant MPN reduces splenomegaly at 3 months. **B.** Reticulocytes in peripheral blood are decreased in Jak2V617F ERK1/2 double knockout (dKO) as compared to Jak2V617F mice with intact ERK reflecting moderated erythropoiesis. **C.** Competitive bone marrow (BM) transplantation 1:1 with wild-type BM showed a smaller Jak2V617F clone by CD45.2 chimerism 8 weeks after transplant if ERK1/2 were ablated than with intact ERK. **D.** The Jak2V617F clone expanded over time with intact ERK1/2, while expansion was blunted in the ERK1/2 dKO setting.

Conclusions: Our data show that targeting ERK1/2 impairs the fitness of the MPN clone by restricting the stem/progenitor compartment and blunting clone expansion with reduced differentiated cell output and moderated cytoses. While targeting ERK could be a promising combination strategy with JAK2 inhibition, it should be restricted to settings of thrombocytosis to prevent induction of thrombocytopenia.

1054

Chronic viral infections induce major disruption of bone marrow stromal cell networks and persistent loss of hematopoietic stem cell function

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Hematopoiesis is a highly demand-adapted and tightly regulated process that is sustained by a rare population of self-renewing, multipotent hematopoietic stem and progenitor cells (HSPCs) residing in specialized microenvironments within bone marrow (BM) cavities. These cavities are built by an intricate network of nonhematopoietic or stromal cells of mesenchymal, neural and vascular origin that jointly build a basic tissue infrastructure throughout the entire BM.
Viral infections act as major stressors to the hematopoietic system, inducing a massive and adaptive response in cellular output. Albeit the effects of viral challenge and ensuing inflammatory responses on hematopoietic cells have been studied, whether viral infections alter BM stromal scaffolds and thus shape hematopoietic responses remains poorly defined. By combining conventional in vitro and in vivo assays with 3D confocal imaging, we herein investigated the structural and functional alterations imposed on the BM after chronic infection with Lymphocytic Choriomeningitis Virus (LCMV).
Our data shows that chronic LCMV infections result in a substantial alteration of the BM endothelial and mesenchymal stromal progenitor cell populations. Moreover, using deep tissue imaging we observed a profound and durable decimation in the number and density of CXCL12-abundant reticular (CAR) cells. Importantly, in-depth analysis showed that after chronic LCMV infection CAR cells are functionally impaired in their support for HSC quiescence. This was accompanied by a profound and sustained reduction in the number of both HSPCs as well as hematopoietic stem cells by phenotype. Competitive repopulation assays revealed a striking and persistent loss of HSC function after chronic LCMV infection. Finally, our results indicate that the observed alterations in the BM are mediated by virus-specific $CD8^+$ T cells and partly dependent on the production of systemic IFNα expression. Chronic infections thus result in persistent damage to HSPC function, which can be explained by an impaired regulatory function of the stromal compartment of the BM.
Considering the long standing clinical observation of an increased transplant rejection within chronically CMV-infected patients, precisely delineating the mechanisms that govern loss of functionality and immune pathology might pave the way for clinical applications in the future.

1061

Treatment with 5-Azacytidine results in expression of long terminal repeat (LTR) elements in peripheral blood mononuclear cells of patients with MDS and AML: first pilot study

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Background: Epigenetic drugs are used for the treatment of hematologic malignancies. By genome wide analysis of transcription start sites (TSS), methylation status and chromatin dynamics, we showed for DNA methyltransferase and histone deacetylase inhibitors (DNMTi and HDACi) global reactivation of long terminal repeat elements (LTRs) in vitro (cell line models) and in vivo (neuroblastoma mouse xenograft model). LTR reactivation is resulting in numerous fusion transcripts that encode novel protein isoforms, which have the potential to influence cell proliferation, seem to be an explanation for the priming effect of epigenetic therapy and might play a role as a potential marker for epigenetic treatment response.
Aims: Investigation of LTR reactivation in sequential peripheral blood samples of AML and MDS patients (pts) upon epigenetic drug treatment with 5-Azacytidine (5-Aza) by qRT-PCR and ddPCR and correlation of expression results with treatment response.
Methods: Sequential RNA samples from peripheral blood mononuclear cells (PBMCs) of three AML and four MDS pts treated with 5-Aza were analyzed. Consensus primers covering the transcript variants of LTR12C as well as particular primers for specific LTR elements were used. Expression of LTRs was analyzed by qRT-PCR technique with SYBRGreen as well as ddPCR technique with EvaGreen. Expression results were associated with treatment response.
Results: In AML and MDS pts, we could detect LTR expression in sequential samples from PBMCs over time with 5-Aza treatment. LTR12C expression values measured by qRT-PCR were confirmed by ddPCR and allowed to assess specific LTR elements. Pts with response to 5-Aza showed increased LTR12C expression whereas patients without treatment response had no increase in LTR12C expression. In contrast to the results from our previous NSCLC cell line model, specific LTR elements were only partially expressed in sequential PBMC samples from AML pts treated with 5-Aza.
Summary/Conclusion: First in-vivo pilot study detecting LTR expression in sequential PBMC samples of AML and MDS pts treated with 5-Aza. In this group of patients, treatment response correlated with LTR expression over several treatment cycles with 5-Aza. These are the first results in AML and MDS pts showing expression of LTRs upon epigenetic drug treatment. Larger patient cohorts are necessary to confirm these results by either PCR- or genome wide-analysis of transcription start sites.

1074

Augmented myeloid biased hematopoietic stem cell induction represents vulnerability to interferon therapy in myeloproliferative neoplasms

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Background: Recent studies using prospective isolation procedures in conjunction with single cell gene expression profiling and single cell transplantation assays of hematopoietic stem and progenitor cells (HSPCs) have identified molecular, cellular and functional heterogeneity within the primitive HSPC subsets. These studies proposed a revised hematopoietic hierarchal model including an early unipotent megakaryopoietic (Mk) pathway bridging stem cells and mature megakaryocytes. However, the extent of HSPC heterogeneity and its contribution to the pathogenesis of hematologic diseases such as myeloproliferative neoplasms (MPN) remain to be elucidated. To test the hypothesis that Mk-biased HSCs are selectively enriched and promote the development of MPN phenotype, we investigated the heterogeneity and lineage potential of HSPC subsets in mutant JAK2 expressing mice.

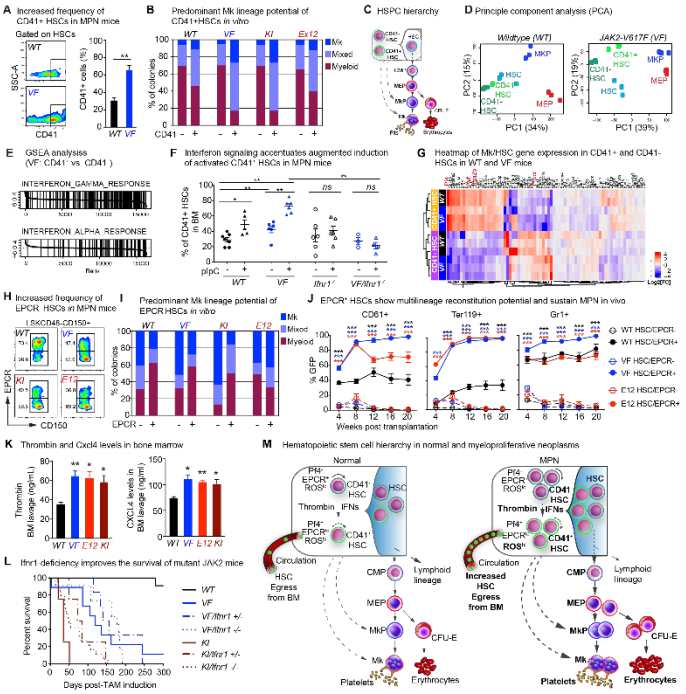

Figure 1. Augmented myeloid biased hematopoietic stem cell induction represents vulnerability to interferon therapy in MPN. A) Increased frequency of CD41+ HSCs in bone marrow of mutant JAK2 expressing mice. **B)** Lineage potential of CD41 HSC subsets in vitro at single cell level. **C)** Schematic of HSPC hierarchy. **D)** Principle component analysis (PCA) showing gene expression similarities and differences between indicated HSPCs subsets from WT and VF mice. **E)** GSEA analysis showing differential regulation of interferon signaling in CD41 HSC subsets. **F)** Ifnr1-deficiency blunts the induction of CD41+ HSCs in MPN mice. **G)** Heatmap showing differentially expressed HSC and MK lineage genes in CD41+ and CD41- HSC subsets from WT and VF mice. **H)** FACS plots showing reduced percentages of EPCR expressing HSCs in mutant JAK2 expressing mice. **I)** Lineage potential of EPCR HSC subsets in vitro at single cell level. **J)** In vivo lineage potential of EPCR HSC subsets in transplant recipient mice. **K)** Thrombin and Cxcl4 levels in bone marrow lavage of WT and MPN mice. **L)** Survival curves of mutant JAK2 expressing mice in the presence and absence of Ifnr1 receptor. **M)** Schematic showing the primitive HSPC hierarchy in normal and MPN mice. Mutant JAK2 induced interferon signaling accentuates the myeloid biased HSC frequency, which represents the majority of circulating HSCs in MPN mice. EPCR-PAR-Thrombin signaling alters their retention in the bone marrow of MPN mouse models.

Results: We found that expression of mutant JAK2 augmented Mk-biased $CD41^+$HSC frequency in MPN mice. These cells showed a predominant Mk bias at the single cell level *in vitro,* but displayed a multilineage potential *in vivo* upon transplantation. Transcriptomic profiling unveiled molecular identity and developmental proximity of $CD41^+$HSCs within the

primitive HSPC hierarchy, and identified them with a CD41⁺EPCR⁻Pf4⁺ cell surface phenotype. We demonstrated that CD41⁺EPCR⁻Pf4⁺ cells represent an activated HSC subset equipped with an active cell cycle state and elevated levels of reactive oxygen species. Furthermore, we found that CD41⁺EPCR⁻Pf4⁺ cells constitute the majority of circulating HSCs, whose frequency is increased 20-fold in MPN mice and are differentially regulated by Thrombin-PAR1-EPCR signaling. Molecular pathway analysis together with *in vivo* genetic approaches revealed that elevated interferon signaling primes the induction of activated CD41⁺EPCR⁻Pf4⁺ HSCs from quiescent CD41⁻EPCR⁺Pf4⁻ in MPN mice. Prolonged treatment with pegylated IFN achieved complete remission of the mutant JAK2 allele in MPN mice by further increasing exhaustion of quiescent CD41⁻EPCR⁺Pf4⁻ HSCs

Conclusions: Mutant JAK2 induced interferon signaling accentuates the myeloid biased HSC frequency and Thrombin-PAR1-EPCR signaling alters their retention in the bone marrow of MPN mouse models. Importantly, our study identified CD41⁻EPCR⁺Pf4⁻ as potential earliest cellular targets of interferon treatment in MPNs and provides a mechanistic rationale of how pegylated IFNα reduces JAK2-V617F allelic burden in the clinical setting.

1080

Resistance to type II JAK2 inhibition in MPN is dependent on targetable MAPK activation and is reversible

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Background: Myeloproliferative neoplasms (MPN) show dysregulated JAK2 signaling. Type I JAK inhibitors as ruxolitinib are often hampered by occurrence of resistance. The novel type II JAK inhibitor CHZ868 effectively inactivates JAK2, reduces the MPN clone and is active in ruxolitinib resistance. Thus, we study whether type II JAK inhibition also elicits resistance and how it could be overcome.

Methods: *JAK2*V617F MPN cells (SET2, UKE-1) were cultured with CHZ868. $IC_{50}$was assessed by proliferation assay, apoptosis induction by cleaved caspase-3 levels and JAK2 signaling by phospho-specific Western. JAK2 was sequenced for resistance mutations.

Results: Long-term exposure to CHZ868 for 16 weeks evoked resistance in MPN cells with 11-fold increased IC50 in SET2 maintained at 0.3uM and 13-fold in SET2 at 0.5uM CHZ868. While CHZ868 effectively induced apoptosis in naive SET2, both CHZ868-resistant SET2 lines were not susceptible to apoptosis induction. Results were confirmed in UKE-1 cells. Resistant MPN cells showed persistent MAPK activation upon CHZ868 with MEK and ERK phosphorylation similar to untreated cells, while STAT3/5 were inhibited. CHZ868 resistant cells were dependent on MEK-ERK activation and highly susceptible to MEK inhibition. Combined JAK2/MEK inhibition with CHZ868 and trametinib abrogated pERK. IC50 was dose-dependently decreased to the level of naive cells both in 0.3uM and 0.5uM CHZ868 resistant SET2. Addition of trametinib potently evoked apoptosis in both resistant SET2 lines to an extent not seen with CHZ868. Sequencing of the JAK2 gene was performed and no resistance mutation was found. Resistant cells gradually resensitized over time upon drug withdrawal with IC50 reduction to 20% at 25 weeks suggesting an adaptive rather than genetic basis of MAPK pathway activation in type II JAK2 inhibitor resistance.

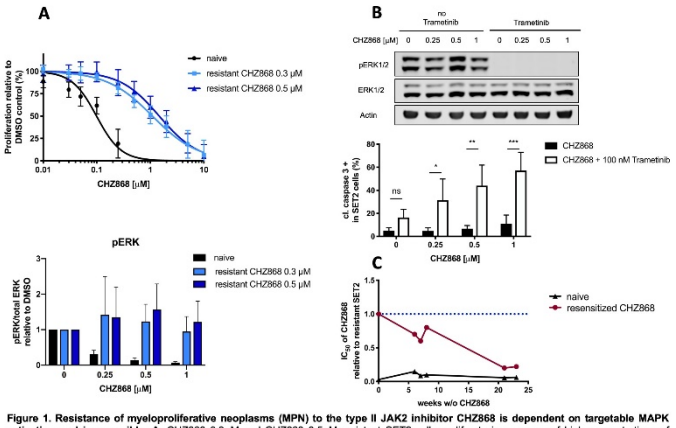

Figure 1. Resistance of myeloproliferative neoplasms (MPN) to the type II JAK2 inhibitor CHZ868 is dependent on targetable MAPK activation and is reversible. A. CHZ868 0.3µM and CHZ868 0.5µM resistant SET2 cells proliferate in presence of high concentrations of CHZ868, while proliferation of inhibitor-naive SET2 is effectively inhibited (top). Densitometric quantification demonstrates that ERK phosphorylation is not inhibited by CHZ868 in both resistant SET2 cell lines, while effectively suppressed in naive SET2 (bottom). **B.** CHZ868 can neither reduce ERK phosphorylation nor induce apoptosis in resistant SET2, whereas addition of trametinib abrogates ERK phosphorylation (top) and effectively induces apoptotic cell death of resistant cells (bottom). **C.** CHZ868 resistant SET2 cells gradually resensitize upon drug withdrawal suggesting adaptive rather than genetic basis of resistance.

Conclusions: Resistance to type II JAK2 inhibition in MPN is dependent on MEK-ERK activation and responsive to trametinib suggesting combined JAK2 and MEK inhibition as a therapeutic approach. Resensitization gradually occurs indicating functional adaptation and suggesting intermittent treatment as an alternative therapeutic strategy.

