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Basel (Switzerland), November 22–24, 2023

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6TH SWISS ONCOLOGY AND HEMATOLOGY CONGRESS (SOHC)

BASEL, NOVEMBER 22–24, 2023

TABLE OF CONTENTS

SSH BEST ABSTRACT & AWARD SESSION – HEMOSTASIS, TRANSFUSION MEDICINE, VASCULAR, LABORATORY MEDICINE, BENIGN HEMATOLOGY

185

Modified Rotating Bed Reactor Compatible with an Industrializable and Scalable Manufacturing of Lab-Grown Platelets

M. Humbert[1], A. Aliotta[2], T. Badalli[2], V. Tâche[2], M. Després[3], Y. Grand[2], P. Jeandet[2], V. Boand[2], C. Rocca[2], F. Khechana[3], T. Braschler[4], O. Naveiras[1,5], E. Dahan[3], L. Burnier[2]

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Introduction: The increasing demand for platelet (PLT) transfusions requires a safe alternative to blood and PLT donation. In vitro production of platelets is currently explored, but achieving a commercially viable solution is hindered by the limitations of large-scale production and costs. This paper introduces a scalable solution that efficiently produces one platelet concentrate (PC) in under two hours, starting from mature megakaryocytes (MK).

Methods: Human CD34+ cells were seeded in a xeno-free medium containing thrombopoietin to differentiate into MK, and transferred into HemostOD's modified rotating bed reactor – consisting of a pod containing microstructures – in a 200 mL bioreactor, for 2 hours at 900 RPM. Control PLTs were both from healthy donors (blood-PLT) or generated using a microfluidic chip (CHIP-PLT). PLT activation was done using ADP + PAR1 agonist peptide and analyzed by flow cytometry (FC). The pharmacokinetic study was done in immunodeficient clodronate-treated mice. Desialylation was measured using R. communis agglutinin. PLT (CD41+CD42b+) were counted by FC.

Results: PLT generated in HemostOD's bioreactor (HOD-PLT) were compared to CHIP-PLT and to blood-PLT. At resting state, 15.2+/-4.1% of CHIP-PLT were CD62P positive, while only 6.6+/-4.0% of HOD-PLTs were positive (mean+/-SD, n = 3, P = 0.057, 2-way ANOVA with Tukey multiple-comparisons test). Phosphatidylserine exposure was significantly reduced in HOD-PLTs (5.1+/-1.1%) compared to CHIP-PLTs (16.5+/-1.3%, P = 0.01). Upon activation, PAC-1 binding and release of CD62P were increased in HOD-PLTs compared to CHIP-PLT (53.5+/-1.0% and 46.0+/-6.6% versus 24.0+/-4.1% and 32.0+/-1.1%, P<0.01). Sialylation (resting) and markers after activation of HOD-PLTs were similar to blood-PLT. After infusion in mice, survival of HOD-PLTs and blood-PLTs were also similar (half-life of 22+/-2 h and 17+/-2.0 h, respectively). Finally, 5,5*10^10 PLTs were produced using 4,5*10^9 MKs, reaching systematically a plateau between 90 and 120 minutes.

Conclusions: Functional platelets are generated in HemostOD's bioreactor in 2 hours, sharing characteristics with blood-PLTs. HemostOD's bioreactor in conjunction with HemostOD's proprietary human MK cell line thus has the potential to enable large-scale production of platelets for clinical use with an unlimited and stable source of megakaryocytes.

274

Genetic ablation of anticoagulant protein S promotes joint health in hemophilia A

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Introduction: Joint damage in hemophilia results from repeated intra-articular bleeds (IAB). With no treatment available to reduce blood-induced synovitis, bleeding prevention is crucial to maintain joint health, but a more targeted therapy is needed. We reported that genetic ablation of protein S (PS) protects hemophilic mice against bleeding, particularly IAB, and that PS reduction by a small interfering RNA prevents acute hemarthrosis in mice with hemophilia A (HA) and improves hemostasis in non-human primates with acquired hemophilia. Further, PS reduction enhanced thrombin generation in human models of HA ex vivo. Here, we characterized the effect of GA of PS on joint health using a mouse model of chronic hemarthrosis (CH).

Methods: HA (F8-/-), HA with genetic ablation of PS (F8-/-Pros1-/-) and WT mice were subjected to CH by 3 joint bleeds induced at 7 days apart. Histology, immunostaining, electron microscopy (EM), joint lavage analyses and RNA sequencing of the synovium were performed in steady-state (SS) and CH.

Results: During CH, joint diameters increased in F8-/- but not in F8-/-Pros1-/- and WT mice. Histology confirmed this result with neither bleeding nor synovitis in F8-/-Pros1-/- mice (Fig.1A). Iron deposition was minimal in F8-/-Pros1-/- with no cartilage erosion. The lining layer (LL) integrity was preserved in F8-/-Pros1-/- with CH as shown by histology and EM. In CH, macrophages were present in the LL of F8-/-Pros1-/- and WT mice, and the subLL of F8-/- mice (Fig.1B). Inflammatory cytokines level was lower in F8-/-Pros1-/- than in F8-/- joint lavage (Fig.1C). In SS, mRNA coding for proteins involved in bone resorption were decreased while those coding for cell communication and bone formation were increased in F8-/-Pros1-/- versus F8-/-mice. Post-CH, F8-/-Pros1-/- mice displayed a positive regulation of genes involved in macrophage activation, bone regeneration and tissue remodelling, and a negative regulation of genes involved in angiogenesis and fibroblast growth. Differently to F8-/- mice, F8-/-Pros1-/- mice synovium displayed increased expression of pleiotrophin (PTN) (Fig.1D), a secreted growth/differentiation factor involved in bone development and tissue repair. PTN was strongly expressed in synovium LL of F8-/-Pros1-/- compared to F8-/- and WT mice (Fig.1E).

Conclusions: These data identify PS as an important modifier of joint health in HA, at least partly via PTN.

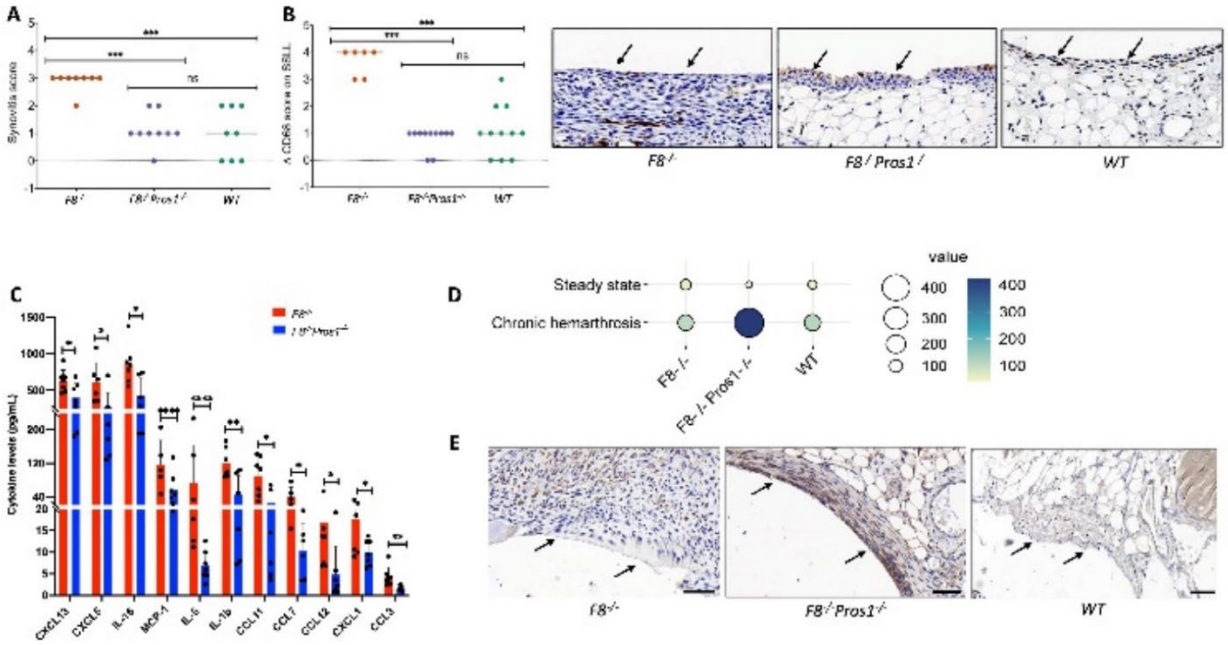

Figure 1. Protein S (PS) genetic ablation improves joint health in a mouse model of chronic hemarthrosis (CH). **A**: Synovitis score in *F8*$^{-/-}$, *F8*$^{-/-}$*Pros1*$^{-/-}$ and *WT* mice evaluated from knee histology after CH. **B**: quantification of macrophages in the sub-synovial lining layer ($p<0.001$). Knees histology sections showing the presence of macrophages in the synovial lining layer in *F8*$^{-/-}$*Pros1*$^{-/-}$ and *WT* but not in *F8*$^{-/-}$ mice (arrows). **C**: Inflammatory cytokine levels in *F8*$^{-/-}$ and *F8*$^{-/-}$*Pros1*$^{-/-}$ knee joint lavages after CH ($p<0.05$). **D**: RNA sequencing data of pleiotrophin (PTN) expression in *F8*$^{-/-}$, *F8*$^{-/-}$*Pros1*$^{-/-}$ and *WT* mice knee synovium at steady state (baseline) and after CH. **E**: Synovial lining layer histology of *F8*$^{-/-}$, *F8*$^{-/-}$*Pros1*$^{-/-}$ and *WT* mice immunostained for PTN, arrows show a strong positive staining in *F8*$^{-/-}$*Pros1*$^{-/-}$ but not in *F8*$^{-/-}$ and *WT* mice, scale bar: 50µm.

SSH/SSMO BEST ABSTRACT & AWARD SESSION – EXPERIMENTAL HEMATOLOGY / ONCOLOGY

183

Mutation-specific CAR T cells as precision cell therapy for IGLV3-21-G110R expressing high-risk chronic lymphocytic leukemia

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Introduction: Stereotyped immunoglobulin (IG) light chain rearrangements using the IGLV3-21 gene with G110R mutation define a subset of chronic lymphocytic leukemia (CLL) patients with aggressive clinical course, poor prognosis and limited therapeutic options. This G110R mutation is a highly specific driver in CLL and has the unique ability to confer autonomous B cell receptor (BCR) signaling via mediating homotypic BCR self-interactions. To advance treatment of these patients, we aimed to develop a chimeric antigen receptor (CAR) T cell product to selectively target the CLL-specific IGLV3-21-G110R neoepitope.

Methods: We translated the IG heavy and light chains of a murine IGLV3-21-G110R-targeting antibody into the single-chain variable fragment (scFv) format and generated murine and humanized second generation CAR constructs. Constructs were transduced into T cells from healthy donors and CLL patients. We performed in vitro co-culture assays using different cell models engineered to express BCRs with IGLV3-21-G110R light chain as well as primary healthy human B and CLL cells to assess killing and specificity via live cell imaging and IFN-γ secretion. We further tested epitope selectivity and safety in xenografts as well as two humanized mouse models after injection of healthy or CLL patient peripheral mononuclear cells (PBMCs) and CAR T cells.

Results: The generated scFv exhibited binding affinities identical to the original antibody. IGLV3-21-G110R targeting CAR T cells generated from healthy donors and CLL patients eradicated IGLV3-21-G110R expressing cell lines and primary CLL cells, but not polyclonal healthy B cells in in vitro co-culture assays as determined in live cell killing assays and IFN-γ secretion. We did not observe differences in efficacy between the murine or humanized CAR backbones. In vivo experiments showed substantially reduced outgrowth of transplanted cell lines or primary CLL patient PBMCs in xenograft models accompanied by prolonged survival and even disease eradication in 17% of mice. Treatment of humanized mouse models with G110R-specific CAR T cells did not reduce B counts after 7-17 days post-injection of PBMCs from healthy individuals.

Conclusions: We designed and demonstrated activity as well as selectivity and safety of the – to our knowledge – first truly tumor-specific, biomarker-driven cellular targeting approach for a hematological malignancy.

SSH/SSMO BEST ABSTRACT & AWARD SESSION – CLINICAL HEMATO-ONCOLOGY

234

Clinical significance of the CD19 L/V174 single nucleotide polymorphism on outcome after FMC63-CAR-T Cell therapy.

K. Seipel[1], M. Abbühl[1], U. Bacher[2], H. Nilius[3], M. Daskalakis[2], T. Pabst[1]

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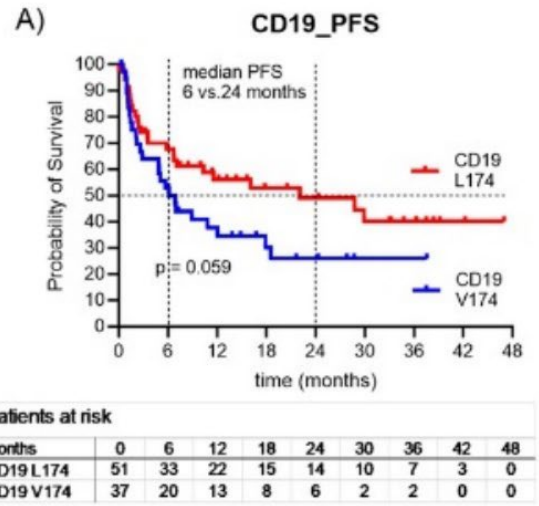
Introduction: Patients with relapsed or refractory diffuse large B-cell lymphoma (r/r DLBCL) undergoing FMC63 based chimeric antigen receptor (CAR)-T cell therapy have response rates of 63–84% with complete response in 43–54%. We hypothesized that common germline variants of the target antigen CD19 elicit differing responses to FMC63-CAR-T cell therapy.

Methods: This single-center retrospective observational study investigated eighty-eight r/r DLBCL patients with a minimum follow-up of at least six months after FMC63-CAR-T cell therapy. The sequence of the CD19 gene spanning exons 3 and 4 was retrospectively determined by Sanger sequencing of the genomic DNA isolated from peripheral blood mononuclear cells of the DLBCL patients before CAR-T cell therapy.

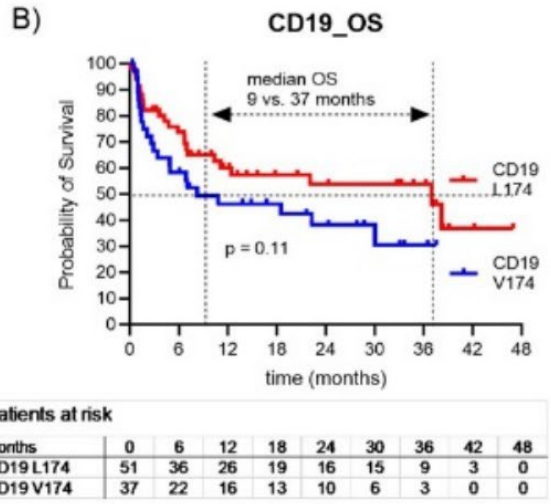
Results: The CD19 gene single nucleotide polymorphism rs2904880 encoding valine instead of leucine at amino acid position 174 of the CD19 antigen was prevalent in 51% of the studied DLBCL patients. Clinical outcome after CAR-T cell therapy was significantly different in CD19 L174 versus V174 carriers: the median progression-free survival was 24 vs. 6 months (p = 0.06), overall survival was 37 vs. 9 months (p = 0.11), complete response rates were 51% vs. 30% (p = 0.05), and refractory disease rates were 14% vs. 32% (p = 0.04).

Conclusions: The single nucleotide polymorphism rs2904880 encoding valine instead of leucine at amino acid position 174 (L174V) of the CD19 antigen affects treatment outcome in patients undergoing FMC63-anti-CD19-CAR-T cell therapy. The CD19 minor allele L174 predicts favorable treatment outcome. Presence of the CD19 minor allele L174 emerges as a potential prognostic factor in FMC63 CAR-T cell therapy. With a global minor allele frequency of 0.29 (ALFA), half of the patients are expected to carry the L174 allele. The minor allele frequency, however, may vary from 0.05 to 0.45 depending on ethnic origin (Asian 0.1, African 0.1, European 0.3, Latin American 0.4). Novel anti-CD19 CAR-T constructs based on other antibodies may improve treatment outcome in CD19 V174 homozygous DLBCL patients.



Patients at risk

months	0	6	12	18	24	30	36	42	48
CD19 L174	51	33	22	15	14	10	7	3	0
CD19 V174	37	20	13	8	6	2	2	0	0



Patients at risk

months	0	6	12	18	24	30	36	42	48
CD19 L174	51	36	26	19	16	15	9	3	0
CD19 V174	37	22	16	13	10	6	3	0	0

238

Pharmacoscopy-guided treatment for acute myeloid leukemia patients that have exhausted all registered therapeutic options – a novel approach to an unmet clinical need

J. Schmid[1], Y. Festl[2], Y. Severin[2], U. Bacher[3], M. Kronig[1], B. Snijder[2], T. Pabst[1]

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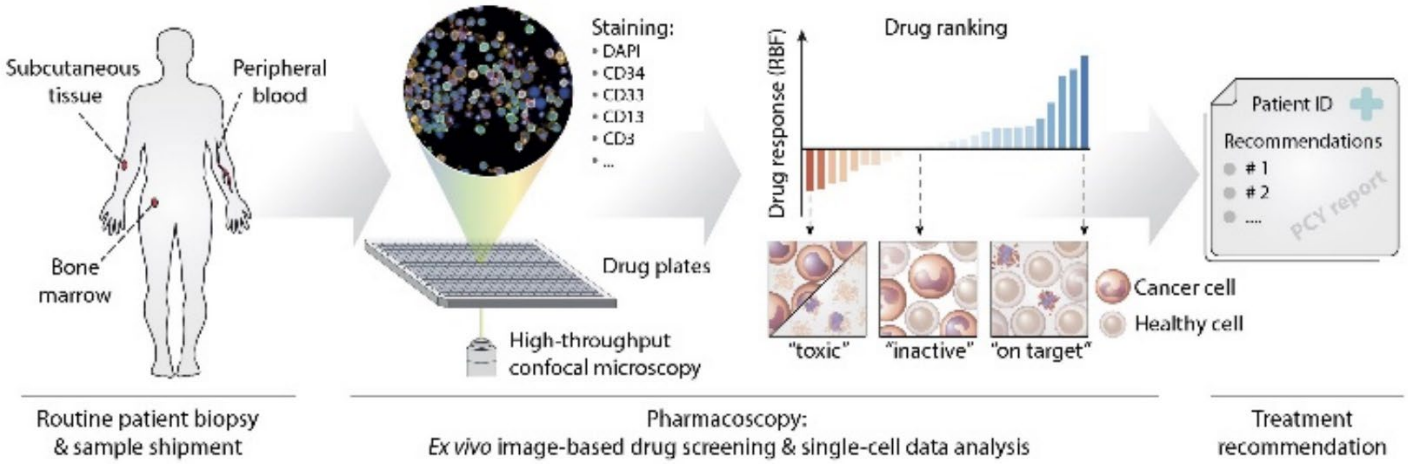
Introduction: Only few registered compounds are currently available for AML patients at relapse. The situation is aggravated by the rapidly progressive nature of AML at relapse which severely restricts the timeframe to select an optimal therapy regimen. Pharmacoscopy, an image-based ex vivo drug screening platform, has previously been suggested as a novel tool for treatment selection in a wide range of haematological malignancies but has never been prospectively tested in AML patients at relapse.

Methods: We subjected patient-derived leukemic samples to pharmacoscopy screening to generate treatment recommendations for 30 relapse settings of 24 AML patients who exhausted all standard therapeutic options (Figure 1). We evaluated if pharmacoscopy can be employed within the narrow timeframe available under an AML relapse setting, how often recommended regimens could be started with adequate financial coverage, and whether they provided durable clinical benefits.

Results: Pharmacoscopy provided therapy recommendations within a median of 5 days from sampling. In 17 out of 30 screening instances (56.7%), patients received a treatment recommended by pharmacoscopy. The top six most recommended drugs in descending order were navitoclax, venetoclax, omacetaxine, cladribine, carfilzomib and panobinostat. Patients receiving a recommended regimen showed higher rates of complete remission (OR 1.62, NS) and longer overall survival (11.41 vs. 6.29 weeks, NS) than those receiving a therapy chosen by conventional methods. A drug regimen's performance during the ex-vivo drug screen can be quantified by the integrated pharmacoscopy score (i-PCY). This score proved to be an excellent predictor of clinical response: Patients receiving a regimen with above-median i-PCY scores showed significantly higher rates of complete remission (OR 3.01, $p < 0.0005$) and significantly longer overall survival (28.6 vs. 8.4 weeks, $p < 0.006$).

Conclusions: Pharmacoscopy can successfully be included in the clinical decision-making process and provides valuable clues when selecting therapy protocols for AML patients at relapse. In the majority of cases, the recommended treatment protocols can be made available within an adequate timeframe under real-world conditions and such protocols prove clinically beneficial with higher rates of complete remission and longer overall survival.



SSH/SSMO BEST ABSTRACT & AWARD SESSION – CLINICAL SOLID TUMOR ONCOLOGY

218

First line (1L) durvalumab in patients with PD-L1 positive, advanced non-small cell lung cancer (NSCLC) with a performance status of 2 (PS2). Primary analysis of the multicenter, single-arm phase II trial SAKK 19/17

M. Mark[1], P. Froesch[2], K. Gysel[3], S. Rothschild[4], A. Addeo[5], C. Ackermann[6], S. Chiquet[3], M. Schneider[3], K. Ribi[3], A. Fischer[1], S. Bastian[1], R. von Moos[7], M. Joerger[8], M. Frueh[8]

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Introduction: The safety and efficacy of 1L durvalumab in PS2 patients (pts) with advanced NSCLC is unknown. Important safety data leading to exclusion of pts with relevant respiratory symptoms have been published as an interim report. Here we present the primary analysis of 1L durvalumab in PS2 pts, unsuitable for combination chemotherapy and PD-L1 expression in ≥25% of tumor cells.

Methods: In this single-arm, multicenter, phase II trial pts with PD-L1 positive (tumor proportional score, TPS ≥25%), advanced NSCLC with PS2, unsuitable for combination chemotherapy determined by the investigators, received a fixed dose of 1500 mg durvalumab every four weeks. The primary endpoint was overall survival (OS) at 6 months. The statistical hypothesis was to improve OS at 6 months from ≤35% to ≥53%. Adverse events (AEs) were assessed according to NCI CTCAE version 5.0.

Results: Forty-eight pts were included (29 males, 19 females). Median age was 76 years (range, 37-87). OS at 6 months was 60% (95% CI: 45-74%). OS at 6 months after the exclusion of pts with initially relevant respiratory symptoms was 67% (95% CI: 46-84%, n = 27) compared to the subgroup of pts without this exclusion criteria who were recruited before the amendment (52%, 95% CI: 30-74%, n = 21). Median OS was 8.5 months (95%CI: 4.4-16.7). Objective response rate and median PFS were 17% (95% CI: 8-30%) and 2.5 months (95% CI: 1.8-7.1). Thirty-three deaths (69%) were observed to date. Ten early fatal events considered not treatment-related occurred during the first 5 weeks of treatment. Four out of the first 7 early fatal events (4/7; 57%) were respiratory failures in pts with advanced symptomatic primary lung tumors. Only 3 more early fatal events occurred after the protocol amendment excluding pts with severe respiratory symptoms. Thirty-nine patients (81%) had an AE grade ≥3 (G3). The most frequent AEs ≥G3 were lung infection (19%), dyspnea (15%) and hypertension (10%), respectively. Treatment-related AEs ≥G3 were reported in 9 pts (19%) and included colonic perforation in one patient (grade 5), colitis in 5 pts (10%), hepatitis and increased lipase in 3 pts each (6%).

Conclusions: 1L durvalumab in PS2 pts with advanced PD-L1 positive (TPS ≥25%) NSCLC is effective and led to a promising 6-month OS of 60%. Four-weekly durvalumab can be safely offered to pts presenting without severe pulmonary symptoms who are not candidates for chemotherapy

ONCOREHA/OPS/PALLIATIVE.CH/SOHC BEST ABSTRACT & AWARD SESSION – SUPPORTIVE & PALLIATIVE CARE, REHABILITATION & SURVIVORSHIP

180

Assisted Suicide in Patients with Pancreatic Cancer: Swiss Data from a 20-year Experience (1999-2018)

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Introduction: The legalization of assisted suicide (AS) is one of the most debated topics in the field of medical ethics worldwide. There is limited data on the long-term development and trends of patients who underwent AS due to pancreatic cancer (PC).

Methods: By using data of the Swiss Federal Statistical Office over a 20-year period from 1999-2018, our analysis is based on:

- All death cases: n = 1´261´923; median age at death: 82 years (Y)

- All cancer-related death cases: n = 323´610 (♂: 55%/♀: 45%); 25.6% of all death cases; median age at death: 74Y.

- All PC-related death cases: n = 21´891 (♀: 52%/♂: 48%); 6.8% of all cancer deaths, and 1.7% of all death cases; median age at death: 74Y.

- All AS death cases: n = 8´738 (♀: 57%/♂: 43%); median age at death: 78Y.

Results: The most common underlying condition for AS was cancer; n = 3´580 (♂: 51%/♀: 49%), 41.0% of all AS cases. The most common subtype was lung cancer (n = 508, 14.2%), followed by colorectal (n = 387, 10.8%), breast (n = 386, 10.8%), and prostate cancer (n = 367, 10.3%). Of the "big five" most common oncologic diseases underlying cancer-related AS, PC was the fifth most frequent subtype (n = 270; ♀: 52%/♂: 48%; 7.5% of all cancer-related AS cases).

During the study period, the number of PC-related AS rose significantly; when analyzing four 5-year periods, this was as follows:

1999-2003, n = 9 → 2004-2008, n = 39: +333% (compared with the preceding period)

2004-2008, n = 39 → 2009-2013, n = 61: +56%;

2009-2013, n = 61 → 2014-2018, n = 161: +56%;

The median age of patients with PC-related AS (73Y) was similar to that of all PC-related deaths (74Y).

The ratio of PC-related AS in relationship with all PC-related deaths increased from 0.2% at the beginning of the study period to 2.4% from 2014-2018. In this most recent study period (2014-2018), the PC ratio was similar compared to that of three of the other four major cancer subtypes (see Figure): 3.0%: breast cancer (♀ only); 2.9%: prostate cancer; 2.8%: colorectal cancer; 2.8%: all other cancer types. Lung cancer showed a slightly lower ratio (1.7%).

Conclusions: During the 20-year study period, the proportion of individuals who chose PC-related AS has increased twelvefold. However, AS among PC patients remains rare and represents only approximately less than 3% of all PC-related deaths.

Keywords: cancer, pancreatic cancer, assisted suicide, end-of-life decision making, Switzerland

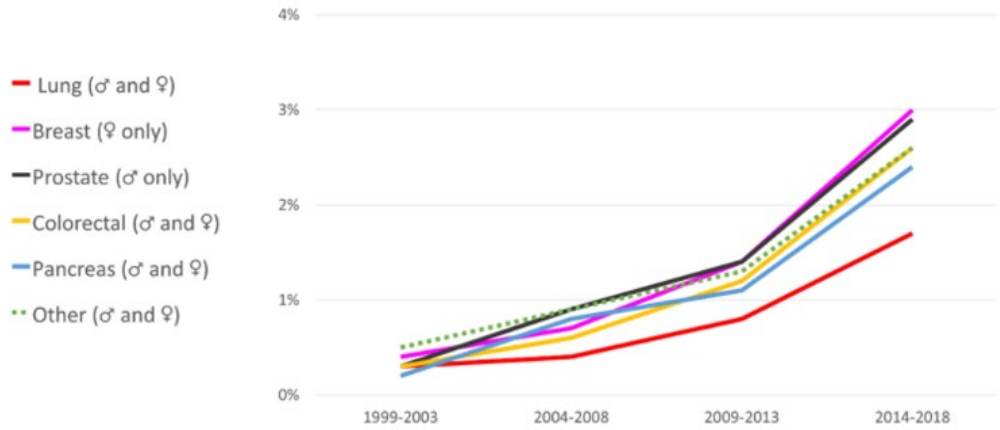

Figure: Development over time of the proportion of cancer-related assisted suicides to cancer-related deaths. Reported are the five most frequent cancer subtypes representing 54% of all cancer-related AS cases.

SSH ORAL PRESENTATION – HEMOSTASIS, TRANSFUSION MEDICINE, VASCULAR, LABORATORY MEDICINE, BENIGN HEMATOLOGY

195

Identification of key regulators of procoagulant COAT platelet generation by quantitative phosphoproteomic analysis and phosphoflow – Focus on VASP

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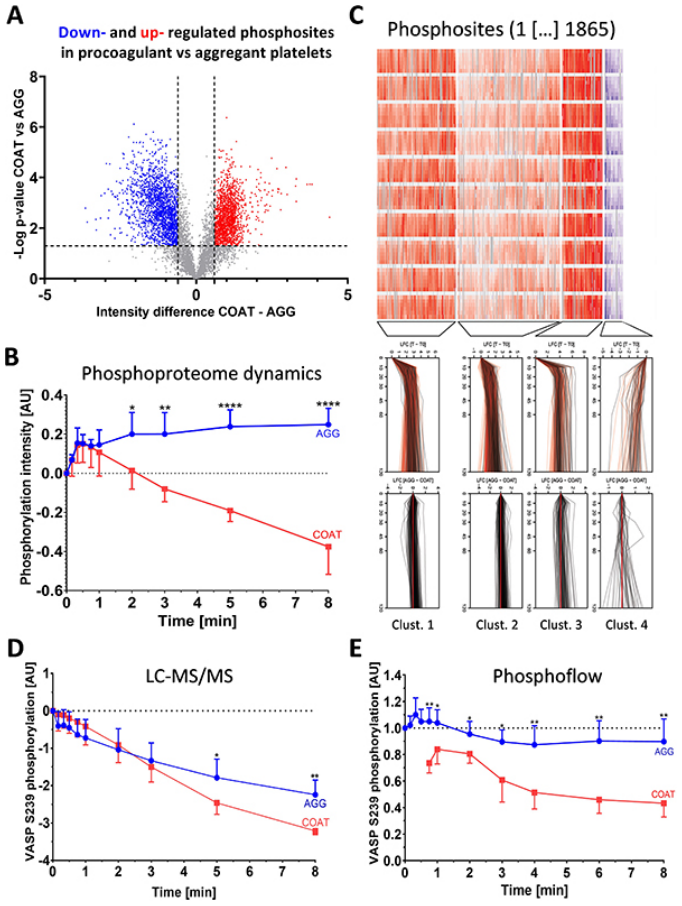
Introduction: Decreased or enhanced procoagulant platelet generation may lead to bleeding or thrombotic events, respectively. The intracellular program underlying the dichotomous generation of aggregating (AGG) and procoagulant (COAT) platelets upon combined activation by Collagen-And-Thrombin is only partially described.

In this study, we investigated the utility of time-lapse phosphoproteomics to find potential regulators of the procoagulant response. Subsequently, we validated the phosphorylation pattern of Vasodilator-Stimulated Phosphoprotein at Serine 239 (VASP S239) with a flow cytometry based intracellular staining technique (phosphoflow).

Methods: Platelets were activated at RT with convulxin plus thrombin in presence or absence of calcium, which generated procoagulant or aggregating phenotypes, respectively. Platelets were sampled at baseline and at different timepoints up to 8 min after activation. The phosphoproteomes of resting, AGG and COAT platelets were analysed by isobaric Tandem-Mass-Tag based Mass Spectrometry strategy. Protein-specific phosphorylation sites (phosphosites) of interest were monitored at different time points in both AGG and COAT phenotypes by phosphoflow.

Results: Upon stimulation, we identified 4223 differently regulated phosphosites corresponding to 1643 unique proteins showing significant regulation (Fig. 1A). Overall, proteins gradually dephosphorylate in procoagulant platelets and hyperphosphorylate in aggregating platelets (Fig 1B). Phosphosites with similar and significant time-dependent phosphorylation changes were clustered in 4 groups (Fig 1C). Interestingly, a dichotomous phosphorylation status was observed for VASP S239 with a significant downregulation in procoagulant platelets (Fig 1D). This observation was also confirmed by phosphoflow (Fig. 1E) which validated the results from the phosphoproteomic analysis. Lower VASP S239 phosphorylation is involved in greater cytoskeleton remodelling, which is reported in the development of the procoagulant response, and adhesive events.

Conclusions: The present study highlights the utility of phosphoproteome analysis to detect time-dependent changes of key molecular regulators of the dichotomous response leading to the generation of procoagulant besides aggregating platelets. We propose that VASP could be an interesting target to modulate the procoagulant response.



224

Role of selected microRNAs in regulation of hemostasis in zebrafish larvae

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Introduction: Thrombosis is the main consequence of cardiovascular diseases and antithrombotics, such as antiplatelet drugs, are a cornerstone for the prevention of the recurrence of these ischemic events. However, the biological impact of antiplatelet drugs on platelet reactivity is variable among patients, leading to the possibility of developing thrombotic or bleeding events. This unfortunate reality results from a poor understanding of the factors regulating thrombocyte function. Several microRNAs (miRNAs) have been associated with platelet reactivity, making them promising candidates for biomarkers of cardiovascular events. However, the mechanism of miRNA-mediated regulation of thrombocyte function remains mostly enigmatic.

Methods: To functionally validate miRNAs as potential biomarkers of platelet function, we developed a zebrafish model based on the real-time, in vivo monitoring of thrombus formation upon laser-induced blood vessel injury in larvae. We focused our attention on miR-96, miR-150 and miR-223, some of the most abundant miRNAs in human platelets, that are associated with platelet reactivity in multiple clinical and/or animal studies. Multiple transgenic zebrafish lines specifically overexpressing these miRNAs in thrombocytes were generated.

Results: The miR-96 and miR-150 manipulations resulted in a decreased number of thrombocyte attachment in the forming thrombus after the venous laser injury. Similar results were also observed in the artery but only in the miR-96-overexpressing fish. While our miR-223 manipulations did not significantly affect the size of the venous thrombus after laser injury, they did however lead to a decrease in the time to first cell adhesion to the injured tissue. Concomitantly, the forming thrombus was less stable, resulting in more cells leaving this site following initial attachment.

Conclusions: The elevated level of each of these miRNAs led to widely different characteristic of the forming thrombus in our assay, in line with a major role of miRNAs in platelet reactivity and thrombus formation. The increased level of miR-96, miR-150 and miR-223 in zebrafish thrombocytes resulted in unique changes in the thrombocytes' properties. Currently, we are working on determining the specific genes and biological pathways targeted by these miRNAs in thrombocytes that leads to the observed phenotypic changes in cellular behavior.

247

Role of MicroRNA-223-3p in regulating platelet-supported thrombin generation

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Introduction: Platelets are anucleate cells mostly involved in hemostasis through their aggregating properties and their ability to promote thrombin generation at their activated surface. Platelet reactivity is variable among individuals and microRNAs (miRNAs) may regulate platelet function. Among platelet-derived miRNAs, miR-223-3p is the most highly expressed. Furthermore, several studies have reported an association between miR-223-3p level and platelet reactivity or recurrence of cardiovascular events, but the impact of miR-223-3p on platelet function is poorly understood.

The objective of the project is to investigate the role of miR-223-3p on platelet reactivity in platelet-like structures (PLS) derived from human hematopoietic stem cells (CD34+).

Methods: MiR-223-3p upregulation and downregulation were carried out by transfecting CD34+-derived megakaryocytes with miR-223-3p mimic or CRISPR/Cas9 complexes, respectively. PLS were then timely collected for functional tests. Phosphatidylserine (PS) exposure was performed using flow cytometry. PLS-supported thrombin generation in human plasma was quantified with the Calibrated Automated Thrombogram using the velocity index. Finally, quantitative polymerase chain reaction was used to quantify the expression of miRNAs and selected mRNAs.

Results: Increased levels of miR-223-3p induced a 30±5% decrease in PS exposure after activation ($n = 7$, $p = 0.001$), and a 10±3% decrease in PLS-supported thrombin generation ($n = 8$, $p = 0.008$), compared to mock condition. These findings were associated with a 47±10% decrease in TMEM16F mRNA expression ($n = 4$, $p = 0.022$), a major contributor in PS exposure. Transfection with CRISPR/Cas9 complexes led to a 56±5% decrease in the expression of miR-223-3p ($n = 7$, $p<0.0001$) and induced a 19±6% increase in PS exposure after activation ($n = 6$, $p = 0.0092$), compared to the negative control.

Conclusions: MiR-223-3p upregulation is associated with a decrease of PS exposure, PLS-supported thrombin generation, and TMEM16F mRNA expression. This observation suggests that miR-223-3p could regulate PLS procoagulant activity through the regulation of TMEM16F. CRISPR/Cas9 editing tool allowed to significantly decrease miR-223-3p and confirmed the involvement of miR-223-3p in PS exposure regulation.

258

Pregnancy Outcomes in Hereditary Thrombotic Thrombocytopenic Purpura – Room for (Further) Improvement

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Introduction: Hereditary thrombotic thrombocytopenic purpura (hTTP) results from bi-allelic ADAMTS13 mutations and severe congenital ADAMTS13 deficiency. Affected patients are particularly vulnerable during infancy and pregnancy, where acute episodes are often severe, presenting with thrombocytopenia, haemolytic anemia and symptoms of organ ischemia. Today, many patients receive regular plasma infusions to prevent morbidity (i.e. strokes) and mortality. We aimed to characterize the impact of hTTP diagnosis and the effect of plasma prophylaxis on pregnancy outcomes.

Methods: Observational study on female patients with confirmed hTTP diagnosis (ADAMTS13 activity <10%, bi-allelic ADAMTS13 mutations) enrolled in the International hTTP Registry (NCT01257269) before June 30, 2023. Documented pregnancies were divided into before (retrospective, incl. index pregnancy) and after (prospective) hTTP diagnosis.

Results: Of the 131 female hTTP patients enrolled, 87 (66.4%) had been pregnant one or several times, resulting in 214 documented pregnancies. Half of these patients (n = 44, 50.6%) received their hTTP diagnosis because of obstetrical complications (index pregnancy) at a median maternal age of 26.7 (IQR, 23.5-31.2). The live-birth rate for the 125 retro- and 89 prospectively followed pregnancies was 52.8% (n = 66) and 79.8% (n = 71), respectively. Miscarriage was the main adverse outcome in retro- and prospectively followed pregnancies (n = 30, 50.8%; vs. n = 13, 72.2%), followed by late abortion (n = 14, 23.7%; n = 4, 22.2%). Stillbirth (n = 7, 11.9%) and neonatal death (n = 8, 13.6%) were observed only before hTTP diagnosis. Continued plasma prophylaxis, or prophylactic plasma infusions started in on-demand treated patients when pregnancy was recognized, increased live-birth rates to 85.7% and 76.2%, respectively, compared to 37.5% in patients without treatment. In 10/88 (11.4%) pregnancies after hTTP diagnosis, aspirin was given in addition (live-birth rate 80%). Acute TTP episodes and occurrence of preeclampsia were lowest in patients on plasma prophylaxis before becoming pregnant.

Conclusions: A diagnosis of hTTP leading to plasma prophylaxis during pregnancy reduces maternal morbidity and increases the live-birth rate considerably. The earlier plasma prophylaxis is started, the larger the positive effect. As hTTP confers an increased risk for preeclampsia, the low prescription rate of aspirin leaves room for improvement.

SSH/SSMO ORAL PRESENTATION – EXPERIMENTAL HEMATOLOGY / ONCOLOGY

188

Detection and Characterization of ASPP2kappa(k) – a central hub of tumorigenesis and drug resistance

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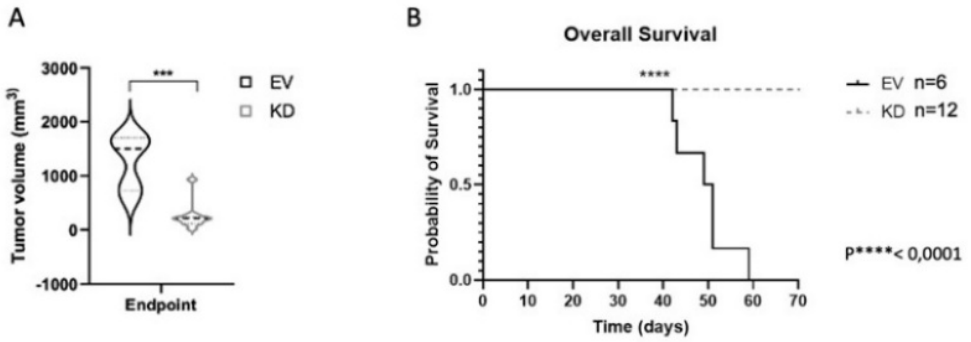
Introduction: ASPP2 is a tumor suppressor that regulates key proteins such as p53, Bcl-2 and NFkB to orchestrate pivotal cancer-related pathways. ASPP2 contains a highly conserved Ank-SH3 domain at its C-terminus, which mediates these interactions. Importantly, we discovered a so far unknown, oncogenic isoform, named ASPP2k, with high prevalence in cancer. This dominant-negative mutant has lost the crucial C-terminus and inhibits ASPP2 WT function, resulting in loss of tumor suppressing capacities. ASPP2k was first detected in acute leukemias, but screening of multiple tumor entities revealed frequent expression of ASPP2k in cancer, going along with a more aggressive tumorbiology and resistance to therapy.

