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Swiss Medical Weekly

Formerly: Schweizerische Medizinische Wochenschrift
An open access, online journal • www.smw.ch

Supplementum 230
ad Swiss Med Wkly
2018;148
June 27, 2018

Swiss Oncology & Hematology Congress (SOHC)

Zurich (Switzerland), June 27–29, 2018

Abstracts

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Abstracts

Index of first authors

www.sohc.ch

Impressum

All communications to:
EMH Swiss Medical Publishers Ltd.
Swiss Medical Weekly
Farnsburgerstrassse 8
CH-4132 Muttenz, Switzerland
Phone +41 61 467 85 55
Fax +41 61 467 85 56
office@smw.ch

Cover photo:
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The Guidelines for authors are published on our website www.smw.ch
Submission to this journal proceeds totally on-line: www.smw.ch

ISSN printed supplement: 1661-6855
ISSN online supplement: 2504–1622

printed in switzerland

SAKK 36/13 – Ibrutinib and bortezomib and ibrutinib maintenance for relapsed/refractory mantle cell lymphoma: an ongoing trial

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Introduction: Mantle-cell lymphoma (MCL) is characterized by frequent relapses. The Bruton's tyrosine kinase inhibitor ibrutinib (IBR) and the proteasome inhibitor bortezomib (BOR) have single agent activity and down-regulate NF-κB activity via different targets. In vitro, this combination provides synergistic cytotoxicity.
Methods: We included MCL patients (pts) with relapsed/refractory disease after ≤2 lines of a non-BOR-containing chemotherapy, and excluded e.g. pts with CNS disease, and in need of anticoagulation. Pts received 6 21-days cycles of IBR+BOR, and IBR maintenance until progression or unacceptable toxicity. To establish the recommended phase II dose, a 3+3 design was used. BOR was given s.c. at 1.3 mg/m^2 (days 1, 4, 8, 11 q3w), and IBR continuously at 420 mg/day (level 1), and 560 mg/day (level 2). Dose-limiting toxicities (DLTs) were assessed in cycle 1: study drug-related adverse events incl. ≥7 missed days of IBR, ≥2 missed BOR doses, delay of >2 weeks of cycle 2, hematological DLTs (ANC <0.5 for ≥7 consecutive days, febrile neutropenia, and G4 thrombocytopenia), and non-hematological DLTs ≥ G3.
Results: In the phase I part, the minimum number of 9 pts was included since no pts had a DLT. The most frequent AEs of the combination treatment were thrombocytopenia (9 pts), peripheral polyneuropathy (PNP), fatigue/anemia (6), diarrhea and injection site reaction (5). The majority of the AEs were G1 & 2. At data cut-off (March 6, 2018), 2 pts were still on maintenance. The median number of cycles administered is 12 (range 3–28). 1 pt went on to stem cell transplantation, 3 pts stopped therapy because of progression (1 at end of cycle 3, 2 during maintenance). The protocol was amended to allow maintenance after 4 cycles.
Conclusions: IBR with twice-weekly BOR at 1.3 mg/m^2 s.c. is safe. We define IBR 560 mg/day as the recommended phase II dose to combine with BOR. The efficacy is tested in the ongoing phase II part; 13 pts have been enrolled, centers in Italy and Germany participate.

The influence of different fever definitions on diagnostics and therapy after diagnosis of fever in neutropenia in children with cancer

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Background/Objectives: There is no uniform definition of the temperature limit defining fever (TLDF) in paediatric cancer patients. This study aimed to investigate the influence of different TLDFs on diagnostics and therapy after fever in neutropenia (FN) diagnosis.
Design/Methods: In a single paediatric cancer center using a high TLDF (39°C tympanic temperature) patients undergoing chemotherapy were observed prospectively (NCT01683370). Temperature measurements and key procedures of diagnostics and therapy during FN were recorded. The effect of applying lower TLDFs (range, 37.5°C to 38.9°C) versus 39.0°C on these procedures was simulated in silicon.
Results: 45 FN episodes were diagnosed in 20 of 39 study patients (maximum, 6 episodes per patient). Of 3391 temperatures measured, 193 were ≥39.0 °C, and 937 ≥38.0 °C. For persisting fever ≥24 hours, additional blood cultures were taken after start of antibiotics in 31 (69%) episodes in reality. This number decreased to 22 (49%) by virtually applying 39.0°C, and increased to 33 for 38.0 °C (73%; plus 11 episodes; plus 24% [95% CI, 13 to 40]). For persisting fever ≥48 hours, intravenous antibiotics were escalated in 25 (56%) episodes in reality. This number decreased to 15 (33%) by virtually applying 39.0°C, and increased to 26 for 38.0°C (58%; plus 11 episodes; plus 24% [95% CI, 13 to 40]). In reality, the median length of stay was 5.7 days (range, 0.8 to 43.4). In 43 episodes with hospital discharge beyond 24 hours, virtually applying 38.0°C instead of 39.0°C led to discharge delay by ≥12 hours in 24 episodes (56% [95% CI, 40 to 71]), with a median delay of 13 hours, and a cumulative delay of 68 days.
Conclusion: Applying lower TLDFs led to relevant increases of diagnostics, therapy, and hospitalization in children with FN. This, in turn, increases treatment-related side effects and costs, and decreases quality of life.

Marked and symptomatic thrombocytosis as initial manifestation of chronic myeloid leukemia

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Background: The clinical picture of chronic myeloid leukemia (CML) *BCR-ABL1*-positive is characterized by a major proliferation of the myeloid lineage and a normal to modestly elevated platelet count. We present two cases with extreme and symptomatic thrombocytosis as an unusual primary manifestation of CML.
Results: In June 2017 and in February 2018, two patients were referred to our hospital with the common laboratory finding of extreme thrombocytosis and initial suspicion of essential thrombocythemia. The first patient was a 43-year-old woman with a history of paresthesia and presyncope, the second patient a 72-year-old man with a gangrenous hallux. While the first patient was anemic, the second patient had a normal hemoglobin concentration. The platelet count was markedly elevated with 4.32 and 2.58 G/l, respectively. Both had only moderate leukocytosis. The referring physician of the first patient had already started treatment with hydroxycarbamide, without any effect on platelet count. Bone marrow examination showed an extreme proliferation of small, hypolobulated megakaryocytes. A *JAK2*mutation was not detected, but cytogenetic analysis revealed the presence of the chromosomal translocation t(9;22) in both cases and RT-PCR detected BCR-ABL1 of the p210 type, subtype e13a2 and e14a2, respectively. Next generation sequencing analysis for 54 commonly mutated genes in myeloid neoplasms showed an additional *ASXL1*mutation in one of the patients. The diagnosis of CML was confirmed and a therapy with nilotinib and dasatinib, respectively, was initiated, which led to a rapid decrease in platelet levels within a month.
Conclusion: Symptomatic thrombocytosis caused by CML is a very rare first manifestation. However, it should be a differential diagnosis in patients with marked thrombocytosis, even when neutrophil blood count is only moderate. It can have significant consequences that may require urgent intervention due to neurologic or ischemic complications. These two cases highlight the remarkable heterogeneity not only between myeloproliferative neoplasms, but also within the same disease entity.

SAKK 35/15: A phase I trial of obinutuzumab with venetoclax in previously untreated follicular lymphoma patients

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Even though the combination of rituximab with chemotherapy is the standard of care for the first-line treatment of follicular lymphoma (FL) patients (pts) in need of systemic therapy, there is increasing evidence that chemotherapy-free treatments may achieve long-lasting disease control. Development of new treatment regimens based on the anti-CD20 monoclonal antibody rituximab, or new antibodies, may represent an alternative to chemo-immunotherapy. Obinutuzumab, a type II anti-CD20 monoclonal antibody has significant clinical activity in chronic lymphocytic leukemia (CLL) and in rituximab-refractory FL pts. In a recently reported trial obinutuzumab-based immunochemotherapy and maintenance therapy resulted in longer progression-free survival than rituximab-based therapy in untreated FL pts. Venetoclax, a Bcl-2 inhibitor has recently shown a good safety profile and activity in CLL and other B-cell lymphomas, including pts with relapsed/refractory FL. Based on their single agent activity and preclinical synergism, we are currently evaluating safety and efficacy of the chemotherapy-free combination of obinutuzumab and venetoclax in previously untreated pts with FL in need of systemic therapy. Untreated pts with FL in need of systemic therapy are eligible. The trial consists of a dose escalation phase to establish the maximum tolerated dose (MTD) using a 3+3 design and a dose expansion phase in which additional pts are accrued to further characterize the toxicity profile and to collect preliminary efficacy data of the combination. The recommended phase II dose (RP2D) is

determined after analyzing the expansion phase. Treatment consists of 6 cycles of 28 days combination therapy, followed by a maintenance therapy with obinutuzumab for 2 years in responding pts. Obinutuzumab is injected i. v. at a flat dose of 1000 mg in cycle 1: day 1, 8, 15, cycles 2–6: day 1 and maintenance: every 2 months. Venetoclax is administered orally once per day, continuously for 6 cycles, at 2 different dose levels: 600 mg and 800 mg. Nine pts have been treated so far, and the trial is ongoing with a planned accrual of 25 patients.

Pediatric fever in neutropenia with bacteremia – pathogen distribution and antibiotic efficacy over time in a retrospective cohort study

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Background: Fever in neutropenia (FN) is a potentially life-threatening complication of chemotherapy in pediatric cancer patients. Current standard of care is emergency hospitalization and empirical initiation of broad-spectrum antibiotic therapy. This single center study evaluated epidemiology of bacteremia in children with FN over a 20-year time period.
Methods: We analyzed in retrospect FN episodes with bacteremia in pediatric cancer patients in a cohort from 1993 to 2012 in a single center not applying antibacterial prophylaxis beyond *Pneumocystis jirovecii* prophylaxis. The distribution of pathogens, efficacy of antibiotic therapy, and their trends over time were assessed. Efficacy of the standard empirical antibiotic therapy in our center (ceftriaxone plus amikacin) was compared to currently recommended antibiotic therapy regimens.
Results: From a total of 712 FN episodes reported, we assessed 136 FN episodes with bacteremia with 198 pathogens isolated in 102 patients. Gram-positive pathogens (127, 64%; 95% CI: 57%–71%) were more common than Gram-negative (71, 36%; 95% CI: 29%–43%). This proportion did not change over time (p = 0.26), but we observed a significant change in Gram-negative pathogen distribution (p = 0.036), mainly due to an increase of *Klebsiella* spp. Coagulase-negative staphylococci (64, 32%), viridans group streptococci (42, 21%), *E. coli* (33, 17%), *Klebsiella* spp. (10, 5%) and *P. aeruginosa* (nine, 5%) were the most common pathogens. No extended spectrum beta-lactamase-producing *Enterobacteriaceae* or methicillin-resistant *S. aureus* were isolated. Comparing the efficacy of empirical antibiotic therapy on isolate level for ceftriaxone plus amikacin (91%; 95% CI: 84%–96%), cefepime (90%; 95% CI: 83%–95%), meropenem (93%; 95% CI: 86%–97%), and piperacillin/tazobactam (89%; 95% CI: 81%–94%), respectively, showed no significant differences.
Conclusion: Over two decades, we detected a relative stable pathogen distribution. No significant trend in efficacy of standard empirical antibiotic therapy was found in our setting. Different recommended antibiotic regimens showed comparable in vitro efficacy.

A rare case of therapy-related acute myeloid leukemia expressing EVI1 with t(11;19) and MLL-MLLT1 after acute B-lymphoblastic leukemia with t(4;11)(q21;q23) and MLL-AFF1

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Introduction: The mixed-lineage leukemia 1 (MLL1) gene (now renamed Lysine [K]-specific MethylTransferase 2A or KMT2A) on chromosome 11q23 recurrently disrupted acute leukemias. We report a patient who developed therapy-related AML (t-AML) carrying a KMT2A-MLLT1 fusion after KMT2A-AFF1+ acute lymphoblastic leukemia.
Case presentation: A 47-year-old patient was diagnosed in 2015 with an acute lymphoblastic leukemia (ALL) with 46,XY,t(4;11)(q21;q23) leading to a KMT2A-AFF1 rearrangement. The patient was treated following the GRAALL-2005 protocol with induction, consolidation, late intensification, maintenance, and intrathecal CNS prophylaxis and achieved early complete MRD-negative remission after induction therapy. Eighteen months later whilst on maintenance treatment with POMP the patient presented with pancytopenia and 25% myeloid blasts in the peripheral blood and diagnosis of a t-AML was made. Molecular analysis revealed t(11;19)(q23;p13.3) with KMTA2A-MLLT1 fusion but t(4;11) negativity with significant expression of EVI1. The patient received 2 cycles of induction chemotherapy (cytarabine and anthracycline-based) followed by an allogeneic hematopoietic stem cell transplantation from a 12/12 HLA-identical unrelated donor in molecular complete remission after conditioning with cyclophosphamide and busulfan and GvHD prophylaxis with ATG, cyclosporine and MTX. The patient molecularly relapsed with KMT2A-MLLT1+ disease three months after allo-HSCT. Despite treatment with with azacitidine (2 cycles) the patient had hematological relapse with 40% blasts in the bone marrow and decreasing donor chimerism six months after allo-HSCT. The patient received high-dose melphalan and donor lymphocyte infusions. Shortly after DLI, the patient developed severe (overall grade IV) acute GvHD of the liver with a maximal bilirubin of 445 µmol/L treated with tacrolimus and steroids (2 mg/kg) without response. Therefore, alemtuzumab, an anti-CD52 monoclonal antibody, was applied, which resulted in GvHD remission. Because of the long-lasting aplasia, the patient received a T-cell depleted stem cell boost three months thereafter without prior conditioning. Actually, the patient is in complete remission with normal bilirubin levels.
Conclusion: We present a rare case of a patient with two different leukemias carrying 2 different KMT2A fusion genes. Of interest here the 2 separate KMT2A translocations which must pinpoint a unique susceptibility in this patient.

Nilotinib combined with lower-intensity chemotherapy for front-line treatment of younger adults with Ph-positive acute lymphoblastic leukemia (ALL): interim analysis of the GRAAPH-2014 trial

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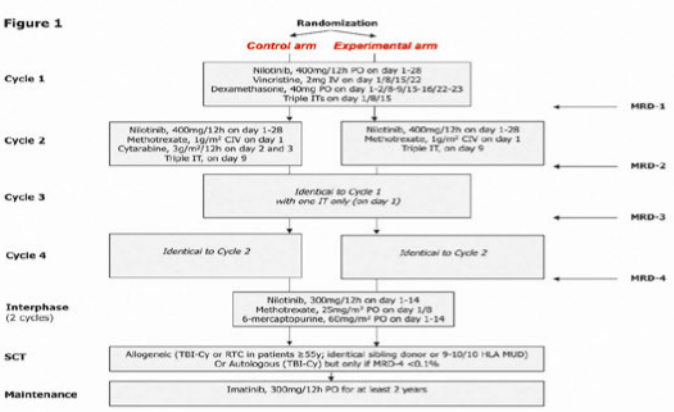
Background: The introduction of TKIs to treat patients with Ph+ ALL allows reducing the intensity of associated chemotherapy. In our previous randomized GRAAPH-2005 trial with imatinib, we have reported better short-term results when patients received lower-intensity chemotherapy during the 1st cycle. In this ongoing GRAAPH-2014 trial with nilotinib, we are pursuing random evaluation of reducing chemotherapy intensity by omitting high-dose cytarabine during the 2nd cycle. We are also repeating cycles 1 and 2 for a total of 4 cycles in order to prolong TKI exposure and reach a deeper response prior to allogeneic or autologous SCT in these patients.
Methods: After a common steroid prephase, patients aged 18–59 years old with newly diagnosed Ph+ ALL were included. Treatments are detailed in figure 1. Primary study endpoint is non-inferiority of major *BCR-ABL1*molecular response (MMolR) in the no-cytarabine arm after cycle 4, defined as *BCR-ABL1/ABL1* ratio <0.1% in the bone marrow. Secondary endpoints are hematologic CR, early mortality, PFS, EFS, OS. An interim analysis was planned after the first 60



patients enrolled became evaluable to make sure that the overall MMolR rate does not significantly differ from the anticipated 80% rate.
Results: 60 patients were randomized between March 2016 and June 2017 with a median follow-up of 14 months. All patients but one who early died from a septic shock achieved hematologic CR after cycle 1 (CR rate, 98%). During the initial 4-cycle period of time, 4 additional CR patients discontinued the planned therapy during cycle 1 (1 septic shock, 1 chest pain, 1 paraplegia, 1 cannabis arteritis) and 1 during cycle 3 (prolonged cytopenia). In intent to treat analysis, the MMolR rate was 43/54 patients (80%) after cycle 2 and 38/41 patients (93%) after cycle 4. Based on these as good as expected MMolR rates, the trial is continuing in order to randomize the 265 patients needed to answer the no-cytarabine question. One year estimate PFS and OS were 84.5% (95% CI, 75.0–95.2) and 95.9% (95% CI, 90.3–100), respectively.
Conclusion: In conclusion, front-line combination of nilotinib and lower-intensity chemotherapy appears to be a promising option to treat adult patients with Ph+ ALL, associated with a high major molecular response rate and a good short-term outcome.

Allogeneic stem cell transplantation in patients with CML-CP in the era of third generation tyrosine kinase inhibitors: a study by the CMWP of the EBMT

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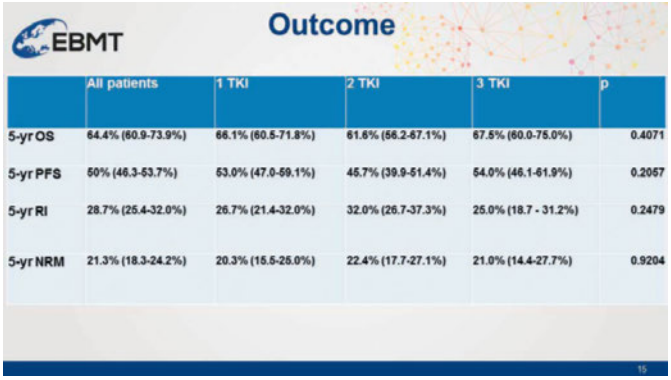
Introduction: Following the introduction of TKI in the early 2000's the use of alloHSCT for CML has dramatically decreased. Imatinib was the 1st TKI introduced and is mostly used as 1st treatment. In case of insufficient response, resistance or intolerance, CML pts can be treated with a 2nd or 3rd generation TKI. Nevertheless there is still a role for alloHSCT for CML. We analysed the impact of the use of 1, 2 or 3 TKI prior to alloHSCT on transplant outcomes in pts whith alloHSCT for CML in CP between 2006 and 2016.
Patients and methods: 3614 pts underwent an alloHSCT for CML, of whom 904 had information on the use of TKI prior to alloHSCT for CML in CP. 778 had Imatinib, 508 dasatinib, 353 nilotinib, 12 bosutinib and 44 ponatinib. 323 had 1, 371 2 and 210 3 TKI prior to transplant. The majority had imatinib as first TKI (n = 747, 82.6%). Transplants were performed in CP1, n = 549, CP2, n = 306, and CP3, n = 49, from HLA id.siblings (n = 345), MUD (n = 516) or others (n = 43). Median age at transplant was 45 (18-71) yrs, 573 pts (61.7%) were male.
Results: The number of TKI given prior to alloHSCT has been increasing over the yrs, median 1 in 2008, 2 in 2010 and 3 in 2012. Pts with only 1 TKI prior to alloHSCT were more likely to have BC at diagnosis (30%) compared with 2 TKI (11%) or 3 TKI (4%). Interval from diagnosis to alloHSCT was longer for pts with 3 TKIs, p <0.001. Median fup was 48.1 (0.5–138) mths. OS for the entire population at 5 yrs was 64.4% (95% CI 60.9–73.9%), PFS 50% (95% CI 46.3–53.7%), RI 28.7% (95% CI 25.4–32.0%) and NRM 21.3% (95% CI 18.3–24.2%). In univariate analysis there was no difference in OS, PFS and RI related to the nb of TKI prior to alloHSCT or to the type of TKI given (p = ns), although there was a tendency for worse OS, PFS or RI for pts who had bosutinib or ponatinib. In multivariable analysis for OS, the number of TKI given did not have any impact. Factors influencing OS were CP2 vs CP1, HR 1.49 (1.14–1.94), p = 0.003 and Karnovsky score >= 90 vs <90 HR 0.66 (0.5–0.85), p = 0.002. Factors influencing



RFS were CP2 vs CP1, HR 1.5 (1.19–1.88), p = 0.001, and Karnovsky score HR 0.77 (0.62–0.97), p = 0.023.
Conclusion: These data suggest that the number of TKI given prior to alloHSCT has no impact on post-transplant outcomes. Pts receiving 3rd Generation TKI might have worse outcomes. Pts in CP1 have better survival than more advanced CML one. The performance status at transplant remains as an important predictive factor in the era of 3rd generation TKI.

SAKK 35/14 trial: Rituximab with or without Ibrutinib for advanced follicular lymphoma in need of first-line therapy

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Background: Previous trials from the Swiss Group for Clinical Cancer Research (SAKK) and the Nordic Lymphoma Group (NLG) showed that first-line rituximab (R) is effective, safe and well tolerated in follicular lymphoma (FL) patients (pts) and that a chemotherapy-free front line strategy can result in long-lasting remission in approx. one third of FL pts, with overall survival similar to chemo-immunotherapy. In this setting of chemotherapy-free strategies, the study of R combined with other immunotherapies or novel targeted agents is obviously relevant. Promising results have been reported for R combined with lenalidomide. Ibrutinib (IBR) and other inhibitors of BCR-signaling are also promising candidates, and there is evidence suggesting synergism between IBR and R. We aim to assess the activity and safety of front-line R+IBR in pts with advanced FL in need of therapy.
Methods: Untreated pts with CD20-positive FL, grade 1-3A, stage II to IV, are eligible if they are in need of systemic therapy. Pts are randomized either to R + IBR or R + placebo. R is administered weekly for 4 infusions on day 1 of week 1, 2, 3, 4 and afterwards in 8-weekly intervals for 12 further infusions. IBR/placebo is administered po (560 mg daily for 24 months), starting 14 days before first R administration. Primary endpoint is complete response (CR) at 24 months; 95 pts per arm are required to detect a difference of 15%, assuming a CR rate of 35% for R monotherapy, with 80% power and alpha at 0.10 (one-sided). 78 pts from Switzerland, Norway, Sweden and Denmark have been randomized until now. A planned interim safety analysis, including the first 26 pts, was performed and the recommendation of the independent data monitoring committee was to continue the trial as planned.

Impact of partial T-cell depletion on an endpoint associated with clinical benefit after initial chronic GvHD treatment

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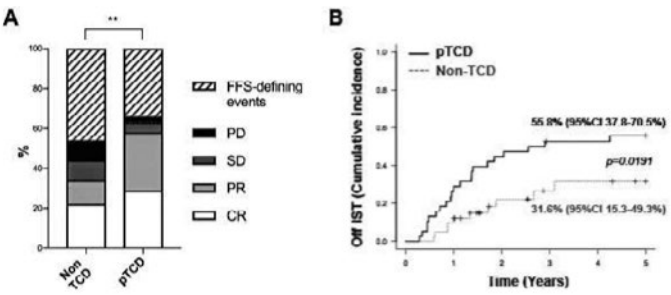
Background: Chronic graft-versus-host disease (cGvHD) is a serious complication of allogeneic hematopoietic stem cell transplantation. Survival with a complete (CR) or partial remission (PR) and no

previous secondary systemic treatment or recurrent malignancy at 1 year after initial systemic therapy for cGvHD is a recently reported composite endpoint associated with clinical benefit (*Martin PJ et al,* Blood 2017). We investigated the effect of partial T-cell depletion (pTCD), a strategy for GvHD prophylaxis, on this endpoint.
Methods: We performed a retrospective analysis on 79 consecutive patients diagnosed with systemic therapy-requiring cGVHD from 2003 to 2016 at our centre. 38 patients received pTCD grafts, consisting of *in vitro*alemtuzumab incubation before infusion followed on day +1 by an add-back of donor T CD3+ cells. 41 patients received non-TCD grafts. Donor lymphocyte infusions were provided at three months to patients receiving pTCD grafts with reduced intensity conditioning and as needed to all patients. Failure free survival (FFS)-defining events were death, disease relapse or secondary systemic treatment. Success was defined as FFS with CR or PR at 1 year of initial treatment. Response rates were compared using the chi-square test. Kaplan-Meier estimates were employed to determine the probability of overall survival (OS) and FFS. Cumulative incidence of treatment cessation was calculated with relapse/death as competitive events and compared using the Gray test.
Results: Success was observed in 36/79 patients (45%). pTCD was associated with a significantly higher endpoint success achievement compared to non-TCD transplantations fig. 1A. Accordingly, patients receiving pTCD had higher 5-year cumulative incidence of systemic treatment interruption (55.8%) compared to patients receiving non-TCD grafts (31.6% p = 0.0191, fig. 1B). Conversely, no effect was observed on 5-year OS and FFS.
Conclusion: pTCD improves the endpoint success achievement in systemic therapy-requiring cGvHD, resulting in an earlier cessation of treatment without affecting OS. These results suggest that pTCD could ameliorate the clinical evolution of cGvHD improving patients' quality of life.



Rare transdifferentiation of lymphoid neoplasms into histiocytic and dendritic cell neoplasms

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Introduction: Histiocytic and dendritic cell (H/DC) neoplasms are very rare hematological disorders and contribute to <1% of lymphoid or soft tissue malignancies. There are very few cases found in literature providing evidence for secondarily derived H/DC neoplasms from lymphoid neoplasms by demonstrating the same IG rearrangements due to clonal relationship. This process of lineage reprogramming, also termed "transdifferentiation" suggests that histiocytic and lymphoid neoplasm originate from a common progenitor which has already undergone IG rearrangement. We present the clinical courses of 4 patients with lymphoid malignancies and subsequent H/DC neoplasm.
Results: Between 2011 and 2017, 4 patients with the diagnosis of H/DC tumors with preceded lymphoid neoplasms were treated in our hospital (characteristics are outlined in table 1). All 4 cases presented a preserved IgH rearrangement for the lymphoid and the H/DC neoplasm, consistent with transdifferentiation. Three patients showed a very aggressive disease course and died shortly after proven transdifferentiation. Long-term survival was achieved only in one patient who was treated with allogeneic stem cell transplantation (allo-HSCT), following radiation and chemotherapy as well as radical surgical amputation of the involved tissue.
Conclusion: Despite progress in understanding the molecular mechanisms underlying the rare phenomenon of secondarily derived H/DC neoplasms, there are no common treatment strategies available due to limited evidence. Transdifferentiated H/DC neoplasms are afflicted by very poor outcome and it should be of high interest to focus on assessing disease course and response to treatment modalities to find successful treatments for this rare disease. To the best of our knowledge, we present the first patient with secondarily derived/ transdifferentiated H/DC neoplasm achieving long-term survival after treatment with allo-HSCT.

Table 1. Patients with both lymphoid neoplasm and H/DC neoplasm

Patient Nr.	Age (y)	Sex	Lymphoid neoplasm/ Type	Lymphoid neoplasm/ Treatment	Time to occurence of H/ DC neoplasm (y)	Treatment of H/DC neoplasm	Clonal IGH rearrangement by PCR	*BRAF*, Exon 15	Outcome/ time after diagnosed H/DC neoplasm
1	75	m	FL grade II	2 # BR	0.5y	Prednisone	+	-	died during work-up/ day 5
2	26	m	B-cell ALL	BMF-90 protocol	10y	surgery/radiation/ CTx -> allo-HCT	+	-	CR/ 6y
3	77	m	CLL	2 # ChP 6 # FCR Ofatumumab	12y	1 # CHOP	+	na	died/ 12d
4	55	f	SMZL	splenectomie	2y	1 # BR 2 # R-CHOP	+	-	died/ 6mo

Abbreviation: FL = follicular lymphoma; CLL = chronic lymphocytic leukemia; ALL = acute lymphoblastic leukemia; SMZL = splenic marginal zone lymphoma; H/DC neoplasm = Histiocytic and dendritic cell neoplasm; # = cycle; BR = bendamustine, rituximab; ChP = chlorambucil/prednisone; FCR = fludarabine, cyclophosphamide, rituximab; R-CHOP = rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; CTx = chemotherapy; allo-HSCT = allogeneic hematopoietic stem cell transplantation; IGH = Immunoglobulin heavy chain; PCR = polymerase chain reaction; na = not available; CR = complete remission.

Blinatumomab – clinical experiences University Hospital Zurich

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Background: Relapsed/refractory (r/r) B-ALL remains a challenge to treat. The median overall survival (OS) in these patients (pts) is 2–6 months with a 5-year OS of <10%. Blinatumomab (Blina) is a bispecific T-cell activating antibody that binds simultaneously to CD3+ T cells and CD19+ cells and thereby attracts and activates T cells towards CD19+ ALL blasts and CD19+ B cells.
Methods: Here, we present our experience treating 12 pts with r/r B-ALL with Blina at the University Hospital in Zurich since 06/2015. Some of these pts were treated within an early access program by Amgen.
Results: Bridging to allogeneic hematopoietic cell transplantation (allo-HCT): for 6 pts Blina was used prior to allo-HCT. Of these 1 patient (pt) had *BCR-ABL1*+ ALL; the majority of pts received 1–2 cycles of treatment. 3 pts achieved molecular CR of the bone marrow (BM); however 1 of these developed extramedullary relapse under Blina treatment. 2 pts were in morphological CR but still MRD+ at the time of allo-HCT. One patient showed no response, was switched to therapy with Inotuzumab, which resulted in a CR prior to HCT.
Palliative treatment: for 3 pts 0.5–4 cycles of Blina were used as a palliative therapy; 2 pts were MRD- after cycle 1; of these one elderly woman stopped the drug after cycle 2 and relapsed 7 months later. One patient with *BCR-ABL1*+ ALL is in molecular CR 27 months after end of therapy, receiving Ponatinib maintenance. 1 pt with 3rd relapse post allo-HCT was progressive after 14 days of Blina and died 1 month later.
Combination with donor lymphocyte infusions (DLI): 3 pts with ALL-relapse (2 of them *BCR-ABL1*+) post allo-HCT were treated with 3-7 cycles Blina in combination with DLI. 1 pt received 4× DLI after cycle 2, 3, 4 and 7. After cycle 1 she was MRD- and is in CR 20 months after end of therapy without Graft-vs-Host Disease (GVHD). The 2. pt received 5 cycles of Blina and 1× DLI after cycle 3. He achieved MRD- after cycle 1; 16 months after end of therapy he is in CR with mild GVHD of the oral cavity. The 3. pt developed early molecular relapse after allo-HCT and was treated with 3 cycles of Blina and 1x DLI. He achieved MRD- after the DLI with no signs of GVHD so far.
Conclusion: Blina is a reasonable treatment choice as a bridging therapy before allo-HCT but also in patients with relapse after allo-HCT, particularly in combination with DLI.

Pneumatosis intestinalis in patients with leukemia – life-threatening emergency or benign incidental finding? Case reports and review of literature

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Introduction: Pneumatosis intestinalis (PI) is defined as air in the wall of the gastrointestinal tract (GIT). It is a rare complication of unknown origin in patients treated for leukemia. For the health care provider it often leads to uncertainty regarding the suitable conservative or surgical treatment. The most common underlying conditions for PI are related to ischemia or bacterial infections. However, the pathophysiology and the most adequate treatment of PI in patients with leukemia is unknown.
Cases: Between 2007 and 2018, three cases of PI in patients with different types of leukemia were diagnosed at the University hospital of Zurich. Two of them were detected coincidentally while performing CT-scans for other reasons and had few or no gastrointestinal symptoms. Both patients did not require surgical treatment. The third patient, suffering from neutropenic enterocolitis, presented with severe

abdominal pain, paralytic ileus, acute kidney failure and beginning hemodynamic instability. CT-scan showed an extensive PI involving the GIT from the esophagus to the distal jejunum. Due to suspicious GIT perforation and bacterial translocation, emergent surgical exploration was performed. During exploration, peritonitis with fibrotic adhesions, fluid-filled bowel loops, and initially ischemic-appearing bowel were found. The entire small bowel was decompressed and the abdominal cavity was irrigated. Postoperatively, the patient was treated in the intensive care unit due to severe inflammation, aspiration and kidney failure with resolving abdominal symptoms.
Discussion/Conclusions: In these three cases, we did not identify a single risk factor causing PI. Naiditch et al., (2010) reported graft-versus-host-disease as a risk factor that two of our three patients suffered from as well. In addition, steroid administration is discussed to increase risk of PI. All of our three patients had recent intake of dexamethason or prednisone. The treatment decision should be based on the clinical presentation and can primarily be conservative. Surgical approaches may target reducing intra-abdominal pressure and ruling out potential GIT perforation.