1082

Dual JAK2 and ERK1/2 kinase inhibition as a potent corrective approach in myeloproliferative neoplasms

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Background: Myeloproliferative neoplasms show constitutively activated JAK2 signaling, but clinical JAK2 inhibitors have limited benefits. We demonstrated that compensatory MAPK pathway activation interferes with JAK2 inhibitor efficacy in vivo. Combined targeting of JAK2 and the MAPK pathway via MEK inhibition improved therapeutic efficacy. We hypothesized that inhibition of ERK1/2 kinases, which are distal in the MAPK pathway and essential for hematopoiesis, could be more potent and less prone to compensatory feedback.

Methods: We assessed ERK inhibitors interfering with ATP binding (CmpdA, SCH772984) or ERK dimerization (DEL-22379) as single agents and in combination with the JAK2 inhibitor ruxolitinib. Inhibitory effects on proliferation and signaling were analyzed in human JAK2V617F mutant cells (SET2) and mouse isogenic cells (Ba/F3) stably expressing EpoR Jak2V617F. ERK inhibition by CmpdA was further studied in vivo in a Jak2V617F mutant mouse model of MPN for its impact on signaling, organomegalies and dysregulated erythropoiesis.

Results: Combined JAK2/ERK inhibition improved suppression of JAK2V617F mutant cell proliferation as compared to ruxolitinib with 2.5- to 7-fold reduced IC50 in SET2 cells and EpoR Jak2V617F Ba/F3 cells, respectively. In Jak2V617F mice, ruxolitinib at 60 mg/kg was not able to inhibit ERK activation as described (Stivala, Codilupi, Brkic et al, J Clin Invest 2019). In contrast, ERK inhibition by CmpdA at 100 mg/kg and combined CmpdA/ruxolitinib suppressed activation of ERK and its substrate RSK in Jak2V617F mouse splenocytes. Combined JAK2/ERK inhibition was superior to ruxolitinib in reduction of spleen size already at 1 week of treatment. Peripheral blood erythroid parameters including hematocrit, hemoglobin and red cell count as well as CD71⁻Ter119⁺erythroid progenitors were reduced by combined JAK2/ERK inhibition, while leukocyte and platelet counts remained in the normal range.

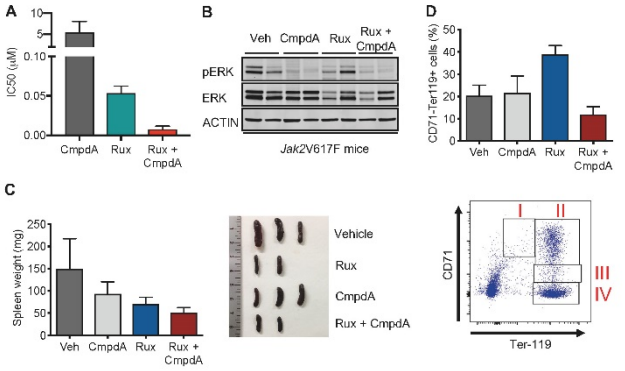

Figure 1. Pharmacologic targeting of ERK1/2 in *JAK2*V617F mutant MPN in vitro and in vivo. Combined ERK (CmpdA) and JAK2 (ruxolitinib) inhibition suppressed proliferation of SET2 cells more effectively than either agent alone reflected by decreased IC50 (**A**). Analysis of downstream signaling in *Jak2*V617F mice showed that ERK inhibition, both as a single agent and in combination with ruxolitinib, suppressed ERK phosphorylation in splenocytes (**B**). Combined JAK2 and ERK inhibition in *Jak2*V617F mice resulted in superior reduction of the spleen size (**C**) as well as CD71+Ter119+ erythroid progenitor cells in the spleen (**D**).

Conclusion: Our data demonstrate that dual inhibition of JAK2 and ERK1/2 increases the corrective potential in MPN models in vitro and in vivo indicating that the MAPK pathway needs to be targeted to increase therapeutic efficacy in MPN.

1090

Functional and structural dynamics of the bone marrow stromal microenvironment after chemotherapy

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As the major site of hematopoiesis, the stepwise generation of mature blood cells of all lineages, the bone marrow (BM) contains the rare population of hematopoietic stem and progenitor cells (HSPCs). To sustain the cell production throughout the entire adult lifespan, the differentiation and proliferation of HSPCs is tightly regulated by intrinsic signals but also by signals from the BM microenvironment. The BM stroma comprises cells of endothelial, mesenchymal and neural origin, which provides signals through direct cell-cell interactions but also through the release of cytokines. While the cytotoxic effects on hematopoietic cells induced by chemotherapeutic agents have been extensively studied, the structural effects, especially the kinetics of the destruction and recovery of the BM microenvironment and its different components are less well understood. We combined advanced flow cytometric protocols, 3D-imaging techniques and newly developed computational tools to investigate effects on murine BM cell populations and the BM microarchitecture, in particular with regard on the vasculature and the dense network of mesenchymal stromal cells.

As previously reported, the analysis by flow cytometry showed a profound loss of HSPCs upon administration of 5-fluorouracil (5-FU) that recovered within 28 days. 3D-imaging revealed that these cytoreductive effects were accompanied by massive changes induced to the BM microenvironment. 5-FU caused severe damage to the integrity of the vascular system, shown by a massive sinusoidal vasodilation and a complete disruption of the vessel walls. By day 7 upon treatment sinusoidal endothelial cells highly proliferated leading to the remodeling of the vascular system and the complete regeneration of the microvascular network after 28 days. Furthermore, 3D-imaging revealed no detectable loss of CXCL12-abundant reticular (CAR) cells but showed profound morphological changes of these cells followed by the disruption of the dense CAR cell network.

Our observations show that, in contrast to hematopoietic cells, mesenchymal stromal cells are less susceptible to the cytoreductive effects upon 5-FU administration and that the BM stroma most likely drive the complex process of rapid and complete regeneration of BM tissues after injury.

1091

TIRAP p.R81C is a novel lymphoma risk variant which enhances cell proliferation via NF-kappaB mediated signaling in B-cells

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Background: Diffuse large B-cell lymphoma (DLBCL) is the most common malignant lymphoma in adults. By gene-expression profiling, it is divided in three cell-of-origin subtypes with distinct molecular and clinical features. Most DLBCL arise sporadically, yet familial clustering is known, suggesting a genetic contribution to disease risk. Familial lymphoma cases are a valuable tool to identify novel lymphoma risk genes. We studied a Swiss/ Japanese family with 2 female siblings affected by a primary mediastinal B-cell lymphoma (PMBL) and a non-germinal center (GC) DLBCL with features of PMBL.

Methods: We performed whole-exome sequencing (WES) on matched tumor (FFPE tissue) and germline DNA (PBMCs) of affected siblings and DNA of their family members. Exomes were enriched using the Illumina TruSeq 62 Mb kit, and sequencing was performed on an Illumina HiSeq2000. GATK Haplotype Caller was used to detect germline variants. To identify lymphoma susceptibility mutations, we focused on rare, putatively harmful variants with an *in silico* predicted link with cancer and malignant lymphomas that are present in the affected siblings.

Results: The somatic landscape of both lymphomas was marked by alterations in multiple players of the JAK-STAT pathway. Consequently, this pathway was constitutively activated as evidenced by high pJAK2 as well as increased nuclear pSTAT3 and pSTAT6 in malignant cells. Potential lymphoma risk variants were identified by WES of the germline DNA derived from siblings and unaffected family members. This analysis revealed a pathogenic variant in *TIRAP*, an upstream regulator of NF-kappaB, in the affected siblings and their mother. We observed increased B-cell proliferation in family members with the TIRAP p.R81C variant. B-cell proliferation correlated with *TIRAP* and NF-kappaB target gene expression, suggesting enhanced NF-kappaB pathway activity in TIRAP p.R81C individuals. *TIRAP* knockdown reduced B-cell survival and NF-kappaB target gene expression, particularly in individuals with TIRAP p.R81C. Functional studies revealed significantly increased NF-kappaB activity and resistance to stress-induced cell-death by TIRAP p.R81C.

Conclusion: The identification of an inherited *TIRAP* variant provides evidence for a novel link between genetic alterations affecting the NF-kappaB pathway and lymphomagenesis. Our work underscores the value of studying familial lymphoma cases.

1101

Function of PU.1 in regulating alternative splicing of cFLIP during differentiation therapy in AML

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PU.1 (*SPI1*) is an essential ETS (E26 transformation-specific) family transcription factor mostly expressed in hematopoietic cells. It is crucial in the terminal differentiation of myeloid (macrophages, neutrophils) and B-lymphoid cells. Attenuated expression of this transcription factor promotes the transformation of myeloid progenitor cells to leukemic blasts, a hallmark of acute myeloid leukemia (AML). PU.1 is known as a direct regulator of myeloid genes, its role in cell death and alternative splicing during differentiation therapy, however, remains scarcely studied. We investigated how PU.1 influences alternative splicing of cFLIP (CASP8 and FADD like apoptosis regulator; CFLAR), which is one of the master anti-apoptotic genes involved in response to all-trans retinoic acid- (ATRA) induced differentiation therapy. Moreover, we are studying the potential interaction of PU.1 with a variety of splice factors, commonly mutated in AML. The impact of splice factor alterations on cell death and differentiation of AML blasts was analysed as well.

First, we observed that knocking down PU.1 specifically increased the mRNA levels of the short but reduced the levels of the full-length cFLIP

isoform. In line with the PU.1 knockdown findings, the induction of PU.1 expression showed a reduction of cFLIP short. These results suggest that PU.1 is involved in cFLIP mRNA splicing and thereby affects cell death responses. Knocking down PU.1 during ATRA-mediated neutrophil differentiation of different AML cells resulted in decreased cell death and differentiation as assessed by CD11b and Annexin V FACS analysis paralleled by increased expression of the anti-apoptotic cFLIP short splice variant. Next, to identify relevant splice factors that cooperate with PU.1 in regulating cFLIP splicing, we performed a genetic knockdown screen using a lentiviral shRNA library targeting splicing factors previously associated with AML pathology. Preliminary data indicate that knocking down the splicing-associated genes *PRPF8* and *LUC7L2* supported ATRA-mediated AML differentiation. Accordingly, high expression of these genes is found in AML compared to healthy myeloid cells. Ongoing experiments aim at quantifying cFLIP splice variant expression in splice factor knockdown AML cell lines. In summary, this study underlines the function of PU.1 in regulating alternative splicing in response to ATRA-treatment by changing gene splice patterns particularly that of the anti-apoptotic gene *cFLIP*.

1103

Quantification and characterization of extracellular vesicles from plasma of patients with acute myeloid leukemia

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Introduction: Extracellular vesicles (EVs) are sub-micrometric membrane fragments released by cells into biological fluids. EVs are considered important mediators of intercellular communication and are the focus of intensive research for their potential use as disease biomarkers and therapeutics[1]. Our group focuses on the quantification and phenotypic characterization of EVs from plasma of acute myeloid leukemia (AML) patients by flow cytometry.
Methods and results: Due to the potential relevance of EV surface signatures in the context of diagnostic applications, we first validated a strategy for quantifying and phenotyping EVs by flow cytometry, based on the "fluorescence triggering" method[2]. We tested the potential use of CD34 as a marker of EVs derived from AML blasts since it is one of the most widely used leukemic surface markers. We measured the concentration of EVs in platelet-free plasma (PFP) from AML patients with blasts being either $CD34^+$ or $CD34^-$ (n = 7 and n = 3, respectively), as well as from healthy blood donors (n = 15). The $CD34^+$AML patients showed a 3-fold higher median $CD34^+$ EV concentration (range: 10 – 80 x 10^3 EVs /µL PFP) compared to the healthy donors and $CD34^-$ AML patients (range: 3 – 10 x 10^3 EVs /µL PFP). The concentration of $CD34^+$ EVs in PFP from $CD34^+$ AML patients correlated with the total white blood cell counts at the time of diagnosis and during follow-up.
To further detect specific AML-derived EV surface signatures, we explored the use of a multiplex bead-based flow cytometry assay, which allows the simultaneous semi-quantitative detection of 37 different potential EV surface markers[3]. First, we used this approach to characterize EVs produced by $CD34^+$ AML cell lines in conditioned medium (CM), and found that CD34+ EVs not only expressed pan-vesicular tetraspanins and integrins, but also specific leukemic cell-associated surface markers (e.g. CD44 and CD133). Second, when analyzing CM of primary cells, the detection of EV surface signatures appeared to be highly specific and reproducible.
Conclusion: We will further use the multiplex assay in combination with fluorescence flow cytometry to quantify and phenotype EVs from AML patients in order to gain information on specific biomarkers for diagnosis and follow-up of the disorder. We further plan to perform functional studies to investigate the influence of EVs on the bone marrow microenvironment.

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1117

CALR mutations in myeloproliferative neoplasms affect chaperone function and differentially impact on client proteins

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Background: Calreticulin (CALR) is an endoplasmatic reticulum (ER)-resident chaperone that folds glycoproteins (GPs) such as the thrombopoietin receptor (TpoR), major histocompatibility complex I (MHC-I) and myeloperoxidase (MPO). *CALR*mutations are found in 20-30% of patients with myeloproliferative neoplasms (MPNs). The mutant variants are secreted from the ER due to the loss of KDEL motif (ER retention signal) and pathologically activate the TpoR. Whether mutations in CALR affect its chaperone function quantitatively (low intracellular levels) or qualitatively (binding affinity to GP) is unknown.
Methods: To assess whether *CALR* mutations impact chaperone function, CALR mutants were expressed in CALR knockout (KO) HL-60 cells or murine CALR KO fibroblasts. MPO protein expression was used as a readout for CALR chaperone function. To identify potentially unfolded proteins in an unbiased approach neutrophils from MPN patients were subjected to limited proteolysis-coupled mass spectrometry (LiP-MS), a technology that allows detecting structural changes in proteins.
Results: Low intracellular concentration of secreted CALR mutants could cause glycoprotein deficiencies in homozygous *CALR*-mutated patients. In line with this assumption, the expression of MPO and MHC-I was reduced in HL-60 CALR KO cells. In homozygous patients however, MPO but not MHC-I expression was reduced showing that GPs react differently to reduced levels of CALR. Restoring intracellular levels of mutant CALR by either blocking its secretion or by expression of a "secretion-resistant" KDEL-positive mutant CALR did not rescue MPO expression indicating that decreased levels of CALR mutants are not responsible for the MPO deficiency. The LiP-screen was able to identify proteins with an aberrant structure occurring in heterozygous patients only (4 proteins), homozygous patients only (83 proteins) or both (28 proteins).
Discussion: *CALR* mutations affect GPs in a client protein-specific manner. Our data suggests that this is not the sole consequence of CALR mislocalization, but rather due to a qualitative defect in chaperone function, which we are currently further assessing. Finally, candidate proteins identified by LiP-MS are evaluated for their contribution to the pathogenesis of CALR mutated MPN.