Methods: > 500 tumors including AML, glioma, sarcoma, breast, colon and lung cancer, were assessed for ASPP2k expression. ASPP2k was stably suppressed or overexpressed in cell lines from these entities and in patient tissue. Induction of apoptosis, cellular proliferation, migration, invasion, colony formation, telomer length, angiogenesis and related pathways were assessed in dependency of ASPP2k. 6 independent, ASPP2k-modified mouse tumor models were established and tumor engraftment, progression and metastasis assessed in vivo.

Results: Representative data, exemplary TNBC: Expression of ASPP2κ was confirmed in virtually all patients. ASPP2κ expressing TNBC cell models displayed impaired induction of apoptosis (avg. IC50s -30%), higher proliferation (avg.+25%), migration (avg.+65%) and invasion (avg.+70%) rates. Knock down (KD) of ASPP2k sensitized cells towards chemotherapy (Doxorubicin avg.+35%) and/or γ-irradiation (avg.+35%) by restoring important p-p53 sites (S15: 2,5x/S46: 2x) and cell cycle regulators (p21: 2x, pRAD: 3x). EMT and angiogenesis were strongly inhibited in ASPP2k KD models, evidenced by downregulation of pro-angiogenic and pro-metastatic players (e.g. VEGF 90%, IL8 60%, Slug 70%, Snail 65%, n-Cadherin 40%). Xenotransplant ASPP2κ KD tumor mouse models confirm attenuated tumor engraftment, growth and metastasis (p = 0.001), resulting in significantly prolonged overall survival (p = 0.0001), Fig.1.

Conclusions: We demonstrate that ASPP2κ is highly expressed in cancer and intimately involved in tumorigenesis and drug resistance, promoting all classical hallmarks of cancer. Importantly, ASPP2k is targetable in vivo and we will provide data evaluating ASPP2κ as a novel target for therapy.

Figure 1: Silencing of ASPP2κ inhibits tumor growth and prolonges survival in vivo



ASPP2κ-silenced TNBC xenotransplant model. (A) tumor volumes at end of the experiment p***<0,001 (B) Highly significant discrepancy in overall survival of mice carrying ASPP2κ expressing (EV) versus ASPP2κ silenced (KD) tumors. P****<0,0001

201

Modulation of the cholesterol or Wnt signaling pathways overcomes TP53 deficiency-associated resistance to CAR T-cell therapy in AML

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Introduction: TP53-mutant myeloid neoplasms are distinct clinicogenomic entities characterized by poor survival. Chimeric antigen receptor (CAR) T-cell therapy might be a promising therapeutic option for TP53mut AML/MDS. However, the AML-intrinsic determinants of efficacy of T-cell-based approaches are largely unknown. We here address the highly relevant question as to whether, and if so, how TP53 deficiency in AML cells might confer resistance to CAR T-cell therapy.

Methods: Taking advantage of a recently developed, CRISPR/Cas9-engineered TP53 isogenic AML cell line harboring TP53 null, missense, or wildtype alleles as well as making use of CD33- and CD123-directed second generation CAR T-cells, we use a combination of in vitro flow cytometry-based co-incubation assays, live-cell imaging, gene expression profiling and an in vivo therapeutic model to address our objective.

Results: We observed increased resistance of TP53mut AML cells to CAR T-cells in vitro (Panel A). CAR T-cells engaging TP53mut AML cells upregulated exhaustion markers resulting in longer time-to-killing (Panel B-C). TP53mut AML xenografted immunodeficient mice treated with CAR T-cells exhibited shorter survival compared to wildtype controls (Panel D). Transcriptional profiling revealed upregulation of the cholesterol pathway in TP53mut AML cells under CAR T-cell attack (Panel E). Simultaneously, CAR T-cells engaging TP53mut AML demonstrated a downregulated Wnt pathway (Panel F). Rational pharmacological targeting of either of these pathways – using simvastatin and BIO-acetoxime, respectively – rescued TP53mut AML cell sensitivity to CAR T-cell-mediated killing (Panel G). Similarly, CRISPR/Cas9-engineering of CAR T-cells to upregulate Wnt pathway signaling constitutively via Regnase-1 knockout rescued TP53mut AML cell sensitivity (Panel H).

Conclusions: We demonstrate that TP53 deficiency in AML cells confers resistance to CAR T-cell therapy by inducing CAR T-cell exhaustion. Furthermore, we identified the cholesterol pathway as a potential therapeutic vulnerability of TP53mut AML cells, and the Wnt pathway as a promising avenue to enhance the efficacy of CAR T-cell therapy. Our data suggest that pharmacological co-interventions or genetic engineering of CAR T-cell therapies may be a strategy towards more efficacious and tolerable cellular therapies for patients with TP53mut myeloid neoplasms.

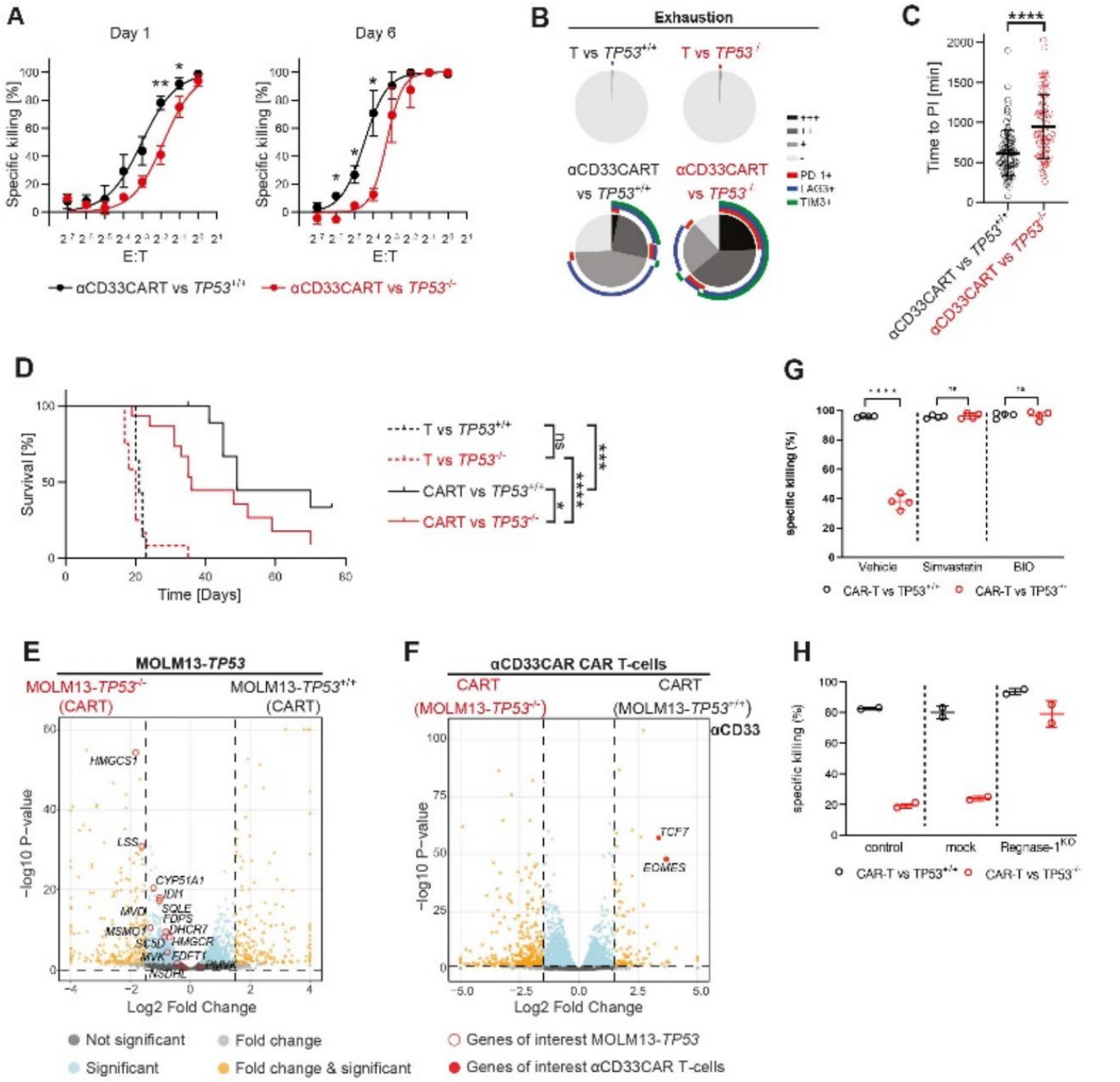

Figure 1: (A) Specific CAR T-cell-mediated killing of MOLM13-TP53+/+ AML cells (black) or MOLM13-TP53-/- AML cells (red) on day 1 and day 6 at the indicated E:T ratios. Pooled results from 3 different healthy T-cell donors are shown, (n=3 biological replicates, *P<.05, **P<0.01, unpaired student t-test). **(B)** Summary of exhaustion markers of CAR T-cells and T-cell controls co-incubated with isogenic MOLM13-TP53 AML cells with wildtype (MOLM13-TP53+/+) or null (MOLM13-TP53-/-) TP53 status, (n=3 biological replicates). **(C)** Summary data showing significantly longer time to propidium iodide influx for CAR T-cells engaging MOLM13-TP53-/- than MOLM13-TP53+/+ AML cells. ****P<.0001; unpaired student t-test. **(D)** Kaplan-Meier survival curve of mice engrafted with luciferase expressing MOLM13-TP53+/+ or MOLM13-TP53-/- AML cells and treated with anti-CD33 CAR T-cells or untransduced T-cell control from three pooled independent experiments. (n=53 mice and n=3 healthy T-cell donors). ns, not significant; *P<.05; ***P<.001; unpaired student t-test. ****P<.0001; Log-rank Mantel-Cox test. **(E)** Volcano plot of differentially expressed genes between MOLM13-TP53+/+(CART) and MOLM13-TP53-/- (CART) highlighting genes involved in cholesterol biosynthesis in MOLM13-TP53-/-(CART) cells. **(F)** Volcano plot of differentially expressed genes between CART(MOLM13-TP53+/+) and CART(MOLM13-TP53-/-) highlighting the master transcription factors EOMES and TCF7. **(G)** Summary data showing specific anti-CD33 CAR T-cell mediated killing of MOLM13-TP53+/+ or MOLM13-TP53-/- AML cells in the presence or absence of simvastatin or BIO-acetoxime, (n=3 biological replicates, ****P<.0001; two-way ANOVA). **(H)** Summary data showing specific killing of MOLM13-TP53+/+ or MOLM13-TP53-/- AML cells mediated by either control, mock-electroporated or Regnase-1-KO anti-CD33 CAR T-cells (at time of submission n=1)

220

Growth differentiation factor 15, a potential biomarker and a therapeutic target in myeloproliferative neoplasms

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Introduction: Despite the growing knowledge on genetic mutations that drive myeloproliferative neoplasms (MPN), effectors downstream of genetic driver activity contributing to disease progression and disease associated complications remain largely unknown. We previously demonstrated that mice expressing MPN driver mutations including JAK2-V617F in hematopoietic system developed not only expected MPN disease associated hematopoietic phenotypes, but also displayed hematopoietic cell intrinsic and systemic metabolic changes that contributed to MPN progression.

Methods: Metabolomics, cytokine arrays and ELISA based analyses were conducted on plasma and bone marrow samples isolated from MPN mouse models and patients to identify factors downstream of genetic drivers promoting MPN progression. Receiver operating curve analysis was performed to assess the sensitivity and specificity of GDF15 for predicting MPN progression in patients. Chromatin immunoprecipitation and immunoblotting was conducted to identify upstream transcriptional regulators of GDF15 expression in MPN cells. Genetic inhibition and pharmacological modulation of GDF15 was performed to assess its relevance in MPN pathogenesis.

Results: We uncovered that expression of MPN driver mutations including mutant JAK2 and CALR-del52 in mice and in human leukemic cell lines leads to elevated levels of growth differentiation factor 15 (GDF15), which was correlated with MPN progression and disease associated systemic metabolic changes including hypoglycemia and body weight reduction. Patients with MPN also showed increased GDF15 levels, with the highest levels found in myelofibrotic (MF) patients. GDF15 levels correlated with mutant allele burden and showed sensitivity and specificity to distinguish MPN patients from healthy controls. At the molecular level, we identified that JAK-STAT signaling induces GDF15 expression through the activation of early growth response 1 (EGR1). Exogenous supplementation of GDF15 promoted murine and human hematopoietic colony growth in vitro and augmented MPN progression in mutant JAK2 mice. Conversely, pharmacological inhibition of GDF15 with a functional neutralizing anti-GDF15 antibody reduced MPN cell growth in humanized mice.

Conclusions: Our findings identify GDF15 as a critical inducer of disease progression and systemic metabolic changes in MPN and highlight it as a diagnostic and therapeutic target in MPN.

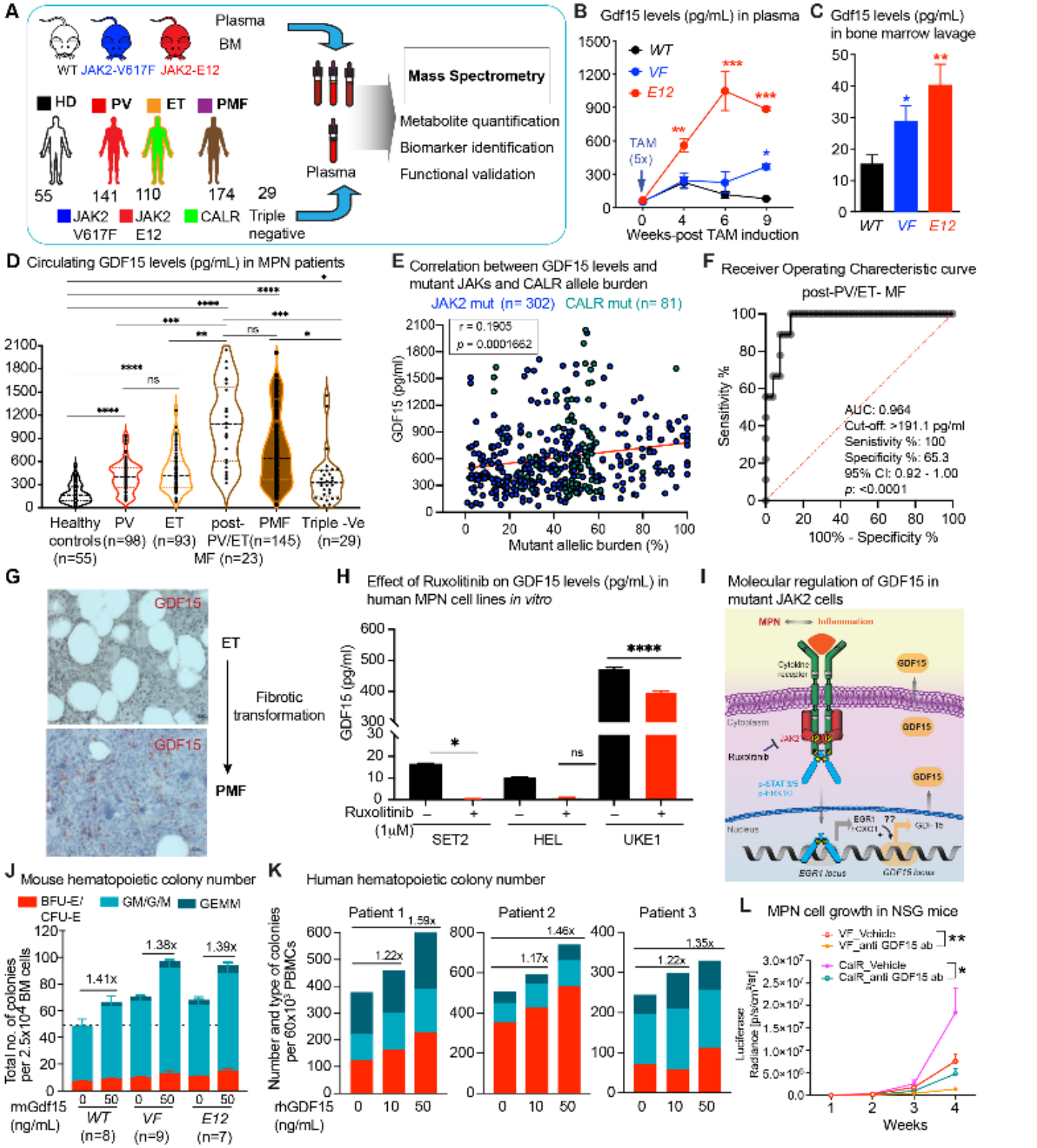

Figure. GDF15 is a functional biomarker and a therapeutic target in MPNs. **A**). Schematic of biomarker identification in MPN mice and patients. **B-C**). Gdf15 levels (pg/mL) in plasma and bone marrow lavage of wildtype (WT) and mutant JAK2 mice. **D**). Gdf15 levels in MPN patients with the indicated diagnosis. **E**). Correlation of GDF15 levels with mutant JAK2 and CALR-del52 allele burden. **F**). Receiver operating curve (ROC) analysis highlighted the sensitivity and specificity of GDF15 as a biomarker for myelofibrosis transformation in MPN patients. **G**). Immunohistochemistry analysis (IHC) for GDF15 expression in bone marrow biopsies of the same patient at ET stage and after transformation to MF. **H**). Ruxolitinib blunted GDF15 expression in mutant JAK2 expressing human leukemia cell lines. **I**). Schematic of molecular regulation of GDF15 induction in mutant JAK2 cells. **J-K**). GDF15 supplementation augmented mouse and human hematopoietic colony growth in vitro. **L**). Treatment with an anti-GDF15 neutralizing antibody reduced the growth of mutant JAK2 and CLAR cells in vivo in NSG mice. *P < .05, **P < .01., ***P < .001. PV: polycythemia vera; ET: essential thrombocythemia; PMF: primary myelofibrosis; AUC: area under curve.

221

Pharmacological modulation by BTK inhibitior induces the upregulation of CD19 and increases sensitivity to CAR T cells in a Model of Marginal Zone Lymphoma (MZL)

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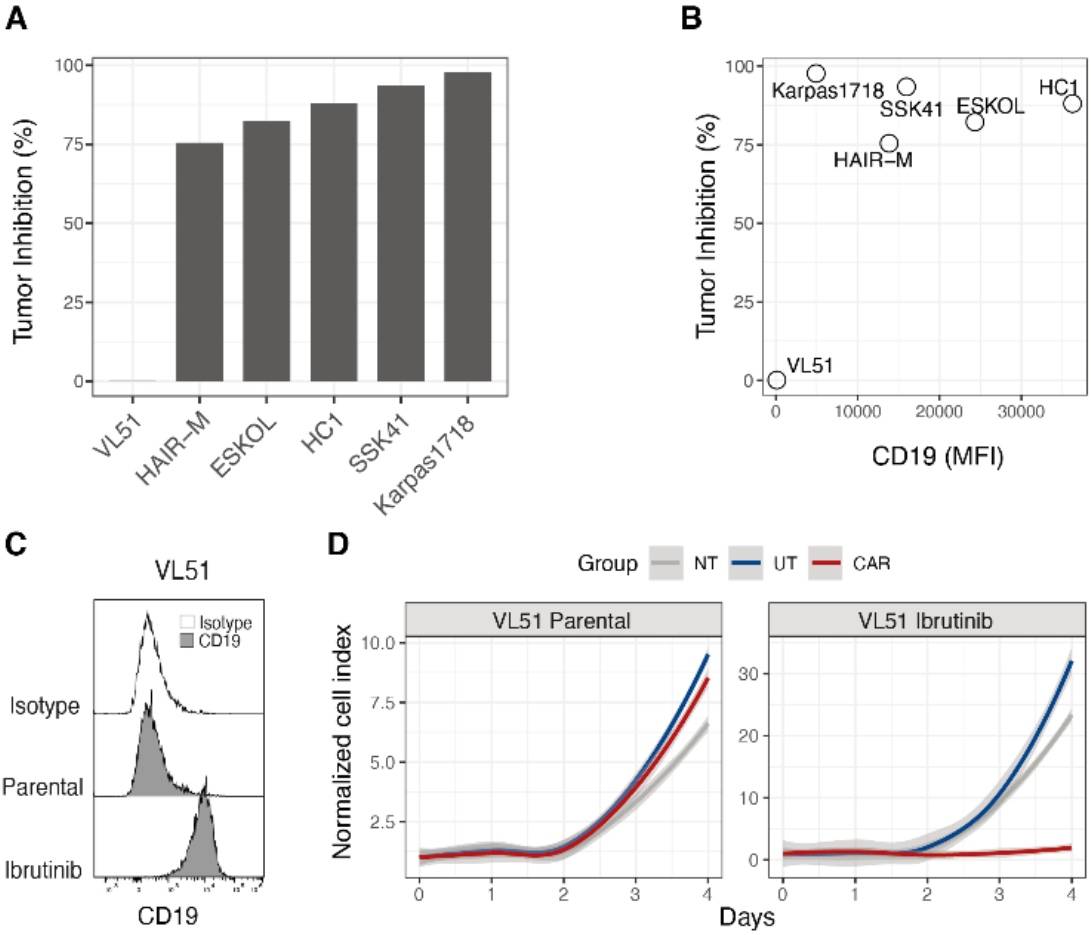
Introduction: Marginal zone lymphomas (MZLs) are characterized by an indolent course, but relapses/progressions are common and might heavily impact patients' survival. Chimeric antigen receptor (CAR) T cells revolutionized the treatment of several relapsing/refractory B cell malignancies but their success in MZL is still limited and none of the currently available products has been approved so far for this indication. Little is known about the reasons behind the limited efficacy of CAR T cells against MZL. In this work we investigated the contribution of CD19 levels in MZL resistance to CAR T cells and we tested a strategy to increase sensitivity to CAR T cells through pharmacological modulation of CD19 expression.

Methods: mCherry/Luciferase-transduced MZL cell lines (VL51, HAIR-M, ESKOL, HC1, SSK41, Karpas1718) were co-cultured with untransduced T (UT) cells or CD19.CD28z CAR T cells at an E:T ratio of 1:1 adjusted to transduction efficacy and tumor burden at different time points was quantified by bioluminescence (BLI). Chronic BTK inhibition was obtained by constantly exposing VL51 to ibrutinib (20 uM) for more than 8 weeks. Epigenetic regulation of CD19 gene expression was analyzed using H3K4me3 CHIPseq and ATACseq.

Results: We first employed a panel of 6 MZL cell lines to test their sensitivity to CD19 CAR T cells. After 3 days of co-culture, all cell lines displayed high sensitivity to CAR T cells with the only exception of VL51 cells that were totally resistant (Figure 1A). VL51 cells displayed the lowest CD19 expression (Figure 1B). Transduction of VL51 cells with truncated CD19 restored their sensitivity to CAR T cells demonstrating that low CD19 expression was their mechanism of resistance. We observed that prolonged BTK inhibition with ibrutinib led to CD19 upregulation on VL51 (Figure 1C). Higher H3K4me3 and higher chromatin accessibility at the CD19 gene transcription starting site were revealed by CHIPseq and ATACseq respectively, pointing to an epigenetic modulation of CD19 expression. According to high CD19 expression, ibrutinib-treated VL51 cells displayed high sensitivity to CAR T cell mediated tumor growth inhibition (Figure 1D).

Conclusions: CD19 modulation by BTK inhibition increased the sensitivity to CAR T cells in a CD19low MZL model. These data support exploring combination therapies for CAR T cell treatment of MZL.



265

Hematopoietic stem cell (HSC) aging drives systemic aging

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Introduction: Aging entails the gradual decline of tissue and stem cell function, potentially impacting homeostasis and contributing to aging. Although the hypothalamus and its stem cells are known to affect systemic aging, it's unclear if other stem cells, especially HSCs, also contribute. In mammals, HSCs maintain blood production but exhibit age-related changes, increased myeloid output and decreased erythroid/lymphoid output, posing a risk for age-related malignancies. This study explores the potential contribution of aging HSCs to organismal aging.

Methods: We created hematopoietic-specific Ribonuclease inhibitor (Rnh1)-deleted mice via cross of conditional knockout (Rnh1fl/fl) with Vav1-iCre mice and an inducible mouse model (Rnh1fl/fl Mx1-Cre+) by crossing Rnh1fl/fl mice with Mx1-Cre strain. We analysed these mice using various methods, including peripheral blood assessment, bone marrow transplantation, competitive transplantation assays, histopathology, RNA-sequencing of FACS sorted HSCs and GMPs, GSEA for aged HSC signatures, cell-cycle analysis, p-CHK1 staining, Co-IP, mass-spec experiments. Further, Molecular docking and confocal microscopy were used to confirm RNH1 colocalizing with identified partner Cyclin-dependent kinase 1 (CDK1). To assess the impact of CDK1 inhibition on HSC aging phenotype polyI:C treated Rnh1fl/fl Mx1-Cre+(Rnh1-/-) BM transplanted mice administered with CDK1 inhibitor RO-3306.

Results: RNH1 gene deletion in adult mouse HSCs caused premature and chronic HSC aging with aberrant HSC expansion, myeloid bias, reduced lymphopoiesis (Fig.1a, b), and diminished repopulating ability. This accelerated HSC aging resulted in premature organismal aging with reduced healthy lifespan, organ hypoplasia, kyphosis, and decreased subcutaneous fat (Fig.1c-h). Global gene expression analysis of HSCs revealed upregulation of cell-cycle and proliferation related genes in Rnh1-/- cells along with significant enrichment of the aged HSC signature in Rnh1-/- HSCs in GSEA (Fig.1i). Mechanistically, RNH1 loss drove cell cycle dysregulation by enhancing CDK1 function, contributing to HSC aging (Fig.1j-p).

Conclusions: Our study highlights vital role of RNH1 in HSC aging and reveal significance of HSCs in mediating systemic aging. We show aged HSCs drive organismal aging and suggest targeting HSC aging as a valuable therapeutic strategy for hematological malignancies and overall healthy aging.

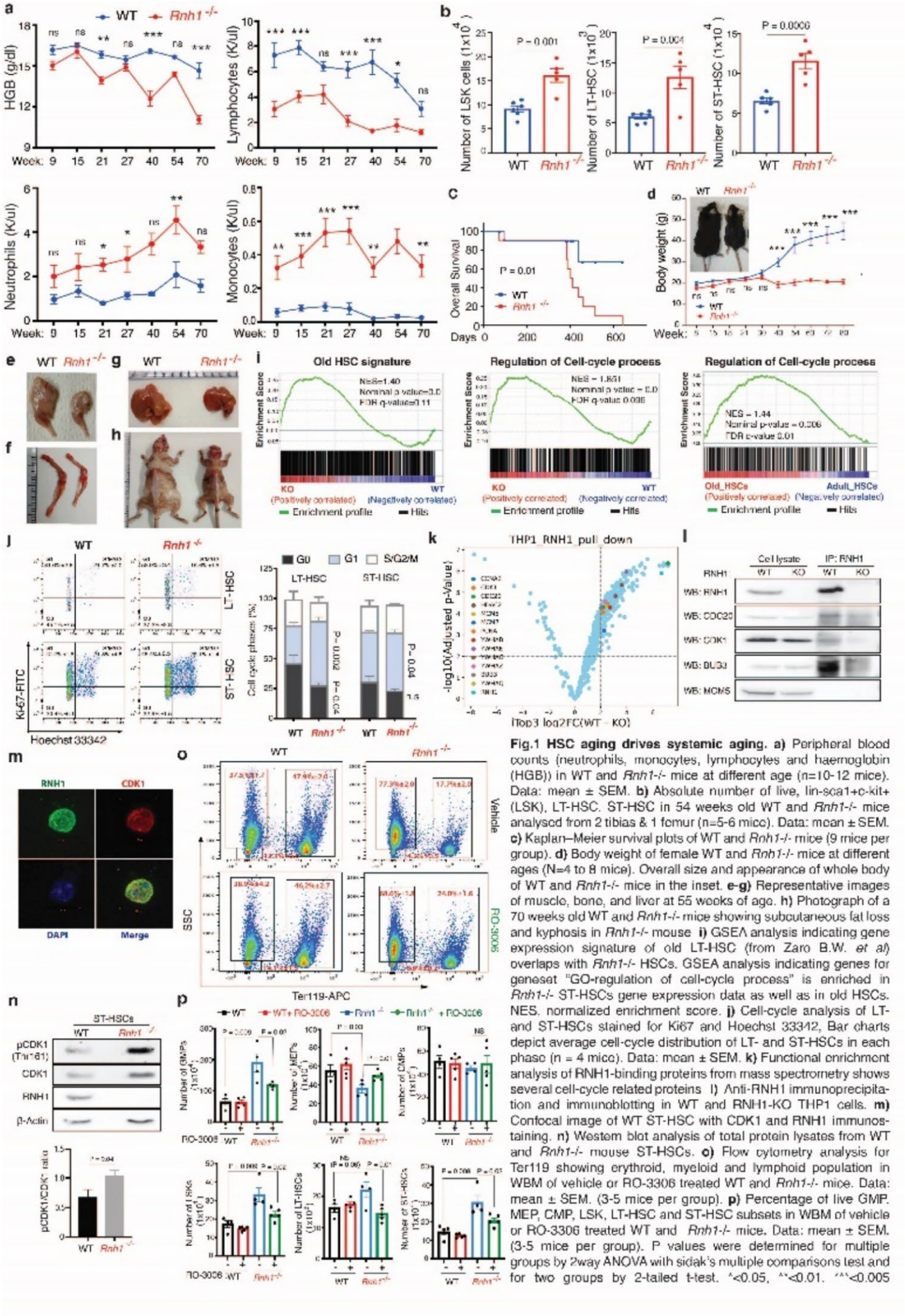

Fig.1 HSC aging drives systemic aging. a) Peripheral blood counts (neutrophils, monocytes, lymphocytes and haemoglobin (HGB)) in WT and *Rnh1-/-* mice at different age (n=10-12 mice). Data: mean ± SEM. **b)** Absolute number of live, lin-sca1+c-kit+ (LSK), LT-HSC. ST-HSC in 54 weeks old WT and *Rnh1-/-* mice analysed from 2 tibias & 1 femur (n=5-6 mice). Data: mean ± SEM. **c)** Kaplan–Meier survival plots of WT and *Rnh1-/-* mice (9 mice per group). **d)** Body weight of female WT and *Rnh1-/-* mice at different ages (N=4 to 8 mice). Overall size and appearance of whole body of WT and *Rnh1-/-* mice in the inset. **e-g)** Representative images of muscle, bone, and liver at 55 weeks of age. **h)** Photograph of a 70 weeks old WT and *Rnh1-/-* mice showing subcutaneous fat loss and kyphosis in *Rnh1-/-* mouse **i)** GSEA analysis indicating gene expression signature of old LT-HSC (from Zaro B.W. *et al*) overlaps with *Rnh1-/-* HSCs. GSEA analysis indicating genes for geneset "GO-regulation of cell-cycle process" is enriched in *Rnh1-/-* ST-HSCs gene expression data as well as in old HSCs. NES. normalized enrichment score. **j)** Cell-cycle analysis of LT- and ST-HSCs stained for Ki67 and Hoechst 33342, Bar charts depict average cell-cycle distribution of LT- and ST-HSCs in each phase (n = 4 mice). Data: mean ± SEM. **k)** Functional enrichment analysis of RNH1-binding proteins from mass spectrometry shows several cell-cycle related proteins **l)** Anti-RNH1 immunoprecipitation and immunoblotting in WT and RNH1-KO THP1 cells. **m)** Confocal image of WT ST-HSC with CDK1 and RNH1 immunostaining. **n)** Western blot analysis of total protein lysates from WT and *Rnh1-/-* mouse ST-HSCs. **o)** Flow cytometry analysis for Ter119 showing erythroid, myeloid and lymphoid population in WBM of vehicle or RO-3306 treated WT and *Rnh1-/-* mice. Data: mean ± SEM. (3-5 mice per group). **p)** Percentage of live GMP, MEP, CMP, LSK, LT-HSC and ST-HSC subsets in WBM of vehicle or RO-3306 treated WT and *Rnh1-/-* mice. Data: mean ± SEM. (3-5 mice per group). P values were determined for multiple groups by 2way ANOVA with sidak's multiple comparisons test and for two groups by 2-tailed t-test. *<0.05, **<0.01. ***<0.005

280

Efficient Combinatorial Adaptor-Mediated Targeting of Acute Myeloid Leukemia with CAR T-Cells

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Introduction: CAR T-cell therapies are efficient in treating B- and plasma-cell malignancies by targeting cell-of-origin antigens, eradicating both tumor cells as well as healthy counterparts. Their loss can be compensated by immunoglobulin substitution until they regenerate from hematopoietic stem and progenitor cells (HSPCs). However, similar on-target, off-tumor cell-of-origin elimination would be detrimental in HSPC-derived malignancies, such as acute myeloid leukemia (AML) as CAR T-cell activity might lead to terminal ablation of hematopoiesis.

To address this challenge, we developed adaptor-mediated CAR T-cells displaying the scFv E2, AdFITC(E2), that binds to fluorescein conjugated to antigen-binding adaptors in diabody format (Db-FM).

Methods: We generated second-generation AdFITC(E2)-CAR T-cells and multiple Db-based adaptors against selected AML antigens. We then tested the Dbs targeting CD33 and CD117 with respect to their ability to mediate CAR T-cell biocidal activity as single adaptors or in combination in vitro and in vivo.

Results: In vitro cytotoxicity assays of healthy-donor-derived AdFITC(E2)-CAR T-cells, both against cell lines (MOLM14-CD117+GFP+Luc+) as well as patient-derived AML blasts, revealed that dual adaptor use significantly improved tumor cell lysis compared to the equimolar concentration of single adaptors. In therapeutic xenogeneic mouse models engrafted with MOLM14 cells where AdFITC(E2)-CAR T-cells in combination with CD117 or CD33 Db-FM administered as single adaptors were as efficient as direct CAR T-cells against the same antigens. Injection of AdFITC(E2)-CAR T-cells and both CD117 and CD33 Db-FM effectively inhibited tumor growth, outperforming monotherapies, and leading in some cases to full AML elimination in bone marrow and blood.

Conclusions: We tested an adaptor CAR T-cell approach using multiple adaptors to modulate and enhance CAR T-cell activity, focusing on AML as a relevant disease model. The high heterogeneity in antigen expression on AML cells supports a combinatorial targeting strategy individualized based on the respective AML immunophenotype. Due to their relatively small molecular weight, Db-based adaptors are rapidly cleared from the body, allowing for rapid control over AdFITC-CAR T-cell on-off activity. We envision that this approach has the clinical potential to improve CAR T-cell safety profiles by enhancing the specificity toward target cells.

SSH/SSMO ORAL PRESENTATION – CLINICAL HEMATO-ONCOLOGY

167

Haploidentical transplant with posttransplant cyclophosphamide vs matched related and unrelated donor transplant in acute myeloid leukemia and myelodysplastic neoplasm

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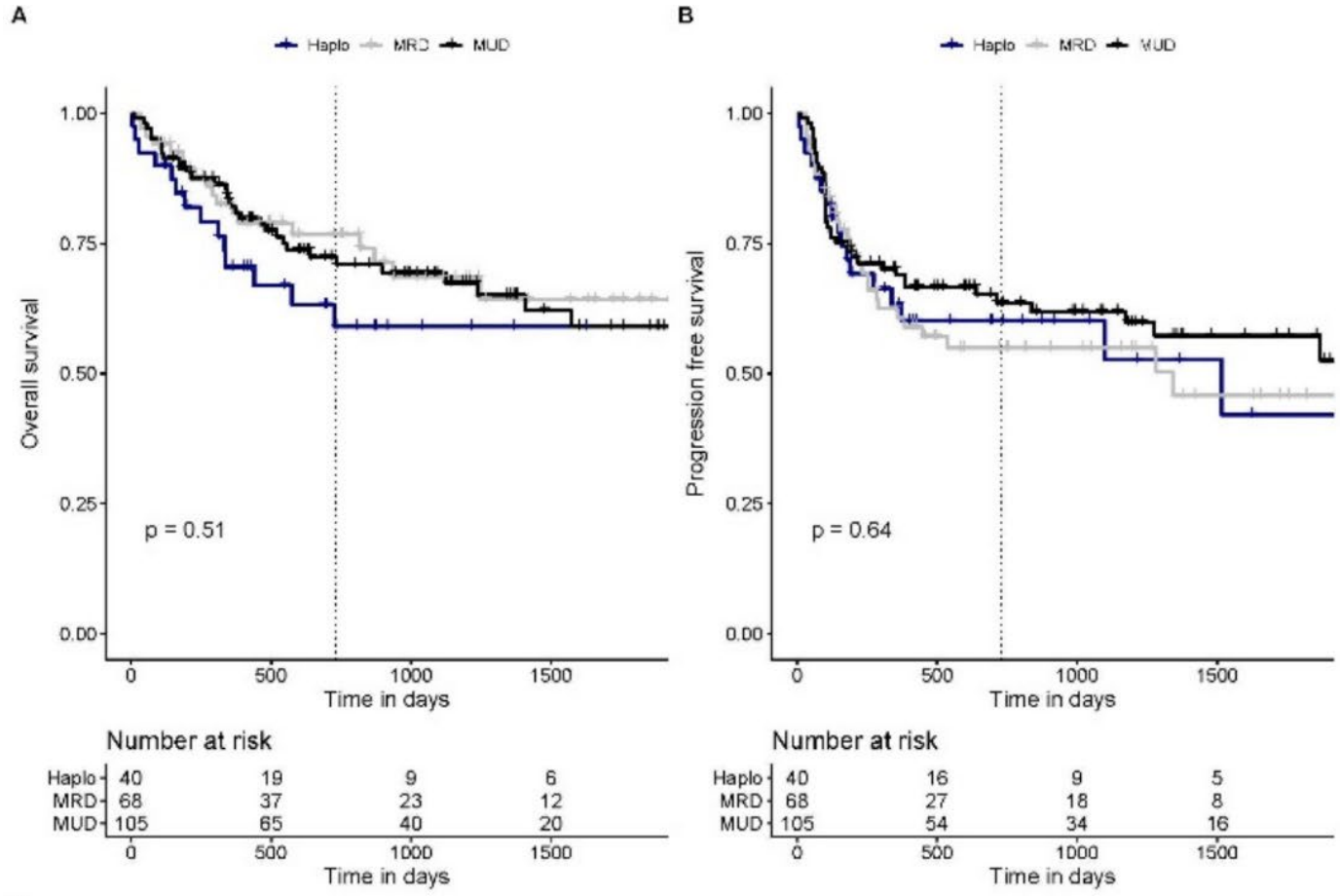
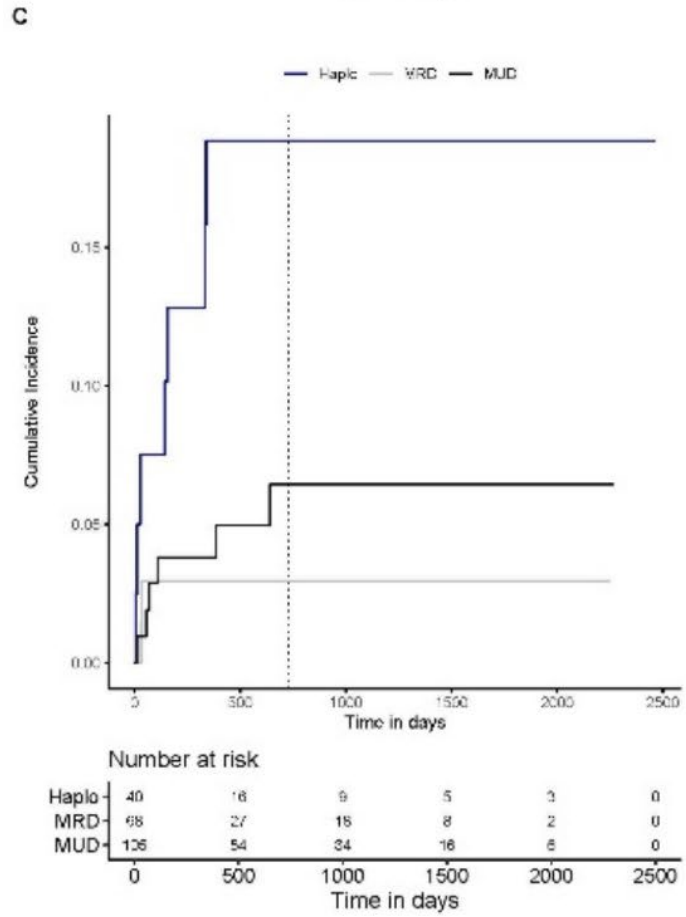
Introduction: Hematopoietic cell transplantation from haploidentical donors (haploHCT) has facilitated treatment of AML and MDS by increasing donor availability and became more feasible since the introduction of post-transplant cyclophosphamide (ptCY). Our objective was to evaluate the efficacy and safety of this regimen compared to standard related (MRD) and unrelated donor transplantation (MUD).