Very long-term follow up of aplastic anemia treated with allogeneic stem cell transplantation and immunosuppressive treatment

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Background: Since the 1970s patients with aplastic anemia (AA) received treatment consisting of allogeneic hematopoietic stem cell transplantation (HSCT) and immunosuppression (IST, originally horse ATG). However, patients may suffer from persistent disease, relapse, clonal complications, GvHD and other late effects of the treatment. To assess very long-term outcome, we compared survival and complications of AA patients treated since 1973.
Methods: Patient charts of 302 AA patients, treated between 1973 and 2017 at the University Hospital Basel, Switzerland, were retrospectively analysed.
Results: Median age at diagnosis was 24 years (IQR: 1–80 years) and median follow up time was 17 years. Overall survival 30 years after the initial treatment was similar in patients treated by HSCT and IST (44% [±14%], respectively 40.3% [±10%]). Patients treated by IST significantly experienced more iron overload (18.1 % versus 10.6%, p = 0.002), cardiovascular events (3.9% and 1.3%, p = 0.011) and clonal evolution to MDS/AML (14.6% versus 1.3%, p = 0.004), whereas in patients treated by HSCT graft versus host disease (GvHD) was the typical complication (acute GvHD Grade II–IV: 36.8%, chronic GvHD 32.9%). Relapse rate incorporating also primary non-engraftment and late graft failure did not significantly differ between the HSCT and IST group (23% versus 33%). At last follow up the majority of long-term survivors were in complete remission (95.8% in the HSCT and 85.5% in the IST group).
Conclusion: The majority of AA survivors experience a good long-term survival and maintain hematopoiesis irrespective of treatment modality, but patients treated with immunosuppression suffer from more complications. Few patients die very late and long-term survival after 10 years is excellent.

Severe neutropenia as immune-mediated adverse event upon exposure to checkpoint inhibitors

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Introduction: Cancer immunotherapy by checkpoint inhibition of the cytotoxic T-lymphocyte-associated Protein 4 and cell death protein 1 has significantly improved the treatment of metastasized melanoma and a rapidly increasing number of other cancer types. However, substantial antitumor effect is often accompanied by immune-related adverse events (irAE), commonly clinically presenting as pneumonitis, colitis, endocrine dysfunction and hepatitis and usually compelling a discontinuation of treatment. There are only few cases in literature documenting neutropenia following checkpoint inhibitors (CPI) as irAE, but this unpleasant condition can lead to critical, probably fatal complications. We report a clinical course of a patient who developed repeatedly severe neutropenia after salvage combination therapy with Ipilimumab/Nivolumab and died due to septic shock but experienced completely remission of the malignant disease.
Results: A 65-year-old male patient with relapsed melanoma was treated with CPI salvage therapy and exhibited severe neutropenia after the third cycle of combination therapy. Bone marrow examination confirmed a CD8 predominant lymphocytosis with significantly diminished myeloid precursors. Discontinuation of CPI and treatment with steroids and G-CSF led to complete recovery of the neutrophil count. Due to the lack of alternative therapy, a re-exposure to Nivolumab monotherapy was installed a month later and unfortunately resulted again in a severe neutropenia. The patient died shortly after in septic shock although steroid and G-CSF therapy was immediately started. The autopsy result demonstrated a severe fungal pneumonia, marked immune related colitis and concurrently revealed an excellent response of the tumor therapy with complete absence of residual melanoma cells.
Conclusion: With the increasing number of cancer patients eligible to CPI therapy, the incidence of severe hematological toxicities may arises substantially in the next years. Clinicians working in the field of cancer immune therapies should be aware oft these potentially lethal irAE and regular blood controls should be considered on regular basis for patients during CPI treatment.

Post-transplantation cyclophosphamide compared to GvHD prophylaxis with ATG in HCT with mismatched unrelated donor

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Background: Post-transplantation cyclophosphamide (PTCy) has been shown to be an effective strategy to prevent GvHD after haploidentical HCT. Mismatched unrelated HCT carries higher risks of GvHD and may benefit from best possible GvHD prophylaxis.
Methods: This study includes 1-antigen HLA-mismatched unrelated donor (9/10 MUD) transplant patients for a hematological disorder between 2010 and 2017 at the Hematology division of the Basel University Hospital. 78 patients were analysed, 20 patients received a PTCy-based GvHD prophylaxis (40mg/kg, day 3+4 after HCT) with cyclophosphamide, cyclosporine A (CyA) and mycophenolatmofetil (MMF), in 58 patients the GvHD conventional prophylaxis consisted of anti-thymocyte globulin (ATG-Fresenius), cyclosporine or tacrolimus, methotrexate (MTX) or MMF. The primary study aim was to compare the cumulative incidence (CI) of acute II–IV GvHD, 1-year chronic GvHD and outcome after HCT [1-year PFS, OS, relapse and NRM)].
Results: The median age in the PTCy- and ATG group was 55 (IQR 44–68) vs. 50 years (IQR 39–60; p = 0.071), respectively (table 1). Median follow-up of surviving patients was 21 months (IQR 13–40 months). Graft source was primarily peripheral blood in the PTCy- (95%) and ATG (91%) group (p = ns), respectively. Incidence of grade II–IV aGvHD in the PTCy group was significantly lower compared to ATG (18% vs. 52%, respectively, p = 0.010). However, the CI of 1-year cGvHD was comparable in both groups (p = 0.451). Patients in the PTCy group showed a trend for a better PFS (p = 0.055), but a significant increased OS (p = 0.035) compared to ATG (fig. 1A-B). Moreover, 1-year relapse and NRM were similar in both groups (fig. 1C-D). Median time to neutrophil engraftment (>500/µl) was comparable in the PTCy- and ATG group (14 days vs. 16 days, respectively, p = 0.169) and also the median time to platelet

Figure 1. 1-year PFS (A) and OS (B), relapse (C) and NRM (D) in the PTCy- vs. ATG-group

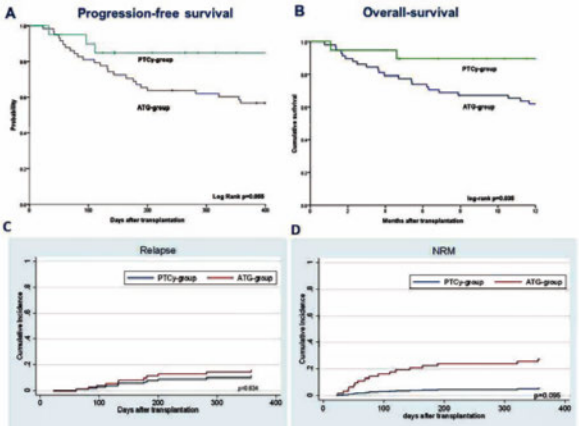

Table 1: Characteristics of patients with PTCy- and ATG-based GvHD prophylaxis

Variable	PTCy group (n = 20)	ATG group (n = 58)	p-values
Diagnosis, n (%)			0.983
Myeloid disorders	12 (60)	36 (62)	
Lymphoid disorders	7 (35)	19 (33)	
Bone marrow failure	1 (5)	3 (5)	
Disease Risk Index, n (%)			0.663
Low	2 (10)	6 (10)	
Intermediate	12 (60)	34 (59)	
High	4 (20)	16 (28)	
Very high	2 (10)	2 (3)	
HLA class mismatch, n (%)			0.418
Class I mismatch (HLA A, B, C)	16 (80)	41 (71)	
Class II mismatch (HLA DRB1, DQB1)	4 (20)	17 (29)	

engraftment (>20'000/µl) showed no significant difference in both groups (15 days vs. 13 days, respectively, p = 0.174).
Conclusion: Our results revealed that PTCy-based prophylaxis (dose of 40 mg/kg) is an effective and safe strategy to prevent aGvHD in 9/10 MUD patients undergoing HCT for hematologic disorders with improved OS and similar results according 1-year relapse and -NRM compared to ATG.

Parents' perceptions of an integrative approach to pediatric oncology treatments: a qualitative study at a University Hospital

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Background: The Department of Pediatric Hematology/Oncology, University Hospital Bern, adopted an integrative approach in addition to standard-of-care oncology therapies. This involves a collaboration with the Institute of Complementary Medicine at the University of Bern. The aim of this study was to investigate parents' experiences about the integrative approach during cancer treatment of their children.
Methods: Ten volunteer parents of childhood cancer survivors treated at our institution were surveyed via semi-structured interviews. Questions to the following elements of an integrative approach (definition of the The Academic Consortium for Integrative Medicine & Health, 2010) were included: focus on the whole person, communication, relationship, inclusion of complementary medicine, and the importance of empirical evidence for complementary methods. Interviews were recorded, transliterated and evaluated with qualitative context analysis (Mayring, 2000).
Results: High satisfaction was reached regarding implementation of a whole person focus, communication and relationship (high satisfaction with treatment, good communication and relationship with medical professionals, being well-informed and taken seriously). Most mentioned negative statements were about impersonal communication and not being enough responsive to the parents emotions. Parents reported that openness to complementary methods depended on personal attitudes of medical professionals. The inter-institutional collaboration was appreciated. Parents wished for a higher level of inclusion of complementary medicine. Being able "to do something" and contribute to the child's well-being ranked higher than scientific evidence of complementary medicine approaches.
Conclusions: Communication, relationship, parent empowerment, and contributing to the child's well-being were perceived as important factors of the integrative oncology approach by parents. Scientific evidence about complementary medicine was ranked less important, suggesting a need for guidance by trained medical personnel in order to avoid possibly harmful self-medication. Needs for improvement could be seen in a more standardized approach to integrative oncology in the future.

M. Waldenstöm coinciding with Schnitzler syndrome

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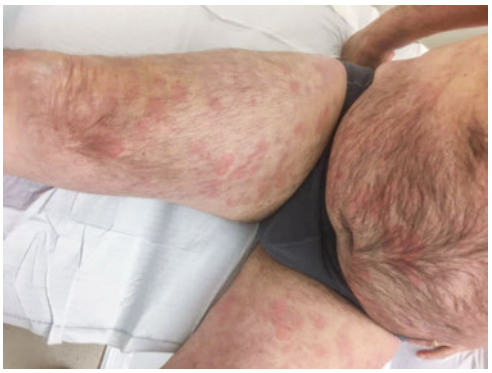
Schnitzler syndrome (Ss) is an aquired, rare and underdiagnosed auto-inflammatory systemic condition with monoclonal IgM gammopathy and neutrophilic urticarial dermatosis as the only two mandatory features. 1 In total, up to 20% of patients develop a manifest lymphoproliferative disorder, beyond a mere MGUS. Inhibition of Interleukin-1 (IL-1) receptor has a dramatic therapeutic effect as depicted in our case report.

Introduction: A 70 year old male with a history of recurrent urticarial rashes for 15 years (fig. 1) with increasing intensity over time was admitted with debilitating exhaustion due to episodes with fever rising above 40 °C as well as profuse sweating and muscle pain. Fever episodes would usually last between two and five hours and were unresponsive to corticosteroids, colchicine and antihistamines.



Case description: Skin biopsies revealed neutrophil infiltrates in all levels of the corium without signs of vessel damage. Infiltration of the bone marrow with lymphoplasmacytic cells and atypical plasmacells (both harboring the same light chain restricition) in conjunction with 6.2 grams of monoclonal IgM kappa paraprotein and positive MYD-88 mutational status lead to diagnosis of M. Waldenström. Diagnosis of Ss was established according to diagnostic criteria by Lipsker et al. [1]. After initiating therapy with the subcutaneous IL-1 receptor antagonist Anakinra, all symptoms vanished within a few hours.
Discussion: In alignment with hereditary auto-inflammatory Syndromes (CINCA, NOMID, Muckle-Wells) patients with Ss constitutively overexpress IL-1 by excessive activation of the inflammasome, an intracellular protein complex in macrophages and neutrophils. Accordingly, IL-1 receptor inhibition is the therapeutic key. Other treatment modalities have not proven to be beneficial. It remains unclear whether the IgM paraprotein in Ss emerges from cytokinic activation (i.e. continuous antigenic stimulation) or inversely, IgM is causative of a prolongued effect of IL-1 (e.g. by lowered clearance). An ongoing observational study hopes to elucidate this question among other unclarities on the exact mechanisms leading to the remarkable clinical presentation of Ss. [2]

References
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Immunophenotypic characterization of autologous, DMSO-treated stem cell transplants

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Background: Recovery of CD34 positive stem cells after thawing can variate considerably, affecting stem cell engraftment. We characterized the cell composition of the autologous transplants after collection in order to find out additional reasons that could explain low CD34 recovery. We test the hypothesis, that increased numbers of leukocyte subsets known to be cytotoxic, such as neutrophils, are responsible for stem cell loss post-thawing.
Patients and methods: Flow cytometry and clinical data of 42 consecutive autologous stem cell transplants were retrospectively analyzed. Stem cell content before freezing and after thawing of the transplants was registered and correlated to neutrophil and platelet engraftment. The influence of leukocyte subsets such as neutrophils, monocytes and lymphocytes on CD34 recovery was assessed. Time to engraftment was correlated to counts of viable CD34 cells pre-freezing and post-thawing.
Results: Viability after stem cell harvest was high for CD34 (99.91% [99.35–100%], as well as for all leukocyte subsets. There was no correlation between CD34 or leukocyte subset viability pre-freezing and CD34 recovery. CD34 viability post-thawing was very heterogeneous (88% [10–98%]). Concentrations of monocytes and lymphocytes in the stem cells product had no influence on CD34 recovery. In contrast, a clear negative correlation between neutrophil concentration in the product and CD34 recovery was found (r = –0.6, p <0.0001). Stem cell engraftment was categorized as "normal" (n = 29) vs. "late" (n = 13) using day 17 as cut-off. Late engrafters

had significantly less viable CD34 cells*10^6/kg BW in the transplant post-thawing, compared to normal engrafters (1.6 [0.3–3.2] vs. 2.4 [0.7–4.9], p = 0.04), whereas there was no difference pre-freezing between these groups (2.4 [2.0–5.5] vs. 3.4 [1.1–8.3], ns).
Conclusion: High neutrophil concentrations in the stem cell products of autologous transplants correlate with poor CD34 recovery post-thawing and delayed engraftment. As viability of neutrophils themselves was very low post-thawing in all products, we postulate that release of cytotoxic substances from apoptotic neutrophils have a detrimental influence on CD34 viability in a dose dependent manner. Although not mandatory by current JACIE standards, CD34 viability measurement post-thawing is a valuable tool for quality control of stem cell products.

NK- and T-cell chimerism after allogeneic stem cell transplants: association to GVHD or relapse

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Introduction: We evaluated the role of peripheral lymphocyte subset recovery and chimerism on transplant outcomes and the impact of transplantation procedures on the immune reconstitution post-transplant.
Patients and Methods: 81 patients were included (median age 55y, median follow-up 493 days). Primary diseases included AML (n = 37), ALL (n = 8), high-risk MDS (n = 19), CML (n = 6), NHL (n = 6), PCM (n = 3) and 2 others; conditioning was myeloablative in 58%. Donors were HLA-matched (n = 49), one-antigen mismatched (n = 23), haploidentical (n = 9), 40 related and 41 unrelated. Correlation between transplant outcome and lymphocyte subset counts, including T cells (CD3+), NK cells (CD56) and activated T cells (ATC), as well as split chimerism of T cells and granulocytes (CD66) at 1 month (1 m) and 3 months (3 m) after transplant was assessed in all patients.
Results: The 2-year OS rate was estimated at 86.4%. Patients with rapid reconstitution of CD56 cells at 1m tended to have improved OS compared to those with a delayed reconstitution (OS-rate 90.2% vs 75%). No significant impact of lymphocyte reconstitution or chimerism at 1m and 3m was found on mortality or relapse. In the multivariate analysis ATG was associated with significantly improved CD56 recovery at 1m (p = 0.001) and better ATC counts at 3m (p = 0.007). On the contrary, ATG induced lower CD3 recovery (p = 0.008) and lower CD4/8 ratio at 3m (p = 0.037). Patients with the post-transplant cyclophosphamide regimen (pTCy) had significantly rapid recovery of both CD56 (p = 0.006) and CD3 (p = 0.038) at 1m but lower ATC counts (p = 0.025) and lower CD4/8 ratio at 3m (p = 0.029). Significantly lower GVHD rates were found in the group with rapid NK cell recovery at 3m (p = 0.018), rapid CD3 recovery at 1m and 3m (p = 0.027 / 0.001) and higher ATC counts at 1m and 3m (p = 0.021 / 0.001). Patients with full HLA-match had significantly higher CD56 counts at 1m (p = 0.004) and higher CD3 counts at 1m and 3m (p = 0.026 and p = 0.029) compared to the non-matched.
Conclusions: Rapid reconstitution of NK-cells and T-cells had no impact on relapse or mortality but co-existed with less GVHD. Both ATG and pTCy as conditioning regimens showed improved CD56 recovery at 1m but only pTCy favored rapid CD3 reconstitution additionally. ATG had a favorable effect on OS-rate without increasing relapse.

The role of MRD in pediatric Acute Myeloid Leukemia: two case reports

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Background: In acute lymphoblastic leukemia (ALL), minimal residual disease (MRD) is an established risk factor. Correspondingly, therapy stratification is guided by routine MRD assessments measured by PCR and flow cytometry (FACS) in both adult and pediatric ALL. In acute myeloid leukemia (AML), however, early response assessment is still based on morphology and the potential value of MRD quantified by different molecular methods is currently studied but not part of clinical routine.
Case Reports: Here we report two pediatric patients, aged 5 and 19 months, diagnosed with MLL-rearranged AML M5 and M4, respectively and morphologically defined non-remission status due to presence of 40% and 45% marrow blasts, respectively, at day 21 after the 1st induction cycle. Surprisingly, MRD by fluorescence in-situ hybridization (FISH) and quantitative PCR of MLL-rearrangement showed complete remission (CR) in both patients at the same time point. Decision on continuation of therapy with the second induction cycle on day 22 for both patients despite lack of hematologic recovery was based on morphology only because MRD analyses were not available immediately. After 2nd and 3rd chemotherapy cycle, molecular MRD confirmed complete remission while morphology showed declining but still elevated blast counts.
Discussion: Induction therapy in pediatric AML aims to achieve CR influencing overall survival. If standard chemotherapy fails this concept, second line therapy has to be discussed, taking into account allogeneic stem cell transplantation. Therefore, MRD assessment in morphologically defined remission failure can help to guide therapy. Current clinical trials are testing different methods to quantify MRD, i. e. by FACS, FISH and PCR, if applicable. Multicenter studies should aim to clarify which MRD method at which time point should guide therapeutic decisions.
Conclusion: These two cases of pediatric AML show that there is a need for rapidly available and comprehensive MRD diagnostics to optimize therapy in situations with discrepancy between morphologic and molecular findings.

Frequency of PNH cells and phenotype of hematopoietic precursors in patients with suspected dysplasia or BM failure syndrome: a study on 120 patients referred to the Geneva University Hospital

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Introduction: PNH (paroxysmal nocturnal hemoglobinuria), a rare clonal hematopoietic stem cell disorder, is characterized by the absence of surface markers bound to the cell membrane by the glycosyl-phosphatidyl-inositol (GPI) anchor. The present study aimed to investigate the frequency and size of PNH clones in patients referred to the Flow Cytometry Laboratory of the Geneva University hospital for a diagnostic work-up of myelodysplasia, aplastic anemia, Coomb's negative hemolytic anemia or an episode of thrombosis or pulmonary embolism of unclear origin.
Methods: Patients ≥18 years were included in the study over a period of two years (10/2015 to 10/2017). A detailed flow cytometric analysis was performed on the peripheral blood and bone marrow samples of the patients in order to characterize the phenotype of myeloid, monocytic and erythroid precursors, as well as of stem cells, and to quantify expression of aberrant surface markers as signs of dysplasia. A panel of 25 antibodies was used to characterize the various stages of hematopoiesis and to delineate different lineages, as well as to measure aberrant markers and establish a dysplasia score (Ogatha score). PNH populations were characterized according to ICCS guidelines.
Results: for 77 patients included in the study the specific analysis for PNH was asked by the referring physician, 45 patients were referred for a suspicion of MDS or aplastic anemia without a specific indication for PNH analysis. Three PNH patients treated with Eculizumab were used as controls. MDS was confirmed in 48 patients, aplastic anemia in 3. In the total 120 patients three new PNH clones were detected in PB samples (one clone with 1.5% and two minor clones at 0.9% and 0.5%, respectively). The PNH phenotype was also found in early hematopoietic precursors (CD34pos and CD34neg) and interestingly in several bone marrow samples from MDS patients where the analysis of peripheral blood samples was normal.
Conclusion: Frequency of MDS/aplastic anemia cases and PNH clones as diagnosed in the Geneva Cytometry laboratory corresponds approximately to the frequency reported in the literature and from other countries. Detailed analysis of the PNH phenotype in hematopoietic precursors might be useful for further studies on the pathophysiology of PNH.

Adverse outcome of AML with aberrant CD16 and CD56 NK cell marker expression

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Introduction: Natural killer (NK) cells are main mediators of antibody-dependent cell-mediated cytotoxicity (ADCC), and CD16 exerts key functions in mediating ADCC response. The prognostic relevance of aberrant expression of myeloid/NK cell associated markers such as

CD16 and CD56 on leukemic cells of AML patients is largely unknown. We hypothesized that AML subtypes co-expressing CD16 and/or CD56 by flowcytometry are associated with an unfavorable course of the disease.
Methods: In this retrospective single center study, we applied univariate survival analysis and multivariate cox regression calculations to assess the prognostic significance of aberrant CD16+ and CD56+ NK cell marker expression in 325 consecutive AML patients at diagnosis before undergoing intensive induction chemotherapy. CD16 and CD56 expression was defined by positivity of more than 20% of the leukemic cells for these markers.
Results: We found that AML patients expressing a combined CD16+/CD56+ phenotype roughly represent 20% of all AML patients. Patients with CD16+/CD56+ expression had a lower probability to achieve complete remission after two induction chemotherapy cycles (52% versus 72%; P = 0.300). AML patients expressing CD56+ predominantly had a monoblastic phenotype (FAB M5) and inferior median event-free (EFS; P = 0.0699) and overall survival (OS; 10.9 versus 20.6 months; P = 0.0132) compared to CD56- patients. Patients with aberrant CD16 expression had worse median EFS (P = 0.062) and OS (13.0 versus 45.9 months; P = 0.028). Finally, EFS for CD16+/CD56+ patients was 5.7 months compared to 7.1 months for CD16-/CD56- patients (P = 0.369), and OS was 10.6 months for CD16+/CD56+ patients as compared to 52.2 months for CD16-/CD56- patients (P = 0.031). In a multivariate analysis, CD16 was identified to have a stronger negative effect on overall survival (hazard ratio (HR) of 2.18), than CD56 expression (HR 1.71).
Conclusions: Our data suggest that AML patients with aberrant CD16 and CD56 expression have an inferior response to induction chemotherapy and adverse survival outcomes. This study provides a rational for developing more effective therapeutic options for this subgroup of patients possibly involving specific inhibition of CD16 and/or CD56.

Allogeneic hematopoietic cell transplantation in patients with GATA2 deficiency

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Introduction: *GATA2* (Guanine-adenine-thymine-adenine 2) deficiency has been identified as the genetic origin to a multifarious disease involving a spectrum of hematologic and non-hematologic anomalies including cytopenias, opportunistic infections and a high risk of developing hematologic malignancies.
Methods: Here, we provide a comprehensive analysis of the available literature on the use of allogeneic hematopoietic cell transplantation (allo-HCT) in patients (pts) with *GATA2* deficiency.
Results/Conclusion: Allo-HCT represents a potentially curative therapy for the abnormal hematopoietic and lymphoid system of patients with GATA2 deficiency. Details of HCT have not been studied extensively with only 2 small prospective studies (n = 13 and n = 22 pts); in addition there are 2 larger datasets, but otherwise only case reports and small case series are available. We compiled data on a total of 178 pts with GATA2 deficiency given an allo-HCT between 4–53 years of age. The majority of pts underwent allo-HCT with the diagnosis of MDS (83%) or AML (10%). Whereas in the earlier years pts preparation was done by reduced intensity conditioning due to concerns of uncontrolled infections and increased toxicity, later myeloablative conditioning was applied, and resulted in more uniform engraftment with reduced risk of relapse. Donors used were matched unrelated (56%), matched related (27%) and haploidentical (12%). At the time of HCT more than 54% of pts had human papilloma virus (HPV) infections; 30% had mycobacterial infections, and 54% *"other bacterial, fungal, parasitical or viral infections".* After allo-HCT the incidence of HPV, mycobacterial, and fungal infections decreased considerably. Post-transplant infections were reported in approximately 24% of pts, but pathogens appeared to be similar to "typical post-HCT infections", while opportunistic infections that manifested prior to HCT did not appear to pose a major problem. Allo-HCT is the treatment of choice in pts with GATA2 deficiency and should be performed before irreversible organ damage or full-blown AML has manifested.

'Amyloidose-Netzwerk Zürich' – an interdiscliplinary collaboration aiming for better care of patients with systemic Amyloidosis

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Introduction: Systemic amyloidosis is a generic term for a disease where the underlying pathomechanism is the misfolding of circulating proteins into the pathognomonic beta-sheet structure leading to extracellular deposition and finally organ dysfunction. To date, more than thirty different proteins are known to be amyloidogenic. In our opinion, systemic amyloidosis is a prototypic disease entity that requires an interdisciplinary diagnostic and therapeutic approach. The "Amyloidose-Netzwerk Zürich" was founded 2013 by a team of specialists from different fields in internal medicine as a platform to exchange knowledge and improve treatment, and a registry was started to collect patient data. In the meantime, large regional tertiary health care providers in Graubunden, Ticino and Sankt Gallen have joined this Collaborative Network.
Methods: In a longitudinal, observational SecuTrialTM based registry patient data are collected retrospectively since 2005 and prospectively since 2013. Demographic information, type of amyloidosis, medical and family history, physical assessment, laboratory results, measures of cardiac, neurological, renal function are assessed at time of diagnosis and in regular follow up visits.
Results: Currently, we have included 128 patients in our registry. AL is the most common type (64.5%), followed by ATTR (20.5%) and AA (3.5%). The registry is characterized by a comprehensive set of data elements that are completed by specialists of various clinical backgrounds and different institutions who administer care to amyloidosis patients.
Conclusion: To our knowledge, this is the first registry collecting patient date with systemic amyloidosis in Switzerland. We consider this a valuable tool for epidemiological research and we will continue our effort to expand the registry nationwide.

Allogeneic hematopoietic stem cells transplantation for non-hodgkin's lymphoma in Switzerland. 30 years of experience 1985–2017

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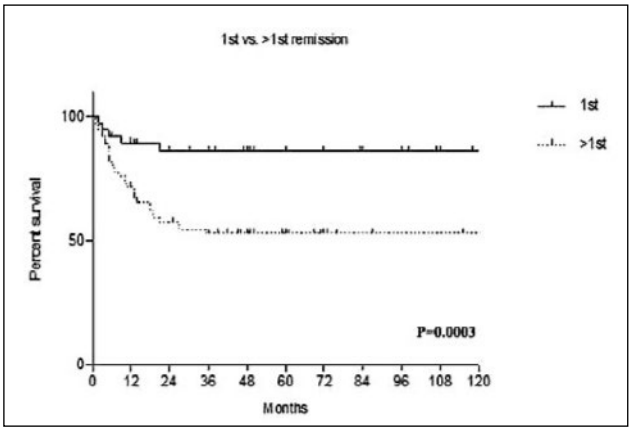
Background: We report the 30 years of Swiss experience (09.1985 – 08.2017) in allo-HSCT for NHL.
Methods: The study included 254 allo-HSCT for NHL from 3 UH of Switzerland (Zurich, Basel and Geneva). CLL (n = 66), DLBCL (n = 60), MCL (n = 31), FL (n = 22), MZL (n = 7), BL (n = 8), WM (n = 6), HCL (n = 1), T-cell, NOS (n = 33), ALTCL (n = 7), AITL (n = 13). OS, DFS, RI and NRM were assessed for: allo-HSCT before and after 2000; prior auto/allo-HSCT; quality and number of remission; Karnofsky index >80%; donor's type and age; stem cell source; graft's T-depletion; RIC vs MAC conditioning; TBI use; presence of a/cGvHD; NHL-type.
Results: Median follow up was 18.5 months (5–72). Median OS = 168 months, DFS = 107 months. Median age at allo-HSCT was 50.7 (43.7–57.2). 5 and 10-yrs OS, DFS and NRM were 57 ± 4%, 53 ± 4%, 23 ± 3% and 51 ± 4%, 46 ± 4%, 25 ± 3.0%. RI at 5 and 10 yrs was 30 ± 3% and 38 ± 4%. In univariate analysis, no prior auto/allo-HSCT, Karnofsky >80%, 1st remission (fig. 1) and CR were associated with improved OS and DFS ($p < 0.05$). TBI use showed a trend toward better OS and DFS ($p = 0.058$ and 0.06). Related donor gaved better OS ($p = 0.017$). In multivariate analysis Karnofsky >80%, CR and PR, no prior auto/allo-HSCT were the most significant factors for OS and DFS ($p < 0.05$). NRM was influenced by donor's type, with the worse survival for the MUD vs IS ($p = 0.001$) and >1st remission status (p =



0.03). The highest RI was found in cases transplanted before the 2000 (p = 0.03) non-being in 1st remission (p = 0.01) and those with aGvHD (p = 0.007).
Conclusion: Our analysis of a mixed-type NHL cohort confirms efficacy of allo- HSCT in pretreated/refractory patients. OS was independent of NHL type and GvHD presence. Karnofsky >80%, remission quality (CR and PR) and absence of heavy pretreatment were associated with better OS and DFS. We interpret carefully these data due to the retrospective analysis; the heterogeneity of NHL and treatment regimen.

Allogeneic stem cell transplantation for peripheral T-cell lymphomas: a study of 284 patients from the Société Francophone de Greffe de Moelle et de Thérapie Cellulaire

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Background: The place of allogeneic hematopoietic stem cell transplantation (allo-HSCT) in Peripheral T cell lymphoma is still a subject of debate.
Methods: Based on the SFGM-TC database, we performed a retrospective multicentric analysis of adult patients who underwent an allo-SCT for PTCL between 2006 and 2014 in 34 centers. Primary cutaneous T cell lymphomas were excluded.
Results: A total of 284 patients with PTCL (NOS-T cell lymphomas: 39%, angioimmunoblastic T lymphomas: 29%, anaplastic T cell lymphomas 15%, others: 17%) were allo-transplanted in a median time of 12.6 months after diagnosis (3-322). Median age at transplant was 50 years (15 to 60) and 67% were males. At the time of transplant, 62% were in complete remission (CR), 27% in partial response (PR) and 11% in progressive disease (PD). Twenty-eight percent were transplanted in front line treatment, 36% after 2 lines of treatment and 35% after 3 or more lines of treatment; 23% had relapsed after a first autologous HSCT. Donors were matched related in 45%, matched unrelated in 36% and alternative in 19% (haplo-identical n = 7, cord blood n = 33, mismatched 9/10, n = 13) and SC source was peripheral blood in 71% of the patients. Reduced intensity regiment was given in 147 patients (52%), myeloablative in 106 (38%) and non myeloablative (NMA) in 27 (10%). Fourteen patients (14%) developed grade III–IV acute GvHd, and 34% developed chronic GvHd (extensive for 13%).