1127

Ribonuclease inhibitor (RNH1) mediated cell cycle regulation is essential for hematopoietic stem cell function and lineage choice

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Background: Cell cycle regulation is critical for hematopoietic stem cell (HSC) self-renewal and cell fate decisions. If not controlled properly it could undermine HSC function and lead to hematological malignancies. Yet, little is known about the molecular events governing cell cycle regulation in HSCs. Recently, we published that Ribonuclease inhibitor (RNH1) regulates erythropoiesis by controlling GATA1 mRNA translation. Here, we report a novel function of RNH1 as HSC cell cycle regulator.
Methods: To study the role of RNH1 in hematopoiesis, we generated hematopoietic-specific knockout mice by backcrossing *Rnh1*$^{FL/FL}$ mice with *Vav1-iCre* and *Mx1-Cre* mice, respectively. Phenotype analysis were performed in different age groups of mice by acquiring blood parameters, histopathology and FACS of different hematopoietic cell populations. Competitive and non-competitive bone marrow transplantation (BMT) experiments and colony forming cell (CFC) assays were performed to check HSC function. LT-HSC and ST-HSC were isolated and genome wide transcriptome analysis was performed to understand the molecular signature. Immunoprecipitation and mass spectrometry analyses were performed to determine RNH1 binding partners. Functional assays such as cell cycle, ROS and cytokine analysis were performed.
Results: *Rnh1*-deficiency resulted in increased numbers of myeloid cells but decreased lymphoid and erythroid cells, resembling emergency myelopoiesis. In line with this, GMP cells are increased but CLP and MEP cells were decreased in the bone marrow. *Rnh1*-deficienct mice survived normally. However, they did not tolerate little stress, such as 35µg LPS administration, which lead to early mortality. Interestingly, we found increased numbers of LT-HSCs and ST-HSCs in *Rnh1*-deficient mice suggesting that RNH1 could affect HSC function. Supporting this *Rnh1*-deficient HSCs failed to engraft lethally irradiated mice in competitive BMT experiments and they also produced less and smaller colonies in CFC assays. Further, transcriptome analysis showed dysregulation of genes related to cell cycle and enrichment of genome instability in *Rnh1*-deficient HSCs. Corroborating this, HSCs showed increased S/G2/M

phase in cell cycle analysis. At molecular level, we found that RNH1 directly binds to cell-division cycle 20 (Cdc20) protein and modulates cell cycle in HSCs.
Conclusion: Our results demonstrate that RNH1 is essential for HSC cell cycle regulation and steady state hematopoiesis.

1135

Prognostic and predictive value of plasma cell-free mutant KRAS in stage IV pancreatic cancer patients

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Introduction: Despite an overall dismal prognosis, both clinical course and treatment response of pancreatic cancer patients are highly heterogeneous. Consequently, novel biomarkers are urgently required to better guide systemic chemotherapy across all stages of disease. Approximately 95% of pancreatic ductal adenocarcinomas harbor activating *KRAS*mutations, which are readily detectable in cell-free DNA extracted from patient plasma (liquid biopsies). The clinical potential of cfKRAS^{mut} as novel non-invasive biomarker for pancreatic cancer has not been sufficiently characterized.
Methods: We collected approximately 400 plasma samples from 50 individual patients undergoing systemic chemotherapy for stage IV pancreatic cancer at our institution. Cell-free DNA was extracted and screened for cfKRAS^{mut}using in-house designed generic discriminatory multiplex digital droplet PCR (ddPCR) assays. Longitudinal follow-up samples were analyzed with mutation-specific single-target assays. cfKRAS^{mut}copy numbers were correlated to treatment response, progression-free and overall survival. CA19-9 levels were analyzed for comparison.
Results: cfKRAS^{mut}correlated with disease stage, overall metastatic burden and the presence of liver metastases. High levels of cfKRAS^{mut}in untreated metastasized patients were associated with inferior treatment response irrespective of chosen chemotherapy regimen. Moreover, cfKRAS^{mut}copy numbers in untreated patients strongly correlated with shorter progression-free and overall survival (PFS and OS). Dynamic changes of cfKRAS^{mut}during 1stline chemotherapy were highly predictive of PFS and OS. cfKRAS^{mut} showed comparable sensitivity and higher specificitywhen compared to CA19-9 levels.
Conclusion: The analysis of cfKRAS^{mut}in patients undergoing systemic treatment for stage IV pancreatic cancer holds considerable clinical potential as novel predictive and prognostic biomarker.

1145

Image-Based Characterization of the Bone Marrow Microenvironment with Deep Learning Methods

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Bone marrow (BM) cavities are the primary sites of blood cell production, which is sustained by a rare population of self-renewing and multipotent hematopoietic stem cells (HSC). Local cues deriving from non-hematopoietic BM stromal cells critically modulate hematopoiesis and HSC maintenance through cell-cell interactions. Among stromal components, perivascular mesenchymal CXCL12-abundant reticular cells (CARc) and endothelial cells lining sinusoidal BM microvessels (sinusoids) have been shown to fulfill prime roles in the orchestration of hematopoietic development. Thus, the study of spatial distributions of different BM components can reveal key information on cellular crosstalk and the molecular mechanisms underlying hematopoietic regulation.
Understanding how cells interact with their microenvironment requires imaging the tridimensional spatial context surrounding them. For this, we have established advanced tissue processing and clearing protocols for the generation of 3D microscopy reconstructions of entire BM cavities with subcellular detail. To generate a high-throughput and unbiased analysis, we have developed a deep learning approach for automatic detection of the observed cellular components, which are then represented as segmented objects. We subsequently used robust spatial statistics to quantify how these segmented structures mutually constrain the available volume and interact with each other within the tissue boundaries.
Applied to our BM datasets, these methods are used to segment 3D sinusoidal microvascular networks with unprecedented speed and accuracy. The sinusoids are seen to occupy 20% of the total BM volume and leave little space for other cellular populations. We use classical segmentation methods to automatically detect the positions of CARc and to report their preferential location in perisinusoidal regions, with 64% of them being in direct contact with the abluminal side of endothelial cell walls.
In the BM, the results suggest that the stromal components have been previously inaccurately characterized, and we have proposed rigorous descriptors of their spatial confinement and cell frequencies. Furthermore, this approach can be used for uncovering novel spatial phenotypes of immunostained cellular components in different organs and conditions.

1148

MN1, hsa-mir-20a-5p and hsa-mir-181b-5p predict poor outcome in AML patients following autologous transplantation

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Background: The meningioma-1 (*MN1*) gene is expressed in hematopoietic progenitor and stem cells and it is suppressed during myeloid differentiation. *MN1* gene expression can be increased in AML cells thereby mediating proliferation and block of myeloid differentiation. Elevated *MN1* gene expression can be due to aberrant gene regulation or due to gene copy number amplification. The prognostic impact of MN1 gene copy amplification in peripheral blood mononuclear cells (PBMC) has not yet been evaluated in AML patients consolidated with autologous stem cell transplantation (ASCT) in first remission.
Methods: We retrospectively analysed 50 PBMC samples of favorable or intermediate-risk AML patients who underwent intensive induction chemotherapy with subsequent ASCT in CR1. *MN1* gene copy numbers and *MN1* gene expression in PBMCs were quantitatively assessed at diagnosis using Taqman copy number and gene expression assays.
Results: Patients with elevated *MN1* gene copy number and/or elevated *MN1* gene expression had an excess relapse risk, with significantly shorter PFS and OS after ASCT. Correlation of *MN1* gene copy number with expression of markers for myeloid differentiation indicated a robust association between *MN1* and *CD34* expression, suggesting *CD34* as a novel *MN1* target gene. Intriguingly, we previously identified a high total CD34+ cell count harvested in a single-day apheresis or a high percentage of CD34+ cells in the autograft to be negative prognostic factors for ASCT in AML patients. Finally, correlation of *MN1* gene expression with expression of non-coding RNAs identified a significant negative association of *MN1* and hsa-mir-20a-5p, as well as hsa-mir181b-5p expression, indicating a negative feed-back regulation of *MN1* gene expression by hsa-miR-20a-5p and hsa-mir-181b-5p.

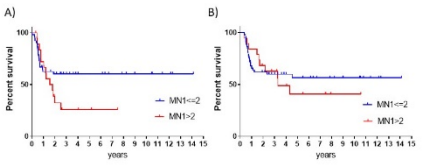

Figure 1: (A) Progression free survival and (B) Overall survival of patients with AML who achieved CR following induction chemotherapy and ASCT, stratified according to MN1 gene copy number at diagnosis. Blue curve: MN1 gene copy numbers of less or equal two (MN1<=2, range 1 to 2); red curve: MN1 gene copy numbers of more than two (MN1>2, range 2.2 to 3) in the mononuclear peripheral blood cells at diagnosis.

Conclusion: In AML patients consolidated with ASCT, high *MN1* gene expression in PBMCs is associated with adverse outcome and may be helpful for risk stratification particularly in the intermediate-risk subgroup. CD34 may represent a target gene of *MN1*. *MN1* offers a potential target for therapeutic intervention, e.g. using miRNA technology, in AML patients.

1150

Mutation-specific dominant negative effects determine phenotype severity in SRP54 deficiency in congenital neutropenia

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Whole exome sequencing analyses are increasingly performed on patients presenting with suspected inherited disease but lacking classical mutations linked to presented phenotypes. Using whole-exome sequencing in *sbds*-negative Shwachman-Diamond Syndrome (SDS) and congenital neutropenia (CN) families, we recently identified three independent patients, each of whom carried a heterozygous *de novo* missense variant of *srp54* (encoding signal recognition particle 54 kDa). The SRP54 protein is a key component of the ribonucleoprotein complex that mediates the co-translational targeting of secretory and membrane proteins to the endoplasmic reticulum (ER). Here, we describe a zebrafish mutant as the very first transgenic *in vivo* model of SRP54 deficiency and translate our previous findings into living organisms. We show that homozygous *srp54* mutant zebrafish die early during development, indicating that complete loss of SRP54 is not compatible with life. Heterozygous siblings, however, are viable and display mild neutropenia but no pancreas defect as shown by whole mount *in situ* hybridization for *mpo* and *lyz* or trypsin, respectively. Mimicking patient genotypes by injecting mutant mRNAs of human SRP54 into heterozygous *srp54* KO mutants aggravates SDS features in a dominant negative manner and induces differential phenotypes resembling those seen in patients. To further investigate SRP54 driven neutrophil defects, we exogenously expressed human SRP54 mutant variants in promyelocytic HL-60 cells and found significantly impaired morphologic differentiation and CD11b surface induction upon ATRA treatment.
Taken together, we here describe a novel disease model of SDS and congenital neutropenia founding on SRP54 as molecular driver. We reveal dominant negative functions of identified SRP54 mutations that vary in the severity of their phenotypic manifestation and we propose defective neutrophil differentiation as the underlying cause of neutropenia.

1151

Lineage tracing of acute myeloid leukemia reveals the impact of hypomethylating agents on chemoresistance selection

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Chemotherapy-resistant cancer recurrence is a major cause of mortality. In acute myeloid leukemia (AML), chemorefractory relapses result from the complex interplay of different levels of intra-tumor heterogeneity (ITH) – genetic, epigenetic and transcriptomic. The dependence of AML chemotherapy-resistant relapses on epigenetic factors is supported by the clinical benefits observed in refractory/ relapsed AML patients treated with DNA methyltransferase inhibitors (DNMTi – hypomethilating drugs). However, the impact of combining DNMTis with chemotherapeutic regimens on the different layers of ITH and its overall effect on de novo AML chemoresistance development remains largely unexplored.
We developed an experimental model system using in vitro lineage tracing (DNA barcodes – BCs) to assess the longitudinal clonal dynamics underlying the emergence of chemoresistance in human AML (hAML) cells in the presence or absence of DNMTi. To assess the contribution of different layers of ITH, we combined lineage tracing with exomic, transcriptomic and phenotypic profiling together with in vivo functional assessment of hAML cells relapsing to chemotherapy.
We found that combining standard chemotherapeutic regimens with low doses of DNMTi (hypomethylating drugs) prevents chemoresistant relapses. Mechanistically, we observed that DNMTi combination shaped dramatically the clonal dynamics and the transcriptomic profile of relapsed cells, while minimally affecing their exome composition. At the lineage tracing level DNMTi led to highest BC-clone depletion and suppressed the outgrowth of a pre-determined set of chemoresistant AML BC-clones with stemness properties, instead favoring the expansion of rarer and unfit chemosensitive clones. In detail, transcriptomic and functional studies showed that cells relapsing to standard chemotherapeutic regimens combined with low doses of DNMTi had lower stemness properties: reduced quiescence, ABC efflux capacity, aldehyde dehydrogenase activity and in vivo leukemia initiating potential. Importantly, we confirmed the capacity of DNMTi combination to suppress stemness-dependent chemoresistance development in both xenotransplantation models and primary AML patient samples.
Collectively, our findings attest the potential of a combinatorial approach of standard chemotherapy with low dose DNMTi to circumvent chemoresistance development in AML treatment, potentially turning this fatal malignancy into a chronic manageable disease.

HEMOSTASIS, TRANSFUSION MEDICINE, VASCULAR, LABORATORY MEDICINE

986

Bernard-Soulier syndrome with neonatal revelation: a rare pathology

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Introduction: Bernard-Soulier Syndrome (BSS) is a hereditary hemorrhagic disorder affecting the megakaryocyte/platelet lineage. It is characterized by a bleeding tendency, giant blood platelets and thrombocytopenia. This syndrome is extremely rare, about 100 cases have been reported in the literature. It is due to a quantitative or functional deficit of the platelet GPIb-V-IX glycoprotein complex.
Methods: A newborn was admitted for thrombocytopenic purpura initially believed to be due to a maternal autoimmune thrombocytopenia. Given the persistence of this thrombocytopenia despite the immunoglobulin treatments put in place, the exploration is launched.
Results: The blood smear revealed macro-thrombocytopenia with giant platelets. The study of platelet function revealed an absence of ristocetin-induced agglutination. The platelet flow cytometry study revealed a reduction in the GPIb level. Correct and early diagnosis of SBS is essential. The unpredictable severity of hemorrhagic symptoms is independent of low levels of GPIb. Our patient needed platelet transfusions. The prognosis is generally good with appropriate treatment.
Conclusion: SBS, also known as hemorrhagic thrombocytic dystrophy, is a rare hereditary disease characterized by inherited autosomal recessive inheritance. With good prophylaxis, a fairly normal quality of life can be maintained. In addition to other therapies, the BS could be a candidate for future gene therapy.

987

Feto-maternal erythrocyte incompatibilities no ABO – no anti-RH 1

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Introduction: Feto-maternal erythrocyte incompatibilities (FMEI) represent an area of perinatal immuno-hematology that is always manifested after birth, but sometimes during pregnancy. They represent 0.5/1000 births. The incompatibilities of anti-KEL1, -RH4, -RH2 specificity are rare, especially since they are not accessible to immunoprophylaxis. The care requires a multidisciplinary approach by specialists of this period of development.
Observation N °1:
A newborn male born from a pregnancy followed and brought to term in a P4D3 (A living child in good health +1 Death in utero + child died at 72h of life), hospitalized for the management of an early neonatal jaundice.
– Blood grouping of the newborn: A Rh + CC ee K negative.
– Blood grouping of the mother: A Rh + CC ee K negative.
– Blood grouping of the father: O Rh + cc Ee K negative.
– Total bilirubin = 175 mg/l, free bilirubin = 170 mg/l, Hemoglobin = 10.6 g/dl
– coombs test ++++ with presence of allo-anti-RH4 antibodies.

Observation N°2:
A newborn male born from a pregnancy followed and completed in a P4D2 (A living child in good health +1 Death in utero + 1 Abortion). He underwent 3 exchange-transfusion in utero. Hospitalized for the management of early neonatal jaundice.
– Blood grouping of the newborn: O Rh + cc ee K negative.
– Mother blood grouping: A Rh + cc ee K negative.
– Blood grouping father: O Rh + CC Ee K negative.

– Bilirubin T = 90 mg / l, free bilirubin = 85 mg/l, hemoglobin = 12 g/dl.
– coombs test +++ with the presence of anti-JK1, anti-RH2 and anti-RH1 allo-antibodies.

Comment: In addition to rhesus FMEI, today the pediatrician is essentially confronted with incompatibilities in the RH4, RH3 and KEL1 groups,which can sometimes be very severe. Interest of the noninvasive antenatal diagnosis of these compatibilities (genotyping of blood groups of the fetus on maternal blood) and that of their fetal anemic complications (Doppler velocimetry of the cerebral artery).