Methods: In our single-center retrospective analysis including 213 patients with AML or MDS, we compare the outcome of haploHCT (n = 40) with ptCY with HCT from HLA-identical MRD (n = 105) and MUD (n = 68).

Results: At 2 years after transplantation, overall survival (OS) after haploHCT was not significantly different (0.59; 95% confidence interval 0.44–0.79) compared to MRD (0.77; 0.67–0.88) and MUD transplantation (0.72; 0.64–0.82, p = 0.51). While progression-free survival (PFS) was also not significantly different (haploHCT: 0.60; 0.46–0.78, MRD: 0.55; 0.44–0.69, MUD: 0.64; 0.55–0.74, p = 0.64), non-relapse mortality (NRM) was significantly higher after haploHCT (0.18; 0.08–0.33) vs. MRD (0.029; 0.005–0.09) and MUD (0.06; 0.02–0.12, p < 0.05). Higher NRM was mainly caused by a higher rate of fatal infections, while deaths related to GvHD or other non-relapse reasons were rare in all groups. As most fatal infections occurred early and were bacterial related, one potential risk factor among many was identified in the significantly longer time to neutrophil engraftment after haploHCT with a median of 16 days (interquartile range; 14.8–20.0) vs. 12 days (10.0–13.0) for MRD and 11 days (10.0–13.0) for MUD (p = 0.01).

Conclusions: Haploidentical transplantation with ptCy shows comparable efficacy and safety compared to MUD and MRD transplantation with standard immunosuppression. Higher NRM could be further addressed by optimising prevention of peri-transplant infectious complications.





214

Efficacy and safety of Daratumumab for the treatment of ABO-incompatible Pure Red Cell aplasia after allogenic HSCT: report from SFGM-TC

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Introduction: Pure Red Cell Aplasia (PRCA) after ABO-mismatched Hematopoietic Stem Cell Transplant (HSCT) remains a challenge for physicians. It occurs in 7 to 10% of major or bi-directional ABO-incompatible HSCT and significantly impairs patients' quality of life due to fatigue, iron-overload and increased risk of transfusion complications. Daratumumab seems the most promising approach given that the physiology of prolonged PRCA is presumably a reflection of residual host plasma cell clones producing isohemagglutinins.

Methods: We performed a retrospective analysis on patients who received Daratumumab to treat ABO-incompatible PRCA in centers affiliated with the Société Francophone de Greffe de Moelle et de Thérapie Cellulaire (SFGM-TC). All patients met the diagnostic criteria for ABO-incompatible PRCA and we defined response as achieving a reticulocytosis > 20 G/L and erythrocyte transfusion independence.

Results: Among our 41 centers, we report 11 patients. Six out of them had previously received one or more treatments such as Rituximab, erythropoietin, or a thrombopoietin receptor agonist, prior to Daratumumab. None received Donor Lymphocyte Infusion or had active graft versus host disease. First infusion of Daratumumab was performed at a median of 255 days from transplant [194 (Q1); 285 (Q3)]. Ten patients (91%) achieved resolution of PRCA after receiving Daratumumab but one patient still requires erythrocyte transfusions. The median time to response was 31 days [17 (Q1); 55 (Q3)]. After a median follow-up of 23 months [3.1 (Q1); 25.4 (Q3)] post initial Daratumumab infusion, all eleven patients remained alive. six patients experienced a grade 2-3 reaction during the first infusion. Three patients had hypogammaglobulinemia (< 5.5 g/L) after Daratumumab treatment and two experienced a lower respiratory tract infection documented with Pneumocystis Jirovecii despite lymphocytes T CD4 above > 200/mm3.

Conclusions: Our retrospective cohort confirms the efficacy of Daratumumab in PRCA, with some patients achieving resolution even after a single dose. We also report the first patient who experienced Daratumumab refractory PRCA with persistent elevated isohemagglutinin titres. A randomized trial is currently recruiting participants in SFGM-TC centers to investigate anti-CD38 strategy for ABO-mismatched PRCA (ClinicalTrial.gov Identifier: NCT05559827).

Patient	Age / Sexe	Disease	Donor Type / Conditionning Regimen	ABO Mismatch (R/D)	Isohemagglutinins at PRCA Diagnosis	Treatment(s) before Daratumumab	Isohemagglutinins before Daratumumab	Delay before Daratumumab (Days/J0)	Daratumumab infusions (Number, IV/SC, gap between 2 infusions)	Response (J1 = First Daratumumab infusion)
1	60 / F	MPN	MUD / RIC	O/A	64		64	278	2 (IV - 3 months)	Day +113 (19 days after 2nd infusion)
2	28 / F	DADA2	MRD / MAC	O/B	32	EPO (6 months)	8	763	1 (IV)	Day +17
3	65 / M	MDS	MMUD / RIC	A/B	1024	EPO (6 months)	1024	494	10 (IV - 2 weeks)	NO
4	63 / M	AML	MUD / RIC	O/AB	64(A) / 128(B)	Rituximab (4) - EPO (6m)	32(A) / 128(B)	182	3 (IV - 1 week)	Day +14
5	37 / F	MDS	HAPLO / MAC	B/A	16	TPO-RAs (6m)	16	210	3 (IV - 3 weeks)	Day +53
6	63 / M	MDS	MUD / RIC	O/A	2048		512	191	1 (SC)	Day +14
7	69 / M	MDS	MUD / RIC	O/A	512	Rituximab (4) - EPO (3m)	512	257	4 (SC - 2 weeks)	Day +92
8	35 / M	AML	MUD / MAC	O/A	128	Rituximab (2)	32	293	6 (IV - 1 week)	Day +35
9	7 / M	SAA	MUD / RIC	O/A	256		64	255	4 (IV - 2 weeks)	Day +56
10	18 / F	AML	HAPLO / MAC	O/B	64		32	176	1 (SC)	Day +18
11	59 / F	AML	MUD / RIC	O/A	128		128	197	2 (SC - 2 weeks)	Day +28

MPN myeloproliferative neoplasm, **DADA2** deficiency of adenosine deaminase 2, **MDS** myelodysplastic neoplasm, **AML** acute myeloid leukemia, **SAA** Severe aplastic anemia, **MUD** matched unrelated donor, **MRD** matched related donor, **MMUD** mismatched unrelated donor, **HAPLO** haploidentical donor, **RIC** reduced intensity conditioning, **MAC** myeloablative conditioning, **EPO** erythropoietin, **TPO-RAs** thrombopoeitin receptor agonists

250

Daratumumab treatment reduces the stem cell mobilization potential in myeloma patients and prolongs engraftment after autologous transplant in myeloma patients.

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Introduction: Autologous stem cell transplantation (ASCT) is part of the standard first-line treatment algorithm for fit myeloma patients. Daratumumab is a CD38 targeting antibody increasingly applied during first-line induction treatment together with lenalidomide, bortezomib and dexamethasone (RVD). The effect of using daratumumab on subsequent stem cell mobilization is poorly investigated.

Methods: In this single-center study, we investigated consecutive multiple myeloma patients who received ASCT between March 2020 and June 2023 after first line induction treatment with (D-RVD) or without daratumumab (RVD). The primary endpoint was the number of circulating CD34+ cells in the peripheral blood at the day of peripheral stem cell collection.

Results: We identified a cohort of 155 consecutive patients, with 45 patients receiving D-RVD as first line treatment and 110 patients had RVD. Patient characteristics at diagnosis and number of induction cycles were comparable. The median duration from the start of mobilization treatment until the first day of apheresis was longer in the D-RVD group (9 days vs. 8 days; p = .0006). Plerixafor was used more frequently in D-RVD compared to RVD patients (38% vs. 28%; p = .3052). The median circulating peripheral CD34+ cell count at the day of apheresis was lower in D-RVD patients (41.37 vs. 52.19 CD34+ cells/mcl blood; p = .0233), whereas median leucocyte counts were comparable. Collection in all patients was successful, but fewer CD34+ cells were collected in D-RVD patients (8.27 vs. 10.22 Mio CD34+ cells/kg; p = .0139), and less CD34+ cells were infused at ASCT in D-RVD patients (3.27 vs 3.6 Mio CD34+ cells/kg; p = .0157). Also, hospitalisation duration tended to be longer in D-RVD patients (23 vs 22 days; p = .0654). The recovery of neutrophils and platelets was prolonged in D-RVD patients (12 vs. 11 days; p = .0164; and 16 vs 14 days; p = .0002; respectively). Finally, the median number of platelet transfusions was higher in D-RVD treated patients (4 vs. 2 platelet transfusions; p = .0015), but not significantly different for erythrocyte transfusions (2 vs 1 transfusions; p = .2837).

Conclusions: Our data suggest that the addition of daratumumab to RVD during first-line induction treatment significantly reduces the stem cell mobilization potential, negatively affects engraftment kinetics, increases transfusion needs and prolongs hospitalization after ASCT.

Parameter	RVD (n=110)		D-RVD (n=45)		P-Value
Mobilization therapy (d), median (range)	8	(8-10)	9	(8-10)	0.0006
Plerixafor used, n (%)	27	(28)	15	(38)	0.3052
Measurements at day of apheresis					
CD34+ x 10^6/L, median (range)	52.19	(3.85-295.14)	41.37	(6.05-115.6)	0.0233
WBC x 10^9/L, median (range)	34.33	(8.97-80.34)	33.07	(16.9-75.49)	0.9526
CD34/WBC (%), median (range)	0.16	(0.02-0.83)	0.13	(0.02-0.45)	0.0463
coll. CD34+ x 10^6/kg BW, median (range)	10.22	(2.39-41.54)	8.27	(3.26-17.37)	0.0139
transp. CD34+ x 10^6/kg BW, median (range)	3.6	(2.05-10.36)	3.27	(1.90-5.15)	0.0157
Hospitalisation duration (d), median (range)	22	(13-51)	23	(18-39)	0.0654
Time to nGC > 0.5 G/L (d), median (range)	11	(9-27)	12	(10-20)	0.0164
Time to Platelets > 20 G/L (d), median (range)	14	(11-20)	16	(11-27)	0.0002
Platelet concentrates, median (range)	2	(1-16)	4	(1-19)	0.0015
Erythrocyte concentrates, median (range)	1	(1-19)	2	(1-8)	0.2837

WBC: white blood cells; BW: body weight; nGC: neutrophil granulocytes.

260

CAR-T cell therapy shows similar efficacy and toxicity in patients with aggressive B-lineage lymphatic malignancies regardless of CNS involvement

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Introduction: Efficacy and toxicity of chimeric antigen receptor T (CAR-T) cell therapy in relapsed/refractory (r/r) B-cell malignancies with central nervous system (CNS) involvement remains understudied. Here we analyzed the outcomes of CAR-T cell therapy in r/r patients with CNS involvement and compared them with patients without CNS disease.

Methods: Retrospective and monocentric comparative analysis of two patient cohorts with r/r DLBCL and B-ALL treated with CAR-T-cell therapy: 18 patients with CNS vs. 70 patients without CNS involvement.

Results: Overall response rates (84% vs. 80%; p = 1.0), progression-free (p = 0.55) and overall survival (p = 0.72) were comparable for both cohorts. The frequency of cytokine release syndrome (CRS) was comparable in the CNS- and non-CNS-cohorts; 78% vs. 80%; p = 0.672. In contrast, immune effector cell-associated neurotoxicity syndrome (ICANS, all grades) was more frequent in patients with CNS manifestation (50% vs. 27%; p = 0.062). In particular, ICANS grade 3 was two-times more frequent in the CNS-cohort (17% vs. 9%; p = 0.382), whereas no grade 4 events were documented.

Conclusions: Our study suggests that CAR-T cell therapy is effective and feasible in patients with r/r B-cell malignancies and CNS manifestation.

SSH/SSMO ORAL PRESENTATION – CLINICAL SOLID TUMOR ONCOLOGY

243

High rate of organ preservation with Papillon contact x-ray radiotherapy in rectal cancer

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Introduction: Rectal cancer typically necessitates a combination of radiotherapy (RT), chemotherapy, and surgery. However, the consequent functional disorders and reduction in quality of life have led to an increasing interest in organ preservation strategies. RT dose escalation would improve the rate of complete responses (CR), but due to the potential toxicity of the surrounding tissues, this strategy remains limited even with modern external beam RT techniques. This study reports on the use of the Papillon, an endocavitary contact RT device, in the treatment of rectal cancer. Papillon delivers low energy X-rays, allowing for significant dose escalation with a favorable toxicity profile.

Methods: Between January 2015 and August 2023, we treated 67 rectal cancer patients with Papillon contact RT in different settings. For this report we assessed the organ-preservation rate and the local control of 23 patients treated with an upfront organ preservation strategy and with a minimum FU of 12 months. Papillon was delivered as a boost to standard RT, with or without chemotherapy. Surgery (TME or local excision) was indicated in case of non-response at 3 months or in case of relapse. Follow up was performed according to the major guidelines at a 3-month interval for the first 2 years and every 6 months thereafter.

Results: All patients achieved a clinical complete response at the first assessment at 6 weeks. After a median FU of 35 months (range 58-12), the organ preservation rate was of 96%

(22/23). The local relapse rate was of 8% (2/23). All of our patients were alive at the last assessment. Ten patients achieved long-term (> 3 year) organ preservation. None of our patients developed grade 3 or more acute or late toxicities.

Conclusions: Our results demonstrate that the addition of Papillon contact RT provides a high rate of local remission with sustained long-term organ preservation and a low toxicity profile. Our results are in line with the recently proffered 3-year results of the OPERA randomized trial. This unique treatment modality may help future patients with rectal cancer benefit from low toxicity RT dose escalation, to achieve complete local response, and avoid surgery. If relapse occurs chance of cure is not compromised.

257

Effect of Staging Intervals on Progression-Free Survival (PFS) in Registration Studies of Oncologic Drugs: A Meta-Analysis

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Introduction: In metastatic breast cancer shorter staging intervals have been associated with lower Hazard Ratios (HR) for PFS suggesting more treatment effects (Dabush et al. 2021). The aim of this study is to corroborate these findings in studies leading to approval of drugs used for palliative treatment of solid tumors in Switzerland between 2010 and 2022.

Methods: Drug approvals were identified from the official Swissmedic Journals and underlying studies were identified from the official drug labels. Data were extracted and recorded in electronic forms, namely data on study design, included patients, disease site, drug class, and HRs for PFS. HRs and 95% confidence intervals (CI) for PFS were pooled in a meta-analysis. Studies were categorized according to the median restaging interval of all included studies. The differences in HRs between trials employing short and long restaging intervals were assessed overall and for prespecified subgroups. Pooled HRs were computed and weighted using generic inverse-variance and random-effect modeling using RevMan v5.4 software.

Results: 113 studies with a total of 66'308 patients were included in the analysis. The median restaging interval was 8 weeks. Overall, longer (i.e. ≥ 8 weeks) restaging intervals were associated with lower HRs than shorter (i.e. <8 weeks) restaging intervals, HR 0.48 (95% CI 0.44 – 0.52) vs. 0.56 (95% CI 0.50 – 0.62), P for subgroup difference 0.03. There was significant heterogeneity (I2 >50%). Subgroup analyses according to type of treatment (immunotherapy, chemotherapy, tyrosine kinase inhibitors) did not show significant differences in HRs according to restaging intervals. Also, no significant differences were found for subgroups according to disease sites with the exception of studies in melanoma where longer restaging intervals were associated higher HRs, 0.58 (95% CI 0.52 – 0.65) vs. 0.44 (95% CI 0.35 – 0.55), P for subgroup difference 0.03 , in contrast to the overall results. Sensitivity analyses are ongoing and will be presented.

Conclusions: In studies leading to approval of oncologic drugs in the palliative setting longer restaging intervals were associated with lower HRs for PFS. Our findings did not confirm results from a previous study in randomized studies in metastatic breast cancer studies. A potential impact of restaging intervals on results for PFS warrants further investigation.

271

Real-world outcomes of patients (pts) with malignant solid tumors treated with immune checkpoint inhibitors (ICI) in relation to smoking status

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Introduction: ICIs are the standard of care for the treatment of different advanced solid organ tumors. Especially in advanced non-small-cell lung cancer, never-smokers were shown to have an inferior outcome when compared to ex-smokers/smokers, suggesting that the smoking status could be a predictive marker for survival benefits under ICI treatment.

Methods: Pts within the Swiss Alpine Tumor Immunology Registry (AlpineTIR) treated with an ICI were differentiated by their smoking status (ex-smokers/smokers versus never-smokers).

Overall survival (OS) and progression-free survival (PFS) from the start of the first ICI treatment were analyzed by smoking status. Further, subgroup analyses for OS and PFS were done for the most common disease entities.

Results: A total of 702 pts were included, from which 455 pts (65%) were ex-smokers/smokers, 213 pts (30%) were never smokers, and 34 pts (5%) had an unknown smoking status. The median follow-up time from the administration of the first ICI to the statistical analysis was 2.7 years (95% CI: 2.3 to 3.2 years). The most frequent tumors were lung cancer (50%), melanoma (13%), renal cell cancer (7%), bladder cancer (6%), and others (24%).

Across all indications, the median OS was 1.7 years (95% CI: 1.4 to 2.6 years) for never-smokers (n = 213) and 1.5 years (95% CI: 1.2 to 1.8 years) for smokers (n = 455) (HR: 1.10, 95% CI: 0.89 – 1.37). The median PFS was 6.3 months (95% CI: 4.4 to 8.3 months) for non-smokers and 6.2 months (95% CI: 5.2 to 7.0 months) for smokers (HR: 1.05, 95% CI: 0.87 – 1.27).

In lung cancer pts, the median OS was 1.4 years (95% CI: 0.8 to 2.8 years) for never-smokers (n = 43) and 1.4 years (95% CI: 1.2 to 1.7 years) for smokers (n = 302) (HR: 1.02, 95% CI: 0.68 – 1.51). In melanoma pts, the median OS was 3.4 years (95% CI: 1.8 to not reached (NR) years) for never-smokers (n = 54) and 1.7 years (95% CI: 0.8 to NR years) for smokers (n = 34) (HR: 1.35, 95% CI: 0.72 – 2.53). There was also no significant OS difference between never-smokers and smokers in renal cell cancer pts or bladder cancer pts.

Conclusions: No survival difference between smokers and non-smokers with metastatic solid organ tumors treated with ICIs could be detected. Interestingly, even in the subgroup of lung cancer pts, no difference was seen. Based on these data, the smoking status should not guide ICI treatment decisions.

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161

Therapy-induced sequelae after breast cancer treatment in oncological rehabilitation – current update of the Scheidegger Breast Cancer Registry (BreCaReg)

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Introduction: During the last years the prognosis of breast cancer has been significantly improved. However, there is still little registry data on the extent to which these mostly multimodal therapy concepts lead to different secondary disorders and toxicities during rehabilitation. To answer this question, a tumor register for breast cancer patients was set up at the Paracelsus Clinic in Scheidegg since 2014.

Methods: In 10.900 cases clinical, sociodemographic and tumor-biological data are evaluated after written informed consent. The psychological stress of 3,750 women was also recorded using the German version of the NCCN distress thermometer (DT).

Results: ER+BC was present in 71.1%, Her2+BC in 17.3% and so-called "triple negative" Breast cancer (TNBC) in 11.5%. 32.4% were pre- , 6.2% peri and 61.4% postmenopausal.

Systemic chemotherapy (CHT) was performed in 56.7% (in 15.3% combined with anti-Her2 antibodies), radiotherapy in 89.1% and anti-hormonal therapy (AHT) in 75%. In almost a quarter of the cases (23.7%) a complete mastectomy was carried out. Patients with TNBC were significantly younger than women with ER+BC (53.4±11.1 vs. 56.2±10.1 years, P<0.001). Therefore, more patients in this subgroup were employed (71.1% vs. 65.3%; P < 0.001), but also somewhat more often disabled (EMR 1.9% vs. 1.4%; P = 0.029).

CHT (91.5 and 93.2 vs. 43%) and mastectomy (26.5 vs. 22.8%) were performed significantly more often in patients with Her2+BC or TNBC. Due to the more aggressive tumor biology (G3, Ki67^, N+), an axillary lymph node resection was performed more frequently in these subgroups. Therefore, the incidence of postoperative lymphedema (LOE) and chemo-induced polyneuropathy (CIPN) was significantly increased in both groups (LOE: 14.6 and 16.8 vs. 13.5%, p = 0.01 / CIPN: 56, 6 vs. 25.4%, p<0.001). Psychological distress (DT ≥ 4/10) was detectable in 66%, with the TNBC group showing the significantly highest values (70.6%; P<0.001).

Conclusions: Our data about over 10.000 breast cancer patients provide a very good evidence of the somatic and psychological complaints during oncological rehabilitation. Patients with TNBC were the most psychologically stressed and sociomedically most relevant group (age, EMR,...). The Scheidegger BreCaReg data can help to create a more precise picture of the rehabilitation of breast cancer patients in order to further develop specific rehabilitation and aftercare concepts.

182

Health-related quality of life in survivors of advanced melanoma treated with anti-PD1-based immune checkpoint inhibitors

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Introduction: Immune checkpoint inhibitors (ICIs) have significantly improved survival in advanced melanoma, resulting in an emerging melanoma survivor's population. However, treatment with ICIs is also associated with immune-related adverse events (irAEs), which might significantly affect patients' long-term overall health-related quality of life (HRQL). This single centre, cross-sectional survey aimed to describe the long-term symptom burden and impact on HRQL using patient-reported outcomes (PROs) in advanced melanoma patients with sustained disease control following treatment with ICIs.

Methods: Patients with stage IIB, III or IV (AJCCv8) melanoma that were treated with anti-PD1-based ICIs between January 2022 and July 2022 and were off-treatment with at least 6 months follow-up since the last infusion were included in a prospective cohort. Eligible candidates had an ongoing treatment response in the metastatic setting or no evidence of disease recurrence in the adjuvant setting. Study participants received a paper-based questionnaire, consisting of the EORTC QLQ-C30, EORTC QLQ-FA12 and the PRO-CTCAE questionnaires.

Results: Of 90 participants, 61 (68%) completed the questionnaire; 40 received single-agent anti-PD1 and 21 anti-PD1/anti-CTLA4. Thirty-three (54%) were treated in the adjuvant setting. At the time of enrolment, 31 (51%) participants had ongoing symptoms or required active treatment for a previous irAE. Overall, 18/61 (30%) participants reported long-term symptoms and trouble in physical and emotional functioning. Physical fatigue was common and interfered with daily activities (n = 12, 20%). In the PRO-CTCAE questionnaire, muscle ache (n = 12, 20%) and joint ache (n = 9, 15%) were commonly reported, whereas sadness (n = 12, 20%) and depression (n = 7, 11%) were also reported. Severe symptoms included dry skin (n = 12, 20%), joint ache (n = 11, 18%), and decreased sexual interest (n = 11, 18%). Despite this, participants reported overall good health (6.00, range 2.00–7.00) and reasonable level of HRQL (6.00, range 3.00–7.00).

Conclusions: Melanoma survivors experience long-term symptoms in physical and psychosocial HRQL domains after ICI treatment. These results underline the importance to address existing gaps in survivorship care, implement these findings in clinical practice and increase awareness for long-term symptoms in this growing number of melanoma survivors.

269

Functional iron deficiency is highly prevalent in patients with malignant diseases but has no significant impact on quality of life

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Introduction: Functional iron deficiency (FID) with and without anaemia, as defined by criteria of the European Society for Medical Oncology (ESMO), is thought to be common in patients with malignancy and might be a therapeutic target with potential improvement of fatigue-specific quali-ty of life (QoL). However, the prevalence of FID in that population is unclear. The goal of this study was to obtain data on the prevalence of FID in patients with oncological and malignant haemato-logical disorders prior to disease-directed therapy and compare QoL in patients with FID, absolute iron deficiency (AID) and without iron deficiency (ID).

Methods: Prospective single-centre observational study assessing the prevalence of ESMO-defined FID in patients aged ≥ 18 years with oncological and malignant haematological disorders within four weeks prior to disease-directed therapy. Patients referred to the outpatient clinic without known iron deficiency, iron supplementation, therapy with erythropoiesis-stimulating agents or red blood cell transfusions eight weeks prior to inclusion were eligible. ESMO-defined FID was characterized by Ferritin ≥ 100µg/L and transferrin saturation < 20%. AID was defined as Ferritin < 100µg/L. QoL-data were assessed using the FACT-An questionnaire.

Results: We screened 160 patients, 146 with complete data were included in the final analysis. The mean age was 62 years (range, 24-87) with a slight preponderance of women (n = 76, 52%). The majority (n = 130, 89%) had solid cancer, of whom 40% (n = 52) had metastatic disease. There were 11% (n = 16) with malignant haematological disease. 30% (n = 44) of the total population had ESMO-defined FID, of which 23% (n = 10) had anaemia (haemoglobin [Hb] < 110g/L as defined by ESMO). ESMO-defined AID was observed in 35% (n = 51) of patients, of which 1.9% (n = 1) had anaemia. The total scores for FACT-An were not significantly different between patients with FID, AID and without iron deficiency.

Conclusions: We observed a high prevalence of ESMO-defined FID of 30% in patients with oncological and ma-lignant haematological disorders prior to disease-directed therapy, most often without anaemia. However, fatigue-specific QoL assessed by FACT-An questionnaire was not significantly different between patients with and without FID.

SSH POSTER PRESENTATION – HEMOSTASIS, TRANSFUSION MEDICINE, VASCULAR, LABORATORY MEDICINE, BENIGN HEMATOLOGY

189

Intrinsic prothrombotic drive modulates the efficacy of direct oral anticoagulants

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Introduction: The “one dose fits all” approach used for direct oral anticoagulants (DOAC) might not be always adequate for patients with complex prothrombotic conditions, as highlighted in triple positive antiphospholipid syndrome. We hypothesize that anticoagulant efficacy of a given DOAC concentration varies as a function of the individual procoagulant potential.

To compare the anticoagulant effect of DOAC on ex-vivo thrombin generation (TG) in plasma from patients with liver cirrhosis (LC) and a prothrombotic (P-LC) or highly-prothrombotic (HP-LC) profile compared to healthy donors (HD), as well as in plasma from obese patients before and after bariatric surgery (BS).

Methods: Plasma samples from patients with LC (n = 71) and HD (n = 10) were spiked with vehicle or DOAC (rivaroxaban, apixaban or edoxaban; 150 ng/mL). Ex-vivo TG was measured with ST-Genesia.

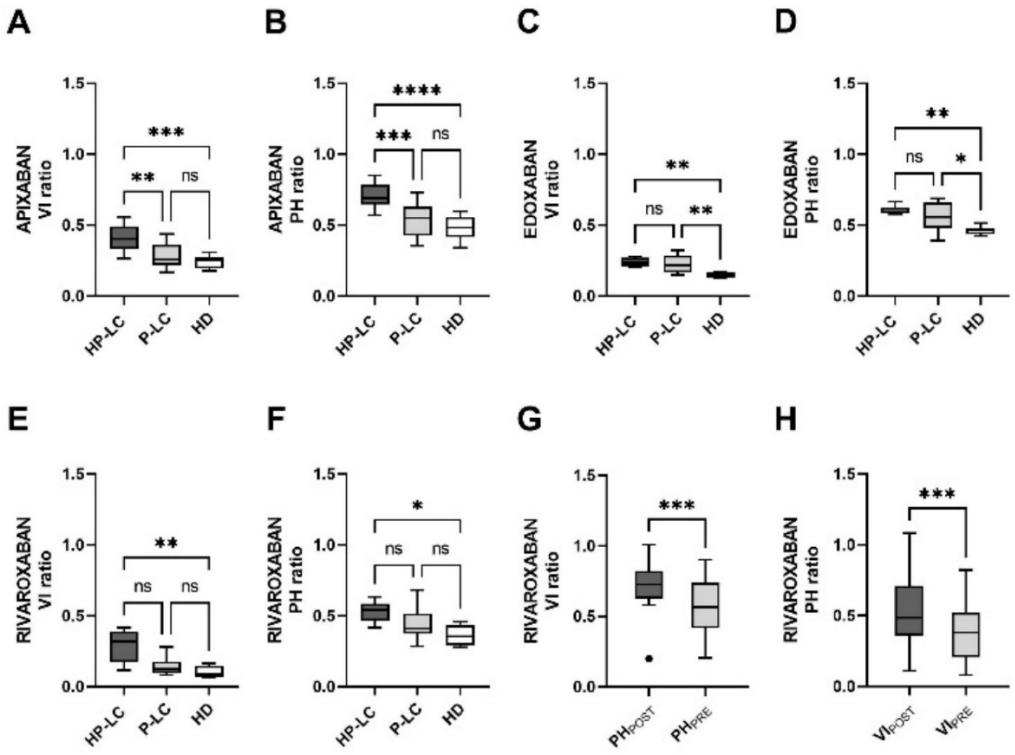
Plasma was collected from obese patients (n = 12, BMI ≥35 kg/m2) receiving rivaroxaban 10 mg 1 day prior and 3 days after BS. TG was assessed by calibrated-automated-thrombogram at baseline and at seven time points during 24h post-rivaroxaban administration.

Velocity index (VI) and peak height (PH) ratios (with/without DOAC) were compared using Kruskal-Wallis test (HD, P-LC, HP-LC) or a parametric paired t-test (pre- vs. post-BS) in samples containing similar rivaroxaban concentrations.

Results: In samples spiked with apixaban, edoxaban and rivaroxaban, significantly higher VI and PH ratios were observed in HP-LC compared to HD (respectively, (median VI ratio 0.39 (HP-LC) vs. 0.25 (HD), p = 0.0002; median PH ratio 0.69 (HP-LC) vs. 0.48 (HD), p<0.0001); (0.25 vs. 0.14 p = 0.0024; 0.62 vs.0.47 p = 0.0015); (0.32 vs. 0.09, p = 0.01; 0.54 vs. 0.35, p = 0.036,)). In P-LC, both ratios were significantly higher compared to HD, only in edoxaban treated samples (0.20 vs. 0.14, p = 0.005; 0.55 vs. 0.47, p = 0.02), but not in apixaban and rivaroxaban treated samples.

In bariatric patients, significantly higher PH and VI ratios (PHpost vs PHpre p = 0.0001, mean of differences = 0.15; VIpost vs VIpre, p = 0.0004; mean of differences = 0.16) were observed after BS, despite similar rivaroxaban concentrations.

Conclusions: Similar DOAC concentrations reach lower anticoagulant efficacy in plasma from individuals with a procoagulant state. These findings raise the question whether a monitoring may be clinically useful in complex prothrombotic conditions.



193

Thrombophilia screening in autoimmune hemolytic anemia

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Introduction: Autoimmune hemolytic anemia (AIHA) is characterized by an increased rate of thrombosis, whose predictors are not completely clarified, although an association with active hemolysis, anemia severity and previous splenectomy has been reported. The value of thrombophilia screening, including congenital and acquired abnormalities, has not been established yet. The aim of this study was to investigate the prevalence of thrombophilia abnormalities in a cohort of AIHA patients.

Methods: AIHA patients have been systematically tested for Factor V Leiden (FVL) and prothrombin (FII) G20210A mutation, protein C (PC), protein S (PS), antithrombin (AT) levels, and the presence of antiphospholipid antibodies (APLA: lupus anticoagulant, anti-cardiolipin and anti-b2-glycoprotein-1 IgG and IgM). Patients' hematological characteristics, additional thrombotic risk factors, history of venous/arterial thrombosis before and after AIHA diagnosis, and anticoagulant/ antiplatelet therapies were recorded.

Results: 59% of 92 enrolled patients were female with a median age at diagnosis of 70.5 years (range 24-89) and were classified as warm AIHA (49%), cold agglutinin disease (31.5%), mixed/atypical AIHA (9.8%), and Evans syndrome (association of AIHA and other immune cytopenia, 8.7%) (Table). Thrombophilia screening resulted positive in 19 patients (20.7%): FVL (1.1%), FII mutation (1.1%), APLA (10.8%), PC deficiency (1.1%), PS deficiency (2.2%), AT deficiency (2.2%), more than one abnormality (2.2%). Twenty-seven patients (28%) received anticoagulant/antiplatelet prophylaxis after AIHA diagnosis. Since AIHA diagnosis, 26 patients (28%) experienced thrombosis (77% venous, 23% arterial), with 33 total events (3 patients had >1 event). Median hemoglobin and LDH at thrombosis were 8.6 g/dL (2-11.7), and 374 U/L (129-1873) respectively, indicating active hemolysis. No relationship was found with inherited thrombophilia abnormalities (3 patients, 8% experienced thrombosis vs 7, 10.5% who did not), whilst a significant association was noted with APLA positivity ($p<0.05$). Two patients with APLA experienced more than 1 event, one had 1 venous and 1 arterial event.

	All patients N=92 (%)	Thrombosis N=26 (%)	No Thrombosis N=66 (%)
AIHA features at diagnosis			
Sex (M/F)	38 (41) / 54 (59)	8 (31) / 18 (69)	30 (45) / 36 (55)
Age, median (range)	70,5 (24-89)	75 (36-87)	69.5 (24-89)
AIHA Type			
Warm	46 (49)	10 (38)	36 (55)
Cold	29 (32)	6 (23)	23 (35)
Mixed/atypical	9 (10)	5 (19)	4 (6)
Evans Syndrome	8 (9)	5 (19)	3 (2)**
Thrombotic complications at/after AIHA diagnosis			
Venous thrombosis		**20 (77)**	
At AIHA diagnosis	-	8 (40)	-
After AIHA diagnosis	-	12 (60)	-
Arterial thrombosis	-	**6 (23)**	-
At AIHA diagnosis	-	3 (50)	--
After AIHA diagnosis	-	3 (50)	
Thrombophilia screening			
Thrombophilia abnormalities	**19 (21)**	**7 (27)**	**12 (18)**
Factor V Leiden	1 (1)	1 (4)	-
prothrombin G20210A mutation	1 (1)	-	1 (1.5)
APLA	10 (11)	5 (19)	5 (8)**
Protein C deficit	1 (1)	-	1 (1.5)
Protein S deficit	2 (2)	-	2 (3)
Antithrombin deficit	2 (2)	1 (4)	1 (1.5)
Combined abnormalities	2 (2)		2 (3)*

Conclusions: Along with disease activity and Evans syndrome, positivity for APLA appears the main predictor of thrombosis in AIHA and is therefore worth of testing, whilst extensive thrombophilia screening encompassing inherited abnormalities appears less useful.

198

Introduction of unbiased diagnostic transcriptome analysis into clinical routine for solid and haematological neoplasms

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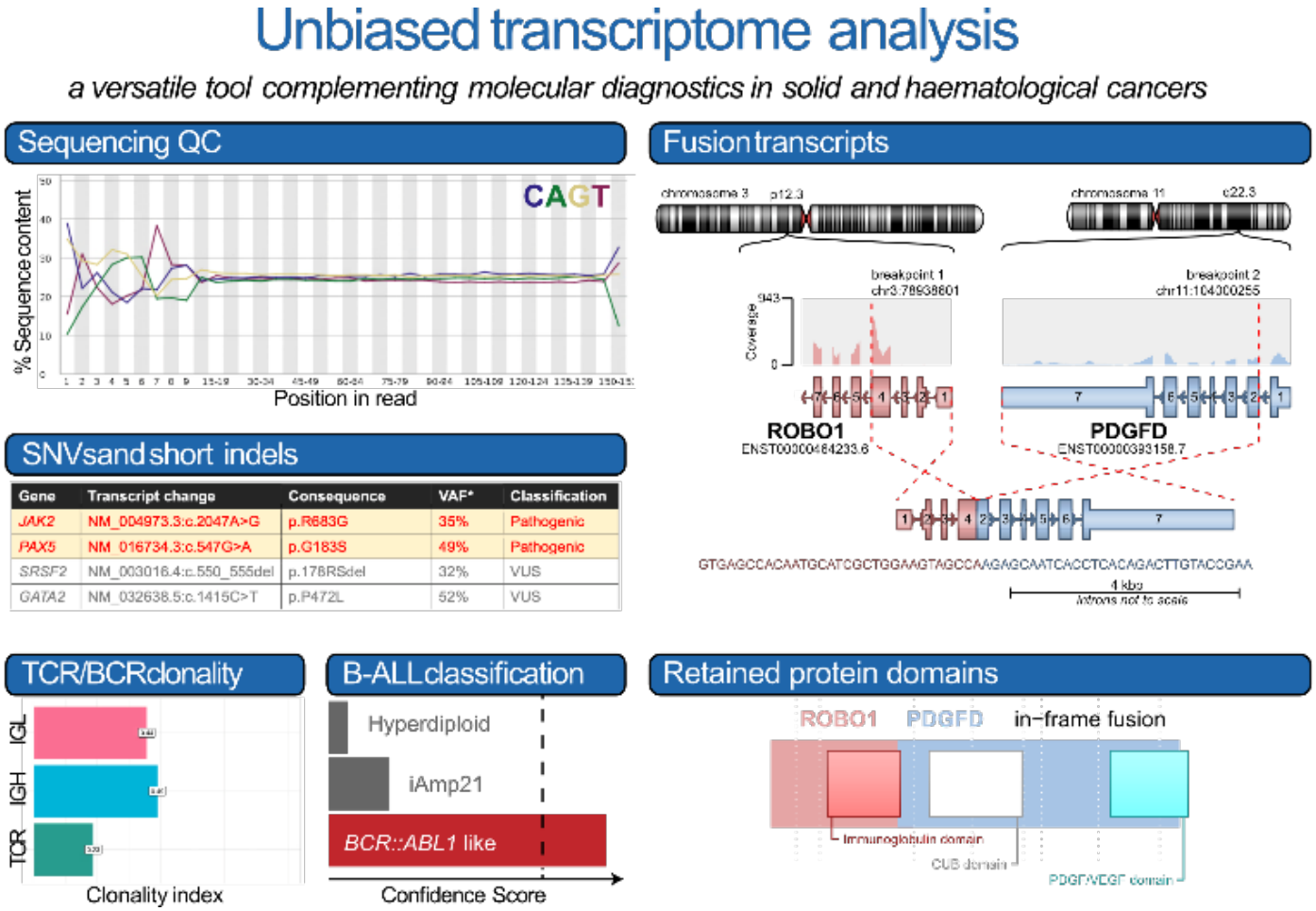
Introduction: Large-scale international sequencing projects have revealed novel oncological entities accessible by targeted therapies. Fusion transcripts are increasingly recognised as targetable driver mutations in sarcoma, epithelial, and other solid tumours. The current WHO-5 classification recommends transcriptome analysis for B-ALL and some MPNs. Targeted NGS panels are available for some of these fusion transcripts; however, as more oncogenic fusions are discovered, these panels must be continuously updated to include relevant genes. Unbiased transcriptome analysis (RNAseq) in a diagnostic setting can overcome the limitations to discover novel fusion transcripts, classify tumours by their transcriptomic profile, determine immune cell clonality as well as identify small nucleotide variants (SNVs).

Methods: A commercial strand-specific library prep was used on low-quality RNA extracted from formalin fixed paraffin embedded (FFPE) tissue biopsies as well as high-quality RNA extracted from fresh bone marrow or blood. Samples were sequenced to at least 25 million 150bp paired-end fragments on an Illumina instrument. We developed a custom analysis pipeline to detect, classify, and visualise fusion transcripts, transcriptomic patterns, SNVs as well as short indels, immune repertoire, and HLA type. This pipeline was validated by in-house samples of tumours with known mutational profile and enriched using published raw sequencing data from B-ALL and solid tumour samples.

Results: We were able to detect all known and several previously not described fusion transcripts from samples, showing that our method is applicable to RNA extracted from FFPE-samples as well as intact cells. Many SNVs and indels were detected, although most truncating variants are under-represented due to nonsense mediated RNA decay. We were able to detect B- and T-cell rearrangement as well as correctly determine high-resolution HLA types for all major MHC classes.

Conclusions: Unbiased transcriptome analysis is a versatile tool complementing conventional molecular diagnostics and is applicable to fixed as well as fresh samples. Fusion transcripts were reliably detected, and several novel previously undescribed potentially targetable fusions were found. As the first institution in Switzerland, we will introduce this novel analysis into the diagnostic setting.



207

Immortalized cell line for megakaryocytes and platelets production

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Introduction: Blood products from donors are used to treat a variety of diseases and conditions that cause cytopenia. However, the blood transfusion system needs to be strengthened because the number of blood donors is decreasing due to low birth rates, an aging population, and contamination by pathogens, and increasing demand due to chronic blood disorders that are common in the elderly population. Platelets play a key role not only in hemostasis and thrombosis, but also in tissue regeneration after injury and in the pathophysiology of inflammation. With a legal expiration date of 5 days, platelet products are difficult to keep in stock. In addition, repeated transfusions are known to induce antibodies to human leukocyte antigens (HLA) on transfused platelets in recipients. Human immortalized hematopoietic progenitor cells would be a potential source to provide a sufficient supply of identical platelet concentrates without loss of responsiveness due to immune rejection, especially in patients with a rare HLA.