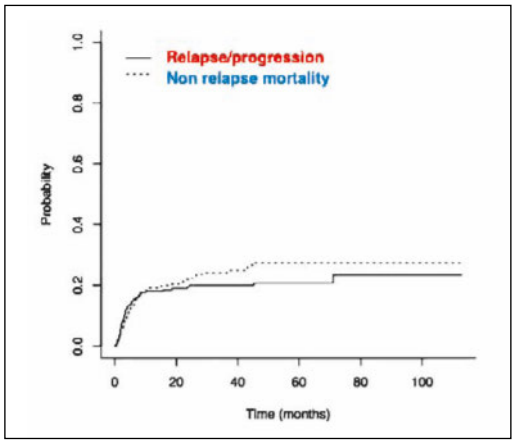

Figure: OS of the 284 allo-SCT for PTCL.

Median follow up was 33 months. One and 2 year -OS were 68% (95% 0.62–0.73) and 64% (95% 0.58–07). Cumulative incidence (CI) of relapse was 18% at 1 year and 22% at 2 years. The median time from transplant to relapse was 94 days and only 10% of the relapse occurred after the first year post transplant. Non relapsse mortality (NRM) was 22% at 1 year and 24% at 2 years. The main causes of death were relapse (35%), infection (27%) or GvHD (22%). In multivariate analysis, 5 year-OS was significantly adversely influenced by the occurrence of grade III–IV aGvhd (HR: 2.52 (1.52–4.19), *p <0.01)*, low karnofsky score at the time of transplant (HR 2.22 (1.32–3.71), *p*: 0.002), cord blood transplant compared to bone marrow (HR 2.01 (1.00–4.01), *p*:0.049). Among thirty patients transplanted in PD, 50% reached CR after allo-HSCT and 2 year-OS was 51% in this subgroup.
Conclusion: This is, to our knowledge, the largest cohort of allo-HSCT patients for T cell lymphoma, showing encouraging results in both MAC and RIC.

Improving sensitivity of FLT3-ITD analysis in patients with AML by investigation of RNA

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Introduction: The multikinase inhibitor midostaurin has become standard of care for younger *FLT3* mutated AML patients requiring timely testing for *FLT3* mutations at diagnosis. Whereas *FLT3*-ITD detection mostly relies on fragment length analysis (FA) with limited sensitivity (2–5%) using genomic DNA, using RNA may be more sensitive.
Methods: We compared *FLT3*-ITD detection by FA in paired DNA and RNA samples from 42 AML patients (33 *FLT3*-ITD-mutated; 9 wildtype) at diagnosis and/or at follow-up. DNA and RNA was extracted from bone marrow (BM) and peripheral blood (PB) followed by *FLT3*-ITD PCR and FA (Leukostrat Assay 2.0; Murphy KM et al., J Mol Diagnostics, 2003). The limit of detection was 5% for DNA according to the manufacturer and 2% for RNA.
Results: Among 82 paired samples (31 BM, 51 PB), *FLT3*-ITD analysis was identical in 85% of the paired samples: 20 (24%) were positive for *FLT3*-ITDs, and 50 (61%) were negative for *FLT3*-ITDs by DNA and RNA. However, 15% of the samples (n = 12) were positive for *FLT3*-ITDs only using RNA (*P* = 0.00053). *FLT3*-ITD/wildtype allelic ratios were higher in RNA compared to DNA analysis in 75% of the patient samples (mean, 16-fold; maximum 226-fold). *FLT3*-ITDs transiently became negative on DNA under therapy in two initially *FLT3*-ITD positive AML patients that later developed *FLT3*-ITD positive overt relapses. In contrast, *FLT3*-ITDs remained detectable using RNA in these patients. Remarkably, one patient had two different *FLT3*-ITDs using DNA detectable only at relapse, whereas these two *FLT3*-ITDs were detectable by RNA analysis already at diagnosis at low levels.
Conclusion: In our study, *FLT3*-ITDs were detectable only by RNA in 15% of all samples investigated (including diagnostic and follow-up samples), whereas genomic DNA did not reveal the mutation. Low *FLT3*-ITD/wt allelic ratios at follow-up under therapy preceding overt relapses may be detectable only on RNA investigation. These results allow improving the indication for FLT3 inhibitor therapy and allo-transplantation.

Secondary chronic neutrophilic leukemia (CNL) after severe aplastic anaemia (SAA) – a case report

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Background: Chronic neutrophilic leukemia (CNL) and aplastic anemia (AA) are both rare diseases. Here we report the case of a 42 years old male patient with a medical history of 20 years of SAA, who developed a secondary CNL and subsequently received an allogeneic hematopoietic stem cell transplantation (HSCT). To the best of our knowledge a secondary CNL after SAA has not yet been described.
Case presentation: After diagnosis of severe aplastic anemia at the age of 22 the subsequent treatment encompassed three cycles of horse ATG and cyclosporine resulting in disease control for nine, three and seven years, respectively. After the third cycle of intensive immunosuppressive therapy, cyclosporine was maintained as long-term therapy. Nineteen years after the initial diagnosis neutrophilia was observed without signs of infection, inflammation or solid tumor.

Bone marrow examination revealed hypercellularity with neutrophilic proliferation and discrete dysplasia of the myeloid cell lineage. Molecular genetics detected a SETBP1, EZH2, RUNX1 and CSF3R mutation (T618I). Integrative diagnosis was made according to WHO classification confirming the diagnosis of CNL. Subsequently, the patient was first treated with Ruxolitinib, showing normalization of the neutrophil counts within 3 months, but treatment had to be stopped due to thrombocytopenia after 6 months. Then allogeneic hematopoietic stem cell transplantation from a 12/12 HLA identical unrelated donor after conditioning with Cyclophosphamid (60 mg/Kg/day for two days) and Busulphan (4 × 0.8 mg/Kg/day for four days) could be performed without relevant toxicity, complications or graft versus host disease (GvHD). The posttransplant follow up (3 months) shows complete remission without any signs of GvHD or other complications.
Conclusion: We describe the first case of CNL that has evolved secondary to SAA nineteen years after the initial diagnosis. Possible pathophysiological mechanisms could include damage of the hematopoetic stem cells by previous cytotoxic therapy or selection of a myeloid clone occurring in aplastic anemia.

Value of regular molecular MRD assessment in NPM1-positive acute myeloid leukemia

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Introduction: NPM1-positive acute myeloid leukemia (AML) encompass ~30% of all adults AML. Prognostic value of NPM1/ABL ratio at minimal residual disease (MRD) 1, 2 and 3 as well as NPM1/ABL ratio immediately before or after allogenic stem-cell transplantation is well established. However, little is known about the relevance of iterative MRD assessment during remission. Furthermore, while cytological relapse is defined as bone marrow blasts count >5%, molecular relapse in NPM1-positive AML is ill defined. We aimed to study the value of NPM1/ABL ratio to predict cytological relapse.
Methods: We present a 10 years retrospective study of all cases of NPM1-positive AML diagnosed at our institution (November 1st 2007 to December 31st 2017).
Results: 36 patients were included (1:1 M:F). Mean age at diagnosis was 56 year-old (range 28-75). 31 patients (88.6%) harbored a normal karyotype, and 17 (47%) a FLT3-ITD mutation. NGS study at diagnosis was available in 18 (50%) cases. All underwent at least 1 cycle of intensive chemotherapy, 6 (17%) autologous, and 15 (42%) allogeneic stem-cell transplant (9 in CR1 and 6 in CR2). Mean number of MRD assessment was 6.7 (range 1–20). Median follow-up was 31.4 months (range 1.5–122.3) and median PFS was 27.8 months (range 1.5–122.3). Eight patients died during follow up, notably 5 of progressive disease, and 1 of treatment-related mortalit. Among them, 1 had autologous, 2 allogeneic stem-cell transplant and 5 harbored the FLT-3 mutation. Ten (36%) patients had cytological relapse. NPM1/ABL ratio ≥1% and ≥0,1% preceded cytological relapse in 7 cases, by a mean of 28 (range 0-68), respectively 56 days (range 0–221). Of note, 3 more patients with progressively increasing MRD were treated with salvage therapy when NPM1/ABL ratio was >1% before cytological relapse ensues. Importantly, 4 patients had an isolated NPM1/ABL ratio between 0,1 and 1% during follow-up (all at MRD4), without having further molecular or cytological progression.
Conclusion: In NPM1-positive AML, regular molecular MRD assessment can predict cytological relapse allowing timely stem cell donor research and salvage therapy initiation. However, the optimal cut-off value for molecular relapse as well as the clinical benefit of early therapy remain to be further investigated.

Isolated thrombocytosis in an elderly female: A very rare case of MDS/MPN-RS-T with SF3B1 and CALR type 1 mutation

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Background: Usually, the distinction between myelodysplastic syndrome and myeloproliferative neoplasm is straightforward, but the diagnosis is challenging if the clinical and morphological features suits both entities. Such "overlap" syndromes are documented in the World Health Organization classification as myelodysplastic/myeloproliferative diseases (MDS/MPD). MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T) is one of these rare overlap entities and is characterized by the presence of thrombocytosis (>450 G/l) and >15% bone marrow ring sideroblasts. It has a strong association with SF3B1 mutations, which are concurrent with JAK2 V617F mutations. We present a rare case of MPN/MDS RS-T with SF3B1 and CALR mutation in a patient with isolated thrombocytosis.
Results: In January 2018, an 82-year old woman was referred to the hospital with thrombocytosis, which was first documented in 2015. Besides a light fatigue, mild hypertonia and psoriasis, she was in good clinical shape. Acetylsalicylic acid was started in October 2017 because of slowly increasing thrombocytosis. Hemoglobin concentration, leukocyte and leukocyte differential count were within normal ranges. A moderate macrocytosis (102fl) and thrombocytosis of 789 G/l were documented. The bone marrow examination showed hypercelullarity with increased megakaryopoiesis with hypolobated nuclei but no clusters. Erythropoiesis was slightly increased without significant signs of dysplasia. Strikingly, 50% ring sideroblasts were revealed by iron staining. The molecular diagnostic work-up confirmed a SF3B1 (Exon 14) mutation. The JAK2 mutation was negative. Instead, a CALR type 1 mutation was detected by specific PCR and direct sequencing and confirmed the diagnosis of MDS/MPN-RS-T.
Conclusion: Although very rarely present, screening for CALR in patients with suspected MDS/MPN-RS-T with negative JAK2 mutation status should be performed to confirm the proper diagnosis. In essential thrombocythemia, CALR mutation is known to exhibit a lower risk of thrombosis and better overall survival. With only 4 reported cases in literature, it is unknown if this also applies to patients with MDS/MPN-RS-T with CALR mutation.

Iron overload impairs autologous stem cell mobilization and survival in AML

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Introduction: Patients with acute myeloid leukemia (AML) undergoing consolidation with autologous stem cell transplantation (ASCT) depend on the successful preceding mobilization of peripheral blood stem cells (PBSC). However, the factors affecting the mobilization potential in AML patients and, in particular, the effects of transfusion-related iron load on PBSC mobilization are largely unknown.
Methods: We investigated the association between varying levels of iron load following red blood cell transfusions and the stem cell mobilization efficacy in consecutive AML patients in first complete remission after two cycles of induction chemotherapy. Serum ferritin levels were used as markers for iron load.
Results: Our cohort comprised 113 AML patients undergoing the mobilization treatment in first complete remission. The median number of transfused red blood cell units until stem cell collection was 22 per patient. While 84 (74.3%) patients had serum ferritin levels exceeding 1'000 µg/l, 26 (23.0%) patients had ferritin levels even above 2'000 µg/l. Iron load positively correlated with the number of preceding red blood cell transfusions and inversely correlated with circulating $CD34^+$ cells ($p = 0.04$) at the day of peripheral stem cell collection. In a multivariate analysis including the factors age, *NPM1* mutational status, LDH levels and ELN risk categories, the effect of iron load on the mobilization potential remained significant. Roughly, we observed that an increase of 100 µg/l in ferritin levels was associated with a decrease of 1'000 $CD34^+$ cells per ml of peripheral blood. In 26 of 113 patients (23.0%), G-CSF stimulation after induction cycle 2 was insufficient, and these patients with primary mobilization failure had higher median ferritin levels compared to patients with successful mobilization (1'694 µg/l versus 1'396 µg/l; $p = 0.03$). Finally, the median progression free survival of patients with ferritin levels above 2'000 µg/l was shorter (47 weeks versus 308 weeks; $p = 0.04$), as was the overall survival (122 weeks versus 319 weeks; $p = 0.04$).
Conclusion: Our data suggest that transfusion-related iron load is an independent prognostic parameter associated with decreased peripheral stem cell mobilization potential and inferior outcome in AML patients undergoing ASCT consolidation.

Natalizumab-induced hyporegenerative anemia due to erythroid maturation arrest

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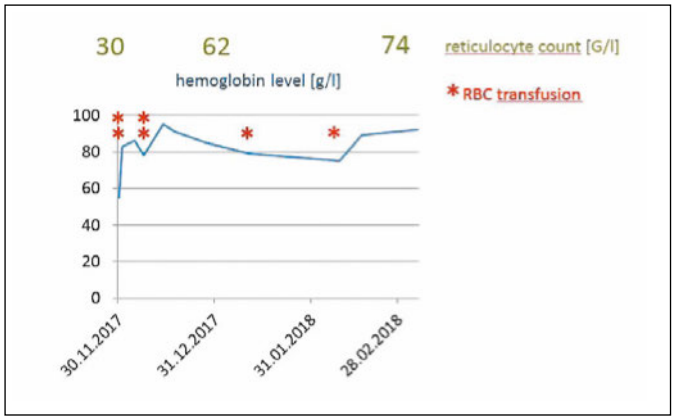
Hyporegenerative anemia in patients with multiple sclerosis (ms) on treatment with the integrin $\alpha4\beta1$-targeting Natalizumab (Nmab) is a recognized side effect [1]. Discontinuation of therapy leads to an



ongoing amelioration of the anemia with marked increase in reticulocyte counts and transfusion independence, as described in a handful of case reports since approval of Nmab treatment [2].
Introduction: We report a case of Natalizumab induced erythroid maturation stop in the bone marrow with the consecutive clinical picture of severe non-regenerative, non-hemolytic anemia after administration of a total of 78 Natalizumab infusions over a period of little more than six years. Cytomorphological findings of the bone marrow tap were unique, showing grouped immature proerythroblasts with no signs of erythroid maturation.
Case description / summary: A 67-year old patient with the aforementioned treatment for ms was assigned to our center in order to investigate on hyporegenerative anemia. In extensive laboratory workup including trephine biopsy, no signs of primary hematopoetic stem-cell disease could be found: immunohematological (AIHA), flowcytometric (PNH), infectious (Parvovirus B19), cytogenetic (marker of clonal hematopoiesis) as well as molecular (Next Generation Sequencing) investigations were blunt. Following low dose corticosteroids the clinical course soon turned out favorable with independence of transfusion and rising reticulocyte counts as depicted in figure 1.
Discussion: This case report highlights the well- established effect in targeted therapy with Natalizumab on integrins expressed on leukocytes as well as erythroid precursor cells: on one hand preventing leukocytes from crossing the blood-brain barrier in ms patients, the same small molecules can cause maturation arrest on erythroid precursors, leading to hyporegenerative reversible anemia in a few documented cases. If in our case the triggering effect is an additional dose-to-effect relationship or a “second hit” event for the anemia to ocurr, must still be investigated.

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2 A.M. Simone et al. Neurology 83, 374–5.

Occurrence and dynamics of HLA-antibodies in the setting of matched related hematopoietic stem cell transplantation

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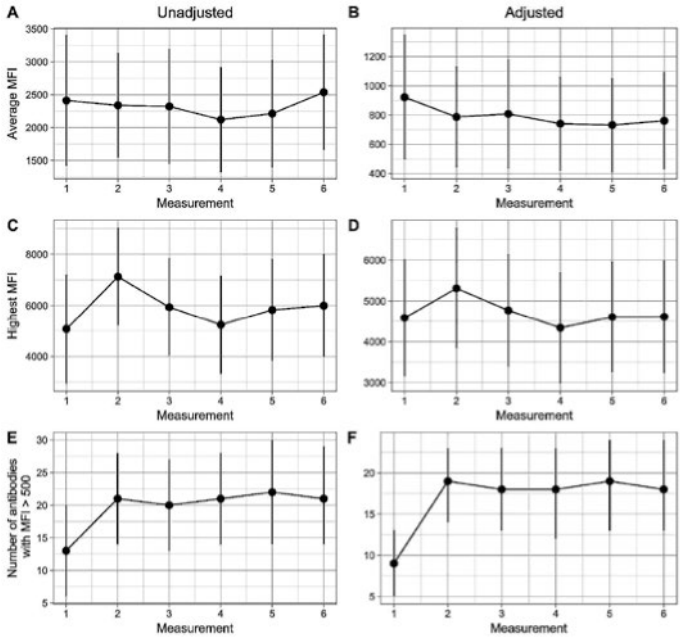
Background: HLA-antibodies are increasingly recognized to play an important role in the setting of HSCT. The aim of the current prospective study was to evaluate occurrence and dynamics of HLA-antibodies after matched related HSCT.
Methods: Patients and their matched related donors were prospectively included in the IRB approved study. HLA-antibodies were determined by Luminex technique at predefined time points. For patients, samples were drawn at baseline, at HSCT and weekly thereafter until 4 weeks after HSCT and for donors at eligibility assessment and at donation. We used generalized estimating equation models of the Gaussian and negative binomial family with log links and robust standard errors to assess temporal trajectories of patients’ average mean fluorescence intensity (MFI), highest MFI, and the number of antibodies with MFI >500.
Results: Among the 50 patients included in the study, 26 (51%) were female and median age at transplantation was 51 years. The majority of patients had AML (37%) and MM (15.7%), received myeloablative conditioning (58.8%) and GvHD prophylaxis consisted mainly of cyclosporine containing regimens. At baseline, HLA-antibodies were detected in 49 patients (98%) (mean number of antibody specifities: 13; range 0–102) and in only 25 donors (50%) (mean number: 6; range 0–51). Overall, both number and MFI of class I antibodies were higher compared to those of class II antibodies. Surprisingly, a considerable increase of the number and intensity of antibodies was observed within a few days, from baseline to the day of transplantation. Thereafter, the number of antibodies as well as MFI-levels remained stable until the end of observation (similar after adjusting for age, gender, diagnosis, see figure). Furthermore, 14 of the 50 patients (28%) developed new HLA-antibodies over the observed time period. Some of the molecular specifities of these antibodies emerging in the patients were the same as to those found in their corresponding donors.
Conclusions: Our data show that HLA antibodies are frequently present in patients undergoing HSCT and that they should be measured at the day of transplantation. Additionally, some patients develop new, including presumably donor-derived antibodies. This might have some impact regarding both transfusion strategies as well as transplant outcome. Since HLA-mismatched HSCT are increasingly performed worldwide, further studies on the significance of HLA-antibodies in these settings are warranted.



Improving survival rates of AML patients following intensive care unit admission

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Introduction: Induction chemotherapy in patients with AML can be associated with life-threatening side effects leading to admission to the intensive care unit (ICU). Patient and disease related factors associated with fatal outcome following ICU admission in AML patients are poorly understood, while the duration of maintaining life-supporting intervention in the ICU can be a matter of intense controversy.
Methods: In this retrospective study, we analyzed all consecutive AML patients between 01/2006 and 12/2016 receiving induction chemotherapy at a single academic center.
Results: During the study period, 76 of 240 (31.7%) AML patients undergoing induction chemotherapy had at least one ICU admission due to critically impaired condition, and 32.9% (25/76) died following ICU admission. Whereas the ICU admission rate remained stable throughout the study period, the mortality rate decreased from 14% (2006–2008) to 3% (2014–2016; p <0.01). We observed no correlation between ICU admission or survival rates and factors including age, blood counts at diagnosis, molecular or cytogenetic abnormalities, and FAB subtypes or ELN risk groups, with the exception of patients with *NPM1* mutations being more likely to survive ICU referrals ($p = 0.0425$). The number of failing organ systems in a given patient negatively correlated with the likelihood to survive ICU admissions ($p < 0.0001$). Septic shock as well as renal, cardiac and pulmonary

failure were each associated with higher probability of death in the ICU. Also, the need of catecholamine support, mechanical ventilation, hemodialysis, and cardio-pulmonary resuscitation were more common in the group of patients dying at the ICU. With increasing ICU duration the survival probability dropped considerably (p <0.0001), but remained above 50% even after 14 days of ICU treatment. Finally, progression free (PFS; p = 0.9415) and overall survival (OS; p = 0.8394) rates remained comparable between ICU surviving patients and patients never needing ICU support.
Conclusion: This study identified factors associated with fatal outcome of ICU admissions in AML patients undergoing induction chemotherapy. Outcome after ICU admission has substantially improved in recent years and surviving the ICU intervention is not affecting AML-related long-term outcome.

BestBits – a pediatric hematology/ oncology journal review and virtual journal club project

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Background: The amount of scientific literature is increasing exponentially. Scanning the appropriate journals for relevant articles and interpreting the impact of new research is challenging. We performed a pilot journal review initiative in Pediatric Hematology and Oncology. Twenty subspecialty fellows and staff participated by screening and appraising relevant articles in 27 journals writing 155 summaries in 5 issues over 1 year. Based on the success of the pilot project, we hypothesized that a website to facilitate literature review, online journal clubs, and education would promote participation, enhance interaction, and create an international resource for relevant literature reviews.
Methods: We created the open access website www.bestbits.ca. Participating reviewers screen assigned journals and select relevant articles to read and appraise using a standardized framework. Reviewers submit manuscript summaries with interpretation online which are then reviewed by at least two editors using pre-defined criteria. Edited journal reviews are posted online in bimonthly issues. We use online participant surveys, website statistics, and editorial review to evaluate participation and gather feedback on learning activities and processes. We analyze number of hours and articles read, proportion of articles read completely and self-efficacy in staying updated of the participants.
Results: We were able to extend the reach of our initiative through creation of the website. So far, 45 participants from seven countries actively participated in the literature review process and contributed to the collection of manuscript summaries. We published 323 review articles, 3 continuous medical education accredited online journal clubs were held, and the number of registered participants increased to 110 from the Americas, Europe, Asia, and Australia.
Conclusion: We significantly extended the reach and increased participation by creating an online platform for literature review. We will continue development of this database and self-assessment educational activities.

An unexpectedly aggressive form of LGL-Leukemia featuring an activating STAT5b mutation

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Large granulocytic leukemia (LGL-L) is a rare clonal lymphoproliferative disorder of T- or NK-cells commonly caused by an activating mutation in the STAT3 gene. The chronic disease features organomegaly, B-symptoms, a plethora of autoimmune phenomena and a massive bone marrow infiltration leading to severe cytopenias. Although incurable, LGL-L usually has a non-aggressive, chronic course with a median survival 5 years after diagnosis of 88% in the United States. Here we present a case of an atypically aggressive and treatment-resistant form of T-LGL-L. The 53 year old male presented with marked cytopenia. repeated episodes of meningeosis leucaemica as well as involvement of the lungs, the spleen, the kidney and the liver. He failed to respond to high dose steroids, oral cyclophosphamide as well two cycles of CHOEP with intrathecal triple therapy and eventually passed away less than 6 months after diagnosis. No classical STAT3 mutation was found, however the patient featured a STAT5b N642H activating mutation. This mutation is commonly found in childhood T-ALL and is associated with a poor prognosis and poor responding to conventional chemotherapy. STAT5b mutated LGL-L may be a separate disease entity, clinically similar to aggressive NK-cell leukemia; however the disease of this patient was unquestionably of T-cell origin with CD3+, CD5+ and CD7+, and a rearrangement of the TCR gamma as well as beta locus. New targeted therapeutic strategies are needed to treat such patients. Since the classical immunosuppressive agents for LGL-L seem to be ineffective in patients suffering from STAT5b mutated LGL-L and other established therapeutic strategies are missing, new therapeutic strategies inferred from preclinical studies as well as from studies of other STAT5b mutated neoplasias of Tcellular origin such as inhibition of JAK and Aurora-Kinases as well as hypomethylating agents may be postulated as possible options for this disease.

CLINICAL SOLID TUMOR ONCOLOGY

Relation of baseline neutrophil-to-lymphocyte ratio to survival and toxicity in head and neck cancer patients treated with (chemo-)radiation

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Purpose: A high neutrophil-to-lymphocyte ratio (NLR) is a marker of systemic inflammation and associated with worse outcomes in several solid tumors. We investigated the prognostic value of NLR in patients with head and neck squamous cell carcinoma (HNSCC) treated with postoperative or definitive (chemo-)radiation (CRT).

Materials and Methods: A retrospective chart review of consecutive patients with locally advanced HNSCC was done. NLR was computed using complete blood counts performed within ten days of treatment start. The prognostic role of NLR was explored with univariable and multivariable Cox regression analyses adjusting for disease-specific

Table 2.
Univariable and multivariable Cox regression analysis of overall survival (60 death events)

		univariable analysis		multivariable analysis	
		HR (95% CI)	P	HR (95% CI)	P
Age	per 10 years older	1.32 (1.03-1.69)	0.026		
Gender	Male	1.17 (0.61-2.25)	0.639		
Smoking status	never smoker (vs. current/past)	0.66 (0.20-2.19)	0.492		
Karnofky Performance Status	per 10 higher	0.76 (0.62-0.92)	0.005	0.74 (0.59-0.94)	0.011
Localization	Larynx or hypopharynx (vs. other)	1.78 (1.06-3.00)	0.030	1.94 (1.10-3.43)	0.023
AJCC stage	IV (vs. I-III)	1.87 (1.01-3.47)	0.045		
Tumor grade	G3 (vs. lower)	0.91 (0.54-1.54)	0.731		
Hemoglobin	per 1g/dL higher	0.89 (0.77-1.04)	0.143		
log NLR	per 1 logNLR higher	1.81 (1.15-2.81)	0.009	1.59 (1.01-2.51)	0.044
log PLR	per 1 logPLR higher	1.62 (0.99-2.63)	0.054		

CI; confidence interval; HR, hazard ratio; logNLR, natural logarithm of neutrophil to lymphocyte ratio; log PLR, natural logarithm of platelet to lymphocyte ratio

Figure 1

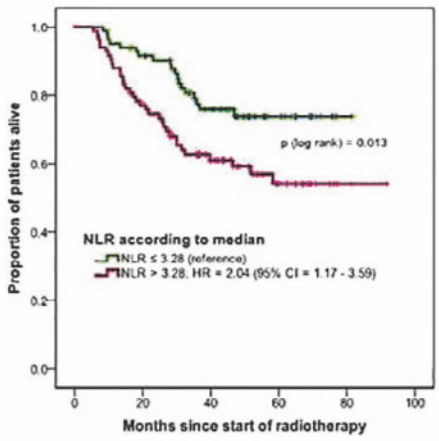
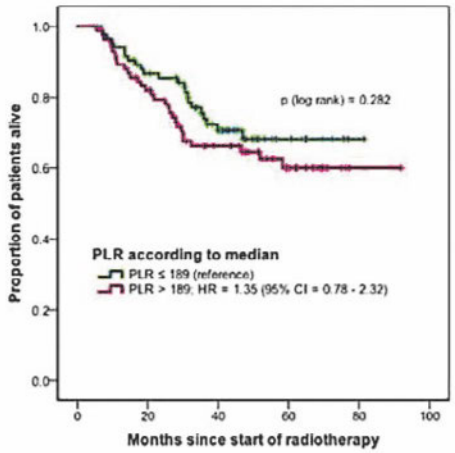


Figure 2



prognostic factors. NLR was assessed as a log-transformed continuous variable (logNLR). Endpoints of interest were overall survival (OS), locoregional recurrence free survival (LRRFS), distant recurrence-free survival (DRFS), and acute toxicity.
Results: We analyzed 186 patients treated from 2007 to 2010. Primary sites were oropharynx (45%), oral cavity (28%), hypopharynx (14%), and larynx (13%). Median follow-up was 49 months for living patients, and 40 months for all patients. NLR was associated with OS (adjusted HR per 1 unit higher logNLR = 1.81 (1.16-2.81), $p = 0.012$), whereas NLR was not associated with LRRFS (HR = 1.49 (0,83–2,68), $p = 0.182$), DRFS (HR = 1.38 (0.65–3.22), $p = 0.4$), or acute toxicity grade 2 or higher.
Conclusion: In HNSCC patients treated with CRT, NLR may be an independent predictor of mortality but not disease-specific outcomes or toxicity. NLR is a readily available biomarker that could improve pre-treatment prognostication and may be used for risk-stratification.

Concurrent hyperthermia and chemoradiotherapy vs. chemoradiotherapy alone in locally advanced pancreatic cancer (HEATPAC)

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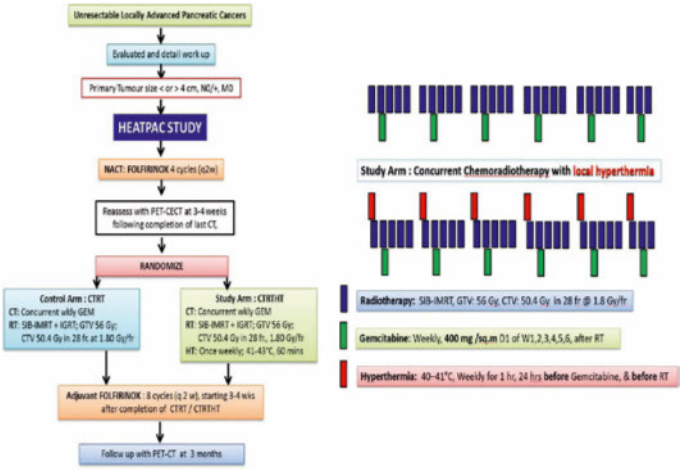
Introduction: Pancreatic cancer has a fatal prognosis with 5-year survival rate of 5%. The outcomes have remained grossly unchanged despite the advances in chemoradiation and advent of newer systemic agents. Therefore novel approaches for the management of the around 80% of the inoperable patients of locally advanced pancreatic cancers (LAPC) are urgently needed. Hyperthermia, a potent radiosensitizer also potentiates the action of gemcitabine, a known radiosensitizer. This phase II randomized trial attempts to explore the feasibility and efficacy of a concurrent thermochemoradiotherapy in comparison to chemoradiotherapy alone following neoadjuvant therapy.
Methods: All patients of LAPC, fulfilling the criteria of unresectable LAPC [1, 2] would be considered to be eligible for enrolment in the study (fig. 1). All eligible patients will receive 4 cycles of neo-adjuvant FOLFIRINOX. At completion of the neo-adjuvant treatment, re-evaluation by PET-CT is performed to rule out metastasis. Thereafter patients will be randomized to the HEATPAC study arms of either a) concurrent chemoradiotherapy with gemcitabine 6w (control arm) or b) locoregional hyperthermia with concurrent chemoradiotherapy with gemcitabine (study arm). Radiotherapy would be delivered using IMRT with 56Gy to gross target volume and 50.4 Gy to clinical target volume. Hyperthermia to 41–43°C would be administered weekly with intraduodenal temperature monitoring with a multi-sensor temperature probe at each session. Following the completion of above therapy, all patients of both groups would receive additional 8 cycles of FOLFIRINOX.
Results/Conclusion: This will be the first study [4] in the field of LAPC with multimodality concept including hyperthermia to chemoradio therapy (CTRT). The expected 1-year baseline overall survival with CTRT alone is considered as 40%. With chemoradiohyperthermia therapy (HTCTRT), a survival advantage of +20% is expected. Considering $\alpha = 0.05$ and $\beta = 0.80$ for sample size computation, a total of 86 patients would be equally randomized into the two treatment groups. This phase II study if found to be safe and effective, would form the basis of a future phase III randomized study [3].



Response to radiotherapy (RT) of brain metastases (BM) in patients with non-small cell lung cancer treated with immunotherapy.