Conclusion: IFME remains valid because of their persistence despite prophylactic measures and because of considerable progress in management methods. Interest in coordinating obstetric-pediatric efforts for immediate management of IFMEs in the birth room and optimize detection of jaundice and phototherapy.

996

Implementation and validation of a collaborative platform for longitudinal biobanking of viable cells and plasma in myelopid neoplasms

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Background: Myeloid malignancies are heterogeneous disorders affecting the proliferation and differentiation of hematopoietic stem cells (HSCs) with a propensity to evolve towards secondary acute myeloid leukemias (sAML). Due to complex genetic interactions and variable clonal evolution, longitudinal collection of biologic material is important to forward our understanding of the pathogenesis.

Aim: To establish a standardized platform for longitudinal collection and storage of viable cells as well as plasma from peripheral blood (PB) and bone marrow (BM) for patients treated for myeloid malignancies at our institution.

Methods: 10-20ml of PB/BM are collected and mononuclear cells (MNCs) isolated using density-gradient centrifugation. 1ml PB/BM are centrifuged and 500ul plasma stored separately at -80°C. Manual and automatized measurement of cell-counts and viability is performed. MNCs are prepared at maximum concentration of 10x10e6 cells in 500 µl freezing-medium (RPMI, 10%DMSO, 20%FCS) using 0.7 ml 2D-barcoded cryotubes (FluidX®). Samples are immediately frozen below -150°C with a predefined temperature curve, in an automatized freezing work bench and centrally stored in a fully-automated biobank (ASKION C-line® system). Health related data is collected on a SecuTrial database.

Results: 122 patient samples were included between March 2017 and January 2019. Samples originated from bone marrow (MNCs n = 108; plasma n = 23), peripheral blood (MNCs n = 57) and leucapheresis samples (n = 9). They were mainly collected at diagnosis and conditions comprised AML (n = 16), MDS/MPN (n = 13), MDS (n = 14), hypoplastic bone-marrow conditions (hypoplastic MDS/aplastic anaemia: n = 5), idiopathic cytopenia of unknown significance (ICUS: n = 26) and others (n = 7). Features of bone-marrow and MNCs are summarized in table 1. We observed cell viabilities ranging from 51-92% in thawed samples without correlation with specific clinical conditions. Clump formation was associated with delay in processing after 24 h.

Diagnoses	AML (n=16)	MDS/MPN (n=13)	MDS (n=14)	Hypoplastic (MDS, AA) (n=5)	ICUS (n=26)
Male/Female	12/4	9/2	8/6	4/1	19/7
Age	68.5 (60 73)	70 (68 77)	72.5 (57 76)	30 (28 55)	68 (62.8 78.5)
Bone marrow features					
- cellularity	hypercellular	hypercellular	hypercellular	hypocellular	normo-/ hypocellular
- initial volume [mL]	10 (7-14.5)	10 (9-15)	12 (11.3-12.9)	8.3 (5.9-11.5)	10.5 (7.3-13.0)
MNCs features					
- cell count [mio/mL]	5.4 (5.2-34.2)	33 (21.9-58.3)	4.7 (4.6-11.1)	no data	6.9 (5-17.5)
- total available cells [mio]	72.8 (35.7-291.3)	510.6 (205.8-728.8)	83.5 (51.1-112.9)	no data	92 (40.9-145.9)
- total isolated cells [mio]	30.1 (15.3-57.2)	42.8 (11.2-78)	7.4 (2.2-30)	14.2 (1.0-30.9)	7.6 (3.5-25.6)
- cell viability before thawing [%]	87 (85.3-91.5)	78 (64-92)	82 (75-95.3)	93.4 (81.8-97.8)	75.4 (61.5-95.8)

Values are shown as medians with IQR.

Table 1: Diagnoses, bone-marrow and MNCs features of biobanked samples

Conclusion: The implementation of a biobank platform for longitudinal collection of viable cells poses numerous challenges at personnel, infrastructural, logistic, technical and financial levels. Such a platform is feasible, but collection procedures must be highly standardized at all levels to minimize heterogeneity of sample quality. Our experiences provide an essential basis for further improvements and will be instrumental to foster collaborative translational research.

1009

Integrated Safety Results from the Phase II and Phase III Studies with Caplacizumab in Patients with Acquired Thrombotic Thrombocytopenic Purpura

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Introduction: During the development of caplacizumab, safety data have been accrued from Phase I,II, and III studies in healthy subjects and patient with acquired thrombotic thrombocytopenic purpura (aTTP) . Given that caplacizumab blocks the interaction of the von Willebrand FactorA1 domain with the GPIb-IX-V platelet receptor, the main expected safety risk is bleeding.

Methods: The objective of this integrated analysis is to characterize the safety and tolerability of caplacizumab based on the pooled Phase I, II, and III data, with a main focus on data from the Phase II and III studies in aTTP. Treatment-emergent adverse events (TEAEs) and clinical laboratory evaluations were evaluated and summarized. Treatment-emergent bleeding was specified as an event of special interest. Data were analysed during the overall study including the follow-up period.

Results: Safety data for caplacizumab have been accrued in 220 aTTP patients. The median duration of exposure to study drug was 35.0 days in the caplacizumab group and 32.5 days in the placebo group. Similar percentages of subjects reported TEAEs in the caplacizumab (96.2%) and placebo (95.5%) group. Events that occurred more frequently (≥5% difference) in the caplacizumab group vs. placebo were epistaxis (29.2% vs. 5.5%; p <0.05), headache (20.8% vs 13.6%) and gingival bleeding (16.0% vs 2.7%; p <0.05). Events that occurred more frequently in the placebo group were TTP (35.5% vs 5.7%; p <0.05), hypokalaemia (20.0% vs 12.3%), and hypertension (12.7% vs 4.7%; p <0.05). Study drug discontinuation due to TEAEs occurred with similar frequencies in both groups and was mainly due to individual events with the exception of TTP. A lower percentage of subjects experienced SAEs in the caplacizumab group vs. placebo. The most frequently reported SAE was TTP in both the caplacizumab (5.7%) and placebo (34.5%) group. A higher percentage of subjects experienced bleeding TEAEs in the caplacizumab group (60.4%vs. 42.7%). Bleeding TEAEs were mainly mucocutaneous, most were self-limited and the majority resolved. Both treatment groups were similar with respect to laboratory values, with very few abnormalities reported as TEAEs.

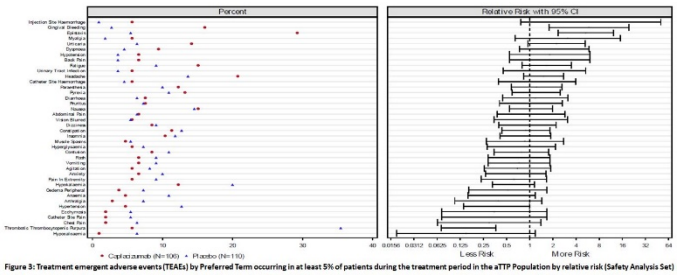

Figure 3: Treatment emergent adverse events (TEAEs) by Preferred Term occurring in at least 5% of patients during the treatment period in the aTTP Population by relative risk (Safety Analysis Set)

Notes: Relative Risk was calculated as: (n/N)Caplacizumab/(n/N)Placebo. The 95% CI was calculated using SAS frequency procedure non-modelling approach; aTTP=acquired thrombotic thrombocytopenic purpura; CI=confidence interval

Conclusions: Bleeding TEAEs (epistaxis and gingival bleeding), were the most common TEAEs in subjects treated with caplacizumab. Results from laboratory tests confirmed the safety profile of caplacizumab. This integrated analysis shows that caplacizumab is well tolerated and has a favourable safety profile.

1011

Laboratory marker of hypercoagulability: a ratio combining thrombin-generation with fibrin-clot-formation

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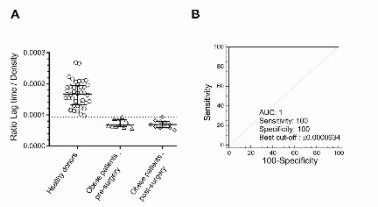
Background: Routine laboratory assays are insensitive to hypercoagulation and unable to predict thrombotic risk. Global hemostasis tests are

a promising strategy to detect procoagulant changes in plasma. Calibrated Automated Thrombogram (CAT) is the gold-standard for measuring thrombin generation and describing coagulation potential. Thrombodynamics (TD) is a new global assay that monitors the temporo-spatial propagation of blood coagulation. In TD, a tissue factor coated activator initiates coagulation, which then propagates through plasma, reflecting the fact that physiologically, initiation and propagation phases of coagulation take place in different regions. TD measures thrombin generation and fibrin clot formation. Here we investigate whether CAT and/or TD can identify the hypercoagulable state in patients with a well-characterized prothrombotic phenotype and which CAT and/or TD parameters could be investigated as potential predictors of thrombotic risk.
Methods: Blood was collected from 12 obese patients (BMI ≥35 kg/m^2), aged 18 or older undergoing bariatric surgery, before (baseline) and after (day 3) surgery. Reference ranges of CAT and TD parameters were established in a control group of 40 healthy donors. Measures of fibrin formation and thrombin generation were performed in platelet free plasma.
Results: Thrombin generation by TD and CAT was significantly higher in obese patients compared to healthy volunteers. Similarly, analysis of fibrin formation by TD showed that clot growth, density, and spontaneous clotting were significantly increased in patients. Nevertheless, none of these measurements could discriminate between normal volunteers and obese patients. Of note, a ratio between thrombin generation's lag time (CAT) and fibrin clot's density (TD) could single out all hypercoagulable plasmas, as shown in the Figure.

Figure



Dot plot (A) and ROC curve analysis (B) of ratio between lag time (CAT) and clot density (TD).

Conclusions: Combining thrombin generation and fibrin-clot formation parameters allows recognizing hypercoagulable plasma and may represent a predictive factor for thrombotic events. A prospective study is needed to validate the performance of this ratio.

1013

Sodium-calcium exchanger reverse mode is required for generation of procoagulant COAT platelets

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Background: Combined activation of platelets (PLTs) with collagen-plus-thrombin induces formation of procoagulant COAT PLTs. The dichotomous intracellular signaling generating a PLT subpopulation displaying procoagulant activity instead of traditional aggregating endpoints is still not fully elucidated. It has been demonstrated that secondary messengers such as calcium and sodium play a critical role in this process. Therefore, we investigated the kinetics of these ions and the role of sodium-calcium exchanger (NCX) during procoagulant PLT formation.
Methods: We characterized distinct PLT populations by flow cytometry after activation with convulxin (CVX, collagen receptor GPVI agonist) and thrombin (THR). Free cytosolic calcium and sodium were measured with specific ion fluorescent indicators (Fluo-3 and ION NaTRIUM Green-2), and Annexin-V co-staining allowed discriminating procoagulant COAT PLTs. NCX function was inhibited with 5-(4-chlorobenzyl)-2'-4'-dimethylbenzamil (CBDMB).
Results: Specific ion fluxes were observed in distinct procoagulant and aggregating PLT subpopulations. High and prolonged intracellular calcium concentration (>1 µM), and transient sodium increase were observed in COAT PLTs, whereas aggregating non-COAT PLTs rapidly decreased their calcium content and maintained higher cytosolic sodium. CBDMB dose-dependently diminished (IC_{50} = 2.85 ±0.3 µM) and delayed (see Figure) generation of COAT PLTs by modulating ion kinetics: calcium efflux was retarded and reduced in aggregating PLTs while sodium efflux was reduced in COAT PLTs. This suggests that both forward and reverse NCX modes are used in CVX-plus-THR-activated PLTs.

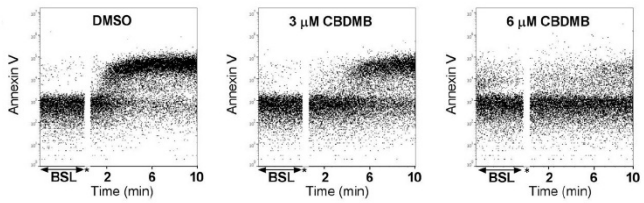

Figure: Time course generation of procoagulant COAT platelets (Annexin-V positive) in presence of DMSO or CBDMB. (*) activation with convulxin-plus-thrombin; baseline, BSL.

Conclusions: This study demonstrates that NCX-dependent calcium influx is required for procoagulant COAT PLT formation. This novel observation shows that non-COAT aggregating PLTs use forward mode NCX (pumping calcium out and moving sodium in), while procoagulant COAT PLTs rely on reverse NCX function (which exchanges cytosolic sodium with extracellular calcium) to achieve an efficient procoagulant signaling.

1016

Women with congenital fibrinogen disorders are at increased risk of obstetrical complications

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Background: Congenital fibrinogen disorders (CFD) are rare quantitative or qualitative fibrinogen anomalies, including afibrinogenemia (A), hypofibrinogenemia (H), dysfibrinogenemia (D) and hypodysfibrinogenemia (HD). As fibrinogen plays an essential role in pregnancy, we addressed the issue of obstetrical complications in women with CFD with a systematic review of the literature.
Methods: A systematic review, restricted to English manuscripts, was conducted according to the PRISMA guidelines. We searched through the MEDLINE database for English articles, published from January 1985 until November 2018, using the following search terms [Mesh]: (`Pregnancy' AND 'afibrinogenemia' or 'hypofibrinogenemia' or 'hypodysfibrinogenemia' or 'dysfibrinogenemia'). A total of 198 articles were identified, 15 articles were added from other sources. Overall, 213 articles were screened and 54 were included in the final analysis.
Results: A total of 188 pregnancies from 70 women were analyzed. The mean age of first pregnancy was 27.3 (±4.3) years old. About half of pregnancies resulted in miscarriage, more specifically in 15 (42.9%), 36 (46.8%), 27 (42.9%) and 4 (30.8%) of A, H, D and HD, respectively. Overall, 62 (88.6%) women had at least one successful pregnancy. Preterm complications were also frequent (33.5%). Metrorrhagia, mainly in the first trimester, was observed in 10 (28.6%), 16 (20.8%), 9 (14.3%) and 3 (23.1%) of A, H, D, HD pregnancies, respectively. Placenta abruption were reported in 8% of the pregnancies. Half of deliveries were performed by caesarean-section. A fibrinogen replacement therapy was introduced in 30% of pregnancies, usually as prophylaxis regimen (81.1%) or on demand (18.9%) due to abnormal bleeding. A total of 24 (12.7%) post-partum were complicated by thrombotic events (3.2%) or post-partum hemorrhage (9.6%).
Conclusion: These results suggest that women with CFD are at high-risk of obstetrical complications, including miscarriage and metrorrhagia, as well as post-partum adverse outcomes. However, systematic reviews are prone to publication bias that may affect the overall prevalence of obstetrical complications in this setting. Prospective international registries may allow to identify more precisely the incidence of obstetrical complication and their management in CFD.