Methods: We propose CD34-derived cell lines that have been genetically modified to retain the proliferative capacity associated with their progenitor-like state. Our target gene discovery approach is based on in silico comparison of RNA sequencing data from genetic blood disorders, i.e. polycythemia vera and essential thrombocythemia. We used an inducible construction to modulate the expression of genes of interest in cord blood CD34+ cells and leukofilter clots.

Results: We developed a protocol to generate megakaryocytes in vitro, they have a large polyploid nucleus with cytoplasmic protrusions indicative of proplatelet activit and they carry glycophorin membrane markers Cd41a, CD42a, CD42b (ie antigens classically found on the surface of megakaryocytes). Upon shear stress, we obtained single cytoplasmic vesicles carrying the membrane markers of mature platelets and the ability to be activated by induction of thrombin. For further functional evaluation, the cells were injected into sublethally irradiated NSG mice and the presence of human platelets in the circulation was demonstrated by cytometry.

Conclusions: In addition to a new cell therapies oriented approach, we provide a model of robust megakaryotic differentiation to deepen our understanding of this complex process. Through further gene editing, we will be able to mimic platelet-associated malignancies.

225

REAL-WORLD EFFECTIVENESS OF ECULIZUMAB, SWITCH TO RAVULIZUMAB AND RAVULIZUMAB AS FIRST THERAPY IN SWISS PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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Introduction: Eculizumab (ECU), Soliris®, a complement component 5 (C5) inhibitor, has been the standard of care for patients with paroxysmal nocturnal hemoglobinuria (PNH). Ravulizumab (RAV), Ultomiris®, is a therapy engineered from ECU with an extended 8-week dosing interval (vs 2-week with ECU), which decreases treatment burden markedly, while providing immediate complete and sustained inhibition of C5. Hence, RAV is expected to become the standard of care to treat PNH. The present work assesses real world effectiveness of ECU and RAV from the PNH Swiss Soliris and Ultomiris Reimbursement Registry (SSURR).

Methods: The SSURR is a prospective, longitudinal, multi-center registry in Switzerland. Patients were recruited and followed in 9 centers. Health-related data were collected at the 1st visit (baseline, before starting therapy), at 3 months (mos) and follow-up (FU) visits every 6 mos. For patients who were switched from ECU to RAV (ECU/RAV), data from the last visit on ECU therapy were used as baseline. Data were collected from 23 Feb 2012 to 3 Oct 2022 on patients treated for ≥12 mos. Data were summarized using descriptive statistics.

Results: At baseline, the median (range) of hemoglobin (Hb) level was 10 g/dL (7.3-14) in ECU (n = 37), 8.1 g/dL (7-13.7) in RAV (n = 7) and 10.5 g/dL (8.1-15) for ECU/RAV (n = 21). At last FU, median Hb in ECU was 11.6 g/dL (9.6-13.4, n = 4), 10.4 g/dL (6.7-12.5, n = 5) in RAV and 10.7 g/dL (8.4-14.8, n = 9) in ECU/RAV treatment (Table 1).

Before inclusion, half of patients required transfusion (transf) in ECU (n = 40) and 57.2% in RAV (n = 7). At 24 mos, 10.3% of patients required transf in ECU (n = 35) and less than half (3/7) at 12 mos in RAV. In ECU/RAV (n = 21), no patient except one required a transf at the 12 mos FU.

Thrombotic events (TEs) before starting treatment were reported in 32.5% (n = 13/40) of patients in ECU and in 14.3% (n = 1/7) in RAV. After starting the treatment, TEs were 2.6%, 2.9% and 3.3% at 24, 36 and 48 mos respectively in ECU. There was no TEs reported for RAV and 1 event for the ECU/RAV.

Conclusions: Since introduction of RAV in Switzerland in Oct 2020, a marked tendency to switch the treatment from ECU to RAV is observed, likewise, for those needing to start therapy, RAV was clearly the drug of election. Although the number of patients is small in ECU, RAV and among switch patients (ECU/RAV), sustained effectiveness is observed.

		ECU	RAV	ECU/RAV[a]
Number of patients at baseline (N)		49	10	30
Number of women at baseline (%)		27 (55%)	3 (30%)	16 (53%)
Median time (in months [P25 - P75]) of treatment for patients with 12 months FU[b]		75.5 (39-102)	14 (13-18)	13 (11-17)
Age (years)		50 (20-81)	67.5 (32-80)	48.5 (21-76)
Hb (in g/dL) at baseline[c]		10 (7.3-14)	8.1 (7-13.7)	10.5 (8.1-15)
Hb (in g/dL) at 12 months[d]		10.7 (7.6-15)	10.4 (8.6-12.5)	11.1 (9.1-14.9)
% change from baseline		+ 7%	+ 28.4%	+ 5.7%
Hb (in g/dL) at last FU[e]		11.6 (9.6-13.4)	10.4 (6.7-12.5)	10.7 (8.4-14.8)
% change from 12 months		+ 8.4%	+ 0%	- 3.6%
LDH (in U/L) at baseline		1 349 (354-3 000)	759 (175-4 267)	247 (194-463)
LDH reduction >60% at 12 months		70% (28/40)	57.1% (4/7)	81% (17/21)
LDH reduction >60% at last FU[f]		100% (4/4)	40% (2/5)	77.8% (7/9)
Number of RBC transfusions at baseline[g] (% of patients)	0	47.5% (19/40)	28.6% (2/7)	90.5% (19/21)
	1 - ≥5	47.5% (19/40)	57.2% (4/7)	4.8% (1/21)
Number of RBC transfusions at 12 months	0	77.5% (31/40)	57.1% (4/7)	90.5% (19/21)
	1 - ≥5	22.5% (9/40)	42.9% (3/7)	4.8% (1/21)
Number of RBC transfusions at last FU[f]	0	-	80% (4/5)	66.7% (6/9)
	1 - ≥5	-	20% (1/5)	22.2% (2/9)
Patients having thrombotic events before treatment (% of patients)		32.5% (13/40)	14.3% (1/7)	0% (0/21)
Patients having thrombotic events at 12 months (% of patients)		0% (0/40)	0% (0/7)	14.3% (3/21)
Patients having thrombotic events at last FU[f] (% of patients)		0% (0/4)	0% (0/5)	0% (0/9)

Data shown as mean Median; (Min-Max) or n% (n/N). – Not enough patients for the last FU visit.

Abbreviations: ECU, Eculizumab; FU, Follow-up; Hb, Hemoglobin; LDH, Lactate Deshydrogenase; n, number of observed cases; N, sample size, PNH, Paroxysmal Nocturnal Hemoglobinuria; RAV, Ravulizumab; RBC: red blood cells transfusion.

[a] Treatment switch from ECU to RAV was conducted irrespectively of time between last ECU and first RAV treatment. The data from the last visit on ECU therapy ("switch visit") are used as baseline. Follow-up duration is counted from the time-point the patients switched and started RAV and continued this treatment. This group includes patients with minimally one follow-up visit at month 12 (after switching to RAV treatment).

[b] Median time of treatment for patients with 12 months FU is based on n=40 patients in the ECU group, n=7 patients for RAV and n=21 patients in the ECU/RAV group respectively.

[c] Hb level at baseline is based on n=37 patients in the ECU group, and n=7 and n=21 patients for RAV and ECU/RAV group respectively.

[d] Hb level at 12 months is based on n=38 patients in the ECU group, and n=7 and n=20 patients for RAV and ECU/RAV group respectively.

[e] Hb level at last FU is based on n=4 patients in ECU group (last FU: 120 months); n=5 and n=9 patients for RAV and ECU/RAV group respectively (last FU: 24 months).

[f] Last FU corresponds to 120 months (ECU group) and 24 months for ECU/RAV and RAV group respectively.

[g] Patients having at least 1 transfusion within 12 months before inclusion.

227

Development, validation, and implementation of a decision support tool for the screening of mild bleeding disorders

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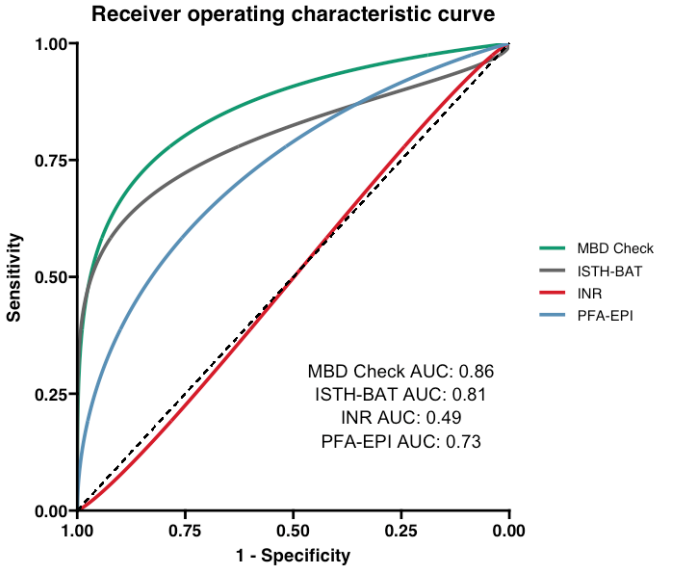
Introduction: Mild bleeding disorders (MBD) are the most common inherited bleeding disorders that often manifest with perioperative hemorrhages. However, practical screening tools are not available. We aimed to develop, externally validate and implement an easy-to-use, machine-learning-based decision support tool for the screening for MBD.

Methods: Detailed clinical and laboratory data were collected in two independent prospective cohort studies including consecutive patients referred for a suspected MBD to specialized outpatient units (n = 555, training cohort; n = 217 validation cohort). The diagnostic workup was done following current guidelines and an expert panel established the final diagnosis. In the training cohort, the items of the ISTH-BAT were simplified by grouping levels with similar response patterns. Predictors were selected using the "Boruta" algorithm and focus group discussions. Multiple machine-learning algorithms were fitted to the data and five algorithms were further tuned. The best-performing model was externally validated in the validation cohort.

Results: The following predictors were selected: (a) activated partial thromboplastin time, (b) PFA-200 closure time (epinephrine/collagen cartridge), (c) sex, and (d) a reduced bleeding history (surgery, tooth extraction, epistaxis, minor wounds, cutaneous bleeding, oral bleeding, postpartum hemorrhage, and

menorrhagia). In the validation cohort, 87.5% of patients with MBD were correctly identified (sensitivity; 95% confidence interval [CI]: 79.9, 93.0), and 54.3% (specificity; 95% CI: 44.3, 64.0) of patients without MBD would have been correctly excluded from further work-up. The AUROC was 0.86 (95% CI: 0.81, 0.90), in contrast to 0.81 (ISTH-BAT, 95% CI: 0.75, 0.87), 0.73 (PFA-200, 95% CI: 0.65, 0.79), and 0.49 (INR, 95% CI: 0.42, 0.55). The decision support tool was implemented on an easy-to-use web application.

Conclusions: We were able to develop, externally validate, and implement an effective decision support tool for screening patients with MBD.



228

Outcomes of patients with suspected heparin-induced thrombocytopenia in a contemporary cohort of patients

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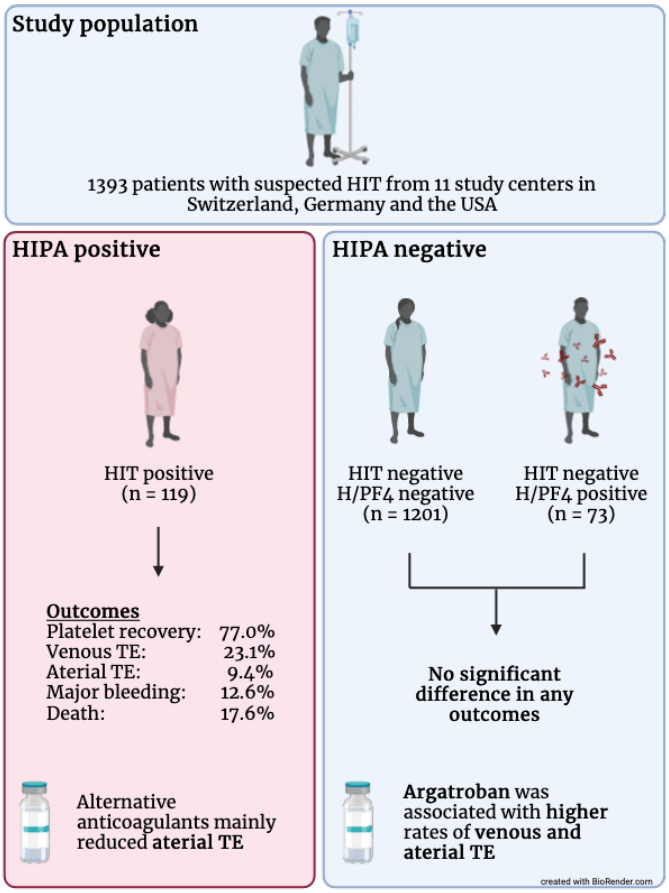
Introduction: Managing patients with suspected heparin-induced thrombocytopenia (HIT) remains a major clinical challenge. Knowledge of the effects of management decisions on clinical outcomes is sparse and treatment recommendations are often based on low certainty. In a prospective multicenter cohort study, we studied the treatment and outcomes of patients with suspected HIT.

Methods: We included consecutive patients with suspected HIT and report the outcomes of (a) patients with HIT, (b) patients without HIT but positive heparin/PF4 antibodies, and (c) patients without HIT. Comprehensive clinical and laboratory data were collected in detail and the washed-platelet heparin-induced platelet activation test (HIPA) served as the reference standard test defining HIT.

Results: Among 1393 patients included in 11 study centers (46% female, median age of 67), HIT was confirmed in 119 patients (prevalence 8.5%). The setting was intensive care unit (37%) and cardiac surgery (32%) in the majority of patients. The predominant treatment was argatroban (70%), and complete platelet recovery was observed in 77% of HIT patients. Of the patients with HIT, 23% developed subsequent venous thromboembolism (TE), 9% arterial TE, and 18% died. Major bleeding occurred in 13% of HIT patients and did not differ significantly between drugs. Treatment with argatroban, bivalirudin or DOAC markedly reduced the risk of subsequent arterial TE. HIT-negative patients with and without H/PF4 antibodies did not differ with regard to any outcome.

Conclusions: Our results indicate that HIT is still a serious disease with a high risk of major adverse events. In the absence of randomized controlled trials, our results add further evidence on the effectiveness of DOAC, argatroban, and bivalirudin treatment.



230

Mitochondrial calcium uniporter as a Key Regulator in the Generation of Procoagulant COAT Platelets

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Introduction: Procoagulant platelets represent a subpopulation of platelets that are phenotypically and functionally different from aggregating platelets. The combined activation of platelets with collagen-plus-thrombin induces the formation of procoagulant COAT platelets, which expose phosphatidylserine (PS) and down-regulate integrin alphaIIb/beta3. This dual agonist stimulation mobilizes high level of cytosolic calcium, which is taken up by mitochondria through mitochondrial calcium uniporter (MCU). A significant rise in mitochondrial calcium results in the depolarization of mitochondrial membrane potential, which triggers the opening of mitochondrial permeability transition pore (mPTP) to achieve the supramaximal cytosolic calcium level required for PS exposure. Thus, in this study, we aimed to explore the function of MCU in the generation and modulation of procoagulant COAT platelets.

Methods: Platelets were activated with thrombin (THR) or convulxin (CVX, collagen GPVI agonist) or a combination of these. Flow cytometry was employed to monitor procoagulant and aggregatory properties of platelets using Annexin-V and PAC-1 binding, respectively. Function of MCU was modulated with specific inhibitors, namely mitoxantrone (MTX) or Ru265.

Results: Our results demonstrated that MTX had non-significant effect on single THR stimulated platelets as this agonist triggered poor procoagulant platelet formation. In contrast, platelets stimulated with single CVX generated moderate procoagulant platelets and MTX showed a significant inhibition in the generation of Annexin V positive platelets. Furthermore, when we co-stimulated the platelets with THR-plus-CVX, our results demonstrated that MTX exhibited significant decrease (-23% ± 1.26) in Annexin V positive platelets and inversely increase (+36% ± 4.6) in PAC-1 positive platelets. Data were replicated and validated by blocking MCU with Ru265.

Conclusions: Altogether, the present study revealed that CVX pathway alone and synergistically with THR enhances the formation of procoagulant platelets through MCU-driven mitochondrial calcium uptake. MCU is as a key regulator in the generation of procoagulant platelets.

232

Inter-center variability in the technique of processing Hematopoietic Progenitor Cells obtained by Apheresis for autologous transplantation in Switzerland

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Introduction: Autologous hematopoietic stem cell transplantation (ASCT) has been performed successfully in Switzerland for more than 30 years.

Standards of the Foundation for the Accreditation of Cellular Therapy (FACT)–Joint Accreditation Committee International Society for Cellular Therapy (JACIE) and of the European Society for Blood and Marrow Transplantation (EBMT) provide us with important guidelines for handling hematopoietic progenitor cells (HPCs) obtained by Apheresis (HPC-A) for ASCT. However, because some aspects of HPC-A processing are not addressed in these guidelines, it can lead to inter-center variations (ICV) in the procedures used.

The objective of this survey is to assess ICV in techniques and materials used to perform processing of HPC-A, understanding the scope of these heterogeneities and focusing on the possibility of unifying procedures among transplantation centers (TCs).

Methods: A 37- item questionnaire was provided in electronic form through Survey Survio and sent to 9 TCs in Switzerland. The survey was not anonymous. Only questions to which all centers responded were evaluated.

Results: From June to July 2023, 7 TCs from Switzerland answered the survey. The participation rate was 77, 7%.

All of responding TCs use standardized procedures (intra-center standardization) in compliance with the FACT-JACIE standards. The ICV identified are summarize in Table 1 which shows these topics as well as the amount of centers which did agree to each particular topic. They were related to number of CD34+ cells collected per graft; the processing system; the time period from thawing to infusion of the material; the quality control tests; the range of storage temperature and the material's disposal policies.

Conclusions: This survey represents a first approach to understand the extent of heterogeneity in the processing of HPC-A material. While this results demonstrate robust homogenization across a number of items, there are a number of variations in less regulated aspects like storage space and disposal of graft, which represent an area of interest for future implementations. Unifying protocols could have a positive impact on processing of HPC-A by promoting comparability between centers (benchmarking) and facilitating discussion on regulatory issues.

A collaborative effort at a national level will be needed to achieve the ambitious goal of harmonizing procedures for HPC-A processing.

Processing of HPC-A	Topic	Results (n= TCs; %)
Collection	▪ The minimum number of CD34+ cells to be harvested is dependent on the patient's pathology ▪ In all conditions, the minimum number of HSPCs to be harvested is ≧2×10⁶ CD34+/kg BW for each graft	n= 3; 42.9% n= 4; 57.1%
Processing	▪ System used to process the material: - Closed system - Semi-open system - Open-system ▪ Maximal delay from thawing to infusion: - From 10 to 30 min (centers thawing their graft in the patient's ward) - From 1 to 2 hours (centers thawing their grafts in the processing facility)	 n= 5; 71.4% n= 1; 14.3% n= 1; 14.3% n= 6; 85.7% n= 1; 14.3%
Quality Control	▪ Stem cell dosage, expressed as CD34+ cell number, by: - Flow cytometry - Tests by Coulter counter and Trypan Blue 0.4% viability test with validated protocols ▪ Viability test after thawing and before the transplantation.	 n= 6, 85.7% n= 4, 57.1% n= 5; 71.4%
Storage	▪ Range of storage temperature	Each processing facility has its own range validated – no agreement between centers (from *-130C° to -196°C)*
Disposal	▪ Expiration date on the label but no agreement on the years (5, 10 or 25 years) ▪ Expiration date on the label not defined ▪ Storage time limit is both disease and age-dependent ▪ Range of age-dependent storage time limit in patient > 75 yrs of age ▪ Written policies on how to handle unused stem cell products.	n= 5; 71.4% n= 2; 28.6% n= 2; 28.6% n= 2; 28.6% n= 3; 42.9%
Unify inter-center protocols	▪ Interest in unifying inter-center protocols on a national scale	n= 6, 85.7%

Table 1. Inter-center variations in processing of HPC-A.

255

D-alloimmunization and serological trap of D variants

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Introduction: Alloimmunization against D antigen is of major concern in case of pregnancy and transfusion. D antigen is highly immunogenic and has many variants. Today, more than 200 variant alleles have been identified. According to the literature, about 1% of Caucasians and 10% of Africans carry a RHD variant allele. Except for the variants Weak 1,2,3, all are considered to confer a risk of alloimmunization. Serologic analyses are limited in their ability to detect variants because of the high sensitivity of the reactants. Therefore, partial D expression may be missed, which can lead to harmful anti-D alloimmunization.

Methods: Using automated methods (IH-1000 ID-DiaClon ABO/D, Bio-Rad) we examined samples of patients and selected all doubtful serological data: intensity just below (+++) the strongest cut-off (++++). Of note, such a sightly decreased intensity (+++), is considered as positive (no variant) by the manufacturer.

From these cases we selected the R0/R0 (Dce/Dce) phenotype of female patients younger than 50 years old and patients requiring phenotype-matched red cell concentrates for D, C/c, E/e and K, according to current international recommendations. We performed genetic analyses of the most common RHD-alleles using PCR (Fluovista; RBC FluoGene DWeak/variant; Inno Train).

Results: Between October 2021 and September 2023, we detected 194 D-positive samples with a slightly decreased intensity. Among them, 40 cases harbored a D variant (25.9%). The detected variants were RHD 4.0 (26 patients), RHD 4.1 (2 patients), RHD 4.2 (12 patients) and RHD Weak D type 5 (1 patient).

Conclusions: The proportion of D variants detected among those slightly reduced reactivities is about 25%. This proportion is higher than expected. These data are clinically relevant for the physicians in the management of pregnancy or transfusion.

In conclusion, when D phenotype is analyzed with a serologic method, we recommend to check all cases of slightly weakened reactivity associated with R0R0 haplotype (Dce/Dce) by a molecular biology method.

261

Thrombin generation parameters accurately predict liver cirrhosis decompensation

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Introduction: Liver cirrhosis (LC) is a complex condition that is associated, among others, with a prothrombotic state and episodes of decompensation. While hypercoagulability is secondary to the altered equilibrium in coagulation factors, the causes of liver decompensation are multiple. The aim of this study was to investigate the relationship between hemostatic parameters and the occurrence of LC decompensation.

Methods: We performed a prospective single-centre study at Lausanne University Hospital (CHUV) including 302 non-anticoagulated adult patients with LC of all aetiologies and stages. The primary outcome was LC decompensation, defined by the development of ascites, encephalopathy, hepato-renal syndrome, spontaneous bacterial peritonitis, or variceal bleeding. We studied clinical and laboratory parameters including in vivo and ex vivo thrombin generation. Statistical analyses were performed using univariate and multivariate logistic regression.

Table 1 : Patient characteristics and results of univariate and multivariate logistic regressions. *Numbers are mean (standard deviation), resp. numbers of patients (percentage). NASH, nonalcoholic steatohepatitis; ETP, endogenous thrombin potential; TM, thrombomodulin.*

		All	Liver cirrhosis decompensation			
			0	1	p-value (univariate)	p-value (multivariate)
	Events	302	235 (77.8%)	67 (22.2%)		
Clinical parameters	Age	58.1 (11.1)	58.2 (10.6)	57.8 (13.0)	0.810	
	Female	66 (21.8)	51 (77.3%)	15 (22.4%)	0.905	
	Aetiology					
	Alcoholic	153 (50.5%)	109 (71.7%)	43 (28.3%)	0.011	
	NASH	25 (8.2%)	18 (72.0%)	7 (28.0%)	0.467	
	HBV	24 (7.9%)	18 (75.0%)	6 (25.0%)	0.730	
	HCV	73 (24.0%)	68 (93.2%)	5 (6.9%)	0.001	
	HBV + HCV	2 (0.6%)	2 (100.0%)	0 (0.0%)	0.000	
	Autoimmune	18 (5.9%)	14 (77.8%)	4 (22.2%)	0.997	
	Others	8 (2.6%)	6 (75.0%)	2 (25.0%)	0.846	
	Porta vein flow >10 (cm/s)	122 (63.2%)	96 (78.7%)	26 (21.3%)	0.519	
	Thrombocytopenia	169 (56.9%)	120 (71.4%)	48 (28.6%)	0.004	
	Portal hypertension	238 (78.6%)	176 (74.3%)	61 (25.7%)	0.007	
	Child-Turcott-Turcott score	5.9 (1.5)	5.49 (1.1)	7.3 (2.0)	0.000	0.028
	MELD score	9.7 (4.1)	8.8 (3.4)	12.7 (4.9)	0.000	
	Ascites					
	Absence	253 (83.8%)	216 (85.4%)	37 (14.6%)		
	Moderate	42 (13.9%)	17 (41.5%)	24 (58.5%)	0.000	
	Refractory	7 (2.3%)	2 (28.6%)	5 (71.4%)	0.002	
	Presence of esophageal varices	175 (59.5%)	124 (71.3%)	50 (28.7%)	0.000	
Laboratory parameters	AST [U/l]	48.7 (35.9)	44.7 (32.5)	62.3 (43.5)	0.001	
	ALT [U/l]	40.5 (32.1)	40.7 (33.8)	39.7 (25.8)	0.821	
	Alkaline phosphatase [U/l]	115.8 (77.2)	103.5 (47.4)	159.2 (129.4	0.000	0.007
	Gamma-GT [U/l]	162.1 (191.2)	143.5 (174.4)	228.8 (231.2)	0.003	0.009
	Total bilirubin [umol/l]	22.6 (29.9)	17.4 (20.0)	40.7 (47.3)	0.000	
	Hemoglobin [g/l]	131.1 (22.4)	136.6 (18.7)	112.7 (23.9)	0.000	0.000
	Thrombocyte count [G/l]	145.5 (74.0)	155.9 (73.8)	109.2 (63.4)	0.000	
	Prothrombin time [%]	77.1 (18.6)	80.9 (16.7)	64.0 (18.7)	0.000	
	aPTT [s]	34.9 (7.0)	33.5 (5.6)	39.2 (9.1)	0.000	
	Fibrinogen [g/l]	2.7 (1.0)	2.9 (1.0)	2.3 (0.9)	0.000	
	Factor V activity [%]	81.9 (30.3)	87.4 (28.9)	63.9 (27.8)	0.000	
	Prothrombin fragment 1 and 2 > normal	83 (12.0%)	57 (68.7%)	26 (31.3%)	0.010	0.043
	Thrombin-antithrombin complexes [pmol/l]	3.6 (3.2)	3.4 (3.3)	4.0 (2.9)	0.208	
	D-dimers [ng/ml]	1415 (2872)	947 (2368)	3003 (3789)	0.000	
	Albumin [g/l]	40.3 (5.6)	41.7 (4.8)	35.5 (6.6)	0.000	
	Creatinin [umol/l]	83.4 (31.9)	80.3 (28.2)	93.4 (41.0)	0.007	
Ex vivo thrombin generation	Lag time normalized	1.2 (0.32)	1.3 (0.3)	1.1 (0.2)	0.002	
	Peak height normalized [%]	77.6 (22.0)	77.9 (22.5)	77.0 (20.0)	0.787	
	Time to peak normalized	1.1 (0.3)	1.2 (0.3)	1.0 (0.2)	0.000	
	ETP normalized [%]	77.4 (16.4)	78.6 (16.2)	73.7 (16.7)	0.035	
	Velocity index normalized [%]	90.4 (41.8)	86.6 (42.7)	103.4 (36.0)	0.005	
	Lag time with TM [min]	2.7 (0.8)	2.7 (0.9)	2.4 (0.4)	0.007	
	Peak height with TM [nM]	164.1 (63.1)	157.8 (64.1)	186.1 (55.0)	0.002	
	Time to peak with TM [min]	4.4 (1.1)	4.5 (1.1)	4.1 (0.6)	0.001	
	ETP with TM [nM*min]	738.9 (288.7)	701.1 (290.1)	871.3 (244.7)	0.000	
	Velocity index with TM [nM/min]	136.2 (70.6)	127.6 (69.4)	165.6 (67.3)	0.000	
	TM-mediated ETP inhibition [%]	37.1 (21.5)	41.7 (20.9)	21.4 (15.2)	0.000	0.002

Results: Among the 302 patients, 67 (22.2%) developed LC decompensation. Results of logistic regression are presented in Table 1. Six parameters were found to be significantly and independently associated with LC decompensation: Child-Turcotte-Pugh score, alkaline phosphatase, gamma-GT, hemoglobin, prothrombin fragments 1 and 2 (F1+2) and thrombomodulin-mediated (TM) endogenous thrombin potential (ETP) inhibition. The combination of these six variables predicted liver decompensation with an area under the ROC curve of 0.93.

Conclusions: We developed a score that accurately predicts LC decompensation. Interestingly, several hemostasis parameters, including in vivo and ex vivo thrombin generation, were found to be significantly associated with the occurrence of LC decompensation in univariate analyses. More specifically, F1+2 and TM inhibition were found to be significantly and independently associated with LC decompensation. This observation supports a major role of a prothrombotic state in the pathophysiology of LC decompensation, suggesting a rationale for the use of anticoagulation as a preventive measure. After external validation, the proposed score shall be used in a large prospective study assessing the utility of targeted prophylactic anticoagulation to prevent decompensation in patients with LC.

272

A role of immunothrombosis in acquired antibody-mediated thrombophilia's: Classical complement and neutrophil activation in APS and HIT

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Introduction: Heparin induced thrombocytopenia (HIT) and antiphospholipid syndrome (APS) are acquired thrombophilias characterized by thromboembolic events in the presence of pathological antibodies (in APS: anti- Cardiolipin, ant- Beta2-Glycoprotein I and Lupus Anticoagulant; in HIT: antibodies to complexes of heparin and platelet factor 4). Complement- (CA) and neutrophil activation (NA) in the form of neutrophil extracellular traps (NETs) play an important role in the pathogenesis of thrombosis, called "immunothrombosis", as seen in PNH. We investigate the role of CA and NA in patients with APS and HIT.

Methods: CA products (C4bc, C3bc) and marks for NA (nucleosomes and human neutrophil elastase-α1-antitrypsin complexes (HNE)) were measured by ELISA in plasma of 34 primary APS- and 26 HIT-patients and 31 healthy controls (HC). Groups have been compared using the Mann-Whitney-Rank Test. Values are indicated as median and range, $p<0.05$ is considered statistically significant.

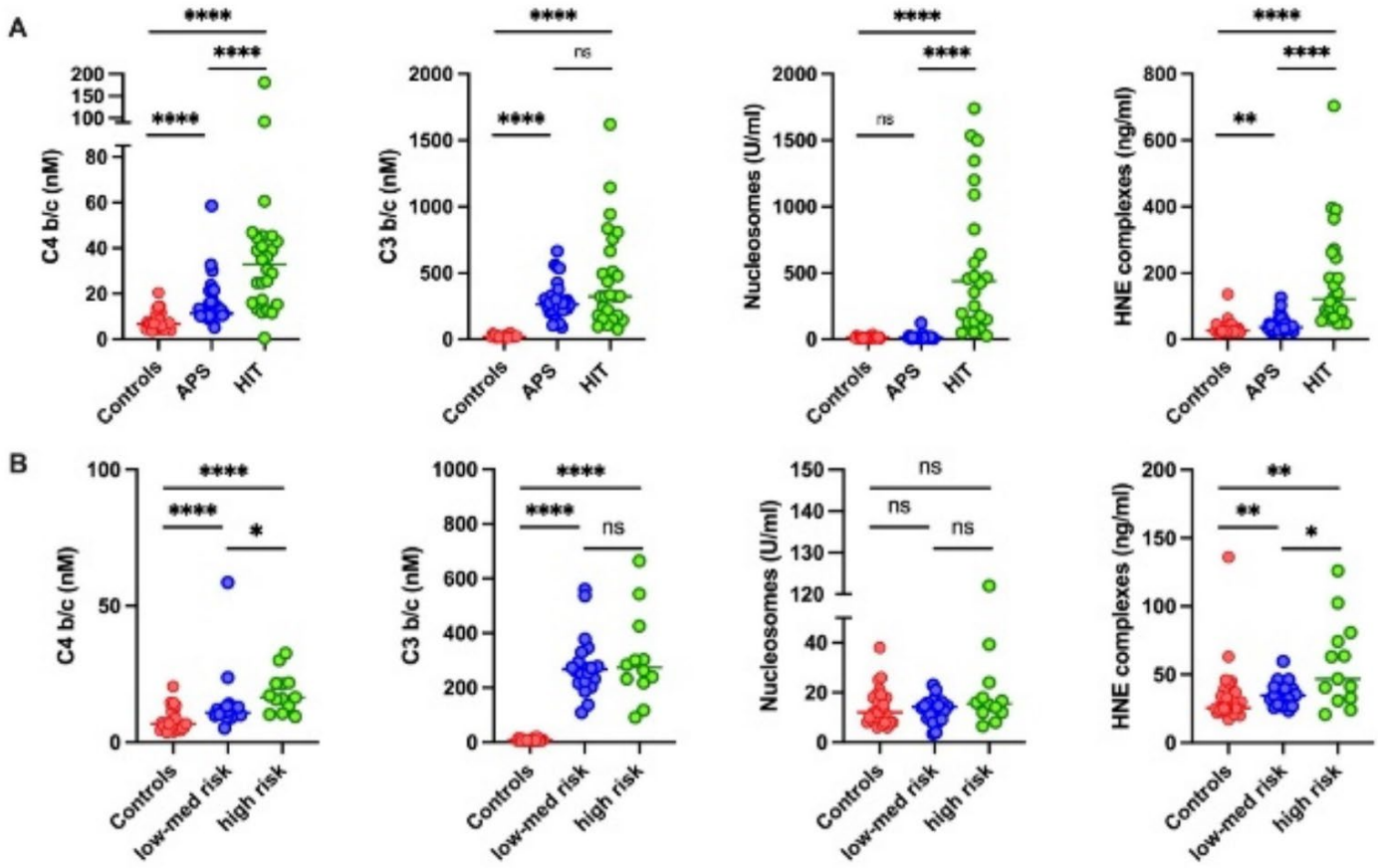
Results: Patients with APS and HIT showed sign. higher levels of CA products as compared to HC. CA of the classical (C4b/c) and alternate pathway (C3b/c) in APS (11,35 nM and 262,5 nM) and in HIT (32,8 nM and 323,5 nM) were sign. higher ($p<0.0001$) as compared to the HC (6.7 nM and 19 nM). C4bc levels were sign. higher in HIT as compared to APS patients ($p<0.001$), there was no difference in C3b/c levels between these two groups. HIT patients had sign. ($p<0.0001$) higher nucleosomes (440 U/ml) and HNE (121 ng/ml)levels as compared to HC (12 U/ml and 25.5 ng/ml). HNE levels were sign. ($p<0.001$) higher in APS patients (36.8 ng/ml) as compared to HC (25.5 ng/ml), no difference was observed in nucleosome levels (12 U/ml vs 14.45 U/ml).

11 APS patients have been stratified as high-risk and 24 APS patients as intermediate/low risk. C4bc levels and HNE were sign. increased in the high-risk patients as compared to the intermediate/low risk group, no difference in C3b/c and nucleosome levels could be observed between the two groups.

CA- and NA- markers in HIT patient with and without thrombosis were not different.

Conclusions: Our results show a significant association between classical CA and NET formation in HIT and APS. In APS, classical CA and NA correlates with APS risk stratification.

Based on the simultaneous raise in nucleosomes and HNE, there is evidence of NET formation in HIT, whereas only weak NA can be found in APS.



SSH/SSMO POSTER PRESENTATION – EXPERIMENTAL HEMATOLOGY / ONCOLOGY

170

CD19-CAR-iNKT cells transactivate NK cells and prevent alloreactivity

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Introduction: Invariant natural killer T (iNKT) cells are a small fraction of T lymphocytes with strong cytotoxic and immunoregulatory properties. We previously showed that human culture-expanded iNKT cells prevent alloreactivity and lyse primary leukemia blasts. Chimeric antigen receptors (CARs) increase the therapeutic benefit of immune effector cells. Here, iNKT cells have several advantages over T cells based on their immunoregulatory properties. In the presented study, we investigated transactivation of NK cells and prevention of alloreactivity through iNKT cells transduced with a CD19-directed CAR.

Methods: iNKT cells were isolated by magnetic cell separation from PBMCs and transduced with a CD19-CAR retrovirus. Transduction efficiency, purity and cell subsets were measured by flow cytometry. A transactivation and cytotoxicity assay has been established to investigate the ability of CD19-CAR-iNKT cells to transactivate primary NK cells. A mixed lymphocyte reaction (MLR) was performed to explore the inhibition of alloreactive CD3+ T cells by CD19-CAR-iNKT cells.

Results: CD19-CAR-iNKT cells are able to transactivate NK cells independent of cell contact: The expression of activation marker CD69 and production of the proinflammatory cytokine IFN-γ were significantly higher in NK cells pre-treated with CD19-CAR-iNKT cells. Consequently, the cytotoxic activity of such NK cells was significantly increased being able to lyse leukemia cells more effectively than without prior transactivation. Adding CD19-CAR-iNKT cells to a MLR resulted in a decreased expression of the T-cell activation marker CD25 on alloreactive CD3+ T lymphocytes stimulated with HLA mismatched dendritic cells. Also, the proliferation of alloreactive CD3+ T lymphocytes was significantly reduced in this setting.

Conclusions: We demonstrate that CD19-CAR-iNKT cells keep their immunoregulatory properties despite transduction with a CAR making them an attractive effector cell population for application after allogeneic HCT. By transactivating NK cells, increasing their cytotoxic activity and suppressing alloreactive T cells, they might further improve outcomes through prevention of both relapse and graft-versus-host disease.

177

Anti-Apoptotic, Non-Glycolytic Function of Hexokinase 3 in Acute Myeloid Leukemia Chemotherapy Response

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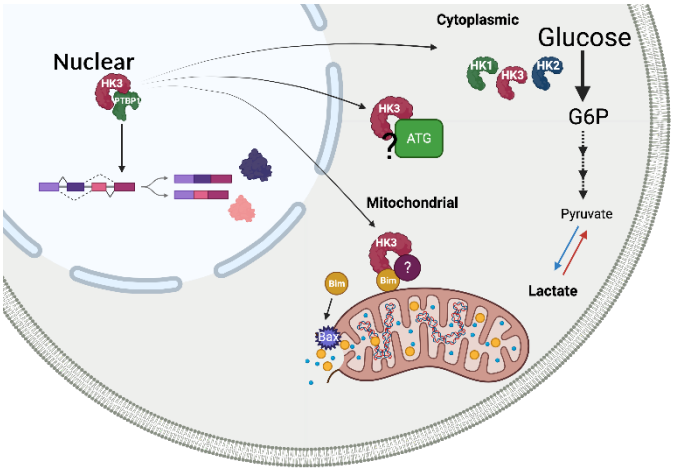
Introduction: AML is a vastly heterogenous disease and despite the available therapeutic options the prognosis of patients remains poor with high rates of relapse. AML therapies include differentiation, conventional chemo- and targeted therapies, or a combination thereof.

Hexokinase (HK) 3, a myeloid-specific member of the Hexokinase family that initiate glycolysis via glucose phosphorylation. In contrast to the widely expressed HK1 and HK2, HK3 is unique to myeloid cells. Depleting HK3 in AML cells reduces viability without affecting glycolysis. We identified the pro-apoptotic Bim protein as a novel interaction partner, shedding light on HK3's anti-apoptotic role. Our focus is unravelling HK3's impact on AML therapy response.

Methods: We generated HK3 knockout in HL60 cells via CRISPR/Cas9 and treated them with the anthracycline Idarubicin. Cell viability was assessed by Annexin V staining measured by flowcytometry. Expression of cleaved Caspase 3, Bim and NOXA was assessed by western blot. Due to the unavailability of specific HK3 antibody, we used CRISPR technology to tag endogenous HK3 with a HiBiT tag. Subsequently, we conducted a Proximity Ligation Assay (PLA) with presumed HK3 interacting partner the splicing factor Polypyrimidine Tract Binding Protein1 (PTBP1). We also employed PLA with the mitochondrial protein TOM20 to determine the subcellular localization of HK3.

Results: Depleting HK3 in AML cells heightens sensitivity to chemotherapeutic treatments. Idarubicin notably increased Annexin V staining, cleaved caspase 3, and NOXA expression in HK3 KO cells compared to controls. Interestingly, HK3-depleted cells primarily expressed the alternatively spliced Bim short isoform. In line, we identified various splice factor proteins via mass spec pull down of HK3. By PLA, we subsequently confirmed HK3's interaction with the splicing factor PTBP1. The latter primarily residing in the nucleus indicates a nuclear role of HK3. Surprisingly, despite lacking a typical mitochondrial binding domain, HK3 can localize to mitochondria, as demonstrated by TOM20 PLA.