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Background: The phase III trials of nivolumab, pembrolizumab or atezolizumab in comparison to standard chemotherapy for advanced NSCLC included a small number of patients with BM. Preclinical studies and clinical data show a synergistic effect of concurrent stereotactic body radiotherapy (SBRT) and immunotherapy. The aim of this study was to evaluate the radiological features of BM upon treatment with this combination.
Methods: We retrospectively reviewed the clinical data and radiological findings from 27 pts with NSCLC receiving immunotherapy (IT) (nivolumab or pembrolizumab) and SBRT or whole brain radiotherapy (WBRT) of BM from June 2015 until August 2017 at the University Hospital of Zürich. Response to RT was assessed with magnetic resonance (MR) in all patients and additionally 18F-Fluorethyltyrosin PET (FET-PET) in 4 pts.
Results: A total of 33 courses of SBRT or WBRT were applied (22 patients received one treatment, 4 patients 2 treatments and 1 patient 3 treatment courses). Radiotherapy was performed in 36% of the cases (12 out of 33) during IT, in 61% (20 out of 33) before and 3% (1 out of 33) after IT. In cases where RT was performed before, the median time to IT was 6.5 months (range: 0.9–36.3). Response evaluation was performed after each course of treatment: in 6 out of 33 treatments (18.2%) a complete response (CR) was achieved, in 14 (42.4%) a partial response (PR), in 2 (6.1%) a mixed response, in 2 pts (6.1%) a progression of disease (PD) and in 4 (12.1%) a pseudoprogression was observed. FET-PET was performed in these cases helping differentiate between pseudoprogression and true progression.

Conclusions: The possibility of peudoprogression (defined as initial enlargement of tumor lesions and then decrease in size) should be considered when evaluating response after concurrent IT and RT of BM form NSCLC. FET-PET could help differenciate pseudoprogression and true progression. In cases of pseudoprogression IT can be continued without additional interventions on BM.

Co-creating a self-management program for breast cancer survivors clinically integrated in Swiss breast centers (COSS pilot study).

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Introduction: The peer-led Cancer Thriving and Surviving Program (CTS) has shown positive effects on cancer survivor's skills, quality of life and clinical outcomes. Until now, the CTS has not been introduced in Swiss cancer care, nor adapted to the growing population of female breast cancer survivors.
Objectives: To adapt the CTS for Breast Cancer Survivors in Switzerland (CTS-BC-CH) and to determine its integration into the clinical pathway of Swiss breast centers.
Methods: From 2016–2017 three workshops, one online rating and one consensus conference were conducted with a transdisciplinary working group (breast cancer survivors, breast care nurses, oncologists, psycho-oncologists, nurse scientists) to identify adaptations of the CTS to breast cancer. An interactive and iterative approach including 5 steps (presenting evidence, collecting ideas, clarification of collected ideas, rating of collected ideas, synthesis) guided the workshops. The online rating after the 1st workshop revealed that all suggested adaptions are relevant for Swiss breast cancer survivors. Final agreement on the CTS-BC-CH content and its clinical integration was sought in an international consensus conference.
Results: The 6-day CTS was extended to 7 days. Adapted contents to address the needs of breast cancer survivors included "My exercise", "Being a woman and having breast cancer" and "My (working) life". New activities were created: e.g. "Take a stand" to help patients in making decisions. Transition from acute treatment to follow-up care was identified as the time point for the introduction of the CTS-BC-CH into the clinical pathway.
Conclusions: The CTS-BC-CH is now ready for pilot testing at two breast centers to evaluate the feasibility of conduction and its preliminary effectiveness on patient outcomes.

Pathologic complete remission after two years of adjuvant Ipilimumab in limited small cell lung cancer

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Small Cell Lung Cancer is an aggressive cancer with poor prognosis and even in limited disease the 5-year survival is below 20%. To date, immunotherapy with immune-checkpoint inhibitors for SCLC have made the greatest advances and preliminary data for pembrolizumab, nivolumab and combination of ipilimumab with nivolumab showed promising antitumour activity. We here describe the case of a patient with limited disease SCLC (cT4 pN1cM0; T4:10 cm with contact to the mediastinum, visceral pleura and infiltration of the main left bronchus), who was included in the ETOP 4-12-STIMULI trial. The patient underwent standard chemoradiotherapy and prophylactic cranial irradiation achieving a partial response; subsequently the patient was randomized to the investigational arm, with adjuvant ipilimumab. The treatment consisted of four doses of ipilimumab 3 mg/kg every three weeks and thereafter eight doses of ipilimumab 3 mg/kg every six weeks for two years. Follow up was done with FDG-PET/CT scans every three months. The treatment was well tolerated and the patient experienced no side effects. On the FDG-PET/CT scans, the primary tumor showed persistence three months after the last dose of ipilimumab, with a size above 7cm, low FDG-uptake (SUVmax 4.1) and no other tumor manifestation. The case was evaluated at the multidisciplinary thoracic-tumorboard for resection. Given the unclear metabolic behavior of the large mass and in order to reduce the risk of pneumonitis, the patient underwent a left upper-lobe resection. The histopathologic analysis showed a necrotic mass with no vital tumor cells, resulting in a ypT0 ypN0 stage, and an immune infiltration. Based on these data, we assume that under immunotherapy an immune reaction occurred leading to the pathological findings as well as the still ongoing complete remission from disease 31 months after diagnosis. The CheckMate 156 showed no efficacy of Ipilimumab alone when added to chemotherapy of extensive SCLC. Based on the CheckMate 012 and 032, with improved efficacy of the combination nivolumab and ipilimumab compared to monotherapy, the STIMULI trial was amended to the combination of nivolumab and Ipilimumab after chemo-radiation. This is the first report of a complete pathological response after adjuvant ipilimumab for limited disease SCLC. This case shows moreover the potential of a multidisciplinary approach in this setting.

PROSPER: A phase 3 study of enzalutamide (ENZA) in men with non-metastatic castration-resistant prostate cancer (nmCRPC)

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Background: Men with nmCRPC and rapidly rising prostate-specific antigen (PSA) are at high risk of developing metastatic (m) CRPC. ENZA improves overall survival (OS) and radiographic progression-free survival in men with mCRPC. We hypothesized that ENZA will improve metastasis-free survival (MFS) in men with nmCRPC.
Methods: Eligible men with nmCRPC, PSA doubling time ≤10 mo and PSA ≥2 ng/mL at screening continued androgen deprivation therapy (ADT) and were randomized 2:1 to ENZA 160 mg or placebo (PBO). The primary endpoint was MFS. Secondary endpoints included time to PSA progression, time to first use of new antineoplastic therapy, OS and safety.
Results: In 1401 men, ENZA significantly prolonged median MFS (36.6 mo vs 14.7 mo [P <.0001]), time to first use of new antineoplastic therapy (39.6 mo vs 17.7 mo [P <.0001]) and time to PSA progression (37.2 mo vs 3.9 mo [P <.0001]) compared to PBO (Table). In the first interim analysis of OS there was a trend in favor of ENZA (hazard ratio [HR] = 0.80; P = .1519). Median duration of treatment was 18.4 mo vs 11.1 mo for ENZA vs PBO. Adverse events (AEs) were higher with ENZA vs PBO (any grade: 87% vs 77%; grade ≥3: 31% vs 23%; serious: 24% vs 18%); 10% with ENZA discontinued treatment due to AE vs 8% with PBO.
Conclusions: In men with nmCRPC and rapidly rising PSA, ENZA treatment resulted in a clinically meaningful and statistically significant 71% reduction in the risk of developing mCRPC. AEs were consistent with the established safety profile of ENZA. NCT02003924

Funding: Astellas/Pfizer
Editorial support: Complete HealthVizion

Baseline Characteristic	ENZA + ADT (n=933)	PBO + ADT (n=468)
Age, median, y	74	73
PSA doubling time <6 mo, no. (%)	715 (77)	361 (77)
Serum PSA, ng/mL	11.1	10.2
Endpoint		
MFS,[a] median (95% CI), mo	36.6 (33.1-NR)	14.7 (14.2-15.0)
HR (95% CI)	0.29 (0.24-0.35)	
P value	<.0001	
Time to first use of new antineoplastic therapy,[a] median (95% CI), mo	39.6 (37.7-NR)	17.7 (16.2-19.7)
HR (95% CI)	0.21 (0.17-0.26)	
P value	<.0001	
Time to PSA progression,[a] median (95% CI), mo	37.2 (33.1-NR)	3.9 (3.8-4.0)
HR (95% CI)	0.07 (0.05-0.08)	
P value	<.0001	
OS,[b] median (95% CI), mo	NR (NR-NR)	NR (NR-NR)
HR (95% CI)	0.80 (0.58-1.09)	
P value	.1519	

[a]Final analysis; [b]interim analysis

CI, confidence interval; NR, not reached

Downstaging of unresectable intrahepatic cholangiocarcinoma by hepatic arterial infusion with floxuridine and systemic chemotherapy with gemcitabine and cisplatin

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Universitätsspital Zürich

Background: Patients with unresectable cholangiocarcinomas (CCC) have a poor prognosis even if palliative systemic chemotherapy is offered. A combined approach of systemic and intrahepatic chemotherapy may improve local control rates and allow downstaging. The aim of the study was to determine the maximum tolerated dose (MTD) of systemic intravenous gemcitabine in combination with intravenous cisplatin and hepatic arterial infusion with floxuridine in patients with unresectable intrahepatic or hilar CCC. Safety, toxicity, response rates and resectability rates after 3 months of combination treatment are reported.
Methods: 12 patients were treated within a 3+3 dose escalation algorithm with 600, 800 or 1000 mg/m^2 gemcitabine and a fixed dose of cisplatin 25 mg/m^2 systemic chemotherapy on day 1, 8 every 3 weeks for 4 cycles and floxuridine 0.2 mg/kg on day 1–14 continous hepatic intra-arterial chemotherapy every 4 weeks for 3 cycles. PET/CT and/or CT scan was performed after 12 weeks.
Results: The MTD of gemcitabine was 800 mg/m^2 in this setting. Dose-limiting toxicities were recurrent biliary tract infections (n = 1) and neutropenic fever (n = 1). Response rates were: 27% partial remission and 73% stable disease. Although none of the patients achieved resectability after 3 months, 3-year-overall survival (OS) was 33%, median OS 21,9 months (1–49) and median progression-free survival 10.5 months (2–40).
Conclusions: Combination of systemic gemcitabine and cisplatin plus intraarterial floxuridine is feasible and appears effective in disease control, but achievement of resectability seems challenging. Randomized trials comparing this combination to gemcitabine/cisplatin alone are warranted.

Changes in PD-L1 expression for non-small cell lung cancer recurrence and correlation with KRAS alteration

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Background: Non-small cell lung cancer (= NSCLC) has the highest mortality rate worldwide. More than 75% of new lung cancer diagnoses are made in patients presenting with distant or regional metastatic disease. Adenocarcinoma is the most common histological type of NSCLC and often presents oncogenic driver alterations. Epidermal growth factor receptor (EGFR), Kirsten rat sarcoma viral oncogene homolog (KRAS) and anaplastic lymphoma kinase (ALK) are the most common alterations in lung adenocarcinoma. We assessed genetic driver alterations and correlation with PD-L1 expression in tumor samples from first diagnosis and at recurrence.
Methods: Patients with NSCLC as well as in biopsy specimens at NSCLC recurrence by immunohistochemical (= IHC) analysis. IHC score was defined of the proportion of tumor cells (= TC) with stained cell membrane. Migration of IHC group was considered as significant change upon PD-L1 expression. Four IHC score groups were defined: TC0 <1%, TC1 ≥1 <5%, TC2 ≥5 <50% and TC3 ≥50%.
Results: 36 patients were included. All patients had a NSCLC with histological type of adenocarcinoma. Median recurrence time was 539 days. 20 patients underwent adjuvant chemotherapy after surgical resection (56%) and 16 patients had no adjuvant chemotherapy (44%). Out of 20 patients receiving adjuvant chemotherapy 7 patients (35%) showed upregulation in PD-L1 expression. In comparison to the subgroup with no adjuvant therapy, only 2 out of 16 (12.5%) patients showed increase in PD-L1 expression. KRAS-alteration on initial tumor was present in 10 out of 36 patients (28%). Among KRAS mutated patients, 7 out of 10 (70%) showed PD-L1 positive either on initial tumor or at recurrence.
Discussion: In our study, we demonstrated that chemotherapy might increase PD-L1 expression in NSCLC specimens. These findings implicate that, in NSCLC, more patients than expected might benefit from immunotherapy at relapse. Furthermore, we demonstrated that KRAS mutation correlates upon PD-L1 expression in lung adenocarcinoma as previously reported.

Investigation of metformin (MET) in patients with castration resistant prostate cancer (CRPC) in combination with enzalutamide (ENZ) vs. ENZ alone. A randomized, open label, phase 2 trial. SAKK 08/14 – IMPROVE

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Background: The current first-line treatment for patients with CRPC and disease progression is either treatment with abiraterone acetate/prednisone, ENZ, or treatment with docetaxel in more symptomatic patients. There is preclinical data on synergism of ENZ and the biguanide MET: studies on mice orthotopically implanted with ENZresistant cells demonstrated that the combination of ENZ and clomipramine or MET significantly reduced tumor growth compared to control groups. Rothermundt et al. previously reported favorable effects of single agent MET in a phase 2 trial: objective PSA responses, disease stabilization and improvement of metabolic endpoints in patients with CRPC. Therefore addition of MET to ENZ might have positive impact on tumor progression, on body composition and insulin sensitivity.
Methods: This is a prospective 1:1 randomized multicenter phase 2 trial. Primary endpoint is disease control (DC) at 15 months. Progression is defined as having 2 of the following events: radiographic progression, symptomatic/clinical progression, or PSA progression. Secondary endpoints include overall response according to modified RECIST v1.1 and PCWG2 recommendations, event-free survival, adverse events, quality of life, pain and overall survival. Translation research comprises liquid biopsy, metabolomics, hyperglycemia, and pyruvate dehydrogenase substudies. Assuming a 20% difference in the DC rate at 15 months (50% vs. 70% in the combination arm) with alpha 0.10 and power 80%, 168 patients are required in total. Eligibility criteria are as follows: asymptomatic or minimally symptomatic mCPRC (adenocarcinoma) documented by imaging, ongoing androgen deprivation therapy (ADT) with GnRH agonists or antagonists or bilateral orchiectomy, total testosterone levels ≤1.7 nmol/L, tumor progression at the time of registration, no prior treatment for mCRPC other than ADT, no history of diabetes and metformin use, and adequate organ function. Patients receive either ENZ 160mg qd in combination with MET 850 mg bd or ENZ 160 mg qd alone. 79 patients have been enrolled since accrual began in March, 2016.

A phase 2 trial of darolutamide maintenance therapy in patients with metastatic castration resistant prostate cancer (mCRPC) previously treated with AR targeting agents and non-progressive on a subsequent taxane (SAKK 08/16)

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Background: Treatment with the AR targeting agents abiraterone or enzalutamide followed by a taxane is currently the most used treatment for men with mCRPC. Further treatment after response to chemotherapy is only indicated in case of disease progression, with limited treatment options available. Darolutamide is a secondgeneration oral androgen receptor antagonist which has demonstrated a good safety profile and antitumor activity in mCRPC. This trial evaluates whether the immediate use of darolutamide after successful chemotherapy can prolong radiographic progression-free survival (rPFS) compared to watchful waiting in patients with mCRPC.
Trial design: This is a multicenter, randomized, double-blind, placebo-controlled phase 2 trial (NCT02933801) conducted in approximately 19 sites in Switzerland and Italy. Patients with mCRPC are required to have been previously treated with abiraterone and/or enzalutamide and have no evidence of disease progression on subsequent docetaxel or cabazitaxel. Patients (N = 88) will be randomized 1:1 to receive 600 mg darolutamide BID or placebo BIDuntil disease progression. Patients will be stratified by country, WHO performance status (0, 1 vs 2), presence/absence of visceral metastases, enzalutamide vs abiraterone vs both prior to chemotherapy, and planned start of trial treatment after last taxane dose (<35 days vs ≥35 days). The primary endpoint is rPFS at 12 weeks after treatment initiation. The secondary endpoints are rPFS, time to PSA progression, time to symptomatic/clinical progression, event-free survival, overall survival, PSA response (30%, 50%, 90%, and best), duration of PSA response (50%), adverse events, and

fatigue. The rPFS rate at 12 weeks after treatment initiation will be compared between the two treatment arms using a one-sided test statistic using the Kaplan-Meier method. Recruitment is ongoing, with the first patient randomized on 20.04.2017.

Evaluation of FDG-PET-CT scan as a predictor of responses to PD-1 blockade in metastatic non-small cell lung cancer

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Immunotherapy with monoclonal antibodies blocking programmed cell death protein 1 (PD-1) has emerged as a new therapy for metastatic non-small cell lung cancer (NSLC). However, only a fraction of patients respond to this treatment. Therefore, predicting responses before starting therapy and identifying responders after starting treatment represents a major clinical challenge. In this study, we evaluate the role of FDG-PET-CT scan to predict responses to PD-1 blockade. Using a large cohort of metastatic NSCLC patients (n = 67) we study whether pre-treatment (cutoff = 60 days before treatment) FDG-PET-CT parameters (metastatic pattern, SUVmax, SUVmean, MTV, TLG of all the lesions) correlate with responder status and survival. In addition, in patients that received FDG-PET-CT before and after therapy (n = 44) we study whether changes in post-therapy (cutoff = 120 days after treatment) FDG-PET-CT signal (variations in metastatic pattern, SUVmax, SUVmean, MTV, TLG of all the lesions) can identify responders to PD-1 blockade and immune side effects. We also detailed describe cases of mix responses, pseudo- and hyper-progressions.

Management of Primary Tectal Plate Low-Grade Glioma (LGG) in Pediatric Patients: Results of the Multicentre Treatment Study SIOP-LGG 2004

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Background: Tectal plate low-grade glioma (LGG) most often present with increased intracranial pressure and sometimes as incidental findings from brain imaging. Prognostic factors predicting outcome are largely unknown.
Methods: From 2004 until 2012, 71 patients with tectal plate LGG from Germany and Switzerland were followed within the SIOP-LGG 2004 study. Median age at diagnosis was 9.7 (range, 0.1–17.5) years, and median follow-up time of surviving patients was 6.3 (interquartile range, 4.9–8.3) years.
Results: A total of 41 out of 71 patients received no tumour treatment (12 with and 29 without biopsy). The 10 year event-free survival rate (EFS) (± SE) for patients with an initial tumour volume of ≤3 cm^3 was 56% (7%), as opposed to 12% (± 8%) for those with tumours >3 cm^3 (p <0.001). The 10-year EFS for patients without contrast enhancement on initial MRI was 52% (± 9%) and for those with enhancement, it was 23% (± 9%) (p = 0.003). The 10-year overall survival rate was 96% (±3%) (death of disease, 1; ventriculoperitoneal shunt infection, 1). Sixtythree (± 89%) patients had at least 1 CSF diversion procedure.
Conclusion: More than half of patients were managed without tumour treatment. Favourable prognostic factors for EFS were small initial tumour volume (≤3 cm^3) and absence of initial contrast enhancement on MRI. Overall survival was excellent.

Overcoming resistance to immunotherapy in a preclinical model and mesothelioma patients

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Background: Combination of immune checkpoint inhibitors with chemotherapy is under investigation for different kind of cancers. We studied the rationale of such a combination for treating mesothelioma, a disease with very limited treatment options.
Methods: We analyzed the expression of PD-L1 in a large cohort of mesothelioma patients (n = 145) and its change upon chemotherapy in paired samples before and after treatment (n = 80). We evaluated the response to the combination of gemcitabine and immune checkpoint inhibitors compared to both treatments alone in a preclinical model of mesothelioma. Finally, we tried this combination in two patients who had not responded to gemcitabine or anti-PD-1 as monotherapy.
Results: PD-L1 expression was heterogeneous in mesothelioma patients and was associated with a worse survival. The expression of PD-L1 did not change significantly upon chemotherapy. The combination of gemcitabine and immune checkpoint inhibitors outperformed immunotherapy alone with regard to tumor control and survival in the pre-clinical mesothelioma model; however, the addition of dexamethasone to gemcitabine and immune checkpoint inhibitors nullified the synergistic clinical response. Further, treatment with gemcitabine plus anti-PD-1 resulted in an objective clinical response in two mesothelioma patients, who were resistant to gemcitabine or anti-PD-1 as monotherapy. In one of the patients we could detect a strong mobilization of T cells to the pleural cavity.
Conclusion: Treatment of mesothelioma with a combination of gemcitabine with immune checkpoint inhibitors is feasible and results in synergistic clinical response compared to single treatment in the absence of steroids.

Prognostic value of exercise in patients with metastatic colorectal cancer undergoing firstline chemotherapy (SAKK 41/14)

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Background: The current pandemic of physical inactivity boosted exercise research worldwide. A link between inactivity and cancer incidence/relapse has been established, particularly for colon cancer, the third most common cancer. However, whether exercise has an impact on disease course and survival in advanced disease is unknown. Exercise modifies key host factors that are determinants of chemotherapy efficacy such as metabolic and immunologic tumor microenvironment, drug tolerability and treatment adherence. Thus, we aim to assess whether a supervised exercise program concomitant to first-line palliative chemotherapy for patients with metastatic colorectal cancer (mCRC) enhances chemotherapy efficacy and, therefore, increases survival and decreases symptom burden as compared to patients treated with chemotherapy alone.
Methods: Patients with newly diagnosed mCRC are stratified (pre-diagnosis physical fitness, RAS-mutational status, primary tumor location, alkaline phosphatase levels) and 1:1 randomly assigned to undergo standard systemic treatment and care-as-usual or standard systemic treatment combined with a 12-week structured physical activity (PA) program with twice weekly supervised, heart-rate guided interval training on a bike ergometer. Both groups undergo regular imaging with CT/MRI in order to assess the 1°endpoint of progressionfree survival (PFS). A total of 524 patients (439 events) are needed to show a clinically meaningful HR of 0.75 for PFS ($\beta = 0.2$; $\alpha = 0.03$). Co-primary endpoint ($\alpha = 0.02$) is self-reported symptom burden as measured by the revised Edmonton Symptom Assessment Scale (rESAS). 54 patients from 17 Swiss and Austrian Centers have been randomized.

Phase 1/2a study of BAL101553, a novel tumor checkpoint controller, administered as 48-h infusion in patients with advanced solid tumors

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Background: BAL101553 is the prodrug of BAL27862, a smallmolecule TCC that binds microtubules and promotes tumor cell death by activation of the spindle assembly checkpoint. In a study of BAL101553 administered as a 2-h infusion on Days 1, 8 and 15 of a 28-day cycle, vascular toxicities observed appeared to be Cmaxrelated. Nonclinical models indicated that the antiproliferative effects of BAL27862 are AUC driven; this trial was intended to determine the recommended phase 2 dose (RP2D) and to assess whether prolonged infusion reduces Cmax-related toxicity while achieving higher AUCs.
Methods: Patients received 48-h infusions of BAL101553 using an elastomeric pump on Days 1, 8 and 15 of consecutive 28-day cycles using a 3+3 dose-escalation design to determine the maximum-tolerated dose (MTD). During Cycle 2, patients received BAL101553 orally QD (Days 15–21) instead of IV to assess oral bioavailability. Adverse events (AEs) were assessed by CTCAEv4.03 grade (G); tumor response by RECIST 1.1 every two cycles; pharmacokinetics were assessed during the first two cycles.
Results: Phase 1 enrollment was completed with 20 patients (7M/13F; median age 60 years) receiving IV BAL101553 at doses of 30, 45, 70 or 90 mg/m^2. Dose-limiting toxicities included transient G3 hypotension at 70 mg/m^2, and reversible G3 hyponatremia, G3 neutropenia, G2 hallucinations and ataxia, at 90 mg/m^2, with no relevant vascular toxicities. Of 16 evaluable patients, one (ovarian cancer) had a confirmed partial response, and one (endometrial cancer) had stable disease for 6 months. BAL27862 exposures (AUC) were near doseproportional. At 70 mg/m^2, the BAL27862 Cycle 1/Day 1 Cmax was 144 ng/mL, and AUC was 8580 ng.h/mL. Oral bioavailability was estimated to be >80%.
Conclusions: The MTD/RP2D in Phase 1 was 70 mg/m^2. At this dose the Cmax was ~2× lower, and AUC ~2× higher, than the 2-h infusion regimen at the RP2D of 30 mg/m^2. There were indications of potential clinical benefits in patients with ovarian and endometrial cancers.

Multimodal Treatment in Operable Stage III Non-Small Cell Lung Cancer using the New TNM Staging Classification Version 8: Long Term Results of a Pooled Analysis of three SAKK trials

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Background: The impact of the 8th edition of the TNM staging system on the optimal treatment choice and the best treatment strategy for stage III non-small cell lung cancer (NSCLC) is unclear. We applied the 8th version of the TNM classification to a pooled analysis of stage III NSCLC trials in order to test its validity and assess long term outcomes and prognostic factors.
Methods: Individual patient data of 368 patients from three very similarly designed trials (SAKK 16/96, SAKK 16/00 and SAKK 16/01) were pooled. Patients with operable stage III NSCLC received preoperative radiotherapy following three cycles of induction cisplatin/docetaxel (tri-modal) or neoadjuvant cisplatin/docetaxel alone (bimodal). Factors associated with improved 5-year overall survival (OS) were evaluated using a logistic regression model.
Results: When applying the 8th TNM staging version*, 162 patients moved from stage IIIA to IIIB* and 5-and 10year OS rates were 41% and 29% for stage IIIA and 35% and 27% for stage IIIB*. When using the 6th version 5- and 10-year OS rates were 38% and 28% for stage IIIA and 36% and 24% for stage IIIB. Factors associated with improved 5-year OS were age, R0 resection and pCR ($p = 0.043$, $p < 0.001$ and $p = 0.009$). There was no difference in the bi- vs. tri-modal group with regards to OS (median: 28 months [95% CI: 21–39 months] vs. 37 months [95% CI: 24–51 months], $p = 0.9$), event-free survival (median: 12 months [95% CI: 9–15 months] vs. 13 months [95% CI: 10–22 months], $p = 0.71$), local recurrence rate (48% vs 44%, $p = 0.61$), and pathologic complete remissions (pCR) rate (15% vs. 16% $p = 0.75$). R0 resection rates were lower in the bi-modal group (69% vs. 87%, $p < 0.001$).
Conclusions: Similarly favourable long term outcomes were observed when the 8th vs. 6th TNM classification was applied. With the exception of the excluded patients with T4* due to multiple lesions in different lobes, multimodality treatment decisions in operable stage III NSCLC can be based on the 8th TNM version in upcoming trials. Tri-modal therapy resulted in higher R0 resection rates but did not improve OS. Younger age, R0 resection and pCR were associated with improved 5-year survival.

EXPERIMENTAL HEMATOLOGY / ONCOLOGY

CALR mutations in myeloproliferative neoplasms induce unfolded protein response and resistance to ER stress

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Introduction: Myeloproliferative neoplasms (MPN) are characterized by a high prevalence of disease-driving mutations in *JAK2*and Calreticulin (*CALR*). Among these, CALR is an endoplasmic reticulum (ER)-resident chaperone involved in folding of glycoproteins (GPs). Mutations result in formation of a novel CALR protein C-terminus and introduction of *CALR* mutations in mouse bone marrow leads to MPN development. *CALR* mutations can result in deficiency of the GP myeloperoxidase (MPO) and activate JAK/STAT signaling. To further explore the loss and gain of function effects of CALR mutations using an unbiased approach, *CALR* mutant and knockout cell lines were compared by proteome analyses.
Methods: CMK and K562 cells were engineered by CRISPR/Cas9 to generate *CALR* mutant and knockout cell lines. The global proteome was analyzed by DIA and TMT workflows. Proteins up- or downregulated in the above conditions were identified by Spectronaut and MSstats. Enriched biological processes were determined by GO analysis. Cell lines were exposed to tunicamycin to increase ER stress. Unfolded protein response (UPR) was determined by Western Blot and qPCR of genes previously described to be involved in UPR.
Results: Proteomes of mutant and knockout cell lines showed significantly altered proteostasis compared to their respective parental

wildtypes. Particularly, *CALR* mutant and knockout showed high similarity of proteome changes in comparison to wildtype suggesting a loss of function effect of *CALR* mutations. The most significantly upregulated biological process was response to ER stress, showing upregulation of many key proteins including BiP/HSPA5. Functional experiments validated upregulation of UPR proteins and higher resistance of cell lines to increases in ER stress.
Conclusions: By an unbiased proteomics approach, we show that CALR mutations cause ER stress, presumably due to loss of CALR chaperone function.

Targeting therapeutic resistance to the novel type II JAK2 inhibition in myeloproliferative neoplasms

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Background: Myeloproliferative neoplasm (MPN) are characterized by constitutively activated JAK2 signaling. Type I JAK2 inhibitors as ruxolitinib show limited clinical benefits and evoke resistance. Thus, we have characterized CHZ868, a novel mode of type II JAK2 inhibition. It blocks inactive JAK2, abrogates type I inhibitor resistance and reduces the mutant clone. Further studies of CHZ868, particularly its potential to evoke resistance, are warranted.
Methods: JAK2V617F mutant SET2 cells were cultured in presence of CHZ868. IC_{50} was determined by proliferation assay and JAK-STAT and MAPK signaling assessed by phospho-specific Western. JAK2 was sequenced for resistance mutations. To assess therapeutic approaches to overcome resistance, apoptosis induction was analyzed by flow cytometry. Resensitization was evaluated upon inhibitor withdrawal.
Results: We observed that chronic exposure to CHZ868 induced resistance in SET2 cells reflected by ~100-fold increased IC_{50}. CHZ868 resistant cells showed maintained activation of the MAPK pathway with increased pMEK1/2 and pERK1/2 levels, while pSTAT3/5 remained responsive to CHZ868. Of note, no resistance mutations were detected and resistant cells resensitized gradually upon drug withdrawal. Combined treatment with CHZ868 and trametinib, a clinical MEK inhibitor, abrogated CHZ868 resistance by blocking proliferation and inducing apoptosis. Ruxolitinib remained active in type II JAK inhibitor resistant cells suggesting distinct mechanisms of resistance.
Conclusions: Our data show that type II JAK2 inhibition by CHZ868 induces resistance in MPN cells based on maintained MEK-ERK signaling in the absence of second-site mutations. Type II JAK inhibitor resistance is reversible and can be overcome by combined JAK/MEK inhibition.

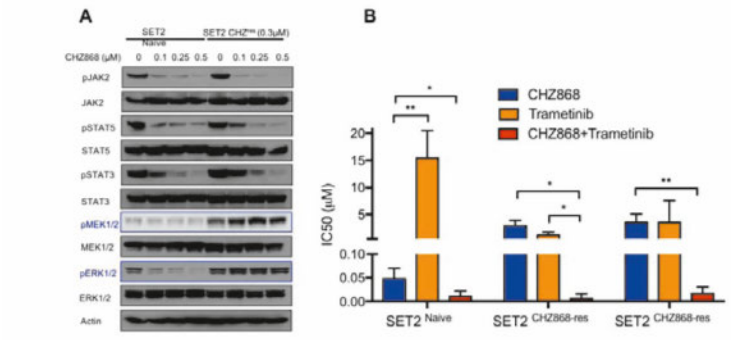

Fig. 1. Acquired resistance to type II JAK2 inhibition is characterized by MEK-ERK activation and abrogated by combined JAK2 and MEK inhibition. A. Type II inhibition by CHZ868 inhibits JAK-STAT and MAPK pathway activation in naive SET2 cells, while in CHZ868-resistant SET2 cells, MEK-ERK activation is maintained. **B.** Type II JAK2 inhibitor-resistant SET2 cells show significantly increased IC_{50} to CHZ868 but remain exquisitely sensitive to combined JAK2 and MEK inhibition by CHZ868 and trametinib.