1020

Impact of miR-204 on human platelets

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Background: Several platelet-derived miRNAs are associated with platelet reactivity (PR) and clinical outcome in cardiovascular patients. We previously showed a correlation between miR-204-5p and PR in stable cardiovascular patients but functional validation is lacking.
Aims: To functionally validate miR-204-5p as a regulator of PR in platelet-like structures (PLS) derived from human hematopoietic stem cells and to address mechanistic issues.
Methods: We used a model where human hematopoietic stem cells are differentiated into megakaryocytes, allowing transfection of miR-204-5p and recovery of subsequent PLS. The functional impact of miR-204-5p was assessed by several methods, including a flow-based assay and a spreading assay on fibrinogen. Transfected megakaryocytes and PLS were morphologically characterized by FACS and microscopy. Quantitative PCR and western blot were used to assess the impact of miR-204-5p on selected gene target. Finally, miR-204-5p plasma level was quantified in an independent cohort of cardiovascular patients.
Results: Functional assays showed an increased reactivity in megakaryocytes and PLS after transfection with miR-204-5p compared to control. In megakaryocytes, miR-204-5p transfection induced cytoskeletal changes, involving regulation of Rho family GTPase through downregulation of cdc42 protein, while megakaryocytes released abnormal chapelets bearing enlarged proplatelet tips. In a cohort of cardiovascular patients treated with aspirin (n = 187), miR-204-5p level was correlated with PR induced by various agonists.
Conclusions: We functionally validated miR-204-5p as a regulator of PR that occurs through actin dynamics regulation. These data support a potential role of miR-204-5p as a biomarker of PR.

1029

The implementation of a ROTEM® sigma-based algorithm for the management of coagulopathic bleeding in a tertiary center

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Introduction: Viscoelastic assays are used for the management of perioperative or traumatic bleeding. Recently, the manufacturer of ROTEM® has introduced the automated sigma model. The change in technology results in new normal rang986es and therefore new cutoff values triggering intervention compared to previous published ROTEM®delta cutoff values. Here, we describe the analysis performed to identify new cutoff values and the specific algorithm for the ROTEM®sigma.
Methods: 78 samples were obtained from 39 patients with various hemostatic defects and 12 healthy volunteers. Chosen standard laboratory test (SLT) cut-offs were fibrinogen (Fi) <1.5 g/l, platelet count (Pltc) <50 G/l or <100 G/l, prothrombin ratio (PR) <80% and activated partial thromboplastin time (aPTT) >37 sec or 1.5x the normal value. PLTEM was calculated as EXTEMA5 – FIBTEMA5. The clinically critical range (CCR) of SLT was defined as the range around the threshold that leads to treatment. Correlation was sought between SLT and ROTEM parameters. The best cut-off for the different ROTEM parameters to identify the chosen SLT thresholds (ROC analysis) were integrated in a step-by-step algorithm (Figure 1).
Results: Correlation between Fi and FIBTEMA5 was very strong (r = 0.94) but was lower in the CCR (0-2.5 g/l) (r = 0.65). The correlation between Fi and EXTEMA5 was weakly moderate (r = 0.42). PLTEMA5 showed very strong correlation (r = 0.96) with Pltc in the CCR (<150 G/L). INTEMCT showed very strong correlation with aPTT (r = 0.84). EXTEMCT correlated moderately with PR (r = -0.58). Cut-off based on ROC curve analysis for different parameters are showed in Table 1.

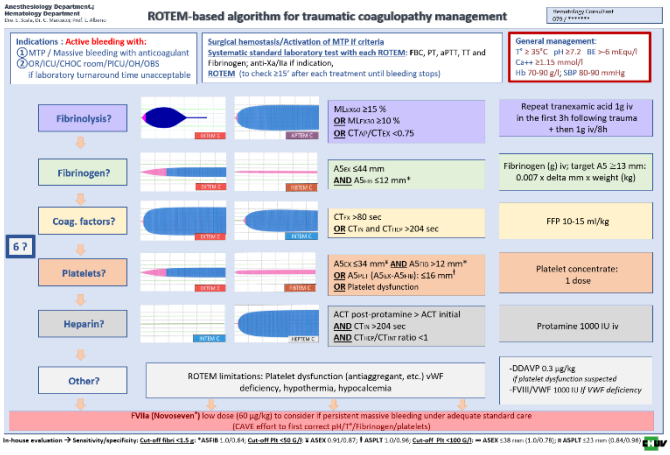

Table 1. ROC analysis for ROTEM® sigma Parameters

ROTEM parameter	SLT cut-off	Sensitivity (%)	Specificity (%)
FIBTEM A5 ≤12 mm	Fi <1.5 g/L	100	63.6
FIBTEM A5 ≤12 mm	Fi <2.0 g/L	100	71.4
EXTEM A5 ≤44 mm	Fi <1.5 g/L	100	38.2
EXTEM A5 ≤44 mm	Fi <2.0 g/L	100	42.9
EXTEM A5 ≤34 mm	Pltc <50 G/L	90.9	87.3
PLTEM A5 ≤16 mm	Pltc <50 G/L	100	96.4
EXTEM A5 ≤38 mm	Pltc <100 G/L	100	78.3
PLTEM A5 ≤23 mm	Pltc <100 G/L	83.9	95.7
CT EXTEM >80 sec	PR <80%	25.0	100
CT INTEM >204 sec	aPTT >37 sec	75.0	97.4
CT INTEM >204 sec	aPTT >55 sec	100	80.0
Combined parameters			
FIBTEM A5 ≤12 mm And EXTEM A5 ≤44 mm	Fi <1.5 g/L	100	77.5

Conclusion: In-house cut-off values of key ROTEM®sigma parameters differ from the published ones for ROTEM®delta. We report the first preliminary ROTEM®sigma-based algorithm for hemorrhage. Diagnostic and therapeutic performances shall be prospectively validated.

1032

PAX5 as a model to study the evolution of transcription factors' regulatory networks

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Background: PAX5 is a B-cell specific transcription factor (TF) that acts as a master regulator of B-cell phenotype through the regulation of lineage-specific genes. The leading role of transposable elements (TEs) in the emergence of new cis-regulatory sequences across the genome of vertebrates during evolution is increasingly recognized. TEs account for 50% of the human DNA, and can spread cis-regulatory sequences (i.e., "TF binding-sites") throughout the genome by a "copy-paste" mechanism at play during gametogenesis and early embryogenesis. TEs are still actively shaping the human genome with an estimated new integrant every 20-50 births. Focusing on early B-cell development, we used PAX5 binding-sites and the genes under its influence to trace back the evolution of its regulatory network.
Methods: We used the results of PAX5 ChIP DNA-Sequencing performed on lymphoblastoid cell linesand lists of PAX5-regulated genes in human Pro-B cells. Batch coordinate conversion between the human genome of those of 47 other species was obtained by whole-genomes alignment using UCSC liftOvertool.

Results: We found that more than 60% PAX5-binding human genomic loci resided within TEs, most of which dated at most to the last common ancestor of mice and human. Amongst 43'763 PAX5-binding sites, 93 were located in the vicinity of gene promoters essential for early B cell development, including 29 at the IgH locus. Phylogenetic analyses determined that the genes of TFs essential for early B-cell development were characterized by the sequential accumulation of PAX5-responsive*cis*-regulatory sequences during evolution. Notably, CBFβ, the promoter of which harbored such elements aged between 29.4 and 159 mio, NFATC1 (105-312 mio), BCL2 (105-159 mio), TCF3 (29.4-312 mio) and ZBTB7A (43.2-105 mio), whereas genes involved in late B cell development such as ID3 and MEF2C, which are respectively responsible for germinal center formation and B cell survival after antigen recognition, were found to harbor TE-embedded PAX5-responsive motifs dating back to 312 and 435 million years, while that ofNOTCH1, an important factor for B-cell activation, contained a similar motif 159 mio y.o.
Conclusions: The evolutionary turnover of TE-embedded PAX5-responsive*cis*-regulatory sequences has played a major role in shaping the humoral arm of the adaptive immune system.

1046

Prospective validation of a rapid and accurate diagnostic algorithm for heparin-induced thrombocytopenia.

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Background: Heparin-induced thrombocytopenia (HIT) carries a high mortality and morbidity if left untreated, while switching unnecessarily to alternative anticoagulants leads to bleeding complications and increased costs. Clinical suspicion of HIT therefore requires rapid laboratory assessment to guide clinical management. We developed a diagnostic algorithm able to accurately confirm or exclude HIT with a short laboratory turn-around-time (TAT).
Methods: Based on our previous derivation studies (05.2014-12.2016, n = 526), we developed an in-house diagnostic algorithm that relies on the Bayesian combination of pre-test clinical probability (4Ts-score) and quantitative results of sequential rapid immunoassays (IAs) for anti-PF4/heparin antibodies [AcuStar HIT-IgG (IgG-specific CLIA) and PaGIA-H/PF4 (PaGIA)] in order to predict the outcome of the functional gold-standard assay (HIPA). We are prospectively validating this algorithm (01.2017-04.2019, n = 492).
Results: 100% negative (NPV) and positive (PPV) predictive cut-off values for a positive HIPA were set at IgG-specific CLIA values of <0.13 U/ml and >3.0 U/ml, respectively. For PaGIA the cut-off titers were <2 and >8, respectively. Likelihood ratios were determined for intermediate results. During the prospective validation study, IgG-specific CLIA was employed as a single IA in 395/492 (80.3%) of cases (TAT 30 min); PaGIA was performed as a second line IA in 97/492 (19.7%) of initially unsolved cases (TAT 60 min). Our Bayesian diagnostic approach could exclude HIT in 432/492 (87.8%) and correctly predict it in 37/492 (7.5%) of patients with suspected HIT, leaving thus only 16/492 (3.3%) of unsolved cases after 60 minutes. In six cases (1.2%), the positive prediction of our algorithm was not confirmed by HIPA. However, laboratory work-ups and clinical evolutions were very suggestive of HIT in all six patients.
Conclusion: The combination of the estimated clinical probability of HIT and the sequential application of two rapid IAs for anti-PF4/heparin antibodies enables a rapid and accurate diagnostic work-up within 60 minutes for more than 95% of patients with suspected HIT.

1076

The new P2Y12 inhibitor cangrelor unreliably inhibits heparin-induced platelet aggregation in the presence of HIT antibodies, an in vitro study

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Introduction: Cardiac surgery in patients with HIT puts the patient at high risk of lethal thrombotic complications if heparin is used during surgery. Two strategies exist to prevent intraoperative platelet aggregation during cardio-pulmonary bypass if anti-PF4/heparin antibodies (HIT-Abs) are present. The first is to use an alternative anticoagulant, the second is to use heparin combined with an antiplatelet agent. The new $P2Y_{12}$ inhibitor cangrelor could be an attractive candidate in this setting and several authors report its successful use. In this in vitro study we evaluated the capacity of cangrelor to inhibit platelet aggregation induced by heparin in the presence of HIT-Abs.
Methods: Platelet poor plasma (PPP) from 30 patients with functional HIT-Abs was mixed with platelet rich plasma (PRP) from healthy donors. Heparin-induced platelet aggregation (HIPA) was measured by light transmission aggregometry (LTA) after adding heparin to achieve a final concentration of 0.5 IU ml^{-1} and compared to samples with normal saline only (negative control) or cangrelor (final concentration 500 ng ml^{-1}) added prior to heparin (treatment).
Results: Heparin 0.5 IU ml^{-1} triggered platelet aggregation in 22 out of 44 PPP-PRP mixtures, with a median aggregation of 85.9% (IQR 69.2 to 90.9). The median aggregation in the corresponding 22 negative controls was 22.1% (IQR 15.9 – 29.7) (p <0.001). Median aggregation in the treatment samples was 28.5% (IQR 19.5 to 51.9): significantly lower than in HIPA positive samples (P <0.001) but higher than in negative control samples (p <0.05) (Figure 1). The mean percentage of inhibition of HIPA by cangrelor was 73.4 ± 34.0%. Cangrelor reduced HIPA by more than 95% in only 10/22 samples (45%) . In 5/22 (22%) the inhibition by cangrelor was less than 50%, and in 3/22 (14%) less than 10%.

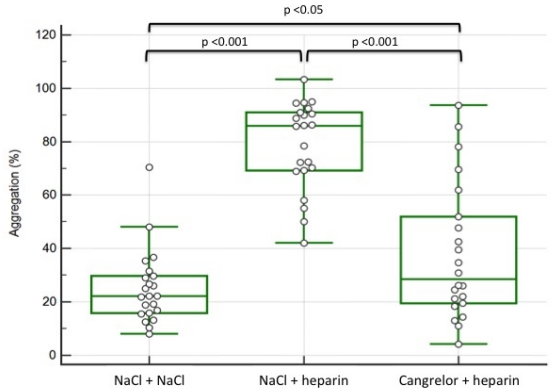

Figure 1: Box plot of the platelet aggregation measured by LTA in the three tested conditions; Spontaneous aggregation (NaCl + NaCl), Heparin-induced aggregation (NaCl + heparin); Effect of cangrelor when added before heparin (Cangrelor + heparin)

Conclusion: In this in vitro study we found that cangrelor unreliably inhibits heparin-induced platelet aggregation in the presence of HIT-Abs. We conclude that cangrelor cannot be used as a standard antiaggregant agent in combination with heparin for cardiac surgery in HIT patients, unless its efficacy has been confirmed in a functional test prior to surgery.

1078

Identification of high platelet reactivity under P2Y12 ADP-receptor blockade: Observation of two platelet populations using the VASP flow cytometric assay and improvement of detection by atypical PFA-P2Y shape of curve

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Introduction: High or low on treatment platelet reactivity under clopidogrel therapy could lead to an increased rate of ischemic or bleeding events, respectively. Platelet inhibition monitoring has been extensively studied in this setting. When comparing the easy and rapid whole blood PFA-P2Y assay to time-consuming reference methods (LTA or VASP), the correlation is moderate to weak. Using these tests, we made two observations (Figure 1): i) The shape of the PFA-P2Y curve is often atypical; ii) Two distinct platelet populations, inhibited and non-inhibited, can be observed during VASP-flow cytometry. Our hypothesis was that the presence of an atypical shape of curve in the PFA-P2Y assay might better predict high platelet reactivity (HPR).
Methods 72 patients on clopidogrel were assessed using two platelet function assays before elective intracerebral stenting, included VASP/$P2Y_{12}$® on an ACCURI C6® flow-cytometer and INNOVANCE PFA-P2Y test cartridge®. Platelet reactivity index (PRI-VASP) was calculated for the whole platelet as well as for the separate populations.
HPR was defined as: PRI-VASP >50% and PFA-P2Y® occlusion time (PFAOT) <106 sec.

Patients were classified in 2 groups according to the PFAOT first and again according to the analysis of the shape of curve (ASC). An atypical shape of curve was defined as an occlusion of >50% with subsequent re-establishment of flow.

Results: HPR was diagnosed in 30/72 (41.7%) using VASP-PRI, 5/72 (6.9%) using PFAOT and 35/72 (48.6%) by the combination of PFAOT and ASC. The kappa coefficient agreement was very weak (0.19) between PRI-VASP and PFAOT but was strong (0.64) between PRI-VASP and PFAOT combined with ASC. ROC analysis of the PFAOT combined with ASC to detect HPR defined by VASP, showed a Youden J index of 0.65 (AUC 0.83) with a sensivity 87% of and a specificity of 79%.The presence of an atypical shape of curve was related to a higher fraction of non-inhibited platelets in the VASP assay.