Conclusions: We speculate that non-canonical HK3 functions in the nucleus and at the mitochondria support AML cell survival in response to cytotoxic therapies. These pro-survival functions of HK3 might be linked to inhibition of Bim function and/or altered gene splicing.

206

Megakaryocyte apoptosis-induced mechanisms in pediatric immune thrombocytopenia

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Introduction: Immune thrombocytopenia (ITP) is an autoimmune bleeding disorder characterized by low platelets counts and a mostly mild- and in rare occasion life threatening bleeding symptoms. Previous studies, also from our group, have demonstrated a role of platelet apoptosis in the pathogenesis of childhood ITP. A mechanistic understanding of the ITP pathogenesis is still lacking, and most treatments increase the platelet counts and prevent the severe clinical manifestations in a limited number of the patients only. In our present study, we aimed to investigate the gene expression and activation mechanisms of key proteins of apoptosis pathways in platelets and in their producers, megakaryocytes, in both newly diagnosed and chronic ITP compared to healthy controls.

Methods: We used the human megakaryoblastic cell line MEG-01, treated with apoptosis chemical inducers or inhibitors (pan-caspase-inhibitor Z-VAD-FMK, ABT737, Rotenone, Rapamycin) and then exposed for 1h with plasma from newly diagnosed and chronic ITP patients and healthy controls. We determined the mRNA levels of apoptosis pathway regulatory genes p53, Bax, Clusterin, Bad, Apaf-1, Caspase -3, -8 GRP94 and GRP78 by qRT-PCR.

Results: We could demonstrate increased expression levels of some apoptotic genes such as GRP78, Bax, Apaf-1, Caspase-8 and Clusterin in ITP platelets and MEG-01 treated cells that could be downregulated by using the pan-caspase-inhibitor.

Conclusions: Our results indicate that a regulation impairment in the apoptosis pathway could play a role in platelet pathophysiology and platelet production by MKs of patients with ITP.

208

The bile acid receptor TGR5 regulates the hematopoietic support capacity of the bone marrow niche

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Introduction: The gut is an emerging regulator of bone marrow (BM) physiology. Several signalling molecules are involved in the communication of the gut with distant organs. Among them, bile acids (BAs), originally classified as lipid solubilizers, have emerged as powerful signalling molecules that act as a relay between the digestive system, the microbiota and the rest of the body. The signalling function of BAs relies on specific receptors, including Takeda-G-protein-receptor-5 (TGR5). TGR5 has potent regulatory effects in immune cells, but its effect on the BM as a primary immune organ remains unknown

Methods: We used flow cytometry and immunohistochemistry to determine the presence of TGR5 in the murine BM using a TGR5:GFP reporter mouse. We analysed the impact of loss of TGR5 on hematopoietic stem and progenitor cells (HSPC) by

flow cytometry and its cell-autonomous effects by competitive transplantation assays. The BM niche was assessed by micro-computed tomography of the calcified tissue and the osmium tetroxide contrast-enhanced BM adipose tissue (BMAT). To evaluate the mechanism at play, we used in vitro assays of BM stroma cell adipocytic differentiation and flow cytometry characterization of adipocyte progenitor cells (CD45-Ter119-CD31-Sca1+CD24-)(APC). Finally, we produced inverse chimeras to evaluate the effect of a Tgr5-/- niche on hematopoietic recovery upon BM transplantation

Results: While TGR5 was present in HSPCs, it was not essential for steady-state haematopoiesis. Tgr5-/- BM cells exhibited a deficit in short-term repopulating capacity upon transplantation that was corrected over time in a wild-type niche. We hypothesized that the original deficit may be caused by an altered BM niche in Tgr5-/- mice. We showed a moderate loss of bone and a marked decrease in BMAT in young, in 1-year old, and in high-fat diet-fed Tgr5-/- mice. This was accompanied by an increase in BM APCs and fibroblast colony-forming units in Tgr5-/- mice. Tgr5-/- BM stroma cells retained adipogenic potential in vitro, suggesting an in vivo differentiation block. Finally, we investigated the effect of the Tgr5-/- BM niche on stress haematopoiesis and found that TGR5 deficiency hastened recovery upon BM transplantation

Conclusions: Our results suggest that TGR5 modulates the BM adipocyte lineage and that strategies aimed at blocking this receptor might be attractive to regulate hematopoietic support by the BM stroma

209

GCN2 protects cancer cells from hypertranslation and loss of metabolic homeostasis

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Introduction: Cancer cells require high levels of protein synthesis to support growth and proliferation. Enhanced translation is driven by oncogenic signalling that expands the translation machinery and relies on intensified ribosome biogenesis (RiBi). However, protein synthesis, including RiBi, is the most resource- and energy-demanding cellular process and needs to be coordinated with metabolic programmes to ensure sufficient supplies of amino acids and ATP. GCN2 is an evolutionarily conserved kinase that activates the Integrated Stress Response (ISR) when intracellular amino acid levels drop. The ISR encompasses a global reduction in translation and increased amino acid uptake and generation. Here, we used a systems biology approach to delineate the multifaceted role of GCN2 in protecting cancer cells from proteostatic and metabolic failure.

Methods: We carried out multiomic analyses (RNA-seq, TMT-labelling based proteomics, Ribo-seq, LC-MS metabolomics) of A375 melanoma cells in which GCN2 was inhibited (GCN2iB) or genetically depleted (shRNA) in cell culture and in vivo, supplemented by puromycinylation assays to quantify translation.

Results: RNAseq and proteomic data of GCN2iB-treated A375 cells showed that GCN2 inhibition induces an augmented translational program in parallel with triggering cell death. Transcriptional and translational responses are dominated by the upregulation of MYC and E2F targets and are accompanied by the repression of genes/proteins involved in glycolysis and lipid metabolism. We confirmed our results in vivo via RNAseq on tumour samples from mice xenografted with A375 cells in which GCN2 was inhibited or knocked down. Ribo-seq demonstrated that, upon GCN2 inhibition, A375 cells expand their translational machinery. Notably, we identified 50 ribosomal genes that are translated at higher rate in GCN2-inhibited cells and whose expression is regulated exclusively at the translational level. GCN2 inhibition/depletion also resulted in a 1.8-fold increase in global protein synthesis, and altered the druggable proteome. Moreover, metabolite profiling of GCN2iB-treated cells revealed a loss of metabolic homeostasis including alterations in glycolysis and both amino acid and lipid metabolism.

Conclusions: Our study uncovers GCN2 as a key translation regulator necessary to maintain proteomic and metabolic homeostasis in cancer cells.

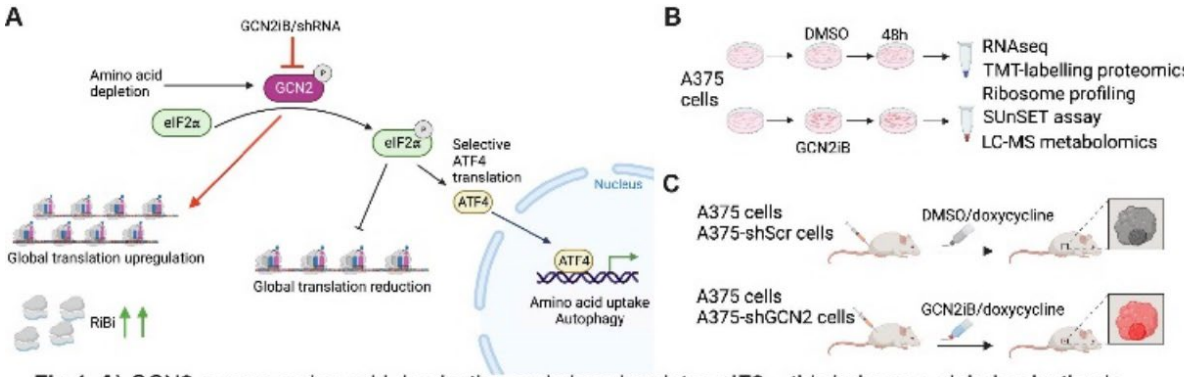

Fig 1. A) GCN2 senses amino acid deprivation and phosphorylates eIF2α, this induces a global reduction in translation but allows the selective translation of ATF4 which in turn activates a stress-adaptation transcriptional program (black arrows). When GCN2 is inhibited or knocked down (red arrows), we observe enhanced protein translation and an increase in ribosome biogenesis (RiBi). **B-C)** Experimental outline: A375 cells were treated with GCN2iB (or DMSO control) for 48h and samples subjected to RNA-seq, TMT-labelling proteomics, Ribo-seq, LC-MS metabolomics and puromycin incorporation (SUnSET) assay **(B)**. For pharmacological inhibition of GCN2 *in vivo*, mice were injected subcutaneously with A375 cells; when tumours reached 125 mm3 volume, GCN2iB was administered orally for 10 days. For genetic depletion of GCN2, mice were injected subcutaneously with A375 cells carrying a doxycycline-inducible shRNA against GCN2 (shGCN2) or shRNA control (shScr), when tumours reached 10 mm3, doxycycline was administered orally for 15 days **(C)**. Created with BioRender.com

244

SHP2 targeting inhibits MAPK pathway activation and improves therapeutic effects of ruxolitinib in myeloproliferative neoplasms

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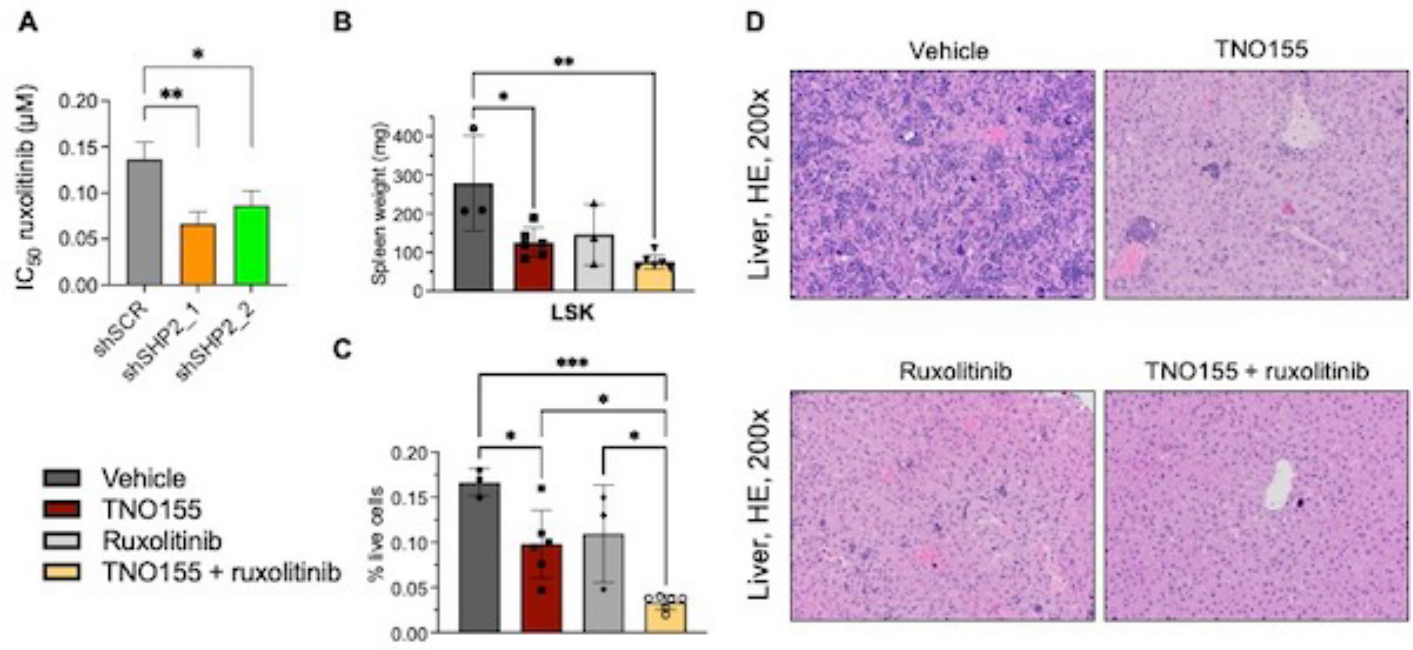
Introduction: Myeloproliferative neoplasms (MPN) are myeloid malignancies characterized by constitutive activation of JAK-STAT signalling due to somatic mutations in JAK2, MPL, or CALR. JAK2 inhibitors are in clinical use, but show limited efficacy due to sustained MAPK pathway activation. We study the protein tyrosine phosphatase SHP2, a known activator of the MAPK pathway, and assess its therapeutic potential in MPN.

Methods: SHP2 depletion was achieved by shRNA-induced knockdown. Two different pharmacologic SHP2 inhibitors, TNO155 and IACS13909, were tested in MPN cell lines. Translational potential of JAK2/SHP2 inhibition with ruxolitinib and TNO155 was studied in Jak2V617F- and MPLW515L-mutant MPN mouse models.

Results: SHP2 was expressed at substantial levels in Jak2V617F-mutant SET2, UKE-1 and Ba/F3 cell lines. SHP2 knockdown and pharmacologic inhibition reduced activation of MAPK pathway kinases including MEK, ERK and RSK as well as MAPK downstream effector DUSP6 in Jak2V617F-mutant Ba/F3 cells. More pronounced effects were observed after combining SHP2 targeting with the JAK2 inhibitor ruxolitinib. When comparing dual SHP2/JAK2 targeting to ruxolitinib as single agent, cell proliferation was inhibited at significantly lower IC50 (A). Anti-proliferative activity of combined JAK2/SHP2 inhibition showed more moderate effects in Jak2 wildtype Ba/F3 cells. The SHP2 inhibitor TNO155 mediated corrective effects on the phenotype of a Jak2V617F mouse model including splenomegaly, erythrocytosis and leucocytosis and reduced hematopoietic progenitor populations in the bone marrow after 1-2 weeks of treatment without signs of toxicity. Phenotype correction was similar in mice treated with TNO155 or ruxolitinib as single agents, while combined inhibition enhanced efficacy (B-C). In a MPLW515L mouse model JAK2/SHP2 inhibitor treatment rapidly normalized leukocyte counts, spleen weight and extramedullary haematopoiesis within a week, which is not seen to this extent with ruxolitinib (D).

Conclusions: Targeting of SHP2 leads to enhanced MAPK suppression, which translates into improved corrective effects on the MPN phenotype. SHP2 therefore presents a promising target in this group of diseases and deserves further evaluation. Future studies will focus on delineating the mechanism of SHP2 in MAPK activation in MPN and consolidating SHP2 as a therapeutic target.



Targeting SHP2 in MPN models enhances efficacy of ruxolitinib. A Shp2 knock-down by shRNA sensitizes Jak2V617F mutant Ba/F3 cells to ruxolitinib. **B-C** Treatment of a Jak2V617F mutant mouse model with TNO155 or ruxolitinib leads to similar reductions in spleen weight **(B)** and hematopoietic stem cells **(C)** whereas combination treatment shows more pronounced effects. **D** Extramedullary hematopoiesis in the liver is strongly reduced in a MPLW515L mouse model after one week of treatment with either TNO155 or ruxolitinib, while liver architecture is almost normalized after combined SHP2/JAK2 inhibitor treatment.

249

Role of ERK1/2 kinases in megakaryopoiesis, thrombopoiesis, platelet function and thrombosis in myeloproliferative neoplasms

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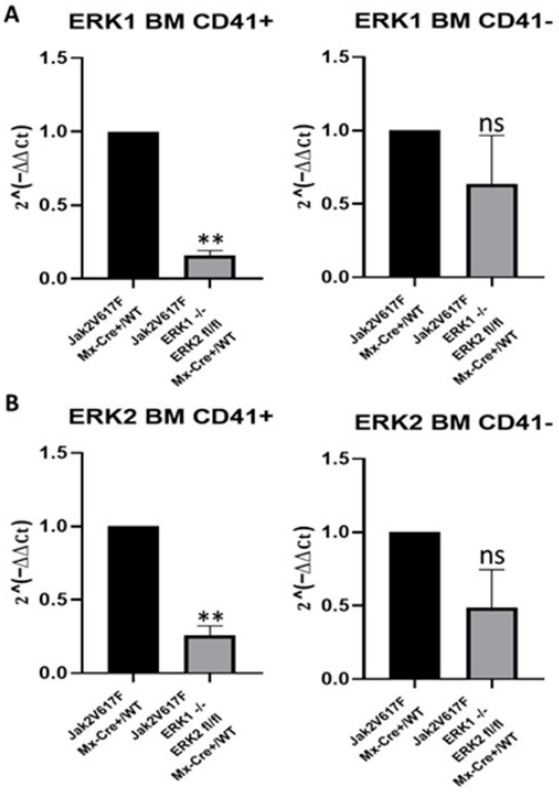
Introduction: Myeloproliferative neoplasms (MPN) are myeloid malignancies driven by the constitutive activation of JAK2 signaling, which are characterized by excessive production of mature myeloid blood cells including megakaryocytes and platelets. Thromboembolic events as well as bleeding complications, which relate to cytoses, are frequent and relevantly contribute to morbidity and mortality of MPN patients. Therefore, platelet production and function are of high interest. Given that ERK1/2, which are distal kinases of the MAPK pathway, are essential for hematopoiesis and remain activated in MPN despite JAK2 inhibitor therapy, we assess the role of ERK1/2 kinases in thrombopoiesis and platelet function in MPN.

Methods: We crossed Jak2 V617F knock-in with ERK1-/-ERK2f/f mice under the control of Mx-1 or PF4-Cre recombinase to generate murine models of constitutive Jak2 activation in ERK1/2 deficient settings. MPN phenotypes are studied with a focus on megakaryopoiesis, thrombopoiesis, platelet function and thrombosis upon ablation of ERK1/2 in presence and absence of JAK2 inhibition with ruxolitinib.

Results: ERK2 expression in Jak2 V617F Mx1-Cre ERK1-/-ERK2f/f mice was preferentially reduced in CD41+ megakaryocyte progenitors and mature megakaryocytes as compared to the CD41- cell fraction 10 days after pIpC induction. A decrease in ERK2 expression by about 17% was observed between Jak2 V617F Mx1-Cre mice with ERK and without ERK in CD41- cells whereas a decrease of ERK2 expression by about 90% was observed in CD41+ cells. Similar results were observed in competitively transplanted mice. Furthermore, we observed that ERK1/2 deficiency reduced production of mature megakaryocytes from CD41+ megakaryocyte progenitors in ex vivo culture conditions. Platelet function will be further assessed by platelet aggregometry and bleeding time studies. In parallel, the lineage-specific Cre recombinase for megakaryo-thrombopoiesis, Pf4-Cre will be used to study the transgenes specifically in megakaryocytes and platelets.

Conclusions: ERK1/2 have been shown to play essential roles to maintain the MPN clone. Our preliminary data suggest that ERK1/2 are relevant for megakaryo- and thrombopoiesis and potential therapeutic targets in regard to platelet function and thrombosis in MPN.



qPCR experiments showing ERK1 (A) and ERK2 (B) mRNA expressions in term of fold change 12 weeks after competitive transplantation in CD41+ and CD41- cells in bone marrow (n=3)

254

Ex vivo bioreactor platform for modeling MPN bone marrow and characterization of SHP2 phosphatase as a therapeutic target

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Introduction: Myeloproliferative neoplasms (MPN) are hematopoietic stem cell disorders with excessive myeloid cell production caused by constitutive activation of JAK2. JAK2 inhibitors are in clinical use but show limited disease-modifying potential. We showed that MAPK activation interferes with JAK2 inhibitor efficacy; interplay of JAK2 and MAPK pathway is not fully clarified. While previous investigations relied mainly on mouse models, which have inherent limitations, we use an ex vivo bone marrow 3D culture system with distinct advantages to enable comprehensive studies of transgenic murine and primary MPN patient cells.

Methods: We use ex vivo bioreactor platform to generate BM niches, derived from MPN Jak2V617F murine model as well as from MPN patients, with complete hematopoietic compartments to evaluate a role of SHP2 phosphatase as mediator of MAPK activation.

Results: Osteoblastic BM niches were generated in three-dimensional porous scaffolds under perfusion flow by culturing murine mesenchymal stromal cells for 3 wks. CD117+ hematopoietic stem cells obtained from Jak2 V617F MPN mouse BM were seeded at 106 cells/ml in IMDM media with EPO 10 U/ml, TPO 10ng/ml, IL3 10ng/ml and SCF 10ng/ml. Ruxolitinib (Rux) and TNO155 were used as JAK2 and SHP2 inhibitors, respectively, and effects assessed at 1 week.

HSPCs successfully colonized engineered niches and engrafted. We observed fully recapitulated hematopoiesis. SHP2 inhibition enhanced inhibitory effect of JAK2 inhibition reflected in reduced total cell numbers. JAK2 inhibition with Rux 0.25uM reduced proliferation in erythroid and megakaryocytic progenitors, whereas SHP2 inhibition with TNO155 2.5uM interfered with expansion of the Lin-Sca-Kit+. Of note, TNO155 preferentially affected differentiation towards the erythroid lineage. A humanized bioreactor system engineered from primary MPN patient cells is being setup.

Conclusions: We report on the generation of MPN BM in bioreactor systems with Jak2V617F stem/progenitors engrafting into engineered niches. We observed that SHP2 inhibition with TNO155 had differential effects to ruxolitinib primarily in LSK and erythroid compartments, which suggests a translational potential of dual JAK2/SHP2 inhibition in MPN.

Conflict of interest: SCM has consulted for and received honoraria from Celgene/BMS, Novartis and GSK and receives research support from Ajax.

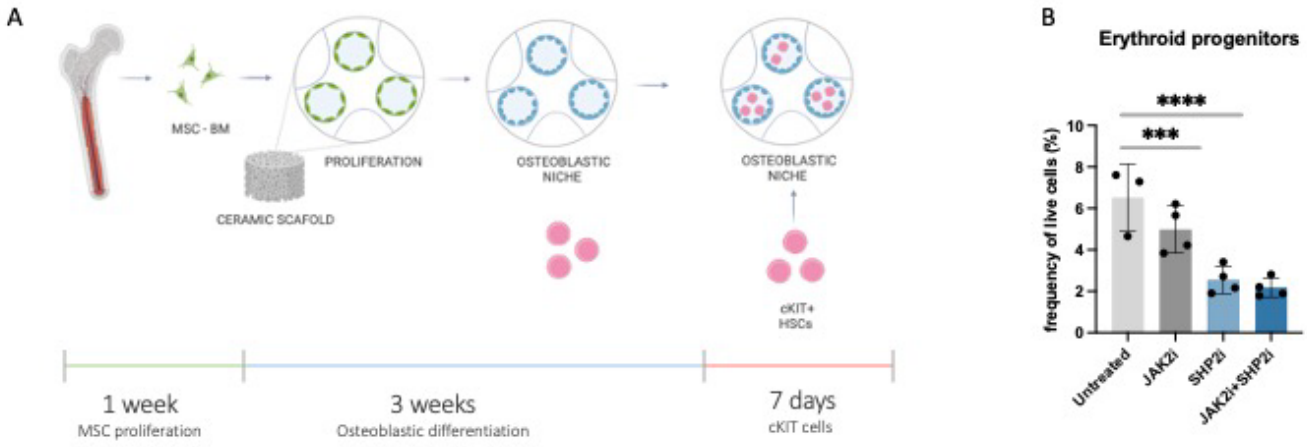

Figure 1: MPN bone marrow in ex vivo bioreactor system creates platform for inhibitor studies. *In the ex vivo bioreactor platform, JAK2 V617F murine stem/progenitor cells (HSPC) successfully colonized osteoblastic niches and expanded. We recapitulated all stages of hematopoiesis (A) and observed efficacy of pharmacologic inhibitors incl JAK2 inhibition with ruxolitinib, SHP2 inhibition with TNO155 and combined JAK2 / SHP2 inhibition in erythroid progenitor compartments (C).*

264

How the phosphatase SHP-1 defines the activation threshold of natural killer cells (NKc) & possible implications for NKc based antitumor therapy

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Introduction: Natural killer (NK) cells can identify and kill virally infected or transformed (malignant) cells. Activation of NK cells depends on signal integration of activating receptors, that recognize cellular stress caused by transformation or infection, and inhibitory receptors (such as KIR), which recognize MHC-I molecules. In mature NK cells, the strong inhibitory KIR signaling provides tolerance towards healthy cells. During maturation, the responsiveness of individual NK cells are set, depending on KIR expression. NK cells are thereby divided in responsive (educated) and non-responsive (uneducated) NK cells. The underlying molecular mechanisms remained elusive.

Methods: We used fluorescence activated single cell sorting and single cell sequencing to compare the gene expression signature of educated and uneducated NK cells. We challenged our findings by flow-cytometry and western blot. Subcellular localization of SHP-1 was assessed using high resolution microscopy. NK cell function was measured by antibody cross-linking of activating receptors or after co-incubation with MHC-deficient tumor cells. SHP-1 was targeted, using CRISPR/Cas9, si-RNA and chemical inhibition. Experiments were performed in mice and human cells.

Results: We found that NK cell tolerance and education were determined by both the expression level and the subcellular localization of the tyrosine phosphatase SHP-1. High resolution microscopy of uneducated NK cells revealed an accumulation of SHP-1 in the activating immune synapse, where it co-localized with F-actin and the signaling adaptor protein SLP-76. Educated NK cells on the other hand showed significantly lower levels of SHP-1 in the immune synapse, which was accompanied by augmented activating receptor signaling. Education was further linked to lower transcription of Ptpn6, the gene encoding SHP-1. When NK cell function was assessed after targeting SHP-1, the functional differences between educated and uneducated NK cells became insignificant.

Conclusions: Our findings have identified SHP-1 as a key phosphatase for the determination of NK cell education & responsiveness. At the same time we show that it can be targeted, allowing us to change the education status. Beyond improving our understanding of fundamental NK cell biology, the present findings may have provided insights to how NK cell signaling can be modified in novel NK cell based therapeutic approaches.

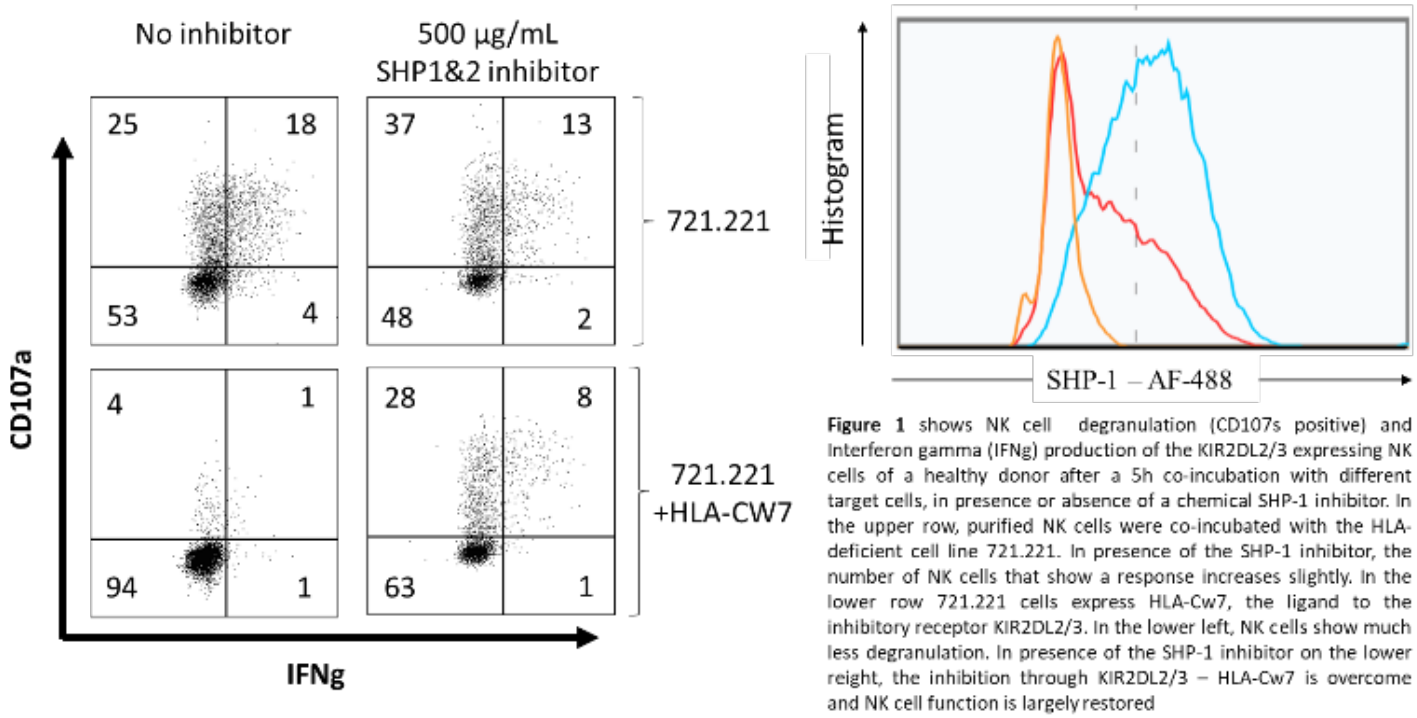

Figure 1 shows NK cell degranulation (CD107s positive) and Interferon gamma (IFNg) production of the KIR2DL2/3 expressing NK cells of a healthy donor after a 5h co-incubation with different target cells, in presence or absence of a chemical SHP-1 inhibitor. In the upper row, purified NK cells were co-incubated with the HLA-deficient cell line 721.221. In presence of the SHP-1 inhibitor, the number of NK cells that show a response increases slightly. In the lower row 721.221 cells express HLA-Cw7, the ligand to the inhibitory receptor KIR2DL2/3. In the lower left, NK cells show much less degranulation. In presence of the SHP-1 inhibitor on the lower reight, the inhibition through KIR2DL2/3 – HLA-Cw7 is overcome and NK cell function is largely restored

270

Exploring the Functional Role of an Alternative mRNA Transcript of the EPO Gene

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Introduction: Erythropoietin (EPO) is the key regulator of erythropoiesis and stimulates the proliferation and differentiation of erythroid progenitor cells into mature erythrocytes. We previously identified a single base deletion in the EPO gene (c.32delG) that causes familial erythrocytosis with elevated EPO levels. This mutation causes a frame shift in an EPO mRNA transcribed from an alternative promoter (P2) located in intron 1 of the EPO gene. This alternative P2 mRNA is normally not coding for a protein, but due to the frameshift becomes the source of excess production of EPO protein in family members who carry the c.32delG mutation. The P2 transcript is conserved between multiple species, suggesting that it could have an as yet unknown physiological function.

Methods: To study the putative physiological function of the P2 transcript, we performed experiments in a human hepatoma cell line, Hep3B, which can produce EPO. We used the CRISPR/Cas9 system to generate mutant Hep3B clones with targeted deletions in intron 1 of the EPO gene. We generated 4 types of mutations in EPO intron 1: deletion of the entire intron 1, deletion of two GATA consensus motifs, the deletion of the P2 transcription start site, and deletion of the homology in the 5'-region of EPO intron 1 that is conserved between human and platypus. Single cell derived clones were generated from targeted Hep3B cells and genotyped by next generation sequencing (NGS) and Sanger DNA sequencing.

Figure 1

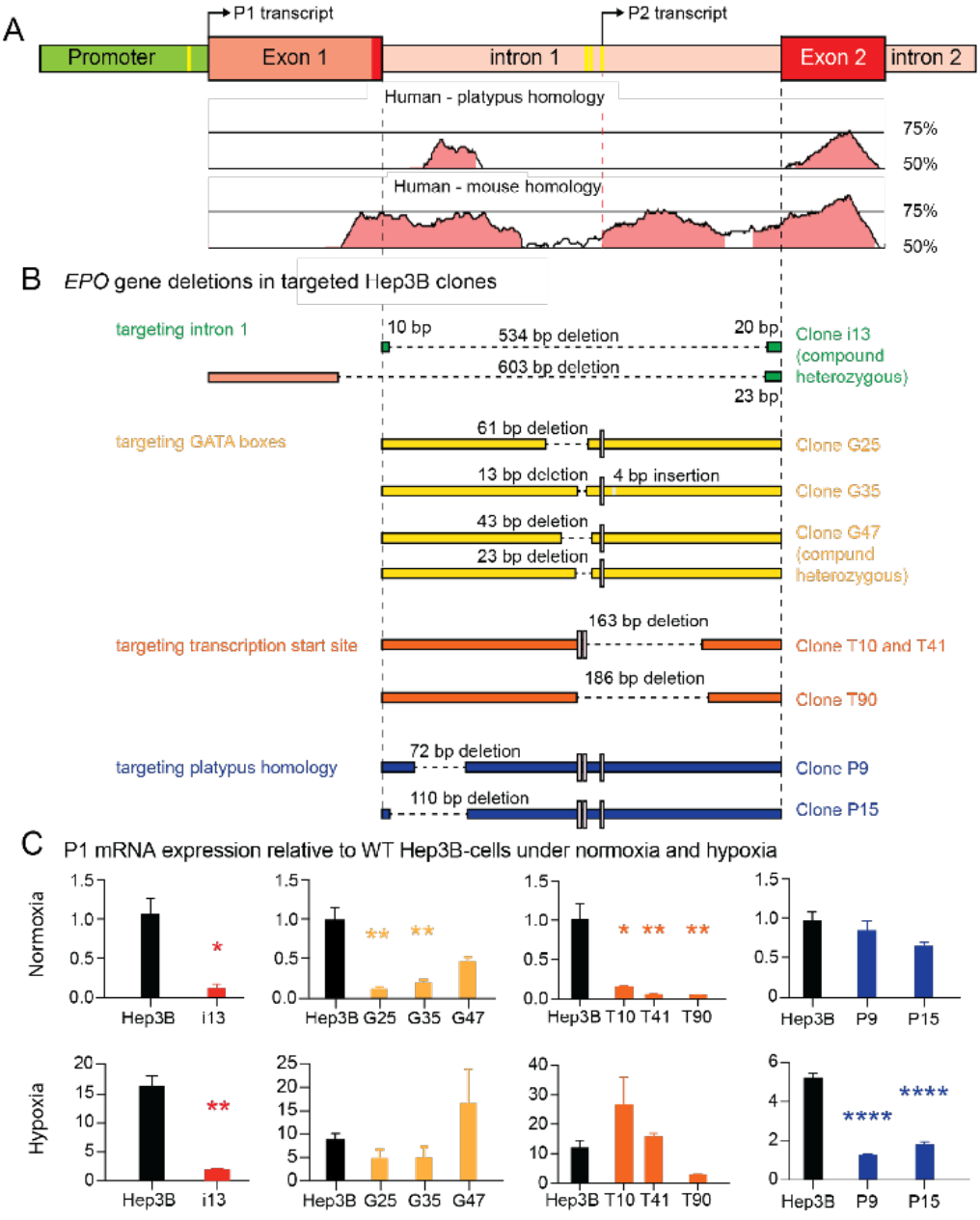

Figure 1. A) Alignment of human, platypus and mouse *EPO* sequences with regions in red showing >70% homology between species. **B)** The genotypes of the clones that resulted from each of the 4 CRISPR targeting strategies are shown. **C)** *EPO* P1 mRNA expression levels under normoxia in targeted clones relative to the expression of wildtype Hep3B cells set as = 1.0 . **D)** *EPO* P1 mRNA expression levels under hypoxia in targeted clones relative to the expression of wildtype Hep3B cells set as = 1.0 .

Results: We obtained 7 clones of interest that had homozygous or compound heterozygous deletions in intron 1 of EPO. In these clones and in the parental Hep3B cells we assessed EPO mRNA expression by RT-qPCR. The deletion of the entire intron 1 greatly reduced P1 EPO mRNA expression under normoxia and under hypoxia. The deletion of the P2 transcription start region reduced EPO P1 transcripts under normoxia, but did not interfere with upregulation under hypoxia. Deleting the region conserved between human and platypus did not affect P1 mRNA expression patterns under normoxia, but reduced P1 mRNA expression under hypoxia.

Conclusions: Our study suggests that the 5' region of intron 1 of EPO contains regulatory elements necessary for upregulation of EPO mRNA expression under hypoxia and the P2 transcript appears to be important to assure normal EPO mRNA expression under normoxia.

276

Reconstitution of NK cell phenotype and function after allogeneic haematopoietic stem cell transplantation (HSCT) – 5-year survival analysis shows distinct phenotypes related to the transplant-outcome

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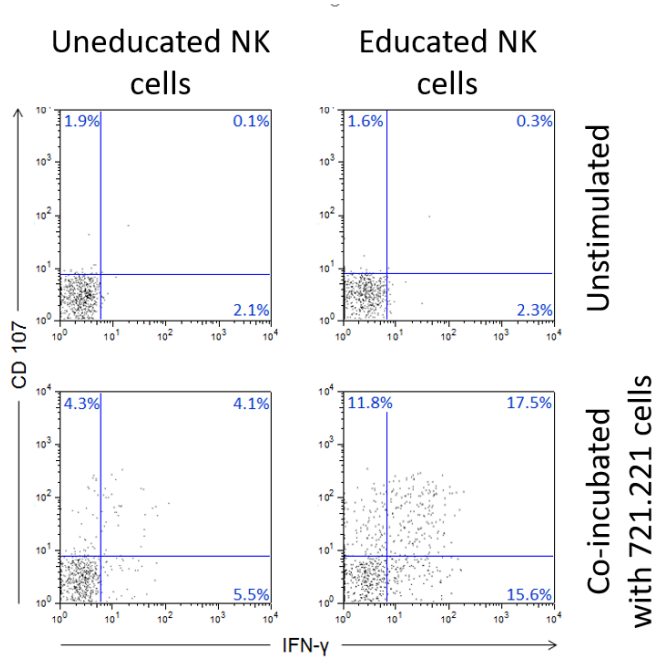
Introduction: Natural killer (NK) cells as a part of natural immunity are specialized on eliminating infected and tumor cells. NK cells thereby integrate the signals of their activating and inhibitory receptors to form an immediate response. Whereas an array of activating receptors detects different signs of cellular stress occurring during viral infection or malignant transformation, the inhibitory receptors (KIR) asses HLA-expression quantitatively. The occurrence of NK cell mediated graft-versus-leukemia effects (GVL) is well documented after haploidentical allogeneic haematopoietic stem cell transplantation (HSCT), if KIR-HLA are mismatched in graft versus host direction. It was suggested that GVL may also occur early after fully KIR-HLA ligand matched HSCT, when NK cell education – a process where NK cell tolerance is established – is disturbed. In the present study we investigate if & when NK cell education is disturbed and how early postransplant NK-cell phenotype is related to overall and disease free survival.

Methods: We included 56 patients, mostly suffering from myeloid malignancies, receiving haematopoietic stem cell transplantation (HSCT) – 23 autologous and 33 HLA-matched allogeneic. Peripheral blood samples were drawn before and one, two, three and six months after transplantation. After density gradient separation of the peripheral blood monoculear cells (PBMC), they were incubated with the HLA deficient target cell 721.221. Subsequently, degranulation (CD107a on the surface) and cytokine production (intracellular IFN-γ production) was assessed by flow-cytometry.

Results: We found that NK cell education is maintained after both autologous and allogeneic HSCT. However, uneducated NK cells display more function and are more similar to educated NK cells after allogeneic than autologous HSCT ($p<0.05$). The present data further suggest that conditioning with ATG and GvHD affect NK cell education. Finally, we found distinct phenotypical and functional NK cell features in long-term (> 5y) survivors.

Conclusions: In conclusion, NK cell function is predicted by the presence of KIR ligands after HSCT. Nevertheless, uneducated NK cells are more responsive after allogeneic than autologous HSCT, which corresponds to previous studies showing a survival advantage in patients lacking KIR ligands after allogeneic transplantation.



SSH/SSMO POSTER PRESENTATION – CLINICAL HEMATO-ONCOLOGY

211

Donor lymphocyte infusions after haploidentical allogeneic haematopoietic stem cell transplantation are associated with inferior survival in myeloid neoplasms

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Introduction: Donor lymphocyte infusions (DLI) are effective for relapses of acute myeloid leukaemia (AML) and myelodysplastic neoplasms (MDS) after allogeneic haematopoietic stem cell transplantation (alloHSCT). However, the resulting risk of graft-versus-host disease (GvHD) is associated with high morbidity and mortality and limits the feasibility of DLI. Although more potential donors are available with the advent of haploidentical (haplo) alloHSCT, there is limited evidence on the efficacy and resulting GvHD risk of haplo DLI. We therefore conducted a retrospective study of patients (pts) with AML or MDS who received DLI at our centre preemptively or with therapeutic intent after alloHSCT to assess efficacy and tolerability.