Characterization of bone marrow graft-versus-host disease post-allogeneic hematopoietic cell transplantation in mice

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Background: Graft-vs-Host Disease (GVHD) is a major clinical problem after allogeneic hematopoietic cell transplantation (HCT). In contrast to classical target organs, alloreactivity directed against the hematopoietic compartment (HC) and non-hematopoietic compartment (NHC) of the bone marrow (BM) has been poorly characterized, even though hematopoietic dysfunction following HCT is frequently observed.
Methods: Here, we studied in MHC-matched, minor-antigen-mismatched mouse HCT models the effects of lethal total body irradiation and HCT of purified HSC alone or in combination with T cells (Tc)/Tc subsets on the HC and NHC of the BM using flow cytometry and 3D-imaging.
Results: At 2 weeks (w) post-HCT recipients given pure HSC had significantly higher BM cellularity with prompt recovery of B cells and granulocytes compared with HSC+Tc recipients. Likewise, alloreactive Tc severely disrupted the NHC: HSC recipients showed prompt recovery of BM endothelial (EC; $CD45^-Ter119^-CD31^+$), and CXCL12-abundant reticular (CAR; $CD45^-Ter119^-CD31^-CD140B^+$) cells. In contrast, HSC+Tc recipients showed delayed recovery with lower EC and CAR cell counts. 3D-imaging revealed rapid recovery of extracellular matrix and sinusoidal vascular structures, with simultaneous disappearance of adipocytes at 2w post allo-HCT in the HSC group, whereas HSC+Tc recipients displayed severe disruption of structural integrity with impaired recovery of BM microvessels and occupation of space by adipocytes. To address the question whether alloreactive Tc directly attack NHC structures, or whether impairment of the NHC results from the inflammatory milieu we performed primary transplants to generate chimeras. In a 2nd HCT we could show that when donor Tc and HC of the recipient were identical, but the NHC was allogeneic, there was still major damage to the NHC. Damage to the NHC was less pronounced when graft Tc were congenic to the NHC, but alloreactive towards the HC.
Conclusion: Delayed recovery of blood regeneration and immune function represent major problems in clinical patient care and contribute significantly to morbidity and mortality. Our experiments help to further characterize "marrow GVHD" and show that alloreactive donor T cells can severely suppress hematopoiesis post-HCT and damage the microarchitecture of the marrow.

Role of SOX9 in neuroendocrine tumours

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The neuroendocrine system is diffused along the body and is responsible for the secretion of multiple proteins important for the regulation of different physiological activities. Neuroendocrine tumours are a very heterogeneous group of tumours that can arise in the lungs, and adrenal glands, among others. Lung tumours are in fact the leading cause of cancer-related deaths, accounting for 18% of them. Peculiarly, 20% of lung cancers exhibit neuroendocrine differentiation. Sry-box transcription factor 9 (SOX9) is a transcription factor important for the organogenesis and development of tissues. In the lungs, the interplay between SOX9 and SOX2 revealed the importance of this mechanism during the branching. Furthermore, SOX9 is also known to promote proliferation and a metastatic phenotype, while inducing endocrine resistance to ER-targeting therapies in breast cancer. Therefore, we aim to understand the role of SOX9 in normal neuroendocrine function and in the development of neuroendocrine lung tumours. We established colon and lung organoids and analysed the expression of either SOX9 alone or SOX9 and SOX2, through immunohistochemistry. We observed that SOX9 is expressed in the bottom of the organoid crypts, similar to what is observed in adult colon crypts. In the lung organoids, SOX9 and SOX2 expression pattern resembles the branching pattern observed during embryonic development, with SOX9 expression being located in the most distal structures whilst SOX2 expression is found in the most proximal structures. We then analysed the expression of SOX9 through immunohistochemistry. In this case, 60% of the 311 patients' samples of neuroendocrine tumours (typical and atypical carcinoid, SCLC, large cell neuroendocrine tumour and mixed types) expressed SOX9. However, survival and relapse did not correlate with the expression of SOX9. When six SCLC cell lines were evaluated for SOX9 and SOX2 expression, different patterns of expression were also found. This observations indicate an important role for SOX9 in both normal and cancer physiology that need to be further clarified.

Intrinsic and extrinsic determinants of Hematopoietic Stem Cells aging

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Lifelong blood production is sustained through a stepwise differentiation program by self-renewing Hematopoietic Stem Cells (HSCs) in bone marrow (BM). Upon aging, HSC numbers increase while self-renewal capacity and bone marrow (BM)–homing ability decreases and differentiation is skewed towards myelopoiesis. We

here tested how extrinsic and intrinsic factors determine HSC behaviour during aging. CFSE-labeled young (8–12 week old) and aged (>2 year old) hematopoietic stem and progenitor cells (HSPCs) were transferred into non-irradiated young or aged recipients. To test biological function of HSCs, quiescent or cycling cells were isolated and transplanted into lethally irradiated mice. These were bled monthly to follow long-term donor engraftment and lineage repopulation. To dissect aging-associated extrinsic factors, we performed antibody based protein arrays and transcriptome analysis with total BM of young versus aged animals. The effects of identified candidates on HSC behavior were further tested in vivo. BM analysis at 8 weeks after tracking showed that young HSPCs proliferated faster than old HSPCs, independently of their environment. In addition, both young and old HSPCs were relatively more dormant in an old versus a young environment. This indicates increased intrinsic and extrinsic drive towards quiescence during ageing. Further, upon lethal irradiation and transplantation of quiescent aged HSCs in young or old recipients, these favoured myelopoietic differentiation, irrespective of the environment exposed to. In contrast, aged HSCs that were cycling in a young environment, showed subsequent balanced lineage repopulation, similar to young HSCs that were cycling within a young or an aged environment. Extrinsic factor screening demonstrated that RANTES, MIP-2, IL-1α and IL-1β are upregulated in aged BM. Furthermore, both, IL-1α and IL-1β drive young HSC towards proliferation, while this effect is mitigated in aged HSCs. Moreover, analysis of aged IL1RI KO mice revealed a reduced aging-associated HSCs phenotype. Our data demonstrate that proliferative history imprints a cell-intrinsic dormancy program on HSCs, which is associated with myeloid-biased differentiation and, at least in natural ageing, with increased IL-1 signaling. Interestingly, this HSC program can in part be "rejuvenated" upon cycling, but not upon dormancy, in young steady-state environments.

The role of SOX10 in melanoma resistance

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When detected early, melanoma can be cured by surgery; however, the prognosis for patients having metastatic melanoma is not promising. In the recent years treatment for patients was revolutionized by usage of targeted kinase inhibitors as vemurafenib, dabrafenib or trametinib. These small molecule inhibitors target specifically kinases from the RAS/RAF/MEK/ERK (MAPK) pathway. Most patients, however, develop resistance against these therapies and succumb to the disease eventually. Sox10 (sex-determining factor 10) is a transcription factor playing a crucial role in the self-renewal of neural crest stem cells as well as in the maintenance of giant congenital nevi and melanoma. To investigate the role of SOX10 in resistance acquisition we cultured several sensitive patient-derived cell lines in presence of the drug (either vemurafenib or binimetinib). Our *in vitro* data reveal that sensitive melanoma cell lines upregulate the expression of SOX10 protein upon treatment with vemurafenib or binimetinib in order to cope with the drug. We are investigating the role of Sox10 in resistance acquisition also *in vivo*. We injected different patient-derived cell lines into immunocompromised mice. There was no correlation found between tumor growth in the immunocompromised mice and *in vitro* SOX10 expression levels of the patient-derived cell lines or their resistance towards BRAF or MEK inhibitors. However, there was a striking difference in MART-1 and TYR expression pattern between the patient-derived sensitive and resistant xenografts. Moreover, we make use of the Tyr::CreER^{T2} Braf^{V600E} Pten$^{fl/fl}$ mouse model combined with either floxed or wildtype Sox10. After induced melanoma formation, the mice are treated with food containing BRAF and MEK inhibitors. Mice receiving control food had to be sacrificed shortly after tumor onset, whereas the tumor regressed completely in mice provided with treatment containing food already few days after treatment start. After several months the mice receiving inhibitor food showed signs of resistance acquisition. Moreover, we found lymph node metastasis in all of these mice. Taken together, our preliminary data show that SOX10 plays an important role in resistance acquisition towards targeted inhibitors *in vitro*. Furthermore, we developed a mouse model to study the process of melanoma resistance acquisition in presence of an intact immune system. However, further investigation is needed to dissect the role of Sox10 in this process.

Acute leukemia cell interactions with their microenvironment: benefits of "protumoral" macrophage reprogramming

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Introduction: Malignant cell interactions with the bone marrow microenvironment may contribute to relapse and drug resistance in acute leukemia (AL). Tumor-associated macrophages can be polarized by myeloblasts from an anti-tumoral (M1) to a pro-tumoral (M2) phenotype. The role of macrophage (MΦ) polarization and reprogramming in AL is not determined. Studies have shown that inhibition of the colony-stimulating factor-1 receptor (CSF1R) signaling can polarize the M2-like MΦs towards the M1 phenotype. Interestingly, a similar effect can be achieved with inhibition of the macrophage migration inhibitory factor (MIF). We hypothesize that targeting malignant cell interactions with M2 MΦs may promote blast apoptosis**.**
Methods: Blasts from BM samples are obtained at diagnosis from patients with AML and cultured in the absence or presence of CSF1R inhibitor or MIF inhibitor for M1-like repolarization with their own BM stroma or on peripheral blood monocytes obtained from healthy donors (HD). Cocultures are analyzed by flow cytometry.
Results: Primary BM cocultures from AML patients exhibited a polarization of macrophages toward the M2-like pro-tumor phenotype and an enhanced growth and survival of primary AML blasts (in 11/13 patients). CSF1R or MIF inhibition dramatically decreased the proportion of M2-like MΦs, and concomitantly the percentage of live blasts. U937 monoblasts cultured in direct contact with M2-polarized healthy donor-derived MΦs are more resistant to the MIF inhibitor (12% cell death) compared to those in contact with M1-like HD-derived MΦs (47% cell death). Blast cell death due to MIF inhibition involves a caspase-dependent mechanism.
Conclusions: Reprogramming the protumoral, M2-like macrophages towards the anti-tumoral M1 phenotype by targeting the CSF1 Receptor and MIF reduces survival and proliferation of malignant blasts from patients with acute myeloid leukemia in bone marrow cocultures. Targeting M2-polarized macrophages may represent a novel approach to sensitize myeloblasts to chemotherapy or targeted therapy.

A network solution for a sustainable national biobank for pediatric cancer to meet high-end quality standards

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The discovery of new biomarkers for early diagnosis and monitoring of diseases is a critical element in the effort towards personalized and precision medicine. The constitution of a large pediatric biobank meets several challenges that the Swiss Pediatric Hematology and Oncology (SPHO) Biobank Network has addressed by endorsing a strategy of collaborations.
For hematological malignancies: biobanking has been integrated in the workflow of the corresponding national reference diagnostic center.
For solid tumors: the concept relies on connecting tissue banks directly at each Swiss Pediatric Oncology Group (SPOG) center, working with local Pathology Institutes and Oncology Laboratories. We harmonized the informed general consent process to serve the needs of both the national and international community and linked the biobank to clinical information collected by the Swiss Childhood Cancer Registry (SCCR). This approach reduces redundancy in the workflows and integrates sample-processing steps into existing clinical infrastructure thereby minimizing the costs for sample storage, quality and data management. By centralizing the legal framework and data management as well as specific components of biobanking, such as storage of normal blood or frozen tissue fragments for patient-derived xenograft generation, the SPHO Biobank Network minimizes the burden for the participating institutions. This project will serve as a model for the Swiss Biobanking Platform that aims to create a large network of biobanks in Switzerland. The next step will be to coordinate the Biobanks SPHO with the SCCR and further datasets. In conclusion, we propose an alternative model to central biobanking for pediatric oncology based on central management, shared governance and supervision and pragmatic selection of institutions for sample processing and storage that will improve the quality and sustainability of biobanking for productive research.

Therapeutic targeting of Clear Cell Sarcoma (CCS)

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Introduction: CCS is an aggressive soft tissue sarcoma with little systemic therapeutic options. CSS is closely related to melanoma, both of which are neural crest derived malignancies. SOX10 is a therapeutic target in melanoma and we have recently shown that it is suppressed by WNT activation. The first aim of this project was therefore to test whether SOX10 is a therapeutic target in CCS. Beyond, we set out to perform an unbiased high-throughput drug screen to identify novel therapeutic options for CCS.
Methods: We assessed SOX10 expression in both CCS cell lines and primary patient biopsies from the European Organization for Research and Treatment of Cancer (EORTC) 90101 "CREATE" trial (1). To study the functional importance of SOX10, we performed shRNA-mediated knock-down (kd) experiments. To assess the effect of WNT signaling activation, we treated CCS cell lines with CHIR-99021, an inhibitor of the negative WNT regulator GSK3a/b. The drug screen was performed using the ActiTarg-K library (960 kinase modulators) on two CCS and one control cell line.
Results and Conclusions: SOX10 is strongly expressed in CCS cell lines and patient samples (25/29; 86.2%). CCS cell lines did show reduced survival upon SOX10-kd, however, as opposed to melanoma, WNT activation had no effect on growth (2). We are currently investigating the mechanism of action. Second, we have successfully performed the drug screen and performed bioinformatic analyses to identify compounds, which efficiently kill both CCS, but not the normal control cell line. The top hits have been confirmed in an expanded cell line panel. We will now aim at identifying the involved signaling pathways and biomarkers of response to the novel drug candidates.

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PPM1D mutations are common in clonal hematopoiesis of indeterminate potential but not in de novo and therapy-related acute myeloid leukemia

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Clonal hematopoiesis of indeterminate significance (CHIP) is an age-related condition characterized by somatic mutations in the blood of otherwise healthy adults and less commonly in children. It has been associated with increased risk of developing hematological malignancies by showing its occurrence in patients with therapy-related myeloid neoplasms (tMN) at the time of their primary cancer diagnosis and before exposure to treatment. Somatic mutations in the p53-inducible protein phosphatase gene *PPM1D* have been described as one of the most recurrent mutations in CHIP and a frequent phenomenon in therapy-related myelodysplastic syndrome (t-MDS). In this study, we analyzed if *PPM1D* mutations commonly seen in CHIP and t-MDS do actually occur in therapy-related acute myeloid leukemia (t-AML) and drive the disease as suggested by the concept to use CHIP a predictive marker, along with the role of those mutations in *de novo* AML. We applied a focused mutational screening of DNA from tumor cells at diagnosis of *de novo* or tAML for *PPM1*Dexon 6. Our study cohort comprised 87 patients with *de novo* AML and 49 patients with tAML. Overall, one patient with *de novo* AML proved mutation positive. Among 49 patients with tAML, mutations in *PPM1D* were not detected at all. Thus, we found that *PPM1D* mutations commonly occurring in CHIP, itself being powerfully associated with the risk of subsequent tMN, are not common in AML whether *de novo* or after prior therapy. Possibly, mutations detectable at the time of primary solid malignancy are not always the ones driving eventual tMN and cells harboring those mutations may not always be part of the dominant clone.

The Metastatic Role of SOX9 in Neuroblastoma

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Introduction: Approximately 50% of neuroblastoma patients suffer from metastatic disease at diagnosis and require intensive treatment. Investigating the molecular basis of neuroblastoma metastasis is crucial to develop an effective therapy. The similarities between neural crest development and neuroblastoma progression have been recognized. Transcription factor SOX9 is involved in cell migration during neural crest delamination, and is implicated in formation and growth of various tumors. Currently, little is known about the role of SOX9 in neuroblastoma pathogenesis.
Methods: Differentially SOX9 expressed neuroblastoma cell lines were chosen to generate overexpression or knockdown clones individually. Functional assays were performed to determine metastatic and tumorigenesis abilities *in vitro*. Orthotopical implantation of cells in mouse adrenal gland were used to evaluate tumorigenicity and metastasis ability *in vivo*. RNA sequencing was used to profile gene expression and analyze SOX9 target genes.
Results: Overexpression of SOX9 significantly enhanced cell migration, invasion, and colony formation. On the other side, knockdown of SOX9 reduced these abilities. In orthotopic model, tumor growth remarkably increased in SOX9 overexpressing compared to control, while it significantly decreased in SOX9 knockdown compared to SK-N-AS control. Interestingly, lung metastasis was detected in 3 of 6 SK-N-AS control mice, and none of 11 SOX9 knockdown mice was found metastasis. By comparing gene expression profile of IMR-5 versus SK-N-AS and SK-N-AS control versus SOX9 knockdown, 1394 genes were identified as SOX9-activated genes and 739 genes were identified as SOX9-repressed genes. Analysis of these genes revealed that SOX9-activated genes were highly related to extracellular matrix organization, cell adhesion, and cell migration. On the other side, SOX9-repressed genes were mainly involved in neuron development and differentiation.
Conclusion: Taken together, these results indicate that SOX9 enhances cell motility and invasive ability of neuroblastoma cells *in vitro*, and promotes tumorigenesis and metastasis in *in vivo* model. Our data point out that SOX9 is a new promising therapeutic target in neuroblastoma.

A novel zebrafish model of congenital neutropenia and Shwachman-Diamond-like phenotype

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Sequencing analyses are increasingly performed on patients presenting with suspected inherited disease but lacking classical mutations linked to the presented phenotype. However, in case a novel mutation is found, its causal contribution to the patients' clinical symptoms is yet unclear and requires further exploration in functional studies. Here we use the zebrafish model to analyze the functional relevance of *SRP54* gene mutations newly identified in patients with unexplained neutropenia including Shwachman-Diamond like disease further involving exocrine pancreas insufficiency. The function of wildtype SRP54 protein was explored in hematopoiesis and pancreas development using two different antisense morpholino oligonucleotides (MO) and a zebrafish srp54 mutant. Reduced neutrophil numbers were observed in MO versus control injected fish when analyzed by WISH for *mpx* or using *Tg*(*lyz:DsRed*) and *Tg*(*mpx:eGFP*) lines. Interestingly, morphants displayed not only quantitatively but also qualitatively impaired neutrophils, which migrated less to injury sites in tail fin injury assays. Supporting these data, in the corresponding Sanger mutant, homozygous fish display severe neutropenia and are not viable, while heterozygous fish survive, but show significantly suppressed neutrophils in comparison to wildtype siblings. Exocrine (trypsin, ptf1a) but not endocrine (insulin a) pancreas impairment was observed in MO versus control injected fish. In srp54 mutant fish, pancreas development showed normal results in heterozygotes and was not assessable in homozygotes which die early (day 2 pf) due to gross developmental defects. Injection with human wt but not mutated mRNA rescued neutropenia and pancreas development in morphants and/or heterozygotes and improved viability of homozygous mutant fish. Together, these *in vivo* analyses support the notion that the newly identified mutations are responsible for the observed phenotype and point out the srp54 zebrafish mutant as opportunity for drug screens. Further genotype-phenotype correlations are underway and will be shown at the meeting.

Generation of mouse CAR T-cells for the depletion of cKIT-positive hematopoietic cells

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Introduction: Allogeneic stem cell transplantation (ASCT) is an effective treatment for hematologic malignancies such as acute myeloid leukemia (AML). However, the required conditioning regiments are highly cytotoxic and contribute substantially to transplant-related morbidity and mortality. As an alternative, the immunological elimination of hematopoiesis might prove to be safer and more efficient. To this end, we strive to develop chimeric antigen receptor T-cells (CARs) with specificity for the stem cell marker cKIT (CD117). Availability of an immunocompetent mouse model is essential to assess safety and applicability prior to potential clinical implementation.
Methods: CARs were produced by retroviral transduction of activated mouse splenocytes and subsequent expansion in vitro. The retroviral plasmid contained a second generation CAR with murine CD3 and 4-1BB domains. The extracellular antigen binding domain (scFv) was generated by phage display and demonstrates high affinity towards mouse cKIT. Functionality of CARs was assessed by cytotoxicity assays using cell lines as well as primary bone marrow. For in vivo studies, 1x107 T-cells where adoptively transferred to syngeneic mice without pre-conditioning.
Results: After stimulation, transduction and expansion, purity of T-cells was >90% and transduction rates were consistently >50%. In co-culture assays, specific elimination of a cKIT-positive cell line was observed in presence of CARs but not in presence of untransduced T-cells. In contrast, cKIT-positive primary bone marrow cells were not eliminated by CARs. Similarly, mice that received CARs did not develop symptoms of bone marrow insufficiency despite persistence of the transferred cells. Flow cytometry revealed limited surface expression of the CAR receptor due to intracellular retention.
Conclusion: The production of mouse CAR T-cells is feasible and elimination of target cells can be achieved. However, the activity of the presented CARs is currently limited by low surface expression of the CAR receptor. The improvement of CAR design is the goal of future studies to provide a valuable model for the hypothesized immunological depletion of hematopoietic stem cells by targeting of cKIT.

WNT-dependent regulation of SOX10 expression in melanoma development

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Melanoma is the most aggressive type of skin cancer, characterized by highly invasive and metastatic features. The high mortality rate is largely due to resistance of melanoma cells to conventional chemotherapy, and despite recent advances in melanoma treatment, including targeted therapies and immune checkpoint inhibitors, melanoma remains a deadly disease. The observation on striking parallels between cancer cells and normal stem cells might lead to fundamental changes in the future therapy against cancer. We have previously demonstrated that Sox10, a neural crest transcription factor, plays a crucial role in the development and maintenance of giant congenital melanocytic nevi and melanoma and we identified SOX10 as a novel promising candidate for melanoma treatment. Interestingly, our data reveal that targeted therapies currently available for melanoma patients do not interfere with SOX10 expression neither *in vitro* nor *in vivo* in human patients. To gain further insight into SOX10-mediated melanoma progression, we have performed a mass spectrometry-based screen to identify proteins interacting with SOX10 in melanoma cells. We show here that SOX10 interacts with β-catenin, a key downstream effector of Wnt signalling pathway. The role of Wnt signaling and β-catenin pathway has been the subject of intensive research in the field of melanoma; however, its exact role has remained highly controversial to date. In this study we demonstrate that inhibition of GSK3β results in ultimate downregulation of SOX10 protein in melanoma cell lines *in vitro* and consequently leads to the death of melanoma cells. Moreover, when Sox10 is suppressed via β-catenin stabilization, tumor formation is delayed and survival is extended in a genetic melanoma mouse model. Our study is the first to demonstrate the protein-mediated regulation of the SOX10-Wnt axis in melanoma biology, shedding light on the molecular mechanism of SOX10 regulation and untangling the controversy around the role of canonical Wnt signaling in melanoma.

Identification of key niche cell types supporting expansive extramedullary hematopoiesis in the fetal liver

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Sustained production of mature blood cell types depends on the continuous self-renewal and differentiation of a rare subset of hematopoietic stem cells (HSCs). During early embryonic development, a small number of HSCs is generated, which subsequently undergo a massive expansion in the fetal liver (FL). Our aim is to dissect the putative stromal cell subsets existing in the FL and determine their contribution to HSC expansion and maintenance. We employed flow cytometry to analyze the identity and phenotypic characteristics of non-hematopoietic stromal components present in the FL. To study the anatomical distribution of the different non-hematopoietic cellular components we employed organ-wide 3D imaging of transgenic reporter mice. Finally, an extensive analysis of gene expression profiles of FACS sorted stromal cell populations was performed to infer mechanisms by which HSCs are extrinsically regulated. The cytokine SCF and chemokine CXCL12 have both been implicated as regulators of HSCs. Using knock-in reporter mice, we determined the cellular subsets, which express these two factors in the FL. SCF and CXCL12 are both contained in the stromal, non-hematopoietic compartment. Developing DLK1-positive hepatoblasts and CD140b-positive mesenchymal cells were characterized by a similarly strong expression of both factors, whereby a third stromal subset, endothelial cells, exhibited only low expression levels. Hepatoblasts showing a cuboidal morphology and the CD140b-positive mesenchymal cells displaying a fibroreticular morphology are uniformly distributed throughout the entire tissue. Of special interest gene expression analysis revealed that SCF gets strongly downregulated at late embryonic time points, coinciding with a decline in extramedullary hematopoiesis in the liver. In summary, our preliminary results point towards a model, where a rapidly expanding hematopoietic system in the FL is enabled by two putative niche populations, hepatoblasts and mesenchymal cells. A decrease in hepatoblast cell numbers, but also a declining expression of the cytokine Kitl accompanied by a rapidly differentiating epithelial tissue might induce a closing HSC niche, that forces hematopoiesis to move to the succeeding hematopoietic organs, the perinatal spleen and postnatal bone marrow.

Dissecting mechanisms that drive 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 in G0 of cell cycle (>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 3–8 weeks, we isolated HSC from recipient mice and analysed their proliferative history and cell cycle status by FACS.
Results: Our data show that after each cell division a small subset of HSC goes into dormancy while the rest immediately re-enters cell cycle. Aged HSC are more prone to return to quiescence after each cycle, compared to young ones. In addition, cells that return to quiescence seem to be hardwired to remain in G0 phase, since most HSC do not re-enter cell cycle even after 2 months. 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.
Conclusions: Our results seem to confirm our initial hypothesis that increased proliferative history with aging or inflammation drive HSC towards quiescence to ensure a balanced turnover of the whole HSC pool at the end of life. This would prevent HSC exhaustion and minimize the risk to develop malignancies. Dysregulation of this drive to quiescence might lead to accumulation of genetic alterations and clonal expansion. To further confirm our findings, we will perform RNA sequencing on non-divided, quiescent HSC from young and aged mice in order to identify novel molecular targets to modulate prospectively in vivo.

Functional validation of microRNA-126-3p as a platelet reactivity regulator using human progenitor cells

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Background: Platelets are an abundant source of microRNAs (miRNAs), which may play a role in the regulation of platelet function. Some miRNAs, such as miR-126-3p, are pointed out as potential biomarkers of platelet reactivity and recurrence of cardiovascular events. However, the biological relevance of these associations remains uncertain and functional validation of these candidate miRNAs on human-derived cells is lacking.
Methods: The functional impact of miRNAs on platelet-like structures derived from human progenitor cells were monitored using a flow-based assay. $CD34^+$-derived megakaryocytes were transfected with miRNA or siRNA and were differentiated in platelet-like structures. Platelet adhesion phenotype was assessed by perfusion of differentiated cells in a microfluidic system under a constant shear rate.
Results: miR-126-3p transfection procedure does not affect megakaryocytes differentiation and platelet-like structures production. Overexpression of miR-126-3p increased platelet-like structures adhesion compared to control. Moreover, miR-126-3p transfection was associated with downregulation of ADAM9, a validated target of miR-126-3p, and of PLXNB2, an actin dynamics regulator. Silencing PLXNB2 led to similar functional results than miR-126-3p transfection.
Conclusions: Taken together, using a flow-based assay, we functionally validate miR-126-3p as a regulator of platelet reactivity on platelet-like structures derived from human progenitor cells. Moreover, PLXNB2 was identified as a platelet reactivity regulator supporting the hypotheses that miR-126-3p modulates platelet reactivity by downregulation of PLXNB2.

FLT3 and MDM2 expression are biomarkers for combined MDM2 and MEK inhibition treatment in acute myeloid leukemia

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Objectives: The tumor suppressor protein p53 is inactivated by mutations in a large variety of cancer cell types. In contrast, *p53* mutations are rare events in acute myeloid leukemia (AML) whereas p53 function is commonly suppressed in AML cells by cellular p53 inhibitors such as MDM2. In addition, *FLT3* growth factor signaling pathways including the MAPK cascade (RAS-RAF-MEK-ERK) are highly active in AML cells. Consequently, combined administration of MDM2 and MEK inhibitors may represent a promising anti-leukemic treatment strategy to be tested *in vitro* and possibly *in vivo*.
Experimental design: In this study, we assessed the anti-leukemic efficacy of the MDM2 antagonist idasanutlin and the MEK inhibitor cobimetinib used as single agents as well as in combination in a representative series of AML cell lines and in primary AML blast cells. AML cell lines and blast cells comprised various AML subtypes with a particular focus on the mutational status of the *TP53, FLT3* and *NPM1* genes. In cytotoxicity assays, we determined the potential to induce apoptosis and cell death, and we aimed to identify biomarkers predicting response to treatment.
Results: We observed considerable differences in the anti-leukemic efficacy of idasanutlin and/or cobimetinib depending on the mutational background of AML cells with regards to the *TP53 and FLT3* genes. AML cells with complex abnormalities and mutated *TP53* were resistant to idasanutlin. AML cells with high sensitivity to treatment with either one of the two compounds as well as to the combined treatment had a normal karyotype and wildtype *TP53*. *FLT3*-ITD cells were more susceptible to idasanutlin, while *FLT3* wildtype cells were more susceptible to cobimetinib. Interestingly, susceptibility of AML cells to the combination treatment correlated with the expression levels of FLT3 and MDM2 protein, but not with the mutational status of FLT3 and NPM1 genes.
Conclusion: Our data indicate that AML cells with normal karyotype (NK) and wildtype status of *TP53* - independent of the *FLT3* and *NPM1* mutation status, but with elevated FLT3 and MDM2 protein levels – emerge to be most sensitive to the combined treatment with cobimetinib and idasanutlin. *FLT3* and *MDM2* are promising biomarkers for treatment response to idasanutlin and cobimetinib in AML.

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

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Introduction: Hematopoiesis is a highly dynamic and tightly regulated process in the bone marrow (BM) sustained by a rare population of self-renewing, multipotent hematopoietic stem and progenitor cells (HSPCs) residing in specialized microenvironments within BM cavities. The basic tissue infrastructure of the BM is provided by stromal cellular networks of mesenchymal, neural and vascular origin, which are critically involved in the fine regulation of hematopoiesis. Albeit the effects of viral challenge and ensuing inflammatory responses on hematopoietic cells have been studied in detail, how viral infections alter BM stromal scaffolds and thus shape hematopoietic responses remains poorly defined. We herein investigated the structural and functional alterations imposed on the BM after chronic infection with Lymphocytic Choriomeningitis Virus (LCMV).
Methods: For multidimensional analyses, we combine conventional *in vitro* and *in vivo* assays with cutting edge 3D confocal imaging technology.
Results: Our data shows that chronic LCMV infections result in a substantial alteration of the BM endothelial and mesenchymal stromal progenitor cell populations and a decrease in their capacity to produce HSPC-sustaining factors. This was accompanied by a strong and sustained reduction in the number of hematopoietic multipotent progenitors as well as hematopoietic stem cells by phenotype. On a functional level, competitive repopulation assays revealed a striking and persistent loss of HSC function after chronic LCMV infection. Finally, preliminary results indicate that this mechanism is governed by CD8 T cells and partially mediated by IFNa.
Conclusion: We herein report that chronic LCMV infections lead to massive alterations in the hematopoietic and stromal compartments in the BM. Intriguingly, the functionality of HSCs and BM mesenchymal stromal cells stays impaired even at time points of immunological exhaustion long after the initial infection.

Transcriptome-proteome correlation in human hematopoietic stem and progenitor cells

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Introduction: Hematopoietic stem cells (HSCs) can self-renew and/or differentiate into various functionally divergent progenitor cell types, such as common myeloid progenitors (CMPs), megakaryocyte-erythrocyte progenitors (MEPs) or granulocyte-macrophage progenitors (GMPs). When the process of self-renewal and differentiation is altered, e.g. upon genetic or epigenetic changes in HSCs, abnormal (pre)leukemic stem cell subpopulations may form, eventually resulting in the onset of hematological malignancies. To gain insight into the physiology and subsequent patho-physiology of self-renewal and differentiation, highly refined analyses of HSCs and downstream progenitor cells are needed.
Methods: We developed an ultra-sensitive mass spectrometric method for robust quantitative proteomic analysis of highly purified, FACS-sorted cell populations and applied this method to quantify the proteome of 25'000 human hematopoietic stem and progenitor cell subpopulations isolated from five healthy donors. The proteomic analyses were complemented by transcriptomic analyses.
Results: A comparison of proteomic and transcriptomic profiles of the respective cell types indicated hematopoietic stem/multipotent progenitor cell-specific divergent regulation of biochemical processes essential for maintaining stemness at the proteome rather than transcriptome level. Specifically, several telomerase maintenance proteins and quiescence-inducing isocitrate dehydrogenase proteins, both assumed to be essential for long-lived stem cells, were found to be upregulated in HSCs on the protein but not on the mRNA level when compared to myeloid progenitor cell subpopulations (CMPs, MEPs, GMPs).