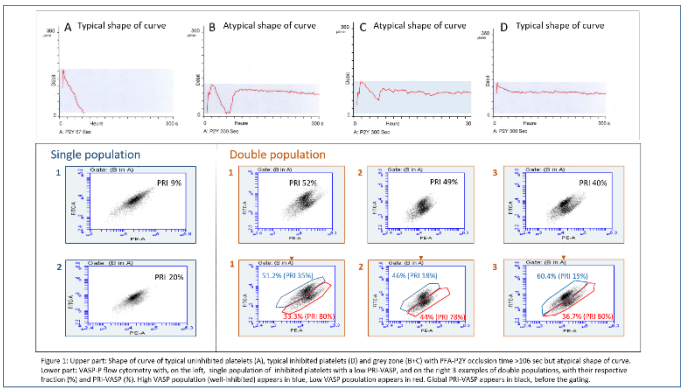

Figure 1: Upper part: Shape of curve of typical uninhibited platelets (A), typical inhibited platelets (D) and grey zone (B+C) with PFA-P2Y occlusion time >106 sec but atypical shape of curve. Lower part: VASP-P flow cytometry with, on the left, single population of inhibited platelets with a low PRI-VASP, and on the right 3 examples of double populations, with their respective fraction (%) and PRI-VASP (%). High VASP population (well-inhibited) appears in blue, Low VASP population appears in red. Global PRI-VASP appears in black, before the gating.

Conclusion: The ability of PFA-P2Y to identify HPR is improved by the analysis of the shape of curve. The atypical shape of curve is significantly associated with a higher fraction of non-inhibited platelets (low VASP population).

1086

Acquired hemophilia A in post-partum: a specific study in the post-partum setting

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Introduction: Acquired post-partum hemophilia A is mostly occurring in primigravidas in the first postpartum weeks. These cases are rare and guidelines are not post-partum specific.

Methods: We present a severe case of post-partum development of a factor VIII inhibitor and an exhaustive review of the literature.

Results/case report: A 36-year-old primigravida, gave birth at term by vaginal delivery. Fourteen days later, she presented abnormal vaginal bleeding and a parietal hematoma, which was surgically drained. Acquired hemophilia A was diagnosed with aPTT 90 seconds, factor VIII (FVIII) <1%, and FVIII inhibitor 10 Bethesda Units. A treatment by bypassing agent (recombinant factor VIIa, rVIIa; 90ug/kg) 3 times a day and oral prednisolone (1mg/kg/d) was started. During the following month, the hematoma progressed together with worsening anemia. Rituximab (750mg (375mg/m2) 1x/week for four weeks) was initiated. The evolution was slowly favorable (anemia stabilization, aPTT 51 seconds, FVIII <1%). Follow-up was characterized by multiple FVIII decreases during the steroid tapering over 15 months. The patient decided against having another child.

The literature search (1946-2019) identified 34 articles describing 123 cases, mostly isolated case reports and small series (<5 cases) with two larger cohorts of 19 and 42 patients. Median age at diagnosis is 30 years (range 17-41). The index pregnancy is usually the first (1st-7th). Symptoms occur within a range of 0 to 308 days after delivery (median 60 days), while only 6 cases (4.9%) occurred prepartum. The most frequent bleeding type is cutaneous (47.5% of reported cases), muscular (35%), peri-partum (32.5%) and hemarthrosis (20%). In most cases, FVIII at diagnosis is undetectable (<1%), the highest value was 8%. Median FVIII inhibitor titer is 16.5 BU/ml (1-621). The preferred treatment is steroids (74%). Bypassing agents are now widely used in the acute phase. Rituximab was first reported efficient in 2005, and is mostly used in refractory or relapsing cases. Only 16 patients (13%) have documented subsequent pregnancies (total 19 pregnancies) and 13 (81%) had hemophilia recurrence defined as decreased FVIII levels.

Conclusion: Post-partum acquired hemophilia A is rare and can be associated with severe bleeding. New treatments, such as Rituximab, have improved the clinical course. However most patients decide against subsequent pregnancies, which are characterized by an alarming 81% of relapse.

1092

Prospective Analysis of Breakthrough Hemolysis in Phase 3 Studies of Ravulizumab Versus Eculizumab in Adults with PNH

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Background: Breakthrough hemolysis (BTH) is the return of hemolytic disease activity during treatment with C5 inhibitors for paroxysmal nocturnal hemoglobinuria (PNH). BTH may be associated with inadequate C5 inhibition or complement activating conditions (eg, infection). Based on literature review and expert consensus, BTH was defined as ≥1 new or worsening symptom/sign of intravascular hemolysis (fatigue, hemoglobinuria, abdominal pain, dyspnea, anemia, major adverse vascular event, dysphagia, or erectile dysfunction) in presence of elevated lactate dehydrogenase (LDH). In 2 large pivotal studies in PNH pts, ravulizumab (RAV) was shown noninferior to eculizumab (ECU) for all key endpoints, including BTH. RAV reduced free C5 levels to <0.5 µg/mL in all pts at all times; ECU did not consistently achieve this level of C5 inhibition. Here we report BTH prevalence and causality in these 2 clinical studies.

Methods: Two phase 3, randomized, open-label, noninferiority, multicenter studies (study 301, NCT02946463; study 302, NCT03056040) included pts ≥18yrs with PNH. Pts in study 301 were naive to C5 inhibitor therapy. Pts in study 302 were stable on ECU treatment for ≥6 months. Pts received weight-based dosing of RAV q8w or the approved ECU dose (900 mg q2w) for 183 days.

Results: In study 301, 246 pts received RAV (n = 125) or ECU (n = 121); in study 302, 195 pts received RAV (n = 97) or ECU (n = 98). In study 301, 5 pts (4%) experienced BTH in the RAV arm vs 13 pts (10.7%) in the ECU arm [95% CI: −6.7% (−14.2%, 0.2%)]. In ECU arm, 7/15 BTH events were associated with inadequate C5 inhibition (free C5 ≥0.5 µg/mL). No BTH events in the RAV arm were due to inadequate C5 inhibition. In the RAV group, 4/5 BTH events, were associated with infection vs 6/15 BTH events in ECU arm. In study 302, no pts in the RAV arm experienced BTH vs 5 pts (5.1%) in the ECU arm [95% CI: −5.1% (−19%, 8. 9%)]. Four of 7 BTH events in the ECU arm were associated with concomitant elevations in free C5. Infection was associated with 3/7 BTH events.

Conclusions: No BTH events in the RAV arm were associated with elevations in free C5. In contrast, pts treated with ECU experienced BTH events with elevations in free C5 suggesting suboptimal C5 control. Similar numbers of pts receiving RAV or ECU experienced infection-related BTH. Differences in BTH rates for RAV vs ECU may be due to improved pharmacodynamics (C5 inhibition throughout the dosing interval) and the optimized ravulizumab dosing regimen.

1096

Thrombin generation and fibrin clot formation in patients with liver cirrhosis: modern global hemostasis assays provide new insights into a procoagulant state

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Introduction: Cirrhotic patients are at increased thrombotic risk despite prolonged routine *in vitro* coagulation assays, *e.g.*prothrombin time and aPTT. This has been proven in clinical studies and *in vitro* using thrombin generation (TG) assessed by Calibrated Automated Thrombogram (CAT). However, *in vitro* studies highlighted some discrepancies. These can be explained by a high inter-laboratory methodological variability and by differences in severity and aetiology of liver cirrhosis. In order to reach reproducible results and to assess the clinical value of TG assays, larger studies using less variable methods and including a prospective follow-up are necessary.
Method: We have initiated a single-centre prospective study recruiting patients with liver cirrhosis Child-Pugh A to C (n = 400). We perform TG measurements using three different assays: 1) CAT, a gold standard method for TG (Fluoroskan Ascent, Thermo Fischer Scientific, Vantaa, Finland); 2) ST Genesia, a very recently marketed automated, standardised and normalised TG assay (Stago, Asnière, France); 3) Thrombodynamics analyser (TD), measuring TG and fibrin formation (Hemacore, Moscow, Russia). Analyses on CAT and ST Genesia are performed with and without thrombomodulin (TM). A clinical follow-up of 12 months is planned. Here, we report the results for the first 60 patients.
Results: We confirm an increased endogenous thrombin potential with TM in cirrhotic patients compared to healthy subjects, highlighting the reduction of the natural anticoagulant proteins C/S in these patients. TD shows already without TM an increased and faster propagation of TG at distance of tissue factor compared to healthy subjects. This observation reflects an increased amplification phase of the coagulation in cirrhotic patients. TD also demonstrates a more rapid fibrin formation with denser clots in liver cirrhosis.
Conclusion: We confirm a prothrombotic state in cirrhotic patients. New insights are: TD shows an increased amplification phase of coagulation in cirrhotic patients, already in absence of TM; this could be explained by reduced natural anticoagulants, mostly antithrombin. TD also shows an increased fibrin formation in liver cirrhosis, likely due to increased TG. The ongoing recruitment of patients should allow to reliably analyse subgroups and clinical parameters associated with TG. The one-year clinical follow-up should allow to assess the ability of these tests to predict thrombo-haemorrhagic complications.

1099

Prevalence of arterial thrombotic events in an international cohort of 123 patients with hereditary thrombotic thrombocytopenic purpura

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Introduction: Congenital thrombotic thrombocytopenic purpura (cTTP) is an ultra-rare autosomal recessive inherited disease characterized by an absent or severely reduced activity of ADAMTS13. In the absence of functional ADAMTS13, large von Willebrand factor multimeric strings are not cleaved into normal-sized ones resulting in the adherence of platelets and formation of occlusive microthrombi, which may lead to arterial thromboembolic events (ATE). At present, little is known about ATE prevalence in cTTP patients.
Methods: The Hereditary TTP Registry is an international open and ambi-directional cohort study on the clinical course and long-term outcome of cTTP. Clinical and laboratory data at enrolment were recorded for each confirmed cTTP patient retrospectively by physicians responsible for patient care. Diagnosis of cTTP is confirmed by a severely deficient ADAMTS13 activity (<10% of normal) in the absence of a functional inhibitor, and the presence of bi-allelic *ADAMTS13*mutations. Enrolment data until the end of 2017 were analyzed, including the occurrence of ATE (stroke, transient ischemic attack (TIA), and myocardial infarction) reported until enrolment. All patients or their legal representatives provided written informed consent before enrolment.
Results: Until the end of 2017, the Registry enrolled 123 confirmed cTTP patients with a median age at enrolment of 26.1 years (range 0-75 years) and a median age of overt disease onset of 20 years (0-70 years). After jaundice, ATE were the second most frequent complications reported. Stroke had occurred in 21%, transient ischemic attack in 10%, and myocardial infarction in 4.2% of patients (n = 120). ATE had occurred in all age groups. From age group >40-50 years onwards, half or more of the cTTP patients had suffered from at least one ATE.
Age of overt disease onset increased with increasing ADAMTS13 activity levels for patients with ATE. For patients without ATE, there was no difference in the age of overt disease onset between patients with undetectable ADAMTS13 activity and those with ADAMTS13 activity 1-<5% (Table 1).

Table 1. Age of overt disease onset related to residual ADAMTS13 activity level in patients with and without ATE occurrence before enrolment

	Residual ADAMTS13 activity (%)		
	< 1%	1 - < 5%	5 – 10 %
cTTP patients with ATE	12 (0-32), n=10	18 (0-68), n=10	47 (24-70), n=2
cTTP patients without ATE	3 (0-56), n=59	3 (0-41), n=22	20 (12-28), n=2

n, number of patients with available values; continuous variables are presented as median [minimum, maximum]

Conclusion: cTTP patients in the Hereditary TTP Registry have a high prevalence of ATE already at a relatively young age. Although the level residual ADAMTS13 activity may not be the only determinant for overt disease onset in cTTP, our data indicate that residual ADAMTS13 may play a role in protecting against arterial thromboembolic events.

1104

Comparison of four liquid biopsy platforms for the detection of circulating tumour DNA

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Background / introduction: Detection of circulating tumour DNA (ctDNA) in liquid biopsy represent a promising new laboratory tool in oncohaematology. Applications include, early detection of cancer, screening for minimal residual disease, and detection of resistance mutation. Currently, more than a dozen technologies are available to perform ctDNA analyses, but no systematic comparison has been conducted to assess the relative efficiency of the various technologies. We conducted a multicentre study assessing pre-analytical (DNA extraction) and analytical (sequencing and variant calling) factors in ctDNA analysis.
Methods: Preanalytical: Blood from a single donor was extracted in four laboratories using different methods: QiaAmp/QiaVac, MinElute, Cobas, and MagMax. Amount and quality of cfDNA, as well as contamination by genomic DNA were checked by fluorimetry, capillary electrophoresis and quantitative PCR.
Analytical: Aliquots of control DNA (Horizon Ltd) containing 8 mutations at various frequencies (5%, 1%, 0.1% and 0%) were sequenced in the 4 laboratories using different library systems, all featuring molecular barcodes and different sequencers: Oncomine + Ion Torrent, Avenio + Illumina and QiaSeq + Illumina. Coverage, duplication rate, sensitivity, specificity and linearity of detection were appraised at the levels of the reads, molecules and variant calling.
Results: Preanalytical: DNA quality and yield were excellent with all systems. Although DNA concentration was higher with MagMax, total DNA yield was similar to other systems.
Analytical: In general, all platforms gave excellent results at 5% and 1% mutation, with 100% sensitivity and specificity, including small indels. Detection of 0.1% mutation (3 mutant molecules in 10 ng DNA) was problematic with all platforms, and none of the variant callers achieved detection of all 8 mutations. Sensitivity was improved by using “whitelist” detection algorithms but did not reach 100%.
Conclusion: There was no major difference in efficiency between the 4 platforms. Mutation detection was achieved perfectly at 5% and 1% mutation frequency but less so at 0.1% due to the very small ctDNA quantity (3 molecules). DNA amount is the most important limiting factor in liquid biopsy analyses emphasizing the importance of optimal preanalytical steps.

1108

Platelet function analysis: a comparison between PRP-Aggregation and FACS before and after activation with TRAP

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Introduction: There are many platelet (PLT) stimuli: adenosine diphosphate (ADP), epinephrine (EPI), thrombin, and collagen (COL). COL and thrombin are the most potent PLT-activators. PLT-aggregation (AG) from platelet rich plasma (PRP) can identify alterations in platelet function. FACS can be a challenging method to study PLT function.
Aim: To describe and compare PLT function analysis performed with PRP-AG according to Born and FACS before and after activation with thrombin receptor activating peptide (TRAP).
Methods: We studied PLT function in 45 patients referred for investigation of bleeding diathesis. In vitro PRP-AG was assessed in all patients. We used standardized PLT-activators in following concentrations: COL (10 µg/ml,2.5 µg/ml); ADP (15 µM,3.75 µM);EPI (26.8 µM,10.3 µM);TRAP(76 µM,38 µM);Ristocetin (1.30 mg/ml, 0.70 mg/ml);arachidonic acid 1.1 µg/ml and thromboxane 2 µM. FACS with and without TRAP-activation (concentration 11mM), was done using PRP. We used monoclonal antibodies against PLT-antigens. (s.Table)
Results: 45 patients (35 females, 8 males), were divided in to diagnostic groups: bleeding disorders without hematological stem cell disease (42 patients) and bleeding disorders with hematological stem cell disease (3 patients). 24 patients (53%) had pathological and 21 (47%) had normal PRP-AG.
Using PLT- FACS before and after TRAP activation, we compared the patients with normal (N-group) and pathological PRP-AG (P-group). As expected, Mann-Whitney-U-Test showed statistically significant better FACS results after activation in the N-group compared to the P-group.
Surprisingly, in the N-group, there is a subgroup of patients in the lower half of the distribution of results (lower than median) showing reduced platelet receptor activation and reduced release reaction. The mean values of the results in subgroup did not differ much from the mean of the results in the P-group (s.Table).