Methods: This is a retrospective, observational cohort study of adult pts aged > 16 years with AML or MDS who received DLI after alloHSCT at our centre between 2002 and 2023. DLI were administered either preemptively in pts with measurable residual disease or therapeutically in pts with overt relapse. The primary endpoint was overall survival (OS). Secondary endpoints included progression-free survival (PFS) and cumulative incidence of acute GvHD and chronic GvHD after DLI.

Results: 57 pts with AML or MDS who received DLI preemptively (n = 7) or therapeutically (n = 50) after alloHSCT were enrolled. With a median follow-up of 516 days, the 1-year OS after DLI was 62.5%. In univariate analysis (UA), patient age, human leukocyte antigen (HLA) match and indication for preemptive DLI were significantly associated with OS. In addition, DLI from a haplo-donor and therapeutic indication were found to be significant risk factors for worse outcome in multivariate analysis (MA). The median OS after DLI, stratified by HLA matching, was 1.7 years for non-haplo and 0.5 years for haplo-donors, respectively (p = 0.003). The incidence of acute GvHD (grade II-IV) and chronic GvHD after DLI was 26.3% and 24.6%, respectively. No association between GvHD and outcome was observed in UA and MA.

Conclusions: To conclude, outcomes after non-haplo DLI and response to preemptively applied DLI were significantly better

in pts with high-risk myeloid neoplasms after alloHSCT in our study population.

235

Dyslipidemia after intensive immunosuppressive therapy versus hematopoietic stem cell transplantation in aplastic anemia: a single-center experience

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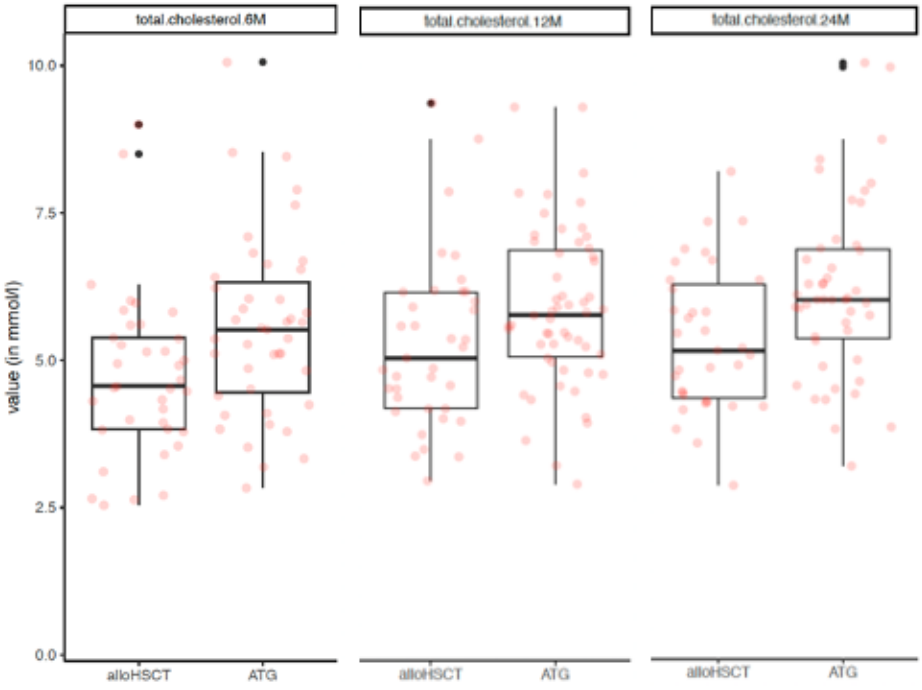
Introduction: Intensive immunosuppressive treatment (IST) and allogeneic hematopoietic stem cell transplantation (HSCT) have significantly improved survival of aplastic anemia (AA) patients, but also induce treatment-related complications. Dyslipidemia and associated cardiovascular events are known to occur more frequently after HSCT, but the influence of IST alone on lipid metabolism is not known. This study analyzed lipid profiles of all patients who underwent either first-line IST or HSCT for AA at our center.

Methods: This retrospective, single-centre cohort study included 109 adult AA patients treated with IST (n = 63) consisting of horse antithymocyte globulin, cyclosporine +/- eltrombopag or HSCT (n = 46), in which lipid values at 6, 12 and 24 months were available for comparison.

Results: Median total cholesterol and LDL after 6, 12 and 24 months were 4.56, 4.72 and 4.48 mmol/l and 2.29, 2.65 and 2.53 mmol/l, respectively after HSCT, compared to 5.52, 5.42 and 5.24 mmol/l and 3.31, 2.91 and 2.96, respectively after IST. There was no significant association between therapy modality and total cholesterol, LDL or triglyceride levels. After HSCT and IST therapy hypercholesterolemia (total cholesterol ≥ 5 mmol/l) was significantly more prevalent (pre- versus posttherapy: 2% vs 15% and 12% vs 26%, respectively, both $p < 0.01$).

Conclusions: Dyslipidemia occurs in an equal higher rate after HSCT and IST in AA. There is a trend for persisting higher lipid values in patients treated with IST alone, emphasizing the need for stringent long-term screening and management of dyslipidemia during AA follow up to potentially improve cardiovascular outcome in these patients.



237

Evaluation of increased melphalan dose for high-dose chemotherapy with TreoMel140 versus TreoMel200 in AML patients before autologous transplant.

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Introduction: Treosulfan and Melphalan prior to autologous stem cell transplantation (ASCT) can be given for consolidation of first remission in patients with acute myeloid leukemia (AML). Whereas the myeloablative dose of treosulfan (14g/m2 on three days) is well established, the optimal dose of melphalan remains a matter of debate, with most reports investigating a lowered dose of 140mg/m2 melphalan. However, it is unclear whether higher melphalan doses are tolerated and preferrable.

Methods: In this single-center study, we investigated two consecutive cohorts of AML patients. We included all AML patients who received treosulfan and melphalan (TreoMel) high-dose chemotherapy before ASCT between August 2019 and August 2023 at a single academic center. All patients received treosulfan 14g/m2 on three days followed by either 140mg/m2 melphalan (TreoMel140; first cohort) until September 2021, or 200mg/m2 melphalan (TreoMel200; later cohort) after September 2021. The endpoints of this study were overall and progression-free survival, and we also assessed toxicities of the two different melphalan doses.

Results: We identified a total of 51 AML patients, with 31 patients treated with TreoMel140 and 20 patients hadTreoMel200. Patient characteristics such as age, blood counts at diagnosis, and time from diagnosis to ASCT were comparable between the two groups. The duration of hospitalization and the adverse event profilels of the two groups were comparable. In particular, no significant differences in toxicities and infection profile were observed. Inevitably, the median follow-up time of the two consecutive cohorts differed. The relapse rates (25% versus 32%; p = .89) and the death rates (15% versus 42%; p = .53) were not different between TreoMel200 versus TreoMel140, as depicted in Figure 1.

Conclusions: Our data suggest that an increased dose of melphalan from 140mg/m2 to 200mg/m2 can be safely added to standard treosulfan for HDCT before ASCT in eligible AML patients in first remission. Longer follow-up and larger cohorts will be needed to assess eventual benefits in leukemia-free and overall survival in AML patients treated with TreoMel200.

Figure 1A: Progression-free survival

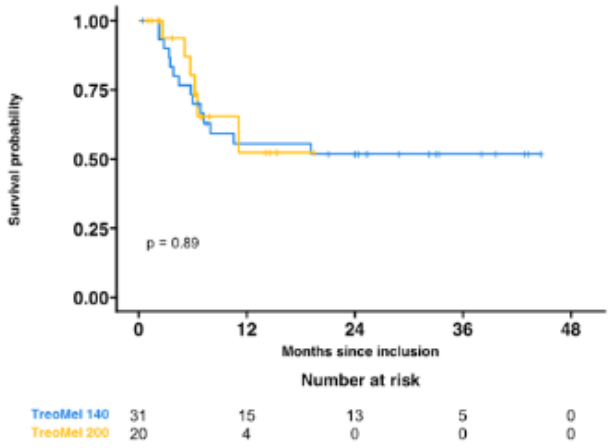
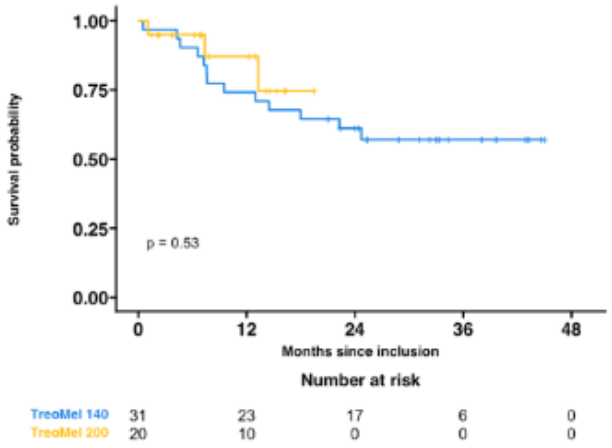


Figure 1B: Overall survival



240

Prophylactic Tocilizumab prior to infusion of anti-CD19 CAR-T cells may reduce the incidence of severe CRS in older lymphoma patients

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Introduction: CAR-T-cell therapies have dramatically changed the treatment landscape for patients with relapsed/refractory aggressive lymphomas and, more recently, plasma cell neoplasia. Treatment tolerability depends on a range of possible side effects such as hematological toxicities, immunodeficiency and infections, cytokine-release-syndrome (CRS) and immune effector-cell associated neurotoxicity syndrome (ICANS). Different to other intensive treatments such as ASCT, CAR-T-cell treatment can be applied independent of age. Still, data on older patients are scarce. Particularly ICANS can prolong the hospitalization, cause additional complications, and may impair the quality of life. Because response rates seem comparable between age groups, meticulous management for toxicities for older patients is warranted.

Methods: We analyzed 5 patients >70 yrs with relapsed/refractory DLBCL who were treated with CD19 directed CAR-T-cells and received prophylactic IL-6 receptor directed antibodies (Tocilizumab) since 2022. CRS and ICANS were graded using ASTCT consensus criteria. Remission status was assessed according to Deauville 5PS. In our center, indication for prophylactic treatment with Tocilizumab was assessed individually and was based on risk factors for severe complications such as age, tumor burden and remission status, co-morbidities, performance status.

Results: All patients were older than 70 yrs. None of the patients achieved a CR prior to CAR-T-cell therapy. 2 patients were diagnosed with Richter's transformation, and 1 patient had a primary CNS lymphoma of DLBCL-type. All patients received one dose of Tocilizumab (8 mg/kg) 2 hours prior to the CAR-T cells. Side effects related to Tocilizumab were not observed. At the end of hospitalization, no patient had developed ≥ grade 3 CRS or ICANS and none required intensive care treatment. All patients were discharged at home. 30 days after CAR-T cells 1 patient achieved a CR, 2 patients had a PR, 1 patient had a PD and one showed a SD.

Conclusions: Given the potential of CAR-T cell therapy to induce CR in heavily pre-treated patients with aggressive hematological malignancies, preventing and effectively managing severe toxicities remain the highest priority. In our case series, we show that in older patients with a high risk for severe CRS and/or ICANS, prophylactic treatment with Tocilizumab is safe and feasible and may reduce the risk for severe CRS.

Diagnosis	D 5PS pre CAR-T	Age	Sex	G8 Score	Prior therapy lines	Product	CRS	ICANS	D 5PS 30 days after CAR-T
DLBCL	SD	85	m	13.5	3	Tisa-cel	no	no	CR
DLBCL	PD	82	m	13.5	2	Tisa-cel	°2	°2	PD
DLBCL of CNS	SD	75	m	16	1	Tisa-cel	°1	°1	PR
DLBCL, RT	SD	77	f	14	2	Axi-cel	no	no	PR
DLBCL, RT	SD	71	m	15	1	Axi-cel	°1	°1	SD

Table 1: Characteristics of patients with Tocilizumab prophylactic treatment prior to CAR-T

242

Current management of mastocytosis: a single center experience

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Introduction: Mastocytosis is a rare disease with clonal expansion of mast cells in various tissues. Presentation is heterogeneous from instances with transient or chronic course to advanced forms of systemic mastocytosis with progressive course. Most patients carry a somatic D816V mutation in exon 17 of KIT. Until recently, treatment options in mastocytosis were limited. However, new KIT D816V-targeting tyrosine kinase inhibitors are now being developed, such as midostaurin and avapritinib, which show high response rates. To foster care of patients with mastocytosis and research, an interdisciplinary outpatient clinic was established.

Methods: 37 patients with various categories of mastocytosis were evaluated in period 2019-2022. Clinical characteristics and treatment of patients were analyzed.

Results: Among 37 patients, 19 (51.4%) were female and 18 (48.6%) male. The group comprised 35 adults and 2 children. Mean age was 48.9±18.7 years (mean±SD), ranging from 1 to 86 years, at time of the first visit. The duration from appearance of first symptoms until first visit was 11.0±12.3 years (mean±SD). Disease categories were as follows: cutaneous mastocytosis, n = 3; mastocytosis in the skin, n = 8; indolent systemic mastocytosis, n = 11; bone marrow mastocytosis, n = 1; smoldering systemic mastocytosis, n = 1; systemic mastocytosis with an associated hematologic neoplasm, n = 3. Eight patients also showed an allergy to Hymenoptera venom (wasp, n = 5; bee, n = 1; wasp and bee, n = 3). In addition to H1-antihistamines and other anti-mediator treatments, the following treatments were administered (multiple treatments per patient possible): midostaurin, n = 5; avapritinib, n = 1; cladribine, n = 1; interferon, n = 1; hydroxyurea, n = 1; allogeneic stem cell transplantation (HCT), n = 1; omalizumab, n = 6; Hymenoptera venom immunotherapy, n = 6. A Swiss Competence Network on Mastocytosis, "Swiss Mastocytosis", was initiated to coordinate the efforts of centers caring for these patients in Switzerland (www.swiss-mastocytosis.ch).

Conclusions: We report on a single center cohort of patients with various categories of mastocytosis seen in an interdisciplinary outpatient clinic. Treatments included KIT-targeting tyrosine kinase inhibitors, allogeneic HCT, omalizumab and allergen-specific immunotherapy. The expert network "Swiss Mastocytosis" will support efforts for patients with mastocytosis and research.

253

Relevance of discrimination of blastic plasmacytoid dendritic cell neoplasm (BPDCN) from related hematologic malignancies

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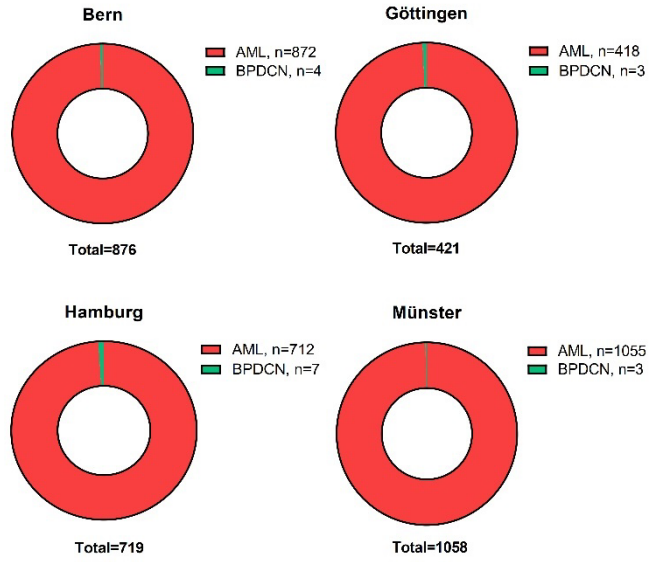
Introduction: Blastic plasmacytoid dendritic cell neoplasm (BPDCN), a rare malignancy originating from plasmacytoid dendritic cells, can mimic both acute leukemia and aggressive T-cell lymphoma. Immediate treatment is essential, particularly for the emergence of newly targeted therapies. However, differential diagnosis of BPDCN remains challenging.

Methods: This retrospective study aimed to highlight the challenges to timely diagnose of BPDCN. We documented the diagnostic and clinical features of 43 BPDCN patients diagnosed at five academic hospitals from 2001-2022.

Results: Frequency of BPDCN diagnosis vs AML was 1:197 cases (Figure 1). The median time from the first documented clinical manifestation to diagnosis of BPDCN was 3 months. Skin (65%) followed by bone marrow (51%) and blood (45%) involvement represented most common sites. Immunophenotyping revealed CD4+, CD45+, CD56+, CD123+, HLA-DR+ and TCL-1+ as most common surface markers. 86% (e.g. CD33) and 83% (e.g. CD7) showed co-expression of myeloid and T-cell markers, respectively. Considering genomic alteration, we identified five genomic alteration (median) per case including

mutational subtypes typically involved in AML: DNA methylation (70%), signal transduction (46%), splicing factors (38%), chromatin modification (32%), transcription factors (32%) and RAS pathway (30%), respectively. Nearly equal proportions of patients (30%) underwent upfront stem cell transplantation (SCT), whether autologous or allogeneic, resulting in favorable overall survival rates, especially for those who underwent allogeneic SCT. (p = 0.0001).

Conclusions: BPDCN is a rare and challenging entity sharing various typical characteristics of other hematological diseases. Comprehensive diagnostics play an essential role to assure appropriate treatment strategies.



259

Efficacy of primary antibiotic prophylaxis in patients with MDS or AML treated with non-intensive agents – A systematic review and meta-analysis

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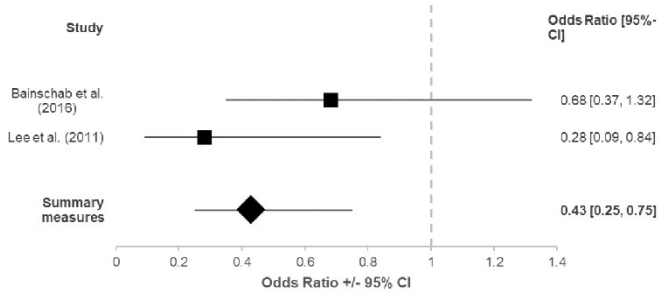
Introduction: Neutropenic patients with myelodysplastic syndromes/neoplasms (MDS) and acute myeloid leukemia (AML) commonly suffer from infections, which results in increased hospital admissions, morbidity and mortality. Antibiotic prophylaxis has a proven effect in reducing infections in hematooncologic patients receiving intensive chemotherapy regimens and is recommended by most hematology/oncology societies. However the lack of controlled prospective trials challenges the extrapolation of these recommendations to elderly MDS or AML patients treated with less intensive regimens. The aim of this work is to summarize published data on the efficacy of primary antibiotic prophylaxis in this patient group.

Methods: We performed a systematic search in PubMed and Embase databases. Inclusion criteria: demographics (adult), type of the studies (original articles), study population (MDS, MDS/MPN or AML patients) and treatment (hypomethylating agent (HMA), low-dose cytarabine (LDC)). Study endpoints: efficacy (incidence of infections or fever, rate of hospitalizations, ICU admissions, death) and safety (side-effects and resistance) of primary antibiotic prophylaxis. The results of the meta-analysis are as odds ratios with 95% confidence intervals.

Results: Two retrospective studies with 68 patients were identified, one with AML (n = 40, Bainschab et al, 2016) and one with MDS patients (n = 28 , Lee et al, 2011) receiving primary antibiotic prophylaxes in 79/215 (25%) and 95/131 (72.5%) of treatment cycles, respectively. The most used antibiotics were Fluorquinolones (92.5% and 95%). A significant reduction of the incidence of infections or febrile episodes (OR 0.43 [0.25-0.75], p<0.003) with a potential reduction in hospital admission, especially in patients with low ANC (< 0.5 G/l), was showed. Additional risk factors for infections were severe cytopenia(s) (grade ≥3), earlier therapy cycles, transfusion dependency, increased LDH and high Charlson Comorbidty Index. Data regarding the safety of antibiotic prophylaxis were not available.

Conclusions: Antibiotic prophylaxis seems to reduce the incidence of infections among MDS or AML patients with high-risk for infections undergoing non-intensive treatment. The lack of prospective controlled randomized trials is an unmet need that should be addressed even more as non-intensive treatments are becoming the standard of care in these patients.



268

Red blood cell deformability in polycythemia vera differs according to the treatment

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Introduction: Polycythemia vera (PV) belongs to the group of myeloproliferative neoplasms (MPNs). The main problem is the elevated risk for thromboembolism. In particular, the increased proportion of red blood cells (RBCs), as well as altered platelet properties seem to have an impact, however, the exact mechanisms remain unclear. Similar to membranopathies, changes in the elasticity of the RBCs may contribute to rheological alterations. This study aimed to assess RBC deformability in patients suffering from PV under various treatments.

Methods: To determine the RBC deformability, we performed ektacytometry measurements using venous blood samples obtained from patients with PV. Ektacytometry is a well-established method for evaluating RBC deformability, where the resulting curve is characterized by the key data points: Omin, Elmax, Ohyper, Elmin, Elmax, Elhyper and the area under the curve (AUC). Patients were stratified into four groups based on their therapies: Group 1: no cytoreductive therapy, Group 2: hydroxyurea treatment, Group 3: ruxolitinib therapy and Group 4: interferon therapy. Here we report the results of RBC deformability assessments for patients with 'no cytoreductive therapy' (Group 1) compared to those receiving hydroxyurea medication (Group 2).

Results: In this analysis, 50 patients were included. Among them, 13 patients had no cytoreductive therapy (Group 1), while

25 patients were treated with hydroxyurea (Group 2). The results revealed a statistically significant difference in two key parameters: The Elmax values were lower in Group 1 (mean 0.579 ± 0.021) compared to the Group 2 (mean 0.592± 0.015) with a p-value of < 0.036. Similarly, the Elhyper values were significantly lower in Group 1 (mean 0.290 ± 0.010) compared to the Group 2 (mean 0.296 ± 0.007) with a p-value of < 0.033. No statistically significant differences were observed in the other indices.

Conclusions: Our study demonstrates statistically significant differences in RBC deformability in PV patients, depending on their current treatment regimen. These variations may play a role in the underlying mechanisms of thrombogenesis. Notably, our results suggest that treatment with hydroxyurea is associated with improved RBC deformability, which may contribute to the observed reduction in the risk of thromboembolic events when compared to the patient group receiving no cytoreductive therapy.

273

Prevalence and prognostic impact of bone marrow fibrosis in acute myeloid leukemia. Preliminary results of a monocentric retrospective study.

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Introduction: Myelofibrosis (MF) is an established independent risk factor in myelodysplastic syndromes. Prevalence and prognostic impact of MF in de novo acute myeloid leukemia (AML) are less well studied.

Methods: We performed a retrospective chart review of patients (pts) diagnosed at the Cantonal Hospital St. Gallen with AML between 2017 and 2021 and having a bone marrow (BM) biopsy at diagnosis. MF was graded according to WHO independently by two pathologists. For survival analysis, allografted patients were censored at the date of transplant.

Results: 105 pts (male n = 63, female n = 42; age median 68 years [IQR 53; 77]) were evaluable for this preliminary analysis with a median follow-up of 15 months (IQR 2; 31). MF was present in 53/105 pts (51%), but was mostly mild (MF-1 46/105 [44%], MF-2 6/105 [6%], MF-3 1/105 [1%]).

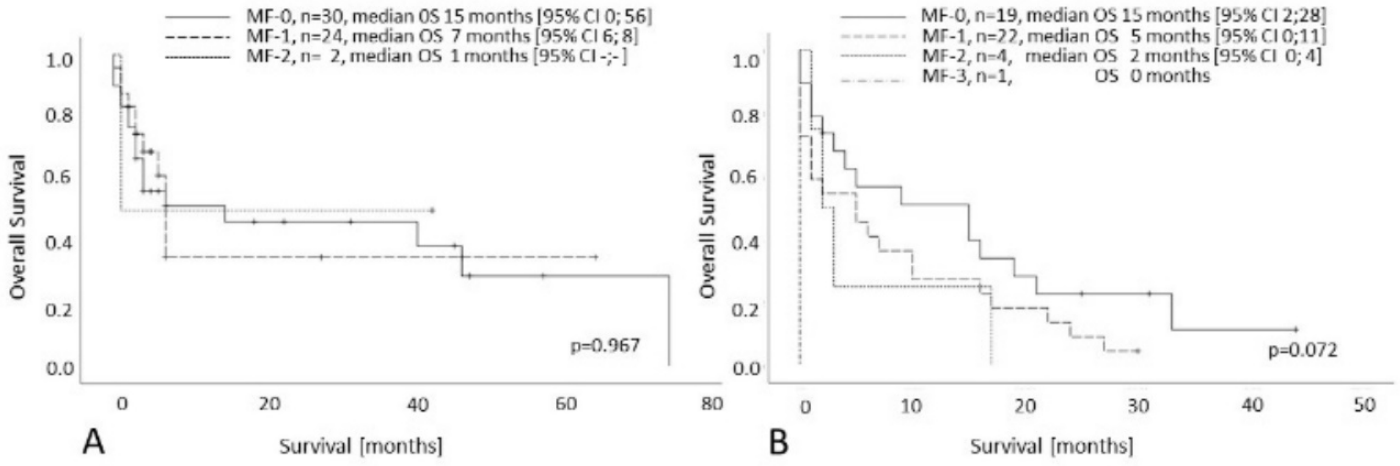
There was no difference with regard to peripheral blood values between pts with and without MF (median haemoglobin 87 vs. 88 g/l [p = 0.981], neutrophils 1.0 vs. 0.9 G/l [p = 0.879], platelets 51 vs. 60 G/l [p = 0.973]).

A classification according to WHO 2016 was available in 90/105 pts (87%). With regard to the frequency of MF grade 2/3 we observed no difference between AML with MDS-related changes, AML with mutated NPM and mutated RUNX1 to the other entities (2/24 vs. 3/66, p = 0.606; 0/17 vs. 5/73, p = 0.579 0/7 vs. 5/83, p = 1.00, respectively).

25 pts received an induction ("7+3"). Time to haematological recovery was not different between pts. with MF≥ 1 and without MF (time to neutrophil recovery median 31 days each, p = 0.243; time to platelet recovery median 26 vs. 23 days, p = 0.347).

Presence or absence of MF did not affect survival in pts receiving induction (Fig. A). In pts treated with BSC or hypomethylating agents, degree of MF was associated with survival differences of borderline significance (median OS MF-0: 15 months [95% CI 2-28], MF-1 OS 5 months [95% CI 0; 11], MF-2 OS 2 months [95% CI 0; 4], Fig. B).

Conclusions: Our small and retrospective series shows a relative high prevalence of MF in AML, which is mostly mild and rarely pronounced (MF ≥2). We were not able to identify a subtype according to WHO2016, that is associated with a higher prevalence of MF. For pts. without intensive treatment, MF may present an additional adverse risk factor. Further studies will focus on the prognostic impact in the context of the ELN risk classification.



282

Real-world (RW) treatment patterns with brentuximab vedotin (BV) in patients with Hodgkin lymphoma (HL) or other CD30+ malignancies in Switzerland

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Introduction: BV is a CD30-directed monoclonal antibody-drug conjugate approved for the treatment of CD30+ lymphomas including HL, systemic anaplastic large cell (sALCL), cutaneous T-cell and peripheral T-cell (PTCL) lymphomas. This non-interventional, observational, multicentre, retrospective study describes characteristics, treatment patterns and outcomes in patients (pts) with CD30+ malignancies who received BV under RW conditions in Switzerland.

Methods: Clinical records (2013–2022) were obtained from a national registry disposed by the Swiss Federal Office of Public Health. The registry included data from pts with a CD30+ malignancy, for whom BV was prescribed and who were aged ≥18 years by end of treatment (EOT). All analyses were exploratory and descriptive. The study was approved by the ethics committee.

Results: In total, data from 212 pts with classical HL, sALCL, or other CD30+ malignancies were analysed. Data were missing for some outcomes, including duration of treatment (DOT) for 67 (32%), reason for EOT for 61 (29%) and overall response for 56 (26%) pts. Among all pts, 61% were male and median age was 58 years (Table). The most common diagnosis was HL in 133 (63%) pts, followed by PTCL in 27 (13%) and angioimmunoblastic T-cell lymphoma in 26 (12%). Pts with HL were younger vs pts with other lymphomas (median 46 vs 61.5–67.0 years), had mostly stage III (29%) or IV (37%) disease and received BV mostly as 1st line of therapy (LoT; 30%) and as part of polychemotherapy (35%). For other lymphomas, BV was given mostly as ≥2nd LoT and as monotherapy. Among the 44 (21%) pts who received BV as monotherapy, 15 (34%) had HL. Moreover, of these 44 pts, 4 (9%), 12 (27%), 12 (27%) and 14 (32%) pts received BV as 1st, 2nd, 3rd and ≥4th LoT, respectively, for a median of 4 treatment cycles; 23 (52%) pts had a response to BV monotherapy, including 11 (25%) with a complete response (CR). Among all 212 pts, a response was observed in 136 (64%) pts, including 91 (43%) with a CR; median DOT was 105 days or 4 cycles (Table).

Conclusions: Under RW conditions, in a cohort of 212 pts in Switzerland, BV was mostly administered to pts with HL, as 1st LoT and as part of polychemotherapy, with a median DOT of 105 days or 4 cycles. The majority of evaluable HL pts had an objective response (64%; including 50% with a CR). This RW study has inherent limitations and missing data sets preclude definitive conclusions.

SSH/SSMO POSTER PRESENTATION – CLINICAL SOLID TUMOR ONCOLOGY

171

Application of a 21-gene recurrence score in a Swiss single center breast cancer population with intermediate oncologic risk. A comparative analysis of chemotherapy before and after TAILORx

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Introduction: TAILORx (July 18, 2018) showed benefit of chemotherapy (CHT) in women <50 years of age with HER2 negative, node negative breast cancer (BC) with a 21-gene intermediate (11-25) recurrence score (RS). The aim of this study was to determine changes in treatment before and after publication of TAILORx at our Swiss Oncology centers.

Methods: This was a retrospective study on 326 estrogen receptor positive, HER2 negative, BC patients treated at Basel University Hospital and Cantonal Hospital Baselland from 2010-2021. BC patients >18 years of age, with node negative disease and an intermediate RS score (11-25) were enrolled. In the cohort before TAILORx (A) 66/106 patients (62%) and in the cohort after TAILORx (B) 58/97 patients (60%) were eligible for the study.

Results: There were no relevant demographic, nor tumor characteristics differences in the two cohorts: median age of 58.9 years in A and 57.2 years in B (p = 0.414), positive menopausal status in 77% of patients in A and 66% in B (p = 0.206), comorbidities in 42% patients in A and 36% in B (p = 0.864). Patients in B had higher ASA scores, with a median score of 3 vs. 2 in A (p = 0.001). Most tumors were 20-21 mm in size in B and A (p = 0.857), with a Ki67 expression of 20% . Mean RS was 17.43 in A and 17.75 in B (p = 0.674).

Most patients had conservative surgery (76% in A and 64% in B, p = 0.114). RT was administered in most patients equally, with a mean dose of 54.15 Gy in A and 50.31 Gy in B (p = 0.021).

There was less CHT application in B vs. A (22% vs. 16%) (p<0.001), and less than recommended by RS guidelines. In the intermediate (RS 11-25) node negative group, 9% (n = 6) of patients received CHT in A, and 5% (n = 3) in B respectively (p = 0.49), although there were 14% (n = 9) in A and 24% (n = 14) in B of patients <50 years of age with an RS 16-25, which would have been eligible for CHT. In A 5 patients refused CHT, while in B no one did (p = 0.49).

Conclusions: Post TAILORx there are less CHT applications, and less than recommended by RS guidelines, in spite of better patient compliance. However, no major changes are observed in terms of treatment applications, nor tumor board decisions. Logistic regression analysis in the entire population shows relevance of age, nodal status and RS when considering CHT, however the tendency for undertreatment points to the emergence of personalized medicine at the oncological forefront.

Table 1. Treatment administration and outcomes in the intermediate RS (11-25) node negative BC population			
	Cohort A	Cohort B	p-value
Number of patients	66 (62%)	58 (60%)	
Menopause	51 (77%)	38 (66%)	0.206
Age ≤ 50 years old	15 (23%)	19 (33%)	0.336
Age ≥ 51 years old	51 (77%)	39 (67%)	0.205
Surgery			
Breast conservative	50 (76%)	37 (64%)	0.114
No surgical reconstruction	28 (42%)	29 (50%)	0.69
CHT	6 (9%)	3 (5%)	0.49
CHT refusal	5 (45%)	0 (0%)	
CHT in premenopausal women with RS >16	1 (16%)	2 (67%)	
RT	46 (70%)	40 (69%)	0.969
RT refusal	4 (8%)	2 (5%)	0.904
Mean RT Dose	54.14 (SD 6.8, IQR 9.52)	50.31 (SD 7.16, IQR 2.6)	0.021
ET	62 (94%)	52 (90%)	0.326
ET refusal	3 (5%)	6 (10%)	
Osteo-oncologic treatment	29 (44%)	23 (40%)	0.91
TB Implementation	50 (76%)	48 (83%)	0.919

Table 2. Logistic regression analysis of chemotherapy (CHT) administration in the entire population according to demographic and tumor characteristics				
	SD	p-Value	Odds Ratio (OR)	95% CI
Age	0.02	0.001	0.93	0.89-0.97
Age ≤50 />50 years old	0.33	0.367	1.35	0.7 - 2.59
Cohort A/Cohort B	0.46	0.102	0.47	0.19-1.16
Comorbidities Yes/No	0.45	0.626	1.24	0.52-2.99
pT	0.35	0.056	1.81	0.92-3.56
pN	0.44	<0.001	4.77	2.03-11-22
Lobular/Ductal Histology	0.32	0.12	0.61	0.33-1.14
KI-67 <20%/>20%	0.49	0.291	0.6	0.23-1.55
Grade 2	0.46	0.376	0.67	0.27-1.63
Grade 3	0.45	0.473	1.39	0.57 - 3.38
Oncotype RS low (0-10)	1.2	<0.001	0	0-0.02
Oncotype RS intermediate (11-25)	0.58	<0.001	0.02	0.01-0.07
Oncotype RS high (≥26)	1.21	<0.001	617.93	57.97 - 6587.16

CHT = Chemotherapy; RS = Recurrence Score; RT = Radiotherapy; ET = Endocrine Therapy; TB = Tumor Board; SD = Standard Deviation; OR = Odds Ratio; CI = Confidence Interval. Note: Percentages were rounded.

179

Precision Oncology Program – Integration of Real World Data in Clinical Decision Making

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Introduction: Cancer is a highly heterogeneous disease, limiting feasibility of conducting controlled trials due to the broad variety of molecular profiles. Real world data (RWD) from structured electronic health records, providing information on cancer types, molecular profiles, treatments and outcomes, may add meaningful information to treatment decisions, within-standard-of-care (SOC) (decision between standards) and beyond SOC (data-driven decision). The Precision Oncology Program (POP) is a collaboration between the University Hospital Zurich (USZ), the University of Zurich (UZH) and F. Hoffmann-La Roche Ltd., integrating patient-matched RWD from a large scale de-identified pan-tumor clinic-genomic database (CGDB) into the Molecular Tumor board (MTB) USZ, and exploring the hypothetical impact of RWD in a first step on informed decision support.

Methods: POP assesses if information from RWD can meet the demands of MTB decision support within / beyond SOC in a clinically relevant turnaround time. USZ cancer patients currently in need of treatment are matched to data from patients in the Flatiron Health-Foundation Medicine Clinico-genomic database to derive insight on therapy and outcomes in a comparable population. This large-scale CGDB includes >100'000 de-identified patients from ~280 US cancer clinics. The development of the matching process and resulting summary reports from CGDB patients is an iterative process, frequently validated by clinicians and improved. Matching algorithms using patient characteristics are developed to reflect cancer phenotypes, while allowing for a clinically meaningful matched cohort size. Summarized information from matched large-scale CGDB patients is presented to the study team at USZ MTB after routine MTB decision to assess the hypothetical impact of RWD on the MTB decision. POP is an approved observational study (BASEC; 2022-02289), prospectively enrolling up to 1500 cancer patients by 2026.

Results: The utility of insights from large-scale CGDB databases will be assessed for overlap/divergence with MTB decision and its ability to provide potential additional personalized information for therapy decisions in-between and beyond SOC.

Conclusions: POP builds on the predictive potential of large-scale RWD databases, which could change how treatment decisions are made in cancer care in the future by pioneering a path of using structured RWD in routine clinical practice.

200

Implementing diagnostic Whole Genome Sequencing: A hydra of hopes and hurdles

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Introduction: The continuous advancement of next generation sequencing (NGS) has made whole genome sequencing (WGS) cost-effective and practical for clinical diagnostics. Although classical targeted NGS panels cover key markers of the most prevalent tumours, studies evaluating WGS in a clinical setting suggest improved off-label treatment options for patients with rare cancers or end-stage diseases. Therefore, we aim to establish WGS for diagnostic purposes.

Methods: Total nucleic acids were extracted from the tumour and matched-control tissue. Next-generation sequencing was performed with an average coverage of 60x for tumour and 30x for normal tissue. In-house bioinformatics pipelines have been established to analyse raw data and identified established and emerging biomarkers.

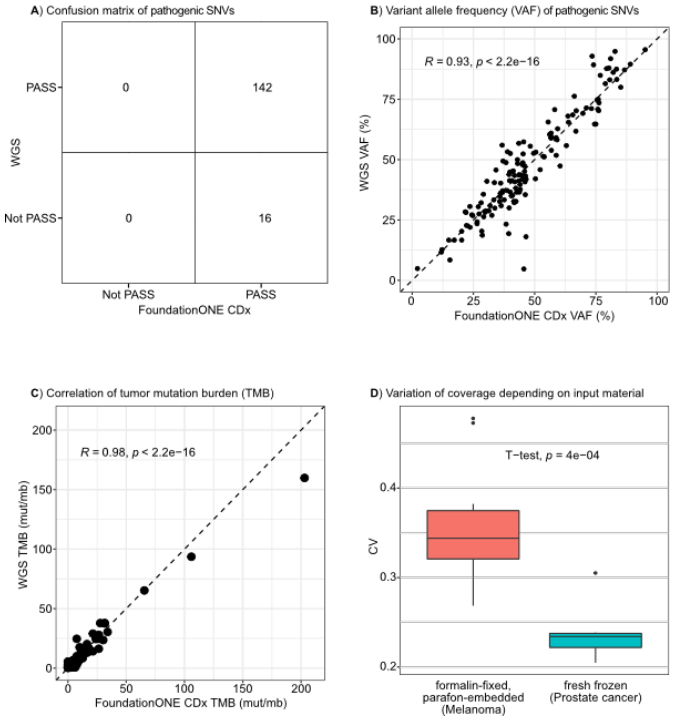
Results: Our primary objective was to compare the outcomes of WGS against FoundationOne CDx as the well-established gold standard of targeted NGS panel in a metastatic melanoma cohort. The majority of short variants (90%) reported by FoundationOne CDx were detected by WGS, and we observed a strong correlation of the variant allele fraction (R2 = 0.93). Our results also showed a strong correlation for TMB (R2 = 0.98) and absolute CNVs. The validation of MSI, HRD and structural

variants are planned following the sequencing of additional cancer entities, where these biomarkers are well represented. Furthermore, WGS enables detecting emerging biomarkers, which currently cannot be validated due to a lack of standards.

We also propose possible solutions concerning regulations for germline testing, automated variant annotation and reimbursement by health insurance companies. Lastly, we created an automated reporting engine that condenses the obtained information to the level of a conventional NGS report.

Conclusions: Based on this experience, we are planning a study employing WGS for cancer patients who have completed therapy. Here, we aim on (1) validating the clinical utility of WGS and (2) gaining additional knowledge with WGS in diagnostic routine.

Figure 1. Validation of whole genome sequencing for tumour diagnostics



239

Genetic Counselling followed by homologous recombination deficiency analysis in patients with newly diagnosed ovarian cancer is feasible and cost-effective in daily practice: A single centre retrospective study.