Conclusion: The divergent mRNA/protein regulation of telomerase maintenance and quiescence-inducing isocitrate dehydrogenase proteins in HSCs illustrates the relevance of generating high quality proteomic data for well-defined cell subpopulations with the goal to identify biological processes that are insufficiently determined by genomic or transcriptomic analyses. The presented approach opens the door for proteomic profiling of relevant disease sample sub-fractions such as (pre)leukemic stem cells in chronic and acute leukemias as well as cancer stem cells from solid tumors and, ultimately, might allow to find therapeutic targets in (pre)leukemic/cancer stem cells.

TRRAP is essential for regulating the accumulation of mutant and wild-type p53 in lymphoma

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Tumors accumulate high levels of mutant p53 (mutp53), which contributes to mutp53 gain-of-function (GOF) properties. The mechanisms that underlie such excessive accumulation are not fully understood. To discover regulators of mutp53 protein accumulation, we performed a large-scale RNA interference (RNAi) screen in a Burkitt's lymphoma (BL) cell line model. We identified TRRAP, a constituent of several histone acetyltransferase (HAT) complexes, as a critical positive regulator of both mutp53 and wild-type p53 (wtp53) levels. TRRAP silencing attenuated p53 accumulation in lymphoma and colon cancer models, suggesting a role for TRRAP across cancer entities and p53 mutations. TRRAP overexpression increased mutp53 levels. Through CRISPR-Cas9 screening, we identified a 109 amino acid region in the N-terminal HEAT repeat region of TRRAP which was crucial for mutp53 stabilization and cell proliferation. Mass spectrometric analysis of the mutp53 interactome indicated that TRRAP silencing caused degradation of mutp53 via the MDM2-proteasome axis. This suggests that TRRAP is vital for maintaining mutp53 levels by shielding it against the natural p53 degradation machinery. To identify drugs that alleviated p53 accumulation similarly to TRRAP silencing, we performed a small molecule drug screen. We found that inhibition of histone deacetylases (HDACs), specifically HDACs1/2/3, decreased p53 levels to a comparable extent as TRRAP knock-down across cell lines. In summary, here we identify TRRAP as a key regulator of p53 levels and link acetylation-modifying complexes to p53 protein stability. Our findings may provide clues for therapeutic targeting of mutp53 in lymphoma and other cancers.

MDM4 is an essential disease driver targeted by 1q gain in Burkitt lymphoma

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Oncogenic MYC activation promotes cellular proliferation in Burkitt lymphoma (BL), but also induces cell cycle arrest and apoptosis mediated by TP53, a tumor suppressor that is mutated in 40% of BL cases. To identify therapeutic targets in BL, we investigated molecular dependencies in BL cell lines using RNAi-based, loss-of-function screening. By integrating genotypic and RNAi data, we identified a number of genotype-specific dependencies including the dependence of TCF3/ID3 mutant cell lines on TCF3 and of MYD88 mutant cell lines on TLR signaling. TP53 wild-type (TP53wt) BL were dependent on MDM4, a negative regulator of TP53. In BL cell lines, MDM4 knockdown induced cell cycle arrest and decreased tumor growth in a xenograft model in a p53-dependent manner, while small molecule inhibition of the MDM4-p53 interaction restored p53 activity resulting in cell cycle arrest. Consistent with the pathogenic effect of MDM4 upregulation in BL, we found that TP53wt BL samples were enriched for gain of chromosome 1q which includes the MDM4 locus. 1q gain was also enriched across non-BL cancer cell lines (n = 789) without TP53 mutation (23% in TP53wt and 12% in TP53mut, p <0.001). In a set of 216 cell lines representing 19 cancer entities from the Achilles project, MDM4 was the strongest genetic dependency in TP53wt cell lines (p <0.001). Our findings show that in TP53wt BL, MDM4-mediated inhibition of TP53 is a mechanism to evade cycle arrest. The data highlights the critical role of p53 as a tumor suppressor in BL, and identifies MDM4 as a key functional target of 1q gain in a wide range of cancers, which is therapeutically targetable.

Spatial analysis of the bone marrow stroma using deep learning

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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 of vascular, mesenchymal or neural origin 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.

A gain-of-function mutation in EPO in familial erythrocytosis

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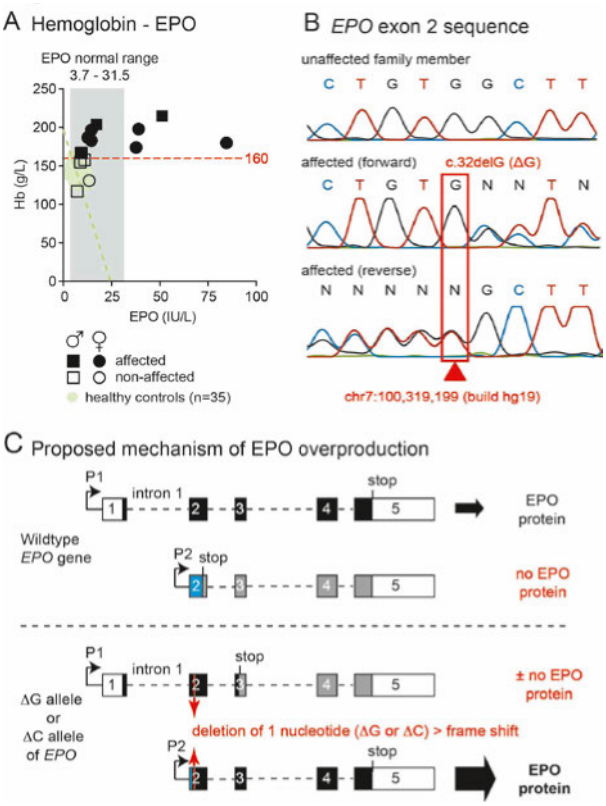
Introduction: Secondary erythrocytosis with elevated erythropoietin (EPO) serum levels is mainly caused by mutations in genes involved in oxygen sensing pathway. We studied a family with autosomal dominant erythrocytosis and elevated EPO serum levels (fig. 1A).
Results: Linkage analysis identified a co-segregating region on chromosome 7q22.1 with a LOD score of 3.3. Targeted sequencing of the co-segregating region revealed a heterozygous single base deletion in exon 2 of *EPO* (c.32 delG, further described as ΔG) as the sole candidate gene mutation (fig. 1B). We used CRISPR to introduce the ΔG mutation into Hep3B cells. Culture supernatants of single-cell-derived clones homozygous for the *EPO* ΔG mutation contained 8-10 times more EPO than parental Hep3B cells or controls and were also capable of stimulating the growth of an EPO-dependent cell line, demonstrating biological activity. 5'-RACE sequencing revealed two types of alternative mRNAs that initiate from a putative promoter (P2) in intron 1 of *EPO* in addition to the transcript originating from the physiologic promoter (P1). Supernatants of HEK293 cells transfected

with P2 ΔG mRNAs contained more EPO than cells transfected with the P1 wildtype cDNA. These supernatants also stimulated the growth of EPO-dependent cells and supported erythroid colony formation of progenitors from human peripheral blood, demonstrating that P2 ΔG transcripts produce an excess of biologically active EPO protein.
Conclusion: Our data indicate that the *EPO* ΔG mutation introduces a frame-shift in exon 2 that interrupts translation of the main *EPO* mRNA transcript, but initiates excess production of EPO protein from (what is normally a non-coding) *EPO*mRNA transcribed from an alternative promoter located in intron 1 (fig. 1C).



Understanding chaperone-mediated autophagy and non-canonical macroautophagy in acute myeloid leukemia differentiation

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Autophagy is a cellular degradation and recycling system. Three main autophagy pathways have been described: Macroautophagy, chaperone mediated autophagy (CMA) and microautophagy. Macroautophagy is characterized by the formation of double-membraned vesicles, so-called autophagosomes that engulf unneeded or harmful components of the cytoplasm. CMA is a selective autophagy pathway that modulates the turnover of soluble cytosolic proteins. In contrast to macroautophagy, CMA cargo deliver directly to the lysosomes. We and others reported that increased macroautophagic activity is key to retinoic acids (RA)-induced granulocytic differentiation of acute myeloid leukemia (AML) cells. We characterized a non-canonical macroautophagy pathway operative during RA-mediated differentiation depending on a variety of ATG genes such as ULK1, VPS34, WIPIs, ATG3, ATG4, ATG16L2, ATG5, ATG7, GATE-16/LC3B, ALFY, DAPK2 and MAP1S, but not on Beclin1 or ATG16L1. Moreover, a number of these ATG genes involved in AML differentiation are significantly downregulated in primary AML patient samples and are transcriptionally regulated by the myeloid transcription factor PU.1. In contrast to macroautophagy genes, key CMA genes such as *HSC70* and *HSP90AA1* are significantly higher expressed in immature normal and leukemic myeloid cells as compared to healthy neutrophils. Accordingly, RA-induced differentiation of AML cells was associated with decreased expression of CMA protein markers (HSC70, HSP90) and CMA substrates as well as decreased LAMP2A and HSC70 co-localization. Interestingly, preliminary data indicate that knocking down *HSP90AA1* allowed for enhanced differentiation. In summary, increased CMA is associated with an immature myeloid phenotype and blocking macroautophagy leads to increased CMA activity upon ATRA treatment while inhibiting CMA enhanced the ATRA differentiation response. Deciphering the particular autophagy pathway(s) active during APL/AML differentiation is a prerequisite to develop novel "autophagy-differentiation therapies" to improve current APL treatment and to expand this approach to additional AML subgroups not responding to RA-therapy.

miRNAs in Sickle Cell Disease

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Background: Sickle Cell Disease (SCD) is a monogenic blood disorder, with a single nucleotide mutation. Because of migration, SCD is seen more frequently in Switzerland. Due to the mutation the RBCs take on a sickled shape, clog small blood vessels and lead to vaso-occlusion, organ ischemia and hemolytic anemia. Specifically recurrent pain crises, chest syndrome and even strokes occur, being life-threatening or compromising the patients' quality of life. The symptoms can ameliorate in the presence of higher amounts of HbF. HbF can be elevated in RBCs of children and adults by pharmacological agents, such as hydroxyurea (HU). Most patients benefit from this treatment, however, the treatment responses are highly variable and the mechanism is not yet well understood. We hypothesize that altered micro-RNA expression might be one effect of HU. Micro-RNAs (miRNAs) are small non-coding ribonucleic acids which play a role in gene expression. MiRNAs control genes post-transcriptionally by binding to mRNAs with (partial) sequence complementarity. They hereby degrade or repress these mRNAs. miRNA profiles hence suggest different gene expression profiles in individuals.
Methods: Previously, we analysed the expression of 380 miRNAs in healthy individuals, SCD patients and SCD patients treated with HU. Clear differences in miRNA expression profiles were observed between these groups. We selected 3 differentially expressed miRNAs and will analyse these in ex vivo erythropoiesis models with and without HU therapy. Additionally we aim to identify their direct target-mRNAs via a pulldown assay.
Conclusion: Our hypothesis is that miRNAs influence gene networks in both SCD and in HU treatment. By identifying target-mRNAs of the selected miRNAs these networks may be elucidated, and shed light on the pathophysiology of SCD as well as on the mechanism of HbF induction by HU. By understanding the regulation of erythropoiesis via miRNAs in patients and healthy individuals we could gain more insight into the mechanism of HU and HbF regulation and ultimately identify new therapeutic targets.

Impact of cancer associated fibroblasts in esophageal adenocarcinomas

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Background: Cancer associated fibroblasts (CAFs) represent a very important component of the tumor microenvironment. CAFs can promote cancer progression through multiple growth factors and signaling pathways, which may represent potential targets for anticancer therapies. Esophageal Adenocarcinomas (EAC) are very aggressive tumors with high rates of resistance to conventional anticancer treatment. In this tissue based ex vivo analysis, we investigated the impact of CAFs in esophageal adenocarcinomas with a special focus on tumors treated with neoadjuvant therapy (nTX) before surgery.
Material and Methods: two case collections of esophageal adenocarcinomas (total n = 310) were investigated using next generation tissue microarrays, generated from formalin fixed paraffin embedded tissue from EAC resection specimens. 112 cases were primary resected carcinomas, 198 cases were resected after nTX. Visualization of CAFs was carried out by immunohistochemical staining for the CAF markers COLL11A1, CD90 and SPARC.
Results: in primary resected EAC, the amount of CAFs was increased in advanced tumor stages and in tumors with aggressive phenotype, e.g. with a significant association with pT category ($p < 0.02$; all markers), lymph node metastases ($p < 0.005$ for COLL11A1 and CD90) and lymphatic vessel invasion ($p < 0.02$ for all markers). Higher CAF counts were also associated with worse survival ($p = 0.05$ for COLL11A1) but this was not independent from other prognostically relevant pathological factors. In the nTX cohort similar significant associations between CAF markers and patho-clinical parameters were observed. The most striking finding was the association between tumor regression and presence of CAFs: tumors with complete and

subtotal regression had significantly lower CAF counts than those who did not substantially respond ($p < 0.05$ for all markers), and CAFs were almost absent in the scars of completely regressive tumors.
Conclusion: this ex vivo analysis highlights the role of CAFs in EAC with increasing amounts of CAFs during tumor progression in treatment naïve tumors and decreasing amount of CAFs following regression after neoadjuvant therapy. This argues for the concept of CAFs as an important factor of tumor promotion and support. Since the tumor stroma is considered a potential target for specific anti-tumor therapy, our results may serve as base for the development of future therapeutic approaches for these highly aggressive tumors.

The whole transcriptional landscape of circulating tumor cells compared to metastases in stage IV breast cancer

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Background: Metastatic breast cancer (MBC) and the circulating cells (CTCs) leading to macrometastasis are inherently different than primary breast cancer, evolving under the selection pressure of systemic therapy. A better understanding of the tumor biology of CTCs compared to metastasis may shed light on treatment opportunities.
Methods: We performed whole transcriptome sequencing (RNA Seq) on fresh metastatic tumor biopsies (mets), CTCs (including 5 patients with follow-up CTC harvest), and peripheral blood (PB) from 21 newly diagnosed MBC patients. CTCs were harvested using the ANGLE Parsortix to isolate cells based on size and deformability independent of cell surface markers. Data were analyzed for breast cancer relevant genes, clinically actionable targets, biological pathways, single nucleotide variants (SNV) and prognostic value using publicly available data sets.
Results: CTCs as a group showed much stronger gene expression of oncogenes, stem cell genes, keratins and mesenchymal markers than did mets from the same patients. Matched patient comparison for 66 potentially clinically actionable genes showed significant correlation between CTCs and mets. The top 50 genes shared between CTC and mets were prognostic of worse overall survival on the TCGA breast cancer dataset. Second time-point analysis demonstrated changes in CTC biology and target expression. Ingenuity pathway analysis was applied to identify canonical pathways and upstream regulators associated with differential gene expression in CTCs and mets. SNV analysis results are currently pending.
Conclusions: Whole transcriptome analysis of CTCs and metastases has prognostic value in Stage IV breast cancer. Our results further showed that RNA Seq of CTCs might be utilized predictively as liquid biopsies to identify molecular alterations that are potentially clinically actionable and could allow for repeated minimally invasive sampling.

Enhanced support of myelofibrosis stem cells in next generation humanized mice

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Introduction: Engraftment of chronic hematopoietic neoplasms, like myelofibrosis (MF), in patient-derived xenograft (PDX) models is poor. We hypothesized that the constitutive expression of human cytokines and growth factors in "next-generation" humanized mice (MISTRG, Rongvaux et al., Nature Biotechnology 2014) may provide a supportive microenvironment for MF stem cell development and could faithfully recapitulate disease phenotype and genetic heterogeneity allowing the development of a pre-clinical MF PDX model.
Methods: Purified peripheral (PB) blood stem and progenitor cells (CD34+) from MF patients were transplanted intra-hepatically into sub-lethally irradiated newborn MISTRG mice and into standard NSG mice as controls. Mice were sacrificed after 5–16 weeks and characterized by flow cytometry, immunohistochemistry and mutational profiling.
Results: The total median engraftment of PB-purified CD34+ cells from seven DIPSS intermediate-2 or high-risk patients was higher in the bone marrow (29.75% vs. 2.36%, $p < 0.0001$), PB (44.10% vs. 0.62%, $p < 0.0001$) and spleen (8.57% vs. 0.44%, $p = 0.014$) of MISTRG compared to NSG mice. Both strains supported substantial human myelo-monocytic and megakaryocytic differentiation. MISTRG mice also engrafted CD34+ cells from 3/3 DIPSS intermediate-1 risk patients. Next generation sequencing data comparing the mutational profile of primary samples to their corresponding engrafted xenografts showed maintenance of the original clonal composition. Finally, purified human MF cells isolated from primary mice showed significant myeloid reconstitution in secondary recipients.
Conclusions/outlook: Overall, these results show that MISTRG mice support robust engraftment of MF-SCs and are able to maintain stemness and the genetic heterogeneity found in patients. The MF PDX model will further be used to understand the disease pathogenesis and asses novel therapeutic agents in order to expedite their transition into clinical trials.

Protein clients are differentially affected by mutations in the endoplasmic reticulum chaperone calreticulin

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Background: *Calreticulin* (*CALR*) is frequently mutated in patients with myeloproliferative neoplasms (MPNs). CALR ensures folding of glycoproteins (GPs) such as the thrombopoietin receptor (TpoR), major histocompatibility complex I (MHC-I) and myeloperoxidase (MPO). All CALR mutants share a mutant-specific C-terminus with type-1 and type-2 variants covering over 80% of the mutational spectrum of *CALR*. Mutant CALR activates the TpoR and consecutively JAK/STAT signaling through a pathologic protein-protein interaction. Furthermore, we have previously shown that MPN patients with homozygous *CALR* mutations develop a maturation defect in MPO. Therefore, we hypothesize that the protein interactome of CALR and the maturation of GPs is altered in the presence of CALR mutants.
Methods: We first aimed to determine how the maturation of known CALR GP clients is affected by *CALR* mutations. Using CRISPR-Cas9 we generated a CALR knockout cell line (HL-60 CALR KO). HL-60 cells express abundant levels of MPO. The expression of MPO and MHC-I on HL-60 CALR KO cells and patients with *CALR* mutations was determined by flow cytometry. To show that MPO and MHC-I expression are directly affected by CALR mutants, rescue experiments with CALR proteins were performed.
Results: The expression of MPO was significantly reduced in HL-60 CALR KO cells resembling the phenotype observed in patients with homozygous *CALR* mutations. In contrast, while MHC-I expression was diminished in HL-60 CALR KO cells, patients with homozygous *CALR* mutations showed normal MHC-I expression. Next, HL-60 CALR KO cells were reconstituted with either wildtype or mutant CALR proteins (type-1 or type-2). Preliminary data indicates that the rescue of MPO expression is more efficient with wildtype CALR compared to both mutant variants. In respect to MHC-I expression, the rescue properties did not differ between wildtype and mutant CALR proteins.
Discussion/Outlook: Together these findings suggest that *CALR* mutations affect the maturation of CALR protein clients in a protein-specific manner.

Functional and structural dynamics of the bone marrow stromal microenvironment after cytoreductive therapies

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Bone marrow (BM) cavities are the primary sites of the high throughput, continuous and tightly regulated production of blood cells during adulthood. This process, called hematopoiesis, is sustained by the proliferation and differentiation of hematopoietic stem and progenitor cells (HSPCs), which are maintained by signals emanating from the BM microenvironment. BM tissues are highly sensitive to myeloablative treatments and the induced cytotoxic effects on rapidly cycling hematopoietic progenitors have been extensively characterized. Nevertheless, the structural effects, especially the kinetics of the destruction and recovery of the BM microenvironment and its different components are less characterized. By combining advanced flow cytometric protocols, 3D-imaging techniques and newly developed computational tools that enabled the quantitative analysis of the images, we could study quantitative changes of the hematopoietic

and especially of the stromal compartment but also investigate effects on the murine BM microarchitecture, in particular with regard on the vasculature. As previously reported, the analysis by flow cytometry showed a profound loss of HSPCs that was accompanied by a similar decrease in stromal cell populations, including endothelial cells and fibroblastic reticular cells. 3D-imaging also revealed a severe damage to the integrity of the vascular system, shown by a complete disruption of the vessel walls following a massive initial sinusoidal vasodilation. The reorganization of the vascular system, starting by day 14 post 5-FU administration, led to a fully regenerated microvascular network 28 days after treatment. In contrast to the analysis by flow cytometry, CXCL12 abundant reticular stromal cells were only slightly affected when quantified by 3D-imaging. Our observations demonstrate that, in contrast to hematopoietic cells, mesenchymal stromal cell populations are highly resistant to cytoreductive damage with 5-FU and most likely drive the complex process of rapid and complete regeneration of BM tissues after injury.

Large-scale RNAi screen identifies the HAT complex member TRRAP as a regulator of mutant p53 accumulation in cancer

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Background: p53 mutation is frequent in aggressive B-cell lymphoma (30–40%). Since tumors often depend on sustained high protein levels of mutant p53 (mutp53), interfering with mutp53 accumulation may be exploitable for cancer therapy.
Methods: To uncover novel regulators of mutp53 levels in an unbiased fashion, we conducted an RNAi screen targeting ~5,000 genes with FACS-based phenotype readout in a mutp53 lymphoma model.
Results: The primary screen hit was TRRAP, a member of several histone acetyltransferase (HAT) complexes. TRRAP knock-down and knock-out reduced mutp53 levels across lymphoma and colon cancer models with a diverse spectrum of p53 mutations, while TRRAP overexpression elevated mutp53 levels. By using a CRISPR/Cas9 mutagenesis approach, we identified a novel TRRAP domain crucial for mutp53 stabilization and cell proliferation. Using mass spectrometry to quantify mutp53-interacting proteins, we found that TRRAP silencing resulted in nuclear export and degradation of mutp53 via the MDM2-proteasome axis. TRRAP silencing also attenuated wildtype p53 (wtp53) stabilization and activity upon genotoxic stress, indicating a role for TRRAP in regulating both mutp53 and wtp53 levels. In search of small molecules modulating mutp53 levels, we found histone deacetylase (HDAC) inhibitors to result in similar degradation and deacetylation of mutp53 as TRRAP knock-down.
Conclusion: Our study links HATs and HDACs to the regulation of p53 protein stability and may provide a basis for therapeutic targeting of mutp53 in lymphoma.

Dissecting the cellular origin of neuroblastoma: Characterization of new progenitor cell populations along differentiation of sympathetic nervous system

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Neuroblastoma (NB) is a pediatric cancer of the sympathetic nervous system, which occurs almost exclusively in infancy and early childhood. It is widely assumed that oncogenic mutations occur in the cells of sympathoadrenal (SA) lineage during prenatal and postnatal life, however, the exact cell of origin has not been yet characterized. The targeted expression of ALKF^{1174L} and MYCN oncogenes driven by a *dopamine beta-hydroxylase* promoters results in NB development. However, these mouse models fail to faithfully recapitulate human NB. Indeed, there is a lack of schwannian stroma, only minimal metastasis formation, failure of mimicking the spontaneous regression and the onset of disease does not correspond to the pediatric characteristic of this tumor. A possible explanation for these discrepancies might be the fact that the oncogene expression is induced in progenitor cells rather than stem cell population. Taken together, these data suggest that there is a strong need to develop more accurate mouse models of NB development and therefore, a comprehensive analysis of the cellular hierarchy within SA lineage is a first step to understand the origin of NB. For this aim, we used several Cre mouse lines where Cre expression is driven by promoters of genes, which are expressed at different levels of differentiation. Through recombination is allowed population-specific transcription of tdTomato reporter, or oncogenes as *MYCN* and ALKF^{1174L}. We used a lineage tracing approach to fate-map different SA and glial progenitors and quantify their contribution to the different derivatives, such as chromaffin cells, sympathetic neurons, and glia. This comprehensive study significantly improved understanding of the cellular origin of NB, since it covered different controversial topics in developmental biology of the SA lineage and connected it with NB tumorigenesis. Contrasting what has been previously suggested, we proved that migrating neural crest stem cells could not be the cellular origin of NB *in vivo*. Fate-tracing of the different progenitors pointed out two interesting multipotent populations that are present after neural crest migration at the beginning of SA differentiation, but not committed yet to such fate. Oncogenes expression in such cells will show if at this stage NB can arise.

ERK and MEK kinases as novel therapeutic targets in myeloproliferative neoplasms based on bypass MAPK pathway activation

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Background: Myeloproliferative neoplasms (MPN) show constitutive JAK2 activation, but JAK2 inhibitors have limited efficacy. We hypothesized that ERK and MEK kinases could be novel therapeutic targets.
Methods: MAPK activation upon JAK2 inhibition with ruxolitinib and CHZ868 was studied in MPN cell lines, primary mouse hematopoietic cells (HC) and patient PBMCs. We screened for mediators of bypass MAPK activation by kinase array, Nanostring and Luminex. MAPK activation was targeted by genetic ERK ablation or MEK inhibition in MPN mice and patient samples.
Results: Type I and II JAK2 inhibition suppressed MAPK activation *in vitro*, but not in MPN mice in vivo. PDGFRα activation and PDGF-AA/BB production persisted upon JAK2 inhibition. PDGF-BB activated ERK in presence of ruxolitinib in mouse and human MPN cells and was blocked by PDGFRα inhibitors. Genetic ERK ablation in MPN mice mitigated polyglobulia and leukocytosis. Combined JAK2/MEK inhibition showed superior reduction of hepatosplenomegaly, blood counts and myeloid progenitors in MPN mice. Dual JAK/MEK inhibition reversed fibrosis to a higher extent than JAK inhibitor monotherapy and was superior in suppressing myelo-erythroid colony formation from MPN patient CD34$^+$ cells.
Conclusions: Our data demonstrate that bypass ERK activation through PDGFRα limits efficacy of JAK inhibition in MPN. We show that genetic targeting of ERK counteracts the MPN phenotype and pharmacologic targeting of JAK2/MEK provides improved therapeutic efficacy by preventing bypass ERK activation suggesting ERK and MEK kinases as therapeutic targets in MPN patients.

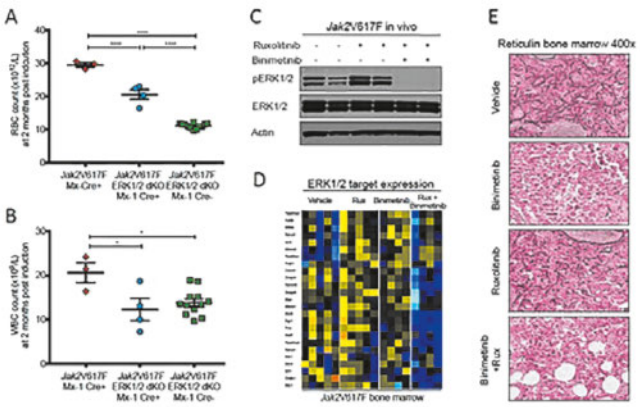

Fig. 1. Genetic and pharmacologic targeting of ERK and MEK kinases provides therapeutic benefit in MPN *in vivo*. Genetic ERK1/2 double knockout (dKO) ameliorates the MPN phenotype in the *Jak2V617F* model with reduced polyglobulia (A) and leukocytosis (B). Pharmacologic inhibition of MEK1/2 by binimetinib and JAK2 by ruxolitinib supresses ERK activation in the *Jak2V617F* MPN model *in vivo* (C). Expression of ERK downstream targets is reduced in *Jak2V617F* bone marrow (D) and fibrosis is resolved (E).

Combined Targeting of Oncogenic JAK2 Signaling and Metabolic Dependencies of Mutant Clones Elicits Synergistic Therapeutic Efficacy in Myeloproliferative Neoplasms

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Background: Myeloproliferative neoplasms (MPNs) are clonal disorders of hematopoietic stem and progenitor cells (HSPCs) characterized by overproduction of erythroid and myeloid lineages. The mechanisms downstream of driver mutations such as JAK2-V617F on MPN initiation and propagation are incompletely understood, and clinically utilized JAK2 inhibitors have limited ability to reduce MPN burden. Mouse models expressing mutant JAK2 exhibit MPN phenotypes and display early mortality. We noticed that these mice also display markedly decreased body weight and adipose tissue. Therefore, we studied the metabolic basis of MPN pathogenesis in these mice and primary samples from MPN patients. Elucidating the unique metabolic demands of MPN cells could be exploited as therapeutic targets in MPN.
Results: We found that activation of mutant JAK2 induces metabolic alterations including adipose atrophy, and resistance to high-fat diet (HFD) induced obesity in mice. Intriguingly, HFD treatment significantly ameliorated early lethality in MPN mice, which was not due to reduction in elevated platelet and erythrocyte numbers. In addition, mice under normal dietary conditions were severely hypoglycemic and showed increased glucose tolerance despite normal insulin levels, and this was correlated to extensive erythrocytosis. Integrated transcriptomics and metabolomics analysis together with metabolic functional assays of MPN propagating HSPCs revealed heightened utilization of nutrients and subversion of metabolic pathway derivatives to biosynthetic processes attributable for expansion of MPN propagating HSPCs and severity of MPN. Some of these metabolic changes were also detected in primary samples from MPN patients highlighting the clinical relevance of our findings. Combined pharmacological targeting of metabolic dependencies of mutant cells and JAK2 activity resulted in marked reduction in splenomegaly and MPN burden (fig. 1).
Conclusions: Our data show that metabolic rewiring in mutant clones is fundamental for MPN initiation and propagation. Activation of mutant JAK2 in the hematopoietic system induced bystander metabolic reprogramming in non-hematopoietic tissues leading to energy crisis, which may contribute to early lethality of MPN mice. Importantly, our study identified therapeutically viable metabolic targets of MPN, and provided the rationale for a “two-pronged” approach of co-targeting distinct metabolic features and oncogenic JAK2 activity in MPN cells.

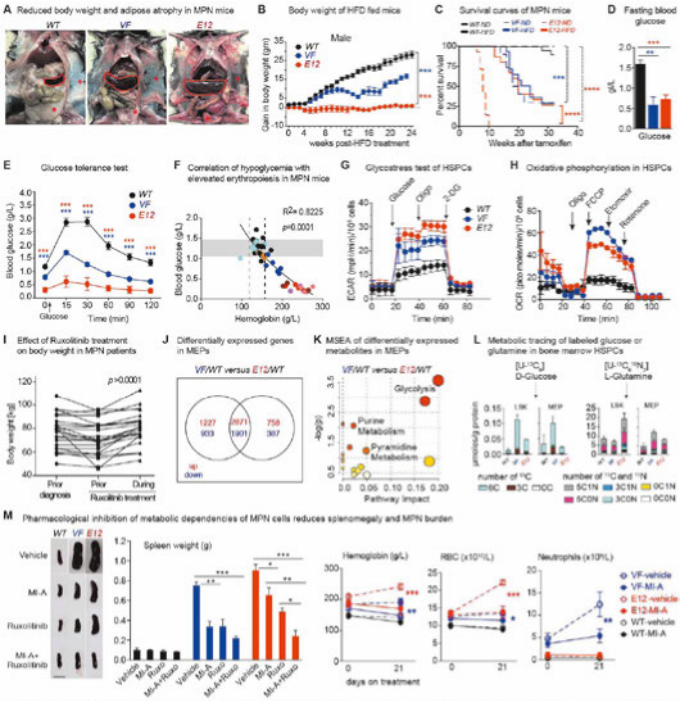

Figure 1. Hematopoietic specific activation of oncogenic JAK2 mutations induces global metabolic reprogramming in mice. **A)** Representative pictures of JAK2;V617F (VF) and JAK2 Exon12 (E12) expressing MPN mice. Asterixs indicates the adipose tissue. **B)** Body weight of MPN mice on high fat diet (HFD). **C)** Kaplan-Meier survival curves of MPN mice exposed to high fat diet. **D)** Fasting glucose levels. **E)** Glucose tolerance test. **F)** Hypoclycemia correlated with erythropoeisis. **G)** Seahorse analysis revealing enhanced glycolytic reliance of MPN HSPCs. **H)** Elevated mitochondrial oxidative phosphorylation levels in mutant JAK2 expressing HSPCs. **I)** Body weight of MPN patient before and after Ruxolitinib treatment. **J)** Venn diagram showing the number of differentially expressed genes in MEP cells from VF and E12 as compared to wild type controls. **K)** Metabolic pathway Enrichment Analysis (MSEA) of differentially expressed metabolites in MEPs. **L)** Metabolic tracing of labeled glucose or glutamine in bone marrow LSK and MEPs. **M)** Pharmacological inhibition of metabolic dependencies of MPN cells reduces splenomegaly and MPN burden.