	CD 61	PAC1	CD 62	CD 63	CD 42a
N-group (n=21)	80 (SD 66)	56 (SD 40)	488 (SD 496)	952 (SD 686)	-19 (SD 16)
P-group (n=24)	43 (SD 48)	29 (SD 33)	186 (SD 182)	329 (SD 348)	-9 (SD 10)
P(M-W)	0.034	0.034	0.023	0.001	0.023
N-subgroup low	33 (SD 8) (n=12)	24 (SD 6) (n=11)	106 (SD 30) (n=10)	413 (SD 97) (n=11)	-8 (SD 5) (n=11)

Conclusion: FACS of platelet function before and after activation with TRAP mainly follows the pattern of PRP-AG. However, in some cases PRP-AG masks the existing defects in platelet receptor activation or release reaction, which could be revealed with FACS.

1114

Addition of Rituximab to ongoing monotherapy of CLL with Venetoclax may improve MRD – A case report

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Introduction: In CLL, the depth of response is an independent predictor of PFS and OS. Combining Venetoclax (Ven) with rituximab (R) results in a higher rate of MRD negativity than Ven alone. The addition of R to Ven monotherapy could induce deeper MRD responses, but there are no reports in literature.
Case report: A 67-year old woman with high risk CLL suffered from PD after pretreatments with FCR, Ofatumumab, Ibrutinib and R+Idelalisib. Ibrutinib had to be ceased due to side effects and Idelalisib+R because of PD. At that time CT-Scan showed no bulk, Lymph node biopsy revealed no Richter´s transformation, FISH detected del 17p (92%), 13q14.2 (13%) and the NGS TP53 (TP53:c.514G>T (81,2%)). Treatment with Ven was started in an outpatient setting. With 10 G/l Lc in peripheral blood no tumor lysis syndrome occurred with oral hydration and allopurinol. After 1 year MRD by FACS in blood was 0.34% (0.43 G/l). R at 500mg/m2 was combined with Ven in the second year of treatment for 12 cycles every 4 weeks. After the third cycle MRD was 0.06% (0.003 G/l). MRD level could be maintained up to 9 cycles (0.007 G/l) with an increase after 12 cycles (0.07G/l). At this time point (2 years Ven and 12 cyles R) therapy was discontinued, following the patient´s request despite Ven being well tolerated.
Discussion: In the CLL8 and CLL10 study PFS was longer for patients with CR or PR when MRD-negativity ($<10^{-4}$ leukemic cells) was achieved (61 months and 54 months, respectively) than for those with MRD-positive CR and MRD-positive PR (35 months and 21 months, respectively). In a phase II study of 107 relapsed/refractory CLL patients with del17 and TP53 mutation, treatment with Ven resulted in an ORR of 80%, of which 21% were MRD negative at a median follow-up of 12 months. In the MURANO trial MRD negativity was 84% with VenR (compared to 23% with Bendamustin+R) at any time point during the trial. Furthermore, treatment with VenR correlates with a prolonged PFS after a median of 24 months (36% vs. 85%), especially in CLL with 17p and/or a TP53 mutation (86% vs. 41%). Therefore we added R to Ven monotherapy in our patient, resulting in a significant reduction of MRD within one year. The low level of MRD may indicate an improved disease control, presumably even after discontinuation of therapy.
Conclusion: The addition of R to ongoing monotherapy with Ven may improve the response and lower level of MRD probable leading to a prolonged PFS and OS in patients with high risk CLL.

1115

HbA1c and Hb variant co-elution: Mass spectrometry method for Hemoglobinopathies diagnosis, an useful tool to complement HPLC method

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Introduction: Hemoglobin (Hb) disorders are the most common monogenetic pathologies. High-performance liquid chromatography (HPLC) is often used to separates Hbs and obtain their relative percentage (HbA, A_2, F and HbA1c). The presence of abnormal Hb is usually detected at this step of the diagnostic process. An accurate phenotype characterization is crucial to select the most appropriate molecular biology technique to confirm the suspected Hb pathology. Unfortunately, due to the limited selectivity of this method (co-elution of several Hbs), only a presumptive Hb identification can be performed and a combination with other methods is mandatory (i.e. isoelectric focusing,…). Techniques commonly used to separate Hbs are mainly based on differences of Hb charge or size. Moreover, some rare variants can co-elute with HbA1c. Such situation could result in two major issues: miss detection of the Hb variant and falsely elevated HbA1c.In this context, mass spectrometry (MS) has a potential role to play. This technique allows to separate ions into gas-phase and measure the mass to charge ratio (i.e. *m*/*z*). Therefore, it provides orthogonal information compared to classical methods. Thanks to their mass difference, Hbs and variants can then be detected. We developed an automated MS platform from sample handling to data acquisition and interpretation, compatible with clinical laboratory practice. Our approach consists in analyzing intact Hbs (without prior digestion) directly by MS and fragment them in order to obtain structural information. In this work, we performed a comparison between HPLC and MS results for samples presenting an elevated HbA1c level.
Method: Three EDTA samples with elevated HbA1c were analyzed. HPLC and MS results were compared (Variant II, Bio-Rad and amaZon speed ETD, Bruker Daltonics).
Results: Elevated HbA1c levels by HPLC were reported for three samples. After usual diagnostic procedure, it was established that these HbA1c were falsely elevated due to co-elution of rare variants (Hb Hope in two cases and Hb K-Woolwich for the third sample). These Hbs are the result of a single point mutation on beta chain. Our MS method could separate these variants from HbA1c and relative percentages could be calculated. As a plus, the method could establish that the detected Hbs were beta chain variants.
Conclusion: Our results suggests that the developed MS workflow is an interesting tool to complement HPLC analysis and allow to add new information.

1116

Feasibility of a new, rapid functional assay for the diagnosis of heparin-induced thrombocytopenia: a standardized flow cytometry assay

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Background: Establishing the diagnosis of heparin-induced thrombocytopenia (HIT) is still a serious clinical problem. Washed platelet assays, which are regarded as gold-standard for the diagnosis of HIT are rarely available in a timely manner. A simple, standardized platelet flow cytometry assay was developed to overcome this important limitation. We aimed to explore the diagnostic utility of a new, standardized platelet flow cytometry assay for the diagnosis of HIT in clinical practice.
Methods: Frozen serum samples of 103 consecutive patients, evaluated for suspected HIT at our institution in 2017, and characterized with 4Ts score, IgG-PF4/heparin ELISA (GTI), HemosIL®Acustar (IgG), as well as heparin-induced platelet activation test (HIPA), were further tested using a flow cytometry assay, determining P-selectin release of donor platelets after incubation with patient's serum (Emo-HIT Confirm®, Emosis, Strasbourg, France). Platelet-rich plasma of one donor was used, and patient's serum was heat-inactivated before determination. Diagnosis of HIT was defined as a positive HIPA result.
Results: HIT was confirmed in 15 out of 103 patients corresponding to a prevalence of 14.6%. HIT Confirm was positive in 11 patients (10.7%), negative in 88 patients (85.4%), and inconclusive in 4 patients (3.9%). Among patients with positive HIT Confirm, median OD of ELISA was 3.1 (range 0.2, 3.8), median Acustar 16.5 CU/ml (0, 128), and median 4Ts score 6 points (range 5, 7.5). Among patients with a negative HIT Confirm, median ELISA was 0.2 (0.1, 2.9), median Acustar 0 (0, 45.6), and median 4Ts score 3 (0, 6). Removing inconclusive results from the analysis, the number of true positives was 9, the number of true negatives 83, the number of false negatives was 5, the number of false positives was 2. This corresponds to a sensitivity of 64.3%, and a specificity of 97.6%.
Conclusions: The proposed standardized flow cytometry assay is able to confirm HIT in most patient samples. Further test modifications might improve sensitivity.

1124

Major surgery in a patient with Haemophilia A with high responder inhibitor (INH) on Emicizumab (Emi)

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Emicizumab, a bispecific monoclonal antibody, enables FIXa to activate FX in the absence of FVIII, and reduces bleeding in hemophiliacs with FVIII INH. Additional treatment with bypassing agents (BPA) may become necessary for occasional breakthrough bleeds, or surgical interventions. As patients on Emi treated with certain BPA for bleedings have experienced severe adverse events (thrombotic microangiopathy (TMA) or thrombosis), current guidelines suggest to postpone major surgery whenever possible until more data are available. Laboratory monitoring requires bovine reagent-based assays. Assessment of thrombin generation (TG) might be helpful.
We report on a 51y old hemophilic with high responder FVIII INH on Emi since March 2018. Before enrolment into the STASEY trial he was on biweekly prophylaxis with Feiba, his annual bleeding rate was >20. On Emi no bleedings occurred, however, early 2019 hip-replacement became inevitable. Preoperative work-up included blood count, routine chemistry, coagulation assays and TG (STGenesia, Stago). We assessed endogenous thrombin potential (ETP), peak height (PH), and spiked patient's plasma with Obizur (porcine rFVIII; doses equivalent to 50, 100, and 200 E/kg body weight), Feiba (25, 50, 75, 100E/kg) and NovoSeven (N7; 60, 90, 140, 270ug/kg). The FVIII INH peaked at >6000BU/ml after the last FVIII exposition in 2005; and was 12.9 BU/ml to human, 12.5 BU/ml to porcine FVIII at present.
Preoperative TG ETP and PH were below the normal range, but normal in plasma spiked with N7 ≥60ug/kg, FEIBA ≥25E/kg, or Obizur ≥100E/kg. We decided to use N7 as BPA, conform to international recommendations. We gave a bolus of 90µg/kg N7 at start of surgery; all subsequent doses were 80µg/kg. During surgery, the dosing interval was 3h, and 4h (d1-d3), 6h (d4-d7) and 8h (d8-d11) afterwards. We used tranexamic acid (3g/24 h), and prophylactic LMW heparin for the entire postoperative period. No blood products were given. The surgeon rated hemostasis as good to excellent, despite reduced TG on d1 and d6. The lowest hemoglobin (99g/L) and platelet count (116G/L) were recorded on d2, the patient had no signs of TMA during the course, and was discharged in good health on d11.
Major orthopedic surgery in patients on Emi is feasible but requires a multidisciplinary team of experts supported by a specialized coagulation laboratory. Our treatment protocol was effective in preventing both excessive bleeding and thromboembolic complications.

1125

Low sensitivity to thrombomodulin correlates with liver stiffness in advanced chronic liver disease

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Background: Advanced chronic liver disease (ACLD) is characterized by changes in the coagulation system that embrace not only hypo-, but also hyper-coagulability. Global hemostatic tests such as thrombin generation (TG) assays are used to evaluate the hemostatic balance, in order to better assess bleeding and thrombotic risks. In addition, procoagulant state in ACLD patients has been demonstrated using modified TG assays with thrombomodulin (TM), a cofactor for protein C activation. Liver stiffness is associated with clinically significant portal hypertension in patients with ACLD. Here, we aimed to investigate whether TG parameters in patients with ACLD could be associated to liver stiffness.
Methods: Blood samples were collected from patients with ACLD (n = 74). LSM was performed by transient elastography (Fibroscan) in each patient. A cut-off of 21 kPa was used to subgroup the studied ACLD patients sample, as a LSM of >21 kPA has been associated with the development of a clinically significant portal hypertension. TG was measured by ST Genesia® Thrombin Generation System (STG) and the Calibrated Automated Thrombography (CAT).
Results: TG was measured by STG in plasma of 34 patients with ACLD using STG Bleedscreen (BLS) and Thromboscreen with/without TM (TS-TM/+TM) assays (STG ACLD group). In addition, TG was measured by CAT with/without TM using PPP low reagent in a subgroup of these patients (n = 18), as well as in an additional group of 22 patients with ACLD (CAT ACLD group). ETP inhibition by TM, evaluated in TS-TM/+TM assays, correlated with LSM (r = -0.54, p = 0.005) and differed among the study groups (STG CLD ≤21 kPa: 57-71; STG CLD >21 kPa: 12-57; p = 0.02). Similarly, ETP inhibition by TM measured by CAT discriminated among LSM groups (CAT CLD ≤21 kPa: 0.1-0.4; CAT CLD >21 kPa: 0.2-0.8; p = 0.049). ETP inhibition by TM measured by STG correlated with ETP inhibition determined by CAT (r = -0.76, p = 0.0003).
Conclusion: Low sensitivity to thrombomodulin correlated with liver stiffness in patients with advanced chronic liver disease. Sensitivity to thrombomodulin measurements determined by ST Genesia® correlated with those obtained with CAT. Further studies are needed to determine whether sensitivity to thrombomodulin and liver stiffness measurements in patients with ACLD could help to identify patients at higher risk of portal vein thrombosis.

1128

Platelet protein S limits venous but not arterial thrombosis propensity by controlling coagulation in the thrombus

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Background: Protein S (PS) is a coagulation inhibitor. In addition to its plasmatic form, PS is stored in platelets (pltPS) and released upon activation. The role of pltPS in thrombosis has not yet been elucidated.
Methods: Conditional PS knockout mice in the megakaryocyte lineage were generated using the Pf4-Cre transgene ($Pros1^{lox/lox}Pf4\text{-}Cre^{+}$).
Results: Rotation thromboelastometry showed that whole blood clots faster in $Pros1^{lox/lox}Pf4\text{-}Cre^{+}$than in $Pros1^{lox/lox}Pf4\text{-}Cre^{-}$mice (P = 0.01), independently of the fibrinogen contribution. Thrombus formation analysis on collagen or collagen/tissue factor surfaces, showed that total thrombogenicity in $Pros1^{lox/lox}Pf4\text{-}Cre^{+}$mice was increased at low but not at high shear rate (P <0.001). In the $FeCl_3$model, occlusive thrombosis in mesenteric arterioles occurred twice faster in $Pros1^{lox/lox}Pf4\text{-}Cre^{+}$than in $Pros1^{lox/lox}Pf4\text{-}Cre^{-}$ mice. In addition, thrombi appeared more extended and displayed a diffuse and intense FXa/thrombin and fibrin staining all over the vessel. Differently, thrombi from $Pros1^{lox/lox}Pf4\text{-}Cre^{-}$mice showed FXa/thrombin and fibrin staining limited at the injury site, covered

by a layer of CD41+ platelets. P-selectin+ platelets were observed at the site of injury in both genotypes. However, they were found in addition on the top of the thrombus only in Pros1$^{lox/lox}$Pf4-Cre^{+}mice. In the vena cava $FeCl_3$model, the thrombus volume was more than three times larger in Pros1$^{lox/lox}$Pf4-Cre^{+} than in Pros1$^{lox/lox}$Pf4-Cre^{-}mice (P = 0.0007). Thrombus histology confirmed the fibrin enrichment compared to Pros1$^{lox/lox}$Pf4-Cre^{-}mice.PS staining was detected in both genotypes, although it was more intense in Pros1$^{lox/lox}$Pf4-Cre^{-}than in Pros1$^{lox/lox}$Pf4-Cre^{+}mice. No difference was observed in thrombus size from both genotypes using the $FeCl_3$model in the carotid artery. Clot contraction was impaired in Pros1$^{lox/lox}$Pf4-Cre^{+}mice. Scanning electron microscopy imaging of platelet rich plasma clots showed highly branched and dense fibrin network at both internal and external clot surface in Pros1$^{lox/lox}$Pf4-Cre^{+}mice. Differently, Pros1$^{lox/lox}$Pf4-Cre^{-} clots comprised less dense fibrin network at the internal surface and displayed a loose fibrin film covering the external surface.

Conclusion: PltPS, by inhibiting FXa/thrombin generation in the thrombus, limits thrombus propensity when the shear rate is low, such as in large veins, but not at high shear, like in large arteries.