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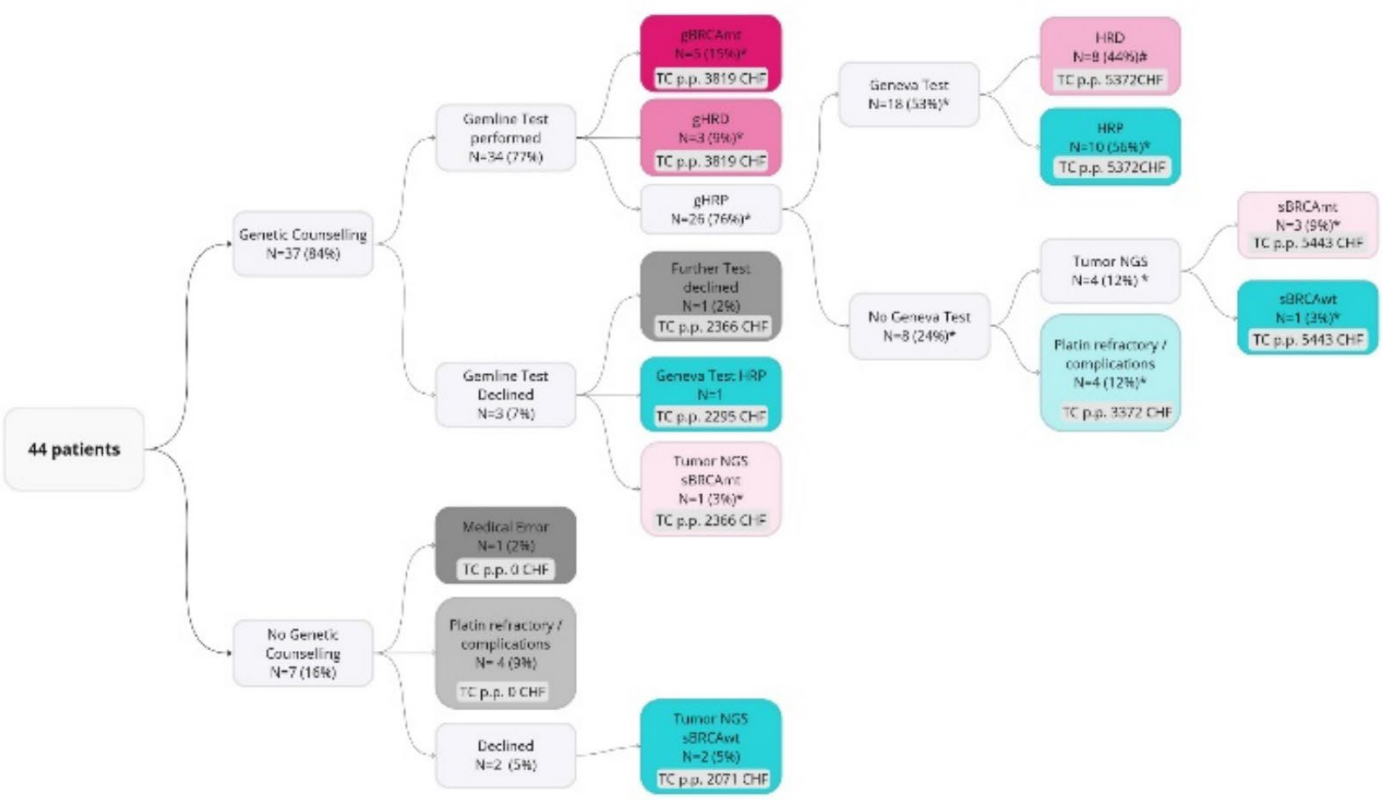
Introduction: Due to its importance for treatment and potential prevention in family members, germline testing of BRCA 1/2 in patients with newly diagnosed high grade seerous ovarian (HGSOC) cancer is decisive and therefore recommended by the American Society of Clinical Oncology (ASCO) Guidelines. Poly (ADP-ribose) polymerase inhibitor (PARPi) maintenance therapy in patients with BRCA mutations and homologous recombination deficient (HRD) tumours substantially improves progression-free survival (PFS) and are licensed in Switzerland for these patients exclusively. Therefore, it is crucial to test patients early while they are receiving adjuvant chemotherapy. This study's objective was to analyse whether genetic counselling followed by HRD testing is feasible in terms of delays in initialisation of maintenance therapy in daily practice in Switzerland.

Methods: A single centre retrospective study of 44 patients with HGSOC of a Federation of Gynecology and Obstetrics (FIGO) stage of IIIA-IVB diagnosed between 12/2020 and 12/2022 was conducted. Outcomes of genetic counselling, germline testing and somatic Geneva test for HRD were collected. To test feasibility and cost effectivity in clinical practice, delays of initiation of maintenance therapy, total testing costs per patient and PFS were analysed.

Results: Of 44 patients with newly diagnosed HGSOC, 37 (84%) received counselling of which 34 (77%) were tested for germline BRCA and other mutations in HRD genes. Thereby 5 (15%) BRCA and 3 (9%) other HRD defining mutations were identified. Of the remaining 26 patients, 11 (42%) had tumours with somatic HRD. Mean time to initialisation of maintenance therapy of 5.2 weeks. PFS at 12 and 24 months did not differ from SOLO1 or PAOLA study population. Mean testing costs per patient were 3592 as compared to 5670 Swiss Franks if a myChoiceCDx HRD assay was applied (p <0.0001).

Conclusions: Genetic counselling to consent patients with newly diagnosed HGSOC for germline testing is the international gold standard. Subsequent somatic HRD analysis complements testing and increases the number of idividuals who will benefit from PARPi maintenance therapy. Contrary to previous assumptions, the procedure does not increase testing costs or delay maintenance therapy. Therefore, all patients should be offered a primary germline analysis. The challenge for the future will be to ensure prompt genetic counselling and testing.



241

Primary and Acquired Resistance to Targeted Treatment in BRAF V600E-mutated Metastatic Colorectal Cancer – PARTACER-Suisse (SAKK 41/23, Trial in Progress)

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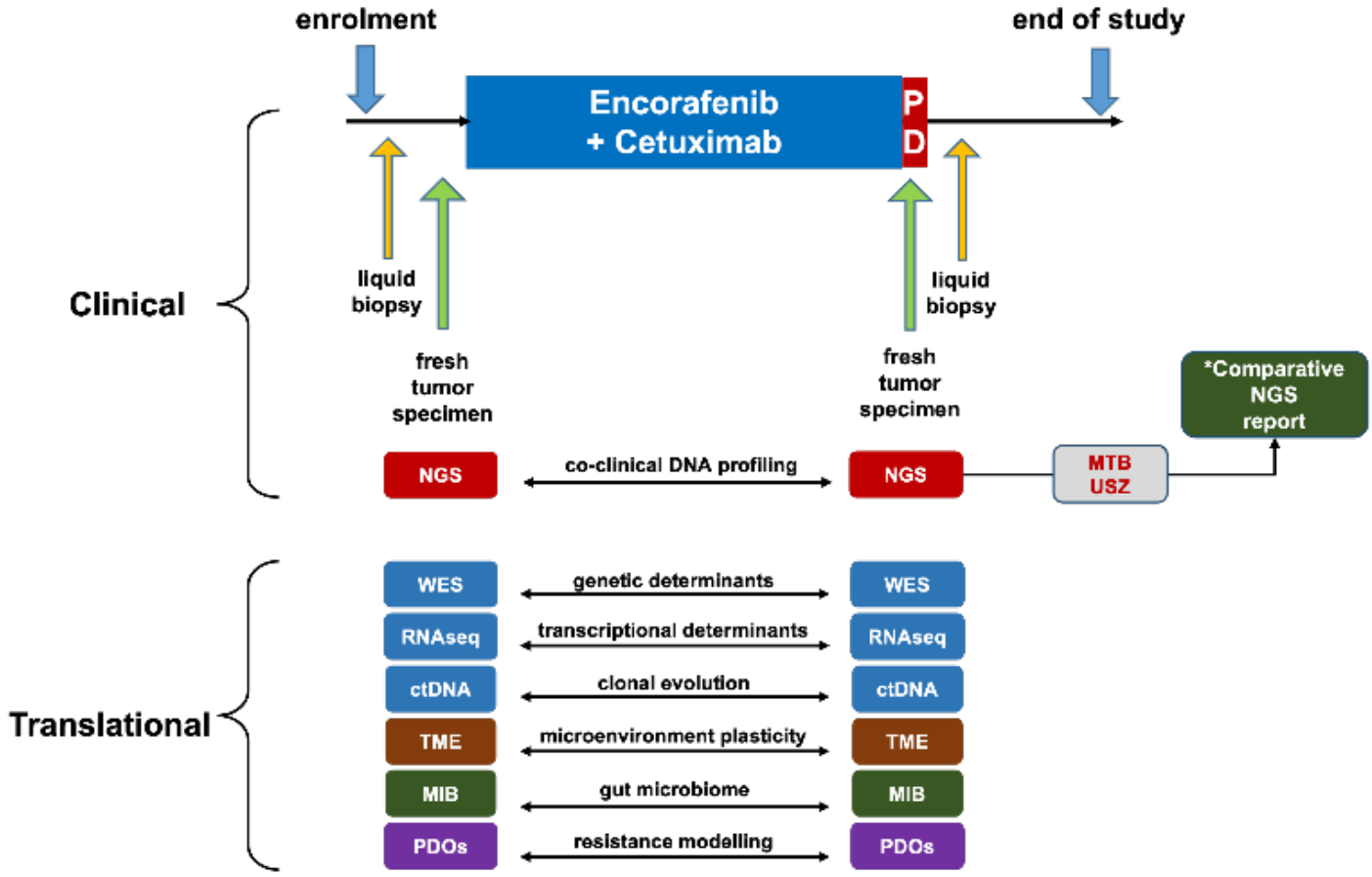
Introduction: BRAF V600E-mutated metastatic colorectal cancer (mCRC) represents the most aggressive subgroup of mCRC with a dismal prognosis. Based on results of the phase III BEACON trial, combined molecularly targeted treatment with cetuximab and encorafenib (CE) is standard-of-care for these patients following 1st line chemotherapy. Acquired resistance to CE often emerges rapidly, leaving patients without well-established further treatment options. We hypothesized that a more comprehensive understanding of the genomic and non-genomic landscape of acquired resistance to CE will help uncover novel treatment targets in this setting. Moreover, the derivation of patient-derived cellular models will allow for the establishment of novel targeting strategies to delay or overcome acquired resistance.

Methods: PARTACER-Suisse is an investigator-initiated prospective HRO chapter 2 multicenter study, enrolling patients undergoing treatment with CE for BRAF V600E-mutated mCRC. PARTACER-Suisse is organized through Swiss Group for Clinical Cancer Research (SAKK, trial number 41/23), 15 study sites across Switzerland have committed to recruiting patients. Recruitment of a total of 30 evaluable patients is planned over a recruitment period of 2.5 years. Patient recruitment for PARTACER-Suisse will commence in Q1/2024.

During the clinical part of the study, pre-treatment and on-progression tissue and liquid biopsies will be collected from patients undergoing treatment with CE at the study sites. Clinical data will be recorded. NGS-based DNA profiling will be performed, and results reported back to the recruiting sites (Figure 1). The translational part of PARTACER-Suisse focuses on multi-omics molecular analyses of paired patient samples and on the generation and exploitation of patient-derived 3D organoids (PDOs) established from pre- and post-treatment biopsies. PDOs will be employed for treatment and resistance modelling in vitro, aiming to pre-clinically establish novel treatment strategies to prevent, delay, modify and overcome acquired resistance to BRAF V600E-targeting in mCRC.

Results: Conclusions: PARTACER-Suisse aims to deliver the first comprehensive molecular analysis of acquired resistance in BRAF V600E mCRC. Through exploitation of patient-derived organoids, our study aims to pre-clinically established novel therapeutic strategies that could then be moved forward to interventional trials.



Abbrevations: NGS, next generation panel sequencing; WES, whole exome sequencing; RNAseq, RNA sequencing; MTB, molecular tumor board; ctDNA, cell-free tumor-derived DNA; TME, tumor microenvironment; MIB, gut microbiome; PDOs, patient-derived organoids;
*** reported to study site**

281

Cost-effectiveness analysis of sotorasib as second-line treatment for patients with KRASG12C mutated metastatic non-small cell lung cancer (mNSCLC) in Switzerland

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Introduction: Sotorasib, a G12C K-RAS protein inhibitor, was compared to docetaxel as a second-line treatment in mNSCLC patients in the CodeBreak200 trial, where it demonstrated a better tolerability without improving overall survival (OS) [1]. We conducted a cost-effectiveness analysis of sotorasib in Swiss mNSCLC patients with KRASG12C mutation who had progressed after first-line platinum and PD-1/PD-L1-based treatment.

Methods: We constructed a partitioned survival model with a discount rate of 3% for costs and quality-adjusted life years (QALYs). We fitted parametric survival curves to the published Kaplan-Meier data and extrapolated survival. QALYs were taken from the CodeBreak100 trial and the literature [2,3]. Costs for drugs, adverse events (AEs), and disease management were assessed from the Swiss statutory health insurance perspective. As the price of sotorasib has not been established in Switzerland, we analysed two scenarios: first, using the published expected monthly UK price, equivalent to Swiss Francs (CHF) 7'870 [4] (dose: 960mg/day). Second, we used ¼ of that price (CHF 1'968), according to the diminished dose (240mg/day) used in the most recent trial [5], under the assumption that ¼ of the sotorasib dose is equally effective and has ¼ of the AEs. Incremental cost-effectiveness ratios (ICER) were compared to a hypothetical willingness-to-pay of CHF 100,000 per QALY gained.

Results: With the lack of an OS benefit, the reduced AEs over a median therapy duration of 5.6 months with sotorasib, practically no difference in QALYs between sotorasib and docetaxel was detected (1.28 QALYs for both treatments). With the ICER calculation hence obsolete, we considered absolute price differences. Total per-patient costs were CHF 145'351 for the strategies representing full sotorasib dose, CHF 83'674 for the ¼ dose, and CHF 73'185 for docetaxel. In the full dose, docetaxel is projected to be CHF 72'166 cheaper per treated patient. When utilizing ¼ of the dose and cost for sotorasib, the cost per treated patient is significantly reduced to a CHF 10'489 difference compared to docetaxel. The results were robust in 99% of probabilistic simulations.

Conclusions: The full dose of sotorasib with the predicted UK price, is not projected to be cost-effective. From our perspective, regarding the better tolerability profile of sotorasib, pricing within the range of ¼ of the dose costs would be reasonable.

ONCOREHA/OPS/PALLIATIVE.CH/SOHC POSTER PRESENTATION – SUPPORTIVE & PALLIATIVE CARE, REHABILITATION & SURVIVORSHIP

160

Incidence and predictive factors for psychological distress in cancer patients in Switzerland during oncological rehabilitation

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Introduction: The cancer diagnosis and the oncological therapy itself lead to somatic symptoms and high psychological distress in the affected patients and in up to 30% to partly chronic mental disorders (depression, anxiety disorders, panic attacks,...). There are currently no published data on the incidence of psychological distress in oncological patients at the beginning of oncological rehabilitation in Switzerland.

Methods: As part of this prospective study, psychological distress at the beginning of oncological rehabilitation was recorded from January 2022 to April 2023 and compared with sociodemographic and clinical data.

In 296 of 400 patients (74%; 52.3% women; mean age 63.5 +/- 12.2) the psychological distress could be recorded using the German version of the 11-scale NCCN distress thermometer.

Results: The average psychological distress in the collective was significantly increased at 5.8 (+/-2.3), significantly increased distress values (DT > = 5/10) could be detected in 74.0% (n = 220). Furthermore, there was a significant difference depending on gender (women DT ≥ 5 in 80%; mean 6 ±2 vs. men DT ≥5 in 68.3%; mean 5.3±2.3; p = 0.009).

While younger women showed a (non-significant) higher distress than older patients (81.4% vs. 78.8%), older men showed higher distress values than younger ones (71.4% vs. 64.3%). The subgroup with the highest distress scores were breast cancer patients (DT ≥ 5 in 92.3%, mean 6.9±1.9), although the proportion of chronic disease stages was significantly lower (P = 0.002) compared to other tumor entities. However, the affected patients reported significant fatigue-like symptoms compared to patients with other tumor entities (P = 0.01).

Conclusions: The incidence of psychological distress at the beginning of oncological rehabilitation is extremely high. Prognostic factors are primarily female gender, diagnosis "breast cancer" and the presence of fatigue symptoms. Therefore, the presence of psychological stress should generally be actively screened at the beginning of rehabilitation and psycho-oncological co-care in oncological rehabilitation in Switzerland should be further expanded.

181

Prevention of taxane chemotherapy induced nail changes and peripheral neuropathy (CIPN) by application of extremity cooling: a prospective single center study with intrapatient comparison

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Introduction: Peripheral neuropathy (CIPN) and nail toxicity (tox) are common side effects of taxanes. Different methods of cryotherapy to prevent these side effects have been tested. We investigated machine-controlled cooling of hand and feet to reduce these common side effects.

Methods: Patients (pts) receiving Docetaxel (Doce) (planned dose ≥300mg/m2) or Paclitaxel (Pacli) (planned dose ≥ 720mg/m"2") in the adjuvant or palliative setting were included. The dominant hand and foot were cooled to approximately 10°C using the Hilotherapy-machine. The contralateral hand and foot were used as intrapatient comparison. Primary endpoint was the occurrence of any nail tox (Doce cohort) or CIPN (Pacli cohort) at any time during and up to 56 days after the end of treatment. We assumed an absolute improvement of 40% in nail tox and 20% in CIPN. With 90% power using a two-sided McNemar test with a significance level of 5%, a total of 30 pts were needed in each group. The intended to treat population (ITT) and the per protocol population (PPP) were analysed. Toxicity was assessed using the CTCAE v5.0 grading system and the PNQ questionnaire.

Results: 69 pts, 21 (9 in the PPP) treated with Doce and 48 (34 in the PPP) treated with Pacli, were included from 08/2020 – 08/2022 in our center. In the Doce cohort, nail tox was not significantly improved by cooling in the ITT (81.0%, vs 85.7%; odds ratio (OR) 0.71, 95% CI: 0.14 – 3.64, p = 0.564) and PPP (100% vs 100%; OR: 1.00, 95% CI: 0.00 – NA, p = NA) but significant benefit across visits over time was found for the ITT (OR: 0.42, 95% CI: 0.18 – 0.98, p = 0.045) but not the PPP (OR: 0.75, 95% CI: 0.30 – 1.86, p = 0.529). In the Pacli cohort, CIPN was numerically better in the ITT (60.9% vs. 71.7%, OR: 0.61, 95% CI: 0.26 – 1.47, p = 0.059) and significantly better in the PPP (67.6% vs. 82.4%, OR: 0.45, 95% CI: 0.14 – 1.40, p-value = 0.025). Differences across visits were significantly in favour of cooling (ITT: OR: 0.28, 95% CI: 0.13 – 0.57, p = 0.001; PPP: OR: 0.25, 95% CI: 0.12–0.55, p = 0.001).

Cooling was well tolerated, only 6 pts (9%) discontinued due to side effects.

Conclusions: Cooling of hand and feet has a clinically meaningful impact on occurrence of CIPN and nail toxicity. Effects are more significant over time and are taxane dose dependent.

187

Patient Reported Outcome Measures (PROMs) in a multidisciplinary lung cancer centre: a value-based healthcare pilot project

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Introduction: Patient reported outcome measures (PROMs) are an integral part of the value-based healthcare (VBHC) concept. PROMs can enable personalized care and potentially improve survival in patients with lung cancer. We report on our early experience with the implementation of PROMs in a multidisciplinary lung cancer centre.

Methods: In 2020, a PROMs program was introduced at our academic lung cancer centre. The project was developed in collaboration with an industry partner as a VBHC pilot project. Following ethics approval, an academic research institute was contracted for analyses of de-identified data.

Following identification at the tumour board, patients with newly diagnosed thoracic malignancies were approached by a dedicated study nurse, who educated patients about the program and led the collection of PROMs data via email, or iPad in personal consultations.

PROMs were assessed at baseline, with follow-ups at 3, 6, 12 and 24 months. Assessments were based on International Consortium for Health Outcomes Measurement (ICHOM) recommendations, and included the European Organization for Research and Treatment of Cancer (EORTC) quality of life (QoL) questionnaires QLQ-C30 and QLQ-LC29. Patient, disease and treatment characteristics were also recorded based on ICHOM recommendations.

Results: From Oct. 2020 to Aug. 2023, a total of 572 patients were screened, of whom 424 were suitable for inclusion. A total of 293 patients were enrolled, of whom 178 (61%) and 115 (39%) were male and female, respectively. Median age was 70 years (range, 21–92). Patients were diagnosed with lung adenocarcinoma (n = 181), squamous cell carcinoma (n = 57), small cell lung cancer (n = 32), and other entities (n = 23). Overall, 144 (49%) patients had early-stage disease, 64 (22%) had locoregionally advanced disease, and 85 (29%) had metastatic disease.

Over the observation period, a total of 878 questionnaires had been completed, with a completion rate of 85%. Reasons for dropouts were death (n = 55), patient refusal (n = 38), loss to follow-up (n = 15), health deterioration (n = 5) and other reasons (n = 17). Results of baseline QoL assessments will be presented at the conference.

Conclusions: A comprehensive PROMs program was successfully implemented in our lung cancer centre, with early experiences indicating a high level of patient interest. We next aim to optimize the integration of PROMs into routine care.

197

Early Warning System for Monitoring of Cancer Patients Using Hybrid Interactive Machine Learning

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Introduction: The use of smartphone app´s in cancer patients undergoing treatment can promote early detection of symptoms and therapy side effects and may be supported by machine learning (ML) for timely adaption of therapies, reduction of AEs and unplanned admissions. We aimed to create an Early Warning System (EWS) to predict situations where supportive interventions become necessary to prevent unplanned visits. For this, standardized electronic patient reported outcome (ePROs) data were analyzed in context with the patient's individual journey. Also, information on well-being, vital parameters, medication, and free text were considered for establishing a hybrid ML model.

Methods: Prediction of unplanned visits was achieved by employing a white-box ML algorithm (i.e., rule learner), which learned rules from patient data (i.e., ePROs, vitals, free text) that were captured from a medical device smartphone app. Those rules indicated situations where patients experienced unplanned visits and, hence, were captured as alert trigger in the EWS. Each rule was evaluated based on a cost matrix, where false negatives (FNs) have higher costs than false positives (FPs, i.e., false alarms). Rules were ranked according to the costs and gave priority to the least expensive ones. Finally, rules with higher priority were reviewed by two oncologists for plausibility check and for extending them with additional conditions.

Results: From a cohort of 214 patients and a more than 16`000 data entries overall, the ML-learned rule set achieved a recall of 28% on the entire dataset and a precision of 15%. While modification by medical experts did not improve these values on the given data set, they expressed confidence that a) they had understood and were able to make sense of the rules and b) the modified rules could be expected to generalize better to presumably unseen data. Te involvement of medical experts allowed to define rule consequences, i.e., actions to be recommended to patients or caregivers that are not necessarily visits to physicians or hospitals. When comparing the effectiveness of the derived rules to rules that our medical experts had formulated without the support of ML– there was no obvious match between the ML-learned rules and those suggested by the physicans.

Conclusions: Learning rules from dynamic ePRO datasets, may be used to establish early warnings for cancer patients in outpatient settings.

204

Patient-Centered Care in Immunotherapy: A Comprehensive Examination of Patient Experiences

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Introduction: Patient-centered healthcare demands a comprehensive understanding of patient experiences. Immune checkpoint inhibitors (ICI) have brought transformative changes to cancer treatment, presenting new opportunities and challenges. The objective was to gain insights into patients' multifaceted experiences to inform patient-centered cancer care strategies.

Methods: A qualitative study applying thematic analysis was conducted from March 2019 to August 2021. Semi-structured interviews with 27 patients diagnosed with advanced melanoma or lung cancer undergoing ICI treatment at a specialized oncology department were conducted. Interviews took place at the beginning of treatment (n = 21), three months (n = 16) and five to six months into treatment (n = 14). Thematic analysis was employed to identify major themes.

Results: The sample included 52% males and 48% patients aged over 65. Three themes were identified.

Perceptions of Treatment: Patients approached their treatment with hope, viewing this new approach as a promising and less aggressive alternative to chemotherapy. Over time questions arose about treatment's effectiveness and its curative or preventive character.

Impact on Daily Life: ICI, which often spanned a year, disrupted daily routines, requiring careful time management and affecting work, family, and health balance. High ICI costs raised concerns about medical expenses, health insurance coverage, long-term financial stability, and the possibility of losing their job due to missed workdays.

Uncertainty was named as a constant companion throughout the six months, contributing to emotional distress. Unknowns included side effects, treatment efficacy, cancer status, or

treatment duration. Waiting for test results, especially related to treatment effectiveness, was a major source of stress. Patients emphasized the importance of receiving clear information right from the start and a transparent communication with healthcare professionals regarding the treatment's implications.

Conclusions: This qualitative exploration highlights the complexity patients face with ICI encompassing physical, emotional, psychological, and financial dimensions. Uncertainties, hopes, and challenges intertwine throughout the journey. Understanding this multidimensional experience is essential. Enhanced self-management support should be integrated in the care pathway to improve patient experiences with these treatments.

263

"SUNSHINE_eHealth: Leveraging Machine Learning for ECOG and One-Year Survival Predictions in High-Risk Oncology Patients"

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Introduction: Early detection of clinical deterioration in oncology patients is crucial and challenging. The Eastern Cooperative Oncology Group (ECOG) performance status is frequently used but is often a subjective assessment open to varied interpretations. This project implemented a telemedicine system to monitor alterations in vital signs, with the aim of evaluating whether such data could predict real-time ECOG performance status and 1-year survival using machine learning models.

Methods: VitalPatch, a Class IIa medical device, was utilized as the monitoring system, continuously tracking seven key parameters (respiratory frequency, oxygen saturation, heart rate, temperature, single-lead ECG data, posture, step count, and fall detection) in oncological high-risk patients over seven days. Laboratory and clinical assessments were also incorporated for comprehensive patient evaluation. We first performed Mann-Whitney U test to identify differences in terms of vital signs between different groups of patients. Then, we trained different machine learning models—logistic regression, random forest classifier, and XGBoost— to predict ECOG PS, C-reactive protein level (CRP) and survival status. To this end ECOG PS was categorized as 'high' (2-3) or 'low' (0-1), C-reactive protein level (CRP) was categorized as 'high' (values >50 mg/L) or 'low' (0-10 mg/L), and survival status was determined one year post-monitoring. Finally, performance of the models was assessed using the F1 weighted score metric and the area under the Receiver Operating Characteristic (ROC AUC).

Results: Analysis of data from 28 patients indicated significant differences in mean heart rates between high and low ECOG PS scores ($p = 0.0094$) and high and low CRP levels ($p = 0.027$). The Random Forest model yielded promising predictions for ECOG (F1 score = 0.9124, ROC AUC = 0.900) and 1-year survival (F1 score = 0.771, ROC AUC = 0.800).

Conclusions: This preliminary analysis indicates substantial potential to develop AI algorithms that utilize vital signs to determine ECOG PS and predict 1-year survival status, with heart rate being notably indicative. These results signify progress in leveraging telemedicine and remote monitoring to enhance patient care, particularly in oncology. Expanded research with larger populations is essential to corroborate these promising findings.

267

What do you think Mr. Patient? Patients views on Quality of life assessment tools in MDS

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Introduction: Myelodysplastic syndromes (MDS) are generally associated with significant morbidity and mortality, and many patients with this disease suffer not only from physical symptoms but also psychosocial manifestations. Quality of life (QoL) assessment through patient-reported outcomes (PROs) is therefore important in the management of these patients. The use of PRO tools to understand the patient's perspective would improve understanding of how their patients are coping with their disease and help to improve their quality of life. The use of the right PRO tool can provide meaningful information in this regard and assist in follow up care. There are however numerous PRO tools currently available for MDS, which may make choosing the right tool sometimes challenging.

The objectives of this study was to evaluate the patients' views and preferences on the currently used PRO tools in MDS.

Methods: In this study, we asked patients with myelodyplastic syndromes treated at our tertiary hospital their preferences regarding the different QoL questionnaires. The most frequently used PRO tools in MDS were evaluated i.e. EORTC QLQ-C30, FACT-An, EQ-5D, Stress-Thermometer and QUALMS. The patients received a separate question sheet where they answered questions and gave their opinions regarding these QoL assessment tools and the tool they most preferred.

Results: 70 MDS and CMML patients took part in the study. The median age was 72 years (Range 41 – 89). 52 patients were male and 18 were female. 43 questionnaires were answered correctly. 45/70 (64%) patients found QoL assessment to be a very important part of management. 19/43 patients (44%) opted for FACT-An as the questionnaire they preferred the most. 9/43 patients (20.9%) favored QUALMS, 7/43 (16.3%) favored the Stress-Thermometer, 5/43 (11.6%) favored EORTC-QLQC-30 while 3/43 (7%) favored EQ-5d.

Conclusions: In this study, FACT-An was the PRO tool most patients preferred. This information may be helpful in the clinical setting as well as in planning studies, in regards to the best QoL questionnaire to use. This study also shows that the patients' views are important when it comes to selecting such tools and this could promote compliance. These results could be used to encourage physicians to include patients in decision making.

278

Boswellia serrata for tumor-related brain edema: a systematic literature review

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Introduction: Tumor-related brain edema (TBE) is a common, distressing complication in patients (pts) with primary brain tumors (PBT) or Brain metastases. Treatment of TBE with corticosteroids is effective, but side effects are common, and they may reduce the effectiveness of tumor directed therapies. Preliminary data suggest that extracts from Indian Frankincense (Boswellia serrata [BS]) can reduce TBE and lead to steroid sparing. Objective: to systematically review peer-reviewed literature for TBE-reducing and steroid-sparing effects of BS.

Methods: Systematic literature review according to PRISMA guidelines. We systematically searched Medline (via pubmed) and clinicaltrials.gov published until August 2022, amended by citation searches of Onkopedia, S3-Guideline for complementary medicine in oncology, and Monographies of BS. Inclusion criteria were original reports (case reports [CR], observational [OS] and controlled studies) from adult pts with TBE due to PBT or metastasis. Data extracted included study design, pts number, tumor type, reduction of TBE and symptoms, steroid tapering and side effects. Due to the heterogeneity of included studies, no formal risk assessment of bias and no meta-analysis was performed.

Results: From 48 identified reports, 44 were excluded (43 not clinical, 1 not adult); one identified by citation search was included. Overall, 102 pts were studied in one randomized, placebo-controlled trial (RCT, during radiotherapy [RT], n = 42), one 3-arm dose-comparing study (preoperative, n = 27), two prospective OS (Glioblastoma during Chemo-RT, n = 20; mixed tumors best supportive care [BSC], n = 12) and one CR. BS was used in doses from 2400 to 4200 mg / day. Reduction of TBE was reported preoperative (only high and moderate, not low dose), during RT, and BSC (except 5/7 Glioblastoma), but remained unclear during Chemo-RT. Reduction of symptoms was reported preoperative and during BSC. Steroid tapering was not studied preoperative, it was only possible in BSC in pts with astrocytoma/metastasis. Side-effects included nausea (n = 1), diarrhea (n = 2) and rash (n = 2).

Conclusions: Available data from heterogeneous studies with generally low methodological quality suggest some promising signals of BS for reduction of TBE, and for symptom reduction and steroid tapering in preoperative and BSC pts, respectively. High-dose BS merits further research considering the high clinical need.

POSTER – HEMOSTASIS, TRANSFUSION MEDICINE, VASCULAR, LABORATORY MEDICINE, BENIGN HEMATOLOGY

158

Epidemiologic study of hereditary bleeding disorders in Dubai ,UAE :A single center experience

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Introduction: Background:

Knowing the frequency of common and rare hereditary bleeding disorders (HBDs) is important in areas where consanguineous marriages are common.

Objective:

Reported bleeding disorders in the Gulf region are scarce, so our study aims to evaluate the epidemiologic features of patients with HBDs in Dubai, UAE.

Methods: In this cross-sectional study, 90 patients with HBDs were evaluated during the period of 2019 to 2022 which was conducted at American Hospital, Dubai (AHD) in January 2023. All patients with confirmed (HDBs) and newly diagnosed, of all ages, and who at least had one follow-up visit at our facility were included. The tool used is medical electronic records designed in an EXCEL.doc categorizing patients based on personal details, Initial symptoms, clinical manifestations, diagnosis, treatment (On-demand or prophylaxis), and if consanguineous marriage is present or not. Individuals with missing data or acquired bleeding disorders were excluded from the study. Coagulation factor assays were done in the USA by IL ACL TOP 700 model.

Results: A total of 90 patients with HBDs including 34 males and 56 females (ages ranging from 15 days to 58 years old) at AHD were evaluated (Table 1). The mean age of participants was 25 years . All types of HBDs and their clinical manifestations are summarized in Table 2. Von Willebrand disease is the most common with 43.3%, and Factor VII is the most prevalent rare bleeding disorder followed by platelet dysfunction.

Conclusions: This is the first comprehensive study that reported HBDs from a referral single center, in Dubai, UAE. Our study showed Von Willebrand disease type 1 is the most common of all HBDs and Factor VII is the highest recorded in rare bleeding disorders. This epidemiologic study guides us to design a proper treatment plan according to the frequency of HBDs in this region where consanguineous marriages are prevalent.

Table 2 : Frequency of factor deficiencies and Initial symptoms at diagnosis of patients with Hereditary bleeding disorders (HBDs),Dubai,UAE.

Factor Deficiency	Number (%)	Major bleeding at diagnosis	On demand: number (%)	Prophylactic: number (%)
Common bleeding disorders				
Von willebrand disease	39 (43.3)	Menorrhagia	29,(74)	10,(26)
FVIII	15,(16.6)	Asymptomatic	5,(33)	10,(67)
FIX	1,(1.1)	Asymptomatic	–	1,(100)
Rare Bleeding Disorders				
FVII	16,(17.8)	Easy bruising/Recurrent bleeding	13,(81)	3,(19)
FX	Not reported	–		–
FXIII	3,(3.4)	Asymptomatic /Post-surgical hemorrhage	1,(33)	2,(67)
FI				
Afibrinogenemia	1,(1.1)	Asymptomatic	1,(100)	–
Congenital hypofibrinogenemia	1,(1.1)	Easy bruising	–	1,(100)
FII	1,(1.1)	Asymptomatic	1,(100)	–
FVIII & FXI	Not reported	–	–	–
FVII &FX	Not reported	–	–	–
VKCFD	1,(1.1)	Asymptomatic	1,(100)	–
FV	1,(1.1)	Asymptomatic	1,(100)	–
FXII	Not reported	–	–	–
Platelet Dysfunction	3,(3.4)	Asymptomatic	3,(100)	–
Combined deficiency				
Von willebrand disease type I, Factor VII	2,(2.2)	Asymptomatic	2,(100)	–
Factor VII ,Factor VIII	1,(1.1)	Asymptomatic	–	1,(100)
Von willebrand disease type I ,Factor IX	1,(1.1)	Fatigue ,tiredness along with dizzy spells	1,(100)	–
Factor IX ,Factor XI	1,(1.1)	Petechia on his lower extremities after birth	1,(100)	–
Bleeding of Unknown Cause (BUC)	3,(3.4)	Bruising	3,(100)	–
Total	90 ,(100)	Asymptomatic	62,(68.8)	28,(31)

192

Involvement of the contact pathway in COVID-19 coagulopathy

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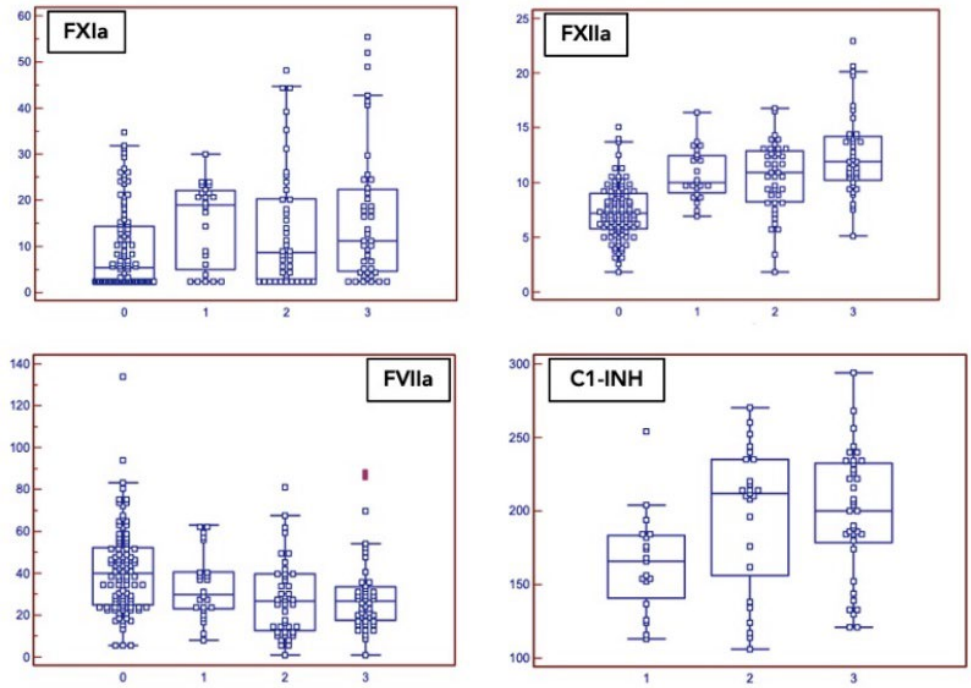
Introduction: A novel acquired coagulopathy characterized by severe procoagulant imbalance is common and associated with the clinical severity in COVID-19 patients. Our study aims to elucidate the underlying mechanisms of coagulation activation in COVID-19 patients.

Methods: Symptomatic COVID-19 patients were consecutively enrolled and stratified into 3 groups based on the intensity of care: low (high-flow oxygen by nasal cannula, 26 patients); intermediate (continuous positive airway pressure, 42 patients); high (mechanical ventilation, 43 patients). Markers of intrinsic (FXIa, FXIIa) and extrinsic (FVIIa) pathway activation and of fibrinolysis (plasminogen and relative activator and inhibitors), D-dimer, Fibrin Monomer (FM), Fibrin Degradation Products (FDP) and C1-inhibitor were tested.

Results: 111 patients were included: 26 at low, 42 intermediate and 43 high care-intensity. Median age was 59±12 (34 patients >65 years); 32 patients (29%) developed a venous thrombosis and 12 (11%) died. Median D-dimer, FDP and FM plasma levels were higher in COVID-19 patients than normal ranges, with a gradient of increase across the three intensity care units; the fibrinolytic pathway parameters were in the normal range. Median plasma levels of FVIIa were lower in COVID-19 patients (27.5 mU/mL) than in controls (40.1 mU/mL) while median plasma levels of FXIIa and FXIa were higher in COVID-19 patients (11.2 and 11.3 mU/mL) than in controls (7.2 and 5.5 mU/mL), with a gradient of increase across the three intensity care units. C1-inhibitor plasma levels were above the normal range in all the 3 COVID-19 patients' groups (Figure).

Conclusions: Our study showed a prevalent activation of the contact pathway over the extrinsic pathway of the coagulation cascade in COVID-19 patients, which is proportional to the clinical severity of the infection, opening the possibility for targeted anticoagulant therapies.



205

Long-standing thrombocytosis and thrombotic events preceding recognition of polycythemia vera: a single-center retrospective study

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Introduction: Patients with polycythemia vera (PV) are at a higher risk for thrombotic events (TE), which may occur before diagnosis or later during the disease. Furthermore, thrombocytosis may be an early marker of PV. Our study aimed to analyze the frequency of TE and the presence of thrombocytosis preceding the diagnosis of PV.

Methods: In this monocentric, retrospective study PV patients aged 18 years or older, who were seen at our institution between January 2008 and December 2018 were included. Baseline demographic, PV diagnosis information, comorbidities, presence and type of TE and blood counts before TE and PV diagnosis were analyzed.

Results: A total of 79 eligible patients were included. There was a slight male predominance (57%) with a median age at PV diagnosis of 69 years. As expected, JAK2V617F was the most prevalent PV mutation (62%). Fifty-two patients (66%) had TE (24 before or concomitant with PV diagnosis, 11 after PV diagnosis and 17 with TE before and after PV diagnosis). Forty-five patients presented arterial thrombosis, 32 had venous thrombosis and 19 patients had both. For patients with TE: median thrombocyte level at PV diagnosis was 479 G/L and at the time of TE 360 G/l, median leucocyte and hemoglobin (hematocrit)

levels at PV diagnosis were 11.4 G/l and 181 g/l (54%), respectively; and at the time of TE 12.5 G/l and 178 g/l (53%), respectively. We collected 61 blood counts performed before PV diagnosis in 13 patients with TE prior or concomitant with PV diagnosis. For these patients, the median time from the first blood count showing thrombocytes >350 G/L or >450 G/l to the diagnosis of PV was 51 months and 17 months, respectively. Three patients had a TE prior to PV diagnosis with a normal thrombocyte count. In these three patients, the median delay between the TE and PV diagnosis was 30 months. For the remaining 10 patients, the median time from the first blood count with thrombocytes >350 G/L or >450 G/l and the TE was 61 months and 5.7 months, respectively. The median time from the first blood count showing Hb>165 g/l to the diagnosis of PV was 20 months and to the TE was 7 months.

Conclusions: Our study shows that thrombocytosis is frequently present for months or even years before the diagnosis of PV and the occurrence of a thrombotic event. The presence of thrombocytosis, even moderate, in the absence of a secondary cause, should evoke the diagnosis of PV.

213

Automated effective algorithm for detecting acquired haemophilia A

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Introduction: Among the various causes of isolated prolonged aPTT, acquired haemophilia A (AHA) is a rare bleeding disorder for which accurate and prompt diagnosis remains challenging. Consequently, there is the need for a standardized approach allowing an automated, rapid, and reliable evaluation of the causes of an isolated prolonged aPTT within large blood sample series.