HEMOSTASIS, TRANSFUSION MEDICINE, VASCULAR, LABORATORY MEDICINE

Validation of the automated ST Genesia for thrombin generation assay: evaluation of variability and reference ranges in a cohort of healthy donors

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Introduction: Global coagulation assays such as thrombin generation (TG) have been proposed to evaluate the balance between pro- and anti-coagulant forces, to better reflect the bleeding and thrombotic risks. Although TG readouts obtained using the calibrated automated thrombin generation (CAT) have been used for several clinical conditions, TG still need standardization and clinical validation. We evaluated the variability of the new standardized reagents for bleeding and thrombophilic conditions using the new automated TG instrument St Genesia (STG) and calculate the normal reference ranges in a cohort of normal donors.
Methods: TG was measured in the frozen platelet-free plasma (PFP) of 120 adult donors (49.2% males and 50.8% females; 20–80 years). Fifteen independent measurements were performed using the same batch of Bleedscreen (BLS) and Thromboscreen (w/wo thrombomodulin, TS-TM/+TM) reagents. Intra- and inter-assay coefficients of variation (CV) were calculated on quality controls (QC) and on 2 PFP samples. To define reference intervals, 2.5th–97.5th percentile were calculated.
Results: Evaluation of the TS-QCs showed a CV below 6% for all the TG parameters (Lag time, Peak, ttPeak, ETP). Similarly, the BLS-QCs showed a CV below 5% for all TG parameters. Preliminary results on the variability of frozen PFP showed the biggest CVs at 10% (intra, n = 5) and 15% (inter, n = 5/days) for both ETP and Peak in BLS. Similarly, we obtained the biggest CVs at 6% (intra, n = 5) and 14% (inter, n = 5/days) for both ETP and Peak in TS+TM/–TM. Evaluation of normal reference ranges for TG parameters in BLS showed normalized ETP and Peak ranging between 36.6–158.2% and 30.1–196.7%, respectively. The normal range of ETP inhibition for TS test was 22.2–86.9%.
Discussion: Our results showed low variability on BLS and TS QCs, however preliminary data on frozen PFP samples did not showed an improvement of intra and inter variability in comparison to published data on CAT.

Utility of platelet function analyzer (PFA) in patients with suspected platelet function disorders

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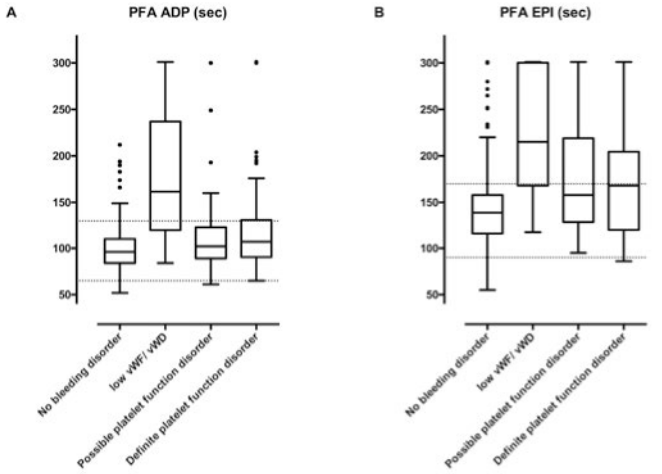
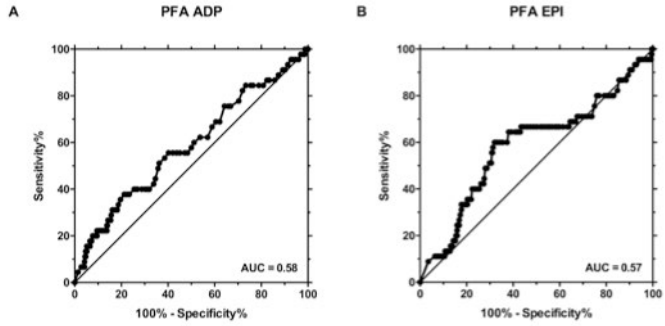
Background: Platelet function analyzer (PFA 100/200) has been introduced as a screening tool for bleeding disorders in the perioperative and outpatient setting. However, the diagnostic performance regarding inherited platelet function disorders (PFD) is not fully established.



Aim: We aimed to assess the diagnostic value of PFA for PFD in clinical practice.
Methods: Data of all consecutive patients referred between January 2012 and March 2017 with a suspected bleeding disorder to an outpatient unit of a university hospital were collected retrospectively. The diagnostic work-up was done according to current guidelines and platelet function was tested using light transmission aggregometry as well as flow cytometry.
Results: PFA (ADP, EPI) was conducted in 473 out of 555 patients referred (median age 41.7 years; inter-quartile range [IQR] 28.0, 60.7; 68.1% female). Possible PFD was diagnosed in 70 patients (12.6%), definite PFD in 48 patients (8.7%), von Willebrand disease or low von Willebrand factor associated with blood group 0 in 43 patients (7.8%), other coagulation disorders in 39 patients (7.0%), and other disorders in 38 patients (6.9%). In patients with possible PFD, median PFA was 102 s (ADP; inter-quartile range [IQR] 89, 123) and 158 s (EPI; IQR 129, 219); in patients with definite PFD 107 s (ADP; IQR 92, 130) and 168 s (EPI; IQR 121, 201); and in patients without bleeding disorders 96 s (ADP; IQR 84, 110) and 139 (EPI; IRQ 117, 158). Area under the ROC curve (AUC; definite PFD) was 0.58 (95% CI 0.48, 0.67) in case of ADP and 0.57 in case of EPI (95% CI 0.48, 0.66).
Conclusions: PFA results were weakly associated with the presence of a PFD. Our results do not support the implementation of PFA for screening of PFD.



Dynamic of peripheral blood schistocytes in GVHD after allogeneic stem cell transplants assessed by digital microscopy

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Background: Early detection of acute graft-versus-host-disease (aGvHD), might help protect patients from transplant-associated thrombotic microangiopathy (TA-TAM). One of the diagnostic criteria for TA-TMA is the quantification of schistocytes in peripheral blood (detected as schistocytes and helmet cells). We tested the diagnostic accuracy of digital microscopy concerning recognition of schistocytes in the setting of endothelial GvHD.
Methods: Peripheral blood smears from 127 allogeneic, 63 autologous HSCT and 31 healthy controls were retrospectively examined before, one month (1m) and at 2-3 months (3m) after transplant. Quantification of schistocytes by digital microscopy (Vision Hema, West-Medica, Wien-Austria) was done out of 5'000 erythrocytes and was correlated to clinical signs of aGVHD/TA-TMA.
Results: In the table schistocyte counts in percent of erythrocytes (mean ± standard deviation) are given. Schistocytes were significantly increased after 3m from the allogeneic (p <0.001) or the autologous HSCT (p <0.001) compared to controls. Statistically significantly higher schistocyte counts were found at 3m in patients with aGvHD (p = 0.027), in patients with an HLA mismatch (p <0.01) and in patients with high levels of lactate dehydrogenase (LDH) (p <0.05), compared to baseline before conditioning. Allogeneic HSCT patients with high schistocyte counts (upper quartile, >0.64%) had significantly more frequently aGvHD (chi-squared test p = 0.024), high LDH levels >225 (chi-squared test p = 0.006) and HLA mismatch (chi-squared test p = 0.035).
Conclusions: Schistocytes can be reliably counted by means of a digital microscopy system and they are increased within the context of aGvHD, probably as an indirect sign of microangiopathy. This might be used as an indicator of endothelial GvHD after allogeneic HSCT, long before a possible TA-TMA syndrome evolves, as patients with GvHD and/or HLA mismatch have more frequently endothelial damage. Appropriate cut-offs for schistocytes have to be defined and validated prospectively.

Group	Baseline	1 Month after HSCT	3 Months after HSCT	Wilcoxon test	Mann Whitney U test
Allogeneic w/o aGVHD (n=70)	0.526 ± 0.6	0.474 ± 0.483	0.433 ± 0.329	N.S.*	P=0.05**
Allogeneic with aGVHD (n=57)	0.430 ± 0.313	0.471 ± 0.343	0.563 ± 0.424	P=0.027*	
Allogeneic w/o HLAmm (n=76)	0.49 ± 0.397	0.471 ± 0.466	0.458 ± 0.327	N.S.*	P=0.005**
Allogeneic with HLAmm (n=51)	0.472 ± 0.613	0.475 ± 0.358	0.6 ± 0.441	P=0.003*	
Autologous (n=63)	0.463 ± 0.56	0.511 ± 0.469	0.519 ± 0.535	N.S.*	
Controls (n=31)	0.095±0.06	0.095±0.06	0.095±0.06		

Europe-wide survey on the use of thrombopoietin agonists for the treatment of aplastic anemia

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Background: Thrombopoietin (TPO) agonists such as eltrombopag (ELT) emerged recently as a novel therapeutic option in the therapy of aplastic anemia (AA). How TPO agonists are used outside of clinical trials in the real-world setting and results of this treatment are not known.
Methods: We conducted a retrospective survey on the use of TPO agonists for AA among EBMT member centers. We included the dataset on ELT-treated patients from France recently published by Lengline *et al.*, Haematologica 2017. 180 patients from 45 centers were reported. We analyzed 151 patients having received at least 30 days ELT and having a follow up of at least 2 months.
Results: The reported ELT treatment episodes were 2012 to mid 2017, 89% of patients are alive at a median follow up of 12 months. ELT was applied both as part of the first-line treatment and in relapsed or refractory patients, either as a monotherapy or in combination with CyA +/-ATG. Compared to lower intensity treatment (ELT alone or in combination with CyA), patients treated with ELT/CyA/ATG in the first line setting were younger and had more severe AA. Overall, most ELT treated patients (89%) were platelet transfusion dependent, suggesting platelet recovery as an important goal of ELT treatment. The overall response rate varied between 42% and 86% across treatment groups, likely reflecting differences in patient selection. Interestingly, the ORR to ELT was similar in relapsed or refractory patients if used alone or in combination with CyA (p = n.s.). Although 53% of the patients stopped ELT, only 16% patients received further non-transplant treatment, showing the lack of meaningful treatment options in patients not responding to IS and ELT. 25 patients (17%) underwent allo-HSCT at a median of 202 days following start of ELT. Whether ELT was useful as a bridge to transplant or was simply applied to relapsed/refractory patients with an indication for allo-HSCT is not known. At the median ELT dose of 150 mg/day, adverse events were infrequent and consistent with the known safety profile of ELT.
Conclusions: ELT, is used widely in Europe to treat AA patients. Although early adoption of this novel therapy led most likely to negative selection of AA patients, the results of ELT treatment in the real world setting are consistent with the reported clinical trial data.

Protein S released from platelets limits thrombus growth

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Background: Protein S (PS) is an anticoagulant acting as cofactor for both APC and TFPI, important enzymes for the inhibition of tenase and prothrombinase complexes. Although the majority of PS circulates in plasma, 2.5% is stored in platelets (pltPS) and released upon activation. Biochemically comparable to plasmatic PS, pltPS role and impact has never been assessed. Since PS deficiency in mouse has shown similarities with the human phenotype, we studied the role of pltPS in mice.
Results: Conditional PS knockout mice in megakaryocyte lineage were generated using the Pf4-Cre transgene ($Pros1^{lox/lox}Pf4\text{-}Cre^{+}$). PS antigenic assays confirmed the specific absence of pltPS. Plasma PS was indeed normal (88.9 ± 4.6%). $Pros1^{lox/lox}Pf4\text{-}Cre^{+}$ were viable, fertile and did not display DIC. In tail clipping model, $Pros1^{lox/lox}Pf4\text{-}Cre^{+}$ showed a decreased bleeding time and blood loss (P = 0.03, P = 0.01) compared to $Pros1^{lox/lox}Pf4\text{-}Cre^{-}$. To evaluate the thrombotic propensity, we induced thrombosis by i.v. tissue factor (TF) injection or by vessel injury triggered by $FeCl_3$. In TF model, the survival rate was reduced by 50% in $Pros1^{lox/lox}Pf4\text{-}Cre^{+}$ as compared to $Pros1^{lox/lox}Pf4\text{-}Cre^{-}$ (46.7% vs 85.8%, n = 14; P = 0.047). In $FeCl_3$ model, occlusive thrombi formed 2 times faster and were larger in $Pros1^{lox/lox}Pf4\text{-}Cre^{+}$. Platelet functions were tested by PFA-200, LTA (collagen, ADP and thrombin) and in vivo by collagen-epinephrine induced thrombosis model without showing any impairment. Ex-vivo TF-initiated thrombin generation demonstrated an increased thrombin potential in PRP in line with the loss of both APC and TFPI dependent activities.
Conclusions: These data demonstrate the unique role of pltPS in limiting thrombus growth.

Chuvash polycythemia in pregnancy, a case report and review of the literature

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Introduction: Erythrocytosis is mostly secondary or caused by clonal erythroid proliferation (polycythemia vera, PV) but genetic defects affecting the oxygen sensing mechanisms, Epo signaling, or hemoglobin affinity for oxygen can cause congenital erythrocytosis.
Methods: We describe diagnostic workup and management of this pathology in pregnant women, based on a case report and a review of literature.
Results/Case report: A Pakistani 27 year-old woman was referred to our center at 10 weeks gestation for erythrocytosis. This condition was known since childhood and present in relatives, suggesting a congenital cause rather than MPN. Accordingly, JAK2 mutations were not found and Epo levels were high (355 pg/ml, N: 26–143). Blood gas analysis was normal, including p50 (27.3 mm Hg, N: 24.7–28.6). Homozygote R200W mutation in the Von Hippel Lindau (VHL) gene led to the diagnosis of Chuvash polycythemia. Therapy with low dose aspirin and phlebotomies to maintain Ht <50% was initiated while monitoring iron reserve to avoid severe deficiency. Initial fetal growth was normal (P50-P90) without sign of placental insufficiency. LMWH was added at 24 weeks gestation after her admission for threatened preterm birth. She delivered a boy at 27 weeks (weight P10-25 for gestational age and Hb in normal range). Placenta pathology showed chorioamnionitis but no sign of thrombotic event. In PV, reported fetal complications include miscarriage (~20%), perinatal mortality (~20%), and preterm birth (~10%) (Robinson. Hematologica, 2005). Maternal complications are rarer, mostly thrombotic and hemorrhagic events. We found 15 pregnancies in 9 patients with congenital erythrocytosis, including the present case, showing 1 first trimester miscarriage, 5 IUGR and/or preterm birth (3 caused by non-hematological factors: twin pregnancy, infection), and 1 maternal thrombotic event.
Conclusion: Pregnancy in congenital erythrocytosis, managed with phlebotomies and low dose aspirin, seems to be associated with fewer complications than in PV although literature data is limited.

Monitoring of unfractionated heparin in clinical practice

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Background: Monitoring of unfractionated heparin (UFH) is recommended by all scientific guidelines. There is however no consensus about the optimal laboratory test to be used in clinical practice.
Objectives: We aimed to investigate the accuracy, reproducibility, and costs of different laboratory assays for monitoring of UFH in clinical practice and to study test utilisation in Switzerland.
Methods: Samples of 254 consecutive patients referred for UFH monitoring in a primary care hospital were analysed with activated partial thromboplastin time (aPTT), thrombin time (TT; high and low thrombin concentration), prothrombinase-induced clotting time (PiCT), and anti-Xa activity. Association with heparin concentration was determined, reproducibility assessed, and costs according to the Swiss health care system calculated. A survey among Swiss hospitals and laboratories was conducted.
Results: In relation to anti-Xa activity, spearman's correlation coefficient (r_s) was 0.68 (95%CI 0.60, 0.75) for aPTT, 0.79 (0.69, 0.86) for TT, and 0.94 (0.93, 0.95) for PiCT. Correlation (r_s) between anti-Xa activity and heparin concentration as determined by spiking plasma samples was 1.0 (1.0, 1.0). Coefficient of variation was at most 5% for PiCT and anti-Xa activity (within-run as well as day-to-day imprecision). Total costs per test were CHF 23.40 for aPTT, CHF 33.30 for TT, CHF 15.70 for PiCT, and CHF 24.15 for anti-Xa activity. Swiss institutions implemented aPTT in 53.2%, TT in 21.6%, anti-Xa activity in 7.2%, PiCT in 1.4%, and more than one test in 16.6%.
Conclusions: Accuracy and reproducibility of PiCT and anti-Xa activity for monitoring of UFH was superior and analytical costs were lower or equal to aPTT and TT. Widespread implementation of PiCT and anti-Xa activity in clinical practice has the potential to improve patient care and reduce health care costs.

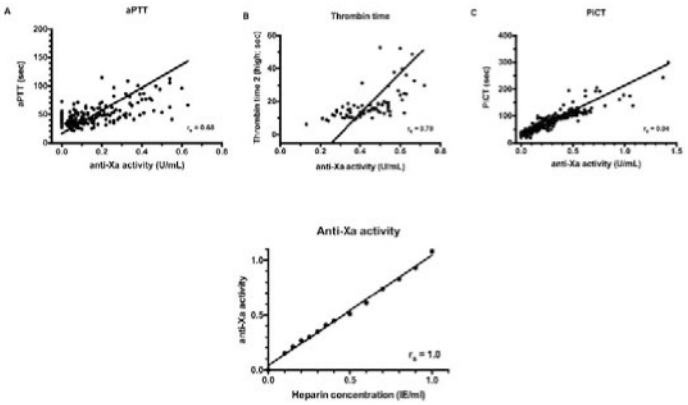

Table 1: Accuracy of the various laboratory tests for heparin monitoring in clinical practice

Assay	Observations	Spearman's correlation coefficient (95% CI)			Regression coefficients[¶]
		All samples	*Unfrozen only*	*Frozen only*	
aPTT*	193	0.68 (0.60, 0.75)	0.62 (0.36, 0.79)	0.71 (0.63, 0.78)	Y=158.6*X+22.3
TT 1 (low)*	163	0.73 (0.65, 0.79)	0.79 (0.66, 0.87)	0.71 (0.60, 0.79)	Y=173.7*X+1.86
TT 2 (high)*	87	0.79 (0.69, 0.86)	0.72 (0.54, 0.84)	0.85 (0.74, 0.92)	Y=94.0*X–24.2
PiCT*	254	0.94 (0.93, 0.95)	0.96 (0.94, 0.97)	0.93 (0.91, 0.95)	Y=189.5*X+24.6
Anti-Xa°	14	1.0 (95% CI 1.0, 1.0)	N/A	N/A	Y=1.005*X+0.03977

aPTT, activated partial thromboplastin time; TT 1, thrombin time 1 (3 U/mL bovine thrombin), TT 2, thrombin time 2 (7.5 U/mL bovine thrombin); PiCT, prothrombinase-induced clotting time;
[¶]Linear regression according to the Deming procedure was used
* with regard to anti-Xa activity
° with regard to a calibration curve derived from dilution studies using heparin-spiked plasma samples

Table 2: Reproducibility of different laboratory tests for heparin monitoring in clinical practice

Assay	Within-run variability	Number of measurements	Day-to-day variability	Number of measurements
	CV		CV	
aPTT	5.7%	10	3.7%	15
TT 2 (high)	14.7%	10	6.1%	15
PiCT	2.5%	10	1.1%	15
Anti-Xa	3.4%	10	5.0%	15

aPTT, activated partial thromboplastin time; CV, coefficient of variation (%); TT 1, thrombin time 1 (3 U/mL bovine thrombin), TT 2, thrombin time 2 (7.5 U/mL bovine thrombin); PiCT, prothrombinase-induced clotting time

Rapid centrifugation for the routine haemostasis laboratory

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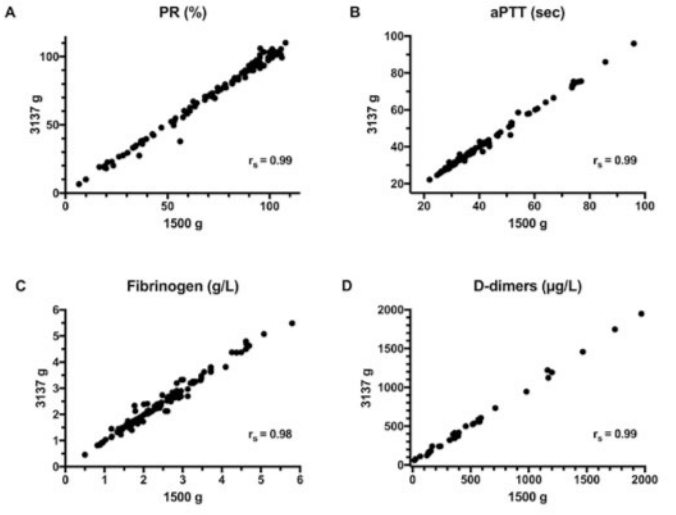
Background: Implementation of full laboratory automation requires short and uniform centrifugation schemes. It is however unclear if this is applicable to the haemostasis laboratory. We aimed to assess the accuracy of measurements obtained with a rapid, high-speed centrifugation scheme in a comprehensive set of haemostasis parameters, and covering the full range of values obtained in clinical practice.
Methods: Two citrated plasma samples were obtained from consecutive patients with suspected abnormal haemostasis parameters and processed with two centrifugation schemes in parallel: 1500 g for 10 minutes and 3137 g for 7 minutes. The following tests were conducted: prothrombin time (n = 125), INR (n = 146), activated partial thromboplastin time (n = 119), thrombin time (n = 105), fibrinogen (n = 125), D-dimers (n = 34), antithrombin (n = 31), anti-Xa activity (n = 30), von Willebrand antigen (n = 25), von Willebrand activity (n = 27), and factors (f)II (n = 69), V (n = 64), VII (n = 64), X (n = 67), VIII (n = 55), IX (n = 37), XI (n = 35), and XIII (n = 20). The flow of the samples is shown in figure 1.
Results: Spearman's rank correlation coefficient was at least 0.95 for all tests but fV (0.92; 95% CI 0.86, 0.95), fIX (0.84; 0.70, 0.91) and fXI (0.79; 0.61, 0.89). A relevant bias of agreement was observed for fVIII (–17.5; 95%CI –22.3, –12.7). Higher or lower limits of agreement were above 15% in case of fV, fVII, fX, fVIII, fIX, fXI, and fXIII as well as von Willebrand activity (see Figure 2 and fig. 3).
Discussion: Whereas a high concordance of haemostasis measurements obtained using a rapid, high-speed centrifugation scheme with an established low speed centrifugation scheme was observed for many routine parameters, some discrepancies were noted in case of fV, fVIII, fIX, and fXI.



Mutational epidemiology of congenital fibrinogen disorders

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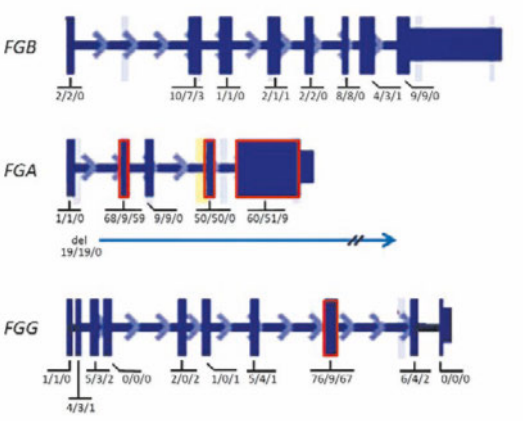
Background: The worldwide allelic distribution of the >250 identified causative mutations of *FGA*, *FGB* or *FGG*leading to congenital fibrinogen disorders (CFD) is not well characterized.
Objectives and patients: Our aim was to evaluate the genetic distribution of CFD mutations in order to develop a genotyping strategy, using data from 266 unrelated CFD patients genotyped at our laboratory (74 afibrinogenemia, 44 hypofibrinogenemia, 141 dysfibrinogenemia and 7 hypodysfibrinogenemia).
Results: Overall, 345 mutated alleles were identified among 187 heterozygous, 63 homozygous and 16 compound heterozygous individuals. Afibrinogenemia was almost always caused by null mutations (98.6%), mainly in *FGA* (85%). Hypofibrinogenemia was mainly caused by missense mutations of *FGB* or *FGG* (54.2%). Dysfibrinogenemias was almost always caused by heterozygous missense mutations (99.3%) in *FGA* and *FGG*. Hotspot mutations were prevalent among quantitative (33.1%) and qualitative fibrinogen disorders (71.1%) and identified in five continents. We found specific mutational clusters in some geographic regions. Screening of *FGA* exons 2, 4, 5 and *FGG* exon 8 and search for the 11kb deletion of *FGA* led to the identification of 80% of mutated alleles.
Conclusions: Using these findings, we propose a step-wise screening strategy that yields a genetic diagnosis in the majority of patients analyzing a minimal number of exons.

Table 1. Demographic and genetic characteristics

	Overall (n=266)	Afib (n= 74)	Hypo (n= 44)	Dysf (n= 141)	HDysf (n= 7)
Demographic					
Men, n (%)	100 (37.6)	35 (47.3)	10 (22.7)	54 (38.3)	1 (14.3)
Age at diagnosis, years, mean (SD)	22.6 (19.22)	3 (4.3)	22.6 (14.9)	32.8 (17.5)	23.4 (10)
Residence					
Europe, n (%)	213 (80.1)	44 (59.5)	33 (75)	130 (92.2)	6 (85.7)
America, n (%)	11 (4.1)	6 (8.1)	3 (6.8)	2 (1.4)	0 (0)
Africa, n (%)	18 (6.7)	9 (12.1)	2 (4.5)	6 (4.3)	1 (14.3)
Asia, n (%)	22 (8.3)	15 (20.3)	5 (11.4)	2 (1.4)	0 (0)
Oceania, n (%)	2 (0.8)	0 (0)	1 (2.3)	1 (0.7)	0 (0)
Status					
Heterozygosity, n (%)	187 (70.3)	0 (0)	40 (90.9)	140 (99.3)	7 (100)
Homozygosity, n (%)	63 (23.7)	58 (78.3)	4 (9.1)	1 (0.7)	0 (0)
Compound het., n (%)	16 (6)	16 (21.7)	0 (0)	0 (0)	0 (0)
FGA, n (%)*	207 (60.0)	126 (85)	13 (27.1)	66 (46.5)	2 (28.6)
Missense mutations, n (%)	64 (18.6)	0 (0)	3 (6.2)	61 (43.0)	0 (0)
Nonsense mutations, n (%)	43 (12.5)	37 (25.0)	4 (8.3)	0 (0)	2 (28.6)
Splice site mutations, n (%)	49 (14.2)	43 (29.0)	5 (10.4)	1 (0.7)	0 (0)
Frameshift mutations, n (%)	27 (7.8)	23 (15.5)	0 (0)	4 (2.8)	0 (0)
Large deletions, n (%)	24 (7.0)	23 (15.5)	1 (2.1)	0 (0)	0 (0)
FGB, n (%)*	38 (11.0)	20 (13.5)	13 (27.1)	4 (2.8)	1 (14.3)
Missense mutations, n (%)	16 (4.6)	2 (1.4)	9 (18.8)	4 (2.8)	1 (14.3)
Nonsense mutations, n (%)	12 (3.5)	10 (6.8)	2 (4.2)	0 (0)	0 (0)
Splice site mutations, n (%)	5 (1.4)	4 (2.7)	1 (2.1)	0 (0)	0 (0)
Frameshift mutations, n (%)	5 (1.4)	4 (2.7)	1 (2.1)	0 (0)	0 (0)
Large deletions, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
FGG, n (%)*	100 (29.0)	2 (1.4)	22 (45.8)	72 (50.7)	4 (57.1)
Missense mutations, n (%)	89 (25.8)	0 (0)	17 (35.4)	70 (49.3)	2 (28.6)
Nonsense mutations, n (%)	1 (0.3)	0 (0)	0 (0)	0 (0)	1 (14,3)
Splice site mutations, n (%)	7 (2.0)	2 (1.4)	4 (8.3)	0 (0)	1 (14.3)
Frameshift mutations, n (%)	3 (0.9)	0 (0)	1 (2.1)	2 (1.4)	0 (0)
Large deletions, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

*: among the mutated alleles (n=345)



Thrombin generating capacity in plasma of elderly patients with venous thromboembolism

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**Equal contribution*

Background&Aim: Predicting the risk of recurrent VTE is a major health issue because thrombosis is subject to a broad range of triggers. Global coagulation assays such as Calibrated Automated Thrombogram (CAT) have been proposed to quantify the composite effect of multiple risk factors. We aimed at investigating the relationship between recurrent VTE and mortality with thrombin generation (TG) in a prospective cohort of elderly VTE patients.
Methods: 565 patients aged ≥65 years 12 months after the first acute, symptomatic VTE episode were selected from the study. TG was measured for each patient sample w/wo exogenous activated protein C (APC) and thrombomodulin (TM), both risk factors for VTE. Statistical analysis was performed separately for patients not under anticoagulation (notAC, n = 232) versus patients under anticoagulation (AC, n = 333). Clinical outcomes were VTE recurrence and mortality.
Results: While AC and notAC patients did not present a different distribution for APC resistance, TM resistance showed a more dispersed distribution for both populations. However, both high APC and TM resistance were not associated with an increased mortality or VTE recurrence.
Conclusion: Despite APC and TM resistance are considered independent risk factors for VTE, we did not find an association with VTE recurrence or mortality in this cohort. Due to the small size of the sample, we might have failed to detect an effect on the selected clinical outcomes. A confirmation of our finding in a larger population is needed.

Utility of the ISTH bleeding assessment tool in patients with suspected inherited platelet function disorders

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Background: Bleeding assessment tools (BAT) has been widely implemented in the work-up of patients with suspected bleeding disorders. However, the utility regarding inherited platelet function disorders is still elusive.
Aim: We aimed to assess the diagnostic value of the BAT of the International Society on Thrombosis and Haemostasis (ISTH) in clinical practice.
Methods: Clinical characteristics and laboratory data of all consecutive patients referred between January 2012 and March 2017 to an outpatient unit of a university hospital with a suspected bleeding disorder were collected. The diagnostic work-up was done according to current recommendations and platelet function was tested using light transmission aggregometry as well as flow cytometry. All patients signed informed consent.
Results: Five hundred fifty-five patients were assessed, 68.1% were female, median age was 41.7 years (inter-quartile range [IQR] 28.0, 60.7). Definite platelet function disorder was diagnosed in 48 patients (8.7%), possible platelet function disorder in 70 patients (12.6%), von Willebrand disease or low von Willebrand factor associated with blood group 0 in 43 patients (7.8%), other coagulation disorders in 39 patients (7.0%), and other disorders in 38 patients (6.9%). Median scoring of the ISTH BAT was 2 in patients without bleeding disorder (IQR 1, 4), 4 in patients with possible platelet function disorder (3, 7), and 7 in patients with definite platelet function disorder (5, 9; p <0.0001; Mann-Whitney U test). Area under the ROC curve (AUC) was 0.80 (95% CI 0.74, 0.86). At a threshold of 4, sensitivity was 87.5% (95% CI 74.8, 95.3) and specificity 55.8% (95% CI 51.4, 60.2).
Conclusions: Presence of a platelet function disorder was associated with relevantly higher BAT scorings compared to patients without an identifiable disorder. Our data suggest that BAT might be a useful screening tool for platelet function disorders.