1129

Thrombin generation measurement using the ST Genesia Thrombin Generation System in a cohort of healthy adults: normal values and variability

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Background: Thrombin generation (TG) assays evaluate the balance between pro- and anticoagulant forces, to better assess bleeding and thrombotic risks. Although TG readouts obtained with the calibrated automated thrombin generation have been investigated in multiple clinical conditions, TG still needs standardization and clinical validation. The new automated TG instrument ST Genesia®(STG, Stago) provides a normalization of TG parameters based on a reference plasma aiming to reduce the inter-laboratory variability and the variability between different measurement runs. In the present study, the STG was evaluated in a healthy adult sample.

Methods: Reference intervals in healthy adults and variability of the new standardized reagents for bleeding (BleedScreen) and thrombophilic (ThromboScreen) conditions were determined using STG.

Results: TG was measured in platelet-free plasma (PFP) samples of 123 healthy adults. Reference intervals were determined for TG parameters. Intra- and inter-assay coefficients of variation were calculated on quality controlsand PFP samples from healthy adults. Oralcontraception possibly influenced TG parameters, resulting in a higher median and a broader reference interval for peak height and ETP in females aged 20 to 49 years than in all other sex- and age-categories. Therefore, we propose the following reference interval categories: males, females <50 years not using OC, females <50 using OC, females ≥50 years. Normalization was effective to reduce the inter-assay variability of quality controls for ETP (BleedScreen assay), and peak height and ETP (ThromboScreen assay without thrombomodulin), but had only little impact on PFP samples variability.

Conclusion: The ST Genesia®Thrombin Generation System appears to be suitable for the accurate measurement of TG in healthy adults. Future studies will comprise pathologic samples to determine how this system can be used in the management of patients with bleeding or thrombotic disorders.

1130

Targeting protein S protects mice with hemophilia A from acute and chronic hemarthrosis

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Background: Hemarthrosis, referring to intra-articular bleeding, is a major hemophilia-related complication. Affected joints display changes in the synovium, bone, cartilage and blood vessels. Current prophylactic treatments are not always effective. Therefore, hemophilia patients can experience breakthrough bleeds. Recently, we established protein S (PS) inhibition as both controller of coagulation and potential therapeutic target in hemophilia. Here, we investigated the efficacy of PS targeting in hemophilic mice and the effect of PS targeting on inflammation using acute (AH) and chronic (CH) hemarthrosis models.

Methods: AH and CH models were applied to F8$^{-/-}$Pros1$^{-/-}$ and F8$^{-/-}$ Pros1$^{+/+}$ mice. For the AH model, mice had 1 single knee-joint injury while 3 knee-injuries were performed in the CH model (1injury/week). Immunohistochemistry (IHC) analysis of injured knee-joints was realized. Inflammatory profile was studied in blood and knee lavages.

Results: F8-/-Pros1-/- mice did not develop AH and CH after knee injuries while F8-/- Pros1+/+ mice developed AH and CH. IHC analysis of joints from F8-/-Pros1+/+ mice with CH showed intra-articular bleeding with hemosiderin deposition and insoluble fibrin, and a higher number of macrophages (anti-CD68) than from F8-/-Pros1-/- mice. In both genotypes, injured joints displayed comparable cartilage erosion. However, synovial hyperplasia and subsynovial proliferation were more extensive in F8-/-Pros1+/+ mice than in F8-/-Pros1-/- mice after CH while vessel number and caliber were comparable in both genotypes indicating no difference in angiogenesis. After AH, F8-/-Pros1-/- and F8-/-Pros1+/+ mice had comparable circulating M1 (inflammatory) and M2 (patrolling) monocytes. In the knee lavage, the number of M1 and M2 monocytes was similar in both genotypes after AH. The amount of monocyte chemotactic protein 1 (MCP-1) and IL-6, cytokines secreted by the inflamed synovium, was less abundant in knee lavage from F8-/-Pros1-/- mice than from F8-/-Pros1+/+ mice after AH (P <0.005).

Conclusion**:** F8-/-Pros1-/- mice were protected against AH and CH. They displayed less intraarticular bleeding, iron deposition and synovial hyperplasia as compared to F8-/-Pros1+/+ mice. Targeting PS also reduced inflammatory cytokines release after AH. This is consistant with the fact that these mice did not develop AH. Further analysis to better understand if the lack of PS affects also the inflammatory process that supports CH is ongoing.

1131

Strengths and caveats of a Bone Marrow Failure syndromes panel by NGS

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Introduction: Next generation sequencing (NGS) allows a relatively fast investigation of large gene panels. One of the important points is the design of the panel itself. We created a Bone Marrow Failure (BMF) panel including 74 genes for the diagnostic assessment of Diamond-Blackfan anemia, telomeropathies, hereditary anemia and neutropenia, Fanconi anemia and hereditary neoplasms. One of the caveats of this method is that permanently new relevant genes are identified requiring panels update. Within the past year, our panel expanded from 63 to 74 genes. NGS does not allow the detection of large deletions or duplications

Method: Patient samples were analyzed by a BMF home-designed NGS panel. Only gene variants of the categories pathogenic, likely pathogenic and variant of uncertain significance (VUS) according to ACMG guidelines 2015 were considered and confirmed by Sanger sequencing. A multidisciplinary platform constituted by hematologists, pediatric hematologists, geneticists and specialists in molecular diagnostics collaborated in the clinical and laboratory results interpretation.

Results: We evaluated 44 patients (55% female). The median age at first presentation and at genetic evaluation were 35 (0.7- 77) and 34 (0.9-67) years respectively. 16/44 (36%) patients had a positive family history. Four patients had congenital BMF, 3 congenital neutropenia, 12 unexplained BMF, 4 telomeropathies, 7 unexplained cytopenias and 14 had malignant hematological diseases with a suspected hereditary neoplasia. In 23(53%) samples, a mutation was identified. In 4 patients we found somatic mutations (TP53, GATA2). Five patients carried likely pathogenic mutations (ELANE, HAX1, WAS, RPL5 and FANCA) allowing therefore diagnosis confirmation. 16(36%) patients carried at least one VUS (4 patients more than one). Within genes frequently carrying VUS we found RTEL1 (N = 4), CUBN (N = 3), BRCA2 (N = 6) and SEC23B (N = 2). AMN, GPI, TCAB1, FANCD2, FANCI, CDAN1, USB1 and ELANE were detected in one case each. As the initial panel did not include SBDN gene, Shwachman Bodian Diamond Syndrome was initially missed in one case. Resequencing with an updated panel allowed diagnosis confirmation.

Conclusions: NGS is a fast high throughput DNA sequencing method that allows diagnosis in BMF. In patients with lifelong conditions, the finding of VUS variants not previously described require further investigation. As consequence of emerging new genes, a permanent update of the panel is mandatory.

1132

Gas6 targeting promotes major bleeding and aggravates inflammation in protein S deficient mice

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Background: Growth arrest-specific gene 6 (Gas6) and protein S (PS) are vitamin K-dependent proteins and ligand for the TAM family of receptor kinases. PS is a natural anticoagulant whose pivotal role is illustrated by purpura fulminans (PF), a life-threatening thrombotic disorder characterized by purpuric skin lesions, disseminated intravascular coagulation and thrombosis. Unlike PS, Gas6 is redundant for hemostasis and its complete deficiency has anti-hemostatic and anti-erythropoietic effects.

Aim: To evaluate if Gas6 deficiency could rebalance hemostasis in PS deficient mice and rescue them from PF.

Methods: *Pros1*$^{+/-}$*Gas6*$^{-/-}$ mice were crossed to obtain *Pros1*$^{-/-}$*Gas6*$^{-/-}$. Embryos genotypic and phenotypic analysis (E14-E19) was realized. Immunohistochemistry, immunofluorescence (IF) of whole mounted embryonic dorsal skin, inflammatory and erythropoietic investigations were performed.

Results: Embryonic mortality was higher in *Pros1*$^{+/-}$*Gas6*$^{-/-}$ than in *Pros1*$^{+/-}$ mattings (6% vs 3% at E14, 21% vs 18% at E16, 30% vs 20% at E17, respectively). Macroscopically, *Pros1*$^{-/-}$*Gas6*$^{-/-}$ embryos displayed maximal bleeding score (3) corresponding to intracranial and full body hemorrhages (65% vs 31% for *Pros1*$^{-/-}$ embryos). Microscopically, *Pros1*$^{-/-}$*Gas6*$^{-/-}$ embryos had a bleeding score 3 while 50% of *Pros1*$^{-/-}$ embryos displayed a milder bleeding score (2). Embryonic major blood vessels of *Pros1*$^{-/-}$*Gas6*$^{-/-}$ mice contained less mature RBC than those of *Pros1*$^{-/-}$ mice. *Erythroid burst–forming units* from fetal liver single cell suspension showed 2 times less colonies in *Pros1*$^{-/-}$*Gas6*$^{-/-}$ than in *Pros1*$^{-/-}$mice confirming altered erythropoiesis in *Pros1*$^{-/-}$*Gas6*$^{-/-}$ mice. Preliminary data measuring CD71/Ter119 erythroid subsets in fetal liver confirmed this finding. Prussian blue staining showed iron deposition in fetal liver and numerous pale RBC in *Pros1*$^{-/-}$*Gas6*$^{-/-}$ vessels indicating iron recycling defect. E15 *Pros1*$^{-/-}$*Gas6*$^{-/-}$ and *Pros1*$^{-/-}$ embryonic dorsal skin IF showed RBC extravasation (anti-VE-Cadherin, anti-Ter119), vascular network outgrowth impairment, with fewer vessel branches (anti-CD31), massively enlarged lymphatic vessels and increased macrophages infiltration indicating ongoing inflammation (anti-Lyve1, F4/80).

Conclusion: Gas6 deficiency did not rebalance hemostasis in *Pros1*$^{-/-}$ embryos and prevent PF. Gas6 and PS combined deficiency leads to a more dramatic phenotype with higher mortality rate, major bleeding, and an erythropoietic defect mimicking anemia of inflammation.

1133

Coincidental detection of type I cryoglobulins by flow cytometry in a patient with paraproteinemia

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Introduction: We report an unusual case of a patient with paraproteinemia, in which we coincidentally detected cryoglobulins by flow cytometry during lymphoma screening. The finding was confirmed by other methods later on.

Patient: 77 year old patient with sensoric polyneuropathy and paraproteinemia IgM kappa. No evidence of lymphadenopathy or organomegaly. Normal blood counts without lymphocytosis.

Methods: 8 colour immunophenotyping for lymphoma screening and characterization was performed using a BD FACS Canto flow cytometer. The following markers were included for characterization of normal and pathological B-cells: CD10, CD19, CD5, CD20, CD23, CD103, CD11c, CD25, CD123, CD180, CD200, FMC7, CD38, IgM, kappa, lambda, CD79b, CD45. Blood smears for microscopic analysis were prepared by ADVIA autoslide. Qualitative detection of cryoglobulins was performed by immunoserology.

Results: As the patient had a known paraproteinemia IgM kappa, we performed immunophenotyping with a panel dedicated to detect B-cell neoplasias. Indeed, among the CD45 bright lymphocytes we found a small B-cell clone with unspecific marker profile and lambda light chain restriction (88% of all B-cells, 14% of lymphocytes, 1% of all events). Additionally, we observed a population of CD45 negative events with bright signals for IgM and kappa light chains (58% of all events), yet negativity for lambda light chains and all other B-cell markers (figure 1A). The negativity of CD45 (pan-leucocyte marker) and markers restricted to the membrane of B-lymphocytes (e.g. CD19, CD20, CD79b) led to the hypothesis, that the population might represent aggregates of soluble immunoglobulins. Subsequently, the patient`s blood smear was screened and showed protein aggregates, compatible with cryoglobulinemia (figure 1 B). Immunoserology was initiated and confirmed cryoglobulins type I.

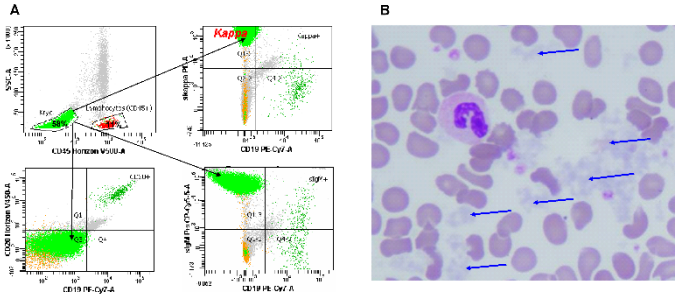

Figure 1: Detection of cryoglobulin in flow cytometry and blood smear

(A) Cryoglobulin (highlighted by light green colouring) appeared negative for CD45, CD19 and CD20 but showed strong signals for Kappa and sIgM by flow cytometry.

(B) Cryoglobulion on blood smear appeared as pale grey aggregates (indicated by blue arrows)

Conclusion: Flow cytometry was able to detect monoclonal immunoglobulin aggregates restricted to IgM kappa, corresponding to type I cryoglobulin in a patient with known IgM kappa paraproteinemia. This case should raise the awareness that coincidental detection of B-cell surface marker negative monoclonal populations by flow cytometry might correspond to cryoglobulins and thus should warrant further diagnostic workup for possible cryoglobulinemia. 24 h

1045

How to utilize argatroban: analysis of 729 treatment days in 32 patients with heparin-induced thrombocytopenia (HIT)

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Background: Argatroban is a reversible direct thrombin inhibitor recommended for patients with HIT. It undergoes hepatic metabolization and biliary excretion. Some aspects of its utilization remain unclear, such as patient-tailored starting dose (sD) and dose adjustments (Da) (µg/kg/min), as well as timing and type of laboratory monitoring.

Methods: We retrospectively studied 32 patients with confirmed HIT treated with argatroban at our institution (08.2014-02.2019), for a total of 729 anticoagulation days. We analyzed plasmatic argatroban concentrations (PAC; µg/mL) at time t' (min) after argatroban sD, the effect of Da on the consecutive PAC, the impact of liver function impairment on argatroban doses, and the role of timing and type of assay on argatroban monitoring.

Results**:** Among patients with normal liver function, argatroban plasmatic half-life was approximately 60 min and steady state PAC was observed after 270 min. Intended sD of 0.5 and 1.0 µg/kg/min reached steady state PAC of approximately 0.45 and 0.85 µg/mL, respectively. Median argatroban Da of +0.06, +0.14 and +0.22 µg/kg/min respectively led to similar and significant median steady state PAC increases of +0.06, +0.15 and +0.21 µg/ml. PAC controls performed 3-6 hours after argatroban Da were significantly higher compared to controls <3 hours after argatroban Da. To reach similar PAC at steady state, patients with serum total bilirubin values above (15)–20 µmol/l required significantly lower argatroban doses compared to patients with lower serum bilirubin levels. We observed a high correlation and a linear relationship between quantitative PAC in µg/ml and correspondent thrombin time values, both assays appearing adequate for argatroban monitoring. In contrast, we demonstrated that activated partial thromboplastin time (aPTT) is not reliable for argatroban anticoagulation monitoring.

Conclusion: Our results provide guidance to physicians on frequent, controversial, and challenging aspects of anticoagulation with argatroban. In particular, we suggest a sD of 1.0 µg/kg/min and Da of 0.2-0.3 µg/kg/min in presence of normal liver function. Dose should be decreased in patients with total bilirubin above (15)–20 µmol/l. Laboratory monitoring should be performed at least 4 hours after sD/Da either by PAC or thrombin time measurement; aPTT should be avoided. These recommendations are expected to improve argatroban dosing and monitoring among patients with HIT. This will be prospectively evaluated.

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