Methods: An in-house laboratory work-up algorithm was developed to detect robustly and efficiently clinically relevant causes of an isolated prolonged aPTT. This algorithm was progressively adapted during two prospective, 12-day and 3-month derivation phases. Subsequently, it was validated during a 3-year analysis period. Investigations included aPTT-related coagulation factors, anti-Xa activity, mixing tests when needed, combined to patient's clinical records.

Results: Of all samples received at the laboratory in a 3-year validation period, our automated algorithm enabled the rapid and accurate identification and evaluation of ~1% (2053/~300'000) blood samples, with an isolated prolonged aPTT. Within these 2053 samples, most cases were explained by contact phase factors deficiency, whether or not in the context of an inflammatory state (51%), and anticoagulant drugs targeting Xa, such as heparin and DOAC (30%). Isolated deficiencies in FVIII or FIX, including several known cases of congenital haemophilia, were observed in 10% and 2%, respectively. Other diagnostic categories involved lupus anticoagulant (0.3%) and von Willebrand disease/acquired von Willebrand syndrome (0.3%). Importantly, 2 cases (0.1%) of AHA were diagnosed out of 2053 isolated prolonged aPTT blood samples in this 3-year timelapse before clinical suspicion was raised. No other cases of AHA were diagnosed during the studied period.

Conclusions: Our in-house automated algorithm allows the rapid and reliable identification of those rare cases of clinically relevant isolated prolonged aPTT, among which a small fraction can cause clinically significant bleeding disorders. Since its implementation, over a 5-year period, the algorithm has detected all new cases of AHA (n = 4), before their clinical suspicion.

216

Thrombotic risk in patients with primary nephrotic syndrome: a better pathophysiological insight to improve current predictive tools

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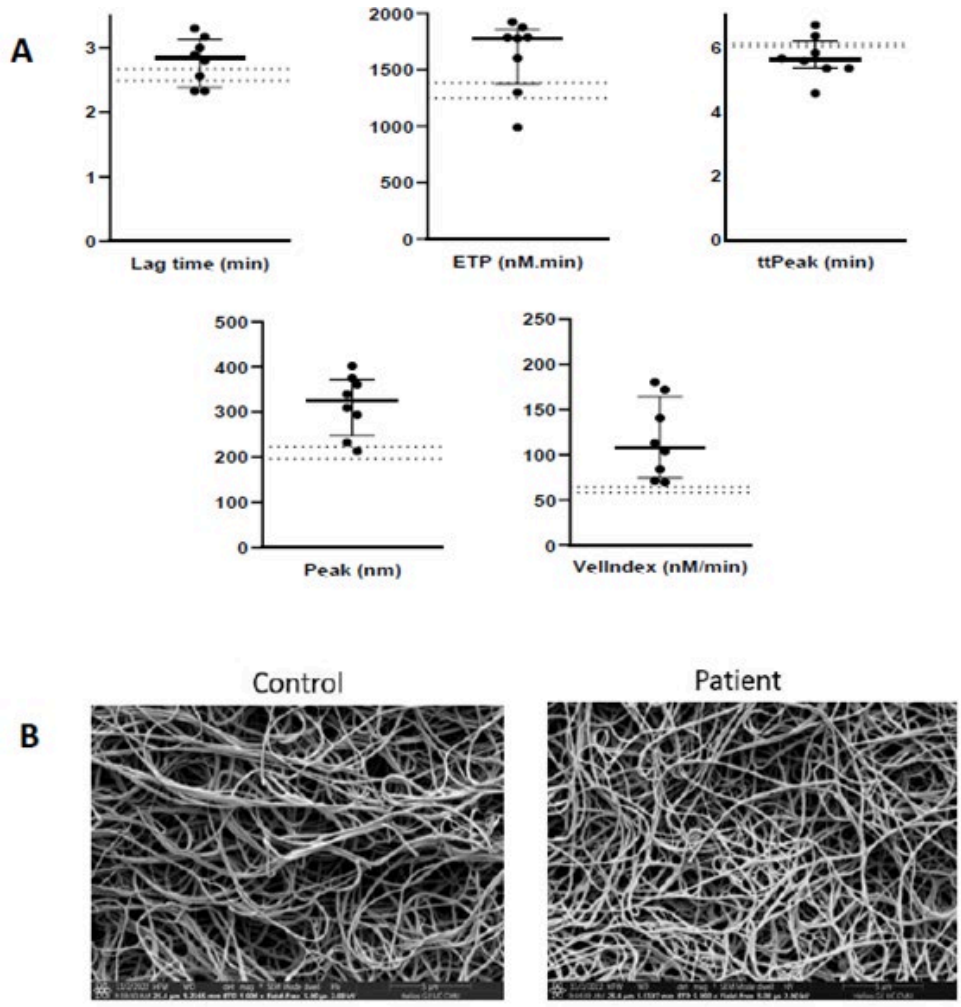
Introduction: Venous thromboembolic disease (VTE) is a well-known complication of nephrotic syndrome (NS) occurring in 25% of patients. The concept of selective or non-selective permeability of the glomerular barrier may explain the variable incidence of VTE in different types of glomerular disorders (GD). There are currently no prospective data that have studied in detail the hemostasis balance between the different diseases of the primary nephrotic spectrum. The aim of our study was to assess the global hemostasis in patients with NS due to primary GD: membranous nephropathy, focal segmental glomerulosclerosis (FSGS), IgA nephropathy (IgAN), or minimal change disease (MCD).

Methods: Patients followed at the nephrology clinic of the McGill University Health Centre and the Geneva University Hospitals are prospectively enrolled. Blood samples were collected within one month from diagnosis or referral. The main exclusion criteria included any anticoagulant medication and immunosuppressive therapy within two weeks and 90 days respectively preceding sample collection. We evaluated the thrombin generation (TG) by Calibrated Automated Thrombography and fibrin clot structure by turbidimetry, permeability and scanning electron microscopy. Patients with different GD and controls (commercial plasma), were compared by a Mann Whitney test at p = 0.05.

Results: We present here preliminary results from 8 patients with a primary GD. Overall, all parameters of TG was markedly increased in patients with primary glomerulopathy compared to controls. Notably, the endogenous thrombin potential was higher in NS (1763 (1451-1831) vs. 1356 (1237-1390) nm.min (p = 0.11)) and the time to peak was lower (5.6 (5.3-6.1) vs. 6.1 (6.0-6.1) minutes (p = 0.14)). Fibrin clot properties indicate a thrombotic phenotype with an increased and faster fibrin polymerization, hypofibrinolysis, reduced permeability , and thinner fibers fibrin (115 (104-154) nm in NS vs. 134 (127-143) nm (p = 0.46) in controls).

Conclusions: Our results converge towards a pro-coagulant state at different levels of hemostasis in patients with NS due to primary GD. To our knowledge such a detailed study of global hemostasis has never been performed before and will allow us to better understand hemostatic balance in nephrotic patients.



217

Ex-vivo supplementation with two different brands of fibrinogen concentrate results in marked difference on fibrin clot network in dysfibrinogenemia.

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Introduction: Fibrinogen concentrates (FC) is usually administered to treat or prevent the risk of bleeding in patients with hereditary dysfibrinogenemia. Several FC are available on the market with similar efficiency and security. The aims of this study were to measure the optimal quantity of fibrinogen to be added to dysfibrinogenemic plasmas of patients in order to improve the clot functionality and structure, and to compare plasma supplementation with two commercial fibrinogen concentrates (Haemocomplettan® (HC) and Fibryga® (FB)).

Methods: Plasmas from seven patients with hereditary dysfibrinogenemia (all with the hotspot mutation Aα Arg35His) were supplemented with several concentrations of HC or FB. The kinetics of fibrin formation and lysis were followed spectrophotometrically at 405 nm. The lag time (min), slope (OD/s)x10-4, MaxAbs (mOD) and T50% (min) were calculated from the curves. The flow through the clots were measured and expressed in terms of area available for flow (Ks: cm2, Darcy constant). Clots were visually inspected by scanning electron microscopy (SEM) and the mean fibers diameter measured (nm).

Results: Independently of the FC, supplementation of dysfibrinogenemic plasma with 2 mg of fibrinogen led to shortening in lag time, increasing of slope, of MaxAbs and T50% and thickening of fibrin fibers similarly to healthy controls. In addition, we observed a decrease in thrombin time suggesting an enhancement of fibrin polymerization. When comparing 2 mg of fibrinogen supplementation with HC or FB, the kinetics of fibrin polymerization was markedly different. Supplementation with FB resulted in higher fibrinogen concentration (3.3 vs 2.8 g/L), more decrease of thrombin time (19.4 vs 22.5 s), shorter lag time (3 vs 3.6 min), higher slope (24.5 vs 8.1 OD/s-4), higher MaxAbs (430 vs 157 OD), thicker fibrine fibers (109 vs 100 nm) but higher permeability (2.5 vs 1.1 cm2x10-8). Similar differences were obtained on healthy control plasma.

Conclusions: In this ex-vivo study, supplementation of 2 mg of FC allows to normalize the fibrin clot properties and structure in dysfibrinogenemic plasma of patients with hotspot mutation. FB had a greater impact than HC for all parameters in dysfibrinogenemic and healthy controls. Future studies will analyze whether such differences are observed for other fibrinogen variants and will determine the impact of FC purity on fibrin clot properties.

233

Targeted management of coexistent severe thrombophilias – A case report of a successful pregnancy despite paroxysmal nocturnal haemoglobinuria and hereditary protein C deficiency

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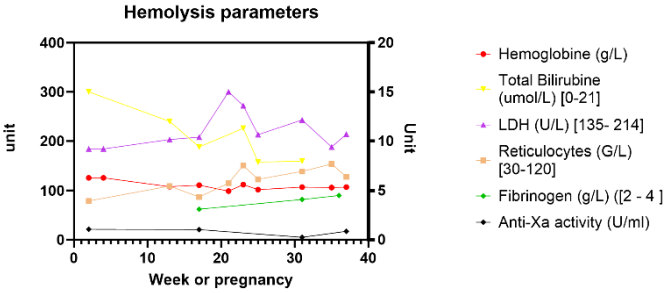
Introduction: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare hematological disorder characterized by the absence of complement regulatory proteins on the surface of erythrocytes, leading to intravascular hemolysis and thrombosis.

Methods: Managing PNH during pregnancy poses significant challenges due to increased risks of morbidity and mortality. This case report describes the obstetric course of a 44-year-old woman with PNH and coexisting hereditary protein C deficiency who had previously experienced multiple thrombotic events and adverse pregnancy outcome (fetal demise caused by placental thrombosis at 24 weeks of gestation).

Results: Eculizumab was introduced at PNH diagnosis and continued throughout pregnancy. Close monitoring of hemolysis and hemostasis parameters was conducted throughout the gestation period. The dosage of eculizumab was increased to 1200 mg every 15 days at the end of first trimester to prevent breakthrough hemolysis. The patient also received full-dose anticoagulation with low molecular weight heparin (LMWH) due to the presence of both arterial and venous thrombotic events, hereditary protein C deficiency, and the hypercoagulable state of PNH. The pregnancy progressed without thrombotic complications or breakthrough hemolysis, and the patient delivered a healthy newborn at 36 weeks gestation. Peripartum anticoagulation management involved switching to intravenous unfractionated heparin to minimize the therapeutic window and thromboembolic risk. Following delivery, therapeutic LMWH was reintroduced for six weeks in the postpartum period.

Conclusions: To the best of our knowledge, this is the first reported case of a positive pregnancy outcome despite PNH in conjunction with hereditary thrombophilia. This case report highlights the importance of a multidisciplinary approach involving hematologists and obstetricians in the management of pregnant women with PNH. Tailored therapy, close monitoring, and comprehensive care are crucial to minimize risks and optimize outcomes. Further research and the development of standardized guidelines are needed to improve the management of pregnant women with PNH and associated thrombotic conditions.



275

Serendipity-1: HbA1c Tina-quant® as potential screening method for Red Blood Cell Diseases

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Introduction: Hemoglobin A1c (HbA1c) is the most common test used for the diagnosis of diabetes, estimating the amount of glucose attached to HbA and showing the average blood glucose level during the past 2 to 3 months. It has been proven that any condition that reduces the erythrocyte survival will erroneously lower the HbA1c results regardless of the method used.

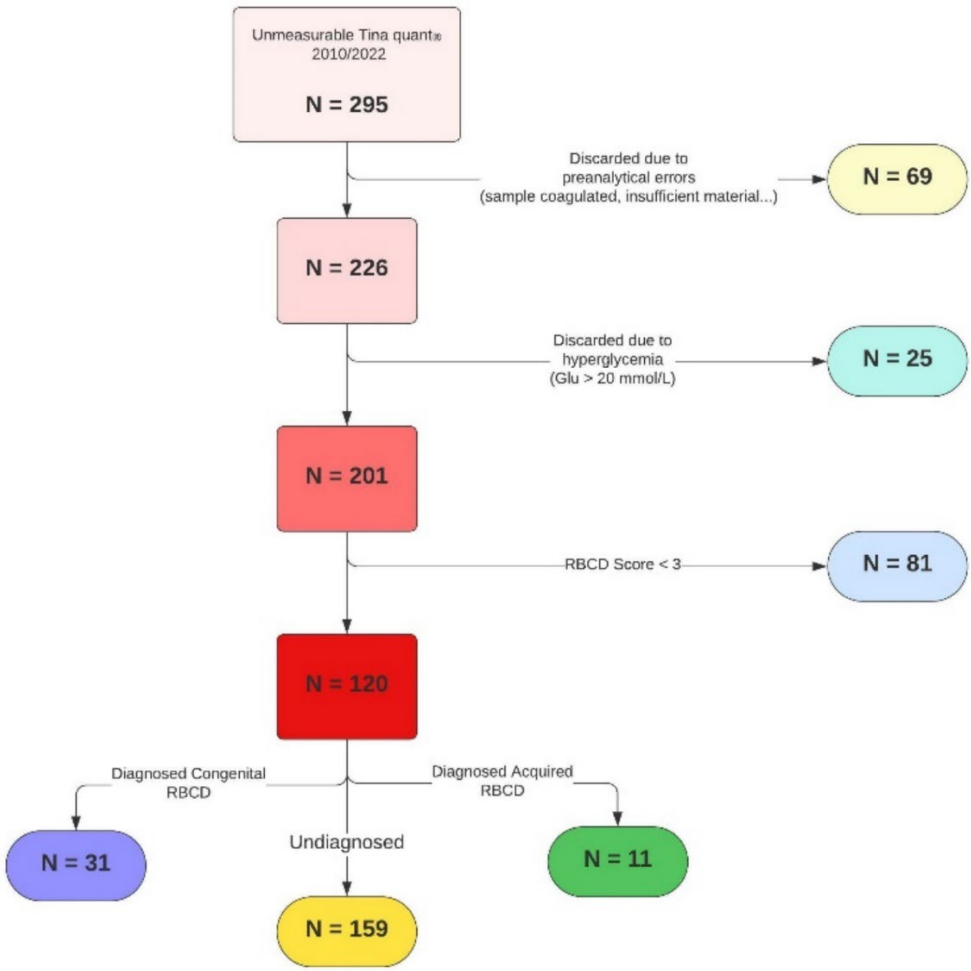
Patients affected by clinically relevant Red Blood Cell Diseases (RBCD) and tested for HbA1c with the immunoturbidometic (IT) assay resulted in a value under the reference spectrum, therefore «unmeasurable». We hypothesize that this method can identify patients affected by RBCD. This retrospective study analyzes causes of unmeasurable HbA1c, measured with the IT assay.

Methods: All the cases tested for HbA1c using Tina-quant® (IT assay) at the central laboratory of our hospital between 01.01.2010 and 31.11.2022 which presented an unmeasurable value were the focus of this study. A hemolytic/dyserythropoesis score evaluating anemia, reticulocytosis, hyperferritinemia, RBC index variations and high normoblasts count was applied (each parameter one point) to identify patients with possible RBCD. Score ≥ 3 was considered high probability. Labormanagement software and the Hospital Database (Microsoft SQL Server management Studio) were queried for score and diagnosis confirmation.

Results: From ca. 89000 patients tested, 295 (0.3%) had unmeasurable HbA1c. Sub-optimal samples (N 69) and hyperglycemia (N 24) were discarded. Of the 201 remaining patients, 81 (40%) had a RBCD score < 3 and none had a previously diagnosed RBCD. Score ≥3: N 31 (15.5%) had a previously diagnosed inherited RBC disease and 11 (5.5%) an acquired one. In 78 (39%) severe anemia was the most frequent explanation.

Furthermore, 29 patients with inherited RBCD were in follow-up in our department in the study period; 27 had an unmeasurable HbA1c and only 2, also affected by insulin-dependent diabetes, had a measurable HbA1c value. There were 4 additional patients with unmeasurable HbA1c and score ≥3, not known in our department but with diagnosed RBCD, according to the clinical files.

Conclusions: The HbA1c IT method combined with the used predictive score demonstrated its potential ability to identify RBCD. New machine learning methods could implement this combination as advantageous cost-effectiveness screening for RBCD.



POSTER – EXPERIMENTAL HEMATOLOGY / ONCOLOGY

266

Biological role of extracellular vesicles in myeloid neoplasms: A systematic review of the current literature

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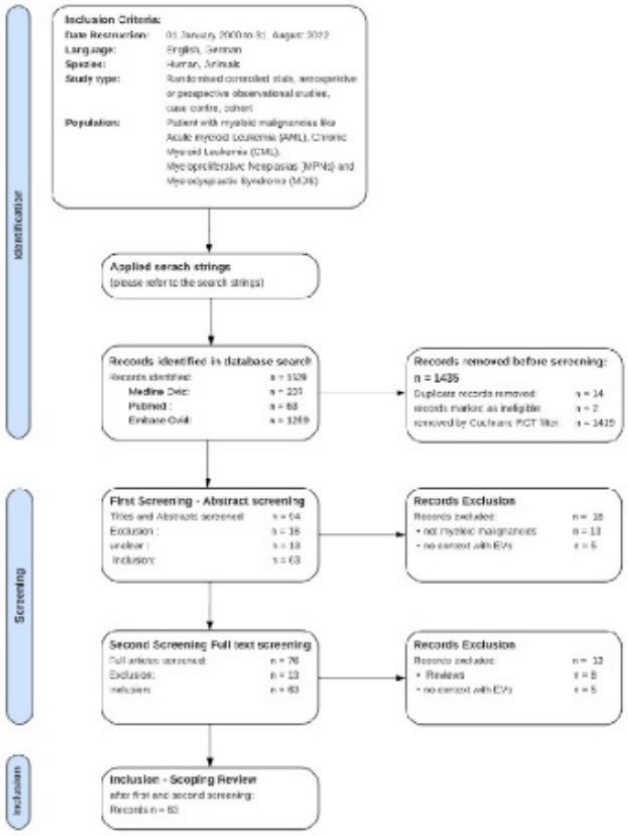
Introduction: Extracellular vesicles (EVs) are lipid-bilayer membrane vesicles secreted by a variety of cells carrying bioactive molecules like proteins, lipids and nucleic acids. They play an important role in the biology of cancer by cell-extrinsic and cell-intrinsic mechanisms. The aim of this review is to summarize the current published literature on the biological role of EVs in myeloid malignancies and to provide an overview of their possible clinical applications.

Methods: We performed a systematic literature research in the Embase, Medline and PubMed Central databases and used a standardized data-extraction form to collect all relevant information.

Results: A total of 63 studies met the inclusion/exclusion criteria and could be categorized into seven main investigational topics regarding their effect (Figure 1). These were prothrombotic activity (n = 13), tumor-microenvironment (n = 14), malignant transformation (n = 5), tumor progression (n = 4), chemotherapy-resistance (n = 5), utility as biomarkers (n = 14) and pharmacotherapeutic role (n = 3). One relevant mechanism how EVs confer their pathophysiological effects is transfering of microRNA (miRNA) from different clonal and non-clonal cells to target the microenvironment, vascular system, as well as immune cells. Examples of all other mechanism identified in our systematic review are summarized in Figure 2. Based on differential abundancy and the content of proteins, lipids and nucleid acids EVs may be suitable as biomarkers for diagnosis, prognosis, and disease monitoring. Finally, their capacity to contain biologically active components could expand their potential use as carriers for the cell-type specific delivery of drugs.

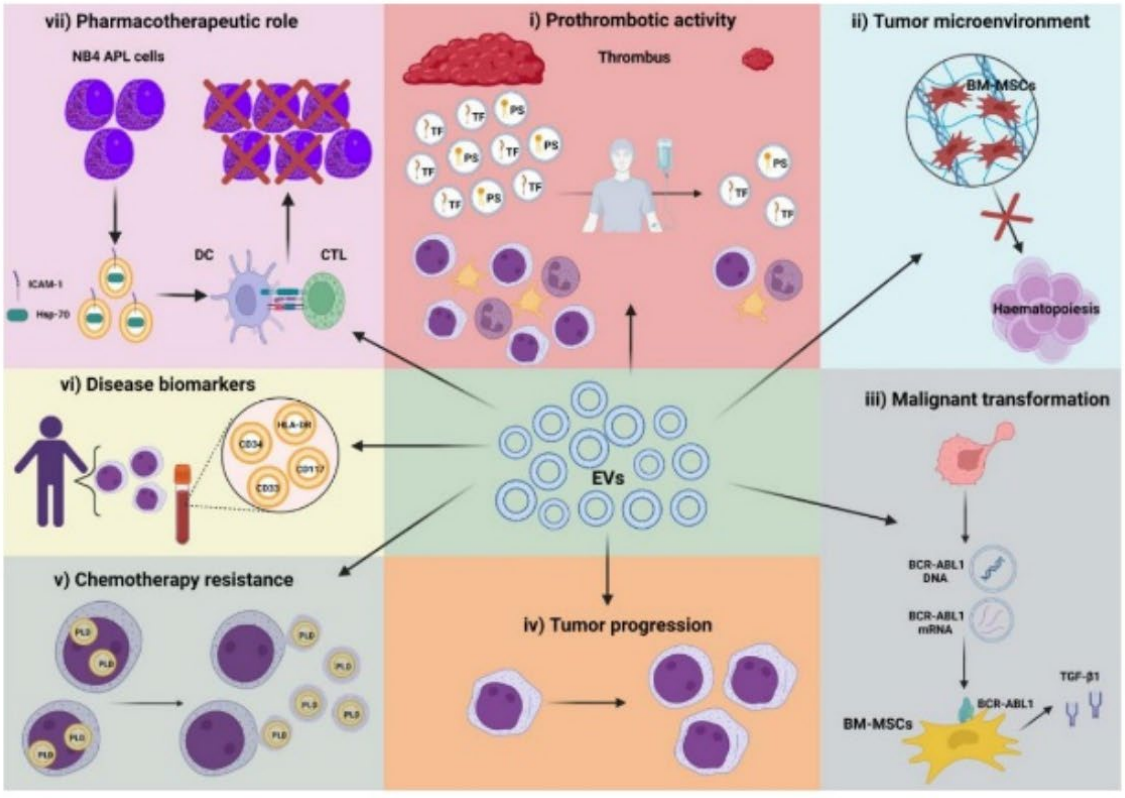
Conclusions: EVs open a new window for the future investigation of novel pathophysiological mechanisms to improve our understanding of the biology of myeloid malignancies and hold promises as biomarkers as well as drug carriers for cell-type specific treatment.

Figure 1. PRISMA flow chart with relevant studies (n=63).



Categories with topics	Number of studies
Prothrombotic activity	**13**
- The connection between EV's and thrombotic Events	11
- How cytoreductive therapy affects the procoagulant activity of EVs	2
The effect of tumor cell EVs on the tumor microenvironment	**14**
- How EVs transfer miRNA to other cells	2
- How EVs can lead to hematopoietic dysfunction	4
- EVs promoting angiogenesis	3
- Bone marrow remodeling	5
Malignant transformation	**5**
Tumor-progression	**4**
- Tumor progression via anti-apoptotic activity of EVs	3
- Tumor progression through immunosuppression by EVs	1
Chemotherapy-resistance	**5**
Biomarkers	**14**
- miRNAs as potential biomarker	6
- EVs count as a biomarker	4
- Mutations and gene transcripts as a biomarker	4
Pharmacotherapeutic role	**3**
Others	**5**

Figure 2. Biological roles of extracellular vesicles in myeloid neoplasms with representative examples



POSTER – CLINICAL HEMATO-ONCOLOGY

199

BCMA-directed bispecific antibodies in plasmablastic lymphoma

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Introduction: Plasmablastic lymphoma (PBL), a rare lymphoma subtype often associated with HIV infection, is characterized by plasmablastic or immunoblastic morphology, high proliferation and at least partial expression of plasma cell markers.

Methods: Here, we present a case of a HIV-positive patient with EBV-associated PBL, who received salvage therapy with the anti-BCMAxCD3 bispecific antibody teclistamab before allogeneic stem cell transplantation (alloSCT) from a donor with high cytotoxic T-cell activity against EBV-infected cells.

Results: Conclusions: The case highlights the need for comprehensive studies on bispecific antibodies in HIV-infected patients and rare disease entities and underscores the importance of donor selection for alloSCT in EBV-associated lymphoma.

223

"Same same but different" – visceral and complicated cutaneous leishmaniasis among lymphoma patients: a case series

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Introduction: Immunocompromised hosts, particularly with cellular immunodeficiency, are at higher risk to reactivate asymptomatic infections with Leishmania spp. We report 2 cases of visceral leishmaniasis with delayed diagnosis due to suspicion of lymphoma recurrence and 1 of treatment refractory cutaneous leishmaniasis complicating completion of lymphoma treatment. All reported a history of travel to the Mediterranean region.

Methods: -

Results:

Case 1: A 54 y.o. male was treated for Hodgkin's lymphoma. During the last chemotherapy cycle he started to experience a new type of fevers – different from the ones he knew from lymphoma. Also, he developed bicytopenia and weight loss of 5kg. In the following 6 months, several diagnostic procedures could not confirm lymphoma relapse. Finally, diagnosis of L. infantum was confirmed through serology and bone marrow biopsy (BMB). Given his recent immunosuppressive treatment, he was treated with liposomal amphotericin B (L-AmB). Daily fevers subsided after the 2nd dose, while splenomegaly and bicytopenia resolved.

Case 2: A 63 y.o. male was diagnosed with lymphoplasmocytic lymphoma and treated with Rituximab-Bendamustin. He was in remission until 8 months later, when he developed recurrent fever, weight loss of 3 kg over 2 months and new onset of progressive pancytopenia. A PET-CT showed hypermetabolic splenomegaly without lymphadenopathy. BMB showed no features of lymphoma recurrence but intracellular organisms in macrophages. PCR for Leishmania spp. was positive. He was traeated with L-AmB for 7 days with resolution of fever after day 3.

Case 3: A 72 y.o. male was treated with two cycles of DA-EPOCH-R chemotherapy for a progressing Burkitt-like lymphoma. In staging PET-CT hypermetabolic activity was detected over the mid upper arm. Biopsy revealed no manifestations of lymphoma, but L. tropica. The lesion worsened and developed secondary central ulceration despite treatment with L-AmB. Second line treatment with local cryotherapy and infiltration of antimonials followed by 2° prophylaxis with L-AmB every three weeks finally lead to resolution of the lesion. Further treatment of his lymphoma was paused, also due to marked asthenia.

Conclusions: Clinicians caring for patients with predominant cellular immunodeficiency should remain aware of this possible and well treatable differential diagnosis that may mimic a relapse or refractory lymphoma.

252

Case series on the use of ropeginterferon alpha-2b for treatment of polycythemia vera

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Introduction: Polycythemia vera (PV) is a myeloproliferative neoplasm characterized by erythrocytosis and mutant JAK2 in most patients. Besides cytoreduction to reduce thromboses, forms of interferon alpha hold potential for disease modification with lower variant allele frequency (VAF). We report first real-world experiences (efficacy and tolerability) with ropeginterferon alpha-2b (Ropeg-INFa2b) which has recently become available in Switzerland

Methods: PV patients (pts) at our center were screened for Ropeg-INFa2b therapy since approval 07/20 to 07/23.

Results: We identified 6 pts (5 males), median age 48y (34-77y). Time since diagnosis at Ropeg-INFa2b start was 7 months to 25 y. All 6 pts had previous cytoreduction including hydroxyurea in 2/6 and pegylated interferon-alpha in 4/6 pts. During treatment, Hematocrit (Hct) was in target range in 5/6 pts with a median Hct 43.8% (35-46%), platelets (Plt) at 401G/l (158-765G/l) and leukocytes 5.7G/l (3.3-9.7G/l). Steady state Ropeg-INFa2b dose varied from 125-300ug/2wks with 250ug/2wks median dose. Median duration on treatment was 15.5 (6-24) months. All patients except one patient (83%) had a good hematological and clinical response. Currently 4/6 pts remain on therapy with counts in target range. Two patients stopped Ropeg-INFa2b, one due to lack of efficacy, and another due to intolerance after 6 months of treatment. Reported side effects were flu-like symptoms (N = 2) and migraine (N = 1). Tolerability in general was good, 3/6 pts did not have side effects. At diagnosis of PV JAK2 V617F VAF was 18-70%. Evaluation of Jak2 V617F VAF in the treatment course is ongoing.

Conclusions: This series shows Ropeg-INFa2b mediates efficacy and favorable tolerability profiles in most PV patients. While courses are heterogenous at this time-point, longer follow-up and increasing number of patients will substantiate real-world experiences with Ropeg-INFa2b for PV therapy

256

Is it myeloma, lymphoma, or monoclonal B cell lymphocytosis? Or all of them? The need of an integrative diagnosis: a case report

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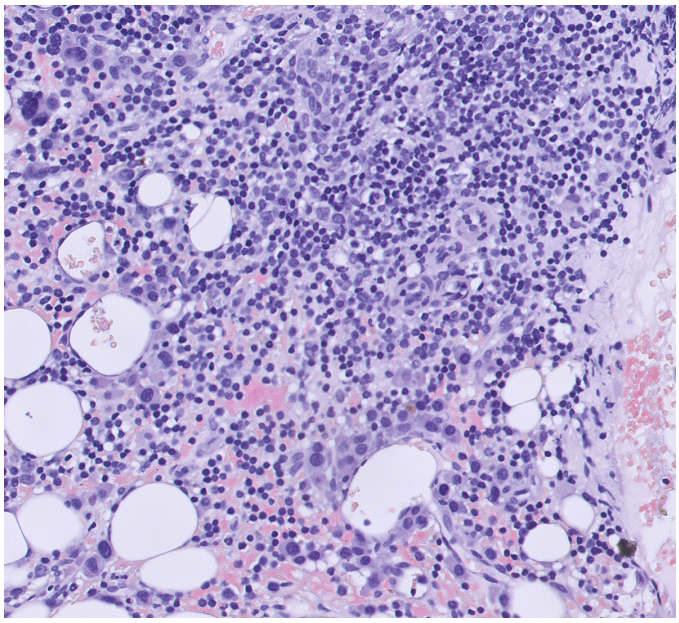
Introduction: The correct diagnosis ist often difficult in haematological malignancies. Often, an integrative approach is needed.

Methods: We present the case of a 66-year old otherwise healthy women presenting with dyspnoe, angina pectoris and fatigue. No bleeding signs, no fever. Weight loss of 6 kg in 12 months. The blood values showed normal white blood cell counts, a normocytic, normochromic, hyporegenerative anemia (hemoglobin 6.1 g/dl), mild thrombocytopenia of 89 G/L; and mild increased LDH (236 U/L). Immunofixation showed biclonal gammopathy IgG lambda and IgM kappa. In the CT scan no lymphadenpathy or organomegaly.; and no osteolysis.

Results: In the bone marrow cytology; increased lymphocytes with suppression of normal hematopoiesis; with suspicion of lymphoma infiltration. 1% plasma cells. Immunophenotyping revealed a mature B-cell population (54%) with expression of CD20, CD22 and CD79b; weak expression of CD19 and negative for CD5, CD10, CD11c, CD23 und FMC7 and light chain restriction kappa. No plasma cells. The results are compatible with splenic marginal zone lymphoma. Further, a monoclonal B-cell population (13%) was found in FACS compatible with CLL/SLL type. FISH showed a 13q14 deletion. Molecular biology showed a MYD88 and TP53 mutation. On the other hand, BM histology revealed a clear infiltration with a biclonal myeloma; 80% lambda-positive, CD138 positive plasma cells; and 30-40% kappa-positive plasma cells (Figure 1). We decided to start therapy with drugs targeting myeloma and lymphoma: Bortezomib, lenalidomide, and dexamethason was started resulting in an increasing hemoglobin level and decreasing M protein. We think the patient has three haematological malignancies: Plasma cell myeloma (lambda), mature non-hodgkin lymphoma (kappa; most likely lymphoplasmacytic lymphoma less likely marginal zone lymphoma) and a monoclonal B cell lymphocytosis of B-CLL type (kappa). MYD88, IgM kappa and the immunophenotype would support the diagnosis of LPL. The 13q14 Deletion and IgG lambda would support the myeloma diagnosis. Possibly, the underestimation of plasma cells in aspirate is due to CD56 expression which has adhesion effects.



Conclusions: Taken together, accurate diagnosis is often challenging in haematological malignancies. An integrative approach including cytomorphology, histology, immunophenotyping, cytogenetics, and molecularbiology are necessary for correct diagnosis.

279

Primary bone marrow lymphoma (PBML): a rare cause of chronic inflammation with diffuse bone marrow hypermetabolism

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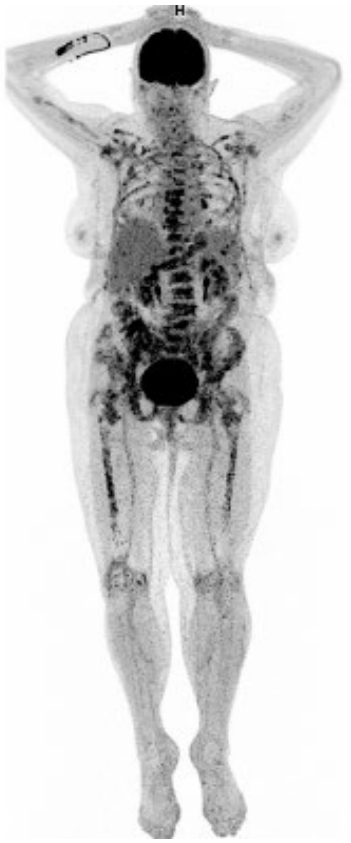
Introduction: Primary bone marrow lymphoma (PBML) is a rare and poorly characterized hematological malignancy, with proliferation of lymphoid cells within the bone marrow, without any evidence of lymphoma in other lymphatic tissues or organs. Patients typically present with non-specific symptoms such as fatigue, fever, night sweats, and weight loss and may develop bone pain or pathological fractures due to bone marrow infiltration. PBML is often associated with unspecific inflammatory syndrome and cytopenias.

Methods: –

Results: An 81-year old patient presented to the emergency with critical symptoms including bilateral chest pain contingent on movement and respiration, dyspnea, and had lost 3 kg in a month. Clinical exams revealed a SpO2 of 88% in ambient air, and pulmonary auscultation unveiled bibasal hypoventilation. Initial tests indicated severe inflammation with CRP at 398 mg/L, ESR at 110 mm/h, ferritin at 2548 µg/L, and LDH at 1159 U/L. Cell blood count showed a normocytic hyporegenerative anemia at 82 g/L, neutrophilia at 5.64 G/L, with normal platelets. PET CT showed a diffuse bone marrow hypermetabolism, without focal lesion. Extensive investigations showed no evident infectious, tumoral, or immunological cause.



She received empirical treatment with Prednisone, leading to a clinical and biological improvement. The bone marrow biopsy revealed a stage IV diffuse large B-cell lymphoma (DLBCL), germinal center-like (Hans), BCL2-, MYC+, highly proliferative (80%), with overexpressed P53, with high R-IPI. The patient underwent 6 cycles of R-CHOP followed by 2 administrations of Rituximab. These were well-tolerated, barring the development of Grade 1 polyneuropathy, associated with significant weight loss at lymphoma diagnosis. Consequently, vincristine in R-CHOP cycles was reduced by half during C5 and omitted during C6. An intermediate PET-CT (post-C2) and treatment-ending PET-CT showed a complete remission (Deauville Score 3). The most recent evaluation, 8 months post-treatment, upheld these findings.

Conclusions: This case underscores the importance of multidisciplinary strategies in diagnosing and treating PBML. It highlights the need for detailed investigations and tailored treatments given the variable presentations of such rare malignancies. This careful approach allows for successful outcomes even when initial prognoses are uncertain, ensuring precise, patient-centric care.

POSTER – CLINICAL SOLID TUMOR ONCOLOGY

196

Phase 3 study of tucatinib, trastuzumab, and modified FOLFOX6 as first-line treatment in HER2+ metastatic colorectal cancer (MOUNTAINEER-03, trial in progress)

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Introduction: Current standard of care (SOC) for treatment of metastatic colorectal cancer (mCRC) is multi-agent chemotherapy, with or without a VEGF or EGFR inhibitor. HER2 amplification occurs in 3%-5% of mCRC patients, and ~5-14% of patients with RAS/BRAF wild-type mCRC tumors. Tucatinib (TUC), a highly selective, HER2-directed tyrosine kinase inhibitor, is approved in multiple regions for HER2+ metastatic breast cancer and is being investigated in gastrointestinal cancers. MOUNTAINEER (NCT03043313) evaluated safety and efficacy of TUC and trastuzumab (TRA) in patients with treatment-refractory RAS wild-type HER2+ mCRC. Results from the primary analysis showed clinically meaningful activity (confirmed objective response rate of 38.1% and median duration of response of 12.4 months) and demonstrated TUC + TRA was well tolerated. MOUNTAINEER-03 will investigate TUC in combination with modified FOLFOX6 and TRA in patients with RAS wild-type HER2+ locally advanced/unresectable or mCRC.

Methods: MOUNTAINEER-03 (NCT05253651) is a global, open-label, randomized, phase 3 study for first-line treatment of HER2+ and RAS wild-type locally advanced/unresectable or mCRC. Approximately 400 adult patients will be randomized 1:1 to the TUC experimental arm (TUC [300 mg PO BID] + TRA + modified FOLFOX6) or the SOC arm (modified FOLFOX6 alone or in combination with either bevacizumab or cetuximab). HER2 status is determined centrally with tissue-based HER2 immunohistochemistry and in situ hybridization. Patients may have received a maximum of 2 doses of mFOLFOX6 in locally advanced/unresectable or metastatic setting prior to randomization and may have received adjuvant treatment if completed >6 months prior to enrollment. Randomization is stratified by primary tumor location (left-sided vs other), liver metastases (presence or absence) and number of doses of mFOLFOX6 chemotherapy prior to randomization (0, 1 or 2). Primary endpoint is progression-free survival per Response Evaluation Criteria in Solid Tumors (RECIST) v1.1, assessed by blinded independent central review (BICR). Key secondary endpoints are overall survival and confirmed objective response rate. Enrollment is ongoing in Europe, and all other regions.

Results: N/A

Conclusions: N/A

POSTER – SUPPORTIVE & PALLIATIVE CARE, REHABILITATION & SURVIVORSHIP

191

Understanding the advanced practice nursing (APN) cancer care workforce: creating their sustainability in Switzerland

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Introduction: The integration of APN roles into cancer service delivery is one important strategy for addressing the increasing patient demands for care. However, healthcare systems are facing a shortfall of nurses, which is mirrored in the global cancer care workforce and in Switzerland. Thus, exploring APN-roles in cancer care is one important step for informing decision-makers about workforce issues that might impinge the sustainable APN-roles integration within the Swiss healthcare system. To better understand the APN-roles in Swiss cancer care this study aimed to describe their characteristics, practice patterns, job satisfaction, and well-being.

Methods: A cross-sectional online survey study was conducted between 12/2021 and 01/2022 in Switzerland. The questionnaire included items exploring APN role characteristics, practice patterns, and validated tools measuring job satisfaction and well-being. Due to the lack of a registry to identify advanced practice nurses, a snowball sampling strategy was used to invite master's prepared nurses working in APN roles in cancer care. Data was analysed with descriptive statistics.

Results: 53 participated in the study. Overall, participants were highly experienced in cancer care and have relevant qualifications in Oncology. Most associated their role with the Advanced Practice Nurse and rarely with the Nurse Practitioner. Cancer services were mostly integrated in hospital settings and in older adult's care. Pneumological, gastrointestinal and urological tumours were the most common diagnosis cared for. APN interventions had multi-components and included activities focusing on patients/families and the healthcare team.

Participants were minimally satisfied with their job due to issues related to autonomous practice, collegiality, and available time for specific tasks. The overall health status was rated 'good' even when burnout scores were high. The majority had not thought about leaving the profession, however, some thought about changing their job.

Conclusions: Strategies to reduce work strain, improve job satisfaction, and address barriers to autonomous practice of Oncology advanced practice nurses will be essential for sustaining the Swiss APN cancer care workforce. Results inform about the need for better workforce planning and policies to strengthen the deployment, recruitment and retention of advanced practice nurses in cancer care.

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