Dichotomous cytosolic mobilization of calcium, sodium, and potassium ions during procoagulant COAT platelet formation

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Background: The combined activation of platelets (PLTs) with **co**llagen **a**nd **t**hrombin induces the formation of procoagulant COAT PLT subpopulation, which retain a coat of prohemostatic α-granule proteins on their surface and express negatively charged phospholipids. While the clinical relevance of COAT PLTs is increasingly recognized, dichotomous intracellular signaling pathways that generate a COAT PLT subpopulation with procoagulant activity instead of traditional aggregation endpoints are still not fully elucidated. As cytosolic ions play important roles as secondary messengers during PLT activation, we developed a continuous flow cytometry kinetic monitoring of calcium, sodium and potassium fluxes to follow the generation of procoagulant COAT PLTs.
Methods: PLTs were preloaded with different ion fluorescent indicators: Fluo-3 AM for calcium, Asante NaTRIUM Green-2 AM for sodium or Asante Potassium Green-2 AM for potassium. After measurement of a stable baseline, PLTs were simultaneously activated with thrombin and convulxin (collagen receptor GPVI agonist) and each ion indicator fluorescence was continuously acquired over time on a flow cytometer up to 10 min. Annexin-V co-staining allowed identifying procoagulant COAT PLT subpopulation.
Results: Annexin-V co-staining revealed dichotomous cytosolic ion mobilizations starting about 2 minutes after an initial common phase. Procoagulant COAT PLTs demonstrated very high and sustained cytosolic calcium concentrations (up to micromolar range), decreased sodium concentrations after a transient increase, and important calcium-dependent potassium efflux. In non-COAT PLTs, calcium levels increased transiently in the high nanomolar range and then declined over time, while sodium levels increased strongly and stayed stable at high levels (>100 mM), and potassium efflux was slower compared to COAT PLTs.
Conclusion: We demonstrated characteristic dichotomous cation mobilization patterns following combined PLT activation by convulxin and thrombin. Our work reveals ion flux kinetics being peculiar to COAT PLT generation and diverging from an initial common aggregating response. This method allows investigating the differential modulation of biological messengers leading to procoagulant response and sharpens our ability to investigate procoagulant PLT pathophysiology.

Severe anemia after trans-catheter arterial chemoembolization – an unusual presentation of hemoglobin Zurich

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Background: Patients carrying the Hb Zurich anomaly show an increased disposition for hemolytic crises under oxidative stress that can be drug-induced by sulfonamides that bind directly to the enlarged binding pocket of Hb Zurich, leading to the formation of methemoglobin and to mechanical hemolysis.
Case description: Herein we describe a case of transfusion-dependent hemolysis in a 78 years old male patient, seven days after trans-catheter arterial chemoembolization (TACE) for hepatic metastasis of a small intestine carcinoid tumor. In immediate temporal relationship to hemolysis was the administration of nifedipine due to hypertensive crisis the night before the marked drop in hemoglobin (126 g/l pre-TACE to 51 g/l) was registered. Blood smear showed bite cells and blister cells and the Brilliant Cresyl Blue stains showed Heinz inclusion bodies (fig. 1). Transfusions and supportive measures led to

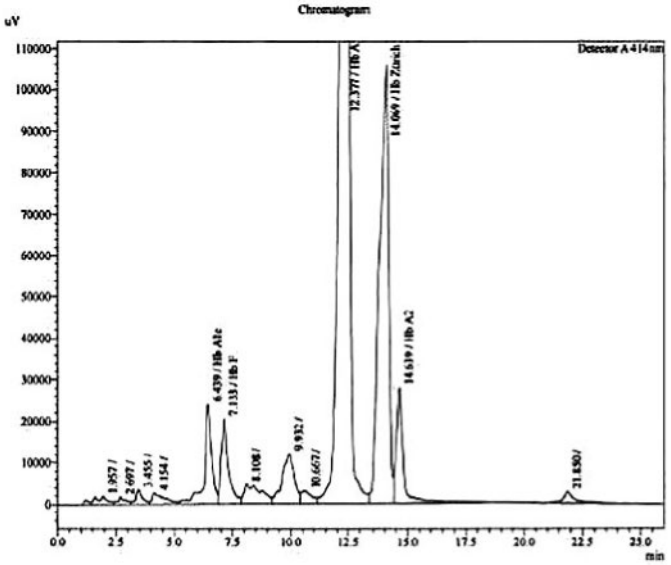

Figure 1
The patient's blood smear with bite and blister cells (upper) and the Brilliant Cresyl Blue stains showing Heinz inclusion bodies (lower).

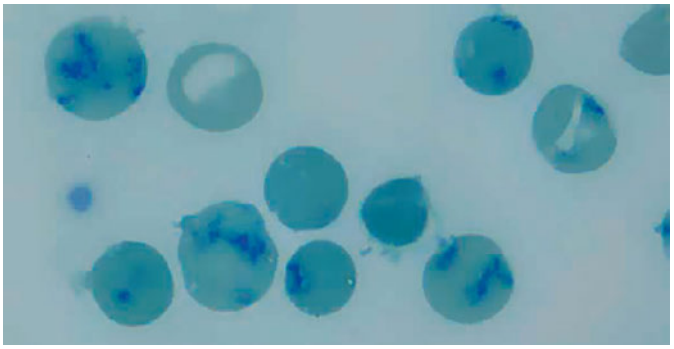

Figure 2
HPLC pattern of our patient with Hb Zurich.

rapid amelioration of the patient's condition and consecutively to timely hospital discharge. The HPLC showed a considerable peak at run time 14.069 minutes, coinciding with the natural migration profile of Hb Zurich (fig. 2). Furthermore, genetic sequencing of the beta globin gene verified the presence of a heterozygous mutation c.191 (A->G), establishing the diagnosis of Hb Zurich.
Conclusion: Hb Zurich was the first unstable hemoglobin to be analyzed structurally. Since the original description, several families with Hb Zurich in Switzerland, the United States, Brazil, and Japan have been described, the actual incidence is unknown. Patients with Hb Zurich are usually asymptomatic. Facing oxidant stress, methemoglobin concentration rises, leading to formation of Heinz bodies and hemolysis.

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

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Introduction: 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 aimed to develop a diagnostic algorithm able to accurately confirm or exclude HIT with a short laboratory turn-around-time (TAT).
Methods: Based on a previous study with 526 patients investigated for HIT suspicion (5.2014–12.2016), we developed an in-house diagnostic algorithm that relies on the Bayesian combination of pre-test clinical probability (4T score) and quantitative results of rapid immunoassays (IAs) for anti-PF4/heparin antibodies (AcuStar HIT-IgG and PaGIA-H/PF4). The functional gold-standard assay HIPA is performed in cases of high 4T score and/or any not negative IAs. We have prospectively validated this algorithm (1.2017–3.2018, n = 247).
Results: 100% negative (NPV) and positive (PPV) predictive cut-off values for a positive HIPA outcome were set at AcuStar HIT-IgG values of <0.13 U/l and >3.0 U/l, respectively. For H/PF4-PaGIA, the cut-off titers were <2 and >8, respectively. Likelihood ratios were determined for intermediate results. During this prospective validation study, AcuStar HIT-IgG was employed as a single IA in 178/247 (72%) of cases (TAT 30 min); H/PF4-PaGIA was performed as a second line IA in 69/247 (28%) of initially unsolved cases (TAT 60 min). Our Bayesian diagnostic approach could exclude HIT in 217/247 (87.9%) and correctly predict it in 22/247 (8.9%) of total suspected patients, leaving thus only 7/247 (2.8%) of unsolved cases after 60 minutes. In only one case, the prediction of our algorithm was not confirmed by HIPA. However, its clinical evolution was very suggestive of HIT.
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 at least 95% of patients with suspected HIT.

Impact of Rivaroxaban on Antithrombin Testing

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Introduction: For the diagnosis of hereditary antithrombin (AT) deficiency, a main genetic determinant of thrombophilia, AT activity is measured. Rivaroxaban (RVX), a direct oral anticoagulant, may interfere with AT testing when using methods based on the inhibition of activated factor Xa (FXa). For this reason, investigating AT in individuals treated with RVX could lead to false normal results, missing an AT deficiency.
Materials and Methods: In order to evaluate the interference of RVX on the measure of AT in our laboratory, we mixed plasma samples with AT deficiency (4 patients and 2 pathological controls) with proband samples with normal AT levels, before and after ingestion of RVX. This allowed knowledge of the expected AT activity in the mixed samples with RVX. The calculated RVX levels in the mixed samples were between 4 and 392 ng/mL (n = 40).
Results: The presence of RVX in the samples lead to a concentration-dependent overestimation of the AT activity up to 53%. An estimate by linear regression analysis indicated that AT activity was increased by 10.5% per 100 ng/mL RVX in plasma. However, with RVX concentrations below 30 ng/mL, the observed absolute increase of AT activity did not exceed 5%.
Conclusion: Our study shows that it is possible to use a method based on the inhibition of the activated factor Xa (FXa) in order to measure the AT activity in plasma samples with a RVX concentration up to 30 ng/mL.
For samples with higher RVX concentrations, it is recommended to use a method based on thrombin inhibition in order to accurately assess AT activity.

Utility of D-dimers in the diagnostic work up of heparin-induced thrombocytopenia (HIT)

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Introduction: The most widely used approach for diagnosing HIT is based on the combination of the 4T score and immunoassays (IAs) detecting anti-PF4/heparin antibodies. In our center, we employ a Bayesian diagnostic algorithm incorporating the 4T score and the magnitude of two sequential rapid IAs. Because HIT is characterized by an activation of the coagulation system, and based on our previous experience, we assessed whether the quantitative result of D-dimers could improve our diagnostic work-up.
Methods: We are currently conducting a prospective validation of our in-house diagnostic algorithm (1.2017–3.2018; n = 247). A confirmatory functional HIPA test is performed for either a high 4T score and/or any not negative IA result(s). Among this cohort, a D-dimer analysis was performed in 113 cases using the INNOVANCE D-Dimer on the CS-5100 System, Siemens. The diagnostic performance of D-dimers was evaluated by ROC analysis, allowing us to determine the AUC, the optimal cut-off value, and the 100% positive (PPV) and negative predictive (NPV) values.
Results: Among the 113 analyzed samples, HIT could be proven by a positive HIPA in 14 patients (12.4%). The AUC of the ROC curve was 0.853 and the optimal cut-off was identified at a D-dimer value of 3'655 ng/ml (sensitivity 100%, specificity 54%). The 100% negative predictive cut-off value (NPV) for a positive HIPA was therefore at 3'655 ng/ml, while the 100% positive predictive cut-off value (PPV) was set at 31'200 ng/ml. Of note, 51/99 (52%) of non-HIT samples were below the 100% NPV cut-off value and only 3/14 (21%) of HIPA positive samples were above the 100% PPV cut-off value.
Conclusion: According to our preliminary data, a D-dimer value below 3'655 ng/ml could be used to exclude HIT. As a note of caution, because of the low sample number the 95% confidence interval of the 100% NPV is wide (77–100%). High D-dimers do not predict a positive HIPA.

Utility of the ISTH bleeding assessment tool in patients with suspected platelet function disorders

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Background: Bleeding assessment tools (BAT) has been widely implemented in the work-up of patients with suspected bleeding disorders. However, the utility regarding inherited platelet function disorders is still elusive.
Aim: We aimed to assess the diagnostic value of the BAT of the International Society on Thrombosis and Haemostasis (ISTH) in clinical practice.
Methods: Clinical characteristics and laboratory data of all consecutive patients referred between January 2012 and March 2017 to an outpatient unit of a university hospital with a suspected bleeding disorder were collected retrospectively. The diagnostic work-up was done according to current guidelines and platelet function was tested using light transmission aggregometry as well as flow cytometry.
Results: Five hundred fifty-five patients were assessed, 68.1% were female, median age was 41.7 years (inter-quartile range [IQR] 28.0, 60.7). Definite platelet function disorder was diagnosed in 48 patients (8.7%), possible platelet function disorder in 70 patients (12.6%), von Willebrand disease or low von Willebrand factor associated with blood group 0 in 43 patients (7.8%), other coagulation disorders in 39 patients (7.0%), and other disorders in 38 patients (6.9%). Median scoring of the ISTH BAT was 2 in patients without bleeding disorder (IQR 1, 4), 4 in patients with possible platelet function disorder (3, 7), and 7 in patients with definite platelet function disorder (5, 9; p <0.0001; Mann-Whitney U test). Area under the ROC curve (AUC) was 0.80 (95% CI 0.74, 0.86). At a threshold of 4, sensitivity was 87.5% (95% CI 74.8, 95.3) and specificity 55.8% (95% CI 51.4, 60.2).
Conclusions: Presence of a platelet function disorder was associated with relevantly higher BAT scorings compared to patients without an identifiable disorder. Our data suggest that BAT might be a useful screening tool for platelet function disorders.

A 27 year old man with extensive postoperative hematomas and a rare genetic disorder

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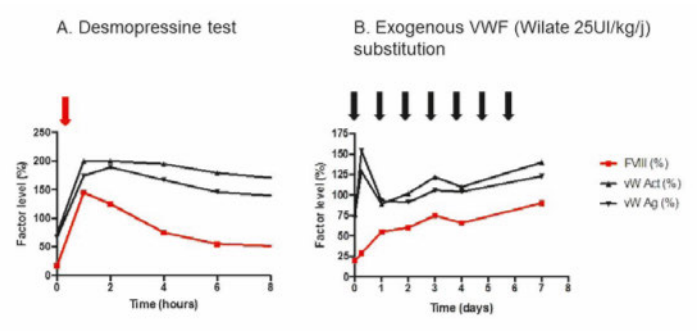
Introduction: Von Willebrand disease (VWD) manifests as quantitative (Types 1 and 3), or qualitative (Type 2) defects of VWF, including adhesiveness (Types 2A, 2B, 2M) and FVIII binding (Type 2N). In VWD 2N the half life of FVIII is reduced from ~12 to ~2 hours. Consequently, patients who have low levels of FVIII and a history of joint, muscle, and postoperative bleeding are often misdiagnosed with hemophilia type A.
Method: We report a recently diagnosed case of VWD 2N to outline diagnostic workup, as well as FVIII and VWF kinetics following desmopressin (DDAVP) and exogenous VWF administrations.
Results/Clinical Case: A 27 year old male was referred to our clinic with a history of repeated extended hematomas of the lower extremity following knee surgery. The ISTH-BAT bleeding score was non-pathological (3/56). Two independent measurements showed slightly prolonged aPTT (40–42 sec.) and markedly reduced activity of FVIII (16%–19%). The rest of the coagulation status was in the normal range including VWF antigen level and activity. The differential diagnosis of mild hemophilia A was ruled out based on i) severe reduction of VWF:FVIII binding assay (23%, reference range >60%), ii) abnormally rapid degradation of FVIII after DDAVP induced increase, as opposed to normal declining kinetics for VWF (fig. 1a), and iii) progressive FVIII level increase after exogenous VWF substitution (FVIII:VWF ratio of <1:10; 25 UI/kg/day) (fig. 1b). Diagnosis was confirmed by molecular genetic testing of the VWF gene showing two heterozygous missense mutations in exons 19 and 20, coding for the FVIII binding site.
Conclusion: Type 2N VWD is a rare genetic disease and misdiagnosis as hemophilia A is relatively frequent. Classical functional testing, however, allows for accurate diagnosis even prior to genetic testing.



Does spatial propagation of blood coagulation predict FXI bleeding phenotype?

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Backround: Bleeding phenotype in patients with FXI deficiency is not predictable with conventional laboratory tests, since FXI levels do not correlate with bleeding tendency. Thrombodynamics assay (TD) is a new global coagulation test that provides information on thrombin generation, clot formation and fibrinolysis. TD could be very useful in a clinical setting to prospectively identify patients with a higher bleeding risk, based on its ability to provide temporo-spatial information on initiation and amplification phases of blood coagulation. Our aim was to evaluate the ability of TD parameters to predict a FXI bleeding phenotype.
Methods: TD assay was performed by measuring thrombin generation (TG), fibrin clot formation and fibrinolysis upon activation with tissue factor immobilized on a plastic surface. We analyzed: 1) a pool of normal plasma spiked with increasing concentration of C7P, a specific inhibitor of FXI, 2) 7 plasmas from FXI patients exhibiting different phenotypes and 3) 15 plasmas from healthy donors. Images of clot growth were recorded every 15 seconds during 60 minutes and parameters describing clot formation, TG and fibrinolysis were calculated.
Results: C7P has a dose response effect on amplification phase of coagulation. In FXI deficient patients with bleeding phenothype, TD parameters describing the amplification phase of coagulation, (amplitude of stationary peak, Ast and rate of thrombin peak propagation, Vt) were reduced. Rate of clot growth (V) was lower or at the lower limit of normal range. In FXI deficient patients without severe bleeding, TD parameters were within normal range. In patients with thrombophilic phenotype, despite a severe FXI deficiency, Ast, Vt and V values were greatly increased; clot density did not correlate with one particular phenotype.
Conclusion: FXI-inhibitor C7P induced a slowdown and a shortening of the propagation phase of coagulation, similarly to FXI-deficient patients with a bleeding phenotype. TD Ast and Vt parameters seems useful laboratory parameters to assess the clinical phenotype of FXI-deficient patients, discriminating bleeding, non-bleeding, and thrombotic phenotypes.

Transient Antiphospholipid antibodies due to chickenpox in an adult female

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Introduction: In women with (recurrent) miscarriages an Antiphospholipid antibody syndrome (APS) should be excluded accordingly to the revised Sapporo/Sydney criteria. Since miscarriages even after the first nine weeks are a quite common phenomenon, several women undergo laboratory testing for Antiphospholipid antibodies (APLA). Transient APLA have been described after bacterial, viral (especially hepatitis C) and parasitic infections as well as after certain drugs. In our patient highly positive APLA were detected shortly after a primary varicella zoster virus (VZV) infection and an APS was suspected by the gynaecologist.
Case presentation: After delivering a healthy child at term our patient suffered three miscarriages (one at 13 weeks) and therefore met the clinical criterion for an APS. A first external analysis of APLA showed extremely elevated anti-beta2-glycoprotein IgM antibodies (427U/ml) and she was referred to our clinic with the suspicion of an APS. However, three weeks before this laboratory testing she had suffered a chickenpox infection and we therefore suspected transient APLA. Seven weeks after the infection the anti-beta2-glycoprotein IgM antibodies had decreased slightly; positive VZV IgM and IgG antibodies confirmed the clinical suspicion of a primary VZV infection. No clinical or laboratory signs of a thromboembolism were detected. Since the patient became pregnant shortly before our consultation and a true APS could not be excluded at that time, we established an antithrombotic treatment with dalteparin and aspirin. Finally 12 weeks

after the first detection of APLA the antibody titers dropped below 40U/l and – since the Sapparo criteria were not met – the antithrombotic treatment was stopped.
Conclusion: In the literature so far only two cases of adults with acute chickenpox and positive APLA have been described, both with thromboembolic complications [1, 2]. In the pediatric population the association is better known [3]. To prevent false diagnosis, unnecessary treatment and further (psychological) stress for these women a thorough clinical investigation for factors contributing to transient APLA is mandatory.

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Accuracy of heparin-induced platelet aggregometry (PAT) for the diagnosis of heparin-induced thrombocytopenia

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Background: Whereas the utility of platelet washed assays such as the heparin-induced platelet activation assay (HIPA) for the diagnosis of heparin-induced thrombocytopenia (HIT) is regarded as high, is the performance of simpler assays such as the heparin-induced platelet aggregometry (PAT) still elusive. We aimed to assess the accuracy of PAT for the diagnosis of HIT.
Methods: Frozen samples of a well-characterized cohort were further analyzed with HIPA. In this previously conducted single-center cohort study, 1291 consecutive patients with suspected HIT were included and samples were analyzed with PAT. The study population was mixed, median age was 67.9 years and 44% of the patients were female. Out of this cohort, 125 consecutive serum samples with a positive polyspecific PF4/heparin ELISA result were examined. HIPA was implemented as previously described and diagnosis of HIT was defined as a positive HIPA, that is a positive reaction in 2/4 donor platelets within 30 minutes.
Results: HIPA was positive in 40 out of 125 patients corresponding to a prevalence of 32%. Median OD (polyspecific ELISA) was 2.87 (IQR 2.4, 3.0) in patients with HIT and 0.79 (IQR 0.68, 2.37) in patients without HIT. The number of true positives was 28, the number of true negatives 85, the number of false-negatives was 12 and the number of false-positives 0. Thus, the sensitivity of PAT for the diagnosis of HIT was 70% and the specificity 100%.
Conclusions: Our investigation suggests that PAT is a valuable test to confirm HIT but it provides limited benefit in ruling-out HIT.

Algorithm-based effective recognition of the causes of isolated prolonged aPTT within large blood sample collections

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Objectives: Among the various causes of isolated prolonged aPTT, acquired hemophilia is a rare disorder for which accurate and prompt diagnosis still remains challenging. Consequently, there is a need for a structured approach allowing the rapid and reliable evaluation of the causes of an isolated prolonged aPTT within large blood sample collections on a daily basis.
Methods: We first performed a comprehensive analysis of 302 blood samples, representative of a typical hospital day using basic hemostasis screening tests (PT, aPTT, TT and anti-FXa activity) combined to the in-depth evaluation of each patient's clinical record and ongoing treatment (e.g. anticoagulation therapy). Based on these data, we next applied an in-house developed algorithm to extract via our database (in 15–20 min) those samples depicting normal PT but prolonged aPTT, present from the 1st day of hospitalization and by excluding therapeutic anticoagulation by anti-Xa. aPTT-related coagulant factors, combined to mixing assays were further performed.
Results: Our analysis revealed that the daily practice of clinical routine hemostasis can be divided into five major categories, including heparin (15%) and vitamin-K antagonist (19%) treatment follow-up, hepatic function follow-up (10%), as well as pre-operative/intervention (23%) and global hemostasis (27%) evaluations. Isolated prolonged aPTT only represented <1% (3/302) of all analyses with one case of acquired FVIII inhibitor. Applying an in-house algorithm, we next identified 52 out of 3235 blood samples with isolated elevated aPTT during 12 days of prospective screening. Among these samples, 52% were due to contact phase activation, confirmed by an underlying infectious disease condition, increased C-reactive protein and aPTT recovery over time. Further 8% were due to pre-analytic artifacts, 12% to FXII deficiency, while 18% to FVIII, FIX or FXI deficiencies, including several known cases of congenital hemophilia. Finally, 12% had no clear etiology, possibly due to deficiency in prekallikrein and/or HMW kininogen. We did not find any lupus anticoagulant.
Conclusions: Our in-house algorithm allows the rapid and reliable identification of those rare cases of isolated prolonged aPTT (<1% of total analyses), among which a small fraction can cause clinically significant bleeding disorders.

Ongoing redistribution of dabigatran requires repetitive application of idarucizumab

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Background / introduction: Dabigatran is an increasingly used direct anticoagulant which does not require routine activity measurement. In severe bleeding or emergency surgery neutralization with a specific antidote can be mandatory.
Methods / results: We report a case of an elderly woman with severe and prolonged gastrointestinal bleeding due to pronounced accumulation of dabigatran used for secondary prevention of unprovoked pulmonary thromboembolism. At presentation, global haemostasis tests like INR, aPTT and TT as well as fibrinogen and factor FXIII activity were out of range. Diluted TT revealed a dabigatran activity of 2044 ng ml^{-1}. A first dose of 5 g idarucizumab reversed dabigatran activity immediately but bleeding continued. Additional ROTEM analysis showed prolonged clotting times (CT) before administration of idarucizumab which normalized after application. Fibrinogen activity had been normal from the beginning shown by maximal clot firmness (MCF). Dabigatran activity started to rise again 4 h after the initial idarucizumab administration and increased to a maximum of 551 ng ml^{-1} at 12 h after the first application of idarucizumab. Due to ongoing bleeding a further dose of 2.5 g idarucizumab was given which again led to a prompt decline of dabigatran activity. Additional local intervention by epinephrine injection achieved no stable haemostasis. In order to stop bleeding we administered idarucizumab at a cumulative dose of 15 g within 84 hours because dabigatran continually redistributed from the extravascular space after each application of idarucizumab as shown by measuring activity as well as free and total dabigatran concentration.
Conclusion: Repetitive administration of idarucizumab might be necessary in patients with pronounced dabigatran accumulation. Different molecular weights, distinct volume distributions and half-lives of the molecules lead to ongoing redistribution phenomena. Dabigatran influences laboratory test differently by thrombin inactivation.

Next generation viscoelasticity assays in cardiothoracic surgery: feasibility of the TEG6s system

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Background: Viscoelastic assays are regarded as important laboratory tests for the monitoring of perioperative hemostasis. Current devices are however associated with important drawbacks, a limited consistency in particular.
Aim: We aimed to assess the feasibility of a new, resonance-based viscoelastic method utilizing the cartridge system in cardiothoracic surgery.
Methods: Twenty-four consecutive patients undergoing major cardiothoracic surgery with extracorporeal circulation (ECC), and managed according to current guidelines using ROTEM® delta were followed in a prospective evaluation study. TEG®6s values were determined in parallel with ROTEM® before ECC (pre-ECC), during ECC (ECC), and after protamine reversal (post-ECC).
Results: Among the 24 patients included, 7 underwent composite graft implantation (29%), 5 received coronary artery bypass grafting (21%), 2 isolated valve surgery (8%), 4 combined operation (17%), and 6 others (25%). TEG®6s provided quantifiable results in most patients pre-ECC and post-ECC, but only R (clotting time) of CKH (kaolin with heparinase) was measurable during ECC (full heparinization). Results are shown in figure 1 (A–C). Spearman's correlation coefficient was 0.8 for fibrinogen levels and MA CFF (functional fibrinogen). Correlation of several TEG®6s parameters was high (0.8 to 0.9) with MCF FIBTEM and low with prothrombin time,

activated partial thromboplastin time, and platelet count. Accuracy of MA CFF for detection of fibrinogen deficiency <1.5 g/L was high (ROC-AUC 0.92).
Conclusions: The TEG®6s device utilizing the resonance viscoelastic test system appears to be a promising concept in cardiothoracic surgery. However, the utility is limited during full heparinization and larger studies are needed before widespread implementation.

Universal anti-Xa assay for rivaroxaban and apixaban plasma concentration

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Background: Fast determination of direct oral anticoagulant (DOAC) plasma concentration is essential in case of bleeding, urgent surgery, or acute stroke. Availability of drug-specific assays is limited to university or other large hospitals while assays calibrated for low molecular weight heparin (LMWH) are available to many smaller hospitals.
Study aim: Accuracy and applicability of a universal anti-Xa assay with LMWH calibrator for the determination of rivaroxaban (RXA) and apixaban (AXA) plasma concentration.
Study design: Retrospective investigation of 625 samples of 432 patients taking RXA and of 73 samples of 42 patients taking AXA. Samples were analyzed by anti-Xa assay calibrated for RXA or AXA, and simultaneously by anti-Xa assay calibrated for LMWH. In addition, normal plasma spiked with RXA or AXA at increasing concentrations was analyzed likewise. Spearman's correlation coefficient (rs) was calculated, and linear regression according to Deming procedure.
Results: For patient samples, (rs) of DOAC-specific assay and LMWH assay was 0.96 for RXA and 0.98 for AXA. Equation for the calculation of DOAC concentrations was y = 128*x-15 for RXA and y = 116*x-21 for AXA. Correlation was less pronounced in samples with very low DOAC concentrations (<50 µg/L). For the spiked samples, (rs) was 1.0 for RXA and for AXA.
Conclusion: Our results indicate that accurate determination of DOAC plasma concentration is feasible using a universal LMWH-calibrated anti-Xa assay. This could prove a valuable tool escpecially for smaller hospitals. A prospective study is needed which besides RXA and AXA should include patients treated with edoxaban.

Germline mutations in a cohort of bone marrow failure patients

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Introduction: Bone marrow failure (BMF) in children and adults is often supposed to be inherited, but in many cases the diagnosis remains unclear. From November 2017 to April 2018 we evaluated a cohort of 18 patients (pat) with BMF with unresolved diagnosis after medical evaluation.
Method: Patients samples were analyzed with NGS using a BMF panel including 63 genes for the diagnostic of Diamond-Blackfan Anemia, Telomeropathies, hereditary Anemia, hereditary Neutropenia and Fanconi Anemia. Only gene variants of the categories pathogenic, likely pathogenic and variant of unknown significance (VUS) according to ACMG guidelines 2015 are mentioned in this abstract. All gene variants (mutations and VUS) were validated by Sanger sequencing.
Results: Ten pat (56%) were male. Median age at first diagnostic and at genetic evaluation were 16 (1–68 years) and 32 (17–73 years) respectively. All pat had chronic cytopenias: 5 (28%) had isolated neutropenia; 3 (17%) had pure red cell aplasia; 3 pat (17%) had bicytopenia and 7 pat (39%) presented with pancytopenia. Two cases presented ≥5% bone marrow blast cells. Telomere length was measured in 8 pat, all presented very short telomere length. In 5 pat, Fanconi anemia was excluded. Three of the 18 patients carried known pathogenic mutations (ELANE, HAX1 and WAS), 11 pat carried at least one VUS, and 4 had no gene variants rated as relevant. In two cases the genetic results allowed to confirm the diagnosis of congenital neutropenia. A WAS mutation was found in a patient with neutropenia, however, this mutation is described in the literature as associated with thrombocytopenia. In patients with suspected Fanconi anemia or presenting features of Dyskeratosis Congenita, the finding of VUS was particularly difficult to interpret. Within genes frequently carrying VUS we found CUBN, BRCA2, and SEC23B.
Conclusions: This result broadens the molecular and clinical portrait of BMF syndromes and contributes to the recognition of disease entities. Using a high-throughput sequencing screen to implement precision medicine can improve patient management and counseling.

Clustering of platelet glycoprotein Ib is inhibited by alpha-linolenic acid as revealed by cryo-electron tomography

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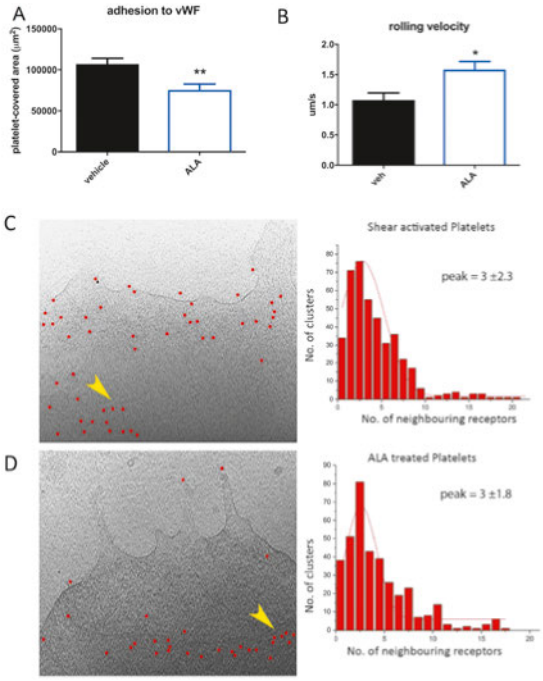
Background: The plant-derived omega-3 fatty acid (n-3 FA) alpha-linolenic acid (ALA) is an abundant alternative to marine-derived n-3 FA with anti-thrombotic properties. In this study, we investigated the impact of ALA on human platelet binding to von Willebrand factor (vWF) under high-shear flow conditions, and GpIb clustering as a potential mechanism of ALA inhibition.
Methods: Human platelets were pre-incubated with vehicle or ALA 30 µM (1 hour at r.t.) before being flowed at 100 dyn/cm^2 over vWF. GpIb distribution was analysed by cryo-elecron tomography by staining resting and sheared platelets and calculating the amount of "neighbors" for each GpIb molecule, defined as the number of gold particles within a radius of 50 nm from each GpIb.
Results: Pre-incubation of whole blood with ALA reduced platelet adhesion to vWF under high-shear flow (area: 106'963 ± 15'892 µm^2 vehicle vs 75'519 ± 16'254 µm^2 ALA, n = 6 p = 0.004). Analysis of GpIb by cryo-ET revealed the formation of GpIb clusters upon shear-activation of platelets (GpIb molecules with more than 15 neighbors) which were not present in non-activated platelets. Pre-incubation with ALA reduced the formation of the biggest complexes (more than 17 neighbors).
Conclusion: Our data show that, under high-shear, platelet GpIb forms high-density clusters of 15–20 complexes in close proximity, possibly representing binding units to multimeric vWF. Pre-incubation with ALA reduces the formation of the larger complexes, explaining at the functional level the reduced platelet adhesion to vWF at high-shear flow. These data provide insight into the mechanisms of the anti-thrombotic effects of ALA in the early phase of arterial thrombosis, making this n-3 FA an attractive cardioprotective agent.



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